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EFEITO DO CLORETO DE SÓDIO NA DIETA, DUREZA E pH DA ÁGUA NA SOBREVIVÊNCIA, CRESCIMENTO E FLUXOS IÔNICOS DE JUVENIS DE JUNDIÁ (*Rhamdia quelen*)

Tese de Doutorado

Carlos Eduardo Copatti

Santa Maria, RS, Brasil
2008

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por

Carlos Eduardo Copatti

Tese apresentada ao Programa de Pós-graduação em Zootecnia, Área de Concentração em Produção Animal – Fisiologia de Peixes, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de

Doutor em Zootecnia

Orientador: Dr. Bernardo Baldisserotto

Santa Maria, RS, Brasil

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Universidade Federal de Santa Maria
Centro de Ciências Rurais
Programa de Pós-graduação em Zootecnia

A Comissão Examinadora, abaixo assinada,
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elaborada por
CARLOS EDUARDO COPATTI

como requisito parcial para obtenção do grau de
Doutor em Zootecnia

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DEDICO

A minha família
e aos meus amigos.

AGRADECIMENTOS

À Deus.

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Com grandes poderes
Vêm grandes responsabilidades
(Ben Parker, tio do Homem-Aranha)

RESUMO

Tese de Doutorado
Programa de Pós-graduação em Produção Animal
Universidade Federal de Santa Maria

EFEITO DO CLORETO DE SÓDIO NA DIETA, DUREZA E pH DA ÁGUA NA SOBREVIVÊNCIA, CRESCIMENTO E FLUXOS IÔNICOS DE JUVENIS DE JUNDIÁ (*Rhamdia quelen*)

Autor: Carlos Eduardo Copatti
Orientador: Bernardo Baldisserotto
Data e local da defesa: Santa Maria, 22 de abril de 2008

A proposta deste estudo foi verificar o efeito da adição de sal na dieta, dureza da água e pH no crescimento, sobrevivência e ionorregulação de juvenis de jundiá (*Rhamdia quelen*). No primeiro experimento, juvenis foram alimentados com dietas suplementadas com 0,0; 0,5; 1,0 e 2,0 % NaCl e expostos aos pH 5,5; 7,0 e 9,0 por 35 dias. No segundo estudo, peixes foram mantidos por 30 dias em três pH (5,5; 7,0 e 9,0) e quatro durezas da água (30, 60, 120 e 180 mg L^{-1} $CaCO_3$). O terceiro experimento investigou os efeitos do pH dentro da faixa de 6.0-8.0 em baixa dureza da água (0, 25 e 50 mg L^{-1} $CaCO_3$) por 32 dias. Nos experimentos 1 e 2, exemplares foram coletados em diferentes momentos para análise dos fluxos iônicos líquidos de Na^+, K^+ e Cl. Em todos os três estudos, crescimento e sobrevivência foram analisados. A água utilizada foi previamente ajustada para o pH e dureza da água apropriados usando NaOH, H_2SO_4 0,5 M e $CaCl_2.2H_2O$, respectivamente. No primeiro experimento, peixes alimentados dietas sem adição de NaCl e expostos a pH 7,0 apresentaram peso, comprimento, SGR e biomassa por tanque significativamente maiores que aqueles mantidos em pH 5,5, e o aumento de NaCl na dieta protegeu contra o impacto da água ácida. A inclusão de sal na dieta reduziu os distúrbios osmorregulatórios nos juvenis expostos a pH ácido ou básico. No segundo trabalho, juvenis expostos a águas alcalinas ou ácidas não tiveram sua sobrevivência afetada, mas o crescimento foi reduzido em água ácida. E finalmente, no terceiro estudo, juvenis expostos a pH 7,0 e 8,0 em dureza zero da água apresentaram mortalidade significativamente maior que aqueles mantidos em pH 6.0. Nos juvenis expostos a 25 e 50 mg L^{-1} $CaCO_3$ a sobrevivência e o crescimento não foram afetados na faixa de pH 6,0-8,0. Portanto, a melhor dureza da água para crescimento e osmorregulação de juvenis de jundiá se encontra entre 30-60 mg L^{-1} $CaCO_3$, e em dureza baixa (próxima de zero) deve-se reduzir o pH da água. Pode-se concluir que a interação de parâmetros como sal na dieta, pH e dureza da água são deveras importantes no cultivo do jundiá, uma vez que alteram o crescimento e a ionorregulação desta espécie.

Palavras-chave: NaCl dietário; pH; dureza da água; jundiá; crescimento; ionorregulação.

ABSTRACT

PhD Thesis
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EFFECT OF DIETARY SODIUM CLORET, WATER HARDNESS AND pH ON SURVIVAL, GROWTH AND IONIC FLUXES OF SILVER CATFISH (*Rhamdia quelen*) JUVENILES

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The purpose of this study was to verify the effect of dietary salt (NaCl) supplementation, water hardness and pH on growth, survival and ionoregulation of silver catfish (*Rhamdia quelen*) juveniles. In the first experiment, juveniles were fed with diets supplemented with 0.0; 0.5; 1.0 e 2.0 % NaCl and exposed to pH 5.5, 7.0 and 9.0 for 35 days. In the second study, fish were maintained for 30 days in three different pH (5.5; 7.0 and 9.0) and four water hardness (30, 60, 120, and 180 mg L^{-1} $CaCO_3$). The third experiment investigated the effects of the 6.0-8.0 pH range at low water hardness (0, 25 and 50 mg L^{-1} $CaCO_3$) for 32 days. In the experiments 1 and 2, fish samples were collected at different moments for analyses of Na^+, K^+ and Cl^- net ion fluxes. In all three studies, growth and survival were analyzed. The water utilized was previously adjusted to the appropriate pH and water hardness using NaOH or H_2SO_4 0.5 M and $CaCl_2.2H_2O$, respectively. In the first experiment, fish fed with diet without NaCl supplementation and exposed to pH 7.0 presented significantly higher weight, length, SGR and biomass per tank than those exposed to pH 5.5, and the increase of dietary NaCl protected against the impact of a water. Dietary salt supplementation contributed to decrease the osmoregulatory disturbances ın the juveniles exposed to acidic or basic pH. In the second study, exposure of juveniles to alkaline or acidic water did not affect survival, but acidic water reduced growth. And, finally, in the third study, juveniles exposed to pH 7.0 and 8.0 at zero water hardness showed significantly higher mortality than those kept at pH 6.0. Survival and growth of juveniles exposed to 25 and 50 mg L^{-1} $CaCO_3$ was not affected in the 6.0-8.0 pH range. Therefore, the best water hardness for silver catfish juveniles growth and osmoregulation is 30-60 mg L^{-1} $CaCO_3$ and at low water hardness (next zero) pH must be reduced. It can be concluded that the interaction of dietary salt, pH and water hardness are very important to silver catfish rearing, because they change growth and ionoregulation in this species.

Key words: dietary NaCl; water hardness; pH; silver catfish; growth; ionoregulation.

LISTA DE TABELAS

Artigo 01: Effect of dietary salt and water pH on growth and net ion fluxes of silver catfish, *Rhamdia quelen*, juveniles

Artigo 02: Interactions of water hardness and pH on growth and net ion fluxes of silver catfish, *Rhamdia quelen*, juveniles

Artigo 03: Low water hardness and pH on growth and survival of silver catfish, *Rhamdia quelen*, juveniles

LISTA DE FIGURAS

Artigo 01: Effect of dietary salt and water pH on growth and net ion fluxes of silver catfish, *Rhamdia quelen*, juveniles

Artigo 02: Interactions of water hardness and pH on growth and net ion fluxes of silver catfish, *Rhamdia quelen*, juveniles

CONSIDERAÇÕES INICIAIS

A presente tese está composta por:

- introdução geral e objetivos;

-três Artigos científicos formatados conforme a revista a serem encaminhados:

a) Effect of dietary salt and pH on growth and net ion fluxes in silver catfish, *Rhamdia quelen*, juveniles;

b) Interactions of water hardness and pH on growth and net ion fluxes in silver catfish, *Rhamdia quelen*, juveniles;

c) Low water hardness and pH affect growth and survival of silver catfish, *Rhamdia quelen*, juveniles;

- conclusões e perspectivas;

- bibliografia geral.

SUMÁRIO

INTRODUÇÃO GERAL

Condições que prezem boa qualidade de água e fornecimento adequado de alimento são metas imprescindíveis para produção e manejo de peixes. Assim como a criação de quaisquer outras espécies, uma cadeia de eventos está relacionada ao cultivo de peixes. Não só a qualidade da água ou a reprodução, mas todos os aspectos relacionados ao cultivo são importantes para a lucratividade envolvida com a piscicultura. Pesquisas com espécies nativas que respondam positivamente às necessidades comerciais, sociais e ambientais devem ser estimuladas, almejando sempre o melhor desenvolvimento das mesmas. A pesquisa é a garantia de informações confiáveis tanto aos produtores, quanto aos consumidores.

No intuito de tais paradigmas, relata-se que, apesar da existência de experimentos relacionando o efeito do sal comum (NaCl) na água com a sobrevivência de juvenis de jundiá (*Rhamdia quelen*, Quoy & Gaimard 1824, Heptapteridae, Siluriformes) (MARCHIORO & BALDISSEROTTO, 1999; GARCIA et al., 2007), bem como vários estudos acerca da nutrição de juvenis desta espécie (COLDEBELLA & RADÜNZ NETO, 2002; COPATTI et al., 2005; LAZZARI et al., 2007; PEDRON et al., 2008), ainda existe a necessidade de relacionar a presença de dietas suplementadas com sal comum com o crescimento e a sobrevivência de juvenis de jundiás, principalmente para avaliação em pH ácidos e alcalinos. Além disso, estudos sobre diferentes durezas da água também podem auxiliar caminhos mais produtivos para o cultivo do jundiá, reforçando a importância de relacionar tal parâmetro com condições de pH neutras, ácidas e alcalinas. Experimentos relatando o desenvolvimento de larvas (LOPES et al., 2001) e juvenis de jundiá (ZAIONS & BALDISSEROTTO, 2000; TOWNSEND & BALDISSEROTTO, 2001; TOWNSEND et al. 2003; COPATTI et al., 2005) têm demonstrado apresentam boa tolerância a pH ácidos e alcalinos. Contudo, acerca do conhecimento dos diferentes ambientes aos quais tal espécie está sujeita, ocorre o interesse em se considerar o efeito de diferentes níveis de dureza com condições ambientais de pH ácido, neutro e alcalino.

Jundiá *Rhamdia quelen*

O jundiá ocorre desde o sudeste do México até a região central da Argentina. O Brasil é o principal produtor, sendo esta uma das principais espécies nativas cultivadas (CRESCÊNCIO, 2005). Tem como característica boca grande, sem dentes, e ao redor da mesma presença de três pares de barbilhões sensitivos, tendo a pele com coloração variada, desde cinza-esverdeado escuro no dorso até esbranquiçado no ventre (STINGELIN et al., 1998). É um peixe que pode suportar invernos rigorosos para crescer vigorosamente no verão. Na aqüicultura, a melhor densidade oscila entre 2-4 peixes m^{-2}, alcançando 600-800 g de peso corporal em oito meses (BARCELLOS et al., 2004) em condições naturais. Além disso, demonstram grande preferência por lugares sombrios no meio natural (SCHULZ & LEUCHTENBERGER, 2006). Apresenta boa rusticidade e resiste bem a grandes oscilações de temperatura (CHIPPARI GOMES et al., 1999) e níveis baixos de oxigênio na água (BRAUN et al., 2006), porém o conforto térmico está entre 18-28 ^{o}C e sua reprodução ocorre preferencialmente em águas com temperatura de 22-25 ^{o}C, coincidindo com o início da primavera (GUEDES, 1980).

A maturidade sexual (citada para sinonímia *Rhamdia hilarii)* é atingida com um ano de idade em ambos os sexos, os machos com 13,4 e as fêmeas com 16,5 cm (NARAHARA et al., 1985). No Brasil, o cultivo do jundiá está aumentando, mas os dados estatísticos são incompletos ou simplesmente inexistentes. Para a maioria dos países da América Latina os dados da produção indicam somente "catfishes" e, conseqüentemente, não é possível identificar quais espécies estão sendo consideradas. A produção dessa espécie no Brasil durante o ano de 2000 foi de 176.000 t, e destas 2.500 t (1,4 %) são referentes ao jundiá (BALDISSEROTTO, 2003b). É uma espécie nativa adaptada a diferentes ambientes, apresenta bons resultados em viveiros de piscicultura, e excelentes características para fins de processamento (CARNEIRO, 2003) e muito apreciada para o consumo humano na Argentina, Brasil e Uruguai, tendo grande importância nos mercados destes países (SALHI et al., 2004).

A melhor faixa de pH para sobrevivência e crescimento de larvas de jundiá ocorre em pH 8,0-8,5 (LOPES et al., 2001), e alevinos dessa espécie (como de muitas outras espécies) sobrevivem em ambiente ácido ou alcalino quando acrescentado Ca^{2+} na água (TOWNSEND & BALDISSEROTTO, 2001). Para larvas, crescimento, sobrevivência e biomassa foram maiores com 30-70 mg L^{-1} $CaCO_3$ em pH 8,25 (TOWNSEND et al., 2003). A concentração letal de NH_3

(96 h) está em 0,4-2,0 mg L^{-1}, em dependência do pH da água (MIRON et al., in press) e o crescimento é reduzido acima de 0,1 mg L^{-1}, para NO_2 a letalidade (96 h) ocorre em torno de 20 mg L^{-1}, mas seus níveis devem ficar abaixo de 0,46 mg L^{-1} para não originar mortalidade em longo prazo (BALDISSEROTTO & RADÜNZ NETO, 2005).

