UNIVERSIDADE FEDERAL DE MINAS GERAIS

RESPOSTAS FISIOLÓGICAS E METABÓLICAS EM JUVENIS DE TAMBAQUIS (*Colossoma macropomum*) EXPOSTOS A SITUAÇÕES DE ESTRESSE: HIPÓXIA E EXPOSIÇÃO AO AR

LUANNA DO CARMO NEVES

BELO HORIZONTE
ESCOLA DE VETERINÁRIA – UFMG
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Tese apresentada ao Programa de Pós-Graduação em Zootecnia da Escola de Veterinária da Universidade Federal de Minas Gerais como requisito para obtenção do grau de Doutora em Zootecnia.

Área de concentração: Produção Animal/Aquacultura.

Prof. Orientador: Dr. Ronald Kennedy Luz.
Coorientadores: Dra. Gisele Cristina Fávero e Dr. Glauber David Almeida Palheta

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ESCOLA DE VETERINÁRIA DA UFMG
COLEGIADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA
Av. Antônio Carlos 6627 - CP 567 - CEP 30123-970 - Belo Horizonte- MG
TELEFONE (31)-3409-2173

www.vet.ufmg.br/academicos/pos-graduacao
E-mail cpgzootec@vet.ufmg.br

ATA DE DEFESA DE TESE DE ALUNO LUANNA DO CARMO NEVES

Às 08:30 horas do dia 28 de maio de 2021, reuniu-se, remotamente, a Comissão Examinadora de Tese, indicada pelo colegiado no dia isso 12/05/2021, para julgar, em exame final, a defesa da tese intitulada: **Respostas fisiológicas e metabólicas em juvenis de tambaquis (*Colossoma macropomum*) expostos a situações de estresse: hipóxia e exposição ao ar** como requisito final para a obtenção do Grau de **Doutor em Zootecnia, área de concentração Produção Animal.** Abrindo a sessão, o Presidente da Comissão, Prof. Ronald Kennedy Luz, após dar a conhecer aos presentes o teor das Normas Regulamentares da Defesa de Tese, passou a palavra ao (a) candidato (a), para apresentação de seu trabalho. Seguiu-se a argüição pelos examinadores, com a respectiva defesa do candidato (a). Logo após, a Comissão se reuniu, sem a presença do candidato e do público, para julgamento da tese, tendo sido atribuídas as seguintes indicações:

	Aprovada	Reprovada
Prof.(a)/Dr.(a) Bernardo Baldisserotto	x	
Prof.(a)/Dr.(a) Cristiano Campos Mattioli	x	
Prof.(a)/Dr.(a) Rodrigo Takata	x	
Prof.(a)/Dr.(a) Rodrigo Yukihiro Gimbo	x	
Prof.(a)/Dr.(a) Ronald Kennedy Luz	x	

Pelas indicações, o (a) candidato (a) foi considerado (a): [x] Aprovado (a)

[ ] Reprovado (a)

Para concluir o Doutorado, o(a) candidato(a) deverá entregar 03 volumes encadernados da versão final da tese acatando, se houver, as modificações sugeridas pela banca, e a comprovação de submissão de pelo menos um artigo científico em periódico recomendado pelo Colegiado dos Cursos. Para tanto terá o prazo máximo de 60 dias a contar da data defesa.

O resultado final, foi comunicado publicamente ao (a) candidato (a) pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora e encaminhada juntamente com um exemplar da tese apresentada para defesa.

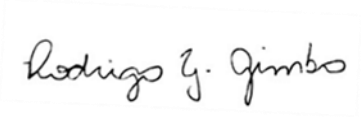
Belo Horizonte, 28 de maio de 2021.

Assinatura dos membros da banca:

Prof°. Dr. Bernardo Baldisserotto

Dr. Cristiano Campos Mattioli

Prof°. Dr. Rodrigo Takata



Prof°. Dr. Rodrigo Yukihiro Gimbo

NORMAS REGULAMENTARES DA DEFESA DE TESE

Art. 61. A seção de defesa de dissertação ou tese será aberta pelo presidente da Comissão Examinadora que dará a conhecer, ao candidato e a todos os presentes, as normas regulamentares que regem a defesa e que constam dos parágrafos subsequentes.

§1º. É vedado ao público qualquer tipo de manifestação durante a defesa da tese.

§2º. O candidato terá 50 (cinquenta) minutos, prorrogáveis a critério da Comissão Examinadora, para fazer a apresentação do seu trabalho de tese.

§3º. Após a apresentação, o candidato será arguido pela Comissão Examinadora, num prazo máximo de 180 minutos.

§4º. A arguição versará sobre aspectos relevantes da dissertação e da tese.

§5º. Terminada a arguição, a Comissão Examinadora reunir-se-á, sem a presença do candidato e do público, para dar o parecer final.

§6º. O parecer final da Comissão será comunicado publicamente ao candidato, pelo presidente, que lavrará Ata de Defesa que, após assinada pelos membros será arquivada na Secretaria do Colegiado.

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Art. 63. No caso de insucesso, poderá o Colegiado do Programa, mediante proposta da Comissão Examinadora, dar oportunidade ao candidato para apresentar novo trabalho, a ser defendido no prazo máximo de 3 (três) meses para alunos de Mestrado e de 6 (seis) meses para alunos de Doutorado, preferencialmente pela mesma comissão.

§ Único. A defesa a que se refere o artigo anterior far-se-á respeitando-se todos os prazos e normas específicas para a defesa de dissertação/tese, previstos neste Regulamento.

“Somos feitos da mesma matéria que nossos sonhos.”

William Shakespeare.

DEDICATÓRIA

Dedico à minha mãe Rute, meu pai José Edson, meu marido, amigo e amor da minha vida João Paulo, minha irmã chatura Luciana, meu cunhado doido André, meus sogros que os tenho como meus pais Mirtes e Orestes e o meu querido e amado Jô.

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RESUMO

O objetivo deste estudo foi avaliar as repostas fisiológicas e metabólicas em juvenis de *Colossoma macropomum* submetidos a estresse por hipóxia e exposição ao ar. No experimento 1 animais foram submetidos ao tratamento de normóxia (6,27 ± 0,42 mg L^{-1}) e de hipóxia (0,92 ± 0,37 mg L^{-1}) por três dias, após este período todos os animais foram mantidos em normóxia por quatro dias. Amostras de sangue foram coletadas 24 e 72 horas após o início da hipóxia e 24, 48 e 96 horas após o reestabelecimento da normóxia. No experimento 2 os animais foram submetidos a testes de exposição ao ar por 30 e 60 minutos, foram coletadas amostras de sangue imediatamente após a exposição, uma, 24 e 48 horas após a exposição. Ambos os experimentos não apresentaram mortalidade. No experimento 1: O maior nível de glicose foi registrado após 24 h (P <0,05); o nível mais alto de lactato estava em 72 de recuperação; e o maior pH sanguíneo foi em 24 e 72 h (P <0,05). A maior concentração de PvCO2 foi após 24 (P <0,05), enquanto em 96 de recuperação foi equivalente ao basal (P> 0,05). A variável PvO2 só foi maior que a basal em 24 h de recuperação (P <0,05). No experimento 2: glicose, houve interação entre os fatores tempo de exposição e diferentes tempos de coleta com valores maiores para a exposição de 60 min em comparação com a exposição de 30 min na coleta de 1h, enquanto após 24h os níveis não diferiram do basal. Outra variável que teve interação foi o lactato com maiores valores para a exposição imediatamente e uma hora após a exposição. O pH sanguíneo variou entre as coletas, com menor valor após 1h em animais expostos por 60 min. Para $PvCO_2$ e PvO_2 houve interação entre os tempos de exposição e os tempos de coleta, eles foram inversamente proporcionais na coleta após 1h. Os valores de HCO_3- diferiram entre os tempos de exposição e variaram entre as coletas. O *C. macropomum* conseguiu recuperar os principais indicadores de estresse (glicose e lactato) nas 48 horas após os diferentes tipos de estresse, restaurando assim sua homeostase.

Palavras-chave: Peixe nativo; Fisiologia; Sangue; Peixes redondos, Gases sanguíneos.

ABSTRACT

The objective of this study was to evaluate the physiological and metabolic responses in juveniles of *Colossoma macropomum* submitted to stress of hypoxia and exposure to air. In experiment 1, animals were subjected to the treatments of normoxia (6.27 ± 0.42 mg L^{-1}) and hypoxia (0.92 ± 0.37 mg L^{-1}) for three days, after which all animals were kept in normoxia for four days. Blood samples were collected 24 and 72 hours after the onset of hypoxia and 24, 48 and 96 hours after the reestablishment of normoxia. In experiment 2 the animals were subjected to tests of exposure to air for 30 and 60 minutes, blood samples were collected immediately after exposure, one, 24 and 48 hours after exposure. Both experiments did not show mortality. In experiment 1: The highest glucose level was recorded after 24 h ($P < 0.05$); the highest lactate level was at 72 recovery; and the highest blood pH was at 24 and 72 h ($P < 0.05$). The highest concentration of $PvCO_2$ was after 24 ($P < 0.05$), while at 96 of recovery it was equivalent to basal ($P > 0.05$). The PvO_2 variable was only greater than the basal at 24 h of recovery ($P < 0.05$). In experiment 2: glucose, there was an interaction between the exposure time and different collection times with higher values for the exposure of 60 min compared to the exposure of 30 min in the collection of 1 hour, while after 24 hours the levels did not differ from basal. Another variable that had an interaction was lactate with higher values for exposure immediately and one hour after exposure. Blood pH varied between collections, with a lower value after 1 h in animals exposed for 60 min. For $PvCO_2$ and PvO_2 there was an interaction between the exposure times and the collection times, they were inversely proportional in the collection after 1h. HCO_3^- values differed between exposure times and varied between collections. *C. macropomum* was able to recover the main stress indicators (glucose and lactate) with in the 48 hours after the different types of stress, thus restoring its homeostasis.

Keywords: Native fish; Physiology; Blood; Round fish, Blood gases.

LISTA DE TABELAS

ARTIGO 1: Physiological and metabolic responses in juvenile *Colossoma macropomum* exposed to hypoxia

Table 1. Mean gasometric values (± standard deviation) of blood samples of *Colossoma macropomum*.

.....45

Table 2. Mean hematological and biochemical values (± standard deviation) of blood samples of *Colossoma macropomum*.

.....47

Table 3. Mean electrolyte values (± standard deviation) of blood samples of *Colossoma macropomum*.

.....49

ARTIGO 2: Physiological responses of juvenile *Colossoma macropomum* after different periods of air exposure

Table 1. Values (mean ± standard deviation) of hematological and biochemistry parameters at times of exposure to air (30 and 60 min) and blood collection (IAE - Immediately after exposure, 1hAE - One hour after exposure, 24hAE - 24 hours after exposure and 48hAE - 48 hours after exposure) for *Colossoma macropomum* submitted to different times of exposure to air. Hematocrit (%), plasma protein (PPT - g dL^{-1}), hemoglobin (HB g dL^{-1}), Triglycerides (TGR mg dL^{-1}), Cholesterol (CLR mg dL^{-1}), glucose (GLI mg dL^{-1}), alanine aminotransferase (ALT - UI), aspartate aminotransferase (AST - UI) and lactate (mmol $L^{-}1$).

.....70

Table 2: Values (mean ± standard deviation) of hematological and biochemical analysis of the times of exposure to air (30 and 60 min) and blood collection (IAE - Immediately after exposure, 1hAE - One hour after exposure, 24hAE - 24 hours after exposure and 48hAE - 48 hours after exposure) compared to basal for *Colossoma macropomum* submitted to different times of exposure to air. Hematocrit (%), total plasma protein (PPT - g dL-1), hemoglobin (HB g dL-1), glucose (GLI mg dL^{-1}), triglycerides (TGR mg dL-1), cholesterol (CLR mg dL-1), alanine aminotransferase (ALT - IU), aspartate aminotransferase (AST - IU) and lactate (mmol $L^{-}1$).

.....73

Table 3. Values (mean ± standard deviation) of blood gas analysis at times of exposure to air (30 and 60 min) and blood collection (IAE - Immediately after exposure, 1hAE - One hour after exposure, 24hAE - 24 hours after exposure and 48hAE - 48 hours after exposure) for *Colossoma macropomum* submitted to different times of exposure to air. Hydrogen potential (pH), partial pressure of carbon dioxide ($PvCO_2$ - mmHg), partial pressure of oxygen (PvO_2 - mmHg), oxygen saturation (sO_2 - %), rate of oxygen (tO_2 - mL dL^{-1}), bicarbonate (HCO_3^- -mmol L-1), standard bicarbonate ($stHCO_3^-$ -mmol L^{-1}) and excess base (BE - mmol L^{-1}).

.....75

Table 4: Values (mean ± standard deviation) of hematological and biochemical analysis of the times of exposure to air (30 and 60 min) and blood collection (IAE - Immediately after exposure, 1hAE - One hour after exposure, 24hAE - 24 hours after exposure and 48hAE - 48 hours after exposure) compared to basal for *Colossoma macropomum* submitted to different times of exposure to air. Hydrogen potential (pH), partial pressure of carbon dioxide ($PvCO_2$ - mmHg), partial pressure of oxygen (PvO_2 - mmHg), oxygen saturation (sO_2 - %), rate of oxygen (tO_2 - mL dL^{-1}), bicarbonate (HCO_3^- -mmol L-1), standard bicarbonate ($stHCO_3^-$ -mmol L^{-1}) and excess base (BE - mmol L^{-1}).

.....78

Table 5: Values (mean ± standard deviation) of hematological and biochemistry parameters at times of exposure to air (30 and 60 min) and blood collection (IAE - Immediately after exposure, 1hAE - One hour after exposure, 24hAE - 24 hours after exposure and 48hAE - 48 hours after exposure) for *Colossoma macropomum* submitted to different times of exposure to air. Potassium (K^+ - mmol L^{-1}), sodium (Na^+ - mmol L^{-1}), calcium (Ca^{2+} - mmol L^{-1}) and chloride (Cl^- -mmol L^{-1}).

.....79

Table 6: Values (mean ± standard deviation) of hematological and biochemical analysis of the times of exposure to air (30 and 60 min) and blood collection (IAE - Immediately after exposure, 1hAE - One hour after exposure, 24hAE - 24 hours after exposure and 48hAE - 48 hours after exposure) compared to basal for *Colossoma macropomum* submitted to different times of exposure to air. Potassium (K^+ - mmol L^{-1}), sodium (Na^+ - mmol L^{-1}), calcium (Ca^{2+} - mmol L^{-1}) and chloride (Cl^- -mmol L^{-1}).

