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CONCENTRAÇÕES GRADUADAS DE CLORIDRATO DE LIDOCAÍNA NA MODULAÇÃO DAS RESPOSTAS COMPORTAMENTAIS, CARDÍACAS E MUSCULARES DO PEIXE DE ÁGUA DOCE AMAZÔNICO TAMBAQUI (*Colossoma macropomum*)

BELÉM-PA
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Documento de Dissertação apresentada ao Programa de Pós-graduação em Farmacologia e Bioquímica do Instituto de Ciências Biológicas da Universidade Federal do Pará como requisito para a obtenção do título de Mestre em Farmacologia e Bioquímica.

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RESUMO

O presente estudo teve como objetivo caracterizar o comportamento e avaliar as respostas eletromiográficas e eletrocardiográficas do tambaqui (C. macropomum) expostos a diferentes concentrações de cloridrato de lidocaína em banhos de imersão e após transferência para água sem anestésico. Para tanto, peixes juvenis (25,38 ± 6,5 g) foram aclimatados em aquários por 15 dias em condições controladas de ambiente e qualidade da água. Quatro concentrações de cloridrato de lidocaína (AL) (150, 175, 200 e 225 mg/L LA) foram usadas em banhos de imersão para avaliar alterações comportamentais, eletromiográficas e eletrocardiográficas durante a indução anestésica de curto prazo (exposição de 5 min) e em uma lavagem. Após a exposição ao LA, e independentemente da concentração, os peixes apresentaram perda do reflexo de endireitamento, juntamente com miorrelaxamento significativo, que foi parcialmente alcançado nas concentrações de 150 e 175 mg/L LA e totalmente alcançado em 200 e 225 mg/L LA. Notavelmente, uma diminuição da frequência cardíaca e alteração do traçado da onda T ocorreram em concentrações mais altas. No entanto, embora protocolos utilizando LA a 225 mg/L ou acima possam exigir maior cuidado no monitoramento, a rápida reversibilidade de tais respostas em peixes em recuperação indica que LA pode ser usado com segurança em banhos de imersão para tambaqui C. macropomum como alternativa a outros anestésicos, especialmente aqueles nos quais importantes efeitos colaterais foram documentados.

Palavras-chave: Anestesia de peixe, sedação, eletromiograma, eletrocardiograma, bem-estar dos peixes.

ABSTRACT

The present study was designed to characterize the behavior and evaluate the electromyographic and electrocardiographic responses of tambaqui (C. macropomum) exposed to different concentrations of lidocaine hydrochloride in immersion baths and after transfer to anesthetic-free water. For this purpose, fish juveniles (25.38 ± 6.5 g) were acclimated to aquariums for 15 days under controlled environmental and water quality conditions. Four concentrations of lidocaine hydrochloride (LA) (150, 175, 200, and 225 mg/L LA) were used in immersion baths to evaluate behavioral, electromyographic, and electrocardiographic changes during short-term anesthetic induction (5-min exposure) and in a washout. Upon exposure to LA, and irrespective of concentration, fish showed loss of the righting reflex, along with significant myorelaxation, which was partially achieved at concentrations of 150 and 175 mg/L LA and fully attained at 200 and 225 mg/L LA. Notably, a decreased heart rate and alteration of the T wave tracing occurred at higher concentrations. Notwithstanding, although protocols using LA at 225 mg/L or above could require greater care in monitoring, the prompt reversibility of such responses in recovering fish indicates that LA can be safely used in immersion baths for tambaqui C. macropomum as an alternative to other anesthetics, especially those in which important side effects have been documented.

Keywords: Fish anesthesia, Sedation, Electromyogram, Electrocardiogram, Fish welfare

LISTA DE FIGURAS

SUMÁRIO

1 VISÃO INTEGRADORA DO PROBLEMA

O Brasil é um país muito visado para a pesca, devido ser um alimento extremamente importante na dieta humana, pois possuem fontes de energias de suma importância para a humanidade como proteína, lipídeos, vitaminas e antioxidantes, com grande variedade de produtos e subprodutos para o consumo humano em que o peixe é o componente principal (JUNIOR, 2014).

O Brasil, por possuir 13,7% de água doce disponível no planeta, caracteriza-se por ser um dos países com maior potencialidade para o desenvolvimento de atividades pesqueiras. Além disso, detém mais de 5 milhões de hectares de águas represadas em reservatórios e hidrelétricas, um total de 8.500 Km de linha costeira, 2,5 milhões de hectares distribuídos em áreas estuarinas e 600.000 hectares de áreas destinadas a carcinicultura, assim como, 4,5 milhões de Km^2 de Zona Econômica Exclusiva (ZEE) para pesca extrativista e da agricultura. Porém, possui uma produção aquícola superior as 562 mil toneladas (FAO, 2016).