Influência do pH

Os peixes, assim como outros organismos, possuem limites de tolerância para diferentes fatores no meio ambiente. Isso ocorre para pH, dureza da água e fatores nutritivos, por exemplo. O conhecimento da faixa ideal dos parâmetros físico-químicos é um fator crucial para incentivar o cultivo de inúmeras espécies de peixes (COPATTI et al., 2005). Contudo, situações de adversidade podem acontecer sempre que os limites situarem-se abaixo ou acima da sua capacidade máxima de suporte. Determinar a capacidade de tolerância em ambiente onde o pH é ácido ou alcalino é importante para indicar o balanço geral de íons dos peixes, registrando se ocorrem perdas ou ganhos iônicos. Apesar das modificações fisiológicas impostas por pH ácidos e alcalinos, vários rios no Brasil sustentam uma impressionante diversidade de peixes. Buckup et al. (2007) registraram a ocorrência de 2.587 espécies de peixes de água doce distribuídos em 39 famílias no Brasil, o que demonstra a alta biodiversidade da região neotropical e afirma o aumento do conhecimento em relação à diversidade de peixes no Brasil.

Zweig et al. (1999) informaram que a acidificação da água pode ocorrer em locais onde os solos contêm cátions ácidos, como Al^{3+}, ou pirita de ferro, os quais em baixas condições de oxigenação formam ácido sulfúrico. Águas alcalinas pode ser conseqüência da presença de fitoplâncton ou “blooms” de plantas aquáticas (WOOD, 2001). Alguns lagos apresentam pH muito alcalino, em consonância com a alta concentração de sais de carbonato e bicarbonato, como ocorre no lago Van, na Turquia (pH 9,8) (DANULAT & SELCUK, 1992), no lago Magadi, no Quênia (pH 10,0) (MAETZ & DE RENZIS, 1978), e em alguns lagos do Pantanal, no Mato Grosso do Sul (pH 10,1) (GALVÃO et al., 2003). Contudo, também é possível encontrar águas com pHs muito ácidos (inferior a 4,0), facilmente verificáveis quando o solo possui grandes quantidades de sulfato ou presença de ácidos fúlvicos e húmicos, como na Bacia Amazônica (WALKER, 1990). Inclusive, peixes dessa região, como matrinxã (*Brycon cephalus*), podem ser encontrados em locais em que o pH varia entre 3,7-4,7 (GONZALEZ, 1996), enquanto que

espécies do Pantanal Mato-Grossense estão sujeitas a valores de pH 11,0 em lagoas sujeitas ao isolamento (ZANIBONI FILHO, 2000). Na região sul do Brasil, onde o cultivo de jundiá é bem desenvolvida, existem ambientes com pH da água abaixo de 5,0, bem como outros em que o pH pode alcançar 9,4 (ZAIONS & BALDISSEROTTO, 2000). O pH da água faz parte de um importante mecanismo de homeostase em animais aquáticos e variações na sua constância são reportadas como causas de distúrbios no equilíbrio ácido-básico e regulação iônica (FREDA & MCDONALD, 1988; WILKIE et al., 1994; WOOD, 2001; ARIDE et al., 2007). Em águas ácidas, os íons H^+ em excesso competem com os íons Ca^{2+} e Na^+ da água, inibindo a sua captura pelo peixe, além de afrouxarem as junções protéicas paracelulares da membrana branquial, de modo que aumenta a perda de íons para o meio. Para viverem nesse tipo de ambiente, sugere-se que algumas espécies controlem o efluxo de íons através da alta afinidade dos íons Ca^{2+} a essas junções paracelulares nas brânquias, funcionando como uma barreira à saída de íons (BALDISSEROTTO, 2003a). Aparentemente, um dos mecanismos de sobrevivência de espécies que vivem em águas de durezas muito baixas, como daquelas do rio Negro é a alta afinidade pelo Ca^{2+}, porque uma leve dureza da água de 0,4-2,0 mg L^{-1} $CaCO_3$ providência suficiente Ca^{2+} para fechar junções paracelulares (GONZALEZ et al., 1998). Contudo, presença grande quantidade de ácidos orgânicos dissolvido na água nessas águas escuras também protege de forma similar contra efeitos deletérios de baixos pH da água (GONZALEZ et al., 2002). Exposição às águas de pH muito alcalino pode causar distúrbios no influxo de Na^+ e Cl^-, causado pela inibição do transporte de íons pelas brânquias (WILKIE et al., 1999). Da mesma forma, a excreção de NH_3 é reduzida e, conseqüentemente, ocorre acúmulo de NH_3 no plasma (WILKIE & WOOD, 1994).

Para juvenis de jundiá, Zaions & Baldisserotto (2000) demonstraram que os mesmos não apresentaram mortalidade significativa na faixa de pH de 4,0-9,0 (dureza de 30,0 mg L^{-1} $CaCO_3$) em 96 h, contudo, verificou-se que a exposição de exemplares desta espécie a águas ácidas e alcalinas provoca uma redução dos níveis corporais de Na^+ e K^+. Juvenis de jundiá desenvolvem-se melhor em pH neutro (7,5) em comparação com pH ácido (5,5) e alcalino (9,0) (COPATTI et al., 2005).

Dureza da água

Os vertebrados são dependentes do Ca^{2+} para formação do esqueleto, coagulação sanguínea, funções musculares e transmissão de impulsos nervosos, bem como demais funções celulares (LOVELL, 1989; COOTE et al., 1996). O Ca^{2+} exerce um papel fundamental na regulação iônica porque influi na permeabilidade das membranas biológicas, evitando o fluxo difusivo e as altas perdas iônicas para a água (GONZAL et al., 1987; WOOD & McDONALD, 1988). Segundo Flik et al. (1995), as fontes internas de cálcio são pouco acessíveis, de modo que a regulação do Ca^{2+} plasmático depende basicamente da ingestão deste íon através da alimentação ou de sua captação via brânquias. Hwang et al. (1996) demonstraram que a principal via de absorção do Ca^{2+} é através das brânquias. Para Steffens (1997), a absorção de minerais da água pelos peixes varia em função da espécie e de alguns fatores ambientais, tais como nível de concentração dos minerais, temperatura e pH da água.

Nas brânquias e na membrana opercular dos peixes existe uma bomba de Ca^{2+} (Ca^{2+}-ATPase) e um cotransportador Na^{+}/Ca^{2+} (presentes nas células de cloreto), transportando o Ca^{2+} de dentro das células para o plasma (BALDISSEROTTO, 2003a), reduzindo sua concentração intracelular, o que facilita a entrada de Ca^{2+} do meio para a célula de cloreto através de um canal de Ca^{2+}. Em águas com pouco cálcio, há aumento das células de cloreto como mecanismo compensatório (FLIK et al., 1995). Em águas ácidas, os íons H^{+} em excesso competem com os íons Ca^{2+} e Na^{+} da água, inibindo a sua captura pelo peixe. Acredita-se que algumas espécies controlem o efluxo de íons através da alta afinidade dos íons Ca^{2+} junto às junções paracelulares das brânquias, agindo como barreira a saída de íons (BALDISSEROTTO, 2003a). Em águas alcalinas o mecanismo ainda não é totalmente entendido.

Trutas arco-íris, *Oncorhynchus mykiss*, mantêm constante o Ca^{2+} plasmático em relação ao Ca^{2+} da água doce. Indivíduos desta espécie expostos a águas com baixa concentração de Ca^{2+} (2,5 mg L^{-1} $CaCO_3$) mostraram um aumento no número das células de cloreto na lamela e superfície apical das brânquias para aumentar a absorção deste íon (PERRY & WOOD, 1985). Cascudo, *Hypostomus tietensis*, traíra, *Hoplias malabaricus*, e jejú, *Hoplerythrinus unitaeniatus*, quando transferidos de 1,7 mg L^{-1} $CaCO_3$ para água destilada ou deionizada também mostraram maior proliferação de células de cloreto nas brânquias, mas a superfície apical em contato direto com o meio externo aumentou só na traíra nos primeiros dias depois da transferência (FERNANDES & PERNA-MARTINS, 2002; MORON et al., 2003). Em exemplares de cascudo mantidos em água destilada, as células de cloreto sofreram modificações que preveniram perda

de íons e favoreceram sua absorção em um meio ambiente muito diluído (FERNANDES & PERNA-MARTINS, 2002). Transferência de traíra de 1,7 para 85 mg L^{-1} $CaCO_3$ também induziu uma proliferação transitória de células de cloreto no epitélio lamelar, mas o mesmo não foi observado no jejú (MORON et al., 2003). Por outro lado, "striped bass" *Morone saxatilis* com 30 e 40 dias tiveram maior sobrevivência em 278 do que 10 mg L^{-1} $CaCO_3$, mas o aumento da dureza da água deve ser feito com $CaCl_2$ e não com $MgCl_2$, mostrando a importância do Ca^{2+} nesse período (GRIZZLE et al., 1990).

Sobrevivência a pH ácidos ou alcalinos é aumentada pela adição de Ca^{2+} na água para jundiás (TOWNSEND & BALDISSEROTTO, 2001) e truta arco-íris (McDONALD et al., 1983; YESAKI & IWAMA, 1992). Townsend et al. (2003) estudaram diversos níveis de dureza da água (30, 70, 150, 300 e 600 mg L^{-1} $CaCO_3$) e constataram que os mesmos não afetam a sobrevivência de juvenis de jundiá expostos aos pH 3,5; 4,0; 7,0 e 9,5 por 96 h, porém o aumento da dureza em pH 3,75; 10,0 e 10,5 resultou em maior sobrevivência, sendo que a melhor dureza para a sobrevivência e crescimento de larvas de jundiá na faixa de pH ótimo está entre 30-70 mg L^{-1} $CaCO_3$ (TOWNSEND et al., 2003). Copatti et al. (2005) verificaram que concentrações de 0,08-0,64 % proporcionam melhor crescimento que concentrações de 0,95-2,39 % Ca^{2+} na ração para esta espécie.

Sal na dieta

Peixes adaptados à água doce ou águas com baixa salinidade apresentam uma perda difusiva de íons para o meio ambiente via brânquias e pele, assim como através de fezes e urina. Esta perda iônica pode ser compensada por um influxo ativo da água para as brânquias (WOOD, 2001), da dieta, via intestino (BIJVELDS et al., 1998), e em algumas espécies como muçum, *Synbranchus marmoratus*, pode também ser complementado pela pele (STIFFLER et al., 1986). Wurts (1992) destaca que a adição de sal comum na água reduz a diferença osmótica entre o meio externo e o plasma dos peixes, diminuindo o estresse no transporte de alevinos e adultos de várias espécies, sendo também importantes para o crescimento. Benefícios de dietas suplementadas com NaCl podem diminuir a perda de íons pelas brânquias e estimular a absorção branquial (D´CRUZ & WOOD, 1998).

O acréscimo de sal na ração é outro fator que pode influenciar no crescimento dos peixes, pois o Na^+ na dieta pode ser tão importante quanto o presente na água para suprir as necessidades fisiológicas dos peixes (SMITH et al., 1995; GARCIA et al., 2007). Em juvenis de truta arco-íris, dietas com alto conteúdo de NaCl induziram a um aumento no número de células de cloreto e Na^+/K^+ ATPase, resultando em aumento na captação de íons (SALMAN & EDDY, 1987), enquanto que dietas com baixos níveis de NaCl aumentaram a conversão alimentar, porém mesmo assim não afetaram o crescimento (SALMAN & EDDY, 1988). A atividade da Na^+/K^+-ATPase no intestino (ceco pilórico e intestino anterior) também foi estimulada pela suplementação de Na^+ dietário em truta arco-íris (PYLE et al., 2003), mas não em "bluegill" *Lepomis macrochirus* (MUSSELMAN et al., 1995).

OBJETIVOS

Objetivos gerais

Testar a sobrevivência, o crescimento e o fluxo iônico corporal de juvenis de jundiá (*R. quelen*), em diferentes pH da água sob duas condições:

- dietas suplementadas com sal comum (NaCl);
- diferentes valores de dureza da água.

Objetivos específicos

- Determinar o efeito das dietas com concentrações variadas de sal comum na sobrevivência e no crescimento de juvenis de jundiá nas faixas de pH estipuladas;
- determinar o efeito de dietas suplementadas com sal comum nos fluxos iônicos corporais do jundiá;
- analisar a sobrevivência e o crescimento de juvenis de jundiás aos valores de dureza da água determinados nas faixas de pH trabalhadas;
- verificar o efeito de diferentes níveis de dureza da água nos fluxos iônicos corporais do jundiá.

Hipóteses

Acredita-se que o desenvolvimento de juvenis de jundiá, especialmente em pH fora da faixa de neutralidade, seja beneficiado pelo aumento da concentração de sal na dieta e pelo aumento da dureza da água.

Artigo 01

Sal na dieta e pH da água no crescimento e ionorregulação de juvenis de jundiá

Effect of dietary salt and water pH on growth and net ion fluxes in juveniles of the silver catfish *Rhamdia quelen*

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Abstract

This study verified the optimum dietary salt level for silver catfish juveniles growth and ion regulation at different pH (5.5, 7.0 and 9.0). The control diet was supplemented with NaCl to yield experimental diets with 0.5, 1.0 and 2.0 % NaCl. Juveniles were collected at 2, 15 and 35 days after the beginning of experiment for analyses of Na^+ net fluxes. Exposure of silver catfish juveniles to alkaline or acidic water did not affect survival. Fish fed with diet without NaCl supplementation and exposed to pH 7.0 showed significantly higher weight, length, specific growth rate and biomass per tank than those exposed to pH 5.5. Ionoregulatory disturbances of silver catfish juveniles are less pronounced at pH 5.5 and 9.0 when fed higher dietary salt supplementation (1.0-2.0 % NaCl), and at pH 7.0 with a low amount quantity of salt in the diet (0.0-0.5 % NaCl dietary supplementation). The increase of dietary NaCl reduced mainly body Na^+ loss and protected against the impact of acidic water on growth.