.....80

LISTA DE FIGURAS

ARTIGO 1: Physiological and metabolic responses in juvenile *Colossoma macropomum* exposed to hypoxia

Figure 1 - Mean values (± standard deviation) of A - Glucose and B - Lactate. Normoxia (basal); 24 hours after initiating hypoxia (24H); 72 hours after initiating hypoxia (72H); 24 hours after reestablishing DO to basal (24R); 48 hours after reestablishing DO to basal (48R) and 72 hours after reestablishing DO to basal (72R). Different letters represent significant differences by Tukey test (P <0.05).
…….43

ARTIGO 2: Physiological responses of juvenile *Colossoma macropomum* after different periods of air exposure

Figure 1 – Breakdown of the interaction (mean ± standard deviation) between the different times of exposure to air and along the collections, in the variables glucose (GLI mg dL-1) and Lactate (mmol L-1). Means followed by different letters differ significantly (P <0.05) between exposure times (30 and 60 min) and across collections according to the Duncan test (P<0.05). Bars represent mean ± standard deviatio.
.....71

Figure 2 – Breakdown of the interaction (mean ± standard deviation) between the different times of exposure to air and along the collections, in the variables partial pressure of carbon dioxide ($PvCO_2$ - mmHg), partial pressure of oxygen (PvO_2 - mmHg) and bicarbonate (HCO_3^- -mmol L-1). Means followed by different letters differ significantly (P <0.05) between exposure times (30 and 60 min) and across collections according to the Duncan test (P<0.05). Bars represent mean ± standard deviation.
.....75

Figure 3: Breakdown of the interaction (mean ± standard deviation) between the different times of exposure to air and along the collections, in the variable sodium (Na^+ mmol L^{-1}). Means followed by different letters differ significantly (P <0.05) between exposure times (30 and 60 min) and across collections according to the Duncan test (P<0.05). Bars represent mean ± standard deviation.
.....79

LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

1hAE: One hour after exposure

24hAE: Twenty-four hours after exposure

48hAE: Forty-eight hours after exposure

%: Percentage

° C: Degrees Celsius

μL: Microliter

μm Micrometre

× g: g force

AE: After exposure

ANOVA: Analysis if variance

ALT: Alanine aminotransferase

APHA: American Public Health Association

AST: Aspartate aminotransferase

BE: Excess base

Ca^{2+}: Calcium

CAPES: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

Cl^{-}: Chloride

cm: Centimeter

CEUA: Comissão de Ética no Uso de Animais

dL: Deciliter

EV: Escola de Veterinária

FAO: Food and Agriculture Organization

h: Hour

g: Grass

HCO_3^{-}: Bicarbonate ion

IAE: Immediately after exposure

IBGE: Instituto Brasileiro de Geografia e estatística

K^+: Potassium

L: Liters

min: Minutes

mg: Milligram

mm: Millimeter

mmHg: Millimeter of mercury

mmol: Millimol

mS: Millisiemens

Na^+: Sodium

pH: hydrogen potential

pO_2: Oxygen partial pressure

pCO_2: partial pressure of carbon dioxide

SRA: Aquaculture recirculation systems

O_2: Oxygen saturation

$stHC0_3^-$: Standard bicarbonate

tO_2: Oxygen rate

UI: International unity

Sumário

1. INTRODUÇÃO GERAL 17
2. REVISÃO BIBLIOGRÁFICA 18
2.1 Hipóxia 18
2.2 Exposição ao ar 21
2.3 Hematologia, bioquímica do sangue e hemogasometria 22
2.5 A espécie *Colossoma macropomum* 26
3. OBJETIVOS 28
3.1 GERAL 28
3.2 ESPECÍFICOS 28
4. REFERÊNCIAS BIBLIOGRÀFICAS 29

5. ARTIGO 1 38
5.1 Introduction 39
5.2 Material and methods 40
5.2.1 Compliance with Ethical Standards 40
5.2.2 Fish and experimental conditions 40
5.2.3 Blood samples for gasometric, hematological and biochemical analyses 42
5.2.4 Statistical analyses 43
5.3 Results 43
5.4 Discussion 49
5.5 Conclusion 54
5.6 Compliance with ethical standards 54
5.7 Funding 54
5.8 Conflict of Interest 54
5.9 References 54

6. ARTIGO 2 63
6.1 Introduction 64
6.2 Material and methods 67
6.2.1 Fish and experimental conditions 67
6.2.2 Air exposure testing with *Colossoma macropomum* 68
6.2.3 Blood samples for gasometric, hematological and biochemical analyses 68
6.2.4 Statistical analyses 70
6.3 Results 70
6.4 Discussion 79
6.5 Conclusions 86
6.6 Acknowledgements 87
6.7 References 87

7. CONSIDERAÇÕES FINAIS 98

1. INTRODUÇÃO GERAL

A produção de peixes na aquicultura teve rápido crescimento no volume de produção e impacto econômico no mundo, contribuindo com 46% do total produzido e 52% do pescado para consumo humano. Com a demanda dos consumidores e o esgotamento da pesca de captura selvagem prevê-se que a aquicultura seja a principal fonte de peixes até 2030 (FAO, 2020). Isso depende da capacidade de expansão e crescimento de maneira sustentável que a indústria de aquicultura reduza sua dependência de peixes selvagens (Fazio, 2019).

Segundo dados Produção da Pecuária Municipal 2019, divulgada pelo Instituto Brasileiro de Geografia e Estatística (IBGE), a piscicultura brasileira alcançou 599,1 mil toneladas em 2019, um crescimento de 3% em relação a 2018. No entanto dados apurados pela Associação Brasileira da Piscicultura ((Peixe BR, 2020)) indicam que produção nacional de peixes apresentou crescimento do total produzido de aproximadamente 4,9% em comparação ao ano anterior. A espécie mais produzida foi a tilápia (*Oreochromis niloticus*), representando 57% da produção nacional. O tambaqui representa a segunda espécie mais produzida no Brasil, segundo dados do IBGE (2019) a produção atingiu 101.079.464 toneladas, estando em primeiro lugar na produção o estado de Rondônia com produção de 40.141.375 toneladas, ficando atrás somente da tilápia (*O. niloticus*). Ainda segundo a PeixeBR (2020) a produção de peixes nativos não apresentou crescimento ente 2018 e 2019 e representou um total produzido de 38%.

Apesar dessa grande diversidade e do potencial de produção, muitas vezes esses animais não são cultivados em sistemas de produção eficientes, o que torna sua produção difícil em grande escala (Sousa e Kato, 2017).

Para se ter sucesso no cultivo de qualquer espécie, é primordial o desenvolvimento e domínio de um pacote tecnológico, o qual deve englobar diversas áreas da produção como sistemas de produção ideal para a espécie, reprodução e larvicultura, sanidade, nutrição e

técnicas de manejo que visem minimizar os estresses, entre outras. Na piscicultura, os peixes são constantemente expostos a diversas condições de estresse devido a diversas práticas de cultivo, como altas densidades, transporte, manejo, como biometrias, entre outros, além das mudanças nos fatores ambientais abióticos como a temperatura, salinidade e pH (Skrzynska et al., 2018) e também variações nas concentrações de oxigênio dissolvido (OD) (Yang et al., 2017; Li et al., 2018; Baldissera et al., 2018).

Nesse sentido, tem merecido destaque o tambaqui, que é um caracídeo nativo da bacia Amazônica e do rio Orinoco, adaptado às condições de cativeiro, aceitando facilmente dietas artificiais, com boas taxas de crescimento e conversão alimentar (Inoue et al., 2011; Stringhetta et al., 2017; Valladão et al., 2018; Fabio et al., 2021; Silva et al., 2021). Embora haja literatura sobre a resistência desses animais em diversas situações de estresse, pouco se conhece sobre a influência de estresse por hipóxia e exposição ao ar na fisiologia de *C. macropomum*. Desse modo, informações e conhecimentos sobre suas variáveis gasométricas, hematológicas e bioquímicas podem proporcionar relevantes indicações de alterações em seu estado fisiológico.

2. REVISÃO BIBLIOGRÁFICA

2.1 Hipóxia

A qualidade da água é composta por parâmetros químicos, físicos e biológicos e o controle desses parâmetros é fundamental para o sucesso do cultivo. Quando dentro da faixa ideal estes parâmetros facilitam a manutenção da saúde dos animais, contribuindo para o processo reprodutivo e estímulo ao crescimento (Abdel-Tawwab et al., 2019).

O oxigênio se destaca dentre os parâmetros críticos para o desempenho zootécnico dos animas, sendo que sua concentração na água de cultivo afeta diretamente os processos

fisiológicos dos organismos aquáticos (Gilmore et al., 2018). Os cuidados no controle da concentração de O_2 devem ser contínuos em sistemas de cultivo aquícolas.

A baixa concentração de oxigênio dissolvido na água (OD) é conhecida como hipóxia e afeta negativamente o comportamento, fisiologia, imunologia e crescimento dos peixes (Jobling, 1995; Lovell, 1998; Mallya, 2007; Pollock et al., 2007; Thorarensen et al. 2010; Baldissera et al., 2018; Burgos-Aceves et al. 2018). Peixes em cativeiro são submetidos a diversas situações de estresse, como densidades inadequadas e confinamento, manejos, qualidade da água variável, inclusive a hipóxia, e diferentemente do ambiente natural, em cativeiro não podem escapar dessas situações (Abdel-Tawwab et al., 2019). Portanto, espécies cultivadas ou com potencial de cultivo devem possuir a capacidade de se adaptar a esses estressores presentes no cultivo (Mallya, 2007; Thorarensen et al., 2010).

Algumas espécies de peixes quando expostos à hipóxia, tem como primeiro comportamento no ambiente a busca por lugares com maiores níveis de oxigênio (Baldisserotto, 2009). Outras espécies, porém, desenvolveram mecanismos compensatórios que podem ser fisiológicos, teciduais e/ou comportamentais que buscam compensar a baixa concentração de OD (Wu, 2002; Wu et al., 2007; Douxfils et al., 2012). A hipóxia pode ter intensidade e duração diferente e em uma exposição moderada os peixes podem ter a capacidade de se adaptar, retornando ao seu estado de homeostase; porém, quando expostos a períodos longos e intensos podem ter uma resposta mal adaptativa, sem conseguir restabelecer a homeostase, como consequência, ter o seu estado de saúde prejudicado (Galhardo e Oliveira, 2006).

O *C. macropomum* criou um mecanismo adaptativo próprio à variação na concentração de oxigênio, composto por alterações comportamentais, morfológicas e fisiológicas expressas como adoção da respiração superficial aquática, expansão labial e alterações no tecido branquial (Val e Almeida-Val, 1999). A hipóxia, a depender do tempo de exposição, intensidade e em menor grau da adaptabilidade da espécie causa asfixia nos animais e, em consequência disso, a

mortalidade (Mallya, 2007). As brânquias de *C. macroporum* submetidos à hipóxia apresentaram alterações na coloração, congestão de sangue, aderência das lamelas branquiais e pequenas hemorragias na parte anterior da cavidade ocular (Mallya, 2007).

Alterações nos níveis hematológicos, bioquímicos e metabólicos dos peixes também foram observados nos animais na condição hipóxica e essas mudanças acontecem através das respostas primária e secundária ao estresse, na tentativa de restabelecer a homeostase (Caldwell e Hinshaw 1994; Delaney e Klesius 2004; Welker et al., 2007; Terova et al., 2008). Para *Acipenser schrenckii* (Ni et al., 2014) e *Astronotus ocellatus* (Baptista et al., 2016) foi registrado aumento dos níveis de glicose plasmática ao longo dos períodos de hipóxia. Isso sugere uma mobilização em resposta aos distúrbios da hipóxia. Este aumento se dá, principalmente, por ação glicogenolítica (quebra do glicogênio estocado no fígado) das catecolaminas (Wendelaar Bonga, 1997). Em hipóxia severa, como já dito, os peixes podem reduzir a locomoção ou a ingestão de alimentos para economizar energia, o que pode não ser suficiente, podendo ocorrer o abrandamento da produção de ATP através da respiração aeróbica, não deixando alternativas para o animal a não ser utilizar as vias anaeróbicas para ajudar com suas necessidades energéticas (Pichavant et al., 2002). Contudo, o metabolismo anaeróbico não é tão eficaz em relação à respiração aeróbica, sendo necessário grande fluxo glicolítico para evitar uma queda prejudicial no ATP celular (Routley et al. 2002; Chabot e Claireaux, 2008). Para esse fim, ocorre o aumento do nível de glicose no sangue como suprimento de combustível anaeróbico em muitos peixes submetidos à hipóxia e como resultado, ocorre ainda mais acúmulo de lactato (Pichavant et al., 2002; Routley et al., 2002; Lushchak et al., 2005). Alguns estudos revelaram os efeitos da hipóxia nos parâmetros sanguíneos, bioquímicos e metabólicos em espécies como *Carassius auratus* (Roesner et al., 2008), *Prochilodus nigricans* (Val et al., 2015), *Micropterus salmoides* (Yang et al., 2017), *Sparus aurata* (Araújo-Luna et al., 2018) e *O. niloticus* (Li et al.,

2018), além de efeitos no sistema imune, como relatados por Baldissera et al. (2018) em *Lophiosilurus alexandri* e Wang et al. (2018) em *O. niloticus.*

2.2 Exposição ao ar

Além da hipóxia, outro fator estressante comum nos manejos é a exposição ao ar, estresse esse prejudicial aos peixes, podendo alterar sua homeostase e quando prolongada pode causar grandes perdas. A exposição ao ar ocorre com frequência na produção de peixes, seja em biometrias, transporte de um sistema para outro ou em despescas. Sabe-se que a exposição ao ar é prejudicial à homeostase e pode ocasionar desarranjos morfológicos, transtornos comportamentais e uma variedade de adaptações metabólicas e fisiológicas aos peixes (Mazeaud et al., 1977; Cooke et al., 2003). Esses desarranjos e a recuperação a eles são influenciados pela duração da exposição ao agente estressor (Suski et al., 2004).

Algumas funções fisiológicas como osmorregulação e regulação ácido-base, circulação, metabolismo e respiração são normalmente alteradas para fazer os ajustes homeostáticos, podendo essas mudanças serem observadas por meio da avaliação de parâmetros sanguíneos (Barton, 2002; Zaragoza et al., 2008; Stewart et al., 2019). A literatura já elencou os parâmetros que são os mais confiáveis e fornecem informações importantes para a avaliação da resposta ao estresse em peixes, dentre os quais podemos destacar, contagem de hemácias, porcentagem de hematócrito, concentração de hemoglobina, glicose no sangue, lactato, pH sanguíneo, CO_2 total e pCO_2 sendo estes tidos como os indicadores mais confiáveis para avaliar a resposta ao estresse em peixes (Wendelaar Bonga, 1997; Roriz et al., 2015; Guan et al., 2017; Mattioli et al., 2019; Stewart et al., 2019).

Vários autores já avaliaram a exposição ao ar em peixes de diferentes espécies por tempos diversos: trutas triploides (*Oncorhynchus mykiss*) e (*Salvelinus fontinalis*) 30 e 40 minutos, respectivamente (Benfey e Biron, 2000); juvenis de garoupas (*Epinephelus coioides*)

expostas durante 60 minutos ao ar (Yokoyama et al., 2006); jeju (*Hoplerythrinus unitaeniatus*) submetidos a 1, 6 e 12 horas de exposição ao ar (Mariano et al., 2009); tilápia nilótica (*O. niloticus*) submetidas a diversos tempos com até 40 minutos de exposição ao ar (Luz et al., 2012); tilápia nilótica (*O. niloticus*) submetidas até 90 minutos de exposição ao ar (Silva et al., 2012); juvenis de pacamã (*Lophiosilurus alexandri*) expostos ao ar por 30 minutos (Mattioli et al., 2019) confirmando que estudos referentes a esta prática, são recorrentes e importantes.

2.3 Hematologia, bioquímica do sangue e hemogasometria

A sobrevivência e o crescimento dos peixes dependem de diversos fatores, tais como os níveis de oxigênio dissolvido na água, temperatura, pH, resíduos nitrogenados, manejo, transporte entre outros (De Castro Oliveira et al., 2007). Quando os peixes são submetidos à alteração de um desses fatores ocorrem modificações no seu metabolismo, o que constituem desvios da homeostase (Barton e Iwama, 1991). Além disso, estes animais expressam um conjunto de respostas fisiológicas frente ao fator de estresse, que consiste no reconhecimento do estressor pelo sistema nervoso central, onde são observadas as reações hormonais do eixo hipotálamo-hipófise-interrenais, com liberação das catecolaminas e do cortisol (Barton e Iwama, 1991).

