CRISTIANO CAMPOS MATTIOLI

RESPOSTAS FISIOLÓGICAS DE *Lophiosilurus alexandri* A DIFERENTES SITUAÇÕES DE ESTRESSE: TESTE DE EXPOSIÇÃO AO AR, CHOQUE TÉRMICO E CHOQUE OSMÓTICO

Tese apresentada ao Programa de Pós-graduação em Zootecnia da Escola de Veterinária da Universidade Federal de Minas Gerais como requisito parcial para obtenção do grau de Doutor em Zootecnia.

Área de concentração: Produção Animal

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“Tudo o que temos de decidir é o que fazer com o tempo que nos é dado”.

(Gandalf)

Aos meus amados pais João Batista e Elaine Torres, a quem devo a vida. Os grandes responsáveis pela pessoa que sou. Vocês são pessoas grandiosas que sempre estiveram ao meu lado nos bons e maus momentos, me mostraram o certo e o errado e me deram o poder de escolha. Pai e mãe vocês são parte de minha alma, e nada mais justo do que dedicar esta conquista a vocês, por todo o apoio e incentivo e por serem assim, meus heróis.

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LISTA DE ABREVIATURAS

ALT.....Alanina Aminotransferase

ANOVA.....Análise de variância

AST.....Aspartato Aminotransferase

BE.....Base Excessiva

Ca^{++}.....Cálcio

Cl^{-}.....Cloreto

FOAL.....Fosfatase Alcalina

HCO_3^{-}.....Bicarbonato

Ht Hematócrito

K^{+}.....Potássio

LAQUA.....Laboratório de Aquacultura da UFMG

LDH.....Lactato Desidrogenase

MDH.....Malato Desidrogenase

Na^{+}.....Sódio

nCa^{++}.....Cálcio ionizado

pH.....Potencial Hidrogênico

PPT.....Proteína Plasmática Total

$PvCO_2$.....Pressão parcial de Dióxido de Carbono

PvO_2 Pressão parcial de Oxigênio

sO_2.....Saturação de Oxigênio

$stHCO_3^-$.....Bicarbonato Padrão

tCO_2.....Taxa de Dióxido de Carbono

tHb.....Hemoglobina total

RESUMO

RESPOSTAS FISIOLÓGICAS DE *Lophiosilurus alexandri* A DIFERENTES SITUAÇÕES DE ESTRESSE: TESTE DE EXPOSIÇÃO AO AR, CHOQUE TÉRMICO E CHOQUE OSMÓTICO

O presente estudo teve como objetivo avaliar as respostas fisiológicas de estresse em juvenis de *Lophiosilurus alexandri* submetidos aos testes de exposição ao ar, choque osmótico e térmico. Para o teste de exposição ao ar foram utilizados 72 juvenis com peso de 361,06 ± 42,82 g. As coletas de sangue foram realizadas nos tempos 0 h: sem exposição ao ar; 0,5 h: animais logo após a exposição ao ar de 30 min (antes de voltar para o tanque); 1,5 (90 minutos), 24, 48 e 96 horas pós exposição ao ar. Para os testes de choque salino e térmico foram utilizados 30 juvenis para cada teste. Dez juvenis não foram submetidos ao teste, permanecendo em condições normais (água doce com temperatura de 28,0 °C). Os demais (20 animais de cada teste) foram submetidos ao choque de exposição ao estresse com (água salinizada a 10,0 g de sal/L ou água resfriada a 18,0 °C). As coletas de sangue foram realizadas nos tempos 0 h: sem exposição ao fator estressor; 1 hora e 24 horas após os testes. No teste de exposição ao ar, após 96 h a sobrevivência foi de 100%. O cortisol e a glicose foram maiores no tempo 0,5 h retornando a valores basais em 48 e 24 h, respectivamente. Já nos testes de choque osmótico e térmico, no tempo de 24 h a sobrevivência foi de 100% em ambos os testes. O cortisol e a glicose foram maiores no tempo de 1 h, apresentando queda em suas concentrações às 24 h. Já a enzima lactato desidrogenase não apresentou diferença no teste de temperatura, mas indicou menores concentrações às 1 e 24 h. Após o estresse no teste por exposição ao ar, a lactato desidrogenase apresentou maiores valores 1,5 h após exposição ao ar, retornando a valores normais em 24 h. Com relação a hematologia e bioquímica sanguínea, a exposição ao ar não afetou ($P>0,05$) o volume globular e a enzima aspartato aminotransferase (AST) ao longo de 96 h. A fosfatase alcalina apresentou os maiores valores ($P<0,05$) 0, 1,5 e 24 h. A proteína total foi semelhante entre 0 e 1,5 h ($P>0,05$) e os menores valores a 96 h. A alanina aminotransferase (ALT) foi maior a 0,5 h. A contagem de leucócitos foi maior a 0,5, 1,5, 48 e 96 h. A contagem de eritrócitos apresentou os maiores valores em 96 h ($P<0,05$). A exposição ao choque térmico não afetou ($P>0,05$) a fosfatase alcalina, proteína plasmática total, o hematócrito, ALT e AST às 1 e 24 h. A fosfatase alcalina e a

proteína total no choque osmótico apresentaram os menores ($P<0,05$) valores a 24 h. Os leucócitos e os eritrócitos apresentaram diferenças após o choque osmótico a partir dos tempos pós teste, diferente do eritrócito do teste de temperatura que não apresentou alteração em 24 h ($P>0,05$). Várias alterações foram registradas na gasometria do sangue (pH, pCO_2, pO_2, taxa de hemoglobina, SO_2, BE tCO_2, HCO_3 e $stHCO_3$) e eletrolíticas (Na^+, Ca^{++}, nCa^{++} e K^+) pós estresses. Juvenis de *L. alexandri* foram capazes de reestabelecer os principais indicadores de estresse (cortisol, glicose), enquanto os demais (hematológicos, bioquímicos e gasométricos) apresentaram variações em sentido compensatório para o reestabelecimento do padrão fisiológico normal.

Palavras-chave: gasometria sanguínea, hematologia, bioquímica.

ABSTRACT

PHYSIOLOGICAL RESPONSES OF *Lophiosilurus alexandri* TO DIFFERENT STRESS SITUATIONS: OF AIR EXPOSURE TEST, THERMAL SHOCK AND OSMOTIC SHOCK

The aim of this study was to evaluate the physiological responses to stress in *Lophiosilurus alexandri* juveniles submitted to air exposure tests, osmotic and thermal shock. 72 juveniles weighing 361.06 ± 42.82 g were used for the air exposure test. Twelve juveniles were not submitted to the test and 60 were submitted to the air exposure test for 30 minutes. Blood samples were taken at 0 h: no air exposure; time 0.5 h: animals shortly after air exposure for 30 min (before returning to the tank); time 1.5 (90 minutes), 24, 48 and 96 hours after air exposure. 30 juveniles were used for the saline and thermal shock tests each. Ten juveniles were not submitted to the test, remaining in normal environmental conditions (freshwater water and water at 28° C, respectively). The remaining 20 animals from each test were submitted to stress shock by two different tests (saline water at 10.0 g salt / L and water cooled at 18.0° C). Blood samples were taken at 0 h: no exposure to the stressor factor; 1 hour and 24 hours after the tests. In the air exposure test, after 96 h survival rate was 100%. Cortisol and glucose were higher at 0.5 h, returning to baseline values at 48 and 24 h. In the osmotic and thermal shock tests, at 24 h the survival rate was of 100% in both tests. In the osmotic and thermal shock tests, cortisol and glucose were higher at 1 h, dropping concentrations at 24 h, respectively. Lactate dehydrogenase showed no difference in the temperature test, but indicated lower concentrations at 1 and 24 h. In the air exposure test, lactate dehydrogenase presented higher values at 1.5 h after exposure to air, returning to normal values in 24 h. Regarding hematology and blood biochemistry, air exposure did not affect ($P > 0.05$) globular volume and aspartate aminotransferase over 96 h. Alkaline phosphatase had the highest ($P < 0.05$) values at 0, 1.5 and 24 h. Total protein was similar between 0 and 1.5 h ($P > 0.05$) and the lowest values at 96 h. Alanine aminotransferase was greater at 0.5 h. Leukocyte was higher at 0.5, 1.5, 48 and 96 h. Erythrocyte presented higher values in 96 h ($P > 0.05$). Considering hematology and blood biochemistry, exposure to thermal shock did not affect ($P > 0.05$) alkaline phosphatase, total plasma protein, hematocrit, ALT and AST at 1 and 24 h. Alkaline phosphatase and total protein from osmotic shock showed lower ($P < 0.05$) values at 24

h. Leukocyte and erythrocyte after the osmotic shock showed differences from the post-test times, different from the erythrocyte from temperature test, which did not change in 24 h (P> 0.05). Several changes were recorded in the blood gas variables (pH, $PvCO_2$, PvO2, hemoglobin, sO2, BE tCO_2, HCO_3^- and $stHCO_3^-$) and electrolytes (Na^+, Ca^{++}, nCa^{++} and K^+) post stresses. Juveniles of *L. alexandri* were able to reestablish the main indicators of stress (cortisol, glucose), while the others (hematological, biochemical and gasometric) presented compensatory variations for normal physiological reestablishment.

Keywords: blood gas analysis, hematology, biochemistry

1. REVISÃO DE LITERATURA

1.1 *Lophiosilurus alexandri* (Pacamã)

O teleósteo *Lophiosilurus alexandri* (Steindachner, 1876) pertence à família Pseudopimelodidae e tem como habitat natural a bacia do rio São Francisco (Shibata, 2003). Conhecido popularmente como pacamã, pacamão ou peixe-sapo, é considerado um animal dócil e de fácil manejo (Travassos, 1960). Observa-se nesta espécie temperamento calmo, de hábito tipicamente noturno (Tenório et al., 2006). Habita regiões de fundo arenoso ou de pedras (Travassos, 1959), pertencendo a mesma ordem de espécies de peixes nativos importantes (Siluriformes), como o surubim *Pseudoplatystoma fasciatum* e o pintado *P. corruscans*, fazendo parte da família de bagres neotropicais de água doce, que ocorrem apenas na América do Sul (Barros et al., 2007).

A espécie caracteriza-se por apresentar cabeça achatada, mandíbula que ultrapassa o maxilar superior, dentes da mandíbula que ficam fora da boca quando fechada (Britski et al., 1986), com olhos pequenos e redondos, barbilhões e dentes pequenos (Tenório, 2003). É um peixe de hábito alimentar carnívoro (Souza et al., 2014) e apresenta desova natural em lagoas (Sato et al., 2003) e em cursos de rios de fluxo lento (Sato e Godinho, 1988). Sua reprodução também pode ser feita através de indução hormonal (Santos et al., 2013) ou por controle térmico da água em laboratório, apresentando cuidado parental com o macho cuidando da massa de ovos (Costa et al., 2015). A fecundidade do pacamã é considerada baixa, o que é característico de espécies de comportamento parado, especialmente as que apresentam cuidado parental (Santos et al., 2013). A sexagem desta espécie pode ser realizada através de análises comparativas da papila genital (Lopes et al., 2013) ou por técnicas de celiotomia (incisão na cavidade abdominal) e celioscopia (exame visual da cavidade abdominal) (Mellilo-Filho et al., 2016).

Há relatos que peixes adultos podem atingir até 8 kg de peso vivo, sendo considerada uma espécie com grande potencial produtivo, a partir das técnicas de cultivo que estão sendo realizadas com sucesso (Cardoso et al., 1996; Costa et al., 2015), apresentando alto valor de mercado por sua carne sem espinhas intramusculares

e de sabor agradável, apreciada pelo consumidor (Marques et al., 2008). Este fato tem levado a prática da pesca predatória às populações selvagens, colocando-a na lista de peixes vulneráveis a extinção (BioBrasil, 2015). Neste sentido, torna-se relevante o repovoamento desta espécie nos reservatórios hidroelétricos localizados na bacia hidrográfica de origem (Meurer et al., 2010). Por essas razões, esforços estão sendo direcionados para o estudo e desenvolvimento de sua reprodução (Barros et al., 2007, Costa et al., 2015), larvicultura e condicionamento alimentar (Santos e Luz, 2009; Luz et al., 2011; Salaro et al., 2015; Cordeiro et al., 2016), alevinagem (Lopes et al., 2013; Souza et al., 2014), transporte (Luz et al., 2013), nutrição (Souza et al., 2013; Figueiredo et al., 2014) e aprimoramento da produção de juvenis em cativeiro (Mattioli, 2017; Costa et al., 2016, Costa et al., 2017).