Keywords: dietary NaCl, acidic water, alkaline water, ion fluxes.

Introduction

Many fish are intolerant to low pH, while others, although more tolerant, will avoid low pH if possible (Graham & Hastings 1984). Growth of most fish populations is affected at pH below 6.0 (Wood & McDonald 1988) or above 9.0 (Boyd 1998). At low pH, acid load through the gills is the source of acid-base disturbance (Wood & McDonald 1988), and there is an increase of H^+ and NH_4^+ excretion by the urine to compensate this problem (Bolner & Baldisserotto 2007).

Silver catfish lives in lakes and deep areas of rivers, mainly quiet waters with sand and mud bottoms. This species occurs from central Argentina to southern Mexico (Gomes et al. 2000). Accepts artificial food and possesses high fertility, fast growth, and good acceptance in the fish market and Brazil is the main producer of this species (Baldisserotto 2003). Silver catfish can survive to acute pH changes within the 4.0-9.0 range without significant mortality (Zaions & Baldisserotto 2000). The best pH range for survival and growth of silver catfish larvae is 8.0-8.5 (Lopes *et al.* 2001), and silver catfish juveniles (as well as many other species) survival in acidic and alkaline pH is improved by the addition of Ca^{2+} to the water (Townsend & Baldisserotto 2001, Copatti et al. 2005), but not growth (Copatti et al. 2005). Dietary salt supplementation (1.2, 2.5, 5.0 and 6.0 % NaCl) for 30 days was ineffective as a therapy for ichthyophthiriasis in silver catfish juveniles (Garcia et al. 2007), but in acidic pH (5.2) for 28 days protected against the deleterious effects in rainbow trout *Oncorhynchus mykiss gairdnerii* (Richardson, 1836) juveniles (D'Cruz & Wood 1998).

The objective of this study was to determine the optimum dietary salt at different pH for survival, growth and ion regulation of silver catfish juveniles.

Materials and Methods

Experimental animals and management conditions

Silver catfish (*Rhamdia quelen*) juveniles (n = 468) were obtained from a fish culture in 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. Stocking density was 0.62 juveniles L^{-1}. After 20 days of

acclimation juveniles (5.30 ± 0.19 g and 8.66 ± 0.11 cm) were then transferred to 36 continuously aerated 40 L polypropylene boxes and kept for 35 days. Thirteen juveniles were placed in each box (0.33 juveniles L^{-1}).

Experimental procedure

Twelve treatments (three pH X four treatment diet) were tested in triplicate. Water pH was fixed at 5.5 (5.47-5.49), 7.0 (6.95-6.99) and 9.0 (9.01-9.03) to 0.0; 0.5; 1.0 and 2.0 % NaCl in the diet. Alkalinity and total ammonia were 3.0-4.5 mg L^{-1} $CaCO_3$ and 1.50-1.74 mg L^{-1}, 19.0-23.0 mg L^{-1} $CaCO_3$ and 1.18-1.39 mg L^{-1}, 51.5-52.5 mg L^{-1} $CaCO_3$ and 1.18-1.67 mg L^{-1} at pH 5.5, 7.0 and 9.0, respectively. Nitrite was below 0.05 mg L^{-1}, water hardness levels 21.6-23.3 mg L^{-1} $CaCO_3$, dissolved oxygen levels 5.38-6.80 mg L^{-1}, and temperature 19-20 ^{o}C. Waterborne Na^+, Cl^- and K^+ levels were (in μmol L^{-1}): 150.6, 228.9 and 260.7, respectively.

Experimental diets

The ingredients were ground in a blender when necessary, followed by hydration with approximately 50.0 % v/w tap water. All diets were prepared based on a feed developed for silver catfish by Coldebella & Radünz Neto (2002), which has sugar cane yeast and soybean meal as its main constituents, and 32.0 % crude protein and 3,500 kcal kg^{-1} digestible energy. Salt was added to the food paste. The resulting paste was mixed and extruded through a pasta maker, air-dried, and broken into small pellets with a grinder.

Actual measured Na^+ concentrations in the four diets (0.0, 0.5, 1.0 and 2.0 % addition of salt) were 3 ± 0.1, 13 ± 0.3, 26 ± 0.6 and 53 ± 1.3 μmol kg^{-1}, respectively; K^+ concentrations were 56 ± 4.4, 50 ± 1.8, 45 ± 0.9 and 42 ± 0.4 μmol kg^{-1}, respectively; and Cl^- concentrations were 108 ± 35, 124 ± 36, 155 ± 38 and 396 ± 118 μmol kg^{-1}, respectively.

Tanks management and water quality

Juveniles were fed once a day, at 8:00 a.m. for 35 days (5.0 % body mass). Uneaten food, as well as other residues and feces were siphoned 30 min after furnishing the food and consequently at least 20.0 % of the water was replaced with water previously adjusted (stabilized two weeks before starting the experimental period) to the appropriate pH using NaOH or H_2SO_4

0.5 M. Whenever necessary, water change was increased to reduce of ammonia and nitrite levels. Dead fish were also daily removed and mortality recorded.

Water pH was monitored several times daily between 7:30 a.m. and 5:30 p.m. with a pH meter Quimis (model 400.A). Total ammonia levels were verified once a week by nesslerization according to Greenberg et al. (1976). Dissolved oxygen levels and temperature were measured daily with oxygen meter YSI (model Y5512 Yellow Springs, USA), and temperature was maintained with the use of an air conditioner in the laboratory. Water hardness was calculated once a week with the EDTA titrimetric method (Greenberg et al. 1976). Alkalinity and nitrite levels were determined once a week according to Boyd (1998).

Biometric analysis

Fifteen days after the beginning of the experiments, ten juveniles per replicate were collected for measurement of weight and length and after returned to the tanks. At the end of the experiment (35 days) all remained juveniles were collected and measured. Specific growth rate (SGR), coefficient of variability (CV) for weight and length and condition factor (CF) were calculated according to Jobling (1994).

Net ion fluxes

Nine fishes (Three by replicate) were randomly selected at 2, 15 and 35 days after the beginning of the experiment and were placed for three hours in individual chambers (100 mL) with aeration and water adjusted to the same pH values requested by the experiment for the determination of Na^+ ion fluxes. After a 10 min settling period, water samples (5 mL) were taken from the chambers at the beginning and end of the exposure time and then stored in plastic tubes at -20 °C for posterior analysis of Na^+ levels. Fish were weighed at the end of the flux experiment. Previous experiments of Rosso et al. (2006) demonstrated that net ion fluxes of juveniles maintained for 24 h in chambers were not significantly different from the fluxes of those which measurements started around 10 min after placing them in the chambers.

Water Na^+ was measured with a B4262 flame spectrophotometer (Micronal, São Paulo, Brazil) and net Na^+ fluxes were calculated according to Baldisserotto & Val (2002):

$Jnet = V([ion]_1 - [ion]_2).(Mt)^{-1}$; where:

$[ion]_1$ and $[ion]_2$ are the bath Na^+ concentrations at the beginning and end of the flux period; V is the bath volume in L; M is the mass of the fish in kg; and t is the duration of the flux period in hours.

Statistical analysis

Homogeneity of variances among the different groups was tested with Levene test. Data of treatment groups presented homogeneous variances and were compared by two-way ANOVA (pH X dietary salt supplementations) followed by the Tukey test. All statistical tests were made with the aid of the software Statistica version 5.1 (1997). Data were expressed as means ± SEM, and the minimum significance level was set at $P < 0.05$.

Results

Survival of silver catfish juveniles was higher than 90.0 % in all treatments, and there were no significant difference among treatments. Water dissolved oxygen, temperature, total ammonia, and nitrite did not show any significant difference among treatments. Up to 15 days growth was also not significantly affected by either pH or dietary NaCl supplementation. Thirty five days after the beginning of the experiment, fish fed with diet without NaCl supplementation and exposed to pH 7.0 presented significantly higher weight, length, biomass per tank and SGR than those exposed to pH 5.5. Dietary NaCl supplementation did not improve growth of fish exposed to pH 7.0 and 9.0, but reduced the deleterious effect of pH 5.5 on growth (Table 1). Condition factor (overall range 0.75-0.93 g cm^{-3}); coefficients of variability for weight (overall range 23.54-36.00 %) and length (overall range 7.88-9.48 %) were not significantly affected by either pH or diets.

At two days of the experiment net Na^+ fluxes were not significantly affected by either pH or diets.

Fifteen days after the beginning of the experiment specimens maintained at pH 9.0 and fed 0.0 and 2.0 % dietary NaCl supplementation showed net Na^+ influxes significantly higher than those fed with other diets at the same pH or with same diets at the other pH (Fig 1A). At 35 days of experiment, the inclusion of the salt reverted the influx or increased the efflux in the treatments with 0.0 and 0.5 % NaCl dietary supplementation, but at pH 9.0 fish fed 1.0 and 2.0 %

NaCl dietary supplementation presented net Na^+ loss significantly higher than those fed with other diets. Individuals exposed to pH 9.0 with 0.0 and 0.5 % NaCl dietary supplementation presented net Na^+ loss significantly higher than those maintained at pH 5.5 and 7.0 and fed the same diet (Fig 1B).

Juveniles exposed to pH 5.5 and 7.0 with 2.0 % NaCl dietary supplementation showed net Cl^- effluxes while fish from all other treatments presented net Cl^- influxes 15 days after the beginning of the experiment (Fig 1C). After 35 days juveniles from all treatments presented net Cl^- effluxes. Fish fed 1.0 % NaCl dietary supplementation showed significantly lower net Cl^- efflux than those kept at pH 7.0 and 9.0 and Juveniles maintained at pH 9.0 fed 2.0 % NaCl dietary supplementation presented net Cl^- efflux significantly lower than those exposed to pH 5.5 and 7.0 (Fig 1D).

Juveniles presented net K^+ effluxes 15 and 35 days after the beginning of the experiment (Fig 1E and 1F). After 15 days, individuals kept at pH 5.5 and fed 0.5 and 1.0 % salt supplementation presented significantly lower net K^+ effluxes than those fed 0.0 and 2.0 % dietary NaCl supplementation. Juveniles exposed to pH 7.0 and fed 0.0 and 1.0 % dietary NaCl supplementation presented significantly lower net K^+ effluxes than those fed with other diets and kept at the same pH. Specimens maintained at pH 7.5 and 9.0 and fed 0.5 % dietary NaCl supplementation showed significantly higher net K^+ efflux than those kept at pH 5.5 and the same diet and those fed with other diets and exposed to the same pH (Fig 1E).At 35 days of experiment, juveniles kept at pH 5.5 and fed diet without NaCl supplementation exhibited significantly higher net K^+ efflux than those exposed to pH 9.0 and fed the same diet. Individuals maintained at pH 5.5 and 9.0 and fed 0.5 % dietary NaCl supplementation showed significantly higher net K^+ efflux than those exposed to pH 7.0 and fed the same diet. Juveniles fed 1.0 and 2.0 % NaCl dietary supplementation and kept at pH 57.0 presented significantly higher net K^+ effluxes than those exposed to pH 5.5 and fed the same diet (Fig 1F).

Discussion

A change in pH is responsible for significant alterations in ion profiles of fish (Matsuo & Val 2002). Exposure to low pH increases branchial Na^+ efflux due to an opening of tight junctions of gill epithelia, increasing ion loss by a paracellular route (Gonzalez 1996). Inhibition

of Na^+ influx is also a typical response of freshwater fishes to low pH (Wood 1989). One of main problems in alkaline waters is the inhibition of ammonia excretion. High water pH also inhibits branchial Na^+/NH_4^+, Cl^-/HCO_3^- and Na^+/H^+ exchangers (the last one due to an internal alkalosis, which decreased availability of H^+ for exchange against Na^+) (Wood 2001).

In the present study silver catfish juveniles exposed to pH 7.0 presented higher weight, length, SGR, and biomass per tank than those exposed to pH 5.5 and without NaCl dietary supplementation. Most species survive to pH from 4.5 to 9.0 (Parra & Baldisserotto 2007), and silver catfish has a similar pattern, because it can survive to acute pH changes within the 4.0-9.0 range without significant mortality and exposure of this species to the 5.5-9.0 pH range did not change whole body Na^+ and K^+ levels (Zaions & Baldisserotto 2000) and survival was also not affected in specimens kept at pH 5.5 or 9.0 for 30 days compared to those exposed to pH 7.5 (Copatti et al. 2005).

The negative influence of acidic pH on fish growth was previously reported in others species, like brook trout, *Salvelinus fontinalis* (Mitchill 1814), and rainbow trout that presented lower growth at acidic water (pH 5.3) than neutral waters (pH 7.0) (Menendez 1976; Rodgers 1984). Some studies proposed that acidic pH may impair growth in rainbow trout due to a decrease on food consumption (D'Cruz & Wood 1998), as was observed in silver catfish (Copatti et al. 2005).