Essas respostas fisiológicas podem ser observadas através de análises hematológicas, as quais são empregadas como ferramentas auxiliares de diagnóstico da saúde dos peixes em cultivo. Através do acompanhamento dessas variáveis é possível avaliar o quadro homeostático em peixes submetidos a situações de estresse (Kori-Siakpere et al., 2006).

O estresse é um conjunto coordenado de respostas fisiológicas e comportamentais frente a qualquer desafio percebido à homeostase ou à alostase (Braithwaite e Ebbesson, 2014). Este pode ser quantificado em respostas primárias, secundárias e terciárias que ocorrem após a ativação do eixo hipotálamo-hipófise-inter-renal (HPI) (Sopinka et al.,2017).

A resposta primária é iniciada pelo sistema nervoso central (SNC) e mediada pelo eixo HPI e pelo sistema nervoso autônomo (SNA), que ao perceber os sinais do ambiente culmina na liberação de hormônios do estresse, corticosteroides e catecolaminas (Wendelaar Bonga, 1997; Barton, 2002). Quando liberados na corrente sanguínea, os hormônios do estresse induzem respostas secundárias nos tecidos-alvo, expressando os receptores apropriados (Mommsen et al., 1999). Geralmente, a secreção de catecolaminas é iniciada logo após o início da resposta ao estresse e é transitoriamente diminuída (Wendelaar Bonga, 1997). Respostas secundárias dos peixes incluem alterações hematológicas, metabólicas, osmorreguladoras, celulares e imunológicas, como aumento dos níveis de glicose e lactato no sangue, aumento da pressão arterial e respiração, alterações na composição iônica, hematócrito, atividade da lisozima e produção de anticorpos (Wendelaar Bonga, 1997). Estes levam a respostas de estresse terciário, como diminuição da ingestão de alimentos, como consequência diminuição do crescimento, resistência a doenças, comportamento alterado, entre outros (Wendelaar Bonga, 1997; Barton, 2002). Devido à importância na cascata fisiológica, a concentração plasmática de glicose é considerada um dos principais indicadores de estresse em peixes. Vários autores revelaram que hipóxia foi responsável por elevar os níveis de catecolaminas, ativando a glicogenólise e a gliconeogênese com um resultado de aumento dos níveis plasmáticos de glicose em *S. aurata* (Henrique et al. 1998); *O. niloticus* (Delaney e Klesius 2004; Abdel-Tawwab et al. 2014, 2015); *Ictalurus punctatus* (Welker et al. 2007); *Ctenopharyngodon idella* (Gan et al. 2013); *A. ocellatus* (Baptista et al., 2016), assim como para lactato em *P. nigricans* (Val et al., 2015) e *O. niloticus* (Li et al., 2018). Esse fato foi relatado em peixes quando estes sofrem exposição ao ar, causando distúrbios fisiológicos, incluindo aumento da glicose como verificado em *Piaractus brachypomus* (Roriz et al., 2015), *Paralichthys olivaceus* (Lim e Hur, 2018) e em *L. alexandri* (Mattioli et al., 2019a).

Tavares-Dias e Moraes (2004) mencionaram que as células vermelhas do sangue transportam oxigênio e gás carbônico por meio da hemoglobina e junto com o hematócrito podem ser indicadores da capacidade de transporte de oxigênio dos peixes, podendo mostrar a concentração de oxigênio que está disponível no ambiente onde o animal se encontra. Segundo Vosyliené (1999), quando a contagem de hemácias e hematócrito diminuem são sinais de anemia e de piora do estado de saúde do peixe. O hematócrito também pode mudar quando há aumento da atividade eritropoiética do baço e do rim proveniente do estresse, sendo que essa mudança pode causar hemoconcentração pela liberação de eritrócitos pelo baço ou hemodiluição, quando os valores de hematócrito reduzem (Tavares-Dias e Moraes, 2004). Estudos demonstraram aumentos rápidos nos glóbulos vermelhos (hemácias), hemoglobina e/ou hematócrito após hipóxia em peixes (Affonso et al. al., 2002; Wells e Baldwin, 2006; Abdel-Tawwab et al., 2014; 2015). Yang et al. (2017) submeteram *M. salmoides* à hipóxia e relataram que a exposição aumentou significativamente o número de hemácias, mas diminuiu a hemoglobina. Araújo-Luna et al. (2018) descobriram que *S. aurata* em condição normóxica (85,4% OD) apresentava níveis significativamente mais baixos de hematócrito quando comparados aos peixes com baixo nível de OD, enquanto não havia diferenças significativas em relação à hemoglobina. O mesmo acontece para a exposição ao ar em *Cyprinus carpio* com variações das respostas hematológicas, como na contagem de hemácias e hemoglobina corpuscular média (Chen et al., 2015).

Além desses parâmetros bioquímicos, as enzimas hepáticas ALT (alanina aminotransferase) e AST (aspartato aminotransferase) também são consideradas indicadores de estresse e, frequentemente, são usadas em diagnóstico de doenças de peixes e na detecção de danos nos tecidos causados por fatores estressantes (Firat et al., 2011). Ambas são enzimas intracelulares, porém ALT é unicamente citoplasmática, enquanto a AST se encontra tanto na forma mitocondrial quanto citoplasmática e ambas são enzimas muito ativas no fígado (Vroon

e Israili, 1990). O aumento da atividade dessas enzimas indica mobilização de aspartato e alanina via gliconeogênese para a produção de glicose para resistir ao estresse e esse aumento também pode ser causado por danos no fígado, pois nesse caso extravasam dos hepatócitos lesionados e alcançam a corrente sanguínea (Harvey et al., 1994), sendo então marcadores bioquímicos de danos hepáticos. *Larimichthys crocea* submetidos ao estresse de hipóxia tiveram as atividades de AST e ALT aumentadas (Harvey et al., 1994).

Do mesmo modo que essas análises hematológicas e bioquímicas, os parâmetros de gasometria sanguínea também são marcadores para o monitoramento da saúde dos peixes (Barton 2002; Cnaani et al., 2004; Tavares Dias et al., 2008). A hemogasometria detecta a eficácia das trocas gasosas respiratórias (oxigenação) e parâmetros metabólicos (equilíbrio ácido-base), como pressão parcial de oxigênio (pO_2), pressão parcial de dióxido de carbono (pCO_2), pH sanguíneo e íon bicarbonato (HCO_3^-) (King, 2000). A associação dessas ferramentas pode auxiliar em avaliação eficaz das condições fisiológicas causadas pelo estresse (Tavares Dias et al., 2008; Barbas et al., 2016; Mattioli et al., 2019a; Mattioli et al., 2019b).

Os gases sanguíneos também sofrem variações. Hipóxia e exposição ao ar podem diminuir o pH do sangue causando a acidose extracelular (Cook et al., 2015), além de afetar outras variáveis gasométricas como aumento da concentração de $PvCO_2$ e diminuição de HCO_3^- (Mattioli et al., 2019b). Variáveis como pH sanguíneo, pressão parcial de dióxido de carbono e oxigênio, íon bicarbornato entre outros, servem para avaliar as condições de estresse por manipulação e resposta à anestésicos (Honorato et al., 2013; Honorato e Nascimento, 2016; Barbas et al., 2020) e também na resposta de exposição ao ar (Mattioli et al., 2019a; Ruiz-Jarabo et al., 2020).

A perturbação do equilíbrio hidromineral também é considerada uma resposta secundária ao estresse. Segundo Wendelaar Bonga (1997) diversos tipos de estressores prejudicam o equilíbrio iônico nos peixes. Alterações nos parâmetros como níveis de potássio,

cloreto, sódio e cálcio podem ser caracterizado como desequilíbrio ácido-base (Mariano et al. 2009).

2.5 A espécie *Colossoma macropomum*

Popularmente conhecido como tambaqui *C. macropomum* (Cuvier, 1816) é um caracídeo nativo da bacia Amazônica e do rio Orinoco, pertencente à superclasse Actinopterygii, ordem Characiformes, família Serrasalminae (Buckup et al., 2007). No ambiente natural possui hábito alimentar onívoro, alimentando-se tanto de matéria animal quanto vegetal, possui tendência de zooplanctófago na fase jovem e de frugívoro na fase adulta (Saint-Paul, 1984). Depois do pirarucu, *Arapaima gigas* (Osteoglossidae), o tambaqui é considerado o segundo maior peixe em escala de água doce da Amazônia (Sousa et al., 2016), podendo atingir 1 m de comprimento e pesar aproximadamente 30 kg (Valladão et al., 2016). Atinge a maturidade sexual entre três e quatro anos de idade, possui desova total, alta fecundidade e ovos semipelágicos (De Lima e Araújo-Lima, 2004).

Muitos pesquisadores e produtores têm intensificado seus esforços para estabelecer um protocolo tecnológico de cultivo desta espécie em cativeiro, incluindo a avaliação da densidade de estocagem em tanques de alvenaria com fluxo contínuo (Costa et al., 2019), densidade em gaiolas (Silva e Fujimoto, 2015), necessidade de proteína após privação alimentar (Santos et al., 2010), frequência alimentar (Souza et al., 2014), uso de anestésicos (Barbas et al., 2014; 2016; de Souza et al., 2019), salinidade da água (Souza-Bastos et al., 2016), pH (Wood et al., 2018), restrição alimentar (Assis et al., 2020). Mais recentemente, bons resultados têm sido verificados também para a larvicultura (Santos et al., 2021), produção de juvenis (Silva et al., 2021) e para fase de crescimento (Lima et al., 2019; Santos et al., 2021) em sistemas de recirculação de água. Além de sua importância na produção, é uma espécie considerada como bom modelo animal para diversas pesquisas como programas de biomonitoramento, anestésicos, entre outros (Corrêa et al., 2007; Marcuschi et al., 2010; Braz-Mota et al., 2015;

Sadauskas-Henrique et al., 2016; Barbas et al., 2016; Barbas et al., 2017a; Barbas et al., 2017b; Baldisserotto et al., 2018).

Embora haja literatura sobre a hematologia e bioquímica de peixes, pouco se conhece sobre a influência de estresse por hipóxia e exposição ao ar na fisiologia de *C. macropomum*. Desse modo, informações e conhecimentos sobre suas variáveis gasométricas, hematológicas e bioquímicas podem proporcionar relevantes indicações de alterações em seu estado fisiológico como um indicativo para melhorar o manejo nas pisciculturas.

3. OBJETIVOS

3.1 GERAL

Avaliar as respostas fisiológicas e metabólicas em juvenis de *Colossoma macropomum* submetidos à condição de hipóxia e exposição ao ar.

3.2 ESPECÍFICOS

- Avaliar sobrevivência dos animais ao longos do experimentos.

-**Artigo 1:** Realizar avaliações gasométricas, hematológicas e bioquímicas sanguíneas em juvenis de tambaqui, submetidos ao estresse por hipóxia em 24 e 72 horas, assim como sua recuperação em 24, 48 e 96 horas.

- **Artigo 2:** Realizar avaliações gasométricas, hematológicas e bioquímicas sanguíneas em juvenis de tambaqui, submetidos ao estresse de exposição ao ar por 30 e 60 minutos e posteriores coletas imediatamente após exposição (IAE), uma hora após exposição ao ar (1hAE), 24 e 48 após exposição ao ar (24hAE e 48hAE respectivamente).

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5. ARTIGO 1

Physiological and metabolic responses in juvenile *Colossoma macropomum* exposed to hypoxia

Running Head

Effects of hypoxia on *Colossoma macropomum*

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Abstract

This study aimed to evaluate hematological, biochemical and gasometric parameters of tambaqui juveniles (*Colossoma macropomum*) exposed to hypoxia and subsequent recovery. Six animals were subjected to normoxia (basal) treatment with dissolved oxygen (DO) 6.27 ± 0.42 mg L^{-1}. Water flow and aeration were reduced for three days (hypoxia), during which DO was 0.92 ± 0.37 mg L^{-1}. Water flow and aeration were then reestablished with DO remaining similar to basal. The treatments were: normoxia - basal; 24 hours after initiating hypoxia (24H); 72 hours after initiating hypoxia (72H); 24 hours after reestablishing normoxia (24R); 48 hours after reestablishing normoxia (48R); and 96 after reestablishing normoxia (96R). The highest glucose level was recorded at 24H ($P < 0.05$); the highest lactate level was at 72R; and the highest blood pH was at 24H and 72H ($P < 0.05$). The highest concentration of $PvCO_2$ was at 24H ($P < 0.05$), while at 96R it was equivalent to basal ($P > 0.05$). The variable PvO_2 was only higher than basal at 24R ($P < 0.05$). Juvenile *C. macropomum* managed to reestablish the main stress indicators (glucose and lactate) at 96R, while the other indicators varied during the study, with homeostatic physiology being reestablished during the recovery period.

Keywords: Hypoxic stress, stress physiology, native fish, tambaqui.

5.1 Introduction

Fish experience many repetitive and chronic stressors when in captivity due to confinement, high density and variation in water quality parameters, including low oxygen levels or hypoxia. Diaz and Rosenberg (1995) define hypoxia in aquatic environments as when the dissolved oxygen (DO) concentration is less than 2.8 mgO_2 L^{-1}, which can negatively affect the behavior, growth, food intake and physiological state of fish (Welker et al., 2007; Bernier et al., 2012; Nguyen et al. 2017; Li et al., 2018; Abdel-Tawwab et al., 2019; Mattioli et al., 2019a), and thus is considered a stressor (Baldissera et al., 2018).

Amazonian fish have developed several adaptive responses to survive low oxygen concentrations. Tambaqui (*Colossoma macropomum*) (Cuvier, 1818) is one such species and has served as a model in several studies on adaptations to hypoxia (Saint-Paul, 1984; Almeida-Val and Val, 1993; Val, 1996; Weber, 1996; Affonso et al., 2002). Tambaqui is native to the Amazon basin and the Orinoco River, and possesses a series of regulatory systems to adapt to hypoxia. Among these mechanisms is breathing on the aquatic surface. These fish do this with great efficiency due to the projection of their lips, which allows capturing O_2-rich surface water

in the branchial region. They are also able to increase the frequency of opercular beats to ensure maximum efficiency at capturing O_2 during hypoxic stress (Sundin et al., 2000). In other species studied the persistence of environmental hypoxia can lead to mortality (Fitzgibbon et al., 2007; Cook and Herbert, 2012; Xiao, 2015; Abdel-Tawwab et al., 2019).

Studies have revealed the effects that hypoxia has on blood, biochemical and metabolic parameters as an increase in glucose in *Oreochromis niloticus* (Li et al., 2018). For *Sparus aurata*, Araújo-Luna et al. (2018) observed an increase in hematocrit, as well as Val and Almeida-Val (2015) for *Prochilodus nigricans* which also showed an increase in hemoglobin and plasma lactate levels. For *Micropterus salmoides* Yang et al. (2017) showed a decrease in hemoglobin. Some studies have also shown effects on the immune system, where *O. niloticus* decreased the phagocytic activity of peripheral blood leukocytes and the production of reactive intracellular oxygen species (Wang et al., 2018). Baldissera et al. (2018) showed that the fish *Lophiosilurus alexandri* got a pro-inflammatory profile after hypoxia.

Previous studies with *C. macropomum* have demonstrated the effect that hypoxia has on hemoglobin concentration, hematocrit, red blood cell count, blood pH and lactate and glucose levels (Saint Paul, 1984; Affonso et al., 2002). These studies reported the possibility of a regulating metabolism that reduces the effects of hypoxia, however, other mechanisms may exist, these studies, however, did not evaluate the recovery of tambaquis. Thus, additional analyses are necessary in order to further clarify adaptations by *C. macropomum* to hypoxia and to determine the recovery time this fish requires to restore its homeostatic state.

Therefore, the present study aimed evaluate the physiological and metabolic responses of juvenile *C. macropomum* exposed to hypoxia and subsequent recovery.