1.2 Estresse em peixes por exposição ao ar

Estudos envolvendo o estresse em peixes têm sido frequentemente realizados no campo da fisiologia de peixes, com enfoque em produção comercial (Tort, 2011). Os comportamentos fisiológicos e metabólicos dos peixes frente a um agente estressor parecem variar de espécie para espécie, sendo que até o momento as informações sobre os efeitos no *L. alexandri* são ainda escassas. Segundo Iwama (1993), em produções intensivas, o estresse dos animais é praticamente inevitável. São conhecidos diversos fatores ambientais que influenciam o estado de saúde dos peixes (Bolasina, 2011). De forma geral, o processo ocorre de duas maneiras diferentes, sendo importante diferenciá-las, como o estresse agudo e o estresse crônico (Moreira et al., 2011). O primeiro está geralmente ligado à sobrevivência, como reação de fuga durante o manejo dos animais em loco ou durante a realização de biometrias que, normalmente, expõem ao ar, submetendo os a um estresse agudo, mas de curta duração (Ferguson e Tufts, 1992). O segundo tipo de estresse é o crônico, considerado como um estado de desconforto prolongado e contínuo, e pode ter alguns efeitos em que as consequências, geralmente são a redução do crescimento, ganho de peso e queda da resistência a diversos parasitas, devido à resposta imunológica deprimida (Iversen et al., 2005).

As respostas aos efeitos estressores externos, que podem ser de natureza física, como a alta densidade populacional, confinamento e exposição ao ar por captura, são

evidenciadas, primeiramente, em nível bioquímico, seguidos por respostas fisiológicas e, por fim, manifestando-se em nível morfológico (Fanouraki et al., 2011). Deste modo, alterações em todos estes níveis são indicativos de estresse e, portanto, parâmetros como cortisol e glicose plasmática são importantes indicadores fisiológicos do grau de estresse em peixes (Bonga, 1997).

De acordo com a literatura, a resposta geral ao estresse em peixes apresenta três níveis, sendo eles, primário, secundário e terciário (Silva et al., 2012), que se iniciam no sistema endócrino e apresentam aumento sucessivo até atingir o organismo como um todo, revelando assim alterações visuais de comportamento (Oba et al., 2009). A resposta primária é compreendida como a ativação dos centros cerebrais, resultando em liberação de catecolaminas (adrenalina e noradrenalina) e corticosteroides (cortisol e cortisona) no plasma (Morgan e Iwama, 1997). A resposta secundária é entendida como a canalização das ações e efeitos imediatos desses hormônios no sangue e tecidos, incluindo o aumento da frequência cardíaca e da tomada de oxigênio, a mobilização de substratos energéticos e, ainda, a perturbação do balanço hidromineral (Silveira et al., 2009). A resposta terciária manifesta-se em nível de população, traduzindo-se em inibição do crescimento, da reprodução e da resposta imunológica (Lima et al., 2006). A limitação da capacidade do animal em tolerar estressores subsequentes ou adicionais também é atribuída a uma manifestação da resposta terciária (Tripathi et al., 2013).

Uma das respostas mais significativas do peixe, após a exposição ao ar, é cessar sua alimentação (Takaoka et al., 2014). Tal comportamento vem acompanhado pelos efeitos assimilatórios das catecolaminas e corticosteroides sobre as reservas energéticas dos tecidos corporais, resultando num reduzido crescimento nos peixes estressados (Bernier, 2006). O cortisol exerce um efeito inibitório sobre a síntese proteica e isto pode ser utilizado como indicador de crescimento somático (Ferguson e Tufts, 1992; Kraul et al., 1993).

Embora exista uma vasta literatura sobre a hematologia e bioquímica de teleósteos (Wilhelm Filho et al., 1992; Walencik e Witeska, 2007; Hrubec e Smith, 2010), pouco se conhece sobre a influência do estresse por exposição ao ar na fisiologia da espécie em questão. Desse modo, informações a partir do estudo de sua natureza podem proporcionar relevantes indicações das alterações do funcionamento do organismo animal.

A exposição ao ar pode provocar estresse nos peixes (Stecyk et al., 2004), resultando em esgotamento do glicogênio hepático, aumento dos níveis de cortisol e glicose, alterações bioquímicas sanguíneas que envolvem a enzima lactato desidrogenase, as proteínas plasmáticas totais e os triglicerídeos plasmáticos (Mahfouz et al., 2015). Na truta-arco-íris *Oncorhynchus mykiss*, tanto a sO_2 (saturação de oxigênio) no sangue, como a quantidade de oxigênio correlacionado a tHB (taxa de hemoglobina total) diminuíram em 80%, durante a exposição ao ar por 60 segundos (Ferguson e Tufts, 1992), causando uma grave ausência de oxigênio sanguíneo (anóxia). A manutenção da função cardíaca sem o equilíbrio adequado de gases requer uma baixa potência cardíaca de costume (Farrel, 1991), e uma baixa demanda de ATP a partir de um elevado potencial glicolítico cardíaco e um meio de lidar com os resíduos anaeróbios (Farrel e Stecyk, 2007). Em curto prazo, os peixes tendem a manter os níveis de oxigênio metabólico pela hiperventilação das brânquias (Randall e Shelton, 1963), elevação da frequência cardíaca, como consequência dos efeitos da liberação de catecolaminas (Henry e Houston, 1984) e compensação cardíaca pela alteração de pressão dos gases arteriais de oxigênio e dióxido de carbono (Keen e Gamperl, 2012), ocasionando o aumento da área de perfusão branquial (Davis, 1972) e troca gasosa nas brânquias (Sollid e Nilsson, 2006).

A difusão de gases nas brânquias dos teleósteos é mais dificultada pela excreção de CO_2 do que pela captação de O_2, como observado em jeju *Hoplerythrin usunitaeniatus* (Lima Boijink et al., 2010). Mecanismos de hiperventilação são respostas ao decréscimo na concentração de oxigênio no sangue, induzido pela acidose sanguínea (Keen e Gamperl, 2012). Em contrapartida, a elevação na concentração de CO_2 no sangue é capaz de desencadear respostas cardiorrespiratórias (Porteus et al., 2012). No entanto, o aumento na pressão de CO_2 arterial e simultânea diminuição do pH são inevitáveis durante o aumento de volume sanguíneo cardíaco, mecanismo este utilizado para alterar o gradiente de difusão entre o sangue e a água, diminuindo a pressão parcial de dióxido de carbono (Milson, 2012). Além destes comportamentos sistêmicos, também ocorrem alterações compensatórias hematológicas, que aumentam a afinidade das moléculas de hemoglobina pelo oxigênio e aumentam a capacidade de transporte de oxigênio do sangue (Lewis et al., 2012).

Esta tentativa de adaptação ocorre pelo resgate de células vermelhas, armazenadas nos tecidos linfoides primários (rim cefálico e timo) e secundários (sistema linfático, baço, tecidos linfoides) (Salinas, 2015), pela precipitação da maturação dos glóbulos vermelhos circulantes (Randall, 1982) e proliferação de novos glóbulos vermelhos (eritropoiese) (Randall e Perry, 1992). A produção de eritrócitos é comum em peixes sob efeito do estresse respiratório (Nikinmaa, 1990). Em carpa comum, *Cyprinus carpio*, foi relatado que a produção de eritrócitos ocasionada por efeito do estresse deriva de diferentes eritroblastos e eritrócitos maduros que se diferenciaram, tornando-se fonte de eletrólitos envolvidos neste processo (Lin, et al., 2011), considerando-se possível que o catabolismo a partir da hiperglicemia resulte em maior disponibilidade de pronto uso da energia, que por sua vez auxilia de forma secundária no controle osmótico (Goniakowska-Witalinska, 1974). Este fato reforça a hipótese de que o comportamento hematológico dos peixes determine a eficiência do transporte de gases dissolvidos no meio sanguíneo para os tecidos (Holland e Forster, 1966).

1.3 Estresse em peixes por choque osmótico e térmico.

Em condições que visam produção intensiva de peixes de água doce, salinizar a água torna-se um manejo viável na criação, devido ao seu baixo custo e pela sua capacidade de equilibrar o gradiente osmótico, reduzindo a difusão de íons para a água e ajudando na produção de muco (Qiang et al., 2012). De acordo com Araújo et al, (2009), os estudos sobre o estado sanguíneo de peixes de água doce em condições de cultivo, têm aumentado nas últimas décadas, já que as informações dos componentes gasométricos, hematológicos e bioquímicos sanguíneos podem ser utilizados como índices de estresse. Em particular, Chew e Kohn (2000) afirmaram que a gasometria ou análise dos gases sanguíneos, que usualmente incluem a pressão parcial de oxigênio (PvO_2), pressão parcial de dióxido de carbono ($PvCO_2$), pH sanguíneo, taxa de hemoglobina e concentrações de bicarbonato (HCO_3^-) podem auxiliar com precisão na avaliação das trocas gasosas respiratórias (oxigenação) e parâmetros metabólicos (equilíbrio ácido-base) de animais produzidos em sistemas intensivos. Desta forma, torna-se essencial a realização de estudos que esclareçam os níveis de estresse para que a espécie trabalhada apresente melhores índices de desempenho (El-Sayed et al., 2003).

Ademais, a utilização do sal tem sido empregada na prática da profilaxia e controle de patologias em peixes de água doce (Garcia et al., 1999, Kolding e Zwieten, 2012). O sal é usado como mitigador de estresse em operações como manejos e transporte (Gomes et al., 2003; Souza-Bastos e Freire, 2009; Breves et al., 2010). No entanto, para juvenis, o uso do sal de forma equivocada pode afetar a excreção de amônia (Altinok e Grizzle, 2004), consumo de oxigênio (Altinok e Grizzle, 2003) e alterar a atividade locomotora, comportamento alimentar e crescimento (Luz et al., 2008; Cnaani e McLean, 2009), bem como a digestibilidade (Harpaz et al., 2005; Perry et al., 2006). Tolerância às mudanças de salinidade dependem de fatores como, por exemplo, fase do desenvolvimento de peixes de couro e de escamas (Niklitschek et al., 2013) e entre espécies. Fatores abióticos como temperatura, pH, resíduos de compostos orgânicos (amônia), podem afetar diretamente a adaptação dos animais ao ambiente salino (Olsen e Hasan, 2012).

Além disso, o uso de baixas salinidades tem proporcionado bons resultados de desempenho e sobrevivência na larvicultura de várias espécies de peixes neotropicais de água doce, em comparação aos registrados com larvas cultivadas em salinidades próximas a zero (Beux e Zaniboni-Filho, 2007; Santos e Luz, 2009; Jomori et al., 2012; Jomori et al., 2013). Como consequência destes resultados, salinidades de até 4 g de sal/L vêm sendo empregadas satisfatoriamente para a larvicultura de pacamã (Luz et al., 2008; Santos e Luz, 2009). Além disso, para o pacamã, ocorre o aumento da tolerância a diferentes gradientes de salinidade, a partir de seu desenvolvimento ontogenético (Luz e Santos, 2008). Segundo Mattioli et al, (2017), juvenis de pacamã apresentam melhores índices de crescimento a 2,5 g de sal/L e não apresentam mortalidade para valores de até 7,5 g de sal/L durante 28 dias, tolerando 10 g de sal/L durante 21 dias.

A salinidade também pode interferir nas atividades morfofisiológicas dos peixes em longo prazo, afetando seu desempenho e perfil metabólico (Deane et al., 2002). Segundo Cho et al. (2005), o contato do peixe a diferentes níveis salinos, em um curto espaço de tempo, pode provocar alterações nos índices de ingestão de água, podendo afetar suas atividades respiratórias e digestivas. No entanto, a possibilidade de uma ação física direta da salinidade no sistema circulatório ainda precisa ser elucidada.

