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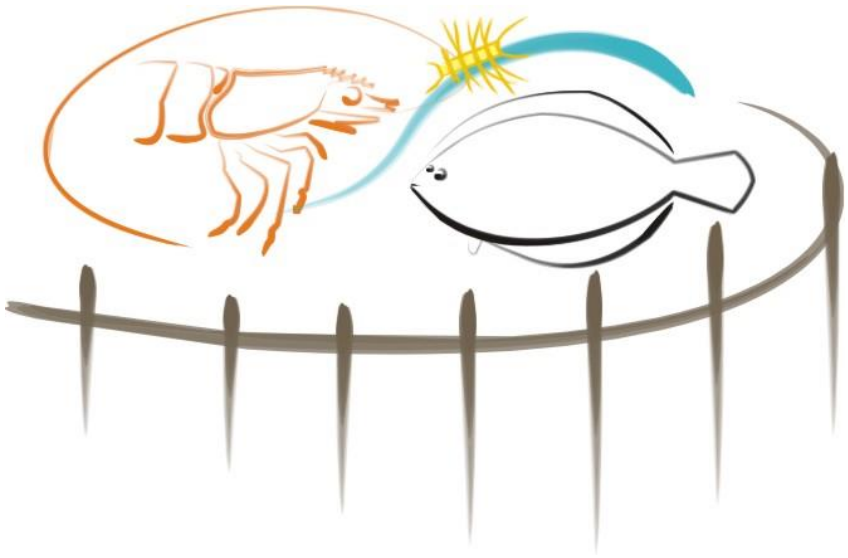

ANESTESIA COM BENZOCAÍNA E MS-222 EM JUVENIS DE TAMBAQUI *Colossoma macropomum*: EFEITOS NOS PARÂMETROS DE ESTRESSE OXIDATIVO

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Dissertação apresentada como parte dos requisitos para a obtenção do grau de mestre em Aquicultura no Programa de Pós Graduação em Aquicultura da Universidade Federal do Rio Grande.

RIO GRANDE, RS

FEVEREIRO DE 2015

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Agradecimentos

Quero agradecer ao meu pai, Heraldo, pela parceria, pelos conselhos, pelas broncas, por não duvidar de mim em nenhum momento, por sua fé, por ser meu pai, por ser meu amigo.

Agradecer a minha mãe, Sandra, pois mesmo tendo deixado ela chorando no portão de casa quando vim fazer o mestrado em Rio Grande, não deixou de me apoiar nessa grande mudança em minha vida. Obrigada pelos abraços, beijos, carinhos! Obrigada por ser a "cola" que mantém nossa família unida. Te amo!

Agradecer a minha irmã, Giulia, por ser minha amiga e que mesmo com seu jeito "relaxado" de viver, está se tornando um grande ser humano! Não sabe o quanto você nos dá orgulho! Te adoro Chu!

Quero agradecer a todos meus amigos de Campo Grande e a minha família que está espalhada por esse país, sempre me mandando boas vibrações. Vocês estão em meus pensamentos!

Quero agradecer ao pessoal do LAC, em especial à Claudinha e ao Aldemar, que além de prestaram serviços ao nosso laboratório, se mostraram grandes amigos, pessoas do bem.

Quero agradecer ao meu amigo Dé Barbas, pois sem ele, esse trabalho com certeza não teria se concretizado. Sua parceria e amizade foram essenciais para meu crescimento e aprendizado. Muito obrigada!

Agradecer aos meus parceiros de sempre, Baiano, Mari, Lucão, Lilian e Daniel. Sem eles, Rio Grande não teria graça!

Obrigada ao pessoal da EMA e do Laboratório de Fisiologia por me ajudarem com meus experimentos e análises. Fizeram a diferença.

E por último, mas não menos importante, quero agradecer ao meu querido orientador, Luciano Garcia, por ter me ajudado a reerguer a cabeça no único momento em que eu poderia ter desistido de tudo, se ofereceu pra ser meu orientador e agüentou todo o estresse que essa nova função lhe gerou! Muito obrigada, professor, amigo! Tomara que eu possa retribuir toda a sua ajuda um dia. Um grande abraço!

Resumo geral

No presente estudo foram avaliados os efeitos de dois anestésicos (benzocaína e metanosulfato de tricaína (MS-222) sobre parâmetros bioquímicos nos tecidos de juvenis de tambaqui *Colossoma macropomum*. Foram utilizados 80 juvenis de tambaqui, os quais foram anestesiados com benzocaína (BZ - 100 ppm) ou MS-222 (TR - 240 ppm). Outros dois grupos, denominados controles: controle água (sem anestesia) e controle álcool (exposição a álcool PA) também passaram pelo mesmo procedimento. Após indução anestésica 10 peixes/anestésico/tempo de coleta foram eutanaziados nos tempos 3, 12 e 24 horas para coleta de amostras de tecidos (brânquias, fígado e cérebro) os quais foram utilizados para a análise da atividade da enzima antioxidante glutationa S-transferase (GST), bem como da lipoperoxidação celular (TBARS) e da capacidade antioxidante total (ACAP), nos diferentes tempos amostrais. Nas brânquias houve um aumento na ACAP no tratamento BZ em relação ao TR e ao grupo controle no tempo 12h. O fígado apresentou uma redução na ACAP no tempo de 12 h para o tratamento TR em relação ao controle. Os tratamentos BZ e TR em 24 h apresentaram aumento na ACAP em relação ao controle. A atividade da enzima GST aumentou nas brânquias para os tratamentos BZ e TR nos tempos de 3 e 12 h em relação ao controle. O fígado apresentou aumento no tratamento BZ em 24 h e para TR nos tempos 3 e 24 h em relação ao controle. Houve uma diminuição do dano lipídico nas brânquias (BZ e TR) e no cérebro (TR) em 24 h em relação ao controle. De acordo com os resultados concluímos que os anestésicos utilizados neste trabalho, nas doses de 100 ppm (BZ) e 240 ppm (TR) não provocaram danos oxidativos nos juvenis de tambaquis, mas são capazes de produzir uma resposta antioxidante para prevenir a lipoperoxidação e provocar a detoxificação do organismo.

Palavras chave: Anestésicos, Estresse oxidativo, Peixe, GST, ACAP, Lipoperoxidação.

Abstract

The present study aimed to evaluate the effects of two anesthetics (benzocaine and tricaine methanesulfonate - MS-222) over biochemical parameters of juvenile tambaqui *Colossoma macropomum* tissues. This study was carried out with 80 juvenile tambaqui were used, which were anesthetized with benzocaine (BZ – 100 ppm) or MS-22 (TR – 240 ppm). Other two grups, named control: control water (without anesthesia) and control alcohol (exposure to ethanol) also went through the same procedure. After anesthetic induction 10 fish/anesthetic/time of sampling were euthanized at 3, 12 and 24 hours after anesthesia for tissue sampling (gills, liver and brain), which were submitted to the analyses of the antioxidant enzyme (GST), as well as cellular lipid peroxidation (TBARS) and total antioxidant capacity (ACAP) at different sampling times. There was an increase in the ACAP on the gills of BZ treatment compared to TR and control group at 12 h. The liver showed a reduction in the ACAP of TR treatment at 12 h compared to control group. The treatments BZ and TR showed an increase of ACAP at 24 h compared to control group. The activity of the GST enzyme increased on the gills for treatments BZ and TR at 3 and 12 h compared to control group. Liver showed increased activity for treatments BZ at 24 h and TR at 3 and 24 h compared to control. There was a decrease of lipid damage on gills (BZ and TR) and brain (TR) at 24 h compared to control group. According to the results it is concluded that the anesthetics, in the doses of 100 ppm (BZ) e 240 ppm (TR) did not cause oxidative damage in juvenile tambaqui, but are capable of producing an antioxidant response to prevent the lipid peroxidation and induce the detoxification of the organism.

Keywords: Anesthetics, Oxidative Stress, Fish, GST, ACAP, Lipid peroxidation

Introdução

Em 2012, foram produzidos pela aquicultura mundial 90,43 milhões de toneladas de organismos aquáticos, sendo que desse montante global a aquicultura continental foi responsável por 41.946 mil toneladas produzidas. Os três maiores produtores mundiais de aquacultura, em toneladas, foram a China (41.108.306), India (4.209.415) e Vietnam (3.085.500) (FAO 2014).

O Brasil, no ranking mundial da aquicultura em 2012, aparece como 12º maior produtor (707.461 toneladas) e entre as Américas é o segundo maior produtor, onde o primeiro é o Chile (1.071.421 toneladas) (FAO 2014).

Em 2013 a piscicultura no Brasil produziu 392.493 mil toneladas, sendo os maiores produtores os estados de Mato Grosso (75.629.524 kg), Paraná (51.143.124 kg) e Ceará (30.669.875 kg) (IBGE 2013). Neste ranking nacional a região Sul do Brasil se destaca por apresentar uma produção total de 22,4% da piscicultura nacional, neste mesmo ano (88.063 mil toneladas). O estado do Rio Grande do Sul, em 2013, obteve uma produção total de 15.679.569 kg, correspondente a 4% da produção total do país.

As espécies mais produzidas a nível nacional, nesse período, foram a tilápia (169.306.011 kg) e o tambaqui (88.718.502 kg), sendo os maiores produtores o Paraná (44.748 toneladas) e Rondônia (18.880 toneladas), respectivamente (IBGE 2013).

O tambaqui *Colossoma macropomum* (Cuvier 1816), da ordem *Characiformes* e família *Characidae*, é uma espécie onívora originária das bacias dos Rios Amazonas e Orinoco. Esta espécie também é conhecida como cachama, gamitana e black pacu; habita águas ricas em nutrientes, de pH entre 4 e 6 e temperaturas entre 25 a 34 ºC. É uma espécie que consegue sobreviver em águas com níveis baixos de oxigênio (≤1 mg

L^{-1}), devido a uma adaptação em seu lábio inferior, chamada de "aiu"capaz de captar oxigênio da atmosfera quando em condições de hipóxia (Baldisserotto & Gomes 2005).

Na natureza esta espécie pode alcançar até 1 m de comprimento e 30 kg de peso vivo (Saint-Paul 1986). As larvas desse peixe se alimentam de zooplâncton e pequenos invertebrados enquanto que os juvenis e adultos podem se alimentar de sementes, frutas, invertebrados e outros animais. Os adultos têm sua primeira maturação entre 3-4 anos de idade e as fêmeas são capazes de produzir em torno de 78 ovos/grama de peso vivo, realizando suas desovas entre outubro e fevereiro (Baldisserotto & Gomes 2005; Araujo-Lima & Goulding 1997).