The present study verified that at 15 and 35 days of experiment silver catfish from almost all treatments presented net Na^+, Cl^- and K^+ effluxes (except net Cl^- influxes in most treatments at 15 days). The ionoregulatory disturbances at the end of the experiment were less pronounced in silver catfish fed 1-2% NaCl dietary supplementation, regardless of pH exposure. This result is in agreements with the explanation of Wood & McDonald (1988) that suggested that the inhibition of Na^+ uptake in fish exposed to acidic pH is associated with competition between H^+ and Na^+ for the transport area of the branchial epithelium. The plasma concentrations of K^+ and Na^+ followed the pH profile, showing a decrease during acid exposure (4.0) and an increase during alkaline exposure (8.0) in tambaqui *Colossoma macropomum* (Cuvier, 1818) and prolonged exposure to alkaline water results in several changes in the blood physiology and in reduced growth of tambaqui, alkaline exposure resulted in more effects severe on the physiology this specie than an acid exposure (Aride et al. 2007). Urine flow rate and urine and plasma pH in silver catfish showed a significant trend to increase with the increase of water pH (4.0, 5.0, 7.5, 8.0 and 9.0).

Silver catfish exposed to pH 4.0 for 24 h presented a significant decrease on plasma Cl^- levels, but only those exposed to water pH 5.0 and 6.0 for 24 h showed reduced plasma Na^+ and K^+ levels compared to those maintained at water pH 7.5 (Bolner & Baldisserotto 2007). Rainbow trout exposed to water pH 4.2 for 24 h also showed a significant decrease on plasma Na^+ and K^+ levels, but not Cl^- (McDonald & Wood 1981).

Rainbow trout exposed to acidic pH (5.2) for 28 days and fed with a low NaCl diet (0.10-0.18 %), independently of energy content, presented a decrease on plasma Na^+ and whole body Na^+ and Cl^- (the last, only the low energy diet), but fish fed with 0.6 % NaCl did not show any ionic imbalance. In our study, the increase of dietary salt apparently decreased Na^+ and K^+ losses of silver catfish exposed to pH 5.5 and pH 9.0. Therefore, is the salt content of the food rather than the energy content that is critical in protecting against the effect of acidic pH (5.2) (D'Cruz & Wood 1998). In acidic water, high H^+ concentrations disrupt the tight junctions of gill epithelia, increasing ion loss by a paracellular route (Wood & McDonald, 1988), and leading to whole body ion loss, as observed in silver catfish (Zaions & Baldisserotto 2000). Under these conditions, dietary salts may become very important in maintaining body ion levels during acid stress (D'Cruz & Wood 1998). Starved rainbow trout (or fed with a very limited diet) showed ionoregulatory changes during exposure to acidic environment (pH 5.2), but when they were fed with adequate amount of salts (263 μmol kg^{-1}) the effect of low pH was reduced or did not occur (D'Cruz et al. 1998). Therefore, dietary salt can replace branchial ion loss because when fish are exposed to acidic pH branchial ion influx is lower and the efflux is higher than in neutral waters, and dietary salt supplementation may help to maintain ionic balance (D'Cruz & Wood 1998). To our knowledge, there are no studies regarding the effects of dietary salt in fish exposed to alkaline waters. The results of the present study demonstrated that the salt has a primordial function in the ionoregulatory balance of silver catfish juveniles.

The intestine (or the pyloric ceca) can absorb ions provided by feeding (Bijvelds et al. 1998; Becker et al. 2006; Ferreira & Baldisserotto 2007). Therefore, diet can be an important ion source for ionoregulatory needs of fish living in hyposaline environments. Dietary salt supplementation can also decrease energy spent on ionoregulation and consequently more will be available for growth (D'Cruz & Wood 1998). Therefore, in present study it was hypothesized that NaCl-supplemented diets would protect against acidic or alkaline pH, compensating ion loss. Dietary NaCl supplementation decreases ionoregulatory disturbances and is not effective to

increase silver catfish growth in neutral and alkaline waters, but improve growth in juveniles exposed to acidic pH.

Rainbow trout fed high NaCl diets (1.8 and 3.0 % Na^+) showed a decrease of 40.8 and 44.0 % on waterborne Na^+ whole body uptake rates relative to controls (0.6 % Na^+). Moreover, Na^+ efflux was 12.0 and 38.0 % higher in fish fed 1.8 and 3.0 % Na^+-enriched diets, respectively (Pyle et al. 2003). Plasma Cl^- levels in bluegill *Lepomis macrochirus* (Rafinesque, 1819) maintained in freshwater and fed diet supplemented with 2.0 or 4.0 % NaCl were also higher than in fish kept in freshwater and fed a diet without NaCl supplementation (Musselman et al. 1995). In present study, results demonstrated ionoregulatory disturbances of silver catfish juveniles are less pronounced at pH 5.5 and 9.0 when fed higher dietary salt supplementation (1.0-2.0 % NaCl), and at pH 7.0 with a lower amount quantity of salt in the diet (0.0-0.5 % NaCl dietary supplementation). Nile tilapia *Oreochromis niloticus* (L., 1758) maintained in freshwater and fed diet supplemented with 8.0 % NaCl for 35 days showed higher growth rate than those fed diet without NaCl supplementation, while dietary NaCl did not change significantly growth rate in fish kept in brackish water (1.0 and 2.0 %) (Fontaínhas-Fernandes et al. 2002).Dietary salt supplementation can improve growth or reduce ionic losses in some species, butin others there is no effect or even present negative effects in their development, as demonstrated by Pyle et al. (2003) with rainbow trout.

It can be concluded that exposure of silver catfish juveniles to alkaline (pH 9.0) or acidic (pH 5.5) water did not affect survival, but fish kept at acidic water and fed a diet without NaCl supplementation presented reduced growth compared to those exposed to neutral pH (pH 7.0). The increase of dietary NaCl protects against the impact of acidic water. To neutral pH is recommended 0.5 % NaCl in the diet, but to acidic or alkaline waters the best salt dietary supplementation is overall 1.0-2.0 % NaCl.

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Table 1 Effect of dietary salt (NaCl) supplementation and pH on biometric parameters of silver catfish after 35 days.

	Dietary NaCl supplementation (%)			
pH	0.0	0.5	1.0	2.0
		Weight (g)		
5.5	$3.98^{B} \pm 0.24$	$4.63^{A} \pm 0.40$	$4.81^{A} \pm 0.45$	$4.48^{A} \pm 0.14$
7.0	$6.67^{A} \pm 0.22$	$5.34^{A} \pm 0.28$	$5.83^{A} \pm 0.35$	$5.75^{A} \pm 0.42$
9.0	$5.41^{AB} \pm 0.37$	$5.06^{A} \pm 0.17$	$5.18^{A} \pm 0.08$	$4.79^{A} \pm 0.40$
		Length (cm)		
5.5	$7.89^{B} \pm 0.16$	$8.46^{A} \pm 0.29$	$8.57^{A} \pm 0.27$	$8.27^{A} \pm 0.09$
7.0	$9.48^{A} \pm 0.08$	$8.89^{A} \pm 0.13$	$8.58^{A} \pm 0.20$	$8.83^{A} \pm 0.17$
9.0	$8.61^{AB} \pm 0.17$	$8.57^{A} \pm 0.06$	$8.65^{A} \pm 0.06$	$8.40^{A} \pm 0.28$
		Biomass per tank (g)		
5.5	$51.70^{B} \pm 3.08$	$60.19^{A} \pm 5.14$	$62.53^{A} \pm 5.86$	$55.20^{A} \pm 2.18$
7.0	$86.75^{A} \pm 2.89$	$69.42^{A} \pm 3.71$	$75.79^{A} \pm 4.66$	$66.55^{A} \pm 7.43$
9.0	$70.37^{AB} \pm 4.82$	$65.82^{A} \pm 2.27$	$65.67^{A} \pm 2.27$	$62.31^{A} \pm 5.18$
		SGR (% day^{-1})		
5.5	$-0.83^{B} \pm 0.17$	$-0.41^{A} \pm 0.24$	$-0.30^{A} \pm 0.27$	$-0.48^{A} \pm 0.05$
7.0	$0.66^{A} \pm 0.09$	$0.01^{A} \pm 0.15$	$0.26^{A} \pm 0.18$	$0.22^{A} \pm 0.22$
9.0	$0.05^{AB} \pm 0.20$	$-0.13^{A} \pm 0.09$	$-0.06^{A} \pm 0.05$	$-0.30^{A} \pm 0.26$

Values are reported as mean ± S.E.M, n = 3. Means identified by different capital letters in the columns were significantly different ($P < 0.05$) as determined by analysis of variance and Tukey comparison of mean values. In the rows, there were no significant difference among different dietary NaCl supplementation for the same pH value.

Figure legend

Figure 1 Net Na^+, Cl^- and K^+ fluxes in *R. quelen* after 15 days (A, C and E respectively) and 35 days (B, D and F respectively) exposure to different pH and dietary NaCl supplementation. Data are expressed as mean ± SEM, n = 9. Positive values indicate net influxes and negative values net effluxes.

Means identified by different capital letters indicate significant difference among pH in the same dietary NaCl supplementation while means identified by different small letters indicate significant difference among different dietary NaCl supplementation at the same pH as determinate by two-way ANOVA and Tukey comparison of mean values ($P < 0.05$).

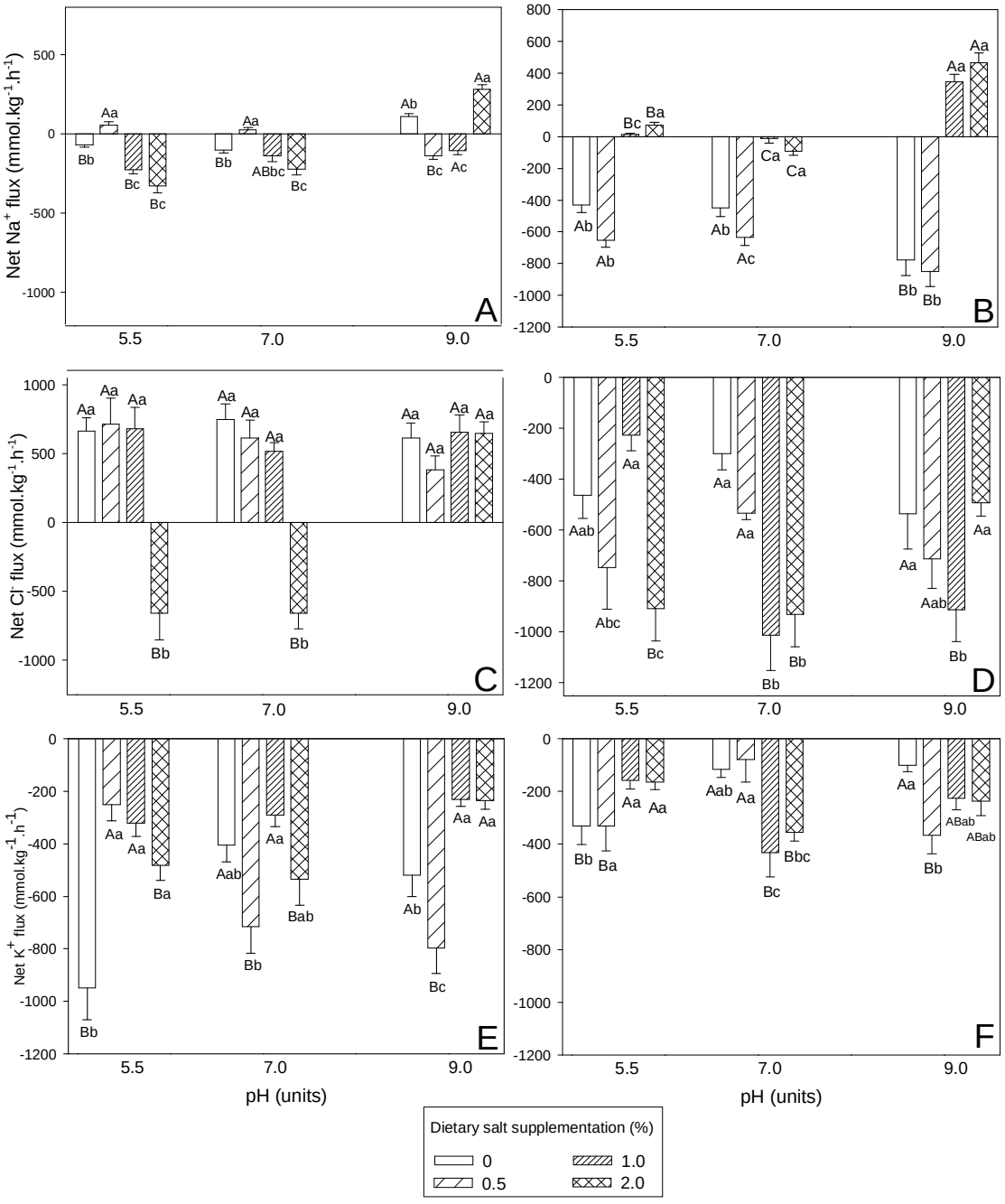
Net Na+ flux (mmol.kg-1.h-1)
Net Cl- flux (mmol.kg-1.h-1)
Net K+ flux (mmol.kg-1.h-1)
A
B
C
D
E
F
5.5
7.0
9.0
pH (units)
Dietary salt supplementation (%)
0
0.5
1.0
2.0

Figure 1

Artigo 02

Dureza e pH da água no crescimento e ionorregulação de juvenis de jundiá

Interactions of water hardness and pH on growth and net ion fluxes in silver catfish, *Rhamdia quelen*, juveniles

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Abstract

This study verified the satisfactory water hardness at different pH for growth and ion regulation in silver catfish juveniles. Fish were maintained for 30 days at three water pH (5.5; 7.0 and 9.0) and four water hardness (30, 60, 120 and 180 mg L^{-1} $CaCO_3$). Juveniles were collected at two and fifteen days for analyses of net Na^+, K^+ and Cl^- - body fluxes. Exposure of silver catfish juveniles to alkaline or acidic water did not affect survival, but reduced growth, and in juveniles at alkaline water the best weight occurred in those kept at the lowest water hardness. At fifteen days of experiment, net Na^+ body fluxes in juveniles maintained at higher water hardness and net Cl^- fluxes at ever water hardness led to net ion loss. Therefore, the best water hardness for silver catfish juvenile growth and ionoregulation is 30-60 mg L^{-1} $CaCO_3$.