Peixes podem desenvolver diversos mecanismos fisiológicos de adaptação, dentre eles, o aumento das células de cloreto presente nas brânquias (Fanouraki et al., 2011), que serão cruciais para a adaptação dos animais em ambientes com variações salinas, desde que a salinidade esteja dentro dos limites de adaptação para cada espécie (Breves et al., 2010). Limites superiores ao nível de tolerância do animal podem ocasionar doenças crônicas graves nas brânquias e alterações fisiológicas prejudiciais ao peixe (Huang et al., 2014). Dessa forma os estudos da tolerância dos níveis de salinidade são de extrema importância para o manejo dos animais, de forma que a implementação do sal durante o cultivo seja benéfica (Santos e Luz, 2009).

Além da salinidade, a temperatura da água também deve ser considerada (Wu et al., 2015). Sabendo-se que os peixes sofrem influência direta da temperatura da água, sendo este um dos principais fatores abióticos que pode afetar diretamente importantes funções fisiológicas e, consequentemente, seu metabolismo, consumo do oxigênio, crescimento e sobrevivência (Jian et al., 2003). Nos peixes, a temperatura corpórea varia de acordo com a do ambiente, sendo denominado como ectotérmico (Sadati et al., 2011), detendo zonas específicas que proporcionam seu desenvolvimento e sobrevivência (Strand et al., 2011).

A temperatura atua sobre as taxas de propagação molecular e reações bioquímicas dos peixes (Kooka et al., 2007), especialmente aquelas associadas a permeabilidade de membranas e funcionamento de células, tecidos e órgãos, afetando indiretamente o suprimento e demanda de energia e, dessa forma, influenciando o comportamento e o crescimento como um todo (Rawles et al., 2012). As neutralizações bioquímicas para os efeitos térmicos podem ser observadas pela continuidade da relação entre os substratos e enzimas metabólicas, bem como a lactato desidrogenase (LDH) e a malato desidrogenase (MDH), evidenciando que mudanças na temperatura propiciaram estratégias de adaptação pelos peixes (Hochachka e Somero, 2002).

A temperatura ideal de produção de peixes neotropicais mantém-se na faixa de 25 a 30º C (Frascá-Scorvo et al., 2007). Nesta faixa de variação, os mecanismos de crescimento muscular desempenham corretamente seu papel, no qual é um processo contínuo por toda a vida do peixe (Assis et al., 2004). Siluriformes apresentam alterações no desempenho produtivo e padrões sanguíneos relacionados às variações na

temperatura de cultivo (Lima et al., 2006), tornando-se interessante entender os efeitos da temperatura sobre o metabolismo dos organismos aquáticos, a fim de compreender que estratégias bioquímicas são adotadas para superar o desafio térmico e como consequência melhorar as técnicas de cultivo (Engin et al., 2013).

2. OBJETIVOS

2.1 Objetivo geral

Avaliar os efeitos dos testes de estresse, por exposição ao ar, choque osmótico e choque térmico nos parâmetros gasométricos, hematológicos e bioquímicos sanguíneos de juvenis de *L. alexandri*.

2.2 Objetivos específicos

Avaliar a tolerância de juvenis de pacamã aos testes de exposição ao ar, choque osmótico e choque térmico;

Avaliar as alterações gasométricas sanguíneas em juvenis de pacamã submetidos aos testes de exposição ao ar, choque osmótico e choque térmico;

Avaliar as alterações hematológicas sanguíneas em juvenis de pacamã submetidos aos testes de exposição ao ar, choque osmótico e choque térmico;

Avaliar as alterações bioquímicas sanguíneas em juvenis de pacamã submetidos aos testes de exposição ao ar, choque osmótico e choque térmico;

Avaliar alterações sanguíneas no período pós estresse e suas interações subsequentes em juvenis de pacamã submetidos aos testes de exposição ao ar, choque osmótico e choque térmico.

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4. Artigo 1

Physiological and metabolic responses of juvenile *Lophiosilurus alexandri* catfish to air exposure

Highlights

1. Cortisol and glucose levels were higher at 0.5 h, after air exposure returning to baseline at 48 and 24 h, respectively, after air exposure.

2. Several changes were recorded in gasometric blood values (pH, $PvCO_2$, PvO_2, total hemoglobin, sO_2, BE tCO_2, HCO_3^- and $stHCO_3^-$) and electrolytes (Na^+, Ca^{++}, nCa^{++} and K^+), after air exposure.

3. Juvenile *L. alexandri* were able to reestablish the main indicators of stress, while other indicators exhibited compensatory variation for normal physiological re-establishment.

Abstract

The present study aimed to evaluate the physiological and metabolic stress responses of juvenile *Lophiosilurus alexandri* submitted to an air exposure test. The study subjects consisted of 72 juveniles weighing 361.06 ± 42.82 g, with 12 not being subjected to the test and 60 subjected to 30 min of air exposure. Blood samples were taken at: 0 h – fish not exposed to air; 0.5 h – fish shortly after exposure to air for 30 min (prior to returning to the tank); 1.5 h (90 min), 24, 48 and 96 h after the initiation of exposure to air for 30 min. After 96 h, survivorship was 100%. Cortisol and glucose levels were higher at 0.5 h, returning to baseline at 48 and 24 h, respectively. Lactate dehydrogenase levels were highest at 1.5 h after exposure to air, returning to normal values in 24 h. Several changes were recorded in gasometric blood values (pH, $PvCO_2$, PvO_2, total hemoglobin, sO_2, BE, tCO_2, HCO_3 and $stHCO_3$) and electrolytes (Na^+, Ca^{++}, nCa^{++} and K^+). With regard to hematology and blood chemistry, exposure to air did not affect globular volume and AST throughout the 96 h of the experiment ($P > 0.05$). The values for alkaline phosphatase were highest ($P < 0.05$) at 0, 1.5 and 24 h. Total protein was similar between 0 and 1.5 h ($P > 0.05$) and lowest at 96 h, while ALT was highest at 0.5 h. Leukocytes were highest at 0.5, 1.5, 48 and 96 h, while erythrocytes were highest at 96 h ($P > 0.05$). After 96 h, juvenile *L. alexandri* were able to reestablish the main indicators of stress (cortisol, glucose and lactate dehydrogenase), while other indicators (hematological, biochemical and gasometric) exhibited compensatory variation for normal physiological re-establishment.

Keywords: physiological stress, blood gasometric analysis, management.

Introduction

Understanding the physiology of fish is vitally important to fish farming since it explains not only the functioning of different body parameters, but also responses to various environmental changes (Baldisserotto et al. 2014).

In intensive pisciculture, situations of stress are constantly present and can affect the productivity of fish by negatively impacting their health and increasing their susceptibility to disease (Diniz and Honorato 2012). Thus, attempts to understand fish welfare have included modeling standards of good practice, guidelines and legislation on how these animals should be handled in captivity (Volpato 2007).

Many factors that individually, or in combination, can impose stress on the physiological system of fish are related to human interference, such as employing routine practices in pisciculture (Luz and Portella 2005; Abreu et al. 2009) like exposure to air during transportation and biometric measurement. When fish are captured, whether by hooks, nets or dip-nets, they attempt to escape, and thus pass through air exposure, resulting in, among other things, an increase in the concentration of lactate dehydrogenase and H^+ ions in their muscle, which can be identified in the bloodstream, and elevated concentrations of cortisol and glucose (Inoue et al. 2008). Exposure to air can also lead to leucopenia, hyperglycemia and ionic alterations (Trushenski et al. 2010), with the time taken to recover to basal physiological conditions depending on the species and stress intensity (Cnaani and McLean 2009).

Another response of fish to air exposure is cessation of feeding (Takaoka et al. 2014). This, together with the catabolic effects of catecholamines and corticosteroids on energy reserves of body tissues (Parra et al. 2013), can result in the loss of immunological system functioning, causing reduced growth and low survival (Adamante et al. 2008). In conditions of chronic stress, cortisol exerts an inhibitory effect on the protein synthesis of muscle tissue and organs, which can be used as an indicator of reduced growth of the fish as a whole (Kraul et al. 1993). Thus, when fish are exposed to acute stressors, a series of responses are initiated, and if stress is long-lasting, high levels of biological organization become affected (Ferguson and Tufts 1992). In this context, understanding the effect of stressors, in order to minimize the

effect on the immune system of fish and improve their physiological condition in intensive pisciculture, is necessary.

The catfish, *Lophiosilurus alexandri*, is a carnivorous fish that has been studied for commercial and restocking purposes. Its reproduction occurs in a piecemeal form and can be manipulated by controlling environmental factors such as temperature (Da-Costa et al. 2015). It has already been successfully raised in larviculture (Santos and Luz 2009; Takata et al. 2014; Cordeiro et al. 2016), as well as through the stage of food conditioning, during which these animals accept formulated feed (Melillo-Filho 2014; Silva et al. 2014; Salaro et al. 2015), nutritional (Figueiredo et al. 2014; Costa et al. 2015; Melo et al. 2016) and behavioral (Kitagawa et al. 2015) studies have also been performed on animals fed with diets.

Thus, the objective of the present study was to evaluate the physiological stress response of juvenile *Lophiosilurus alexandri* subjected to air exposure.

Material and Methods

Ethical approval

All experimental protocols were approved by the Committee for Ethics in Animal Experimentation of the Universidade Federal de Minas Gerais (approval reference number: 280/2016). The study complied with the ethical principles under which Experimental Physiology operates, and the experiments complied with the journal's animal ethics principles and regulation checklist (Grundy 2015).

Fish

Juvenile *L. alexandri*, previously conditioned to accept formulated diets, were maintained in the Laboratório de Aquicultura of the Escola de Veterinária of the Universidade Federal de Minas Gerais, in 400 L tanks mounted in a recirculating-water system. Fish were feed a commercial extruded diet (2.6 mm in diameter, 36% maximum crude protein, 17.56 kJ gross energy, 8.35% ether extract; 4% maximum crude fiber, 15% maximum mineral material, 2% minimum calcium and 1% minimum phosphorus; manufacture's data), offered at 8.00 and 16.00 h until they reached satiety. The photoperiod was maintained at 10 h of light and 14 h of darkness.

During the pre-experimental maintenance period of the animals, mean air temperature and relative humidity was maintained at 29 ± 1 ºC and 75% respectively. Water temperature was maintained at 28 ± 0.5 ºC, dissolved oxygen kept above 5 mg L^{-1}, and pH and conductivity at 6.9 ± 0.1 and 0.2 ± 0.2 mS cm^{-1}, respectively, as measured by a YSI multiparameter probe (Model 6920 V2, Xylem Analytics' global brands, Letchworth, United Kingdom). Osmolarity of the water, measured using Osmomat 030® equipment (Gonotec Gmbh, Berlim, Alemanha), was 7.1 ± 6.2 mOsmol L^{-1}, and total ammonia concentration remained below 1.0 mg L^{-1}, using the methodology described in Standard Methods 20th Ed (APHA 1998).

Air exposure testing in *Lophiosilurus alexandri*

To test air exposure effect, 72 individual juveniles of *L. alexandri* weighing 361.06 ± 42.82 g, were used. Prior to the test, fish were fasted for 24 h. Twelve juveniles were selected to not-undergo testing (0 h) while 60 individuals were submitted to air exposure testing for 30 min. For the test, animals were captured with a dip-net and maintained kept in individual dry containment tank, out of the water for 30 min, after which they were placed in four 400 L tanks (12 animals per tank). The 30 min time of exposure to air was based on preliminary management tests. Blood samples were collected and represented in the following manner: Time 0 h – fish that had not been submitted to the air exposure test; Time 0.5 h – fish shortly after air exposure for 30 min (before returning to the tank); Time 1.5 h – fish 90 min after initiation of exposure to air for 30 min; Time 24 h – fish 24 h after the initiation of exposure to air for 30 min; Time 48 h – fish 48 h after the initiation of exposure to air for 30 min; and Time 96 h – fish 96 h after the initiation of exposure to air for 30 min (n =12 for each sampling time).

During observations, the animals were maintained under the same conditions described previously; however, during the entire 96 h the fish were not fed.

Gasometric, hematological and biochemical analyses

Blood collection was performed by caudal vertebral venous puncture, with ventral access.