5.2 Material and methods

5.2.1 Compliance with Ethical Standards

The experimental protocol was approved by Comissão de Ética no Uso de Animais (CEUA) of Universidade Federal de Minas Gerais (approval number: 296/2019).

5.2.2 Fish and experimental conditions

Thirty-six juvenile *C. macropomum* (Cuvier, 1818) (51.82±7.01 g and 15.59±0.94 cm) originating from creation at Laboratório de Aquacultura of the Escola de Veterinária of

Universidade Federal de Minas Gerais, were acclimatized to experimental units for 15 days (Wood et al, 2017; Wood et al., 2018; De Souza et al., 2019). The experimental units comprised six 30-L tanks, with six animals each, in a recirculating aquaculture system (RAS) with biological and mechanical filters. The fish were fed to apparent satiety twice a day (08:00 and 16:00 h), with commercial extruded diet (4 mm in diameter, 32% crude protein, Laguna®). During this period the water quality remained at: DO 6.27 ± 0.42 mg L^{-1}; temperature 28.02 ± 0.42 °C; pH 7.52 ± 0.14; conductivity 0.33 ± 0.04 mS cm^{-1}; salinity 0.16 ± 0.02 g of salt L^{-1}; and total ammonia 0.33±0.13 mg L^{-1}. Dissolved oxygen concentration and temperature were measured using a digital oximeter (Ecosense®, model DO200A); pH, conductivity and salinity were measured with a multiparameter analyzer (Hanna®, model Combo pH & EC Waterproof); and total ammonia was analyzed using a commercial kit (LabconTest®).

Each animal was considered a replicate in a completely randomized experimental design. Six animals from one tank were used as a control (normoxia/basal), which were fasted for 12 hours prior to collection. Water flow and aeration of the remaining five tanks were reduced for three days such that the water quality parameters for hypoxia were: DO = 0.92 ± 0.37 mg L^{-1}; temperature = 28.28 ± 0.35 °C; pH = 7.41 ± 0.27; conductivity = 0.31 ± 0.06 mS cm^{-1}; salinity = 0.16 ± 0.02 g of salt L^{-1}; and total ammonia = 0.36 ± 0.12 mg L^{-1}. Water flow and aeration were the reestablished after the three-day with the water parameters remaining similar to those of the acclimatization period. Is the treatments related to the sampling-time, as shown below: normoxia - basal; 24 hours after initiating hypoxia (24H); 72 hours after initiating hypoxia (72H); 24 hours after reestablishing normoxia (24R); 48 hours after reestablishing normoxia (48R) and 96 hours after reestablishing normoxia (96R). Six animals of the same tank were collected for each treatment (n=6 for each sampling time). Survival was observed throughout the experiment.

It has been reported that *C. macropomum* performs shallow aquatic breathing when under hypoxia, which is facilitated by the development of cutaneous edema of the lower lips (Val and Almeida-Val, 1995). Saint-Paul (1988) reported that these lips do not participate in gas exchange, but they function as mechanical structures that optimize bringing oxygen-rich surface water to the gills. Thus, the anatomy of animals exposed to hypoxia was observed every 30 minutes until lip expansion was noted, after which observations were made twice a day until the end of exposure. The animals were also observed every 30 minutes during recovery to verify the lip regression. During treatments the animals were fed twice daily until apparent satiety with the same commercial diet described previously for acclimatization, the observation

regarding the feeding was performed in the period of 30 minutes. Leftover feed, feces and other debris were removed daily after feeding. Fish to be collected were fasted for 24 hours (except for normoxia/basal fish, which were fasted 12 hours) prior to collection.

5.2.3 Blood samples for gasometric, hematological and biochemical analyses

Blood samples were collected by caudal venipuncture (Saint-Paul, 1984). Fish were contained in an appropriate damp cloth during sampling and two blood samples were collected from each animal. The first sample, 300 µL of blood removed using heparinized syringes, was used for gas analysis and for determining the following parameters: pH, tO_2 (oxygen rate, mmol L^{-1}), PvO_2 (venous oxygen partial pressure, mmHg), $PvCO_2$ (venous carbon dioxide partial pressure, mmHg), sO2 (oxygen saturation, %), lactate (mmol L^{-1}) and concentrations of sodium Na^+ (mmol L^{-1}), potassium K^+ (mmol L^{-1}), calcium Ca^{2+} (mmol L^{-1}) and chloride Cl^- (mmol L^{-1}). These data were used to measured: $HC0_3^-$ (bicarbonate, mmol L^{-1}), $stHC0_3^-$ (standard bicarbonate, mmol L^{-1}) and BE (base excess, mmol L^{-1}). These analyses were performed using a blood gas meter (ABL800 BASIC analyzer - Radiometer®), with water temperature and oxygen saturation being corrected in the equipment to correspond to the experimental treatments.

The second sample, 1 mL of blood collected in heparinized syringes, was used for hematocrit (%) evaluation using capillary tubes filled with to two thirds with previously homogenized blood. The capillary tubes were centrifuged for 10 min at 10,867 x g (Micro SPIN 1000) and then read in a microhematocrit using an appropriate scale. Plasma protein (g dL^{-1}) was measured using a hand-held refractometer (RHC 200-ATC, Huake Instrument Co. Ltd.; www.instrument-china.com) while hemoglobin concentration (g dL^{-1}) was determined by cyanomethahemoglobin reaction using a commercial kit (reference no. K023-1 QUIBASA Química Básica Ltda. Bioclin - www.bioclin.com).

Sodium heparin (HEPAMAX-S® Cotia, São Paulo, Brazil) was used at 10% µL mL^{-1} of blood. The aliquots of heparinized whole blood remaining after the hematological analyses were centrifuged at 1165 x g por 10 minutes (Centrifuge Spinlab SL-5AM) for plasma separation and determination of glucose (mg dL^{-1}) by glucose oxidase test (GOD Trinder) (reference no. K082-2); monoreagent triglycerides (mg dL^{-1}) by Trinder reaction (reference no. K117); and cholesterol (mg dL^{-1}) by the Trinder enzyme method using a commercial kit (reference no. K083-3). The enzymes AST – aspartate-aminotransferase (UI) (reference no. K034-1) and ALT – alanine-aminotransferase (UI) (reference no. K035-1) were determined by

the Reitmamm and Frankel method. All the cited tests were analyzed by the Bioclin kit (www.bioclin.com QUIBASA Química Básica Ltda, Belo Horizonte, Minas Gerais, Brazil). All analyses were read on a spectrophotometer (Biocrom Libra S22 – analiticaweb.com.br).

5.2.4 Statistical analyses

Data were analyzed for normality and homoscedasticity of variances using the Shapiro-Wilk and Levene tests, respectively. Data were then submitted to analysis of variance (one-way ANOVA) followed by Tukey's test at 5% probability. Data are presented as means ± standard deviation. Statistical analyses were performed with InfoStat® version 1.0 software.

5.3 Results

Survival during the experiment was 100%. The animals did not feed during 24H, 72H and 24R, but resumed feeding after 48R. Lip expansion was observed during the three days of hypoxia, which returned to normal after restoring DO levels.

The highest glucose value was recorded at 24H ($P < 0.05$) (Fig. 1A), which returned to basal levels at 72H ($P < 0.05$). Glucose was lower than basal at 24R and 48R ($P < 0.05$), and similar to basal at 96R ($P > 0.05$).

Lactate increased at 24H with a higher value by 72H compared to basal ($P < 0.05$) (Fig 1B). All collections during the recovery period had lactate levels similar to that of basal.

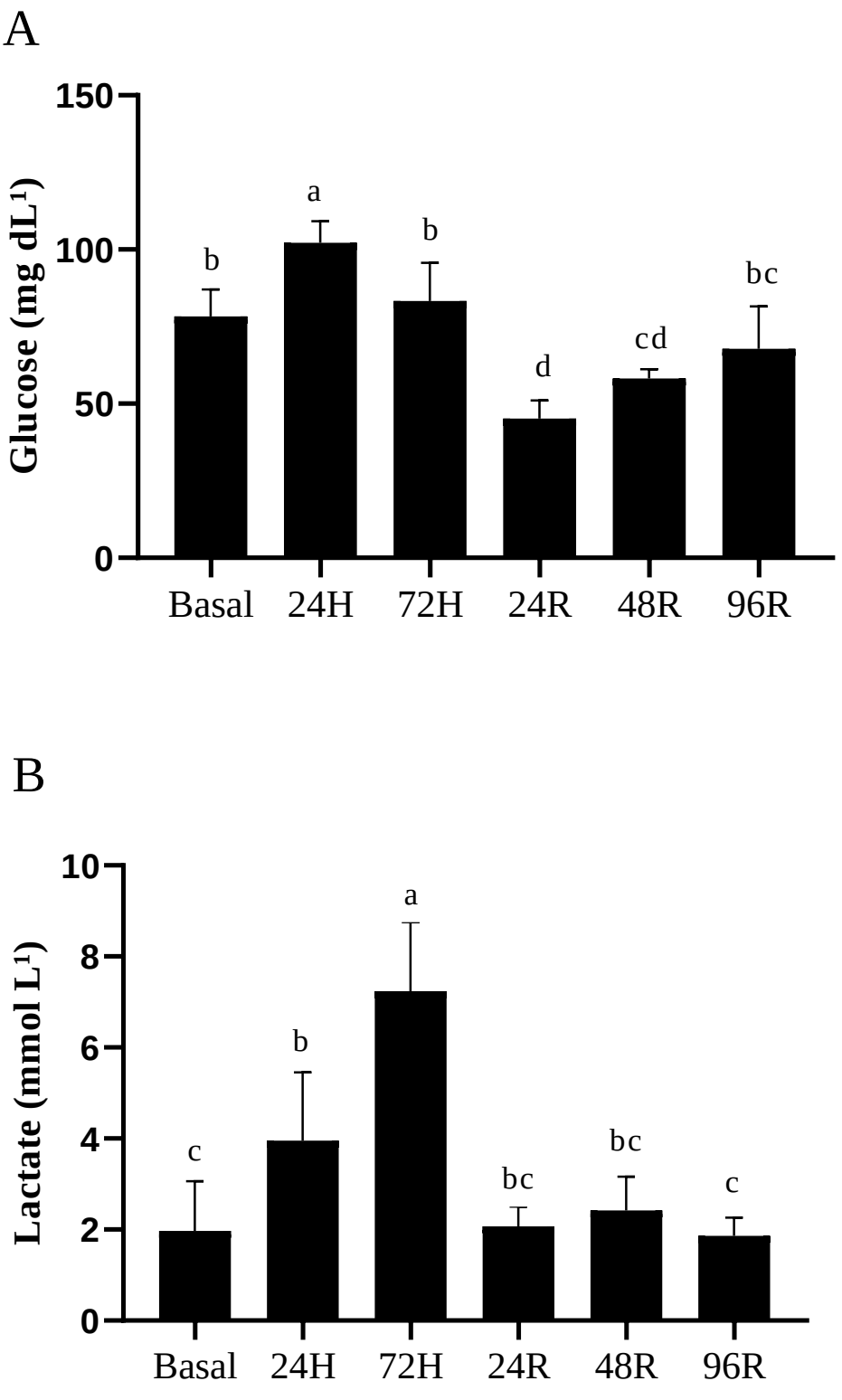

Figure 1 - Mean values (± standard deviation) of A - Glucose and B - Lactate. Normoxia (basal); 24 hours after initiating hypoxia (24H); 72 hours after initiating hypoxia (72H); 24 hours after reestablishing DO to basal (24R); 48 hours after reestablishi

Table 1 presents the results of the hemogasometric analysis. There were lower values for pH ($P < 0.05$) at 24R and higher values to 24H and 72H; however, 48R, and 96R were similar to basal ($P>0.05$). The concentration of $PvCO_2$ was highest at 24H than basal ($P < 0.05$), and equal to basal at 96R ($P > 0.05$). The highest PVO_2 value was observed at 24R ($P < 0.05$). The concentration of sO_2 was higher at 24R than basal ($P < 0.05$). There was an increase of BE at 24H and 72H ($P < 0.05$), compared to basal, with subsequent recovery to near basal at 24R, 48R and 96R. There were no differences between basal, hypoxia and recovery for tO_2 ($P > 0.05$). For HCO_3^- the highest values were at 24H and 72H ($P < 0.05$), then subsequently returned to basal values at 24R and 96R ($P > 0.05$). The behavior of $stHCO_3^-$ was similar to that of HCO_3^- with highest values at 24H ($P < 0.05$), followed by 72H and similar values to basal at 24R and 96R ($P > 0.05$).

Table 1. Mean gasometric values (± standard deviation) of blood samples of *Colossoma macropomum.*

Time	Basal	24H	72H	24R	48R	96R
pH	7.46±0.06^{ab}	7.57±0.07^{a}	7.58±0.06^{a}	7.39±0.05^{b}	7.49±0.07^{ab}	7.48±0.07^{ab}
$PvCO_2$ (mmHg)	7.52±0.94^{b}	10.53±1.90^{a}	8.17±1.59^{ab}	8.65±0.40^{ab}	8.83±1.49^{ab}	6.37±0.81^{b}
PvO_2 (mmHg)	13.52±3.22^{b}	7.86±1.15^{b}	7.58±2.88^{b}	30.70±15.54^{a}	14.73±3.75^{b}	16.87±6.95^{b}
sO_2 (%)	41.67±11.56^{b}	54.62±8.74^{ab}	52.55±14.17^{ab}	71.32±10.95^{a}	52.96±8.90^{ab}	55.18±13.52^{ab}
BE (mmol L^{-1})	-19.28±0.50^{ab}	-12.47±1.20^{d}	-14.88±1.97^{cd}	-20.17±1.20^{a}	-17.07±1.29^{bc}	-19.77±1.35^{a}
tO_2 (mmol L^{-1})	3.67±1.01^{a}	5.67±1.36^{a}	4.95±1.66^{a}	5.52±1.98^{a}	4.97±0.74^{a}	5.13±1.56^{a}
HCO_3^- (mmol L^{-1})	5.88±0.31^{c}	10.58±0.76^{a}	8.45±1.33^{b}	5.85±0.56^{c}	7.48±0.80^{b}	5.15±0.43^{c}
$stHCO_3^-$ (mmol L^{-1})	9.30±0.50^{c}	14.10±0.90^{a}	12.40±1.38^{ab}	9.06±0.77^{c}	10.88±0.86^{bc}	9.12±0.97^{c}

Different letters in the same row indicate significant differences between treatment groups by ANOVA followed by Tukey's post-hoc test ($P < 0.05$). Normoxia (basal); 24 hours after initiating hypoxia (24H); 72 hours after initiating hypoxia (72H); 24 hours after reestablishing DO to basal (24R); 48 hours after reestablishing DO to basal (48R) and 72 hours after reestablishing DO to basal (72R).

Table 2 presents the results of the hematological and biochemical analyses. The highest value for hematocrit was at 24H compared to 96R ($P < 0.05$). Plasma protein decreased at 72H compared to basal ($P < 0.05$) and remained low in recovery. Hypoxia did not alter hemoglobin (HB) ($P > 0.05$). Plasma triglycerides were similar to basal through hypoxia exposure, but reduced during recovery, with the lowest value at 96R. Plasma cholesterol did not change during hypoxia, but at 96R values were lower than during hypoxia ($P < 0.05$). The highest values for the enzymes ALT and AST through hypoxia were at 72H ($P < 0.05$), while at 24R and 48R the values were intermediate between those of basal and 72H; values at 96R were equal to basal ($P < 0.05$).

Table 2. Mean hematological and biochemical values (± standard deviation) of blood samples of *Colossoma macropomum*.