É bem adaptada às condições de cativeiro, aceitando rações artificiais, com índices desejáveis de crescimento e conversão alimentar (Inoue *et al*. 2011). Sua reprodução em cativeiro é realizada por indução hormonal (glândula pituitária) e após a eclosão (4-6 dias) faz-se a transferência das larvas para viveiros adubados, onde se alimentarão de ração e zooplâncton até virarem alevinos e poderem ser remanejados (Baldisserotto & Gomes 2005).

Suas características favoráveis de crescimento e rusticidade fizeram com que fosse possível seu cruzamento com outras espécies da mesma família, para agregar ainda mais qualidades aos novos animais originados, como por exemplo, tambacu e paqui (fêmea tambaqui x macho pacu e fêmea pacu x macho tambaqui), cachameta (fêmea tambaqui x macho pacu-manteiga) e pacogama (fêmea pirapitiga x macho tambaqui) (Baldisserotto & Gomes 2005).

A grande produção desta espécie merece um cuidado especial durante as práticas de manejo, tais como biometrias, coleta de sangue e transporte, as quais são necessárias para o monitoramento do crescimento e estado geral de sanidade dos animais. Devido a

isso, os anestésicos são importantes, pois ajudam a reduzir o estresse e mortalidade provocados por esses procedimentos (Gomes *et al.* 2001; Inoue *et al*. 2011; Sneddon 2012). Além disso, a utilização dos anestésicos pode evitar alterações fisiológicas nos peixes, as quais podem desencadear doenças e, dependendo da saúde do animal, bem como da intensidade e duração do estresse ou de exposição a estressores, os efeitos deletérios podem ser consideráveis (Weber 2011).

Sendo assim, os anestésicos naturais ou sintéticos, de uso veterinário e/ou humano passaram a ser usados na aquicultura a fim de facilitar o manejo durante o cultivo (Zahl *et al*. 2010). Entretanto, a necessidade de um melhor conhecimento sobre a fisiologia da resposta a utilização destas substâncias nos organismos aquáticos e a importância do tratamento anestésico para manter o bem-estar animal devem ser melhor entendidos para as diferentes espécies de peixes.

Algumas características devem ser levadas em consideração durante a escolha de um anestésico, como por exemplo, ter baixo custo, ser atóxico, ser de fácil administração (Treves-Brown, 2000). Além disso, a indução anestésica em peixes, deve ocorrer antes de 3 minutos e sua recuperação antes de 10 minutos (Park et al., 2008).

Muitos compostos químicos são utilizados para a anestesia de peixes, dentre os quais temos o MS-222 (metanosulfato de tricaína), benzocaína, quinaldina, 2-fenoxietanol, metomidato e Aqui-S™ (Coyle *et al*. 2004). Dentre esses, os mais comumente utilizados são o MS-222 e a benzocaína (Gomes *et al.* 2001; Sneddon 2012; Vera *et al*. 2013).

A tricaína (também conhecida como MS-222 ou metanosulfato de tricaína - $C_9H_{11}NO_2$ + CH_3SO_3H) é um isômero da benzocaína com um radical sulfonato, comercializada na forma de um pó branco cristalino (droga 100% pura), e pode ser

diluída em água (Popovic *et al.* 2012) e lipídios, porém quando adicionada à água diminui o pH, necessitando a adição de um agente tamponante ($NaHCO_3$) (Sneddon 2012).

A tricaína foi originalmente produzida como um analgésico local e tem sido usada dessa forma desde então (Popovic *et al*. 2012). Esse composto causa depressão no sistema nervoso central e hipóxia, paralisando os peixes (MCTI 2013), e é metabolizada pelo fígado (Wayson *et al*. 1976). Para juvenis de tambaqui (15,1 ± 3,1 g; 10,1 ± 0,8 cm) a dose efetiva de tricaína, que induz a anestesia, sem ocasionar mortalidade é de 240 ppm (Barbas *et al*. comunicação pessoal). Adicionar o peso e tamanho dos peixes.

Outro anestésico comumente utilizado, a benzocaína (p-aminobenzoato de etila - $C_9H_{11}NO_2$), é o anestésico mais utilizado no Brasil, principalmente por ser de fácil obtenção, baixo custo e seguro ao usuário (Gimbo *et al.* 2008). É um anestésico de uso humano local, embora atue de forma sistêmica em peixes, agindo no sistema nervoso central (Okamura *et al.* 2010), onde bloqueia os canais de sódio, reduzindo os potenciais de ação dos nervos (Arias 1999).

Muitos estudos já foram feitos sobre o potencial anestésico da benzocaína em peixes, como carpa (Heo & Shin 2010), matrinxã (Inoue *et al.* 2004), pampo (Okamoto *et al.* 2009) e robalo peva (Souza *et al.* 2012). Gomes *et al.* (2001) avaliaram o efeito anestésico da benzocaína no tambaqui e concluiu que a concentração de 100 ppm de benzocaína, durante mais de 30 minutos, não causa mortalidade e a dose é eficaz para anestesiar os animais.

Apesar de a anestesia ser capaz de atenuar distúrbios bioquímicos e fisiológicos em peixes causados pelas práticas de manejo, o próprio anestésico pode induzir efeitos que causem alterações nos parâmetros bioquímicos e de estresse oxidativo (Velisek *et*

al. 2011). Estas alterações podem ocorrer devido à hipóxia ocasionada quando os animais são anestesiados e, além disso, a re-oxigenação que pode ocasionar uma situação de hiperóxia no momento que os animais são colocados em condições normais de oxigênio dissolvido, na recuperação (Azambuja *et al*., 2011).

As alterações fisiológicas causadas pela utilização dos anestésicos podem desencadear uma situação de estresse oxidativo (Halliwell & Gutteridge 1999), o qual se caracteriza por um desequilíbrio entre a produção de pró-oxidantes e as defesas antioxidantes a nível intracelular. Os pró-oxidantes ou ROS (Espécies Reativas do Oxigênio) são formados a partir da redução do oxigênio nos tecidos (Belló *et al*. 2000) e produzidos em maior quantidade quando um organismo é exposto a compostos xenobióticos (Tew & Ronai 1999) ou situações de estresse. São responsáveis por causar danos a proteínas celulares, lipídios, ácidos nucléicos, entre outras estruturas, levando a danos permanentes a tecidos e órgãos (Storey 1996; Lushchak *et al*. 2005; Fogaça & Sant'Ana, 2009).

Entretanto, os peixes assim como outros organismos dependentes de oxigênio, desenvolveram uma resposta ao estresse oxidativo, uma forma de combate as ROS, chamada de defesa primária ou antioxidantes primários. Essa defesa é constituída por antioxidantes de baixo peso molecular (vitaminas E, K e C, aminoácidos e peptídeos) e enzimas (catalase, superóxido dismutase e enzimas glutationa-dependentes) (Martínez-Álvarez *et al*. 2005).

Dentre estas enzimas, a glutationa S-transferase (GST) é um exemplo de enzima antioxidante capaz de catalisar a reação de agentes alquilantes com seu grupo –SH, tornando seus produtos mais solúveis à água e, após outros processos, produz o ácido

mercaptúrico, produto final a ser excretado pela urina, constituindo, então, o início do processo de detoxificação desses agentes através da enzima GST (Habig *et al*. 1974).

Lushchak *et al.* (2001) encontraram alterações no valor da GST nos rins e músculo de *Caurassius auratus* quando estes animais foram expostos a situações de anoxia e reoxigenação. Estudos com a espécie *Metynnis argenteus* exposta ao agroquímico paclobutrazol (3 e 30 mg L^1 de ingrediente ativo) e *Geophagus brasiliensis* provenientes de um rio poluído (Rio Tubarão), apresentaram alterações nos valores da GST (Jonsson *et al.* 2002; Osório *et al*. 2014).

Além das enzimas antioxidantes, outra forma de se avaliar o estresse oxidativo é através da quantificação de danos celulares, a partir da lipoperoxidação das células, causada pelo uso de xenobióticos. A peroxidação lipídica se resume na incorporação de oxigênio molecular em ácidos graxos poliinsaturados (PUFA) produzindo hidroperóxidos (Janero 1990). Para a quantificação desse dano utiliza-se o método de fluorimetria de TBARS (Substâncias Reativas ao Ácido Tiobarbitúrico), que consiste na reação do malondialdeído (ácido fraco de cadeia curta) com ácido tiobarbitúrico para dar acesso à peroxidação lipídica de materiais biológicos (Draper *et al*. 1993).

Estes danos já foram verificados por Wilhelm Filho *et al*. (2001), que observaram maior dano lipídico (TBARS elevado) e maior atividade da enzima catalase em exemplares de *Geophagus brasilienses* coletados em área poluída de um Rio no estado de Santa Catarina, demostrando, assim, que os peixes da área poluída sofreram estresse oxidativo. Em trabalho com a espécie *Rhamdia quelen* submetida a transporte com óleo essenial de *Lippia Alba* os autores evidenciaram que ocorreram alterações nos níveis de lipoperoxidação de brânquias, fígado e cérebro (Azambuja *et al*. 2011), bem como para brânquias, fígado, cérebro, músculo e intestino da espécie *Onchorhyncus*

mykiss submetidas à anestesia com quatro diferentes anestésicos (MS-222, óleo de cravo, 2-phenoxyethanol e Propiscina) (Velisek *et al*. 2011), reforçando ainda mais o fato de que exposição à xenobióticos, pode causar geração de ROS e lipoperoxidação.

Em técnica desenvolvida por Regoli & Winston (1999) se destaca por quantificar a capacidade total de eliminação de oxiradicais (TOSC) capaz de estabelecer uma resposta antioxidante integrada de um tecido ou organismo contra tipos específicos de espécies reativas de oxigênio (radicais peroxil, hidroxil e peroxinitrito). Essa é uma forma mais limitada e menos abrangente de se obter resultados de resposta antioxidante de um tecido. Visando determinar a capacidade antioxidante total de tecidos, Amado *et al*. (2009) propuseram uma metodologia para determinar a capacidade total contra os radicais peroxil (ACAP), fornecendo um melhor entendimento sobre a resistência de organismos à toxicidade causada pelas ROS.

Este método (ACAP) descrito por Amado *et al*. (2009) consiste em detectar espécies reativas de oxigênio por fluorimetria com a utilização H2DCF-DA (diacetato de 2′,7′-diclorofluorescina) como substrato e um gerador de radicais peroxil ABAP [dicloridrato 2,2′-azobis (2 metilpropionamidina)] e a capacidade total de absorbância de peroxiradicais é monitorada pelo sinal de fluorescência emitido pela reação entre ROS e H2DCF-DA.