Two blood samplings were performed on each animal. In the first, 500 μL of blood was collected without anticoagulant. From this volume, 300 μL was used to analyze the following parameters: pH, tCO_2 (carbon dioxide rate), PvO_2 (partial venous oxygen pressure), $PvCO_2$ (partial venous carbon dioxide pressure), tHB (total hemoglobin), sO_2 (oxygen saturation), and concentrations of Na^+, K^+, Ca^{++} and nCa^{++} (ionized calcium standardized by pH). With these data, the following were calculated: $HC0_3^-$ (bicarbonate), $stHC0_3^-$ (standard bicarbonate) and BE (base excess) using an OPTI CCA Cassettes kit and an OPTI CCA-TS analyzer (OPTI Medical Systems, Inc®, USA; www.optimedical.com). This equipment uses optical fluorescence and reflectance as a reference, thus eliminating the use of electrodes and contact points. The remaining 200 μL of the blood sample was centrifuged at 1,500×g for 1 minute and 2,000×g for 4 min for separation of the liquid fraction. The sample was then frozen at -80 ºC, for serum stabilization, and subsequently analyzed for plasma cortisol levels, which were determined using a commercially available enzyme-linked immunosorbent assay kit ELISA (Elabscience®, USA; www.elabscience.com, Houston, EUA), according to procedures validated for fish (Barcellos et al. 2010).

The second 500 μL blood sample of each animal, was used for hematological and biochemical analyses. Sodium heparin (10% μL mL^{-1} of blood) was used for this sample. Approximately 50 μL was used for globular volume (VG) analysis of previously homogenized blood in capillary tubes (approximately two-thirds filled). These capillary tubes were centrifuged at 10,867×g for 5 min, by the microhaematocrit method (SPIN 1000, Microspin®, USA; www.hettichlab.com). The reading was performed on the appropriate card, matching the plasma meniscus with the upper line of the ruler (line 100) and the lower end of the erythrocyte portion lined-up with the lower line of the ruler (line 0), so that the resulting VG is indicated by the value of the line. Immediately after collection, aliquots of 10 μl of blood were added to 1 mL of Dacie dilution solution in the ratio of 1:50 (Jain 1986), for total erythrocyte and leukocyte counts using a hemocytometer Neubauer chamber. Hematological procedures were performed within 6 h after blood collection.

Erythrocyte and leukocyte counts using a hemocytometer were performed under an optical microscope at 40x magnification within 24 h after collection, which was made possible by the presence of formaldehyde in the Dacie solution. The reading was

made according to procedures for mammals, with some adaptations. The leukocytes were counted in the four lateral squares, each with sixteen sub-squares. The erythrocytes were counted in the single central square with twenty-five sub-squares. Once the homogeneity, distribution and cellular quantity were observed, five diagonal squares were counted, as is usually done in standard mammalian analyses.

At the end of the hematological analyses, the remaining aliquots of heparinized whole blood were centrifuged at 1,500×g for 1 min and 2,000×g for 4 min for separation of the liquid fraction. This centrifugation protocol was adopted in order to minimize the occurrence of hemolysis. The biochemical profile was then determined for plasma samples using an automatic device Cobas-Mira Plus (Roche Diagnostic Systems®, USA; www.usdiagnostics.roche.com). The studied analytes were: total plasmatic proteins (PPT), glucose, alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), alkaline phosphatase and lactate dehydrogenase (LDH), using commercial kits (Synermed International Inc®, USA; www.synermedinc.com).

Data management and statistical analysis

Data were submitted to the Kolmogorov-Smirnov test for normality. The variable $PvCO_2$ underwent logarithmic transformation, and the data submitted to an analysis of variance (ANOVA) and Tukey test at 5% probability.

Results

Ninety-six hours after the 30 min air exposure test, survival was 100%.

Cortisol and glucose data are presented in Figure 1. The highest values for cortisol were at 30 min, immediately after exposure to air, and prior to being returned to the water, and returned to baseline values 48 h after the test. The highest values for glucose were also at 30 min, returning to baseline values 24 h after the test.

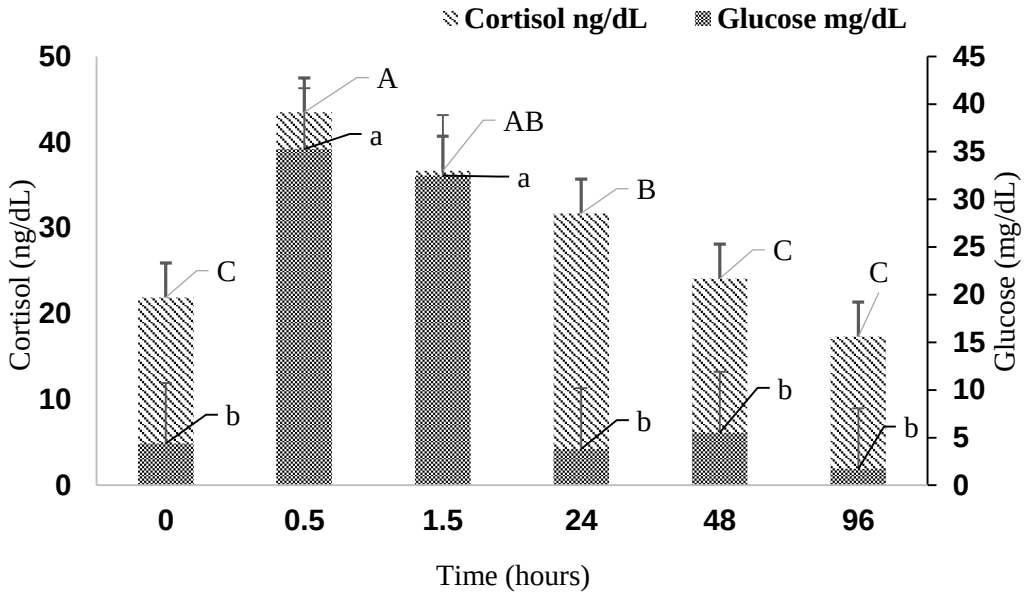

Figure 1. Mean values (± standard deviation) of cortisol and glucose. Different letters, upper case for cortisol and lowercase for glucose, represent a significant differences according to the Tukey test ($P < 0.05$).

The highest values for lactate dehydrogenase (LDH) were at 1.5 h after the test, returning to normal values at 24 h (Figure 2). After 48 h the LDH increased again, subsequently reducing at the end of 96 h.

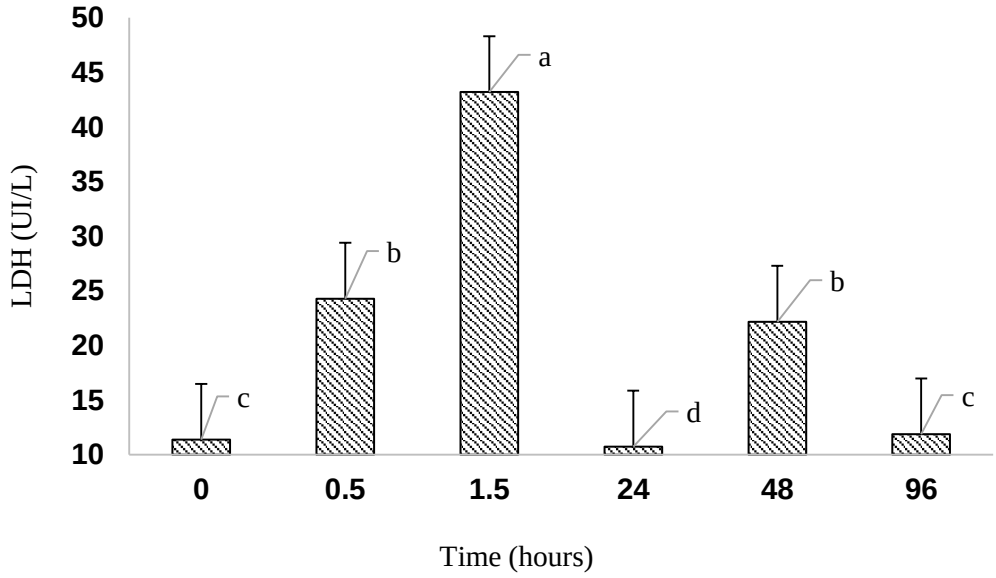

Figure 2. Mean values (± standard deviation) of lactate dehydrogenase (LDH). Different letters represent significant differences according to the Tukey test ($P < 0.05$).

The values obtained from blood gas analysis are shown in Table 1. The values for pH were lowest ($P < 0.05$) immediately after exposure to air (0.5 h), subsequently increasing up to 24 h. At 48 h the pH was similar to initial values ($P > 0.05$), while at 96 h it exhibited higher values ($P < 0.05$), but similar to the values at 24 h ($P > 0.05$).

The concentration of $PvCO_2$ was highest 0.5 h after exposure to air ($P < 0.05$), while PvO_2 remained similar to 0 h after 0.5 h ($P > 0.05$), with highest values 1.5 h after the test ($P < 0.05$), after which it reduced until 96 h. Hemoglobin rate (tHb) varied overtime, with higher values at 0.5, 1.5 and 48 h after the test and lower values at 24 and 96 h ($P < 0.05$).

The concentration of sO_2 exhibited higher values at 1.5 h, which remained similar through 48 h ($P > 0.05$), and reducing at 96 h, yet remaining above the values of 0 and 0.5 h ($P < 0.05$). The BE exhibited higher values ($P < 0.05$) at 0.5 and 1.5 h, while at 48 h it was lower ($P < 0.05$), yet similar to the values of 0 h ($P > 0.05$). Concentrations of tCO_2 and $HC0_3^-$ exhibited lower values at 1.5 h ($P < 0.05$). The values at 0, 0.5 and 48 h were higher and similar to each other ($P > 0.05$). The $stHCO_3^-$ exhibited a reduction from 0 to 1.5 h, returning to initial values at 48 h after the test.

Table 1. Mean gasometric values (± standard deviation) of hydrogen potential (pH), partial carbon dioxide pressure ($PvCO_2$ - mmHg), partial oxygen pressure (PvO_2 - mmHg), total hemoglobin (tHb - g dL^{-1}), oxygen saturation (sO_2 - %), base excess (BE - mmol L^{-1}), carbon dioxide rate (tCO_2 - mmol L^{-1}), bicarbonate (HCO_3^- - mmol L^{-1}) and standard bicarbonate ($stHCO_3^-$ - mmol L^{-1}) of blood samples of *Lophiosilurus alexandri*.

Time	**0h**	**0.5h**	**1.5h**	**24h**	**48h**	**96h**
pH	7.30±0.06b	6.98±0.05d	7.06±0.11c	7.48±0.04a	7.31±0.09b	7.41±0.03a
$PvCO_2$	11.92±0.99b	22.17±2.21a	12.33±1.82b	*	11.25±1.81b	*
PvO_2	28.50±8.70cd	23.16±5.11d	77.08±25.18a	60.58±21.86ab	44.92±24.08bcd	49.08±18.55bc
tHb	10.79±1.24abc	12.18±1.47a	11.07±1.19abc	9.39±1.50c	11.17±1.45ab	9.52±1.60bc
sO_2	59.80±0.93c	59.74±0.97c	81.58±3.91a	78.16±9.17ab	82.07±9.49a	71.39±7.26b
BE	-18.12±2.06b	-25.14±1.35a	-24.49±2.35a	*	-18.47±1.00b	*
tCO_2	5.97±0.74a	5.74±0.45a	3.95±0.82b	*	5.73±0.47a	*
HCO_3^-	5.61±0.72a	5.06±0.41a	3.60±0.78b	*	5.63±0.48a	*
$stHCO_3^-$	9.52±1.16a	6.74±0.62b	5.41±1.39c	*	8.91±0.59a	*

Different letters in a row represent significant differences (Tukey test $P < 0.05$).

* Indicates that the reading was beyond the capacity of the equipment.

Table 2 presents the results of the hematological and biochemical analyses. Exposure to air did not affect ($P > 0.05$) AST and globular volume of juvenile individuals of *L. alexandri* throughout the 96 h. Alkaline phosphate exhibited higher values ($P < 0.05$) at 0, 1.5 and 24 h after the test, which were all similar to one another ($P > 0.05$), while lower values ($P < 0.05$) were recorded 96 h after the test; the values at 0.5 and 48 h were intermediate. The PPT exhibited similar values from 0 to 1.5 h ($P > 0.05$) and lower values at 96 h ($P < 0.05$). The ALT was higher at 0.5 h, subsequently decreasing to 96 h ($P < 0.05$). Leukocytes exhibited higher values ($P < 0.05$) for 0.5, 1.5, 48 and 96 h, which were all similar ($P > 0.05$), while lower values were recorded at 0 and 24 h after the test. Erythrocytes exhibited higher values at 96 h after the test ($P > 0.05$), followed by 1.5 h; 0, 0.5, 24 and 48 h had lower values, which were all similar.