A piscicultura continental é uma atividade com potencial elevado, tendo como finalidade o cultivo de peixes, favorecido pelas grandes variedades naturais de espécie existentes e com alto valor no mercado. Pesquisas afirmam, que a região amazônica, por conter cerca de 68% de área hídrica superficial do país, é responsável por 45,7% da produção aquícola continental nacional (SCORVO-FILHO, 2003; SILVEIRA et al., 2009).

A piscicultura é uma atividade em grande expansão nacional, pois em piscicultura, os peixes são submetidos a diversos fatores estressantes como, por exemplo, manejo, transporte, biometria e reprodução induzida. O uso de agente ante-estresse é uma prática comum na aquicultura moderna, devido os anestésicos ser utilizados para induzir os peixes a anestesia durante o manejo, transporte, seleção, marcação dentre outros. Dessa forma, o uso de anestésico não somente ajuda a impedir danos aos peixes, mas também atenua os níveis de estresse (ROSS, 2008; ROUBACH et al., 2005).

Além disso, a escolha de um ativador depende principalmente da sua ativação de indução e imobilização com rápida recuperação. Entre as substâncias utilizadas, estão como drogas sintéticas o Éter dietílico, Benzodiazepinas, Halotano, Lidocaína,

Cetamina, Medetomidina, Propofol, Dióxido de carbono e Benzocaína. Sendo a lidocaína o principal Anestésico local por ser definido como uma droga que pode

bloquear de forma reversível a transmissão do estímulo nervoso no local onde for aplicado, sem ocasionar alterações no nível desconsciência, uma vez que, estudos comprovaram que a lidocaína é muito eficaz quanto sua ação comparado aos outros anestésicos local, devido seus valores de pKa que são mais altos e também pelo custo do anestesio ser baixo (BEECHAM et al., 2021).

Uma alternativa, frente às drogas sintéticas, pode ser obtida com a utilização dos produtos naturais, tais como óleos essenciais de plantas, que possuem um elevado potencial na utilização como anestésico na aquicultura. Um exemplo desse produto é o óleo de cravo, pesquisado em muitos estudos, que tem em sua composição 70 a 90% de eugenol (como principal componente ativo), proporcionando eficiência na indução anestésica, bem como, um bom custo-benefício (WALSH; PEASE, 2002; IVERSEN et al., 2003; KING et al., 2005; MYLONAS et al., 2005; ROUBACH et al., 2005; CUNHA; ROSA, 2006; HAJEK et al., 2006; BARBOSA et al., 2007). Outros produtos naturais, também utilizados em estudos, são o mentol (GONÇALVES, et al.,2008), os óleos essências das plantas erva cidreira (Lippia alba) e a alfavaca - Ocimum gratissimum (CUNHA et al., 2010; AZAMBUJA et al., 2011; SILVA et al., 2012), o extrato ceroso de jambu, S. acmella, também conhecido como agrião-do-Pará (BARBAS et al., 2017), e o óleo essencial de canela-amarela Nectandra grandiflora (BARBAS et al., 2017).

Embora muitos dos fármacos atualmente apresentados como novos anestésicos para peixes e/ou relaxantes musculares (ROSS; ROSS, 2008),para o avanço nos estudos da piscicultura, o conhecimentos sobre a biologia das espécies, dependendo de cada cultivo, favorecem a compreensão da fisiologia e consequentemente do funcionamento dos sistemas orgânicos, o que permite um melhor entendimento das relações e repostas fisiológicas dos animais (SCORVO-FILHO ,2003 ; SILVEIRA et al. 2009),

Dentre os fatores eletrofisiológicos temos, o eletromiograma que detecta, registra e analisa os sinais elétricos da contração do músculo esquelético e pode medir a extensão do relaxamento ou contração muscular alcançada durante a exposição a substâncias xenobióticas em organismos (FUJIMOTO et al., 2017). Enquanto o eletrocardiograma (ECG) consiste em registrar os impulsos gerados pelo tecido do

marcapasso cardíaco (GUIMARÃES et al., 2003). Ondas de ECG (P, Q, R, S e T) e intervalos de tempo (PR, QRS, ST e QT) foram usados para diferenciar peixes saudáveis e doentes (LANGHEINRICH et al., 2003).

Portanto, os produtos anestésicos são de suma importância para o auxílio à piscicultura, deve ser realizada de forma eficiente tanto do ponto de vista biológico, uma vez que, anestesiar os peixes sem causar nenhum problema no crescimento e na reprodução, quanto econômico, visa a utilização de uma dose correta de anestésico, tornando fundamental para evitar desperdícios do produto ou a morte dos peixes por overdoses. Ressaltando que todas essas questões devem está de acordo com comitê de ética (ROUBACH; GOMES, 2001).

Assim, o presente estudo será realizado para investigar quais as concentrações ideais do cloridrato de lidocaína deve ser utilizado sobre os efeitos eletromiográficos, eletrocardiográficos e comportamentais para indicar a segurança da droga na anestesia do tambaqui (Colossoma macropomum).