Para exemplares da espécie *Poecilia vivipara* exposta a 10, 20 e 200 µg L^{-1} do hidrocarbono aromático policíclico fenantreno (poluente), alterações foram observadas na capacidade antioxidante total de brânquia, fígado e músculo (Machado *et al*. 2014). Harayashiki *et al*. (2013) também encontraram alterações na capacidade antioxidante dessa mesma espécie exposta a 130 e 700 µg L^{-1} do herbicida glifosato. Em outro estudo, Abujamara et al. (2014) relataram alterações nos valores para ACAP entre

outras formas antioxidantes quando anêmonas *Bunodosoma cangicum* pré-expostas a cobre antes de passarem por alterações de oxigênio.

Diferentes defesas antioxidantes enzimáticas foram estudadas em diferentes situações de exposição e em diferentes espécies. Marcon & Wilhelm Filho (1999) avaliaram níveis de SOD e catalase no fígado, sangue e plasma de tambaquis em ambiente natural. Outros autores encontraram essas respostas enzimáticas em espécies expostas a diferentes compostos químicos, como por exemplo, piava (*Leporinus obtusidens*) (Miron *et al*. 2008) e jundiá (*Rhamdia quelen*) (Ferreira *et al*. 2010) expostos a agroquímicos e truta marrom (*Salmo truta*) (Hansen *et al*. 2006), white seabream (*Diplodus sargus*) (Ferreira *et al*. 2008) e jundiá (*Rhamdia quelen*) (Pretto *et al*. 2011) expostos a metais pesados.

Contudo, ainda há a necessidade de se verificar se a utilização de anestésicos comumente usados na aquicultura, afetam os peixes, provocando assim, um estresse oxidativo nestes animais e prejudicando a produtividade e os lucros ao criador. Dentre eles podemos citar os trabalhos de Azambuja *et al*. (2011) e Velisek *et al*. (2011), onde estes autores realizaram estudos com transporte de jundiá após anestesia com *Lippia Alba* e a comparação de quatro anestésicos (MS-222, óleo de cravo, 2-fenoxietanol, propiscina) em biomarcadores de estresse oxidativo em trutas arco-íris, respectivamente.

Diante destes fatores é fundamental estudarmos os efeitos ocasionados nos tecidos (lipoperoxidação e estresse oxidativo) pelos dois anestésicos mais utilizados na aquicultura mundial e brasileira, em juvenis de tambaqui, para obtermos informações sobre possíveis danos gerados a esta espécie pela exposição a estes dois anestésicos. Esta abordagem deverá proporcionar dados importantes para o manejo do tambaqui,

beneficiando uma grande gama de piscicultores e proporcionando um melhor bem estar aos animais no momento do manejo.

Objetivos

Objetivo geral

O presente trabalho tem como objetivo geral avaliar alterações em parâmetros de estresse oxidativo em diferentes tecidos de juvenis de tambaqui *Colossoma macropomum* submetidos à anestesia com benzocaína e MS-222.

Objetivos específicos

Verificar possíveis alterações na atividade de lipoperoxidação celular (TBARS) no fígado, brânquias e cérebro de juvenis de tambaqui após serem submetidos à anestesia com benzocaína e tricaína (MS-222);

Avaliar os efeitos ocasionados na capacidade antioxidante total (ACAP) e na atividade da enzima glutationa S-transferase (GST) no fígado, brânquias e cérebro de juvenis de tambaqui após serem submetidos a anestesia com benzocaína e tricaína (MS-222).

Referências Bibliográficas

AMADO, LL, ML GARCIA, PB RAMOS, RF FREITAS, B ZAFALON, JRL FERREIRA, JS YUNES & JM MONSERRAT. 2009. A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: Application to evaluate microcystins toxicity. *Science of the total Environment*, 407:2115 – 2123.

ARAUJO-LIMA, C & M GOULDING. 1997. So fruitful a fish. Ecology, conservation, and aquaculture of the Amazon's tambaqui. *Columbia University Press*, New York, NewYork, USA.

ARIAS, HR. 1999. Role of local anesthetics on both cholinergic and serotonergic ionotropic receptors. *Neuroscience and Biobehavioral Reviews*,23:817-843.

AZAMBUJA, CR, J MATTIAZZI, APK RIFFEL, IA FINAMOR, LO GARCIA, CG HELDWEIN, BM HEIZMANN, B BALDISSEROTO, MA PAVANATO, SF LLESUY. 2011. Effect of the essencial oil of *Lippia alba* on oxidative stress parameters in silver catfish (*Rhamdia quelen*) subjected to transport. *Aquaculture*, 319:156-161.

BALDISSEROTTO, B & LC GOMES. 2005. Espécies nativas para piscicultura no Brasil. Editora UFSM, 468p.

BELLÓ, ARR, E FORTES, A BELLÓ-KLEIN, AA BELLÓ, SF LLESUY, RB ROBALDO & A BIANCHINI. 2000. Lipid peroxidation induced by *Clinostomum detruncatum* in muscle of the freshwater fish *Rhamdia quelen*. *Diseases of Aquatic Organisms*, 42:233-236.

DRAPER, HH, EJ SQUIRES, H MAHMOODI, J WU, S AGARWAL & M HADLEY. 1993. A comparative evaluation of thiobarbituric acid methods for the determination

of malondialdehyde in biological materials. *Free Radical Biology & Medicine,* l:353-363.

FAO.FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. 2012. *The State of World Fisheries and Aquaculture 2012*, Rome, 230p.

FERREIRA, D, AC MOTTA, LC KREUTZ, C TONI, VL LORO, LJG BARCELLOS. 2010. Assessmente of oxidative stress in *Rhamdia quelen* exposed to agrichemicals. *Chemosfere*, 79:914:921.

FERREIRA, M, M CAETANO, J COSTA, P POUSÃO-FERREIRA, C VALE & MA REIS-HENRIQUES. 2008. Metal accumulation and oxidative stress responses in, cultured and wild, white seabream from Northwest Atlantic. *Science of the total Environment*, 407:638-646.

FOGAÇA, FHS & LS SANT'ANA. 2009. Oxidação lipídica em peixes: mecanismo de ação e prevenção (artigo de revisão). *Archives of Veterinary Science,* 14:117-127.

GIMBO, RY, MV SAITA, AFN GOLÇALVES & LS TAKAHASHI. 2008. Diferentes concentrações de benzocaína na indução anestésica do lambari-do-rabo-amarelo (*Astyanax altiparanae*). *Revista Brasileira de Saúde e Produção Animal*, 9:350-357.

GOMES, LC, AR CHIPPARI-GOMES, NP LOPES, R ROUBACH & CARM ARAUJO-LIMA. 2001. Efficacy of benzocaine as an anesthetic in juvenile tambaqui *Colossoma macropomum*. *Journal of the Aquaculture Society*, 32:426-431.

HABIG, WH, MJ PABTS & WB JACOBI. 1974. Glutationa-S-Transferase. The first enzymatic step in mercapturic acid formation. *The Journal of Biological Chemistry*, 249:7130-7439.

HALLIWELL, B & JMC GUTTERIDGE. 1999. Free radicals in biology and medicine. 3rd ed, Oxford University Press.

HANSEN, BH, S RØMMA, ØA GARMO, PA OLSVIK & RA ANDERSEN. 2006. Antioxidative stress proteins and their gene expression in brown trout (*Salmo trutta*) from three rivers with different heavy metal levels. *Comparative Biochemistry and Physiology, Part C*, 143:263-274.

HARAYASHIKI, CAY, AS VARELA JUNIOR, AAS MACHADO, LC CABRERA, EG PRIMEL, A BIANCHINI & CD CORICINI. 2013. Toxic effects of the herbicide Roundup in the guppy *Poecilia vivipara* acclimated to fresh water. *Aquatic Toxicology*, 142-143:176-184.

HEO, G. J. & G SHIN. 2010. Efficacy of benzocaine as an anaesthetic for Crucian carp (*Carassius carassius*) (*short communication*). *Veterinary Anaesthesia and Analgesia* 37:132-135.

IBGE: INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA, 2013 *Produção da pecuária municipal*. Ministério do Planejamento, Orçamento e Gestão, Rio de Janeiro, Brasil, 41:34-94.

INOUE, LAKA, A HACKBARTH & G MORAES. 2004. Avaliação dos anestésicos 2-phenoxyethanol e benzocaína no manejo de matrinxã *Brycon cephalus* (Günther, 1869). *Biodiversidade Pampeana,* 2:10-15.

INOUE, LAKA, CL BOIJINK, PT RIBEIRO, AMD DA SILVA & EG AFFONSO. 2011. Avaliação de respostas metabólicas do tambaqui exposto ao eugenol em banhos anestésicos. *Acta Amazonica,* 41: 327-332.

JANERO, DR. 1990. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury (review article). *Free Radical Biology & Medicine*, 9:515-540.

JONSSON, CM, VL FERRACINI, LC PARAÍBA, M RANGEL & SR AGUIAR. 2002. Alterações bioquímicas e acúmulo em pacus (*Metynnis argenteus*) expostos ao paclobutrazol. *Scientia Agricola*, 59:441:446.

LUSHCHAK, VI, LP LUSHCHAK, A MOTA, M HERMES-LIMA. 2001. Oxidative stress and antioxidant defenses in goldfish *Carassius auratus* during anoxia and reoxygenation. *American Journal of Physiology*, 280:100-107.

LUSHCHAK, VI, TV BAGNYUKOVA, VV HUSAK, LI LUZHNA, OV LUSHCHAK & KB STOREY. 2005. Hyperoxia results in transient oxidative stress and an adaptive resposnse by antioxidant enzymes in goldfish tissues. *The International Journal of Biochemistry & Cell Biology*, 37:1670-1680.

MACHADO, AAS, MLM HOFF, RD KLEIN, GJ CORDEIRO, JML AVILA, PG COSTA & A BIANCHINI. 2014. Oxidative stress and DNA damage responses to phenanthrene exposure in the estuarine guppy *Poecilia vivipara*. *Marine Environmental Research*, 98:96-105.

MARCON, JL & D WIHELM FILHO. 1999. Antioxidants process of the wild tambaqui, *Colossoma macropomum* (Osteichthyes, Serrasalmidae) from the Amazon. *Comparative and Biochemistry and Physiology Part C*, 123:257-263.