Table 2. Mean hematological and biochemical values (± standard deviation) of alkaline phosphatase (UI L^{-1}), total plasma protein (PPT - g dL^{-1}), alanine aminotransferase (ALT - UI L^{-1}), aspartate aminotransferase (AST - UI L^{-1}), globular volume (VG - %), leukocytes (x10^4 μL^{-1}) and erythrocytes (x10^6 μL^{-1}) of blood samples of *Lophiosilurus alexandri.*

Time	**0h**	**0.5h**	**1.5h**	**24h**	**48h**	**96h**
Alkaline phosphatase	33.10±7.11a	29.76±8.88ab	33.71±8.73a	34.89±5.82a	27.98±6.06ab	20.93±7.25b
PPT	3.01±0.27a	3.13±0.54a	3.26±0.42a	2.72±0.35ab	2.88±0.37ab	2.39±0.72b
ALT	6.07±2.31abc	7.61±3.20a	7.19±1.84ab	4.63±2.48bc	4.47±2.00bc	3.39±2.45c
AST	153.01±81.00a	140.98±37.10a	147.02±49.95a	108.76±39.64a	121.47±35.06a	117.36±46.10a
VG	21.91±2.71a	23.75±3.16a	24.58±0.79a	21.00±3.64a	24.17±3.54a	22.00±4.00a
Leukocytes	20.36±1.70b	35.10±2.13a	30.40±5.42a	19.86±4.37b	33.10±3.53a	31.10±5.69a
Erythrocytes	1.06±0.33c	1.12±0.48c	1.28±0.87b	1.09±0.24c	1.11±0.49c	1.59±0.17a

Different letters in a row represent significant differences (Tukey test $P < 0.05$).

With respect to electrolytes (Table 3), blood sodium exhibited lower concentrations at 24 and 96 h than at the other time periods ($P < 0.05$). Potassium had higher values 0.5 h after the exposure to air ($P < 0.05$), while the values were all similar to each other for the other time periods ($P > 0.05$). Blood calcium was higher at 0.5 h, intermediate at 48 h and lower at 0, 1.5, 24 and 96 h after the test ($P < 0.05$). Calcium ions (nCa^{++}) exhibited lower values 0.5 and 1.5 h after the test, and higher values at 24 h 0.5 ($P < 0.05$).

Table 3. Mean electrolyte values (± standard deviation) for sodium (Na^+ - mmol L^{-1}), potassium (K^+ - mmol L^{-1}), calcium (Ca^{++} - mmol L^{-1}) and ionized calcium (nCa^{++} - mmol L^{-1}) of blood samples of *Lophiosilurus alexandri*.

Time	**0h**	**0.5h**	**1.5h**	**24h**	**48h**	**96h**
Na^+	128.66±1.43a	129.58±1.50a	128.16±2.65a	125.08±2.64b	128.33±2.96a	125.42±1.93b
K^+	3.47±0.29b	4.77±0.56a	3.75±0.46b	3.20±0.47b	3.39±0.62b	3.30±0.32b
Ca^{++}	1.31±0.04b	1.38±0.02a	1.31±0.05b	1.29±0.03b	1.33±0.05ab	1.31±0.02b
nCa^{++}	1.25±0.05c	1.11±0.03d	1.11±0.06d	1.35±0.03a	1.35±0.05bc	1.28±0.02ab

Different letters in a row represent significant differences (Tukey test $P < 0.05$).

Discussion

The 30 min air exposure test did not cause mortality of juvenile *L. alexandri* after 96 h. This finding demonstrates the resistance of this animal to this type of test, which represents a mild intensity of stress. Although there is no data in the literature, this resistance is widely reported by fishermen who capture this species in the natural environment. In a previous work, Stoot et al. (2014) considered that moderate interaction with stressors can induce adaptive responses in fish, which restores homeostasis of the organism. However, if they were subjected to intense or prolonged stressors, the response could become ill adapted, with negative consequences for overall health, as was noted by Pottinger and Carrick (1999) for rainbow trout, *Oncorhynchus mykiss*, submitted to a prolonged stress test of environmental modification (3 h of confinement). In addition, long air exposure times, 3 h (Buttle et al. 1996) and 1 h (Martins et al. 2006), also did not cause mortality in the African catfish *Clarias gariepinus*, as was the case with *L. alexandri*.

The responses of *L. alexandri* to air exposure included higher cortisol levels after 30 min, before being returned to water. However, the cortisol levels returned to baseline values after 48 h. Leclercq et al. (2014) reported that plasma cortisol levels can return to basal levels in 24 h for the ballan-wrasse, *Labrus bergylta*. Thus, the magnitude of change in corticosteroid level in response to stress can vary widely among fish, even within the same species (Barton and Iwama 1991). Therefore, in a given population there may be fish that respond with a considerably high level of cortisol, as well as fish that respond more discreetly, yet both exhibiting similar performance (Weil et al. 2001). Analysis of salmonids under resting conditions found the cortisol level to be around 10 ng mL^{-1} (Mommsen et al. 1999), while in the silver-scaled *Leuciscus cephalus* it was approximately 250 ng mL^{-1} (Pottinger et al. 2000) and in *Brycon amazonicus*, it was around 90 ng mL^{-1} (Martins da Rocha et al. 2004). However, in situations of acute stress, such as with animal manipulation, there may be a considerable increase in plasma cortisol, reaching a concentration of approximately six-fold, from 2.90 to 17.24 $x10^4$ ng mL^{-1} in *Hoplerythrinus unitaeniatus* (Mariano et al. 2009) and 40 to 200 ng mL^{-1} in salmonids (Madaro et al. 2016). In the present study, the mean resting

cortisol level exhibited by *L. alexandri* was 21.9 ng mL^{-1}, while at maximum level it was 43.48 ng mL^{-1}, representing an increase of approximately two-fold.

In the present study, glucose had a stress response similar to that of cortisol. Mariano et al. (2009) subjected *H. unitaeniatus* to 12 h of aerial exposure and found the glucose level to double (203.26 to 413.53 ng mL^{-1}) after the 6 h, followed by a gradual return to basal levels with fish kept in the aquatic environment. However, in the present experiment with *L. alexandri*, glucose levels returned to values close to the control 24 h after the test, confirming that stress response is species-specific, besides there were significant differences in the magnitude of the stress tests. For other species, shorter times of exposure to air can increase glucose levels, such as increased glucose levels 1 h after a 3 min exposure for the gilt-head sea bream, *Sparus aurata* (Arends et al. 1999), while in *O. mykiss*, 30 seconds of air exposure was sufficient to increase basal glucose concentration (Sloman et al. 2001).

Exposure to a stressor agent can, in fact, trigger intense physical activity (Begg and Pankhurst 2004), which, in the case of insufficient oxygenation, is also fed via anaerobic glycolysis, leading to LDH accumulation in white musculature and, by diffusion, into the blood stream (Wood 1991). Plasma LDH level is therefore considered a useful indicator of anaerobiosis, which increases with forced swimming exercise and, in many cases, exhaustive stress exercises (Wood 1991; Begg and Pankhurst 2004). Consequently, secondary responses to stress typically include measurable changes in blood LDH levels (Barton 2002). This was verified for *L. alexandri* when LDH peaked at 1.5 h after exposure to air, returning to normal values 24 h after the test, but yet varying up to 96 h. Variation in LDH differs from the behavior of cortisol and glucose due to the aerobic metabolism of the animal possibly being maintained by transport efficiency and maintenance of O_2, associated with hematological adjustments during air exposure. In this way, for *L. alexandri*, the primary response of cortisol and the secondary response of glucose and LDH can be used as indicators of stress in analyses. For *L. bergylta*, Leclercq et al. (2014) found that cortisol exhibited its greatest increase after 1 min of air exposure, compared to glucose and LDH, indicating that the behavior of these variables in this study was consistent with what was observed in the present study.

The mean values of PvO_2 and $PvCO_2$ for *L. alexandri* ranged from 23.16 to 77.08 and 11.25 to 22.17 mmHg, respectively, according to post-stress time. The higher PvO_2 value at 1.5 h after air exposure can be attributed to an has some kind of aerial respiration to increase in common opercular beats in the early stages after exposure to air, which is a stress indicator. The effect of the action of catecholamines on the cardiorespiratory system is the dilation of blood vessels of the gills by increasing blood flow in order to increase the perfusion of the lamellae and to facilitate the exchange of gasses, thus favoring the greater distribution of gasses and their use by the corporal tissues (Brauner et al. 2000). Silver catfish, *Rhamdia quelen,* submitted to three different water temperatures (15, 23 and 30 ºC), exhibited PvO_2 and $PvCO_2$ values that varied between 5.0 ± 20.0 and 6.0 ± 13.0 mmHg, respectively (Gomes et al. 2011). According to these authors, gasometric values were also influenced by the type of fish studied and differences between arterial and venous blood. In addition, for *Colossoma macropomum*, exposure to different water pH (3.0, 4.0, 5.0 and 6.5) also exhibited variation, through arterial blood collection, with mean PvO_2 and $PvCO_2$ values of 34.0 ± 46.0 and 4.7 ± 5.8 mmHg, respectively (Wood et al. 1998), demonstrating that other management factors may also affect these parameters. Indeed, hypoxia tests with *Salminus maxillosus,* reported PvO_2 values similar to those of the present study (Souza et al. 2001).

In general, the behavior of the pH of the venous blood of *L. alexandri* in this work was similar to that of another species of catfish, *Hypostomus aff. Pyreneusi*, submitted to hypoxia stress testing, (Scott et al. 2017). However, in the present study the lowest pH values were recorded when $PvCO_2$ values were highest and PvO_2 values were lowest (0.5 h after exposure to air), as reported by Gomes et al. (2011). A reduction of blood pH was also documented in *H. unitaeniatus* submitted to a test of 1, 6 and 12 h of air exposure, characterized by metabolic parameters (acid-base imbalance) (Mariano et al. 2009).

In this study, respiratory acidosis and, consequently, metabolic acidosis were recorded, since pH and plasma bicarbonate values were altered in relation to the control group, characterized by the decrease of pH, reduction of sO_2 and HCO_3^- levels and an increase of $PvCO_2$. The variable HCO_3^- permitted the evaluation of the response of *L. alexandri* to variation in pH in the blood and its influence on blood protein that carries

oxygen. Bicarbonate and hemoglobin are the main immediate sources of buffering, with bicarbonate accounting for more than 50% of the extracellular buffer capacity (Milson 2012), a fact that may explain its lower values at the points of greatest stress, in an attempt to maintain suitable pH levels. Normally, changes in bicarbonate concentrations are directly related to elevated $PvCO_2$ because the compensatory mechanism raises bicarbonate rates in order to neutralize excess CO_2 present in blood (Damsgaard et al. 2015a). However, *L. alexandri* exhibited contradictory behavior, with a drop in plasma concentration of plasmatic HCO_3^- and $stHCO_3^-$ 1.5 h after stress. Hemoglobin changes with the degree of oxygenation, and since O_2 is poorly water-soluble only a small amount dissolves in plasma, the remainder being transported by hemoglobin (hemoglobin saturation) (Damsgaard et al. 2015b). Thus, the O_2 content of blood is determined mostly by oxyhemoglobin saturation (Keen and Gamperl 2012), with the effect of exposure to air to being the highest level of hemoglobin soon after the stress to the animal, as found in this work.