1.1 Anestésicos no manejo de peixes

A anestesia em peixes geralmente é realizada por imersão, ou seja, o composto anestésico é adicionado na água, provocando perda de sensação parcial ou total do corpo resultando em depressão nervosa, ocasionado por um fármaco (agentes químicos ou físicos). Esses anestésicos locais fazem parte da única classe de drogas que pode bloquear totalmente os impulsos nociceptivos de atingir o córtex cerebral, sendo fundamentais na medicina veterinária e regularmente úteis para produzir e manter a anestesia geral em peixes e anfíbios (CHATGNY et al., 2017; BARLETTA; REED, 2019).

A seleção de anestésicos a serem usados em peixes é complexa. O processo de decisão é baseado na qualidade da indução, manutenção e recuperação da anestesia. Idealmente, a dose de anestésico selecionada deve induzir à inconsciência muito rapidamente e sem estresse acentuado; embora geralmente haja alguma agitação, que é causada pelas propriedades ligeiramente irritantes da maioria destes fármacos (ROSS; ROSS, 2008; MARTINS et al., 2018). Alterações significativas nos parâmetros cardiovasculares e respiratórios têm sérias implicações no curso e na recuperação da anestesia. A anestesia também deve permitir uma recuperação rápida

e completa, com peixes apresentando comportamentos normais, como padrões normais de natação em uma coluna d'água e respostas positivas aos alimentos (NEIFFER; STAMPER, 2009; MARTINS et al., 2018).

Os anestésicos também podem ser induzidos de maneira injetáveis, apesar da maioria das vezes ser administrada na água. Logo, quando essa espécie é utilizada na forma de banho, o anestésico entra através das brânquias e da pele no sistema circulatório, bloqueando algumas ações reflexas. Uma vez realizada a absorção branquial, os anestésicos se difundem para corrente sanguínea na lamela secundária, colaborando para drenagem desse ao sangue arterial eferente, o que caracteriza-se como uma rota mais curta para o sistema nervoso central (MARTINS et al., 2018).

Diante disso, observa-se que, a eficiência anestésica também sofre influência das características físico-químicas das águas, como por exemplo, salinidade, temperatura, pH, entre outros, assim como, fatores biológicos, a saber variações na taxa metabólica, teor de lipídios, tamanho, peso, relação entre área branquial e corporal, além das influências associadas às diferentes espécies (SUMMERFELT; SMITH, 1990).

De modo geral, a indução anestésica deve ser feita de forma rápida e com hiperatividade baixa ou nenhuma. Além disso, o nível de redução da atividade neuronal, a amplitude das modificações elétricas cardíacas ou musculares que um determinado anestésico pode determinar são difíceis de avaliar em peixes, além de raramente investigados. Poucos estudos utilizaram abordagem analítica adequada, como avaliação eletrofisiológica de peixes submetidos à anestesia, o que permite uma avaliação mais adequada em profundidade do processo anestésico em si (LAMBOOIJ et al., 2002; ROBB e ROTH, 2003; BARBAS et al., 2017).

1.1.1 LIDOCAÍNA

A lidocaína, por sua vez, foi desenvolvida na primeira metade do século XX. Entre 1943 e 1946 essa droga foi sintetizada pela primeira vez por Nils Lofgren e Bengt Lundquist, o qual demostrou segurança superior em relação aos agentes anestésico mais antigo (CALATAYUD; GONZÁLEZ, 2003; BEECHAM et al., 2021). Além disso, a lidocaína é muito eficaz quanto sua ação comparado aos outros

anestésicos local, devido seus valores pKa que são mais altos. Essa droga consegue minimizar efeitos colaterais,

por conter acidose, que tem como função diminuir a proporção de moléculas de lidocaína não ionizadas, uma diminuição mais rápida da concentração de lidocaína devido ao aumento do fluxo sanguíneo e potencialmente também pelo aumento da produção de mediadores inflamatórios como o peroxinitrito, que atuam diretamente nos canais de sódio (BEECHAM et al., 2021).

A lidocaína é um anestésico local do tipo amida que atua bloqueando reversivelmente os canais de sódio dependentes de voltagem (CSDV) nos tecidos neuronais, interrompendo assim a despolarização e a transmissão sináptica. A afinidade da lidocaína pelos CSDV varia de acordo com o estado do canal e com o pH do meio, sendo alta quando o canal está aberto e em pH fisiológico (pH de 7,4) e baixa quando está fechado ou em acidose tecidual, como em inflamações por exemplo. (SOTO; GONZÁLEZ; CALERO, 2018; BEECHAM et al., 2021).

A lidocaína é o mais conhecido entre os anestesio, devido ser um medicamento antiarrítmico e por possuir propriedades analgésicas em várias condições patológicas. Essas características favorecem a sua atuação como bloqueando de maneira reversível nos tecidos neuronais nos canais de sódio dependentes de voltagem (CSDV), anulando a despolarização e a transmissão sináptica (HERMANNS et al., 2019).