MARTÍNEZ-ÁLVAREZ, RM, AE MORALES & A SANZ. 2005. Antioxidant defenses in fish: Biotic and abiotic factors (review). *Fish Biology and Fisheries*, 15:75-88.

MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E INOVAÇÃO (MCTI). 2013. Diretrizes da prática de eutanásia do CONCEA. *Conselho Nacional de Controle de Experimentação Animal*. Portaria N. 596. 54 p.

MIRON, DS, A PRETTO, M CRESTANI, L GLUSCZAK, MR SCHETINGER, VL LORO & VM MORSCH. 2008. Biochemical effects of clomazone herbicide on piava (*Leporinus obtusidens*). *Chemosfere*, 74:1-5.

OKAMOTO, MH, MB TESSER, LR LOUZADA, RA DOS SANTOS, LA SAMPAIO. 2009. Benzocaína e eugenol como anestésicos para juvenis do pampo *Trachinotus marginatus*. *Ciência Rural*, 39:866-870.

OKAMURA, D, FG DE ARAÚJO, PVE ROSA, RTF DE FREITAS, LDS MURGAS & MP CESAR. 2010. Influência da concentração de benzocaína e do comprimento de peixes na anestesia e na recuperação de tilápias-do-nilo. *Revista Brasileira de Zootecnia*, 39:971-976.

OSÓRIO, FHT, LFO SILVA, LDS PIANCINI, ACB AZEVEDO, S LIEBEL, FY YAMAMOTO, VP PHILIPPI, MLS OLIVEIRA, CF ORTOLANI-MACHADO, F FILIPAK NETO, MM CESTARI, HCS ASSIS & CAO RIBEIRO. 2014. Water quality assessment of the Tubarão River through chemical analysis and biomarkers in the Neotropical fish *Geophagus brasiliensis*. *Environ. Sci. Pollut. Res.*, 21:9145-9160.

PARK, MO, WJ HUR, SY IM, DW SEOL, J LEE, IS PARK. 2008. Anaesthetic efficacy and physiological responses to clove oil-anaesthetized kelp grouper *Epinephelus bruneus*. *Aquaculture Research*, 39:877-884.

POPOVIC, NT, I STRUNJAK-PEROVIC, R COZ-RAKOVAC, J BARISIC, M JADAN, AP BERAKOVIC & RS KLOBUCAR, 2012. Tricaine methane-sulfonate (MS-222). *Journal of Applied Ichthyology*, 28:553-564.

PRETTO, A, VL LORO, B BALDISSEROTO, MA PAVANATO, BS MORAES, C MENEZES, R CATTANEO, B CLASEN, IA FINAMOR & V DRESSLER. 2011.

Effects of water cadmium concentrations on bioaccumulation and various oxidative stress parameters in *Rhamdia quelen*. *Archives of Environmental Contamination and Toxicology*, 60: 309-318.

REGOLI, F & GW WINSTON. 1999. Quantification of total oxidant scavenging capacity of antioxidants for peroxynitrite, peroxyl radicals, and hydroxyl radicals. *Toxicology and Applied Pharmacology*, 156:96–105.

SAINT-PAUL, U. 1986. Potential for aquaculture of South American freshwater fishes: A review. *Aquaculture*, 54:205-240.

SNEDDON, LU. 2012. Clinical Anesthesia and Analgesia in Fish. *Journal of Exotic Pet Medicine*, 21:32-43.

SOUZA, RAR DE, CVA DE CARVALHO, FF NUNES, BR SCOPEL, JD GUARIZI & MY TSUZUKI. 2012. Efeito comparativo da benzocaína, mentol e eugenol como anestésicos para juvenis de robalo peva. *Boletim do Instituto de Pesca*, 38:247-255.

STOREY, KB. 1996. Oxidative stress: animal adaptations in nature. *Brazilian Journal of Medical and Biological Research*, 29:1715-1733.

TEW, KD & Z RONAI. 1999. GST function in drug and stress response (*mini-review*). *Drug Resistance Updates*, 2:143-147.

TREVES-BROWN, KM. 2000. Anaesthesia. Em: Treves-Brown, K.M. (Ed.), Anaesthetics in Applied Fish Pharmacology. Kluwer Academic Publishers, Dordrecht, Netherlands, 206–217p.

VELISEK, J, A STARA, Z-H LI, S SILOVSKA & J TUREK. 2011. Comparison of the effects of four anesthetics on blood biochemical profiles and oxidative stress biomarkers in rainbow trout. *Aquaculture*, 310: 369-275.

VERA, LM, A MONTOYA & FJ SÁNCHEZ-VÁZQUEZ. 2013. Effectiveness of the anaesthetic MS-222 in gilthead seabream, Sparusaurata: Effect of feeding time and day–night variations in plasma MS-222 concentration and GST activity. *Physiology & Behavior*, 110-111: 51-57.

WAYSON, KA, H DOWNES, RK LYNN & N GERBER. 1976. Anesthetic effects and elimination of tricaine methanesulphonate (MS-222) in terrestrial vertebrates. *Comparative Biochemistry and Physiology C*, 55:37-41.

WEBER III, ES. 2011. Fish analgesia: pain, stress, fear aversion, or nociception? Veterinary Clinics of North America: *Exotic Animal Practice Journal*, 14:21-32.

WILHELM FILHO, D, MA TORRES, TB TRIBESS, RC PEDROSA & CHL SOARES. Influence of season and pollution on the antioxidant defenses of the cichlid. *Brazilian Journal of Medical and Biological* Research, 34: 719-726.

fish acará (*Geophagus brasiliensis*)

ZAHL, IH, A KIESSLING, OB SAMUELSEN & RE OLSEN. 2010. Anesthesia induces stress in Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus*). *Fish Physiology Biochemistry*, 36: 719-730.

Capítulo I

Anesthesia with benzocaine and MS-222 on juvenile tambaqui *Colossoma macropomum*: effect on oxidative stress parameters

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Artigo a ser submetido para a revista científica Aquaculture Research

Abstract

The present study aimed to evaluate the effects of two anesthetics (benzocaine and tricaine methanesulfonate - MS-222) over biochemical parameters of juvenile tambaqui *Colossoma macropomum* tissues. This study was carried out with 80 juvenile tambaqui were used, which were anesthetized with benzocaine (BZ – 100 ppm) or MS-22 (TR – 240 ppm). Other two grups, named control: control water (without anesthesia) and control alcohol (exposure to ethanol) also went through the same procedure. After anesthetic induction 10 fish/anesthetic/time of sampling were euthanized at 3, 12 and 24 hours after anesthesia for tissue sampling (gills, liver and brain), which were submitted to the analyses of the antioxidant enzyme (GST), as well as cellular lipid peroxidation (TBARS) and total antioxidant capacity (ACAP) at different sampling times. There was an increase in the ACAP on the gills of BZ treatment compared to TR and control group at 12 h. The liver showed a reduction in the ACAP of TR treatment at 12 h compared to control group. The treatments BZ and TR showed an increase of ACAP at 24 h compared to control group. The activity of the GST enzyme increased on the gills for treatments BZ and TR at 3 and 12 h compared to control group. Liver showed increased activity for treatments BZ at 24 h and TR at 3 and 24 h compared to control. There was a decrease of lipid damage on gills (BZ and TR) and brain (TR) at 24 h compared to control group. The results demonstrate that the anesthetics did not cause oxidative damage in juvenile tambaqui, but are capable of producing an antioxidant response to prevent the lipid peroxidation and induce the detoxification of the organism.

Keywords: Anesthetics, Oxidative Stress, Fish, GST, ACAP, Lipid peroxidation

Introduction

Tambaqui *Colossoma macropomum* (Cuvier 1816) of *Characiformes* order and *Characidae* family is an omnivorous species indigenous to the basins of Amazon and Orinoco rivers (Araujo-Lima & Goulding, 1997). In natural environment this species can reach up to 1 meter in length and 30 kg of live weight (Saint-Paul 1986). It is well adapted to captive conditions, accepting artificial diets with good growth rates and feed conversion (Inoue, Boijink, Ribeiro, Silva, and Affonso, 2011). In Brazil is the second most produced species (88,718,502 kg) (IBGE 2013).

The vast production of this species requires special attention during handling practices, such as biometrics, blood sampling and transportation which are necessary to monitor the growth and general animal health. Due to these reasons, anesthetics are important to aid to reduce stress and mortality caused by these procedures (Gomes, Chippari-Gomes, Lopes, Roubach, and Araujo-Lima, 2001; Inoue *et al*. 2011; Sneddon 2012). Furthermore, the use of anesthetic can prevent physiological changes in fish which can trigger various diseases and deleterious effects (Weber III 2011).

Many chemical compounds are used for fish anesthesia, among which the most commonly used are MS-222 (tricaine methanesulfonate) and benzocaine; besides those, there is still quinaldine, 2- phenoxyethanol, metomidate and Aqui-S™ (Gomes *et al.* 2001; Coyle, Durborow, and Tidwell, 2004; Sneddon 2012; Vera, Montoya, and Sánchez-Vázquez, 2013).

The tricaine, also known as MS-222 or tricaine methanesulfonate is a benzocaine isomer with a sulfonate radical. This substance may be dissolved in water and lipids (Popovic, Strunjak-Perovic, Coz-Rakovac, Barisic, Jadan, Berakovic, and Klobucar, 2012). For juvenile tambaqui the effective dose of tricaine that induces

anesthesia without causing mortality is 240 ppm (Barbas *et al*. p*ersonal communication*).

Another anesthetic, benzocaine (ethyl p-aminobenzoate), is the most commonly used anesthetic in Brazil, mainly because it is ease to purchase, safe to users and have low cost (Gimbo, Saita, Golçalves, and Takahashi, 2008). Many studies have been conducted on the anesthetic potential of benzocaine for different species, among which the crucian carp *Carassius carassius* (Linnaeus, 1758**)** (Heo and Shin 2010), matrinxã, *Brycon cephalus* (Günther, 1869) (Inoue, Hackbarth, and Moraes, 2004), pompano, *Trachinotus marginatus* (Cuvier 1932) (Okamoto, Tesser, Louzada, Santos, and Sampaio, 2009) and fat snook, *Centropomus parallelus* (Poey 1860) (Souza, Carvalho, Nunes, Scopel, Guarizi, and Tsuzuki, 2012). Gomes *et al.* (2001) evaluated the anesthetic effect of benzocaine on tambaqui, with an effective concentration of 100 ppm for this species, moreover, it does not cause any mortality.