Keywords: growth, acidic water, alkaline water, calcium, sodium fluxes, chloride fluxes

Introduction

Water acidification may occur in places where the soil contains acidic cations, as Al^{3+} or iron pyrite, which in oxygenating conditions forms sulfuric acid (Zweig, Morton & Stewart 1999). Alkaline waters may be consequence of phytoplankton or aquatic plants blooms, which decrease the CO_2 available in the water during daylight (Wood 2001).

Calcium is important for ion regulation of freshwater fish because it influences the permeability of biological membranes, preventing the diffusive efflux and high ionic loss to water (Wood & McDonald 1988). It is also essential for several biological processes such as bone construction, blood coagulation, and other cellular functions (Flik, Verbost & Bonga 1995). Freshwater fish take up Ca^{2+} predominantly by the gills, and even those fed a Ca^{2+} deficient diet grow normally if there is enough waterborne Ca^{2+} to be absorbed (Flik & Verbost 1995). However, in low Ca^{2+} water the relative contribution of the food increases like a mechanism of compensation (Steffens 1997).

Silver catfish, *Rhamdia quelen* (Quoy & Gaimard, 1824, Heptapteridae), occurs from southern Mexico to central Argentina. Brazil is the main producer of this species, which was the fifth most raised native species in 2004 (Crescêncio 2005). This species can reach 600-800 g in eight months in aquaculture systems (Barcellos, Kreutz, Quevedo, Fioreze, Cericato, Soso, Fagundes, Conrad, Baldissera, Bruschi & Ritter 2004). In southern Brazil, where the culture of silver catfish is increasing, surface waters can sometimes present pH lower than 5.0 and in underground waters used by fish farmers pH can reach 9.4 (Zaions & Baldisserotto 2000). The best pH range for survival and growth of silver catfish larvae is 8.0-8.5 (Lopes, Silva & Baldisserotto 2001), and juveniles survival in acidic and alkaline pH is improved by the addition of Ca^{2+} to the water (Townsend & Baldisserotto 2001). Higher larvae growth, survival, and biomass were obtained at 30-70 mg L^{-1} $CaCO_3$ at pH 8.25 (Townsend, Silva & Baldisserotto 2003). Growth of juveniles of this species is higher at pH 7.0 compared to acidic (pH 5.5) or alkaline (pH 9.0) soft water and dietary Ca^{2+} supplementation does not protect against pH change (Copatti, Coldebella, Radünz Neto, Garcia, Rocha & Baldisserotto 2005).

Therefore, the objective of this study was to determine the satisfactory water hardness at different pH for survival, growth and ion regulation in silver catfish juveniles.

Materials and Methods

Experimental animals and management conditions

Four hundred and sixty eight silver catfish juveniles were obtained from the fish culture Bela Vista in 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. Stocking density was 0.62 juveniles L^{-1}.

After 15 days of acclimation, juveniles (2.08 ± 0.20 g and 6.01 ± 0.18 cm) were then transferred to 36 continuously aerated 40 L polypropylene boxes and kept for 30 days. Thirteen juveniles were placed in each box (0.33 juveniles L^{-1}).

Twelve treatments (three pH X four water hardness), were tested in triplicate. Water pH was fixed at 5.5 (5.41-5.56), 7.0 (7.02-7.06) and 9.0 (8.85-9.05) and water hardness at 30 (30.44-31.24), 60 (58.33-60.39), 120 (118.06-122.24), and 180 (178.89-180.28) mg L^{-1} $CaCO_3$.

Alkalinity and total ammonia were 4-5 mg L^{-1} $CaCO_3$ and 0.68-0.95 mg L^{-1}, 19-23 mg L^{-1} $CaCO_3$ and 0.76-1.41 mg L^{-1}, 51-52.5 mg L^{-1} $CaCO_3$ and 0.83-1.78 mg L^{-1} at pH 5.5, 7.0 and 9.0, respectively. Nitrite was below 0.05 mg L^{-1}, dissolved oxygen levels 5.24-6.47 mg L^{-1} and temperature 21-22 ^{o}C. Waterborne Na^{+}, Cl^{-} and K^{+} levels were 150.6, 228.9 and 260.7 μmol L^{-1}, respectively.

Tanks management and water quality

The diet offered was a commercial feed (Vicente Alimentos S.A. Presidente Prudente/SP, Brazil) with 3.5 % Ca^{2+}, 28.0 % crude protein and 3,500 kcal kg^{-1} digestible energy according to manufacturer. The juveniles were fed once a day, at 8:00 a.m., for 30 days, at a ratio of 5.0 % body mass. Uneaten food, as well as other residues and feces were siphoned 30 min after furnishing the food and consequently at least 20.0 % of the water was replaced with water previously adjusted to the appropriate pH and water hardness using NaOH or H_2SO_4 0.5 M and $CaCl_2.2H_2O$, respectively. Whenever necessary, water change was increased to reduce ammonia and nitrite levels. Dead fish were also daily removed and mortality recorded.

Water pH was monitored several times daily between 7:30 a.m. and 5:30 p.m. with a pH meter Quimis (model 400.A). Water hardness was measured every two or three days with the

EDTA titrimetric method and total ammonia levels were verified once a week by nesslerization according to Greenberg, Taras & Rand (1976) and non-ionized ammonia levels were calculated according to Piper, Mcelwain, Orine, McCraren, Fowler & Leonard (1982). Dissolved oxygen levels and temperature were measured daily with oxygen meter YSI (model Y5512 Yellow Springs, USA), and laboratory temperature was maintained with the use of an air conditioner. Levels of total alkalinity and nitrite were determined once a week according to Boyd (1998).

Net ion fluxes

Three fishes (n = 3) were randomly selected from each replicate at two and fifteen days after the beginning of the experiment and were placed in individual chambers (100 mL) with aeration and water adjusted to same conditions of the experiment to the determination of the net ion fluxes.

After a 10 min settling period, water samples (5 mL) were taken from the chambers at the beginning and 3 h later and then stored in plastic tubes at -20 °C for posterior ionic analysis (Na^+, Cl^-, and K^+ concentrations). Fish were weighed at the end of the experiment. Previous experiments of Rosso, Bolner & Baldisserotto (2006) demonstrated that net ion fluxes of juveniles maintained for 24 h in chambers were not significantly different from the fluxes of those which measurements started around 10 min after placing them in the chambers.

Water Na^+ and K^+ levels were measured with a B4262 flame spectrophotometer (Micronal, São Paulo, Brazil) and Cl^- levels by the colorimetric assay described by Zall, Fisher & Garner (1956). Net ion fluxes were calculated according to Baldisserotto & Val (2002):

$Jnet = V([ion]_1 - [ion]_2).(Mt)^{-1}$, where:

$[ion]_1$ and $[ion]_2$ are the bath ion concentrations at the beginning and end of the flux period, in order; V is the bath volume (L); M is the mass of the fish (kg); and t is the duration of the flux period (h).

Biometric analysis

Fifteen days after the beginning of the experiment, ten juveniles per replicate were collected for measurement of weight and length and after returned to the tanks. At 30 days all remained juveniles were collected and measured. Specific growth rate (SGR), coefficient of

variability (CV) for weight and length, and condition factor (CF) were calculated according to Jobling (1994).

Statistical analysis

Data were expressed as mean ± SEM. Homogeneity of variances among groups was tested with the Levene test. Mean length, weight, biomass, SGR, CV for weight and length, CF, survival and net ion fluxes presented homogenous variances and then data were compared by two-way ANOVA (pH X water hardness) followed by the Tukey test, using the Software Statistica version 5.1 (1997) and the minimum significance level was set at $P < 0.05$.

Results

Survival of silver catfish juveniles was higher than 92.7 % in all treatments, and there were no significant differences among treatments. Dissolved oxygen, temperature, alkalinity, total ammonia, and nitrite did not show any significant difference among treatments. Fifteen days after the beginning of the experiment, weight and length were already significantly lower in juveniles exposed to pH 9.0 than in those kept at pH 7.0 and 60 mg L^{-1} $CaCO_3$. Weight of juveniles exposed to pH 7.0 was significantly higher than those maintained at pH 9.0 and 180 mg L^{-1} $CaCO_3$ and at pH 5.5 and 30, 60 and 180 mg L^{-1} $CaCO_3$. In addition, juveniles exposed to pH 9.0 and 30 mg L^{-1} $CaCO_3$ presented significantly higher weight than those kept at the same pH and other water hardness. Length to juveniles exposed to pH 7.0 and 30 mg L^{-1} $CaCO_3$ was significantly higher than those maintained at pH 5.5. After 30 days of experiment weight of individuals kept at pH 7.0 was significantly higher than those exposed to pH 5.5 and 30, 60 and 180 mg L^{-1} $CaCO_3$ and at pH 9.0 and 60 and 180 mg L^{-1} $CaCO_3$. Juveniles exposed to pH 9.0 and 30 mg L^{-1} $CaCO_3$ presented significantly higher weight than those kept at the same pH and other water hardness. At the end of 30 days of experiment specimens maintained at pH 7.0 showed significantly higher length than those exposed to pH 5.5 and 30 and 180 mg L^{-1} $CaCO_3$ and to pH 9.0 and 60 mg L^{-1} $CaCO_3$ (Table 1).

Biomass per tank and SGR were significantly higher in juveniles exposed to pH 7.0 than those maintained at pH 9.0 and 60 mg L^{-1} $CaCO_3$ and at pH 5.5 and 30 mg L^{-1} $CaCO_3$ (15 and 30 days). In addition, after 30 days of experiment fish exposed to pH 5.5 presented significantly

lower biomass per tank and SGR than those kept at pH 7.0 and 60 mg L^{-1} $CaCO_3$ and at pH 9.0 and 30 mg L^{-1} $CaCO_3$ and lower SGR than those kept at pH 7.0 and 180 mg L^{-1} $CaCO_3$. Besides, juveniles exposed to pH 9.0 and 30 mg L^{-1} $CaCO_3$ presented significantly higher biomass per tank than those kept at other water hardness and the same pH (Table 2).

Condition factor presented significantly higher values in fish exposed to pH 9.0 than those maintained at pH 7.0 and 30 mg L^{-1} $CaCO_3$ or to pH 9.0 and 120 mg L^{-1} $CaCO_3$ (Table 2). After 30 days of experiment, coefficients of variability for weight (overall range 20.18-35.00 %) and length (overall range 8.15-12.26 %) were not significantly affected by either pH or water hardness, except that coefficient of variability for weight was significantly lower in juveniles exposed to pH 9.0 and 120 mg L^{-1} $CaCO_3$ than those kept at 30 mg L^{-1} $CaCO_3$ and the same pH.

Two days after the beginning of the experiment juveniles exposed to pH 7.0 did not show significant difference on net Na^+ influx among the different water hardness. Juveniles exposed to pH 5.5 presented a significant increase of net Na^+ loss with the increase of water hardness. However, juveniles exposed to pH 9.0 and 30 mg L^{-1} $CaCO_3$ showed net Na^+ influx lower than those kept at 180 mg L^{-1} $CaCO_3$ (Fig 1A). However, after 15 days of experiment occurred an inversion of the fluxes at pH 7.0 and 9.0 and maintenance of effluxes at pH 5.5 to 120 and 180 mg L^{-1} $CaCO_3$ (Fig 1B). At 2 days of the experiment net Na^+ influxes in juveniles maintained at pH 7.0 and 9.0 were significantly higher than in those exposed to pH 5.5, except juveniles maintained at pH 5.5 and 30 mg L^{-1} $CaCO_3$ (Fig 1A). Moreover, at 15 days of the experiment, juveniles exposed to 30 and 60 mg L^{-1} $CaCO_3$ and pH 9.0 showed significantly higher net Na^+ influx than those maintained at pH 5.5 and 7.0. However, to same pH and 120 mg L^{-1} $CaCO_3$ juveniles presented significantly higher net Na^+ efflux than those kept at pH 7.0 and the same water hardness (Fig 1B).

Juveniles maintained at pH 7.0 and 9.0 presented net Cl^- influxes at all water hardness, but among those kept at pH 5.5 only juveniles exposed to 60 mg L^{-1} $CaCO_3$ showed net Cl^- influx at two days of experiment. Fifteen days after the beginning of the experiment, in mosttreatments juveniles presented net Cl^- effluxes (Fig 1C and 1 D). Two days after the beginning of the experiment net Cl^- influxes in juveniles maintained at 60 mg L^{-1} $CaCO_3$ were significantly higher than of those exposed to other water hardness regardless of pH, except juveniles maintained at pH 7.0 and 120 mg L^{-1} $CaCO_3$, that showed net Cl^- influx also significantly higher than those kept at 30 and 180 mg L^{-1} $CaCO_3$ at the same pH. Silver catfish exposed to pH 7.0 and 9.0 at 30

mg L^{-1} $CaCO_3$ presented net Cl^- influxes significantly higher than those maintained at pH 5.5 and the same hardness. Juveniles exposed to pH 9.0 at 60 and 180 mg L^{-1} $CaCO_3$ presented net Cl^- influxes significantly higher than those maintained at pH 5.5 and 7.0 at the same hardness (Fig 1C). Fifteen days after the beginning of the experiment juveniles maintained at pH 5.5 and 120 mg L^{-1} $CaCO_3$ showed significantly lower net Cl^- efflux than those exposed to the same pH at 30 and 180 mg L^{-1} $CaCO_3$. Juveniles kept at pH 9.0 showed significantly higher net Cl^- efflux at 60 mg L^{-1} $CaCO_3$ than those maintained at other water hardness and the same pH. Fish exposed to pH 9.0 and 30 and 180 mg L^{-1} $CaCO_3$ presented significantly lower net Cl^- loss than those maintained at the same water hardness and pH 5.5 and 7.0. In addition, juveniles exposed to pH 5.5 and 120 mg L^{-1} $CaCO_3$ showed significantly lower net Cl^- efflux than those kept at pH 7.0 and the same water hardness (Fig 1D).