1.2 Testes farmacológicos utilizando peixes como modelo animal

1.2.1 ELETROCARDIOGRAMA

O eletrocardiograma também é chamado de ECG ou eletrocardiografia. É um exame que avalia a atividade elétrica do coração por meio de eletrodos fixados na pele, uma vez, que fornece monitoração e análise quali-quantitativa da segurança de anestésicos em peixes através da caracterização da frequência cardíaca, das ondas P e T, da amplitude e duração do complexo QRS e duração dos intervalos QT e RR (ROSS, 2001; NEIFFER; STAMPER, 2009; CHOI et al., 2010).

Diante disso, o ECG corresponde à soma dos potenciais de ação cardíacos que emanam da superfície do corpo. Embora existam alguns estudos sobre a monitorização cardiorrespiratória de peixes sob anestesia estes ainda são

relativamente escassos (SANDBLOM et al., 2013;SETH et al., 2013; BARBAS et al., 2017).

Segundo Ross e Ross (2008), o ECG juntamente com a monitoração da capacidade respiratória permitirá a verificação de impactos como arritmia, bradicardia excessiva, parada cardíaca e hipoventilação grave durante a indução, manutenção e retorno da anestesia. Esse monitoramento acabará por esclarecer a interação entre as respostas cardíaca e ventilatória e esclarecer a compatibilidade de um determinado anestésico com a vida, principalmente nos casos em que novos produtos são propostos para anestesia de peixes. Assim, o monitoramento desses marcadores é importante para a avaliação do processo anestésico propriamente (GUIMARÃES et al., 2003; GANONG, 2003).

1.2.2 ELETROMIOGRAMA

A extensão do relaxamento muscular alcançado durante a anestesia dos peixes pode ser medida objetivamente pelo eletromiograma (EMG), que corresponde a técnica de detecção, registro e análise do sinal elétrico proveniente da contração muscular. Dessa forma, essa técnica pode ser realizada de duas maneiras, eletromiografia de superfície (que facilita a captação do sinal mioeletrico a partir de eletrodos exposto na pele), ou eletromiografia de profundidade (constitui de uma técnica invasiva, na qual eletrodos, do tipo agulha ou fio, são introduzidos) (RIBEIRO, 2013)

Portanto, a eletromiografia tem a função de avaliar atividade fisiológica muscular do peixe após o uso do anestésico, por meio de eletrodos são implantados em músculos dorsais do animal (HAYSASHIDA et al., 2013) .

1.2.3 *C. MACROPOMUS* COMO MODELO ANIMAL EM ESTUDOS FARMACOLÓGICOS

O tambaqui (*Colossoma macropomum*) é a segunda espécie mais cultivada no Brasil com uma produção de 139.209 toneladas, sendo distribuída especialmente nas regiões norte, nordeste e centro-oeste, possuindo grande destaque na América latina. Seu crescimento na Amazônia ocorreu devido à implementação do Programa de Desenvolvimento da Aquicultura pelo governo do Amazonas na década de 80, sendo

que a partir daí a produção do tambaqui tem crescido e se expandido em todos os estados da região norte, tornando-se a segunda espécie mais cultivada no país (JUNIOR, 2009, IBAMA, 2014).

Segundo Fortes-Silva (2018), o Tambaqui (*C. macropomum*) é uma das espécies de peixes nativos na América do Sul, cultivados com valor comercial e socioeconômico para fins agrícola. Essa espécie é onívora, nativos das bacias dos rios Amazonas e Orinoco. Em ambiente natural esta espécie pode atingir até um metro de comprimento e 30 kg de peso. A adaptação das espécies em cativeiro, apresenta boas taxas de crescimento e conversão alimentar. Essa espécie é rústica e resistente a patógenos, seja em águas com baixas concentrações de oxigênio dissolvido e elevadas taxas de nitrito, tendo uma boa adaptação na faixa de temperaturas entre 26 e 35 ºC possui um rendimento de carne de 63% e a rentabilidade da criação, varia de 19 a 40 (Figura 1). Espécie Colossoma macropomum.

Figura 1 – Espécie Colossoma macropomum.

Fonte: Elaborado pelo autor do trabalho.

O sucesso de criação do manejo de Tambaqui (*C. macropomum*), se da a partir de suas boas qualidades zootécnicas; a existência de um sistema de criação parcialmente definido; a disponibilidade de ração adequadamente formulada; a viabilidade econômica da sua criação; e a existência de protocolos de manejos básicos como transporte e anestesio. Além disso, se adapta facilmente em cativeiros, tem fácil manejo, aceita bem rações, grãos e boa conversão alimentar. Diante disso, a eficácia em sua criação esta relacionada ao equilíbrio entre eficiência de utilização do alimento e maximização do crescimento (IBAMA, 2014).