Although anesthesia is able to mitigate biochemical and physiological disorders in fish caused by handling practices, the very anesthetic may induce effects that cause changes in biochemical parameters and oxidative stress (Velisek, Stara, Li, Silovska, and Turek, 2011). These changes may occur due to hypoxia caused when fish are anesthetized and, moreover, due to re-oxygenation that may lead to a hyperoxia situation at the moment fish are placed under normal conditions of dissolved oxygen on recovery (Azambuja, Mattiazzi, Riffel, Finamor, Garcia, Heldwein, Heizmann, Baldisseroto, Pavanato, and Llesuy, 2011).

These physiological changes caused by the use of anesthetics may trigger an oxidative stress scenario (Halliwell and Gutteridge 1999), which is characterized by an imbalance between the production of pro oxidants and the antioxidant defenses inside

cells. The pro oxidants or Reactive Oxygen Species (ROS) are formed from the oxygen reduction in the tissues (Belló, Fortes, Belló-Klein, Belló, Llesuy, Robaldo, and Bianchini, 2000) and produced in greater amounts when an organism is exposed to xenobiotics (Tew and Ronai 1999) or submitted to stress scenarios. They are responsible for causing damage to cellular proteins, lipids, nucleic acids among other structures, leading to permanent damage in tissues and organs (Storey 1996; Lushchak, Bagnyukova, Husak, Luzhna, Lushchak, and Storey, 2005; Fogaça and Sant'Ana, 2009).

Therefore, our study aims to assess whether there are changes on oxidative stress parameters on gills, liver and brain of juvenile tambaqui after anesthesia with benzocaine and MS-222.

Material and methods

Fish were purchased from a commercial fish farm (Rio Preto da Eva, Amazon, northern Brazil) and maintained under acclimation at the Continental Aquaculture Laboratory – LAC/FURG (Rio Grande, RS, southern Brazil) for 30 days in five tanks of 250 L capacity with clean freshwater, arranged in a recirculation system.

Water parameters (mean ± SE) were maintained as follows: Temperature (T) (27.3 ± 0.14 ºC) and Dissolved Oxygen (DO) (6.7 ± 0.06 mg L^{-1}) were measured daily using an oxygen meter (Yellow Springs Instruments YSI DO200A); pH (7.5 ± 0.03) was also measured daily using a pH meter (Hanna Instruments HI 8424). Total ammonia nitrogen (TAN) (0.8 ± 0.28 mg L^{-1}) was determined according to Eaton, Clesceri, Rice, and Greenberg, (2005), and nitrite (0.03 ± 0.01 mg L^{-1}) and total alkalinity (70.0 ± 0.1 mg $CaCO_3$ L^{-1}) were evaluated in accordance to Boyd (1998). A 12 L/12D photoperiod

was fixed. During experimental period the water quality showed the follows values: temperature, dissolved oxygen, alkalinity and pH presented the same acclimation values; TAN and nitrite showed the values of 0.04 ± 0.01 mg L^{-1} and 0.0 mg L^{-1}, respectively. Fish were fed twice a day (09:00 and 16:00 h) at 2% biomass with 28% crude protein commercial feed. All food and feces remains were siphoned daily and the water restored under the same conditions as in the tanks (20% of system total amount of water).

Two anesthetics were used in the experiment: tricaine methanesulphonate (Sigma Chemical E10521 - St Louis, MO, USA) and benzocaine (Sigma Chemical E1501 - St Louis, MO, USA). Tricaine was dissolved directly in the anesthesia water and buffered with $NaHCO_3$ at a 1:1 rate to achieve a neutral pH. Benzocaine was previously dissolved in ethanol (1:10) to make a stock solution before being added to the anesthesia water.

Tambaqui juveniles (40 fish, 45.83 ± 1.66 g) were anesthetized with a 100 ppm benzocaine concentration previously established for the species by Gomes *et al*. (2001). Another group of 40 fish (46.78 ± 1.35 g) were anesthetized with a concentration of 240 ppm of tricaine methanesulphonate, known as the appropriate dosage to induce anesthesia in tambaqui (Barbas *et al*. *personal communication*). In both trials, fish were anesthetized individually in 30 L aquarium containing 10 L of the acclimation tanks water. Each fish was kept in the aquarium until they had lost total equilibrium and opercular movement (less than 3 minutes in the aquarium) and put through recovery in 40 L tanks, in groups of 10 fish each, with water under the same conditions as the acclimation tanks. The fish remained in those tanks until time of sampling.

The sampling of tissues occurred in three periods of time after the fish were exposed to the anesthetics: 3, 12 and 24 hours. For each treatment (anesthetic) and time of sampling a group of 10 fish was used. Also a group of 10 non-anesthetized fish was used as a control group. To verify if the ethanol used to dissolve benzocaine could cause any biochemical alteration in the fish tissues, another group was kept for 3 minutes (maximum time to induce anesthesia according to Park, Hur, Im, Seol, Lee, and Park, 2008) in a 30 L aquarium containing 10 L of the acclimation tanks water with the maximum dosage of ethanol used to dissolve benzocaine. The ethanol group also went through a 3 minutes recovery, and only then the tissues were collected.

Fish were randomly captured with a net, euthanized with a blow to the head, and only then gills, liver and brain were sampled. The tissues were maintained in an ultrafreezer (-80 ºC) and then homogenized at a rate of 1:5 (w/v) in GCL buffer (Tris-HCl – 100 mM; EDTA – 2 mM; and $MgCl_2.6H_2O$ – 5mM). The supernatants resulting from the centrifugation of the homogenates (10,000 x*g*, 20 minutes, 4ºC) were used for all analyses. After homogenization, total protein content was determined through Biuret method. Thereafter, samples of gill and liver were diluted with GCL buffer to 2.0 mg protein mL^{-1} and brain to 0.5 mg protein mL^{-1} for posterior analysis of total antioxidant capacity against peroxyl radicals.

Total antioxidant capacity against peroxyl radicals was determined according to the method described by Amado, Garcia, Ramos, Freitas Zafalon, Ferreira, Yunes, and Monserrat, (2009). The fluorescence was determined through a microplate reader (Victor 2, Perkin Elmer), at 37ºC (excitation: 488 nm; emission: 525nm) with readings every 5 minutes, for 30 minutes. The results were expressed as a relative area (the

difference between the area with and without ABAP divided by the area without ABAP).

The lipid peroxidation of all tissues was measured using the methodology described by Oakes and Van Der Kraak (2003). To determine the thiobarbituric acid reactive substances (TBARS) by the quantification of MDA (malondialdehyde), 20 µl of BHT solution (67µM), 150 µl 20% acetic acid solution, 150 µl 0.8 % TBA solution, 50 µl Milli-Q H_2O, and 20 µl of 8.1% SDS were added to 30 µl of each sample homogenate before being heated to 95 ºC with water bath for 30 minutes. Thereafter, 100 µl of Milli-Q H_2O and 500 µl of nbutanol and pryridine (15:1 v/v) solution were added to the final solution. The remaining supernatant of centrifugation (3000 rpm, 10 minutes, 15 ºC) was used to determine the fluorescence (553 nm) and the results were expressed as nmol MDA mg protein^{-1}.

To determine the levels of GST produced in each tissue collected, the methodology described by Habig, W.H, Pabts, M.J. and Jacobi, W.B., (1964) was used. A potassium phosphate buffer (KH_2PO_4 – 0.05 M; K_2HPO_4 – 0.05 M; and Milli-Q H_2O; pH = 7.0) was used as substrate (previously heated to 25 ºC with water bath) for 1-Chloro-2,4-dinitrobenzene (CDNB) (50 mM solution with ethanol) and reduced glutathione (GSH – 25 mM). The CDNB and GSH were added to 10 µl of each sample homogenate in a transparent 96 well microplate (340 nm, microplate reader). The results were expressed as nmol mg protein^{-1} minute^{-1}.

The experimental design was completely randomized with 4 treatments (benzocaine, MS-222, control group and alcohol group) and three times of collection (3, 12 and 24 hours after anesthesia), each treatment with 10 repetitions (10 fish), totaling 80 animals.

All collected data from analyzes were expressed as means ± standard deviation. To verify normality and homocedasticity of variances, data were submitted to Kolmogorov-Smirnov and Levene tests, respectively. When at least one of those conditions was not met, the necessary mathematical transformations were performed. Thereafter the data were submitted to a Two-Way ANOVA (time and treatment) followed by Tukey test. When ANOVA assumptions were not met, even after transformation, Kruskall-Wallis non-parametric test was performed followed by Mann-Whithney multiple comparison test. All analyzes were performed with a minimum significance level of 95% ($p < 0.05$).

Results

Total Antioxidant Capacity - ACAP

The results of the ACAP analyses in the gills of tambaquis demonstrated that there was a significant increase in antioxidant capacity of fish anesthetized with benzocaine compared to those exposed to tricaine and control group at the 12 hour mark post anesthesia (PA). There was no difference between times for fish exposed to tricaine. However, for fish exposed to benzocaine, there was a greater antioxidant capacity at 12 h compared to 24 h PA (Figure 1A).

In relation to the liver, there were no significant differences between treatments in any of the times tested, neither over time for each treatment. Meanwhile, fish anesthetized with tricaine showed a significant decrease of the antioxidant capacity compared to control at the 12 h PA (Figure 1B).

Like the liver, the brain also did not show significant differences between treatments in none of the time tested, neither over time for each treatment. Nevertheless,

at 24 h PA the fish anesthetized with benzocaine and tricaine were significantly different from control group, showing a greater antioxidant capacity (Figure 1C).

Glutathione S-transferase - GST

The GST activity on gills of tambaqui did not show statistical differences between treatments in none of the times, the same occurring over time for each treatment. In this same tissue was observed a significantly increase on the GST activity at 3 and 12 h PA of animals anesthetized with benzocaine and tricaine compared to control (Figure 2A).

The liver of the fish anesthetized with benzocaine or tricaine did not show significant differences between them at the same time. The group exposed to benzocaine showed significant increasing on the GST activity compared to control only at 24 h PA, not showing changes in the enzymatic activity over time. There was an increase in the GST activity for tricaine treatment compared to control group at 3 and 24 h PA, being the 24 h activity significantly higher than 12 h PA (Figure 2B).

The GST activity on brain of tambaqui did not suffer any changes over time for tricaine treatment. Also no differences occurred between benzocaine and tricaine within each time, neither between treatments and control group. However, the benzocaine treatment showed a GST activity significantly greater at 12 and 24 h compared to 3 h PA (Figure 2C).