Two days after the beginning of the experiment net K^+ fluxes were not significantly different from zero (data not shown), and at 15 days specimens kept at pH 5.5 and 9.0 presented significantly higher net K^+ effluxes at 30 mg L^{-1} $CaCO_3$ tan those exposed to pH 7.0 and the same water hardness. Juveniles exposed to 60, 120 and 180 mg L^{-1} $CaCO_3$ did not show any significant net K^+ loss (except those maintained at pH 7.0 and 180 mg L^{-1} $CaCO_3$) (Fig 1E).

Discussion

Water pH plays an important role in the maintenance of fish homeostasis in fish and pH changes are reported to cause disturbances in acid-base and ion regulation (Freda & McDonald 1988; Wilkie, Wright, Iwama & Wood 1994; Zaions & Baldisserotto 2000; Wood 2001; Aride, Roubach & Val 2007). Water hardness higher than 100 mg L^{-1} $CaCO_3$ increased survival of silver catfish exposed to very acidic (3.75) and alkaline (10.0) pH (Townsend & Baldisserotto 2001), but in larvae maintained at neutral water (pH 7.0) water hardness of 150 mg L^{-1} $CaCO_3$ or higher impaired survival (Townsend *et al.* 2003).

Most fishes presented net ion loss in acidic water. Wilson, Wood, Gonzalez, Patrick, Bergman, Narahara & Val (1999) described significant net losses of the plasma concentrations of Na^+, K^+ and Cl^-, but not of Ca^{2+}, in response to acid (pH 3.5-4.0) exposure in three Amazonian fish. Plasma Na^+ and K^+ concentrations followed the pH profile, decreasing during acid exposure (pH 4.0) and increasing during alkaline exposure (pH 8.0) in tambaqui *Colossoma macropomum* (Cuvier, 1818) (Aride *et al.* 2007). Similar reductions in plasma Na^+ and K^+ were observed in

common carp *Cyprinus carpio* (L. 1758) and Nile tilapia *Oreochromis niloticus* (L. 1758) after transference from pH 7.3 to pH 4.0 (Van Dijk, Van Den Thillart, Balm, & Wendelaar Bonga 1993). However, silver catfish juveniles maintained at water hardness of 30 mg L^{-1} $CaCO_3$ for 96 h did not show any significant mortality or change in whole body Na^+ and K^+ within the 5.5-9.0 pH range (Zaions & Baldisserotto 2000). In addition, in the present study the ionic losses occurred not in response to pH, but to water hardness, because water hardness higher than 60 mg L^{-1} $CaCO_3$ induced net Na^+ loss in all tested pH.

The present study demonstrated that exposure of silver catfish juveniles to acidic (pH 5.5) water and low water hardness (30 mg L^{-1} $CaCO_3$) for 30 days reduced significantly weight, biomass per tank and SGR compared to those exposed to pH 7.0 and the same water hardness. Silver catfish kept at pH 5.5 at higher water hardness (60-180 mg L^{-1} $CaCO_3$) did not show any significant increase in these parameters compared to those maintained at 30 mg L^{-1} $CaCO_3$, but only SGR remained significantly lower than of those exposed to pH 7.0. Similar results in others studies related that fishes exposed to neutral water (or next) presented higher growth. Brook trout *Salvelinus fontinalis* (Mitchill 1814), juveniles and rainbow trout *Oncorhynchus mykiss gairdnerii* (Richardson, 1836), presented lower growth at acidic water (pH 5.3) than neutral waters (Menendez 1976; Rodgers 1984). Brook trout juveniles presented lower growth at pH 5.5, 6.0 and 6.5 than at pH 7.1 (Menendez 1976), and at pH 4.2-5.0 than 5.2-6.5 (Norrgren & Degerman 1992). Rainbow trout showed better growth at neutral pH (7.2) than at acidic pH (4.4) (Nelson 1982). Alkaline exposure (pH 8.0) resulted in more severe effects on blood physiology and reduced growth in tambaqui than acid exposure (pH 4.0) (Aride *et al.* 2007).

Townsend *et al.* (2003) described water hardness of 30-70 mg L^{-1} $CaCO_3$ and pH 8.2 as the best for survival, growth, and biomass gain for silver catfish larvae. The results of the present study demonstrated that water hardness did not affect survival of silver catfish juveniles maintained at pH 5.5 and 9.0. Exposure of the same species to pH 5.5 or 9.0 also did not affect survival compared to pH 7.5, and growth is lower at acidic (5.5) or alkaline (9.0) soft water compared to neutral water (Copatti *et al.* 2005).

Rainbow trout exposed to low waterborne Ca^{2+} (2.5 mg L^{-1} $CaCO_3$) showed an increase in number of chloride cells on lamellae and large apical surfaces to increase ion uptake (Perry & Wood 1985). It is possible that highly alkaline conditions in soft water causes the leaching of Ca^{2+} from the gill epithelium, as occurs in fish exposed to acidic waters (Lauren & McDonald

1986), so the protective effect of high waterborne Ca^{2+} becomes more important. Most teleosts exposed to acidic or alkaline waters showed higher survival in hard than in soft waters (Freda & McDonald 1988; Yesaki & Iwama 1992; Townsend & Baldisserotto 2001) and in our present study although had been hypothesized that the increase of the water hardness could improve growth of the silver catfish juveniles exposed to acidic or alkaline pH, this was not verified in this experiment. Rainbow trout exposed to pH 3.0 or 3.2 presented higher survival at water hardness of 165 mg L^{-1} $CaCO_3$ than those exposed to the same pH and at water hardness of 10 mg L^{-1} $CaCO_3$ (McDonald, Hobe & Wood 1980). The same species transferred from pH 6.8 to 10.1 showed lower Na^+ and Cl^- plasma levels at 4.0 mg L^{-1} $CaCO_3$, but when maintained at 320 mg L^{-1} $CaCO_3$ did not change plasma ion levels and showed higher survival at pH 10.1 (Yesaki & Iwama 1992).

It can be concluded that exposure of silver catfish juveniles to alkaline or acidic water did not affect survival, but reduced growth, and at alkaline water the best weight occurred at the lowest water hardness. Moreover, ionoregulatory disturbances are more pronounced at higher water hardness. Therefore, the best water hardness for silver catfish juvenile growth and ionoregulation is 30-60 mg L^{-1} $CaCO_3$.

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Figure legends

Figure 1 Net Na^+ and Cl^- fluxes of *R. quelen* after two (A, C respectively) and fifteen days (B, D respectively) and net K^+ fluxes after fifteen days (E) of exposed to different water hardness and pH. Potassium fluxes of all treatments were not significantly difference from zero after two days. Data expressed as mean ± SEM, n = 9. Positive values indicate net influxes and negative values net effluxes.

Means identified by different capital letters indicate significant difference among pH (5.5; 7.0 and 9.0) in the same water hardness while means identified by different small letters indicate significant difference among different water hardness in the same pH as determined by two-way ANOVA and Tukey comparison of mean values ($P < 0.05$).

Table 1 Effect of water hardness and pH on silver catfish weight and length.

	Water hardness (mg $CaCO_3$ L^{-1})			
	30	60	120	180
	Weight (g)			
pH	15 days			
5.5	$2.32^{Ba} \pm 0.14$	$2.93^{Aba} \pm 0.06$	$2.72^{Aa} \pm 0.22$	$2.79^{Aa} \pm 0.28$
7.0	$3.05^{Aa} \pm 0.14$	$3.40^{Aa} \pm 0.14$	$2.86^{Aa} \pm 0.12$	$2.84^{Aa} \pm 0.14$
9.0	$2.93^{ABa} \pm 0.06$	$2.43^{Ba} \pm 0.04$	$2.73^{Aa} \pm 0.12$	$2.77^{Aa} \pm 0.04$
	30 days			
5.5	$2.45^{Ba} \pm 0.11$	$2.80^{Ba} \pm 0.04$	$2.71^{Aa} \pm 0.22$	$2.56^{Ba} \pm 0.17$
7.0	$3.34^{Aa} \pm 0.16$	$3.52^{Aa} \pm 0.17$	$2.93^{Aa} \pm 0.13$	$3.32^{Aa} \pm 0.16$
9.0	$3.26^{Aa} \pm 0.08$	$2.50^{Bb} \pm 0.04$	$2.59^{Ab} \pm 0.07$	$2.57^{Bb} \pm 0.02$
	Length (cm)			
pH	15 days			
5.5	$6.36^{Aa} \pm 0.13$	$6.87^{ABa} \pm 0.01$	$6.77^{Aa} \pm 0.08$	$6.58^{Aa} \pm 0.29$
7.0	$6.88^{Aa} \pm 0.12$	$7.13^{Aa} \pm 0.08$	$6.83^{Aa} \pm 0.13$	$6.79^{Aa} \pm 0.06$
9.0	$6.63^{Aa} \pm 0.17$	$6.48^{Ba} \pm 0.03$	$6.77^{Aa} \pm 0.10$	$6.67^{Aa} \pm 0.06$
	30 days			
5.5	$6.61^{Ba} \pm 0.12$	$6.92^{ABa} \pm 0.06$	$6.83^{Aa} \pm 0.18$	$6.61^{Ba} \pm 0.11$
7.0	$7.44^{Aa} \pm 0.11$	$7.38^{Aa} \pm 0.08$	$7.04^{Aa} \pm 0.11$	$7.30^{Aa} \pm 0.07$
9.0	$7.08^{ABa} \pm 0.11$	$6.69^{Ba} \pm 0.02$	$6.88^{Aa} \pm 0.61$	$6.80^{ABa} \pm 0.17$

Values are reported as mean ± S.E.M, n = 3. Means identified by different capital letters in the columns (in the same period of time) or small letters in the rows were significantly different ($P < 0.05$) as determined by two-way ANOVA and Tukey comparison of mean values.

Table 2 Effect of water hardness and pH on silver catfish standard growth rate (SGR), biomass per tank and condition factor (CF).

	Water hardness (mg $CaCO_3$ L^{-1})			
	30	60	120	180
	SGR (% day^{-1})			
pH	15 days			
5.5	$0.72^{Ba} \pm 0.40$	$2.30^{ABa} \pm 0.14$	$1.75^{Aa} \pm 0.54$	$1.90^{Aa} \pm 0.66$
7.0	$2.55^{Aa} \pm 0.31$	$3.27^{Aa} \pm 0.38$	$2.11^{Aa} \pm 0.28$	$2.06^{Aa} \pm 0.32$
9.0	$2.30^{ABa} \pm 0.14$	$1.05^{Ba} \pm 0.10$	$1.82^{Aa} \pm 0.30$	$1.93^{Aa} \pm 0.09$
	30 days			
5.5	$0.54^{Ba} \pm 0.14$	$1.00^{Ba} \pm 0.04$	$0.86^{Aa} \pm 0.26$	$0.68^{Ba} \pm 0.22$
7.0	$1.57^{Aa} \pm 0.16$	$1.75^{Aa} \pm 0.17$	$1.14^{Aa} \pm 0.14$	$1.55^{Aa} \pm 0.16$
9.0	$1.50^{Aa} \pm 0.08$	$0.62^{Ba} \pm 0.05$	$0.74^{Aa} \pm 0.08$	$0.85^{ABa} \pm 0.02$
	Biomass per tank (g)			
pH	15 days			
5.5	$27.79^{Ba} \pm 1.76$	$36.17^{ABa} \pm 1.17$	$33.74^{Aa} \pm 4.24$	$36.27^{Aa} \pm 3.61$
7.0	$39.65^{Aa} \pm 1.87$	$44.16^{Aa} \pm 1.84$	$37.14^{Aa} \pm 1.58$	$34.92^{Aa} \pm 1.18$
9.0	$38.13^{ABa} \pm 0.79$	$31.59^{Ba} \pm 0.46$	$34.66^{Aa} \pm 2.13$	$36.05^{Aa} \pm 0.50$
	30 days			
5.5	$29.30^{Ba} \pm 1.38$	$34.56^{Ba} \pm 0.66$	$33.52^{Aa} \pm 3.95$	$33.24^{Aa} \pm 2.24$
7.0	$43.38^{Aa} \pm 2.10$	$45.80^{Aa} \pm 2.27$	$38.05^{Aa} \pm 1.66$	$40.80^{Aa} \pm 0.90$
9.0	$42.34^{Aa} \pm 1.01$	$32.50^{Bb} \pm 0.52$	$32.88^{Ab} \pm 1.60$	$32.80^{Ab} \pm 0.24$
	CF (g cm^{-3})			
pH	30 days			
5.5	$0.84^{ABa} \pm 0.01$	$0.85^{Aa} \pm 0.01$	$0.84^{Aa} \pm 0.01$	$0.88^{Aa} \pm 0.01$
7.0	$0.81^{Ba} \pm 0.01$	$0.88^{Aa} \pm 0.01$	$0.84^{Aa} \pm 0.01$	$0.85^{Aa} \pm 0.01$
9.0	$0.92^{Aa} \pm 0.02$	$0.84^{Aab} \pm 0.02$	$0.80^{Ab} \pm 0.02$	$0.85^{Aab} \pm 0.01$

Values are reported as mean ± S.E.M, n = 3. Means identified by different capital letters in the columns (in the same period of time) or small letters in the rows were significantly different ($P < 0.05$) as determined by two-way ANOVA and Tukey comparison of mean values.