A produção em tanques é a melhor prática devido à possibilidade do manejo da qualidade da água e do melhor aproveitamento da ração, que não ficará em contato com a terra. Por se tratar de um material impermeável, impossibilita o contato da água

com o solo e permite a retirada de matéria orgânica acumulada no fundo, que poderia contaminar a água e colocar em risco a saúde e o desenvolvimento correto dos animais. Periodicamente, a cada safra é possível remover toda a matéria orgânica acumulada durante o ciclo produtivo dos peixes e, ao mesmo tempo, aumentar a areação e a capacidade de biomassa dentro dos tanques. Entre os tipos de tanques mais eficazes para a produção da espécie esta o tanque revestido, que permite montar sistemas ambientalmente corretos para tratar a água e fazer o controle sanitário, que resultam no aumento e na intensificação da produção.

1.3 Objetivos

1.3.1 GERAL

Avaliar o relaxamento muscular e atividade elétrica do coração do tambaqui e relacionar ao comportamento durante o tempo de contato em banho de imersão.

1.3.2 ESPECÍFICOS

- Avaliar a latência para a perda do reflexo de postura durante banho de imersão nas concentrações de Cloridrato de lidocaína em 150 mg L^{-1}, 175 mg L^{-1}, 200 mg L^{-1} e 225 mg L^{-1}, e latência para a recuperação do reflexo de postura após o contato;
- Avaliar a atividade Eletromiográfica durante o contato com concentrações 150 mg L^{-1}, 175 mg L^{-1}, 200 mg L^{-1} e 225 mg L^{-1}, durante indução e recuperação da anestesia.
- Avaliar as características eletrocardiográficas durante a indução e recuperação: frequência cardíaca, Intervalo P-Q, Intervalo R-R, Intervalo Q-T, e duração de QRS.

2. ARTIGO 1: CONCENTRAÇÕES GRADUADAS DE CLORIDRATO DE LIDOCAÍNA NA MODULAÇÃO DAS RESPOSTAS COMPORTAMENTAIS, CARDÍACAS E MUSCULARES DO PEIXE DE ÁGUA DOCE AMAZÔNICO TAMBAQUI (COLOSSOMA MACROPOMUM) [1]

Graded concentrations of lidocaine hydrochloride in the modulation of behavioral, cardiac, and muscular responses of the Amazon freshwater fish tambaqui (*Colossoma macropomum*)

Abstract

The present study was designed to characterize the behavior and evaluate the electromyographic and electrocardiographic responses of tambaqui (C. macropomum) exposed to different concentrations of lidocaine hydrochloride in immersion baths and after transfer to anesthetic-free water. For this purpose, fish juveniles (25.38 ± 6.5 g) were acclimated to aquariums for 15 days under controlled environmental and water quality conditions. Four concentrations of lidocaine hydrochloride (LA) (150, 175, 200, and 225 mg/L LA) were used in immersion baths to evaluate behavioral, electromyographic, and electrocardiographic changes during short-term anesthetic induction (5-min exposure) and in a washout. Upon exposure to LA, and irrespective of concentration, fish showed loss of the righting reflex, along with significant myorelaxation, which was partially achieved at concentrations of 150 and 175 mg/L LA and fully attained at 200 and 225 mg/L LA. Notably, a decreased heart rate and alteration of the T wave tracing occurred at higher concentrations. Notwithstanding, although protocols using LA at 225 mg/L or above could require greater care in monitoring, the prompt reversibility of such responses in recovering fish indicates that LA can be safely used in immersion baths for tambaqui C. macropomum as an alternative to other anesthetics, especially those in which important side effects have been documented.

Keywords: Fish anestesia, Sedation, Electromyogram, Electrocardiogram, Fish welfare.

2.1 Introduction

Anesthetics represent a unique class of drugs capable of completely blocking nociceptive impulses in the cerebral cortex. They are essential in experimental research, and veterinary medical practice and have been increasingly used to produce and maintain general anesthesia in fish and amphibians, and also for fish euthanasia and welfare purposes (Aydın and Barbas, 2020; Barbas et al., 2021; Barbas et al.,

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2017; Barletta and Reed, 2019; Chatigny et al., 2018). While deep anesthesia is important for more invasive procedures in fish, including surgeries and euthanasia, sedation, i.e. superficial anesthesia, is a more suitable condition for transportation, short-term handling, and animal experimentation. However, adequate sedation should be carefully observed, as slightly over sedation doses can also cause fish to lose equilibrium, sink to the bottom, pile up and finally suffocate (Yanar and Kumlu, 2001).

Lidocaine is a well-known local anesthetic and has been reported as a general anesthetic for fish (Carrasco et al., 1984). Moreover, this drug presents antiarrhythmic and analgesic properties (Hermanns et al., 2019). Lidocaine is an amide-type local anesthetic that reversibly blocks voltage-gated sodium channels (VGSCs) in neuronal tissues, thereby interrupting depolarization and synaptic transmission. Its affinity for VGSCs varies according to the state of the channel and pH of the medium, being high when the channel is open (at around pH 7.4) and low when it is closed or in tissue acidosis, such as during inflammation (Soto et al., 2018; Beecham et al., 2021).