Time	Basal	24H	72H	24R	48R	96R
Hematocrit (%)	20.80±2.93^{ab}	21.83±4.34^{a}	17.50±3.30^{ab}	18.50±3.50^{ab}	17.33±2.29^{ab}	15.00±3.65^{b}
Total plasma protein (g dL^{-1})	4.90±0.12^{a}	4.80±0.18^{ab}	4.40±0.22^{c}	4.38±0.20^{bc}	4.30±0.15^{c}	4.35±0.33^{c}
Hemoglobin (g dL^{-1})	5.21±0.23^{a}	5.01±0.18^{a}	4.65±0.56^{a}	4.45±0.77^{a}	4.67±0.40^{a}	4.44±1.06^{a}
Triglycerides (mg dL^{-1})	243.82±26.95^{a}	241.46±51.75^{a}	200.67±13.20^{ab}	166.32±19.60^{bc}	116.17±14.14^{cd}	96.07±4.91^{d}
Cholesterol (mg dL^{-1})	95.61±5.12^{ab}	94.52±14.42^{ab}	98.07±4.58^{a}	81.85±7.14^{bc}	82.52±9.38^{bc}	77.38±8.44^{c}
ALT (UI)	11.92±3.59^{b}	15.64±3.85^{b}	41.28±20.66^{a}	23.28±4.80^{ab}	27.09±8.34^{ab}	9.80±1.83^{b}
AST (UI)	20.00±8.45^{b}	31.57±8.90^{b}	53.77±15.99^{a}	37.82±8.39^{ab}	36.94±8.37^{ab}	32.29±3.95^{b}

Different letters in the same row indicate significant differences between treatment groups by ANOVA followed by Tukey's post-hoc test ($P < 0.05$). Normoxia (basal); 24 hours after initiating hypoxia (24H); 72 hours after initiating hypoxia (72H); 24 hours after reestablishing DO to basal (24R); 48 hours after reestablishing DO to basal (48R) and 72 hours after reestablishing DO to basal (72R).

Results for electrolytes are provided in Table 3. Values for K^+ were lower than basal at 72H, and higher values at 24R ($P < 0.05$). At 72H, Na^+ levels were lower than basal values ($P < 0.05$), but these levels returned to basal levels during reoxygenation. The electrolyte Ca^{2+} was not influenced by hypoxia or recovery. Values of Cl^- decreased at 24H, and even more so at 72H, in relation to basal ($P < 0.05$); Cl^- increased at 24R, 48R and 96R, in relation to hypoxia, but remained below basal.

Table 3. Mean electrolyte values (± standard deviation) of blood samples of *Colossoma macropomum*.

Time	Basal	24H	72H	24R	48R	96R
K^+ (mmol L^{-1})	4.57 ± 0.34^{b}	4.92±0.69^{ab}	3.13±0.65^{c}	5.76±0.59^{a}	4.36±0.26^{b}	4.57±0.61^{b}
Na^+ (mmol L^{-1})	155.50± 7.30^{a}	150.33± 4.35^{ab}	143.83±6.64^{b}	156.00±6.09^{a}	156.66±4.45^{a}	156.33±6.39^{a}
Ca^{2+}(mmol L^{-1})	0.66± 0.14^{a}	0.75± 0.23^{a}	0.64±0.14^{a}	0.63±0.15^{a}	0.72±0.20^{a}	0.69±0.23^{a}
Cl^-(mmol L^{-1})	144.67± 2.21^{a}	126.33± 2.56^{c}	118.50±5.57^{d}	134.83±1.47^{b}	137.33±2.33^{b}	136.33±1.49^{b}

Different letters in the same row indicate significant differences between treatment groups by ANOVA followed by Tukey's post-hoc test ($P < 0.05$). Normoxia (basal); 24 hours after initiating hypoxia (24H); 72 hours after initiating hypoxia (72H); 24 hours after reestablishing DO to basal (24R); 48 hours after reestablishing DO to basal (48R) and 72 hours after reestablishing DO to basal (72R).

5.4 Discussion

Colossoma macropomum is known for its resistance to environmental conditions, especially the hypoxic conditions typical of its region of origin (Saint-Paul 1984; Rantin and Kalinin, 2004; Wood et al., 1998; Wilson, et al., 1999; Chagas and Val 2006; Florindo et al., 2006; Robertson et al., 2015). In this study animals survived for three days with an average DO of 0.92 mg L^{-1} and subsequently recovered homeostasis. Available DO is an important environmental parameter for aquatic organisms. According to Long et al. (2015), in general, the hypoxic level for fish is 2 mg L^{-1}; however, as seen in the present study, this can vary among species.

The study animals exhibited expanded lips during hypoxia, which allows them to capture oxygen present in surface water. Saint-Paul (1984) also observed these characteristics when subjecting *C. macropomum* to a DO concentration of 0.5 mg L^{-1} for 60 minutes and reported the large branchial surface area facilitating gas exchange in waters with low oxygen concentrations. In the present study, glucose reached its highest values at 24H, and then decreased until 72H. Similar results were recorded for *Acipenser schrenckii* (Brandt, 1869) exposed to hypoxia with DO of 1 mg L^{-1} for evaluations at 30 minutes and one hour after hypoxic stress (Ni et al., 2014). The same occurred for the Amazonian fish *Astronotus ocellatus*

(Agassiz, 1831) exposed to an average DO concentration of 0.52 mg L^{-1} during three hours of hypoxia (Baptista et al., 2016). This initial increase is mainly due to the glycogenolytic action (breakdown of stored glycogen in the liver) of catecholamines (Wendelaar Bonga, 1997). Glycogen metabolism occurs especially in unstable environments (Bacca et al., 2005; Li et al., 2018). When water DO cannot meet the oxygen requirement of aerobic glycolysis, normal physiological function and metabolic rate cannot be maintained (Richards, 2011). Some authors have reported that hypoxia is associated with the activation of anaerobic metabolism and that anaerobic glycolysis would meet the high energy requirements of animals during stress from hypoxia (Bie et al., 1998; Bartrons & Caro 2007; Speers-Roesch et al., 2010). In the present study, however, the basal glucose level was reestablished during recovery, along with the reestablishment of feeding.

Due to the low ATP yield of glycolysis, substrates such as glycogen and glucose are substantially consumed, leading to lactate accumulation (Richards, 2011; Genz et al., 2013). In the present study, lactate increased at 24H, reaching its highest value at 72H, compared to basal ($P < 0.05$). This increase was also observed in *O. niloticus* exposed to hypoxia (OD 0.7 mg L^{-1}) for six hours (Li et al., 2018). All samples taken during the recovery period had lactate values similar to basal. The dependence on anaerobic metabolism, as indicated by the accumulation of blood lactate in *C. macropomum*, shows that this species could not maintain the aerobic pathway during hypoxia. Therefore, the use of anaerobic metabolism may explain its survival under hypoxia conditions (Duncan, 1998; Affonso et al., 2002).

Both glucose and lactate were high for *C. macropomum* at 24H. Baptista et al. (2016) obtained similar results for *Astronotus ocellatus* and reported that this species undergoes blood adjustments that optimize the transport and supply of oxygen to priority tissues. In contrast, anaerobic activation is related to increased creatine phosphate hydrolysis and higher use of glycogen stores in liver and muscle, which are used by activating glycogenolysis, resulting in higher glucose and subsequently lactate in plasma during acute hypoxia (Muusze et al., 1998). This could explain what happened with *C. macropomum* in the present work.

Mean values of $PvCO_2$ and PvO_2 for *C. macropomum* ranged from 6.37 to 10.53 and 7.58 to 30.70 mmHg, respectively, according to hypoxia and recovery time. The highest PvO_2 value was for 24R, which can be attributed to the fact that the animals become more agitated and increased opercular beats after the return of oxygen, as noted for *L. alexandri* exposed essential oil of *Aloysia triphylla* used as an anesthetic (Becker et al., 2017) and also in another work where Mattioli et al., (2019b) exposed this species to the air. Increased blood flow in the

gills favors the dilatation of blood vessels, which increases perfusion of the lamellae, thereby facilitating gas exchange and its use in tissues (Brauner et al., 2000). Gomes et al (2011) reported that blood gas values may vary according to the type of fish studied. Furthermore, for *C. macropomum* exposed to water with different pH values, values of $PvCO_2$ and PvO_2 were found to range from 4.7 to 5.8 and 34.0 to 46.0 mmHg, respectively (Wood et al., 1998), which are similar to those of the present study. In another study with *L. alexandri* subjected to air exposure stress for 30 minutes, the $PvCO_2$ value without air exposure averaged 11.92 mmHg and obtained its highest value of 22.17 mmHg after 30 minutes of exposure (Mattioli et al., 2019b). The same authors found the PvO_2 for fish not exposed to air to be 28.50 mmHg, reaching its highest value of 77.08 mmHg one hour and 30 minutes after exposure. Thus, it is evident that other stressors and different environmental conditions may also affect these parameters.

The blood pH values of the present study oscillated during hypoxia and recovery. At 72H pH value was higher than at 24R. This differs from what was reported for *P. nigricans* by Val et al. (2015), who found pH values to decrease over time for animals subjected to one to five hours of hypoxia, and returning to basal value after six hours of recovery. The same reduction in pH was observed for *L. alexandri* submitted to air exposure for 30 minutes (Mattioli et al., 2019b). These authors reported an acid-base imbalance, with the fish suffering respiratory acidosis and, as a consequence, metabolic acidosis, since pH and plasma bicarbonate values were altered in relation to the control group. For *C. macropomum* in the present study, hypoxia times provided a significant increase in bicarbonate (HCO_3^-) and pH levels, which supposedly prevented acidosis and kept pH above neutrality. However, at 24R the pH value was lower than that of basal animals, with HCO_3^- also decreasing at the same time, although values were equal to the basal group at the end of the recovery period.

In this study, plasma protein decreased at 72H and these values did not return to basal values during recovery. It consists basically of albumin and globulins, these are the structural part of most organs, in addition to representing the nutritional and metabolic status of fish, as well as, indirectly, the level of nonspecific immunity (Ortuno et al., 2001; Ni et al., 2014). Its decrease in the present study can be explained by the animals' fasting, which associated with the fish's energy expenditure to deal with hypoxia stress, leaving less energy available for other biological functions such as immunity (Douxfils et al., 2012; Segner et al., 2012).

Previous studies have shown hematological changes in fish being induced by management stress and exposure to sulfide or hypoxia (Benfey and Biron, 2000; Affonso et al.,

2002). Hemoglobin of the juvenile *C. macropomum* of the present study did not differ significantly among basal, hypoxia and recovery times. Silva et al. (2012) submitted *O. niloticus* to air-exposure for 30, 60 and 90 minutes and also did not observe variation in hemoglobin concentrations in relation to the control (without air exposure). Hematological indicators may present hemoconcentration or hemodilution of erythrocytes as a result of stressors. Hemoconcentration is a means of increasing the oxygen carrying capacity of blood when energy demand is high (Caldwell and Hinshaw, 1994; Carvalho and Fernandes, 2006). Other stress responses may compromise iron absorption, inhibit hemoglobin synthesis or cause competition for the oxygen binding site, which may cause hemodilution or anemia in fish and, thus, reduced capacity for transporting oxygen to tissues (Affonso et al., 2002; Jung et al., 2003; Das et al., 2006). The results for hemoglobin reflect a metabolic adaptation of this species to hypoxia, indicating that the animals were able to cope with the stress condition.

The lowest value found for cholesterol was 96R compared to basal. Cholesterol is a steroid that acts as a precursor to hormone synthesis (Payne and Hales, 2004). The amount of cholesterol in fish plasma can determine their health (Messina et al., 2013). The observed decreased in cholesterol demonstrates that it was consumed to obtain pregnenolone, which is a precursor in cortisol synthesis, which in turn would act on the physiology of the animal by promoting metabolic changes such as increases in glucose and lactate levels by anaerobic pathways (Silva et al., 2012).

Triglycerides exhibited the same behavior as cholesterol, but especially a decrease during the recovery period. Triglycerides are used as an energy source for different metabolic processes (Pavlidis et al., 2007; Tolussi et al., 2010). In this case, the reduction of triglycerides may be explained by the energy needed to restore homeostasis.

The enzymes AST and ALT behaved similarly, with higher values at 72H, and recovery to basal at 96R. In addition to acting on the regulation of amino acid metabolism, serum enzymatic activities are markers of cell damage or toxicity by reflecting changes in the function of various tissues and organs that respond to environmental stress, and, thus, are important signals of the integrity of cell membrane function (Nelson and Cox, 2014). According to Shahsavani et al. (2010), some studies have shown that increased intracellular levels of reactive oxygen species (ROS) can lead to lipid peroxidation, resulting in higher permeability of liver-cell membranes. This, in turn, may cause the release of liver enzymes, such as AST and ALT. In addition, factors such as toxic drugs and chemicals, metabolic disorders, and even hypoxia can lead to hepatocyte injury and, as a consequence, ALT leakage, thereby increasing its level

circulating in blood (Thrall et al., 2015). ALT is known to be responsible for the removal and transfer of the amino group of the amino acid alanine to α-ketoglutarate (α-keto acid), forming glutamate and pyruvate (Voet and Voet, 2013). AST is responsible for the removal and transfer of the amino group of the amino acid aspartate to α-ketoglutarate, forming glutamate and oxaloacetate (Voet and Voet, 2013). Pyruvate is a substrate for gluconeogenesis, and so increased ALT activity may indicate a greater need for glucose formation, requiring mobilization of energy reserves, as seen in the present study. Thus, we can suggest that changes in AST and ALT values may be considered as biomarkers of liver injury (Shahsavani et al., 2010), and since these enzymes are involved in amino acid catabolism (Gaylord et al., 2006), their levels also may indicate their use for energy production — a process to be evaluated for *C. macropomum* in future studies.

Calcium was not influenced by hypoxia of *C. macropomum*. Moreira et al. (2015) submitted juvenile *O. niloticus* to transport stress and anesthesia and also observed little variation in calcium levels. Ion-regulatory homeostasis is critical for ensuring proper cell function (Hwang et al., 2011). Similarly, stress as a consequence of routine aquaculture management is known to affect ionization regulation in fish (Ashley, 2007). Values of potassium and sodium were lower at 72H ($P < 0.05$), which differs from the results reported by Mattioli et al. (2019b) who submitted *L. alexandri* to air exposure for 30 minutes and found the highest values for potassium at 30 minutes of exposure, after which it decreased, while there were no differences in sodium among treatments. Thus, responses can vary among species and types of stress. In addition, *C. macropomum* is a species that lives in the water column while *L. alexandri* lives on the bottom. Values of Cl^- decreased at 24H compared to basal, followed by even lower values at 72H. These results show a change in ionic homeostasis of *C. macropomum* as a result of decreases in these ions. The juveniles, however, were able to maintain calcium balance, which is ideal for the operation of neural muscular functions and maintenance of homeostasis (Baldisserotto, 2003). Fish have increased blood flow and gill permeability during stress, mainly due to the action of catecholamines and cortisol, which enables the transport of oxygen to meet the biological demand of tissues. However, alteration of gill permeability leads to blood electrolyte loss and osmoregulatory disturbances in freshwater fish (McDonald and Milligan 1997; Silva et al., 2012), as recorded in the present study during hypoxia.

5.5 Conclusion

Colossoma macropomum addresses hypoxia using anatomical, physiological, biochemical and metabolic adjustments to improve oxygen transfer. The varied values analyzed in this study indicated that physiological response plays an essential role in hypoxic stress. Rapid adjustments in glucose and lactate levels were adequate, mainly via anaerobic metabolism, which plays an important role in providing energy when these fish cope with stress from hypoxia.