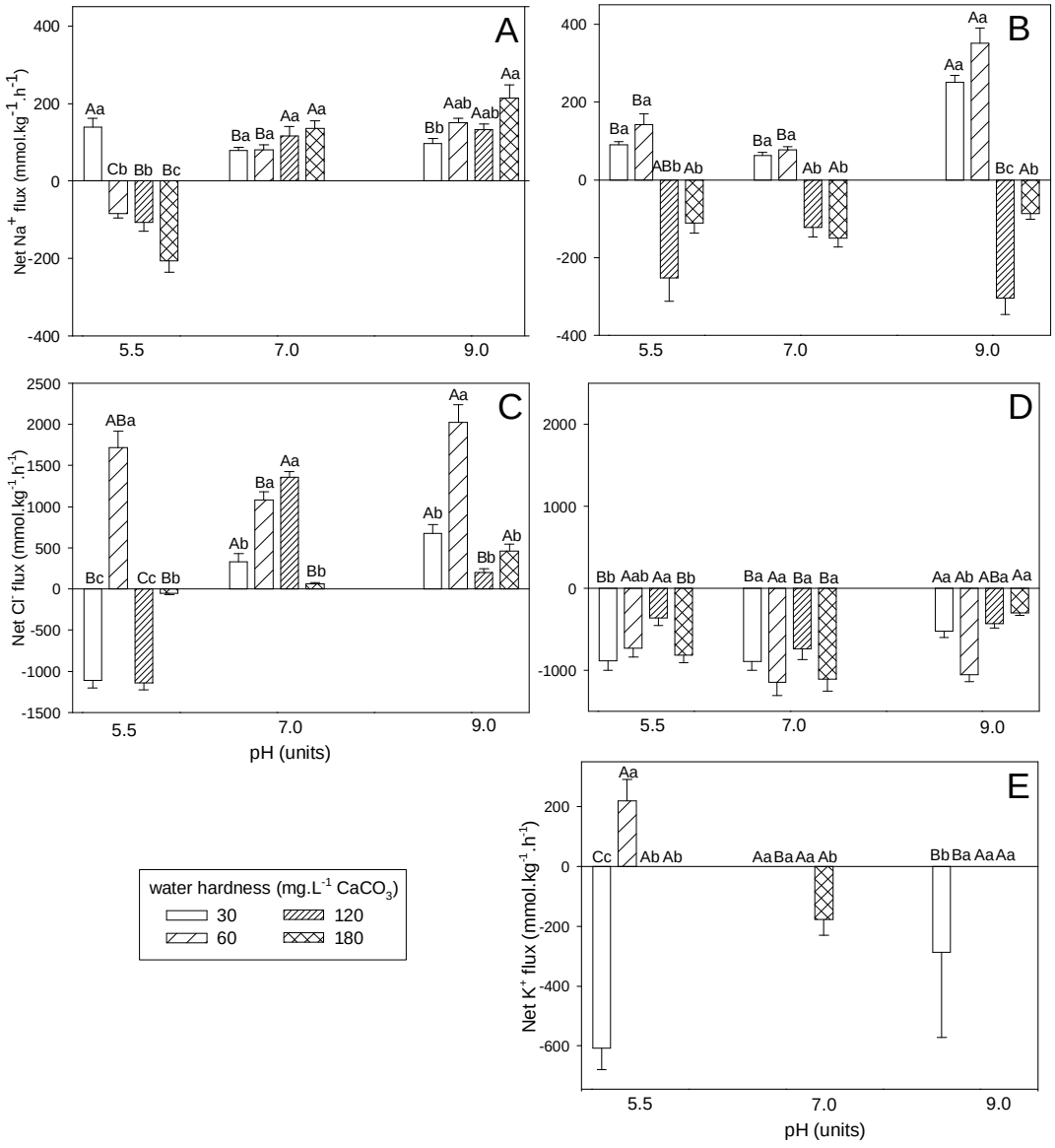
A
B
C
D
E
Net Na+ flux (mmol.kg-1.h-1)
Net Cl- flux (mmol.kg-1.h-1)
Net K+ flux (mmol.kg-1.h-1)
pH (units)
5.5
7.0
9.0
water hardness (mg.L-1 CaCO3)
30
60
120
180

Figure 1

Artigo 03

Dureza baixa e pH da água no crescimento e sobrevivência de juvenis de jundiá

Low water hardness and pH affect growth and survival of silver catfish, Rhamdia quelen, juveniles

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Abstract

The objective of this study was to investigate the effects of exposure to low water hardness (0, 25 and 50 mg L^{-1} $CaCO_3$) into the 6.0-8.0 pH range to silver catfish juveniles survival and growth. Juveniles kept at zero water hardness presented higher mortality at pH 7.0 and 8.0 than those submitted to other treatments. Weight of juveniles exposed to pH 6.0 and zero water hardness was significantly higher than those kept at the same water hardness and other pH. Survival and growth of juveniles exposed to 25 and 50 mg L^{-1} $CaCO_3$ was not affected in the 6.0-8.0 pH range. Therefore, the best water hardness for silver catfish juveniles growth and ionoregulation is 25-50 mg L^{-1} $CaCO_3$ and at low water hardness (next zero) pH must be reduced.

Keywords: alkaline or acid water; calcium; growth; mortality.

Introduction

Ca^{2+} is important for ionic regulation of freshwater fish because it influences the permeability of biological membranes, preventing the diffusive efflux and high ionic loss to water (Wood and McDonald 1988). Furthermore, Ca^{2+} plays a crucial role in numerous other physiological and biochemical processes such as muscular contraction, vision, blood coagulation, regulation of enzymatic reactions, modulation of permeability and excitability of plasma membranes, neutral and intercellular communication and intracellular signaling (Riccardi 2000). The relevance of the branchial tissue for Ca^{2+} uptake has been demonstrated in a large variety of fish species, and the gill epithelia is probably the most important site for Ca^{2+} uptake in fish (Evans et al. 2005).

Several lines of evidence indicate that the main cause of fish mortality in acid waters is due to the failure in gill ionoregulation (Milligan and Wood 1982), and consequent loss of Na^+, Cl^- and Ca^{2+} (Heath 1995). In fish exposed to low pH, acid load through the gills is the source of acid-base disturbance, and there is an increase of H^+ and NH_4^+ excretion by the urine to compensate this problem (Bolner and Baldisserotto 2007). Mortality of fishes exposed to acidic soft water seems related to a decrease of around 50.0 % plasma ion levels, mainly Na^+ and Cl^- (Freda and McDonald 1988). The main problems in alkaline waters are the inhibition of ammonia excretion and increase of CO_2 excretion (Heath 1995). High pH also inhibits branchial Na^+/NH_4^+ and Cl^-/HCO_3^- exchangers (Wilkie and Wood 1996). The exposure of fish to stress situations, either natural or artificial results in a series of adjustments through homeostatic mechanisms (Val 1996) and can affect growth performance. An increase in environmental pH affects fish ionoregulation and has been associated with an increase in mucus secretion (Wood et al. 1998).

Husbandry of silver catfish, Rhamdia quelen Quoy and Gaimard, 1824 (Heptapteridae), is spreading in Brazil. Fish farmers are interested in the culture of this species because of its good growth rate, omnivorous feeding habit, high fertilization and hatching rates, and acceptance by the consumers (Gomes et al. 2000; Fracalossi et al. 2004). Silver catfish can survive to acute pH changes within the 4.0-9.0 range without significant mortality (Zaions and Baldisserotto 2000). Exposure to low pH (5.5-6.0) reduced length and weight of silver catfish larvae compared to those maintained at pH 8.0-8.5 (Lopes et al. 2001), and growth of juveniles of this species is lower in acidic (pH 5.5) or alkaline (pH 9.0) soft water compared to neutral water (pH 7.0) (Copatti et al. 2005). In juveniles survival in acidic and alkaline pH is improved by the addition

of Ca^{2+} to the water (Townsend and Baldisserotto 2001), but no studies regarding water hardness and growth of juveniles of this species was performed. Therefore, this study verified the effects of the 6.0-8.0 pH range, at low water hardness in the survival and growth of silver catfish juveniles. This pH range was studied because is considered the best for fish culture (Boyd 1998) and is within a pH range not tested for silver catfish growth.

Materials and methods

Experimental animals and management conditions

Two hundred and forty three silver catfish juveniles were obtained from the fish culture Bela Vista in 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. Stocking density was 0.33 juveniles L^{-1}.

After 15 days of acclimation, juveniles (0.603 ± 0.07 g and 4.25 ± 0.17 cm) were then transferred to 27 continuously aerated 40 L polypropylene boxes and kept for 32 days. Nine juveniles were placed in each box (0.225 juveniles L^{-1}).

Nine treatments (three pH X three water hardness), were tested in triplicate. Water pH was fixed at 6.0 (5.95-6.02), 7.0 (6.95-7.02) and 8.0 (7.94-8.04) and water hardness at 0 (0.0-2.57), 25 (24.97-26.43) and 50 (48.76-52.48) mg L^{-1} $CaCO_3$.

Alkalinity and total ammonia were 5-6 mg L^{-1} $CaCO_3$ and 0.41-0.54 mg L^{-1}, 8-12.5 mg L^{-1} $CaCO_3$ and 0.33-0.36 mg L^{-1}, 31-33 mg L^{-1} $CaCO_3$ and 0.27-0.34 mg L^{-1} at pH 6.0, 7.0 and 8.0, respectively. Nitrite was below 0.05 mg L^{-1}, dissolved oxygen levels 8.56-8.65 mg L^{-1} and temperature 22.4-23.1 ^{o}C.

Tanks management and water quality

The diet offered was a fish commercial feed (Vicente Alimentos S.A., Presidente Prudente/SP, Brazil) with 3.5 % Ca^{2+}, 28.00 % crude protein and 3,500 kcal kg^{-1} digestible energy energy according to manufacturer. The juveniles were fed once a day, at 8:00 a.m., for 32 days, at a ratio of 5.0 % body mass. Uneaten food, as well as other residues and feces were siphoned 30 min after furnishing the food and consequently at least 20.0 % of the water was

replaced with water previously adjusted to the appropriate pH and water hardness using NaOH or H_2SO_4 0.5 M and $CaCl_2.2H_2O$, respectively. Treatments with water hardness zero were obtained using distilled water. Whenever necessary, water change was increased to reduce ammonia and nitrite levels. Dead fish were daily removed and mortality recorded.

Water pH was monitored several times daily between 7:30 a.m. and 5:30 p.m. with a pH meter Quimis (model 400.A). Water hardness was calculated every two or three days with the EDTA titrimetric method and total ammonia levels were verified once a week by nesslerization according to Greenberg et al. (1976) and non-ionized ammonia levels were calculated according to Piper et al. (1982). Dissolved oxygen levels and temperature were measured daily with oxygen meter YSI (model Y5512 Yellow Springs, USA), and laboratory temperature was maintained with the use of an air conditioner. Levels of total alkalinity and nitrite were determined once a week according to Boyd (1998).

Biometric analysis

Twenty days after the beginning of the experiments, nine juveniles per replicate were collected for measurement of weight and length and after returned to the tanks. At the end of the experiment (32 days) all remained juveniles were collected and measured. Specific growth rate (SGR), coefficient of variability (CV) for weight and length and condition factor (CF) were calculated according to Jobling (1994).

Statistical analysis

Data are expressed as mean $\pm$ SEM. Homogeneity of variances among groups was tested with the Levene test. Mean length, weight, biomass, SGR, CV for weight and length, CF and survival of the treatment groups were compared by two-way ANOVA (pH X water hardness) followed by the Tukey test, using the Software Statistica version 5.1 (1997). The minimum significance level was set at $P < 0.05$.

Results

Dissolved oxygen, temperature, total ammonia, and nitrite did not show any significant difference among treatments. Twenty days after the beginning of the experiment there was no

significant difference of mortality among treatments. However after 32 days, juveniles exposed to pH 7.0 and 8.0 at zero water hardness presented significantly higher mortality than those submitted to the other treatments (Table 1). After 32 days of experiment, coefficients of variability for weight (overall range 15.37-33.99 %) and length (overall range 5.46-10.72 %) were not significantly affected by either pH or water hardness

Twenty days after the beginning of the experiment, weight and length were significantly higher in juveniles exposed to pH 6.0 than in those kept at pH 7.0 and 8.0 at 50 mg L^{-1} $CaCO_3$. Length of juveniles exposed to pH 6.0 and 25 mg L^{-1} $CaCO_3$ was significantly lower than of those maintained at the same pH and 50 mg L^{-1} $CaCO_3$. At 32 days of experiment there was no significant difference in length among treatments, but weight of juveniles exposed to pH 6.0 and zero water hardness was significantly higher than those kept at the same water hardness and other pH (Table 2).

Biomass per tank and SGR showed significantly higher values in juveniles exposed to pH 6.0 than those maintained at pH 7.0 and 8.0 at 50 mg L^{-1} $CaCO_3$ after 20 days of experiment and those kept at pH 7.0 and 8.0 at zero water hardness after 32 days of experiment. In addition, after 32 days of experiment, fish exposed to pH 7.0 and zero water hardness showed lower biomass per tank than those kept at 25 and 50 mg L^{-1} $CaCO_3$. Condition factor presented significantly lower values in fish exposed to pH 7.0 at zero water hardness than those maintained at pH 7.0 and 50 mg L^{-1} $CaCO_3$ and those at pH 6.0 and zero water hardness (Table 3).