The selection of anesthetics to be used on fish is rather complex and based on market availability, cost, quality of the anesthesia induction, maintenance, and recovery. Preferably, the anesthetic dose should induce full-body immobility and unconsciousness, at least presumably, without causing important side effects or physiological stress to the fish throughout or post-anesthesia (Ross and Ross, 2008; Zahl et al., 2012; Barbas et al., 2016). Notwithstanding, some agitation usually occurs during induction, which is caused by the mildly irritating properties of most anesthetics (Ross and Ross, 2008; Martins et al., 2018). Moreover, significant changes in cardiovascular and respiratory parameters may occur in the course of and during recovery from anesthesia. Ideally, a good anesthesia process should allow for a quick and complete recovery of normal behavior after fish is transferred to anesthetic-free water, with the resumption of the righting reflex, normal ventilation, swimming movements (Barbas et al., 2021), and positive response to refeeding (Neiffer and Stamper, 2009; Martins et al., 2018).

The level of depression in neuronal activity, and the amplitude of cardiac or muscle electrical changes resulting from a particular anesthetic are difficult to assess in fish and not frequently reported. Few studies have used a more adequate analytical approach, e.g., electrophysiological assessment in fish undergoing anesthesia, which could best clarify their responses to anesthesia exposure (Lambooij et al., 2002; Robb

and Roth, 2003; Barbas et al., 2017, 2021). The electrocardiogram allows for the monitoring and quali-quantitative analysis of the anesthetized fish through characterization of the tracing patterns, heart rate, amplitude and duration of the QRS complex, and length of the PQ, QT, and RR intervals (Ross, 2001; Neiffer and Stamper, 2009; Choi et al., 2010; Cantanhˆede et al., 2021). Additionally, electromyography can be used to assess the muscle contraction activity of fish exposed to anesthetic baths (Hayashida et al., 2013; Fujimoto et al., 2017; Barbas et al., 2021).

Previous studies have shown that lidocaine-hydrochloride decreased oxygen consumption and ammonia excretion in other teleosts, and did not result in adverse side effects (Park et al., 2009; Chatigny et al., 2018). Lidocaine has a short half-life in the environment (Kostrubiak et al., 2020). Information on the concerted behavioral, electromyographic, and electrocardiographic changes during short-term exposure to lidocaine-hydrochloride has never been reported in fish. Such refined information could improve the usability of lidocaine, prevent fish mortality and minimize physiological stress during the handling and transportation of live fish.

Tambaqui (*Colossoma macropomum*) is currently one of the main reared native fish species in South America, presenting high commercial and socioeconomic value (Sandre et al., 2017; Fortes-Silva et al., 2018). It is an omnivorous species native to the Amazon and Orinoco river basins. This species can reach up to one meter in length and 30 kg in weight in natural environments. It is well adapted to captivity conditions, and easily accepts artificial diets, showing a good growth rate and feed conversion ratio (Saint-Paul, 1986; Araujo-Lima and Goulding, 1997; Inoue et al., 2011). Tambaqui has been used as a promising live model in fish anesthetic studies (Barbas et al., 2017; De Souza et al., 2019; Barbas et al., 2021; Cantanhêde et al., 2021).

The present study was carried out to characterize the behavior and evaluate the electromyographic and electrocardiographic changes in tambaqui (*C. macropomum*) exposed to different concentrations of lidocaine hydrochloride in short-term immersion baths.

2.2 Material and methods

2.2.1 EXPERIMENTAL ANIMALS

Juveniles of *Colossoma macropomum* were purchased from a commercial local farm. The experiment was conducted at the facilities of the Laboratory of Pharmacology and Toxicology of Natural Products at the Federal University of Par´a (UFPA). Fish were stocked in aquariums with controlled room temperature (25 to 28 ◦C) and photoperiod (12/12 h Light/Dark). Feeding was carried out twice a day for apparent satiety on a commercial diet (32% crude protein and 7% crude fat). After feeding, aquariums were siphoned to remove uneaten food and feces, and the water was partially renewed (approximately 20% of the total volume) with tap water of the same origin. During the 15-day acclimation period, every 48 h the water quality variables such as water temperature (◦C); pH; dissolved oxygen (DO) (Multi-parameter meter AK87); total ammonia nitrogen - TAN (NH4+ + NH3 120 + mg/L) and total hardness were monitored and maintained as follows: Temperature 26.4 ± 1.0 ◦C; pH 7.5 ± 0.5; OD = 5.06 ± 0.1 mg/L; TAN 0.1 ± 0.04 mg/L (UNESCO, 1983) and Total Hardness 41.7 ± 0.08 mg CaCO3 L^{-1} according to Adad guidelines (Adad, 1982). The mean values of all parameters are in line with those observed in the natural environment for *C. macropomum* (Araujo-Lima and Goulding, 1997). The project was approved by the ethics committee of UFPA CEUA nº 8,887,260,721 (ID 001747).