Lipid Peroxidation - TBARS

There were no significant differences between treatments at the same periods of time, neither among the same treatment over the different times in relation to the levels

of lipid peroxidation of the tambaquis gills. Except at the 24 h PA, when occurred a significant decrease in the levels of lipid peroxidation of the fish anesthetized with benzocaine and tricaine compared to control group (Figure 3A).

The liver also did not show differences between benzocaine and tricaine treatment, neither the treatments compared to control group at the same period of time. The levels of lipid peroxidation of the benzocaine treatment did not vary over time. Otherwise, the tricaine treatment showed a significant decrease in the lipid peroxidation level at 24 h PA compared to 3 h PA (Figure 3B).

The levels of lipid peroxidation on the brain did not show any differences between treatments at the same period of time, except at 24 h PA when a significant decrease occurred in the lipid peroxidation levels in fish anesthetized with tricaine compared to control. Fish anesthetized with benzocaine showed a significantly increase in the lipid peroxidation levels at 12 h compared to 3 h PA (Figure 3C).

Discussion

The oxidative stress is caused by an imbalance between the generation of pro oxidants and antioxidants in an organism, and may be triggered by physiological changes (Halliwell & Gutteridge 1999) generated by stress or by exposure to pollutants or xenobiotics (Tew & Ronai 1999). Various physiological changes of an organism are responsible for pro oxidants generation (ROS – Reactive Oxigen Species), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Storey 1996). To prevent ROS attack, fish developed an antioxidant defense mechanism composed of non enzymatic (α-tocopherol, β-carotene, ascorbic acid) and enzymatic agents (catalase, superoxide dismutase, glutathione S-transferase) (Gutteridge 1995).

In the present study we observed increases of GST activity on gills and liver of tambaquis exposed to benzocaine and tricaine, which can be explained as an attempt of these tissues to detoxify the anesthetics/xenobiotics through the activation of an enzymatic antioxidant response improved the oxidative status of tambaqui. The GST increase was also described to Salbego, Becker, Parodi, Zeppenfeld, Gonçalves, Loro, Morsch, Schetinger, Maldaner, Morel, & Baldisserotto, (2015) for silver catfish, *Rhamdia quelen* (Quoy & Gaimard, 1824) (420.1 ± 8.8 g and 21.2 ± 2.3 cm) submitted to transportation with methanolic extract of *Condalia Buxifolia* (sedative) added to the water. An increase in the GST activity of liver of *Rhamdia quelen* submitted to anesthesia with essential oil of *Aloysia triphylla* (135 and 180 mg L^{-1}) was also described by Gressler, Riffel, Parodi, Saccol, Koakoski, Costa, Pavanato, Heinzmann, Caron, Schmidt, Llesuy, Barcellos & Baldisserotto (2012), but the same authors verified that this species, when anesthetized with tricaine (150 and 300 mg L^{-1}) showed a decrease in the liver GST activity. It is important to reinforce that the enzyme GST (glutathione S-transferase) has detoxification functions by catalyzing the conjugation of reduced glutathione (GSH) to nucleophilic xenobiotics or ROS damaged cellular components (Storey 1996).

Because tambaquis underwent anesthesia in less than a three minutes period, these fish gills were not exposed for too long to the anesthetics. Because of this fact, the GST activity on gills was observed until 12 h PA. However, on liver, both tricaine and benzocaine showed increases on GTS activity in the periods of 3 and 24 h PA compared to control, and at 12 h this activity did not differ from control. This later enzymatic response (24 h PA) showed on liver may be explained by the fact that this is a very metabolically active organ and has a high detoxifying potential (Tkachenko, H.,

Kurhaluk, N., Grudniewska, J. & Andriichuk, A., 2014), and its detoxifying activity remains active in the organism for a higher period of time.

The GST activity on liver (tricaine) and brain (benzocaine) also showed changes over time after anesthesia. These changes suggest that enzymes in this species may suffer influence of a circadian rhythm and might have their activity changed over the day (Vera *et al*. 2013). The same authors verified, for the first time, that samples of gilthead seabream, *Sparus aurata* (Linnaeus, 1758) submitted to different feeding regimes (in the presence and absence of light) and anesthesia (MS-222) showed changes in the effectiveness of MS-222 and in the GST activity on liver according to the circadian rhythm. In our study, the increase in the GST activity indicates that this enzyme functioned on the detoxification of the fish organism submitted to the anesthetics benzocaine and tricaine, thereby assisting in maintaining the balance between pro oxidants and antioxidants.

Despite the fact that enzymatic activities are possible indicators of oxidative stress generation, each organ, tissue, and organism produces antioxidants responses in different ways (Amado *et al*. 2009). Because of this fact, our study conducted ACAP analyzes, thereby observing that an increase in the antioxidant capacity occurred in gills of fish 12 h PA with benzocaine. This may be related to the fact that total antioxidant capacity also incorporates enzymatic activities, including GST, that showed greater activity in fish anesthetized with benzocaine and tricaine until 12 h PA. These results allow us to state that fish anesthetized with these two anesthetic substances do not exhibit prolonged changes, indicating minimal tissue damage in the studied species.

However, in general, the antioxidant capacity of the treatments in liver remained similar to group control over time, as in the study conducted by Harayashiki, Varela

Junior, Machado, Cabrera, Primel, Bianchini, & Coricini, (2013) that, by testing two Roundup concentrations (130 and 700 μg L^{-1} of glyphosate) in male (0.54 ± 0.06 g, 3.8 ± 1.2 cm) and female (0.41 ± 0.03 g; 3.5 ± 0.9 cm) guppy, *Poecilia vivipara* (Bloch & Schneider, 1801) found no differences in the antioxidant capacity of liver and gills, concluding that, besides the herbicide does not change biochemical parameters of this species it causes differences between genders in these parameters responses.

The increasing in the total antioxidant capacity in brain of tambaquis in 24 h PA may have occurred, either because the anesthetic induces an antioxidant response, improved the oxidative status in this period of time or reduces the generation of ROS in this tissue.

One way to verify if a xenobiotic was able to cause oxidative stress in an organism is the evaluation of cell damage, especially in relation to cell membranes, composed of lipoprotein susceptible to ROS action. ROS have deleterious effects as oxidation of proteins, DNA and steroids components, besides being able to cause peroxidation of cell membrane lipids (Martínez-Álvarez, Morales, & Sanz, 2005). This lipid peroxidation of membranes (attack against polyunsaturated fatty acids) causes changes in its molecules, thereby modifying its fluidity and ionic control (Storey 1996). The reactive oxygen species are capable of reacting with a hydrogen atom of a methylen group (-CH_2-) of lipid membranes, weakening the C-H bond and allowing the remaining hydrogen to be removed, thereby compromising the composition and structure of cellular membrane (Gutteridge 1995).

The gills of the fish anesthetized with benzocaine and tricaine showed a decrease in lipid peroxidation (LPO) at 24 h PA. This result may be explained by increase to the enzyme GST that, also in the period of 24 h PA maintained its activity similar to the

control group after increasing at the first two sampling times (3 and 12 h). The decrease in LPO at 24 h PA must have occurred due to the use of the GST enzyme in the gill produced in the first moments PA as an antioxidant response and also because, probably, the tissue had no longer the presence of the anesthetic after 24 hours, the GST activity was reduced. Therefore, the enzymatic activity of GST is directly related to the LPO (measured by TBARS) reduction, indicating lower ROS production.

In the liver the levels of LPO were not changed due to anesthetic exposure, but fish anesthetized with tricaine varied over time, and decreased lipid peroxidation at 24 h compared to 3 h PA. These results may be related to the high detoxifying capacity functions of the liver along with the activity of the GST enzyme, was able to reduce the LPO over time after the administration of anesthetic. Differently from the present study, Gressler *et al.* (2012) observed a decrease in the lipid peroxidation on the liver of *Rhamdia quelen* during recovery after the fish were anesthetized with 300 mg L^{-1} of tricaine.

The decrease in the fish brain LPO levels anesthetized with tricaine at the 24 h PA occurs due to the increase of the antioxidant capacity observed during this same period. It can be considered that ACAP and LPO function together, because the total antioxidant capacity is related to antioxidants activities in general (enzymatic and non-enzymatic) and the lipid peroxidation acts as an indicator of the balance between ROS/ACAP (Machado, Hoff, Klein, Cardozo, Giacomim, Pinho, & Bianchini, 2013). In general, the antioxidants responses studied in the present work were responsible for maintaining the lipid peroxidation levels similar to or lower than the control group in all tissues over time, demonstrating that both anesthetics studied do not interfere positively in oxidative damage and LPO.

Velisek *et al*. (2011) compared the effects of four anesthetics (MS-222, clove oil, 2-phenoxyethanol e Propiscin) over oxidative stress biomarkers in the species rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) (107.78±22.11 g; 226.74±13.06 mm). The authors, as well as the present study, conducted the lipid peroxidation analyzes in liver, brain and gills until 24 hours after exposure to the anesthetics. Brain and liver of trout exposed to MS-222 (24 h PA) showed the highest levels of LPO compared to control group, leading to a conclusion that the use of Propiscin may be better than MS-222 because it causes less damage. However, trout did not show changes in gills, differently from our study when gills and brain of tambaqui anesthetized with MS-222 showed lower levels than control group at 24 h PA, while the liver did not show any changes. Zeppenfeld, Toni, Becker, Miron, Parodi, Heinzmann, Barcellos, Koakoski, Rosa, Loro, Cunha, and Baldisserotto, (2014) evaluated the LPO level in the liver of *Rhamdia quelen* after 6 hours of transportation with two different doses of the *Aloysia triphylla* essential oil and the results of both doses were lower than group control, concluding that it is convenient to use this anesthetic for transportation without causing any damage.

In conclusion, the use of the effective doses of the anesthetic benzocaine (100 ppm) and tricaine (240 ppm) for tambaqui did not cause oxidative damage to the gills, liver and brain. However, the presence of these xenobiotic in the fish tissues was capable of inducing an antioxidant response sufficient to prevent lipid peroxidation and cause the organism's detoxification.

References

Amado, L.L., Garcia, M.L., Ramos, P.B., Freitas R.F., Zafalon, B., Ferreira, J.R.L., Yunes, J.S. & Monserrat, J.M. (2009). A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: Application to evaluate microcystins toxicity. *Science of the total Environment* **40,** 2115-2123.