For *L. alexandri* there was a decrease in total plasma protein 96 h after the test. This fact demonstrates that the determination of plasma and its fractions is of great clinical importance because these concentrations can be altered, mainly, by changes in plasma volume, in which the fluid output is caused by osmotic imbalance between extracellular and intracellular compartments of plasma, and any stress that induces such an imbalance can lead a decline in plasma protein (Barton and Iwama 1991). An increase in blood count (leukocytes and erythrocytes) was also recorded, showing that the fish subjected to stress exhibited regulation of the rate of gill ventilation in the aquatic environment in order to normalize the concentration of CO_2. As blood flows through tissues, CO_2 diffuses into the plasma and erythrocytes where carbonic acid forms and then dissociates into H^+ ions and bicarbonate (Chen et al. 2015; Joyce et al. 2015). As observed in this work, Chen et al. (2015) reported that in carp, *Cyprinus carpio*, exposed to air for 4 h, leukocytes and erythrocytes are directly related to the modeling action of cortisol, suggesting that stress induces the production of new cells to replace old ones, inducing immunological activation quantitatively reflecting the hematological response to prolonged air exposure.

Juveniles of *L. alexandri* exhibited variation in their concentrations of serum enzymes, alkaline phosphatase and ALT, with their lowest values being at 96 h after

stress. These levels of enzymes can be considered one of the markers of hepatic dysfunction (Rehulka 2000; Talas and Gulhan 2009). The decline of these analysts in the blood plasma of *L. alexandri* in the present study is similar to that found for rainbow trout, *O. mykiss*, subjected to increased stress (Tahmasebi-Kohyani et al. 2012), and supports the behavior of stress physiology, based on alkaline phosphatase concentrations of *Arapaima gigas*, in response to environmental changes during cultivation, allowing the identification and prevention of adverse health conditions (Bezerra et al. 2014).

The imbalance of electrolytes (Na^+, K^+, Ca^{++} and nCa^{++}) in *L. alexandri* was evident from observations under stress. With the elevation of $PvCO_2$, fish enter into a state correlated to dyspnea, which causes a rapid increase in respiratory acidosis and, due to CO_2 narcosis, may cause a decrease in cardiac output, hypotension and depression of the central nervous system (Joyce et al. 2015). Since fish gills have multiple purposes, including promoting gas exchange and osmotic, ionic and hematological regulation, exposure to air can cause adverse secondary reactions such as acidosis and osmotic stress due to respiratory arrest impairing gas an ion exchange between blood and water (Gholipourkanani et al. 2013). In this sense, when determining the increase of gill permeability by metabolic dysfunction after stress, Souza et al. (2001) found that it results in an influx of water and an output of blood ions to the external medium, in the case of freshwater fish, affecting osmotic and ionic regulation. Values of Na^+ and K^+ in *Pollachius virens*, subjected to acute stress from fishing and capture, also underwent significant changes after the physical stress of escape, due to the interaction of cortisol with cellular ionic metabolism, causing osmotic resistance of the fish (Roth and Rotabakk 2012). However, these effects observed in *L. alexandri* were at reversible levels, as shown during the post-stress recovery period, demonstrating that the animal sought to maintain blood gas equilibrium for the tissues and restore osmoregulatory balance.

Conclusions

Juveniles of *L. alexandri* exhibited resistance to a 30 minutes air exposure test. In addition, they were able to re-establish their normal physiology according to primary

stress indicators (cortisol, glucose and lactate dehydrogenase), while other indicators (hematological, biochemical and gasometric) exhibited compensatory changes in accordance with normal physiological establishment.

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5. Artigo 2

Response of juvenile *Lophiosilurus alexandri* to osmotic and thermic shock

Abstract

The objective of the present study was to evaluate the physiological responses to stress exhibited by juvenile *Lophiosilurus alexandri* submitted to osmotic and thermic shock. Thirty juveniles were used for each test, of which 10 were not subjected to stress and remained in normal conditions (fresh water at 28.0 °C). The others were submitted to stress shock (saline water of 10.0 g of salt/L or water cooled to 18.0 °C). Blood samples were taken at 0 h (no exposure to the stress factor) and 1h and 24 h after the tests. At 24 h, the survivorship was 100% in both tests. In both the osmotic and thermic shock tests, cortisol and glucose levels were higher at 1 h, but then decreased until 24 h. Lactate dehydrogenase showed differences in the temperature test, but had lower concentrations at 1 and 24 h. Difference were recorded in blood gas variables (pH, $PvCO_2$, PvO_2, hemoglobin, sO_2, BE, tCO_2, HCO_3^- and $stHCO_3^-$) and electrolytes (Na^+, Ca^{++}, nCa^{++} and K^+) in both experiments. With regard to hematology and blood biochemistry, exposure to thermal shock did not affect ($P>0.05$) alkaline phosphatase, total plasma protein, hematocrit, ALT and AST at 1 h and 24 h. Alkaline phosphatase and total protein of the osmotic shock were lowest ($P<0.05$) at 24 h. Leukocyte and erythrocyte counts exhibited differences after osmotic shock, in contrast to erythrocyte counts of the temperature test, which did not change in 24 h ($P>0,05$). Juveniles of *L. alexandri* were able to reestablish the main indicators of stress (cortisol, glucose), while the others (hematological, biochemical and gasometric) varied in compensation for normal physiological reestablishment

Keywords: chemico-physiological stress, blood gasometry, hematology

Introduction

Conditions that aim to produce freshwater fish regularly involve biochemical and physiological changes to the animal (Abass et al., 2016), which, during their interaction with management and environmental variability, require organic adjustments to reach homeostatic equilibrium (Nguyen et al., 2017). Species are able to occupy artificial habitats due to homeostatic adaptations, such as enzymatic interactions with thermal variation (Da Costa et al., 2016) and the reduction of body fluid concentration to values compatible with cellular functioning in response to saline shock (Mattioli et al., 2017).

The levels of thermal and saline comfort of each species of fish should be provided to the interested public in order to ensure that animals are in their wellness zone (Bui et al., 2010). Water temperature and salinity are important environmental variables because they affect fish metabolism and, consequently, oxygen consumption, osmotic control and, mainly, growth (Jian et al., 2003). Thus, it is essential that studies be performed that clarify levels of stress caused by environmental changes, particularly because temperature affects secretion and responsiveness of target organs to gonadotropic hormones in freshwater fish (Fraile et al., 1994).

Freshwater fish experience alterations to muscle development and blood patterns in response to variation in temperature (Assis et al., 2004; Lima et al., 2006), which makes understanding their effects on the metabolism of aquatic organisms of interest because it can help to understand what biochemical strategies can be adopted to overcome the challenge of thermic shock (Engin et al., 2013). The optimal growth rate for species used in fish farming in Brazil is achieved at temperatures between 25.0 and 32.0°C (Rebouças et al., 2014).

Osmoregulatory interactions in freshwater fish is one of the most basic principles to understand (Garcia et al., 2007), since salt is a therapeutic product used on a large scale through prophylactic baths and in combating disease and sickness (Fiúza et al., 2015). Some species of catfish, for example, may suffer from stress and metabolic interference due to their physiological inability to cope with acute osmotic shock (Su et

al., 2013). Enzymatic and hormonal constituents have been explored as potential stress indicators for innumerable fish species, since they are considered highly sensitive and reliable parameters (Tang et al., 2001).

The carnivorous species *Lophiosilurus alexandri* (Steindachner, 1876), of the order Siluriformes, is considered an important fish species of the São Francisco river basin due to its ecological and social relevance as a result of increasing demand due to the preference of fishers (Costa et al., 2015). In addition, studies related to temperature (Takata et al., 2014; Da Costa et al., 2016), salinity (Santos and Luz, 2009; Salaro et al., 2015; Mattioli et al., 2017) and both (Martins et al., 2014), aid the theoretical understanding of this species. However, the standard hematological behavior of fish under stress from environmental interference demonstrates the need to increase knowledge from blood gas analyses of Siluriformes (Lutz and Nilsson, 1997). In this sense, such information about biochemical variables under the effect of thermic and saline shock can provide relevant indications of alterations to the physiological state and adaptation of blood gasometry of *L. alexandri.* The present study aimed to evaluate the physiological responses to stress by juvenile *L. alexandri* submitted to osmotic and thermic shock tests.

Material and Methods

Ethics committee approval

All of the experimental protocols performed in this study were approved by the Comitê de Ética em Experimentação Animal (Ethics Committee for Animal Experimentation) of the Universidade Federal de Minas Gerais (approval reference number: 77/2017) and followed the animal ethics and regulatory checklist (Grundy, 2015).

Fish

Juvenile *L. alexandri*, previously conditioned to accept formulated diets, were kept in the Laboratório de Aquacultura (Aquaculture Laboratory) of the Escola de

Veterinária (Veterinary School) of the Universidade Federal de Minas Gerais in 400-L tanks maintained in a water recirculation system. The diet consisted of a commercial extruded diet (2.6 mm in diameter, 45% maximum crude protein, 4% maximum crude fiber, 15% maximum mineral matter, 8% minimum ethereal extract, 2% minimum calcium and 1% minimum phosphorous; data from the manufacturer), offered at 8:00 and 16:00 h, until they satiety. The photoperiod was maintained at 10 hours of light and 14 hours of dark.

During the pre-experimental maintenance of the animals, the mean water temperature was 28.5 ± 0.5 ºC, dissolved oxygen was maintained above 4.5 mg/L, and the pH and conductivity had values of 6.7 ± 0.2 and 0.3 ± 0.1 mS/cm, respectively, measured using a YSI multi-parameter probe (Model 6920 V2, Xylem Analytics global brands). The osmolarity of the water, measured using Osmomat 030® equipment (Gonotec Gmbh, Berlim, Alemanha), was 7.3 ± 4.8 mOsmol/L. The concentrations of total ammonia were maintained below 0.5 mg/L, using the method of Standard Methods 20th Ed (APHA, 1998).

Experiment 1. Effect of osmotic shock on juvenile L. alexandri

The osmotic shock experiment was performed using 30 juvenile *L. alexandri* weighing of 49.21 ± 4.91 grams and measuring 15.5 ± 59 centimeters in length. The animals were stored in three 180-L water tanks (10 animals per tank) with a water temperature of 28.0 ± 0.5 °C and supplemental aeration using a porous stone that maintained the oxygen level above 5 mg/L. The juveniles were acclimated to the experimental conditions for one week, being fed twice a day as previously described and with daily water renewal of 100%.

The animals were fasted for 24 hours prior to and during the the experiment. Each animal was considered a replicate in a completely randomized experimental design with three treatments of 10 replicates each. Two 180-L tanks were prepared with 10.0 g of salt/L, and a third tank with freshwater. The freshwater tank was used as the starting point (hour 0), and blood was collected from its 10 fish at the time when the osmotic shock of the fish of the other tanks took place. Juveniles were transferred

directly from freshwater to the two saline tanks and had blood samples collected (n = 10 per collection) at 1 h and 24 h after osmotic shock.

Non-iodized course salt, the type exported by the company MARISAL LTDA (ingredients: sodium chloride and anti-humectant INS 535 sodium ferrocyanide), was used to salinize the water. Temperature, dissolve oxygen concentration, salinity, conductivity and pH of the water were measured before and after water renewal to correct for corrections due to water loss from evaporation. The tanks were siphoned twice during the 24 h period (08:00 and 16:00 h), removing 50% of the total volume each time, thus summing to 100% of the volume at the end of the experiment (24 h), with the water being renewed having the same experimental conditions. The parameters of water quality and total ammonia concentration (methodology Standard Methods 20th Ed, APHA, 1998) were measured prior to changing the water.

Experiment 2. Effect of thermic shock on juvenile L. alexandri

The thermic shock experiment was performed using 30 juvenile *L. alexandri* weighing 68.28 ± 12.08 grams and measuring 16.7 ± 9.7 centimeters in length. The animals were stored in three 180-L water tanks (10 animals per tank) with a water temperature of 28.0 ± 0.3 °C and supplemental aeration using a porous stone that maintained the oxygen level above 5 mg/L. The juveniles were acclimated to the experimental conditions for one week, being fed twice a day and with water renewal of 100%, as described previously for Experiment 1.

After acclimatization, the juveniles were fasted for 24 hours prior to the thermal shock test was performed. For the thermal chock test, two 180-L tanks were prepared with a water temperature of 18.0 ± 0.2 °C, based on other studies of temperature variation already mentioned. A completely randomized design with three treatments and 10 replicates was used, with each animal being considered a replicate. A third tank remained at the temperature of 28.0 ± 0.3 °C and blood was collected from them when the temperature shock of the fish of the other tanks took place (hour 0). Juveniles were transferred directly from water at 28.0 °C to the two tanks with water at 18.0 °C and had blood samples collected (n - 10 per collection) at 1 h and 24 h after thermal shock.