5.6 Compliance with ethical standards

The experiment was carried out at Laboratório de Aquacultura of Universidade Federal de Minas Gerais (LAQUA), Brazil, and was approved by the Comitê de Ética em Uso de Animais (Ethics Committee on Animal Use) of UFMG (protocol 296/2019).

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5.8 Conflict of Interest

The authors declare that they have no conflict of interest.

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6. ARTIGO 2

Physiological responses of juvenile *Colossoma macropomum* after different periods of air exposure

Major reviews periódico Aquaculture

Abstract

The objective of this work was to evaluate the physiological responses of juvenile *Colossoma macropomum* exposed to air for different periods of time. Juveniles weighing 74.78± 17.39 g were acclimated for 15 days in 30-L tanks in a recirculating aquaculture system (RAS) at a density of 6 animals/tank. The animals were then fasted for 24 hours prior to the air exposure test and six juveniles were captured for blood collection to establish basal levels of the parameters. Next, 24 animals were exposed to air for 30 min and the other 24 for 60 min. Blood samples were taken from six animals of each exposure treatment immediately after exposure (IAE), and the other animals returned to their tanks. Subsequent blood samples were taken for six animals (n = 6) per exposure time at 1 hour (1hAE), 24 hours (24hAE) and 48 hours (48hAE) after air exposure. No mortality was recorded. Hematocrit and hemoglobin did not vary according to air exposure time, collection time or the interaction between these two factors. There was an interaction of the factors times of exposure to air and times of collection for glucose where was higher for the 60 min exposure compared to the 30 min exposure at the 1hAE collection, while after 24hAE the levels did not differ from basal. For lactate there was also an interaction between the factors being higher for the 60 min exposure compared to the 30 min exposure at IAE and 1hAE. Blood pH varied among the collections, with a lower value at 1hAE for the 60 min exposure. The parameters $PvCO_2$ (venous carbon dioxide partial pressure) and PvO_2 (venous oxygen partial pressure) were inversely proportional; while $PvCO_2$ increased, PvO_2 decreased after collection 1hAE. Values of HCO_3^- (bicarbonate) differed between exposure times and varied among collections. The electrolytes Cl^- (Chloride) and K^+ (Potassium) varied among collections in relation to basal, however, they did not differ between exposure times. The value for Na^+ (Sodium) for the 60 min exposure at IAE was higher than basal, and only 24hAE was different from basal for both exposures. Values of Ca^{2+} (Calcium) differed between exposure times at 24hAE, however, these did not differ from basal. Exposure

to air caused physiological biochemical, gasometric and electrolytic in juvenile *C. macropomum*. These animals can be considered resistant to this stress due to their ability to restore physiological.

Keywords: Tambaqui, blood gases, stress, physiology, hematological parameters, electrolytes.

6.1 Introduction

Aquaculture constantly exposes fish to various stress conditions due to cultivation practices such as high densities, transport and handling, among others, in addition to changes in abiotic environmental factors such as temperature, salinity and pH (Skrzynska et al., 2018). Another stressful management practice that is common in fish farming is biometry and the selection of animals, when there is a reduction in the level of water in the tank, capture, transport and exposure to air, with the latter being directly linked to fish breathing (Eslamloo et al., 2014; Lim and Hur, 2018). However, these management practices are frequently done without an understanding of how these stressors can physiologically affect fish (Roriz et al., 2015; Lim and Hur, 2018).

Some physiological adjustments developed by fish can be quantified during stressful situations by several parameters such as cortisol, glucose, plasma lactate, hematocrit, plasma protein, hemoglobin and electrolytes, among others (Wendelaar Bonga, 1997). Like these hematological and biochemical analyses, blood gas parameters can also serve as markers for monitoring fish health (Barton 2002; Cnaani et al., 2004; Tavares Dias et al., 2008). Hemogasometry detects the effectiveness of respiratory gas exchange (oxygenation) and metabolic parameters (acid-base balance), such as the partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), blood pH and bicarbonate ion (HCO_3^-) (King, 2000). The combination of these tools helps to effectively assess physiological conditions caused by stress (Tavares Dias et al., 2008; Barbas et al., 2016; Mattioli et al., 2019a, b).

Exposure to air can lead to severe physiological disorders in fish, including increased glucose, as reported by Roriz et al. (2015) for *Piaractus brachypomus*; Lim and Hur (2018) for *Paralichthys olivaceus* and Mattioli et al. (2019a) for *Lophiosilurus alexandri*, with this last author also finding high values of plasma lactate. Chen et al. (2015) reported variation in hematological responses for *Cyprinus carpio* when subjected to stress to exposure to air. Blood gases also vary when subjected to stress. According to Cook et al. (2015), exposure to air can lower blood pH causing extracellular acidosis, with juvenile *L. alexandri* experiencing a drop in pH shortly after exposure, in addition to affecting other gasometric variables such as increased $PvCO_2$ concentration and decreased HCO_3^- (Mattioli et al., 2019b).

Tambaqui, *Colossoma macropomum*, (Cuvier, 1816) is a native characid of the Amazon basin and the Orinoco river. The species adapts well to captive conditions, easily accepting artificial diets with good growth rates and feed conversion. It is the main native species produced commercially in Brazil and one of the most bred and commercialized species in South America (Inoue et al. 2011; Stringhetta et al., 2017; Valladão et al., 2018). Many researchers and producers have increased their efforts to establish a technological protocol for culturing this species in captivity, including the evaluation of stocking density in masonry tanks with continuous flow (Costa et al., 2019), density in cages (Silva and Fujimoto, 2015), protein requirement after food deprivation (Santos et al., 2010), feeding frequency (Souza et al., 2014), recirculating aquaculture system for the cultivation of juveniles (Lima et al., 2019), use of anesthetics (Barbas et al., 2016; de Souza et al., 2019), water salinity (Souza-Bastos et al., 2016), pH (Wood et al., 2018), food restriction (Assis et al., 2020) and stocking density in a recirculating aquaculture system with air exposure test (Silva et al., 2021). The same authors have shown that juveniles of *C. macropomum* are extremely resistant a 75 min the air exposure test without it affecting animal survival. However, little is known about how this management practice affects the physiological responses of *C. macropomum*. Thus, the objective of this work

was to evaluate the physiological responses of juvenile *C. macropomum* exposed to the air for different periods of time.

6.2 Material and methods

The experimental protocol was approved by Comissão de Ética no Uso de Animais (CEUA) of Universidade Federal de Minas Gerais (approval number: 183/2019).

6.2.1 Fish and experimental conditions

The studied animals were purchased from Fazenda Tataueira, in the municipality Peixe - Boi, state of Pará, Brazil, and kept in Laboratório de Aquacultura of the Escola de Veterinária of Universidade Federal de Minas Gerais. The fish, aged 60 days post-hatching, were stocked in 16 circular cultivation tanks of 28-L of useful volume and were fed an extruded commercial diet (1.7mm Supra juvenile® pellet, 46% crude protein, 3% crude fiber and 8% ethereal stratum). When the animals reached an average weight of 10 g, they were transferred to 1,000-L tanks and fed the same diet with a gradual change to commercial feed (Laguna ® 4 mm, 32% crude protein, 5% crude fiber and 5% ethereal stratum), until they reached the initial weight for the experiment. The juvenile *C. macropomum* (n = 54) (74.78±17.39 g and 16.89±1.09 cm) were transferred to nine 30-L tanks in a recirculating aquaculture system (RAS), with biological and mechanical filters, at a density of six animals/tank and for a total of 54 animals. The conditions of the RAS were as follows: temperature of 28.71±0.26°C, pH of 7.13±0.37, dissolved oxygen of 6.64±0.32 mg L^{-1}, salinity of 0.14±0.03 g of L^{-1} salt, conductivity of 0.29±0.06 mS cm^{-1} and total ammonia of 0.32±0.11 mg L^{-1}. Dissolved oxygen concentration and temperature were measured using a digital oximeter (Ecosense®, model DO200A); pH, conductivity and salinity were measured using a multiparameter analyzer (Hanna®, Combo pH & EC Waterproof model HI98130 high range - Sigma-Aldrich Labware); and total ammonia

was analyzed using a commercial kit (LabconTest®). The fish were fed with extruded commercial feed (Laguna®, 4mm in diameter, 32% crude protein, 5% crude fiber, 5% ether extract and 14% mineral ash) (08:00 and 16:00 h) until apparent satiety. The animals were acclimatized to experimental units for 15 days.

6.2.2 Air exposure testing with *Colossoma macropomum*

After the acclimatization period, the juveniles were fasted for 24 hours prior to the air exposure test (AE). Six juveniles were then captured for blood collection to determine basal parameter levels. Next, 24 animals were exposed to air for 30 min and the other 24 for 60 min. An individual person was responsible for each tank and the work took place in a synchronized manner, with all animals (average 17 cm in length) being removed from their tank, dried with cloth and packed in an individual 30x20-cm plastic box. Another work team was responsible for drawing blood immediately after air exposure (IAE; n = 6 animals per exposure time, 30 and 60 min), while the others were responsible for returning the animals to their respective 30-L tanks (1hAE, 24hAE and 48hAE; six animals per tank). And so the collection occurred in the other times after the exhibition. Additional blood samples were taken (n = 6 animals per exposure time, 30 and 60 min) at 1-hour (1hAE), 24-hours (24hAE) and 48 hours (48hAE) after air exposure treatments. Each animal was considered a replicate and used only once. Thus, the experimental design consisted of a basal treatment, two air exposure treatments (30 and 60 min) and four post-exposure blood collection times (IAE, 1, 24 and 48-hours post-AE). Animal survival was monitored throughout the experiment.

6.2.3 Blood samples for gasometric, hematological and biochemical analyses

Blood samples were collected by caudal venipuncture (Saint-Paul, 1984). Fish were contained in an appropriate damp cloth during sampling and two blood samples were collected

from each animal. The first sample, 300 μL of blood, was removed using a heparinized syringe and used for gas analysis and for determining the following parameters: pH, tO_2 (oxygen rate, mmol L^{-1}), PvO_2 (venous oxygen partial pressure, mmHg), $PvCO_2$ (venous carbon dioxide partial pressure, mmHg), sO2 (oxygen saturation, %), lactate (mmol L^{-1}) and concentrations of sodium Na^+ (mmol L^{-1}), potassium K^+ (mmol L^{-1}), calcium Ca^{2+} (mmol L^{-1}) and chloride Cl^- (mmol L^{-1}). These data were used to determine: $HC0_3^-$ (bicarbonate, mmol L^{-1}), $stHC0_3^-$ (standard bicarbonate, mmol L^{-1}) and BE (base excess, mmol L^{-1}). These analyses were performed using a blood gas meter (ABL800 BASIC analyzer - Radiometer®), with water temperature and oxygen saturation being corrected in the equipment to correspond to the experimental treatments.

The second sample, 1 mL of blood, was collected in a heparinized syringe and used for hematocrit (%) evaluation using capillary tubes filled with previously homogenized blood. The capillary tubes were centrifuged for 10 min at 10,867 × *g* (Micro SPIN 1000) and then read in a microhematocrit using an appropriate scale. Plasma protein (g dL-1) was measured using a hand-held refractometer (RHC 200-ATC, Huake Instrument Co., Ltd.; www.instrument-china.com) while hemoglobin concentration (g dL-1) was determined by cyanomethahemoglobin reaction using a commercial kit (reference no. K023-1 QUIBASA Química Básica Ltda. Bioclin - www.bioclin.com).

Sodium heparin (HEPAMAX-S® Cotia, São Paulo, Brazil) was used at 10% mL^{-1} of blood. At the end of the hematological analyses, the remaining aliquots of heparinized whole blood were centrifuged at 4,000 × *g* for 10 minutes (Centrifuge Spinlab SL-5AM) for plasma separation and determination of glucose (mg dL^{-1}) by glucose oxidase test (GOD Trinder) (reference no. K082-2); monoreagent triglycerides (mg dL^{-1}) by Trinder reaction (reference no. K117); and cholesterol (mg dL^{-1}) by the Trinder enzyme method using a commercial kit (reference no. K083-3). The enzymes AST – aspartate-aminotransferase - UI (reference no.

K034-1) and ALT – alanine-aminotransferase - UI (reference no. K035-1) were determined by the Reitmamm and Frankel method. All the cited tests were analyzed by the Bioclin kit (www.bioclin.com QUIBASA Química Básica Ltda, Belo Horizonte, Minas Gerais, Brazil). All analyses were read on a spectrophotometer (Biocrom Libra S22 – analiticaweb.com.br).

6.2.4 Statistical analyses

All data were tested for normality (Shapiro-Wilk) and homogeneity of variances (Bartlett). Two-way ANOVA followed by Duncan's post-hoc test ($P < 0.05$) were used to compare air exposure times (30 and 60 minutes) immediately after exposure (IAE) and 1, 24 and 48 hours after exposure. One-way ANOVA followed by post-hoc T - test ($P < 0.05$) were applied for comparisons between basal (without air exposure) and collection times for both exposure times (30 and 60 min). Statistical analyses were performed using RStudio Version 1.2.5033.

6.3 Results

No mortality was recorded throughout the experiment. Hematocrit and hemoglobin did not differ for air exposure time, collection time nor for the interaction between these factors ($P > 0.05$) (Table 1). Only hemoglobin differed from basal for 60E24hAE ($P < 0.05$). An effect on plasma protein was found only for collection time, with lower values at IAE ($P<0.05$); only 60EIAE was similar to basal ($P>0.05$). An effect on triglycerides was found only for collection time ($P<0.0026$), with lower values at IAE and 1hAE and the highest at 48hAE; significant differences from basal were observed only at 30EIAE and 60EIAE ($P<0.05$). Cholesterol was also affected only by collection time ($P<0.0001$), with lower values at IAE and 1hAE; 60E24hAE and exposure times 30 and 60 min at 48hAE ($P<0.05$) differed from basal. Glucose

had an effect of air exposure time, collection time and their interaction ($P<0.05$), with the highest values being for 30EIAE, 60EIAE and 60E1hAE ($P<0.05$). Glucose values differed from basal at IAE for both exposure times and at 1hAE for 60 min of exposure ($P<0.05$), with the other exposure and collection times being similar to basal ($P>0.05$). Lactate was also affected by exposure time, collection time and their interaction ($P<0.05$), with the highest value for 60E1hAE and the lowest for 30E24h, 20E48hAE, 60E24hAE and 60E48hAE. Lactate was lower at 30EIAE than at 60EIAE. All exposure times and collection times for lactate differed from basal, being longer for both times of exposure to air in IAE and 1hIA and shorter for collections in 24hAE and 48hAE ($P<0.05$). The enzyme ALT had an effect only for collection time ($P<0.0236$), with higher values at 1hAE and 24hAE ($P<0.05$). There were no differences in ALT compared to basal. ($P>0.05$). The enzyme AST also had an effect only for the collection time ($P<0.0003$), with higher values at 24hAE. ALT differed from basal only for 30E24hAE and 30E48hAE ($P< 0.05$).

Table 1. Values (mean ± standard deviation) of hematological and biochemical parameters for air exposure time (30 and 60 min) and blood collection time (IAE - immediately after exposure, 1hAE - One hour after exposure, 24hAE - 24 hours after exposure and 48hAE - 48 hours after exposure) for *Colossoma macropomum*.