Azambuja, C.R, Mattiazzi, J., Riffel, A.P.K., Finamor, I.A., Garcia, L.O., Heldwein, C.G., Heizmann, B.M., Baldisseroto, B., Pavanato, M.A. & Llesuy, S.F. (2011). Effect of the essencial oil of *Lippia alba* on oxidative stress parameters in silver catfish (*Rhamdia quelen*) subjected to transport. *Aquaculture* **319,** 156-161.

Araujo-Lima, C. & Goulding, M. (1997) So fruitful a fish. Ecology, conservation and aquaculture of the Amazon's tambaqui. New York: Columbia University Press. 191 p.

Belló, A.R.R, Fortes, E., Belló-Klein, A., Belló, A.A., Llesuy, S.F., Robaldo, R.B. & Bianchini, A. (2000) Lipid peroxidation induced by *Clinostomum detruncatum* in muscle of the freshwater fish *Rhamdia quelen*. *Diseases of Aquatic Organisms* **42**, 233-236.

Boyd, C.E. 1998. Water quality management for pond fish culture: research and development. *International Center for Aquaculture and Aquatic Environment* **43,** 1-37.

Coyle, S.D, Durborow, R.M & Tidwell, J.H. (2004) Anesthetics in aquaculture. *Southern Regional Aquaculture Center* (SRAC). Publication 3900.

Eaton, A.D, Clesceri, L.S., Rice, E.W & Greenberg, A.E. (2005) Standard methods for the examination of water and wastewater. 21st ed, Springfield: American Public Health Association.

Fogaça, F.H.S & Sant'ana, L.S. (2009) Oxidação lipídica em peixes: mecanismo de ação e prevenção (artigo de revisão). *Archives of Veterinary Science* **14,** 117-127.

Gimbo, R.Y, Saita, M.V., Golçalves, A.F.N. & Takahashi, L.S. (2008) Diferentes concentrações de benzocaína na indução anestésica do lambari-do-rabo-amarelo (*Astyanax altiparanae*). *Revista Brasileira de Saúde e Produção Animal* **9**, 350-357.

Gomes, L.C., Chippari-Gomes, A.R., Lopes, N.P., Roubach, R. & Araujo-Lima, C.A.R.M. (2001) Efficacy of benzocaine as an anesthetic in juvenile tambaqui *Colossoma macropomum*. *Journal of the Aquaculture Society* **32,** 426-431.

Gressler, L.T.. Riffel, A.P.K, Parodi, T.V., Saccol, E.M.H., Koakoski, G., Costa, S.T., Pavanato, M.A., Heinzmann, B.M., Caron, B., Schmidt, D., Llesuy, S.F., Barcellos L.J.G. & Baldisserotto, B. (2012) Silver catfish Rhamdia quelen immersion anaesthesia with essential oil Aloysia triphylla (L'Herit) Britton or tricaine methanesulfonate: effect on stress response and antioxidant status. Aquaculture Research 1-12.

Gutteridge, J.M.C. (1995) Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clin. Chem* **41,** 1819-1828.

Habig, W.H, Pabts, M.J. & Jacobi, W.B. (1974) Glutationa-S-Transferase. The first enzymatic step in mercapturic acid formation. *The Journal of Biological Chemistry* **249,** 7130-7439.

Halliwell, B. & Gutteridge, J.M.C. (1999) Free radicals in biology and medicine. 3rd ed, Oxford University Press.

Harayashiki, C.A.Y, Varela Junior, A.S., Machado, A.A.S., Cabrera, L.C., Primel, E.G., Bianchini, A. & Coricini, C.D. (2013) Toxic effects of the herbicide Roundup in the

guppy *Poecilia vivipara* acclimated to fresh water. *Aquatic Toxicology* **142-143**:176-184.

Heo, G. J. & Shin, G. (2010) Efficacy of benzocaine as an anaesthetic for Crucian carp (*Carassius carassius*) (*short communication*). *Veterinary Anaesthesia and Analgesia* **37**, 132-135.

IBGE: Instituto Brasileiro de Geografia e Estatística, (2013) Produção da pecuária municipal (Available in ftp://ftp.ibge.gov.br/Producao_Pecuaria/Producao_da_Pecuaria_Municipal/2012/ppm2012.pdf) Ministério do Planejamento, Orçamento e Gestão, Rio de Janeiro, Brazil **41**, 34-94.

Inoue, L.A.K.A., Hackbarth, A. & Moraes, G. (2004) Avaliação dos anestésicos 2-phenoxyethanol e benzocaína no manejo de matrinxã *Brycon cephalus* (Günther, 1869). *Biodiversidade Pampeana* **2,** 10-15.

Inoue, L.A.K.A., Boijink, C.L., Ribeiro, P.T., Silva, A.M.D. & Affonso, E.G. (2011) Avaliação de respostas metabólicas do tambaqui exposto ao eugenol em banhos anestésicos. *Acta Amazonica* **41**, 327-332.

Lushchak, V.I., Bagnyukova, T.V., Husak, V.V., Luzhna, L.I., Lushchak, O.V. & Storey, K.B. (2005) Hyperoxia results in transient oxidative stress and an adaptive resposnse by antioxidant enzymes in goldfish tissues. *The International Journal of Biochemistry & Cell Biology* **37**, 1670-1680.

Machado, A.A.S, Hoff, M.L.M., Klein, R.D., Cardozo, J.G., Giacomim, M.M., Pinho, G.L.L. & Bianchini, A. (2013) Biomarkers of waterborne copper exposure in the

guppy *Poecilia vivipara* acclimated to salt water. *Aquatic Toxicology* **138-139**, 60-69.

Martínez-Álvarez, R.M., Morales, A.E. & Sanz, A. (2005) Antioxidant defenses in fish: Biotic and abiotic factor. *Fish Biology and Fisheries* **15**, 75-88.

Okamoto, M.H, Tesser, M.B., Louzada, L.R., Santos, R.A & Sampaio, L.A. (2009). Benzocaína e eugenol como anestésicos para juvenis do pampo *Trachinotus marginatus*. *Ciência Rural* **39**, 866-870.

Oakes, K.D. & Kraak, V.D. (2003) Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations. *Aquatic Toxicology* **63**, 447-463.

Park, M.O, Hur, W.J., Im, S.Y., Seol, D.W., Lee, J. & Park, I.S. (2008) Anaesthetic efficacy and physiological responses to clove oil-anaesthetized kelp grouper *Epinephelus bruneus*. *Aquaculture Research* **39**, 877-884.

Popovic, N.T, Strunjak-Perovic, I., Coz-Rakovac, R., Barisic, J., Jadan, M., Berakovic, A.P. & Klobucar, RS. (2012) Tricaine methane-sulfonate (MS-222). *Journal of Applied Ichthyology* **28**, 553-564.

Saint-Paul, U. (1986) Potential for aquaculture of South American freshwater fishes: A review. *Aquaculture* **54**, 205-240.

Salbego, J., Becker, A.G., Parodi, T.V., Zeppenfeld, C.C., Gonçalves, J.F., Loro, V.L., Morsch, V.M.M., Schetinger, M.R.C., Maldaner, G., Morel, A.F. & Baldisserotto, B. (2015) Methanolic extract of *Condalia buxifolia* added to transport water alters biochemical parameters of the silver catfish *Rhamdia quelen*. *Aquaculture* **437**, 46-50.

Sneddon, L.U. (2012) Clinical anesthesia and analgesia in fish. *Journal of Exotic Pet Medicine* **21**, 32-43.

Souza, R.A.R., Carvalho, C.V.A., Nunes, F.F., Scopel, B.R., Guarizi, J.D. & Tsuzuki, M.Y. (2012) Efeito comparativo da benzocaína, mentol e eugenol como anestésicos para juvenis de robalo peva. *Boletim do Instituto de Pesca* **38**, 247-255.

Storey, K.B. (1996) Oxidative stress: animal adaptations in nature. *Brazilian Journal of Medical and Biological Research* **29**, 1715-1733.

Tew, K.D. & Ronai, Z. (1999) GST function in drug and stress response. *Drug Resistance Updates* **2**, 143-147.

Tkachenko, H., Kurhaluk, N., Grudniewska, J. & Andriichuk, A. (2014) Tissue-specific responses of oxidative stress biomarkers and antioxidant defenses in rainbow trout *Oncorhynchus mykiss* during a vaccination against furunculosis. *Fish Physiol Biochem* **40**, 1289–1300.

Velisek, J, Stara, A., Li, Z-H., Silovska, S. & Turek, J. (2011) Comparison of the effects of four anesthetics on blood biochemical profiles and oxidative stress biomarkers in rainbow trout. *Aquaculture* **310**, 369-275.

Vera, L.M, Montoya, A. & Sánchez-Vázquez, F.J. (2013) Effectiveness of the anaesthetic MS-222 in gilthead seabream, *Sparus aurata*: Effect of feeding time and day–night variations in plasma MS-222 concentration and GST activity. *Physiology & Behavior* **110-111**, 51-57.

Weber III, E.S. (2011) Fish analgesia: pain, stress, fear aversion, or nociception? Veterinary Clinics of North America: *Exotic Animal Practice Journal* **14**, 21-32.

Zeppenfeld, C.C, Toni, C., Becker, A.G., Miron, D.S., Parodi, T.V., Heinzmann, B.M., Barcellos, L.J.G., Koakoski, G., Rosa, J.G.S., Loro, V.L., Cunha, M.A. &

Baldisserotto, B. (2014) Physiological and biochemical responses of silver catfish, *Rhamdia quelen*, after transport in water with essential oil of *Aloysia triphylla* (L'Herit) Britton. *Aquaculture* **418-419**, 101-107.

Figure 1. Total antioxidant capacity (ACAP) of gills (A), liver (B) and brain (C) of juvenile *Colossoma macropomum* anesthetized with benzocaine or tricaine. Values are expressed as means ± SD. Different lowercase letters indicate significant differences between treatments in the same time of collection; different capital letters indicate significant differences in the same treatment throughout the time; asterisk indicates significant difference between treatment and control ($P<0.05$).

Figure 2. Activity of the enzyme glutathione S-transferase (GST) of gills (A), liver (B) and brain (C) of juvenile *Colossoma macropomum* anesthetized with benzocaine or tricaine. Values are expressed as means ± SD. Different lowercase letters indicate significant differences between treatments in the same time of collection; different capital letters indicate significant differences in the same treatment throughout the time; asterisk indicates significant difference between treatment and control ($P<0.05$).