During the 24 hours of the experiment, the tanks received artificial aeration maintaining the oxygen level above 5 mg/L. The fish were not fed during the experimental period. The tanks were siphoned twice a day (08:00 and 16:00 h), removing 50% of the total volume each time, thus summing to 100% of the volume at the end of the experiment (24 h), with the water being renewed having the same experimental conditions.

Gasometric, hematological and biochemical analyses

Blood was collected from fish by caudal vertebral venipuncture with ventral access from fish restrained with a damp cloth. Two blood samples were taken from each animal. For the first sample, 500 μL of blood was taken without anticoagulant. From this volume, 300 μL were used to determine pH, tCO_2 (carbon dioxide rate), PvO_2 (partial venous oxygen pressure), $PvCO_2$ (partial venous carbon dioxide pressure), tHB (total hemoglobin rate), sO_2 (oxygen saturation), Na^+, K^+, Ca^{++}, and nCa^{++} (ionized calcium standardized by pH). The following were calculated with an OPTI CCA Cassettes kit and an OPTI CCA-TS analyzer (OPTI Medical Systems, Inc®, USA; www.optimedical.com): HCO_3^- (bicarbonate), $stHCO_3^-$ (standard bicarbonate) and BE (base excess). This equipment uses optical fluorescence and reflectance as a reference in releasing results, thus eliminating the use of electrodes and contact points. The remainder of the sample (200 μL of blood) was centrifuged at 1000 rpm for 1 minute and 3000 rpm for 4 minutes for separation of the liquid fraction (Mattioli et al., 2017). The samples were then frozen at -80.0 °C for serum stabilization and subsequent analysis of plasma cortisol levels, which were determined using a commercially available enzyme-linked immunosorbent assay kit (ELISA), according to procedures validated for fish (Barcellos et al., 2010).

From the second sample (also 500 μL of blood) of each animal, hematological and biochemical analyses were performed. Sodium heparin (10% μL/μL of blood) was used for this sample. Approximately 50 μL was destined for hematocrit (Ht) analysis, from capillary tubes filled to approximately 2/3 volume with previously homogenized blood. These capillary tubes were centrifuged for 15 min. at 10000 rpm. The reading was performed on the appropriate card by matching the plasma meniscus with the upper

line of the ruler (line 100), and the lower end of the erythrocyte portion with the lower line of the ruler (line 0), such that the result is indicated by the value of the line. Immediately after collection, 10 μl of blood was aliquoted into 1 mL of Dacie dilution solution in the proportion of 1:50 (Jain, 1986), for counting total erythrocytes and leukocytes using a hemocytometer within 24 hours after collection following adaptations of Mattioli et al, (2017). Hematological procedures were performed within six hours after collection.

Upon completion of the hematological analyses, the remaining aliquots of heparinized whole blood were centrifuged at 1000 rpm for 1 minute and 3000 rpm for 4 minutes to separate the liquid fraction. This centrifugation protocol was adopted in order to minimize the occurrence of hemolysis. The biochemical profile was performed using an automatic device (Cobas-Mira Plus) with plasma samples. The following analytes were assessed using commercial kits (Synermed International Inc®): total plasma protein (PPT) glucose, alanine aminotransferase (ALT) aspartate aminotransferase (AST), alkaline phosphatase (FOAL) and lactate dehydrogenase (LDH),

Statistical analysis

Data were submitted to the Kolmogorov-Smirnov normality test. The variable $PvCO_2$ underwent logarithmic transformation. Subsequently, the data were submitted to analysis of variance (ANOVA) and Tukey test at 5% probability.

Results

Experiment 1. Effect of osmotic shock on juvenile L. alexandri

After 24 hours of exposure to osmotic shock, fish survivorship was 100%. Cortisol exhibited higher values 1 h after osmotic shock ($P<0.05$) (Table 1). Glucose also exhibited higher values 1 hour after the shock, but returned to baseline values at 24 h ($P<0.05$).

The enzyme LDH exhibited no difference between 1 h and 24 h after exposure to osmotic shock ($P>0.05$) (Table 1). Exposure to saline water did not affect ($P>0.05$) ALT and Ht throughout the 24 hours, whereas FOAL had a lower value ($P<0.05$) at 24 h after the test compared to that at the initial time. The values for PPT were similar at 0 h and 1 h ($P>0.05$) and lower at 24 h ($P<0.05$), whereas AST was higher at 24 h ($P<0.05$) and had similar values at 0 h and 1 h ($P>0.05$). The leukocyte count was lower ($P<0.05$) at 1 h, and similar between 0 h and 24 h ($P>0.05$). Erythrocyte count was lower at 1 h and 24 h after osmotic shock ($P<0.05$).

Table 1. Means (± standard deviation) for hematological and biochemical values of alkaline phosphatase (FOAL - UI/L), lactate dehydrogenase (LDH - UI/L), cortisol (ng/dL), glucose (mg/dL), total plasma proteins (PPT - g/dL), alanine aminotransferase (ALT - UI/L), aspartate aminotransferase (AST - UI/L), hematocrit (Ht - %), leukocytes ($x10^4/\mu L$) and erythrhocytes ($x10^6/\mu L$) of blood samples of juvenile *Lophiosilurus alexandri* submitted to osmotic shock.

	Time		
	0 h	**1 h**	**24 h**
FOAL	33.13±7.59a	31.71±3.43a	19.75±3.33b
LDH	9.48±0.69a	9.42±1.54a	8.51±1.39a
Cortisol	15.31±0.57c	36.24±6.73a	24.61±0.47b
Glucose	0.19±0.09b	2.91±1.38a	0.25±0.22b
PPT	2.38±0.19a	2.54±0.10a	2.19±0.21b
ALT	5.30±1.06a	6.90±2.28a	7.45±2.37a
AST	127.87±24.47b	156.64±34.89ab	183.60±66.52a
Ht	20.77±2.58a	19.81±3.48a	20.50±2.67a
Leukocytes	45.28±9.26a	34.94±5.68b	44.96±14.25a
Erythrocytes	10.06±0.31a	7.45±0.11b	7.89±0.91b

Different letters in a row represent significant differences according to Tukey test ($P<0.05$).

The values obtained from the blood gas analysis are shown in Table 2. After exposure to salinized water, pH was lower ($P<0.05$) at 1 h and 24 h. The concentration of $PvCO_2$ did not differ significantly after exposure to saline ($P>0.05$), while PvO_2 had at higher concentrations at 1 h and 24 h ($P<0.05$). The hemoglobin rate (tHb) varied over time, with a higher value at 24 h post-exposure ($P<0.05$). The sO_2 level decreased at 1 h, and was similar at 24 h ($P<0.05$). The levels of BE, tCO_2, HCO_3^- and $stHCO_3^-$ did not exhibit significant differences ($P>0.05$) among the times analyzed.

Table 2. Means (± standard deviation) for the gasometric values of hydrogen potential (pH), partial venous carbon dioxide pressure ($PvCO_2$ - mmHg), partial venous oxygen pressure (PvO_2 - mmHg), total hemoglobin rate (tHb - g/dL), oxygen saturation (sO_2 - %), base excess (BE - mmol/L), carbon dioxide rate (tCO_2 - mmol/L), bicarbonate (HCO_3^- - mmol/L) and standard bicarbonate ($stHCO_3^-$ - mmol/L) of the blood samples of *Lophiosilurus alexandri* submitted to osmotic shock.

	Time		
	0 h	**1 h**	**24 h**
pH	7.42±0.05a	7.37±0.05b	7.35±0.07b
$PvCO_2$	11.11±1.05a	9.81±1.60a	*
PvO_2	61.11±17.53a	46.18±11.38b	45.70±6.14b
tHb	8.52±1.42b	8.21±0.76b	9.70±0.79a
sO_2	76.22±11.32a	65.63±8.64b	64.10±7.09b
BE	-13.68±2.41a	-16.30±2.43a	*
tCO_2	6.51±2.91a	5.60±1.53a	*
HCO_3^-	6.32±2.90a	5.32±1.51a	*
$stHCO_3^-$	10.92±1.94a	9.31±1.56a	*

Different letters in a row represent significant differences according to Tukey test ($P<0.05$).

* indicates the reading was outside of the capacity of the equipment.

Regarding electrolytes (Table 3), blood sodium had a higher concentration at 24 h than at the other times ($P<0.05$). The values for potassium, calcium and ionized calcium (nCa^{++}) did not differ significantly ($P>0.05$) among the times measured in the experiment.

Table 3. Means (± standard deviation) for electrolyte values of sodium (Na^+ - mmol/L), potassium (K^+ - mmol/L), calcium (Ca^{++} - mmol/L) and ionized calcium (nCa^{++} - mmol/L) for blood samples of *Lophiosilurus alexandri* submitted to osmotic shock.

	Time		
	0 h	**1 h**	**24 h**
Na^+	127.01±1.73b	128.18±2.31b	151.50±3.50a
K^+	3.13±0.45a	3.04±0.48a	3.13±0.39a
Ca^{++}	1.29±0.02a	1.28±0.01a	1.32±0.08a
nCa^{++}	1.30±0.03a	1.26±0.02a	1.29±0.08a

Different letters in a row represent significant differences according to Tukey test ($P<0.05$).

Experiment 2. Effect of thermic shock on juveniles of L. alexandri

After 24 hours of exposure to low temperature, fish survivorship was 100%. Data for cortisol and glucose are presented in Table 4. Cortisol exhibited a higher value at 1 h after thermal shock ($P<0.05$) than at 0 h and 24 h. Glucose also exhibited a higher value at 1 h ($P<0.05$) than at 0 h, and remained high until 24 h ($P<0,05$).

Table 4 also presents the results for the hematological and biochemical analyses. The enzyme LDH exhibited higher values at 1 h and 24 h of exposure (Table 4), compared with 0 h ($P<0.05$). The thermic shock did not affect ($P>0.05$) FOAL, PPT, ALT, AST and Ht of juvenile *L. alexandri* throughout the 24 hours of the experiment. The leukocyte count was similar between 1 h and 24 h ($P>0.05$), which were lower ($P<0.05$) than at 0 h. The erythrocyte count showed no significant differences among the times analyzed ($P>0.05$).

Table 4. Means (± standard deviation) for hematological and biochemical values of alkaline phosphatase (FOAL - UI/L), total plasma proteins (PPT - g/dL), lactase dehydrogenase (LDH - UI/L), alanine aminotransferase (ALT - UI/L), aspartate aminotransferase (AST - UI/L), glucose (mg/dL), cortisol (ng/dL), hematocrit (Ht - %), leukocytes ($x10^4/\mu L$) and erythrocytes ($x10^6/\mu L$) of blood samples of juvenile *Lophiosilurus alexandri* submitted to thermic shock.

	Time		
	0 h	**1 h**	**24 h**
FOAL	33.39±7.20a	29.50±7.08a	25.37±4.27a
PPT	2.41±0.19a	2.25±0.23a	2.41±0.19a
LDH	9.39±0.71a	7.04±1.86b	6.71±1.09b
ALT	5.37±1.02a	6.08±2.14a	5.69±2.48a
AST	128.61±23.18a	160.07±61.74a	138.28±63.50a
Glucose	0.18±0.09c	13.61±2.06a	6.39±2.85b
Cortisol	15.38±0.58c	31.27±0.92a	18.26±0.71b
Ht	20.30±2.86a	20.90±2.18a	20.40±1.83a
Leukocytes	44.16±0.94a	30.88±0.76b	29.92±0.79b
Erythrocytes	9.70±3.05a	9.49±3.08a	7.88±0.77a

Different letters in a row represent significant differences according to Tukey test ($P<0.05$).

The values obtained for blood gas analysis are shown in Table 5. After thermic shock, pH was lower at 1 h ($P<0.05$), and at 24 h was similar to initial values ($P>0.05$). The concentration of $PvCO_2$ did not differ between 0 h and 1 h ($P>0.05$), but at 24 h it was outside of the measurement capacity of the equipment. The concentration of PvO_2 was similar at 0 h and 1 h ($P>0.05$), but was higher at 24 h after the test ($P<0.05$). The hemoglobin rate (tHb) did not vary over the 24 hour period ($P>0.05$). The values for sO_2 were higher at 1 h than at 0 h, and remained so until 24 h ($P>0.05$). The level of BE had a higher value ($P<0.05$) at 0 h compared to 1 h. The analytes tCO_2, HCO_3^- and $stHCO_3^-$ did not exhibit significant differences ($P>0.05$) among the times analyzed.