2.2.2 EXPERIMENTAL DESIGN

2.2.2.1 Exposure to Lidocaine Hydrochloride (LA)

Tambaqui juveniles (25.38 ± 6.5 g) were randomly assigned to different groups for the evaluation of behavioral, electromyographic, and electrocardiographic responses. Groups were divided as follows: a) control fish; and fish exposed to b) 150 mg/L LA, c) 175 mg/L LA, d) 200 mg/L LA, and e) 225 mg/L LA. Measurements were performed during induction and recovery for each concentration. Each recording lasted 5 min and nine fish were used (n = 9 per group), totaling 117 animals.

2.2.2.2 Behavioral assessment

For the evaluation of behavior fish were individually exposed to the previously mentioned concentrations, and the latency, i.e., time to loss of the posture reflex, characterized by lateral decubitus (≥ 15 s) was registered. Subsequently, fish were transferred to LA-free water, whereby the latency to the recovery of the posture reflex was recorded. Fish were considered recovered if they could maintain the righting reflex for a minimum of 15 s.

2.2.2.3 Electromyographic analysis (EMG)

To evaluate the muscle activity, electrodes were implanted in the dorsal muscle, following the methodology of Barbas et al. (2017). Briefly, for the EMG recordings, electrodes were affixed to the muscle, 5.0 mm below the dorsal fin. Fish were placed in aquariums inside a Faraday cage and subjected to 5-min baths using LA solution at 150, 175, 200, and 225 mg/L. Then, recovery responses in washout were observed and evaluated.

2.2.2.4 Electrocardiogram analysis (ECG)

For the cardiac monitoring, the electrodes were built using separate stainless-steel rods of 0.3 mm in diameter and 5.0 mm in length. The affixation of the reference electrode followed the indication of the cardiac vector, being ventrally positioned at 0.2 mm from the end of the opercular cavity, whereas the recording electrode was affixed 2.0 mm below the pectoral fin. Subsequently, electrodes were connected to a high-impedance amplifier. From the records, heart rate in beats per minute (BPM), QRS duration (ms), PQ, RR (ms), and QT intervals (ms) were analyzed (Figure 7 A and B).

2.2.2.5 Recordings and analyses

The electrodes were connected to a digital data acquisition system through a high input impedance differential amplifier (Grass Technologies, Model P511), adjusted with 0.3 and 300 Hz filtering, with 2000× amplification, and monitored by an oscilloscope (Protek, Model 6510). Recordings were continuously digitalized at a rate

of 1 kHz using a data acquisition board (National Instruments, Austin, TX), and stored on a hard disk for later processing using specialized software (LabVIEW express). The analysis of the acquired signals was performed by a tool built in the Python programming language version 2.7. Numpy and Scipy libraries were used for mathematical processing and the Matplolib library was used for the elaboration of histograms. The graphical interface was developed using the PyQt4 library. Amplitude graphs show differences in potential between the reference and recording electrodes. Signal records were observed at a rate of 1000 samples per second.

2.2.3 STATISTICAL ANALYSIS

After verifying compliance with the assumptions of normality and homogeneity of variances through the Kolmogorov-Smirnov and Levene tests, pair-wise comparisons between mean amplitudes (power) were made using one-way ANOVA, respectively followed by Tukey's post hoc test. The GraphPad Prism 8® software was used for the analyses and a *p*-value <0.05 was considered statistically significant in all cases (Zar, 1996).

2.3 Results

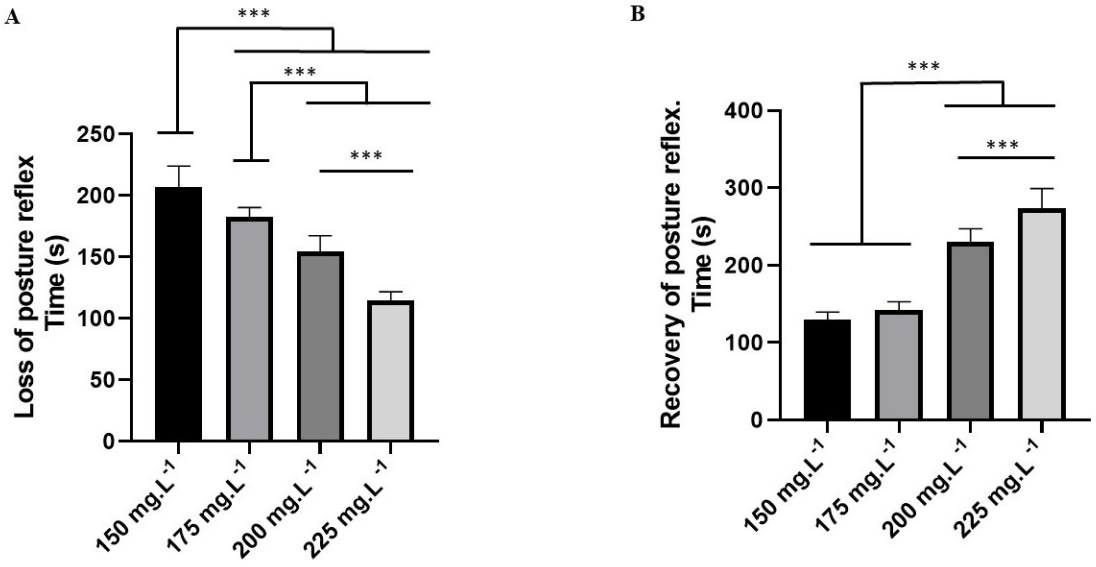
2.3.1 DOSE-DEPENDENT BEHAVIORAL ANALYSIS OF LIDOCAINE