	Hematocrit	**PPT**	**HB**	**TGR**	**CLR**	**GLU**	**ALT**	**AST**	**Lactate**
Means for basal									
Basal	21.17±3.89	4.20±0.15	5.76±0.56	213.19±17.69	69.47±5.32	75.58±14.93	8.72±4.01	11.68±2.39	2.82±0.72
Means of exposure (E)									
30 minutes	18.67±0.71^{a}	4.89±0.06^{a}	6.39±0.59^{a}	201.72±14.15^{a}	82.31±2.89^{a}	103.38±6.57	13.27±1.12^{a}	25.36±3.84^{a}	4.77±3.87
60 minutes	19.57±0.73^{a}	4.89±0.06^{a}	6.27±0.91^{a}	225.53±15.94^{a}	89.17±2.89^{a}	123.21±6.57	11.94±1.27^{a}	14.91±2.90^{b}	3.57±2.96
Means of collections (C)									
IAE	20.00±1.01^{a}	4.49±0.08^{b}	6.35±0.73^{a}	170.38±20.487^{b}	73.07±4.09^{b}	176.87±43.16	9.60±1.56^{b}	8.35±4.86^{c}	8.05±2.72
1hAE	18.08±1.01^{a}	4.96±0.08^{a}	6.48±0.92^{a}	192.43±19.53^{b}	72.72±4.09^{b}	128.37±50.95	15.48±4.60^{a}	13.81±4.00^{c}	10.97±1.43
24hAE	18.73±1.06^{a}	5.10±0.08^{a}	6.31±0.63^{a}	227.22±27.61^{ab}	95.03±4.09^{a}	79.69±12.54	14.97±7.75^{a}	41.06±7.53^{a}	1.07±0.79
48hAE	19.58±1.01^{a}	5.01±0.08^{a}	6.23±0.78^{a}	265.32±19.53^{a}	102.14±4.09^{a}	68.25±9.63	10.77±2.78^{b}	29.74±3.44^{b}	1.78±0.80
Means for E × C									
30EIAE	18.67±4.61	4.60±0.24*	6.68±0.34	161.43±7.30*	72.62±16.22	175.58±42.76^{a*}	8.94±1.18	10.32±1.61	6.14±2.40^{c*}
60EIAE	21.33±2.13	4.38±0.20	6.01±0.85	179.32±23.74*	73.52±9.59	178.15±43.52^{a*}	10.27±1.62	8.35±4.19	9.65±1.78^{b*}
30E1hAE	17.33±3.50	4.92±0.36*	6.59±0.45	200.65±26.30	76.45±14.88	92.97±30.85^{b}	16.15±4.10	13.64±2.38	10.02±1.30^{b*}
60E1hAE	18.83±3.13	5.00±0.12*	6.36±1.20	184.20±10.86	68.99±16.03	163.76±41.65^{a*}	14.92±4.92	13.98±4.38	11.92±0.78^{a*}
30E24hAE	18.00±2.58	4.97±0.14*	5.85±0.30	227.22±60.06	84.10±4.68	77.27±11.64^{b}	17.09±1.85	26.50±6.86*	1.03±0.37^{d*}
60E24hAE	19.60±2.42	5.23±0.29*	6.77±0.53*	288.61±63.03	105.97±14.38*	82.11±12.94^{b}	12.85±2.32	26.22±9.42	1.10±1.06^{d*}
30E48hAE	20.67±4.38	5.07±0.36*	6.49±0.72	217.56±57.71	96.08±13.50*	67.69±7.76^{b}	10.90±3.58	21.40±6.85*	2.13±0.84^{d*}
60E48hAE	18.50±0.96	4.95±0.14*	5.96±0.94	313.08±131.95	108.20±9.73*	68.81±11.17^{b}	10.64±1.60	22.41±11.03	1.42±0.56^{d*}
P-value Exposure (E)	0.3839^{ns}	0.9579^{ns}	0.5725^{ns}	0.0522^{ns}	0.1014^{ns}	0.0389$^{\downarrow}$	0.4862^{ns}	0.0916^{ns}	0.0056$^{\downarrow}$

P-value Collection (C)	0.5545^{ns}	<0.0001$^{↓↓}$	0.8897^{ns}	0.0026$^{↓}$	<0.0001$^{↓↓}$	<0.0001$^{↓↓}$	0.0236$^{↓}$	0.0003$^{↓}$	<0.0001$^{↓↓}$
P-value Interaction (ExC)	0.3647^{ns}	0.1489^{ns}	0.0559^{ns}	0.2127^{ns}	0.0769^{ns}	0.0282$^{↓}$	0.6400^{ns}	0.4614^{ns}	0.0036$^{↓}$
CV (%)	18.30	5.56	11.96	30.36	16.53	28.39	40.83	54.30	25.68

Means followed by different letters in a column differ by Duncan's test (P <0,05). * Asterisks indicate a significant difference compared to baseline by the T test (P<0.05). CV = coefficient of variation. (↓P<0.05) (↓↓P<0.0001) (ns not significant). Hematocrit (%), plasma protein (PPT - g dL^{-1}), hemoglobin (HB g dL^{-1}), Triglycerides (TGR mg dL^{-1}), Cholesterol (CLR mg dL^{-1}), glucose (GLU mg dL^{-1}), alanine aminotransferase (ALT - UI), aspartate aminotransferase (AST - UI) and lactate (mmol L^{-1}).

Blood pH only had an effect for collection time ($P<0.0001$), with the lowest value at IAE followed by at 1hAE, and higher values at the other collection times (Table 2). Blood pH differed from basal for all exposure times and collection times ($P<0.05$). $PvCO_2$ had an effect of air exposure time, collection time and their interaction ($P< 0.05$), with the highest value for 60EIAE and the lowest for 30E48hAE and 60E48hAE. $PvCO_2$ differed from basal for all treatments. PvO_2 differed for collection time and the interaction between exposure time and collection time ($P<0.05$), with the highest value being for 60E24hAE and the lowest for 60EIAE. PvO_2 did not differ from basal for any of the treatments ($P>0.05$). The variables sO_2 and tO_2 had similar behavior and were affected only by collection time ($P<0.0001$ for both), with lower values at IAE and higher values at 24hAE ($p<0.05$). sO_2 differed from basal for 30EIAE, 60EIAE, 30E24hAE and 60E24AE, whereas tO_2 differed for 60EIAE, 30E24hAE and 60E24hAE. HCO_3^- had an effect of air exposure time, collection time and their interaction ($P<0.05$), with the highest value being for 60E24hAE and the lowest for 30IAE, 30E1hAE and 60E1hAE. HCO_3^- differed from basal for all treatments ($P<0.05$). The same behavior was found for $stHCO_3^-$ and BE, with an effect only for collection time ($P<0.0001$ for both), with higher values for 24hAE and 48hAE compared to IAE and 1hAE ($P<0.05$). $stHCO_3^-$ and BE differed from basal for 30EIAE, 30E1hAE, 60EIAE, 60E1hAE and 30E48hAE.

Table 2. Values (mean ± standard deviation) of blood gas analysis at times of air exposure time (30 and 60 min) and blood collection time (IAE - immediately after exposure, 1hAE - One hour after exposure, 24hAE - 24 hours after exposure and 48hAE - 48 hours after exposure) for *Colossoma macropomum* submitted.

	pH	**$PvCO_2$**	**PvO_2**	**sO_2**	**tO_2**	**HCO^-_3**	**$StHCO^-_3$**	**BE**
Means of Basal								
Basal	7.23±0.09	27.33±1.53	19.80±6.78	36.07±8.35	2.45±0.67	12.95±2.46	12.47±2.55	-14.55±3.52
Means of exposure (E)								
30 minutes	7.26±0.02^{a}	9.03±0.37	25.38±2.79	50.93±3.27^{a}	3.88±0.34^{a}	4.32±0.21	6.86±0.25^{a}	-23.24±0.38^{a}
60 minutes	7.12±0.02^{a}	16.64±0.41	19.37±3.25	38.26±3.92^{a}	2.94±0.41^{a}	4.62±0.24	6.26±0.29^{a}	-24.13±0.44^{a}
Means of collections (C)								
IAE	6.77±0.10^{c}	27.88±10.14	18.20±8.70	22.38±12.33^{c}	1.75±0.92^{c}	4.60±1.54	4.09±0.37^{b}	-27.56±1.80^{b}
1hAE	7.14±0.10^{b}	6.69±1.35	31.56±10.28	52.65±15.76^{b}	3.58±1.68^{b}	2.52±0.36	4.63±0.35^{b}	-26.52±0.95^{b}
24hAE	7.52±0.10^{a}	7.62±1.56	36.46±24.33	66.22±11.89^{a}	5.67±1.69^{a}	6.81±1.18	10.10±0.48^{a}	-17.36±2.21^{a}
48hAE	7.53±0.08^{a}	5.94±1.38	16.60±6.11	51.37±17.65^{b}	4.13±1.63^{ab}	5.47±1.28	9.33±0.34^{a}	-19.30±2.20^{a}
Means for E × C								
30EIAE	6.82±0.06*	17.04±2.33^{b*}	24.28±8.33^{cb}	30.58±9.57*	2.38±0.65	3.26±0.61^{c*}	3.58±0.54*	-28.46±0.95*
60EIAE	6.72±0.11*	36.92±2.14^{a*}	13.13±4.96^{c}	14.18±8.82*	1.12±0.69*	5.72±1.15^{b*}	4.60±1.06*	-26.82±1.99*
30E1hAE	7.19±0.10*	6.52±1.44^{cd*}	33.13±9.85^{b}	54.75±13.91	3.92±1.93	2.77±0.17^{c*}	5.07±0.66*	-25.95±0.87*
60E1hAE	7.08±0.05*	6.87±1.24^{cd*}	29.68±10.48^{cb}	50.12±17.39	3.18±.21	2.27±0.33^{c*}	4.20±0.38*	-27.20±0.46*
30E24hAE	7.52±0.08*	6.80±0.91^{cd*}	26.22±8.75^{cb}	66.13±11.00*	5.52±2.08*	6.18±0.75^{b*}	10.10±1.17	-18.33±1.73
60E24hAE	7.51±0.12*	8.43±1.64^{c*}	51.83±31.18^{a}	66.32±12.88*	5.90±0.76*	7.43±1.19^{a*}	11.50±1.60	-16.20±2.17
30E48hAE	7.50±0.10*	5.75±1.31^{d*}	17.90±5.65^{cb}	52.27±16.51	3.72±1.33	5.05±1.19^{b*}	8.68±1.40*	-20.23±2.22*

60E48hAE	7.55±0.05*	6.13±1.41^{d*}	15.30±6.28^{cb}	50.47±18.68	4.53±1.79	5.88±1.23^{b*}	9.98±1.02	-18.37±1.73
P-value Exposure (E)	0.1178^{ns}	<0.0001$^{↓↓}$	0.6078^{ns}	0.2397^{ns}	0.6928^{ns}	0.0015$^{↓}$	0.0518^{ns}	0.0515^{ns}
P-value Collect (C)	0.0001$^{↓↓}$	<0.0001$^{↓↓}$	0.0010$^{↓}$	<0.0001$^{↓↓}$	<0.0001$^{↓↓}$	<0.0001$^{↓↓}$	<0.0001$^{↓↓}$	0.0001$^{↓↓}$
P-value Interaction (E × C)	0.1571^{ns}	<0.0001$^{↓↓}$	0.0214$^{↓}$	0.6298^{ns}	0.4279^{ns}	0.0118$^{↓}$	0.0965^{ns}	0.1225^{ns}
CV (%)	1.33	14.98	52.80	32.04	42.81	20.85	15.99	8.07

Means followed by different letters in a column differ by Duncan's test (P <0,05). * Asterisks indicate a significant difference compared to baseline by the T test (P<0.05). CV = coefficient of variation. (↓P<0.05) (↓↓P<0.0001) (ns not significant). Hydrogen potential (pH), partial pressure of carbon dioxide ($PvCO_2$ - mmHg), partial pressure of oxygen (PvO_2 - mmHg), oxygen saturation (sO_2 - %), rate of oxygen (tO_2 - mL dL^{-1}), bicarbonate (HCO_3^- -mmol L^{-1}), standard bicarbonate ($stHCO_3^-$ -mmol L^{-1}) and excess base (BE - mmol L^{-1}).

K^+ did not have an effect for air exposure time, collection time or their interaction ($P>0.05$) (Table 3), and differed from basal for 30EIAE, 60EIAE, 30E1hAE, 60E1hAE and 60E24hAE ($P<0.05$). Na^+ had an effect for collection time and the interaction between collection time and air exposure time ($P<0.05$), with the highest value being for collection and the lowest for 60E24hAE. Na^+ differed from basal for 60EIAE, 30E24hAE and 60E24hAE. Ca^{2+} had an effect for air exposure time and collection time ($P<0.05$), with the highest values being at 60 minutes of air exposure and at IAE. Ca^{2+} only differed from basal for 60E24hAE ($P<0.05$). Cl^- had an effect only for collection time ($P<0.0011$), with the highest value being at IAE. Ca^{2+} only differed from basal for 30EIAE and 60EIAE ($P>0.05$).

Table 3. Values (mean ± standard deviation) of blood electrolytes at times of air exposure time (30 and 60 min) and blood collection time (IAE - immediately after exposure, 1hAE - One hour after exposure, 24hAE - 24 hours after exposure and 48hAE - 48 hours after exposure) for *Colossoma macropomum*.

	K^+	Na^+	Ca^{2+}	Cl^-
Means of Basal				
Basal	3.82±0.90	166.67±3.77	0.59±0.16	152.00±1.73
Means of exposure (E)				
30 minutes	5.38±0.24^{a}	161.52±1.08	0.52±0.03^{b}	143.35±0.95^{a}
60 minutes	5.42±0.26^{a}	164.72±1.21	0.63±0.04^{a}	144.94 ±1.06^{a}
Means of collections (C)				
IAE	5.44±0.77^{a}	164.53±7.52	0.69±0.05^{a}	148.78±1.37^{a}
1hAE	5.38±0.53^{a}	164.33±3.70	0.50±0.05^{b}	141.67±1.30^{b}
24hAE	4.95±1.01^{a}	159.33±5.14	0.48±0.07^{b}	144.67±1.84^{b}
48hAE	5.60±1.55^{a}	161.58±4.96	0.56±0.05^{ab}	141.33±1.30^{b}
Means for E × C				
30EIAE	5.44±0.65*	159.40±6.74^{bc}	0.61± 0.11	145.40± 7.79
60EIAE	5.43±0.86*	169.67±4.23^{a*}	0.77±0.29	152.17±2.79
30E1hAE	5.38±0.60*	164.67±4.71^{ab}	0.47±0.10	142.50±2.69*
60E1hAE	5.37±0.46*	164.00±2.24^{ab}	0.52±0.05	140.83±1.57*
30E24hAE	4.95±0.79	159.33±4.19^{bc*}	0.48±0.09	144.67±2.05*
60E24hAE	5.67±1.07*	154.83±5.01^{c*}	0.75±0.14*	141.83±2.48*
30E48hAE	5.73±2.04	162.67±2.92^{b}	0.51±0.06	140.83±7.24*
60E48hAE	5.47±0.79	160.50±6.18^{bc}	0.61±0.20	141.83±1.34*
P-value exposure (E)	0.7471^{ns}	0.6289^{ns}	0.0047$^{⸸}$	0.5399^{ns}
P-value Coleta (C)	0.9303^{ns}	0.0035$^{⸸}$	0.0474$^{⸸}$	0.0011$^{⸸}$
P-value Interaction (E × C)	0.7353^{ns}	0.0081$^{⸸}$	0.3922^{ns}	0.0713^{ns}
CV (%)	20.70	3.18	27.92	3.14

Means followed by different letters in a column differ by Duncan's test (p <0,05). * Asterisks indicate a significant difference compared to baseline by the T test (P<0.05). CV = coefficient of variation. (⸸P<0.05) (⸸⸸P<0.0001) (ns not significant). Potassium (K^+ - mmol L^{-1}), sodium (Na^+ - mmol L^{-1}), calcium (Ca^{2+} - mmol L^{-1}) and chloride (Cl^- -mmol L^{-1}).