Table 5. Means (± standard deviation) for the gasometric values of hydrogen potential (pH), partial venous carbon dioxide pressure ($PvCO_2$ - mmHg), partial venous oxygen pressure (PvO_2 - mmHg), total hemoglobin rate (tHb - g/dL), oxygen saturation (sO_2 - %), bases excess (BE - mmol/L), carbon dioxide rate (tCO_2 - mmol/L), bicarbonate (HCO_3^- - mmol/L) and standard bicarbonate ($stHCO_3^-$ - mmol/L) of blood samples of *Lophiosilurus alexandri* submitted to thermic shock.

	Time		
	0 h	**1 h**	**24 h**
pH	7.43±0.06a	7.28±0.03b	7.47±0.03a
$PvCO_2$	10.7±1.33a	11.10±0.87a	*
PvO_2	61.50±16.57b	78.10±17.14b	107.80±27.38a
tHb	8.42±1.38a	7.48±0.88a	8.23±0.90a
sO_2	76.30±10.67b	86.90±4.93a	88.50±4.24a
BE	-14.24±3.54a	-19.17±1.83b	*
tCO_2	6.61±2.76a	5.54±0.42a	*
HCO_3^-	6.43±2.76a	5.20±0.42a	*
$stHCO_3^-$	10.92±1.81a	8.60±0.57a	*

Different letters in a row represent significant differences according to Tukey test ($P<0.05$).

* indicates the reading was outside of the capacity of the equipment.

Regarding electrolytes (Table 6), blood sodium and calcium had lower concentrations at 24 h ($P<0.05$), than at 1 h. The fish submitted to thermic shock (1 h and 24 h) had a decrease in K^+ concentration compared to 0 h ($P<0.05$). Ionized calcium (nCa^{++}) had its lowest values at 1 h ($P<0.05$) and values similar to 0 h at 24 h ($P>0.05$).

Table 6. Means (± standard deviaiton) for electrolyte values of sodium (Na^+ - mmol/L), potassium (K^+ - mmol/L), calcium (Ca^{++} - mmol/L) and ionized calcium (nCa^{++} - mmol/L) for blood samples of *Lophiosilurus alexandri* submitted to thermic shock.

	Time		
	0 h	**1 h**	**24 h**
Na^+	126.90±1.66ab	127.80±1.75a	124.40±4.37b
K^+	3.15±0.43a	2.75±0.15b	2.52±0.28b
Ca^{++}	1.29±0.02ab	1.32±0.02a	1.27±0.03b
nCa^{++}	1.31±0.03a	1.24±0.03b	1.31±0.04a

Different letters in a row represent significant differences according to Tukey test ($P<0.05$).

Discussion

The results of this work demonstrate that osmotic and thermic shock lead to changes in the gasometric, hematological and biochemical parameters of juvenile *L. alexandri*. However, the shocks did not affect the survival 24 hours after the tests, demonstrating that the effect is inferred in the well being of *L. alexandri*. Similar results were observed for the species *Pangasianodon hypophthalmus* with the effects of salinity (Nguyen et al., 2014) and temperature (Nguyen et al., 2017), demonstrating that species of Siluriformes have also been investigated. Juvenile *Rhamdia quelen* weighing 1.7 g tolerated salinities of up to 9 g of salt/L of common sea salt for 96 hours (Marchioro and Baldisserotto, 1999). The high survival rate recorded in the present study can be attributed to adequate fish management and good water quality parameters.

The water quality parameters recorded in the present study are within the known range for this fish, as previously reported by Costa et al. (2015). The perception of a stressor depends on the previous experience of an animal and how it assesses a new situation by considering the memory of past events and the possible strategies available to deal with disruption (Ursin and Eriksen, 2004). Not only can differences between species modulate responses to stressors, but the magnitude of a stress response also depends on the individual's perception of the situation (Galhardo et al., 2011). Therefore, what is stressful to one individual may prove to be an innocuous stimulus to another, depending on past experiences; furthermore, the same animal may respond differently to the same stressor at different stages and contexts throughout life (Galhardo & Oliveira, 2009). In addition, catfish are resistant by nature (Kumar et al., 2012) and can thrive in adverse ecological conditions (Sarma et al., 2012). However, Mattioli et al. (2017) observed that for juvenile *L. alexandri* weighing 28.6 ± 1.3 grams, the salinity of 11.67 g of salt/L becomes lethal with 24 hours of exposure.

Previous studies have evaluated the implications of these environmental variables, with possible physiological interactions in fish, from both hematological and biochemical perspectives. Most of these studies, however, were carried out mainly in an attempt to improve aquaculture production systems, and were not just looking for the effects of climate change. Nguyen et al. (2017) compared the effects of the thermic

amplitude of 10 °C and salinities of up to 12 g of salt/L on *Pangasianodon hypophthalmus* and observed significant effects mainly in plasma concentrations of cortisol and glucose, both of which increased during the initial phase of the tests, but then declined and stabilized at the end of the 24-hour analysis time, which is similar to the results obtained in the present study. The return of plasma concentrations, especially that of cortisol, of *L. alexandri* to close to the basal concentrations prior to the test, suggest that this species has the ability to acclimate to changes in the medium.

Changes in blood biochemical parameters can be regulated by hormones involved in stress response, which are related to changes in the concentrations of blood constituents (Randall and Perry, 1992). For example, it has already been shown that stress in fish causes a primary response involving neurohormonal stimulation, resulting in an increase in the secretion of corticosteroids and catecholamines (Karşi and Yildiz, 2005). These primary responses to stress cause a number of physiological changes known as "secondary effects" (Foo and Lam, 1993). Increased plasma glucose level is used as an indicator of secondary response to stress (Mommsen et al. 1999). Jeanette et al. (2007) reported a significant increase in plasma glucose levels in *Oreochromis mossambicus* with changes in salinity and temperature, which they attributed to an increased energy demand to maintain hydro-mineral equilibrium at higher salinity and thermal metabolism due to stress response, as observed in the present study. However, for *Clarias batrachus* the blood glucose level was lower in 8 g of salt/L at 96 hours after exposure (Sarma et al., 2012), which the authors attributed to the possible use of circulating blood glucose due to the reduction of appetite at higher salinity (Plaut, 1998; Martínez-Álvarez et al., 2002).

The changes in FOAL observed in the present study probably caused disturbances in AST concentrations after osmotic shock, indicating probable hepatic and non-osseous disorder (Garavini et al., 1981), since the values for potassium, calcium and ionized calcium were similar ($P>0.05$). The effects on LDH after heat shock may be indicative of the existence and severity of acute tissue damage due to stress, indicating effects from disturbances due to fasting and vigorous exercise due to thermal shock (Chatzifotis et al., 2017).

In fish, in general, stress-related hormones, after being secreted into circulation, may activate hematological responses linked to biochemical constituents, thereby mitigating the harmful effects associated with acute stressors, such as hematocrit (Reid, 1998; Perry et al., 1999). As observed in *Clarias batrachus* by Saha et al. (2011), the actions of osmotic shock induced changes in the concentrations of blood constituents, such as glucose, initially causing gluconeogenesis under environmental hypertonicity, possibly occurring as a consequence of changes in the state of hydration and cellular volume of different types of plasma proteins, leukocytes and erythrocytes. Thus, when biochemically analyzing the increase of blood glucose from stress tests, help was observed in maintaining glucose homeostasis and, thus, an adequate energy supply to support the metabolic demands of the transport of ions by leukocytes and other metabolic processes altered significantly under red blood cells.

The complex metabolism resulting from the effects of saline and thermal shock, along with plasma gasometry, reflect disturbances in the metabolic, hormonal and respiratory rate of fish, as was observed for *L. alexandri*. Changes in the interactions of plasmatic oxygen and carbon dioxide have been observed in mammals (Nikinmaa, 2001). Juvenile *Hypophthalmichthys molitrix* of 45 ± 10 mm in length exhibited variation in O_2 consumption and locomotor activity when exposed to different salinities and temperatures. Temperatures below 8°C and above 24°C, combined with salinities above 10 g of salt/L, can be lethal to juveniles The minimum standard metabolic rate was observed in salinities of 3 and 4 g of salt/L and a maximum resistance to salinity was between 18 and 22 °C (Von Oertzen, 1985). Juvenile *Ictalurus punctatus* weighing 2.7 g exhibited a reduction in the rate of oxygen consumption with increasing salinity of freshwater up to 9 g of salt/L. However, no fish mortality or behavioral changes were observed at the salt concentrations mentioned (Altinok and Grizzle, 2001).

Increased stress-associated hormones elevate blood O_2 transport by increasing the carrying capacity (Wells and Weber, 1990) and improve the binding affinity of Hb-O_2 via alkalinization of erythrocytes (Nikinmaa, 2001). However, this effect is much more pronounced in fish than in mammals, and this mechanism promotes the release of O_2 from hemoglobin to respiratory tissues (Brauner and Randall, 1998), which is consistent with the results of the present study. This is because in vitro evidence has shown that respiratory diffusion of CO_2 within teleost red blood cells occurs more rapidly than the

release of O_2 from hemoglobin by diffusion in response to a change in the PvO2 gradient (Pelster and Randall, 1998), as observed with osmotic shock. Consequently, a transient drop in plasma pH and erythrocyte concentration followed by catalyzed CO_2 hydration may elucidate these effects and produce high plasma PvO_2 values (McKenzie et al., 2004).

The effects on electrolytic concentrations observed with saline and thermic shock are probably due to the change in Na^+ levels in the blood and, consequently, in osmotic pressure, with an influence on glucose and cortisol levels and Na^+/K^+-ATPase activity of chloride cells in fish. Individuals of *Ctenopharyngodon idella* weighing 120 g exposed to a salinity of 10 g of salt/L exhibited changes in secondary stress responses, such as sodium and potassium levels, which increased with exposure to a saline environment, while calcium levels were not affected (Yavuzcan-Yıldız and Kırkağaç-Uzbilek, 2001). The increase of salinity for this same species led to an increase in the total concentration of ions in the plasma (Maceina et al., 1980). These parameters may change when individuals are exposed to different levels of salinity and change in ionic permeability due to temperature (Perry and Bernier, 1999; Piaia et al., 1999; Parsons, 2007).

Conclusions

Juvenile *L. alexandri* were able to reestablish the main indicators of stress (cortisol, glucose), while the others (hematological, biochemical and gastrometric) exhibited variation in compensation for normal metabolic reestablishment. This species has the physiological ability to restore acid-base balance after osmotic and thermic shock and the associated acute stress.

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6. CONSIDERAÇÕES FINAIS

Com a realização destes trabalhos pode-se considerar que:

Não foi observado mortalidade em juvenis de *L. alexandri* submetidos aos testes de exposição ao ar de 30 minutos, choque de salinidade de 10 g de sal/L e choque térmico por redução da temperatura da água de 10 °C.

Os juvenis de pacamã demonstraram a partir de análises sanguíneas, que foram capazes de reestabelecer os principais indicadores de estresse (cortisol, glicose), enquanto os demais (hematológicos, bioquímicos e gasométricos) apresentaram variações em sentido compensatório para a convalescença do organismo.

Variáveis gasométricas, hematológicas e bioquímicas podem ser utilizadas como indicativos do estado metabólico de juvenis de pacamãs criados em ambiente fechado.

Por conta de todas estas particularidades metabólicas do animal pós-estresse, observa-se diferentes tipos de modelos de adaptação e aclimatação em exposições a situações de extenuação por meios bióticos e abióticos.

Dentre todos os processos fisiológicos e metabólicos do estresse observados aqui nestes trabalhos, verifica-se a necessidade de pesquisas em outras espécies, já que o estresse é considerado um processo altamente custoso energeticamente. Assim, estas análises podem ser utilizadas como ferramentas importantes de estudo para a compreensão da influência dos fatores intrínsecos aos sistemas produtivos.