Figure 3. Levels of lipid peroxidation (TBARS) of gills (A), liver (B) and brain (C) of juvenile *Colossoma macropomum* anesthetized with benzocaine or tricaine. Values are expressed as means ± SD. Different lowercase letters indicate significant differences between treatments in the same time of collection; different capital letters indicate significant differences in the same treatment throughout the time; asterisk indicates significant difference between treatment and control ($P<0.05$).

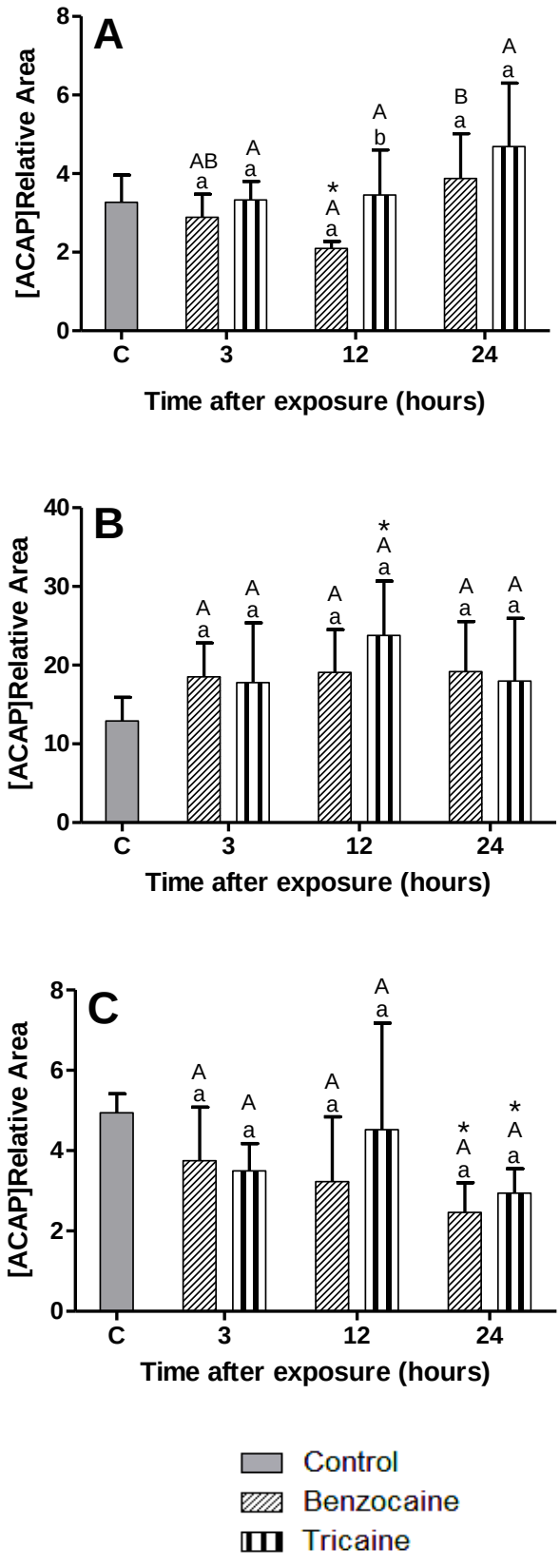
A
[ACAP]Relative Area
8
6
4
2
0
C
3
12
24
Time after exposure (hours)
AB a
A a
*A a
A b
B a
A a
B
[ACAP]Relative Area
40
30
20
10
0
C
3
12
24
Time after exposure (hours)
A a
A a
A a
*A a
A a
A a
C
[ACAP]Relative Area
8
6
4
2
0
C
3
12
24
Time after exposure (hours)
A a
A a
A a
A a
*A a
*A a
Control
Benzocaine
Tricaine

Figure 1

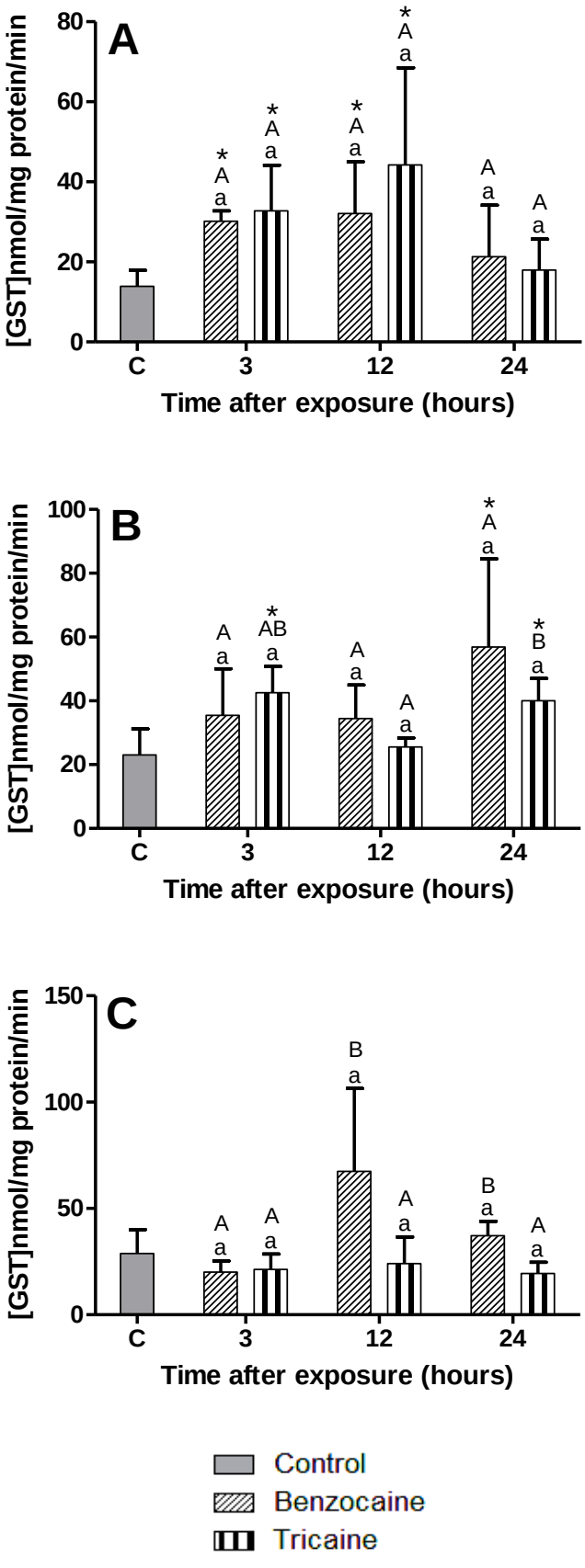
A
[GST]nmol/mg protein/min
80
60
40
20
0
C
3
12
24
Time after exposure (hours)
B
100
80
60
40
20
0
C
3
12
24
Time after exposure (hours)
C
150
100
50
0
C
3
12
24
Time after exposure (hours)
Control
Benzocaine
Tricaine

Figure 2

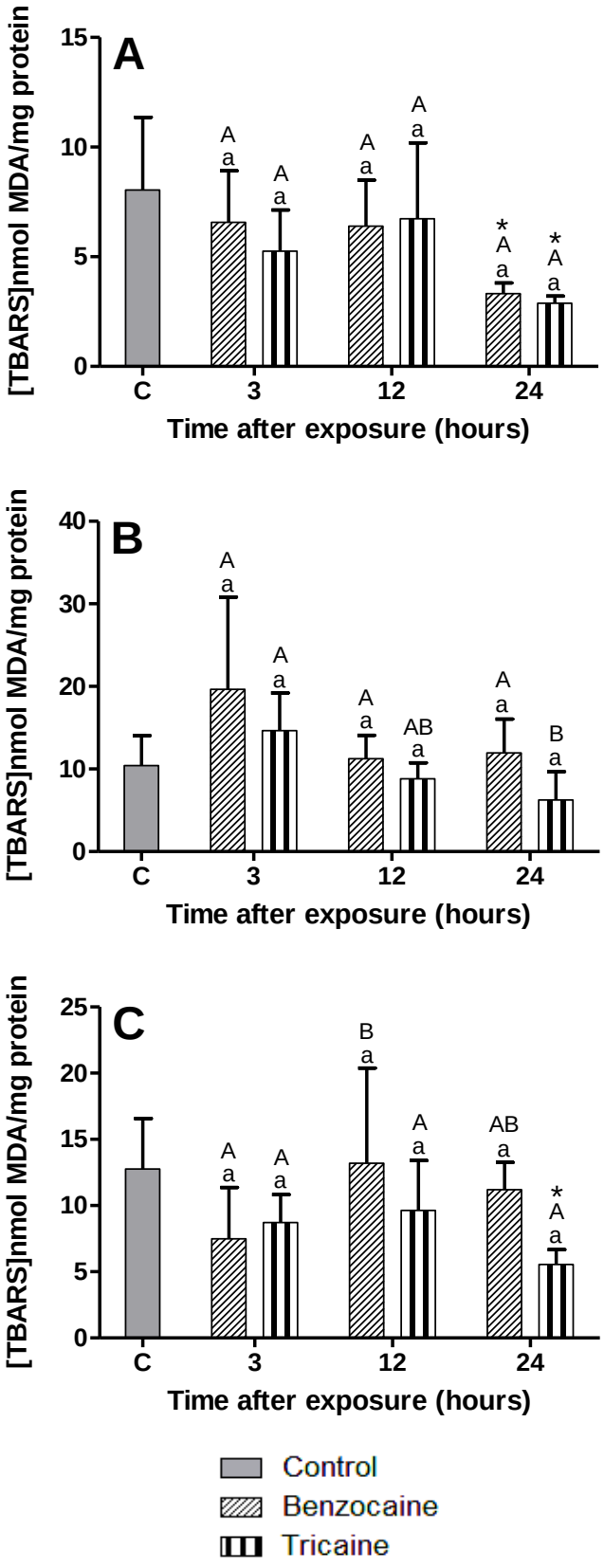
A
[TBARS]nmol MDA/mg protein
15
10
5
0
C
3
12
24
Time after exposure (hours)
B
40
30
20
10
C
25
Control
Benzocaine
Tricaine

Figure 3

Conclusões Gerais

- Os anestésicos utilizados no presente estudo, benzocaína (100 ppm) e tricaína (240 ppm), alteram os parâmetros de estresse oxidativo nos juvenis de tambaqui *Colossoma macropomum*;
- Portanto, com base nos resultados obtidos no presente estudo, benzocaína (100 ppm) e tricaína (240 ppm) podem ser utilizados para tambaqui, em suas doses eficazes de anestesia, sem causar danos oxidativos, podendo ainda beneficiar os animais pela diminuição da lipoperoxidação.