6.4 Discussion

Colossoma macropomum is known to have a robust physiology that has the capacity to cope with different stress conditions, such as hypoxia (Saint-Paul, 1984) and variation in pH (Wood et al., 1998), among others. The present study encountered no mortality during air exposure (30 to 60 min) or even for the subsequent 48 h. These results confirm the resistance of this animal to this type of stress, which is a desirable factor for production species that are constantly subjected to handling when exposure to air can occur, such as with biometrics, capture and transport. Another Neotropical species, *L. alexandri*, but with benthic behavior, also experienced no mortality of juveniles for 96 h after being subjected to 30 min of air exposure (Mattioli et al., 2019a).

In the cultivation environment, management procedures, such as transport and air exposure, can trigger stress in fish (Peters et al., 1984; Hur, 2018; Hur et al., 2019; Mattioli et al., 2019a). Primary stress responses are triggered by cortisol and catecholamines, which are precursors to secondary responses to stress (Barton, 2002). Catecholamines induce cardiorespiratory changes in animals that aim to increase the oxygen uptake and transport capacity (Nilsson and Sundin, 1998). To increase the distribution capacity of oxygen captured in the gills, catecholamines induce the production of red blood cells (Oba, 2009). Hematocrit and hemoglobin are known indicators of the oxygen transport capacity of fish (Tavares-Dias and Moraes, 2004). According to Iwama et al. (1995), stress can increase hematocrit levels by 10–15%. In the present study, however, this increase was not observed relative to basal since there were no differences between exposure times for all collection times.

Hemoglobin did not differ between air exposure times nor among collection times. Values differed from basal only for the 60 min exposure at the 24hAE collection. This differs from what was found for *Oreochromis niloticus*, which had no differences in hemoglobin when exposed to air for 30, 60 and 90 min with subsequent recovery (Silva et al., 2012).

Plasma protein is included in the body's physiological adaptation to stressful situations (De Smet and Blust, 2001) and is a good indicator of stress response, as well as, indirectly, the level of nonspecific immunity (Ortuno et al. 2001; Ni et al. 2014). Plasma protein is included in the body's physiological adaptation to stressful situations (De Smet and Blust, 2001) and is a good indicator of stress response, as well as, indirectly, the level of nonspecific immunity (Ortuno et al. 2001; Ni et al. 2014). In the present study, protein values varied among collection times, with higher values at 1hAE, 24hAE and 48hAE; only 60 min of exposure at IAE did not differ statistically from basal, with lower values compared to the other treatments. This increase may indicate the mobilization of proteins to meet the energy need required by the stress of air exposure (Vargas-Chacoff et al., 2016). The same was found with *C. macropomum* in different waters (water from the Rio Negro and underground water from Instituto Nacional de Pesquisas da Amazônia - INPA) 30 min after three minutes of air exposure (Ruiz-Jarabo et al., 2020). A contradictory result was reported for *O. niloticus*, with no changes reported for 30, 60 and 90 min of air exposure (Silva et al., 2012). Abdel et al. (2019) suggested that fish increase specific proteins, such as lysozyme or complement proteins, that increase immunity levels to deal with stress. Further studies are needed to better clarify this increased exposure to air in *C. macropomum*.

The responses of *C. macropomum* to different air exposures showed a significant increase in glucose for 60 min compared to 30 min at collection 1hAE. In addition, glucose took longer to return to basal after 60 min of exposure. Comparisons among collections revealed a decrease in glucose levels for 30 min of exposure at 1hAE; however, for 60 min of exposure the decrease occurred only at 24hAE. Comparisons to basal revealed that the time of air exposure influenced the fish, with values at 1hAE for 30 min exposure being equal to basal. However, for 60 min of exposure this only happened at the 24hAE collection. The hypothalamic-pituitary-interrenal axis (HPI) is the main mechanism that animals use to mobilize energy in the face of a stressful

situation. During stress, the HPI axis regulates the release of cortisol hormone, which eventually causes glycogen break down in fish (Pankhurst, 2011). Energy expenditure occurs because of this breakdown of hepatic glycogen, with effects on the liver stimulating glycogenolysis and thus increasing plasma glucose (Pankhurst, 2011). Several authors have reported this increase in glucose with the stress of air exposure for different lengths of time. Five minutes of air exposure of *Piaractus mesopotamicus* resulted in increased plasma glucose, while after 24 h of exposure the values were equal to the control (Abreu et al., 2008). This same increase occurred in *Piaractus brachypomus* exposed to air for 60 min. According to the authors, the animals were unable to restore control values even after 24 h of recovery (Roriz et al., 2015). This result is contrary to the present study, where juvenile *C. macropomum* restored basal levels 24 h after exposure.

When energy demand is high, obtaining energy through aerobic respiration alone is not sufficient. In such cases the body uses the anaerobic route to supply this energy demand, the main metabolite of which is lactate (Brandão et al., 2006, Barbosa et al., 2007). The present study found important differences for lactate between the exposure times for *C. macropomum* at collections IAE and 1hAE, with significantly higher values for 60 min exposure compared to 30 min. The increase in lactate shows that the anaerobic glycolytic pathway is active; that is, glucose is being degraded with the decrease in oxygen levels for use by the body, thus guaranteeing available energy to meet demand (Wood, 1991; Barton, 2002; Begg and Pankhurst, 2004). The results of the present study confirm that plasma lactate levels are important indicators of anaerobiosis, since it increases as the animal undergoes stressful and exhausting stress exercises (Begg and Pankhurst, 2004; Mattioli et al., 2019a). Other authors have also reported an increase in lactate in animals subjected to air exposure, such as *Astyanax altiparanae* for 5 min (Pereira-da-Silva e Oliveira, 2016) and *L. alexandri* for 30 min (Mattioli et al., 2019a). Ruiz-Jarabo et al. (2020) also recorded an increase in plasma lactate for *C.*

macropomum submitted to 3 min of air exposure in different waters (water of the Rio Negro and underground water of Instituto Nacional de Pesquisas da Amazônia - INPA), with these remaining high after 120 min of recovery. These data confirm that one hour of recovery time of is not sufficient to restore homeostasis of *C. macropomum*. However, in the present study, lactate levels decreased after 24hAE. The observed increase in lactate shows that the stress caused by air exposure induces hypoxia with a consequent increase in anaerobic glycolysis. *Colossoma macropomum* submitted to stress due to hypoxia showed an increase in lactate, thus suggesting the use of anaerobic metabolic adjustment patterns for this species (do Carmo Neves et al., 2020).

Although triglycerides varied among collection times, they never differed from basal. Cholesterol is a steroid that acts as a precursor in the synthesis of hormones (Payne and Hales, 2004). Cholesterol spikes are due to the consumption of pregnenolone, a precursor of cortisol, which in turn promotes metabolic changes (Messina et al., 2013), such as increases in glucose and lactate levels by anaerobic pathways (Silva et al. 2012). In the present study, cholesterol was only affected by collection time, with the lowest values being at IAE and 1hAE. Cholesterol differed significantly from basal for the exposure time of 60 min at 24hAE and 30 and 60 min at 48hAE. Silva et al. (2012) found contradictory results, with no changes in cholesterol values for *O. niloticus* exposed to air for 30, 60 and 90 min, but lower values than the control after 60 min of recovery from 90 min of exposure.

The enzymes AST and ALT are important indicators of stress and assist in the diagnosis of diseases and tissue damage caused by various stressors (Firat et al., 2011). ALT and AST had their highest values for the collection at 24hAE and differed from basal for 30 min of air exposure at 24hAE and 48hAE. ALT values did not differ from basal for the different exposure times and collection times. The AST enzyme has higher concentrations in the liver, heart muscle and skeletal muscle and lower concentrations in the kidneys and pancreas. The ALT enzyme is

found mainly in the liver, somewhat in the kidneys and in low concentrations in heart and skeletal muscle (Nelson and Cox, 2014). Increases in these enzymes may be related to some cellular damage in these tissues, such as damage to the liver when injured hepatocytes overflow and reach the bloodstream (Harvey et al., 1994).

Along with blood and biochemical parameters, blood gas analysis is an important tool for assessing physiological conditions since it shows the effectiveness of respiratory gas exchange (oxygenation) and metabolic parameters (acid-base balance) (King, 2000; Tavares Dias et al., 2008; Araújo et al., 2009). Variables such as blood pH, partial pressure of carbon dioxide and oxygen, and bicarbonate ion, among others, serve to assess stress conditions due to handling and response to anesthetics (Honorato et al., 2013; Honorato and Nascimento, 2016; Barbas et al., 2020) as well as air exposure (Mattioli et al., 2019a; Ruiz-Jarabo et al., 2020).

The blood pH values observed here were lowest at IAE followed by the 1hAE collection. This behavior was similar to that found for *L. alexandri* exposed to air for 30 min (Mattioli et al., 2019), and for *C. macropomum* in different waters exposed for 3 min (Ruiz-Jarabo et al., 2020). A reduction in blood pH was also described by Mariano et al. (2009) for *Hoplerythrinus unitaeniatus* exposed to air for one, six and 12 h. According to these same authors, low pH levels after stress can be characterized as an acid-base imbalance. The highest pH values were found at 24hAE and 48hAE for both exposure times, in relation to basal, for *C. macropomum*. Gilmour (2001) reports that increased pH occurs when there is an increase in ventilation in response to hypoxia, which can cause respiratory alkalosis, a fact also verified for *C. macropomum* submitted to the anesthetic *Spilanthes acmella*, when respiratory alkalosis occurred in the first 24 h of recovery (Barbas et al., 2016).

The parameters $PvCO_2$ and PvO_2 were inversely proportional; while $PvCO_2$ increased, PvO_2 decreased at the IAE collection, when $PvCO_2$ differed between exposure times (30 and 60 min). PvO_2 differed between exposure times (30 and 60 min) at the 24hAE collection. $PvCO_2$

varied between exposure times and among collection times compared to basal, while PvO_2 did not. The basic function of the system is to supply oxygen to the tissues and remove carbon dioxide that is produced by cellular metabolism, thereby regulating the gas exchange process to maintain relatively constant partial pressures ($PvCO_2$ and PvO_2) (Gilmour, 2001).

The concentrations of HCO_3^- varied among collections with differences between 30 and 60 min of exposure at the first collections, increasing at 24hAE. This variation was not observed by Ruiz-Jarabo et al. (2020) for *C. macropomum* exposed to air for 3 min. The length of air exposure in the present study was much longer, which may explain the variation in responses throughout the collections, with the concentrations of HCO_3^- trying to establish a homeostatic balance. The measurement of HCO_3^- makes it possible to evaluate an organism's response to the fluctuation of blood pH. An increase in CO_2 causes the formation of carbonic acid (H_2CO_3). Then, by the action of carbonic anhydrase, the production HCO_3^- increases, which acts as a physiological buffer (Honorato and Nascimento, 2016). Here, for *C. macropomum*, there was a decrease in collections in relation to basal, which suggests that these animals went through respiratory acidosis defined by a decrease in pH and an increase in $PvCO_2$ in collections with greater stress and decreases in HCO_3^- in all collections. The same decrease occurred for *L. alexandri* in a collection one and a half hours after air stress of 30 min (Mattioli et al., 2019a).

In this sense, sO_2 behaved in such a way as to sustain the condition of respiratory acidosis, compared to basal, at the IAE collection for exposure times of 30 and 60 min. There was an increase at 24hAE for both exposure times (30 and 60 min), while at 48hAE these values were already equal to basal.

The lowest values for tO_2 were for IAE, while at 24hAE the 30 and 60 min exposures differed from basal. Damsgaard et al. (2015) reported that hemoglobin changes with the degree of oxygenation. However, in the present study, there was slight variation in hemoglobin concentration and a decrease in tO2 only at the IAE collection. The oxygen content of the blood

is established, above all, by oxyhemoglobin saturation (Keen and Gamperl, 2012), and as it remained stable the same happened with the percentage of tO_2.

The perturbation of hydromineral balance is also considered a secondary response to stress. According to Wendelaar Bonga (1997), several types of stressors impair the ionic balance in fish.

Potassium values differed from basal at collections IAE and 1hAE for both 30 and 60 min of exposure and at 24hAE for 60 min exposure, unlike what happened with *O. niloticus* with the exposure times of 30, 60 and 90 min and recovery, when these levels did not differ (Silva et al., 2012), suggesting species-specific responses. Fish gills have several functions, including the promotion of gas exchange and osmotic, ionic and hematological regulation. The stress of air exposure can cause acidosis and osmotic stress as a result of respiratory interruption and ionic exchange between water and blood (G holipo ur kananiand Ahadizadeh 2013). When assessing increased branchial permeability due to metabolic dysfunction after stress in freshwater fish, Souza et al. (2001) observed that, as a consequence, there is an influx of water and a production of blood ions and their release to the external environment. The present study found these effects to be at reversible levels in *C. macropomum* exposed to air for 30 min and 60 min, as shown by the 48hAE collection, thus demonstrating that the animal sought to maintain the balance of blood gases in the tissues and restore osmoregulatory balance. The same did not occur for animals submitted to 60 min of exposure.

Chloride levels for *C. macropomum* decreased relative to basal at the 1hAE collection for both exposure times. This change is favored by increased branchial permeability driven by catecholamine, which is responsible for the exchange of chloride with the environment (McDonald and Milligan, 1997). A decrease in plasma chloride concentration was also reported by Abreu et al. (2009) for *P. mesopotamicus* after capture stress.

There was variation in sodium levels showing that juvenile *C. macropomum* submitted to the two periods of air exposure managed to maintain sodium balance under acute stress. However, at 24hAE these levels decreased in relation to basal, and after 48 hours of exposure reached basal. Sodium is known to be the main ion in the blood and its loss occurs after stressful conditions due to the passive diffusion of ions between the blood and the environment, with vasodilation of the branchial blood vessels also occurring (Gratzek and Reinert, 1984). Juvenile *C. macropomum* behaved differently in the study of Ruiz-Jarabo et al. (2020), in which they were exposed to air for three minutes in different waters and sodium levels decreased shortly after stress and recovered basal after 120 minutes.

To maintain organic homeostasis and neuromuscular functions, the balance of calcium levels must be kept stable (Shiau and Hwang 1993, Björnsson et al. 1999). Juvenile *C. macropomum* showed variation in calcium values among collection times. However, these values did not differ from basal; only the 60 min exposure at 24hAE differed from basal with higher values, returning after 48hAE. The results indicate that juvenile *C. macropomum* exposed to air for 30 and 60 min sought to maintain and restore osmoregulatory balance.

6.5 Conclusions

Air exposure caused hematological, biochemical, gasometric and electrolytic changes in juvenile *C. macropomum*, which were resistant to stress because they were able to restore physiological homeostasis within 48 h after air exposure. This information is relevant to understanding stress conditions and their physiological consequences for *C. macropomum* and will help in management and in choosing measures that minimize stressors and their harmful effects in cultivation.

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7. CONSIDERAÇÕES FINAIS

O peixe nativo Colossoma macropomum é conhecido pela sua resistência a variações do ambiente. Para o enfrentamento da hipóxia e exposição ao ar, os animais passaram por ajustes fisiológicos, metabólicos, anatômicos e bioquímicos para compensar a falta de oxigênio. Pode-se observar nestes estudos que os animais passaram por rápidos ajustes nas variáveis glicose e lactato, decorrentes do uso do metabolismo anaeróbio, que exerceu um papel importante no fornecimento de energia para o peixe conseguir passar pelo momento de estresse.

Maiores estudos são necessários a fim de elucidar ainda mais as respostas fisiológicas desse animal quando submetidos ao estresse por hipóxia e exposição ao ar.