Discussion

Neutral and lightly alkaline pH have been recommended by a series of authors as being appropriate for the cultivation of commercial freshwater fish (Boyd 1998) and growth of most fish populations is affected at pH below 6.0 (Wood and McDonald 1988). Most teleosts species survive to acute pH changes down to 4.0-5.0 or up to 9.0-10.0, but exposure to more acidic or alkaline waters is lethal within a few hours (Parra and Baldisserotto 2007). The present study verified that silver catfish juveniles maintained in very soft water (near zero water hardness) showed lower mortality at pH 6.0 (3.70 %) than those kept at pH 7.0 (62.96 %) and 8.0 (40.74 %) after 32 days of experiment, demonstrating that at near zero water hardness the use of slightly acidic water (pH 6.0) is advantageous to Rhamdia quelen juveniles. Other authors also verified

that this species can resist to acidic pH when water hardness was between 20-70 mg L^{-1} $CaCO_3$, but the best growth occurred at slightly alkaline pH (Lopes et al. 2001; Townsend and Baldisserotto 2001; Copatti et al. 2005).

On the other hand, the increase of water hardness up to 25 mg L^{-1} $CaCO_3$ was enough to reduce mortality in silver catfish. This effect of water hardness was expected because most teleosts exposed to very acidic or alkaline waters showed higher survival in hard than in soft waters (Freda and McDonald 1988; Yesaki and Iwama 1992; Towsend and Baldisserotto 2001). Growth of silver catfish juveniles kept at pH 6.0 was not affected by water hardness. This was in agreement with the fact that larvae survival in striped bass Morone saxatilis (Walbaum, 1792) and Oreochromis mossambicus raised at optimum pH (6.6-6.8) were not affected by water hardness of 3-250 and 3-96 mg L^{-1} $CaCO_3$, respectively (Grizzle et al. 1992, Hwang et al., 1996). However, results in other species were different, because white bass female X sunshine bass male juveniles died in a few hours in water with 5-6 mg L^{-1} $CaCO_3$ at circumneutral pH and increase of water hardness to 210 mg L^{-1} $CaCO_3$ increased survival to 64.0 % (Grizzle and Mauldin 1999). Juveniles of striped bass and Morone hybrid bass (M. chrysops X M. saxatilis) had 80-99 % survival compared with 16 % survival for a group of fish without additional calcium in the water before (20 to 45-100 mg L^{-1} $CaCO_3$) or after (10 to 70-200 mg L^{-1} $CaCO_3$) harvest (pH 7.0) (Grizzle et al. 1985).

In zero water hardness, silver catfish juveniles presented higher growth at pH 6.0 than at pH 7.0 and 8.0. Prolonged exposure to slightly alkaline water (pH 8.0) soft water resulted in several changes in the blood physiology and reduced growth in tambaqui Colossoma macropomum (Cuvier, 1818) after 40 days of the exposition (Aride et al. 2007). However, acidic water (pH 5.2-5.5) impaired growth in rainbow trout and silver catfish at water hardness 2.5 and 20 mg L^{-1} $CaCO_3$, respectively (D'Cruz and Wood 1998; Copatti et al. 2005).

Very soft hardness (zero) decreased growth of silver catfish exposed to pH 7.0 and 8.0 compared to higher hardness (25 and 50 mg L^{-1} $CaCO_3$). However, in rainbow trout, the average growth rate of fish maintained in water of higher Ca^{2+} concentrations (40 mg L^{-1} $CaCO_3$) was significantly higher than that of fish kept at 5 mg L^{-1} $CaCO_3$ and pH 5.3 or 6.5 (Rodgers 1984). In white bass, Morone chrysops (Rafinesque, 1820) X sunshine bass M. saxatilis, and tilapia, Oreochromis mossambicus (Peters, 1852), growth was not affected by different Ca^{2+} concentrations (2 to 96 mg L^{-1} $CaCO_3$ and pH 6.9) (Seals et al. 1994; Hwang et al. 1996).

Channel catfish Ictalurus punctatus (Rafinesque, 1818) swim-up fry exposed to 0, 1, 5, 10, and 100 mg L^{-1} $CaCO_3$ (pH 7.0) showed that concentrations higher than 10 mg L^{-1} $CaCO_3$ afforded no significant benefit, so a minimum calcium concentrations (10 mg L^{-1} $CaCO_3$) was recommended (Tucker and Steeby 1993). The same authors observed an abnormal behavior (fry appeared lethargic and were spread out over the bottom) in water with low Ca^{2+} concentration (below 5 mg L^{-1} $CaCO_3$). Townsend et al. (2003) conclude as the best for survival, growth, and biomass gain for larvae of the same species this study water hardness of 30-70 mg L^{-1} $CaCO_3$ at pH around 8.2.

Our data allow concluding that water hardness of 25-50 mg L^{-1} $CaCO_3$ is indicated to raise silver catfish juveniles at the 6.0-8.0 pH range, but at low water hardness (next zero) pH must be reduced to improve survival and growth.

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Table 1 Effect of water hardness and pH on silver catfish mortality (%).

	Water hardness (mg $CaCO_3$ L^{-1})		
	0	25	50
pH	20 days		
6.0	$3.70^{Aa} \pm 3.70$	$3.70^{Aa} \pm 3.70$	$0.00^{Aa} \pm 0.00$
7.0	$22.22^{Aa} \pm 11.11$	$11.11^{Aa} \pm 6.40$	$0.00^{Aa} \pm 0.00$
8.0	$3.70^{Aa} \pm 3.70$	$0.00^{Aa} \pm 0.00$	$11.11^{Aa} \pm 7.41$
	32 days		
6.0	$3.70^{Aa} \pm 3.70$	$3.70^{Aa} \pm 3.70$	$0.00^{Aa} \pm 0.00$
7.0	$62.96^{Bb} \pm 19.60$	$11.11^{Aa} \pm 6.42$	$3.70^{Aa} \pm 3.70$
8.0	$40.74^{Bb} \pm 3.70$	$0.00^{Aa} \pm 0.00$	$11.11^{Aa} \pm 6.40$

Values are reported as mean ± S.E.M, n = 3. Means identified by different capital letters in the columns (in the same period of time) or small letters in the rows were significantly different ($P < 0.05$) as determined by two-way ANOVA and Tukey comparison of mean values.

Table 2 Effect of water hardness and pH on silver catfish weight and length.

	Water hardness (mg $CaCO_3$ L^{-1})		
	0	25	50
	Weight (g)		
pH	20 days		
6.0	$0.71^{Aa} \pm 0.09$	$0.60^{Aa} \pm 0.05$	$0.82^{Aa} \pm 0.08$
7.0	$0.55^{Aa} \pm 0.04$	$0.64^{Aa} \pm 0.01$	$0.55^{Ba} \pm 0.05$
8.0	$0.49^{Aa} \pm 0.02$	$0.58^{Aa} \pm 0.06$	$0.59^{Ba} \pm 0.01$
	32 days		
6.0	$0.79^{Aa} \pm 0.07$	$0.69^{Aa} \pm 0.08$	$0.90^{Aa} \pm 0.09$
7.0	$0.48^{Ba} \pm 0.02$	$0.69^{Aa} \pm 0.02$	$0.63^{Aa} \pm 0.07$
8.0	$0.48^{Ba} \pm 0.03$	$0.66^{Aa} \pm 0.09$	$0.69^{Aa} \pm 0.02$
	Length (cm)		
pH	20 days		
6.0	$4.49^{Aab} \pm 0.24$	$4.22^{Ab} \pm 0.15$	$5.64^{Aa} \pm 0.78$
7.0	$4.17^{Aa} \pm 0.07$	$4.29^{Aa} \pm 0.03$	$4.12^{Ba} \pm 0.12$
8.0	$4.03^{Aa} \pm 0.08$	$4.30^{Aa} \pm 0.13$	$4.23^{Ba} \pm 0.04$
	32 days		
6.0	$4.65^{Aa} \pm 0.19$	$4.53^{Aa} \pm 0.18$	$4.83^{Aa} \pm 0.18$
7.0	$4.34^{Aa} \pm 0.28$	$4.52^{Aa} \pm 0.03$	$4.30^{Aa} \pm 0.16$
8.0	$4.15^{Aa} \pm 0.07$	$4.39^{Aa} \pm 0.21$	$4.52^{Aa} \pm 0.05$

Values are reported as mean ± S.E.M, n = 3. Means identified by different capital letters in the columns (in the same period of time) or small letters in the rows were significantly different ($P < 0.05$) as determined by two-way ANOVA and Tukey comparison of mean values.

Table 3 Effect of water hardness and pH on silver catfish standard growth rate (SGR), biomass per tank and condition factor (CF).

	Water hardness (mg $CaCO_3$ L^{-1})		
	0	25	50
	SGR (% day^{-1})		
pH	20 days		
6.0	$0.76^{Aa} \pm 0.62$	$-0.09^{Aa} \pm 0.44$	$1.51^{Aa} \pm 0.50$
7.0	$-0.40^{Aa} \pm 0.40$	$0.32^{Aa} \pm 0.07$	$-0.50^{Ba} \pm 0.42$
8.0	$-1.08^{Aa} \pm 0.21$	$-0.22^{Aa} \pm 0.52$	$-0.08^{ABa} \pm 0.07$
	32 days		
6.0	$0.82^{Aa} \pm 0.25$	$0.38^{Aa} \pm 0.35$	$1.21^{Aa} \pm 0.31$
7.0	$-0.72^{Ba} \pm 0.13$	$0.40^{Aa} \pm 0.07$	$0.12^{Aa} \pm 0.34$
8.0	$-0.74^{Ba} \pm 0.17$	$0.23^{Aa} \pm 0.41$	$0.40^{Aa} \pm 0.11$
	Biomass per tank (g)		
pH	20 days		
6.0	$6.21^{Aa} \pm 0.96$	$5.12^{Aa} \pm 0.56$	$7.41^{Aa} \pm 0.73$
7.0	$3.93^{Aa} \pm 0.80$	$5.16^{Aa} \pm 0.44$	$4.95^{Ba} \pm 0.42$
8.0	$4.22^{Aa} \pm 0.23$	$5.25^{Aa} \pm 0.57$	$4.36^{ABa} \pm 0.12$
	32 days		
6.0	$6.88^{Aa} \pm 0.78$	$5.99^{Aa} \pm 0.79$	$8.07^{Aa} \pm 0.78$
7.0	$1.62^{Bb} \pm 1.07$	$5.49^{Aa} \pm 0.41$	$5.52^{Aa} \pm 0.78$
8.0	$2.53^{Ba} \pm 0.06$	$5.94^{Aa} \pm 0.82$	$5.07^{Aa} \pm 0.62$
	CF (g cm^{-3})		
pH	32 days		
6.0	$0.78^{Aa} \pm 0.03$	$0.74^{Aa} \pm 0.01$	$0.79^{Aa} \pm 0.02$
7.0	$0.60^{Bb} \pm 0.12$	$0.74^{Aab} \pm 0.01$	$0.79^{Aa} \pm 0.03$
8.0	$0.66^{ABa} \pm 0.01$	$0.77^{Aa} \pm 0.02$	$0.74^{Aa} \pm 0.01$

Values are reported as mean ± S.E.M, n = 3. Means identified by different capital letters in the columns (in the same period of time) or small letters in the rows were significantly different ($P < 0.05$) as determined by two-way ANOVA and Tukey comparison of mean values.

CONCLUSÕES E PERSPECTIVAS

As espécies de peixes vivem em ambientes com concentrações iônicas diferentes das do seu plasma sanguíneo, sendo que alterações na ionorregulação podem reduzir sua sobrevivência e crescimento. A interação de parâmetros como sal na dieta, pH e dureza da água abordam a possibilidade de redução de estresses causados por diferentes métodos de cultivo e alterações na qualidade da água.

A presente pesquisa procurou contribuir em vários aspectos para o cultivo de juvenis de jundiá: fornecimento de informações nutricionais sobre dosagem de sal para um crescimento mais promissor; relação entre a dosagem dietética de sal e diferentes pH da água; melhores níveis de dureza considerando ambientes de pH ácido, alcalino e próximo à neutralidade; análise do crescimento e sobrevivência sob durezas da água extremamente baixas; efeito do pH na sobrevivência e no crescimento e a possibilidade do NaCl introduzido na ração auxiliar no desenvolvimento de tais indivíduos em ambientes de pH extremos e; verificação de perdas e ganhos iônicos sob diferentes condições de dureza da água e sal disponível na ração em diferentes níveis de pH.

De forma resumida, o estudo permitiu concluir para juvenis de jundiá, que:

- A exposição em águas ácidas (pH 5,5) ou alcalinas (pH 9,0) não afeta a sobrevivência, entretanto reduz o crescimento;

- a melhor dureza se encontra entre 25-60 mg L^{-1} $CaCO_3$;

- distúrbios de ionorregulaçãosão mais pronunciados em águas de alta dureza;

- em dureza zero da água, exposição a pH 7,0 e 8,0 reduz a sobrevivência e o crescimento, mas o mesmo não acontece em pH 6,0;

- o aumento de sal na ração protege contra o impacto de águas ácidas (5,5) no crescimento e o melhor crescimento registra-se em águas neutras ou alcalinas (7,0 e 9,0), independentemente da quantidade de sal na ração;

- a inclusão de sal na dieta contribui para reduzir os distúrbios ionorregulatórios em águas ácidas e alcalinas.

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