Behavioral analysis showed that lidocaine caused a dose-dependent loss of posture reflex, and the higher the dose the faster the reflex loss occurred. Fish exposed to 150 mg/L LA showed loss of posture in 206 ± 17 s whereas fish exposed to 175 mg/L took a significantly shorter time to lose reflex (183 ± 7 s). Fish treated with 200 mg/L LA showed an average loss of posture reflex of 154 ± 12 s, being significantly faster than groups receiving 150 and 175 mg/L LA. For the group treated with 225 mg/L LA the average latency was even shorter (114 ± 7 s) compared to groups submitted to lower concentrations ($F(3,32) = 98.01$; $P < 0.0001$) (Figure 2A).

Recovery of the posture reflex in fish exposed to 150 mg/L LA occurred in 129 ± 10 s, being similar to the recovery time of fish exposed to 175 mg/L LA (142 ± 11 s, $p > 0.05$). The group exposed to 200 mg/L LA presented an average recovery of 230 ± 17 s, which was longer than those recovery times of fish bathed at 150 mg/L or 175

mg/L LA. Fish exposed to 225 mg/L LA recovered the posture reflex in 274 ± 25 s, which is longer relative to the other groups (F (3, 32) = 150 0.7; $p < 0.0001$) (Figure 2B).

Figure 2 – Mean latencies (s) for loss of posture reflex during immersion baths of tambaqui juveniles, Colossoma macropomum using different concentrations of lidocaine (LA) (A). Recovery of the posture reflex upon exposure to different concentrations of LA (B) (ANOVA followed by Tukey's test; *** P < 0.0001).

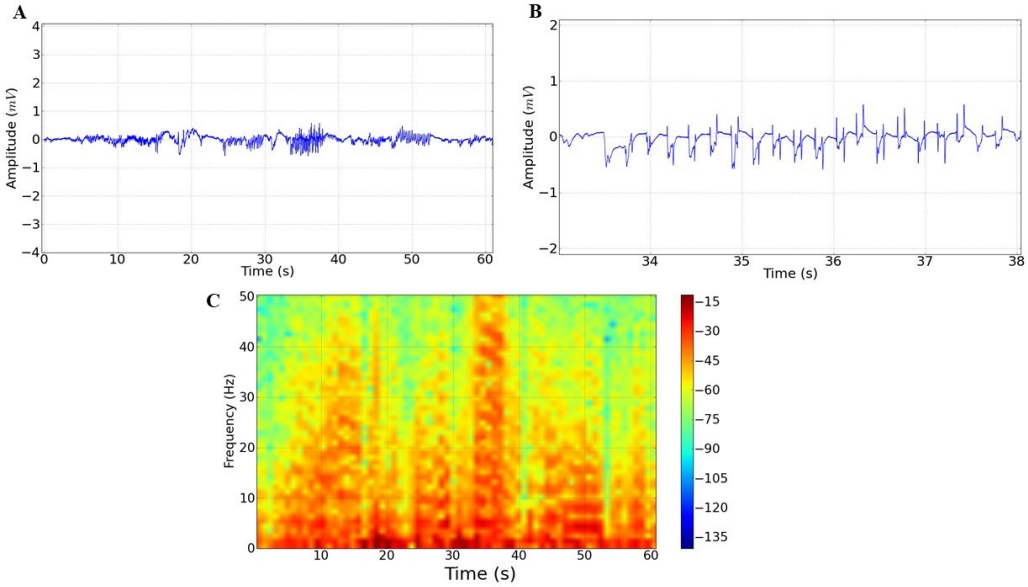


Fonte: Elaborada pelo autor do trabalho.

2.3.2 DORSAL MUSCLE CONTRACTION ACTIVITY IN TAMBAQUI C. MACROPOMUM SUBJECTED TO IMMERSION BATHS WITH LIDOCAINE

The dorsal muscle activity of fish during swimming showed amplitudes below 1 mV (Figure 3A). The 5-s amplification of the record showed the muscle contraction tracing pattern (Figure 3B). Power distribution during muscle contraction in frequencies of up to 50 Hz (the reddish colour indicates a more powerful signal in the spectrogram of frequency) (Figure 3C).

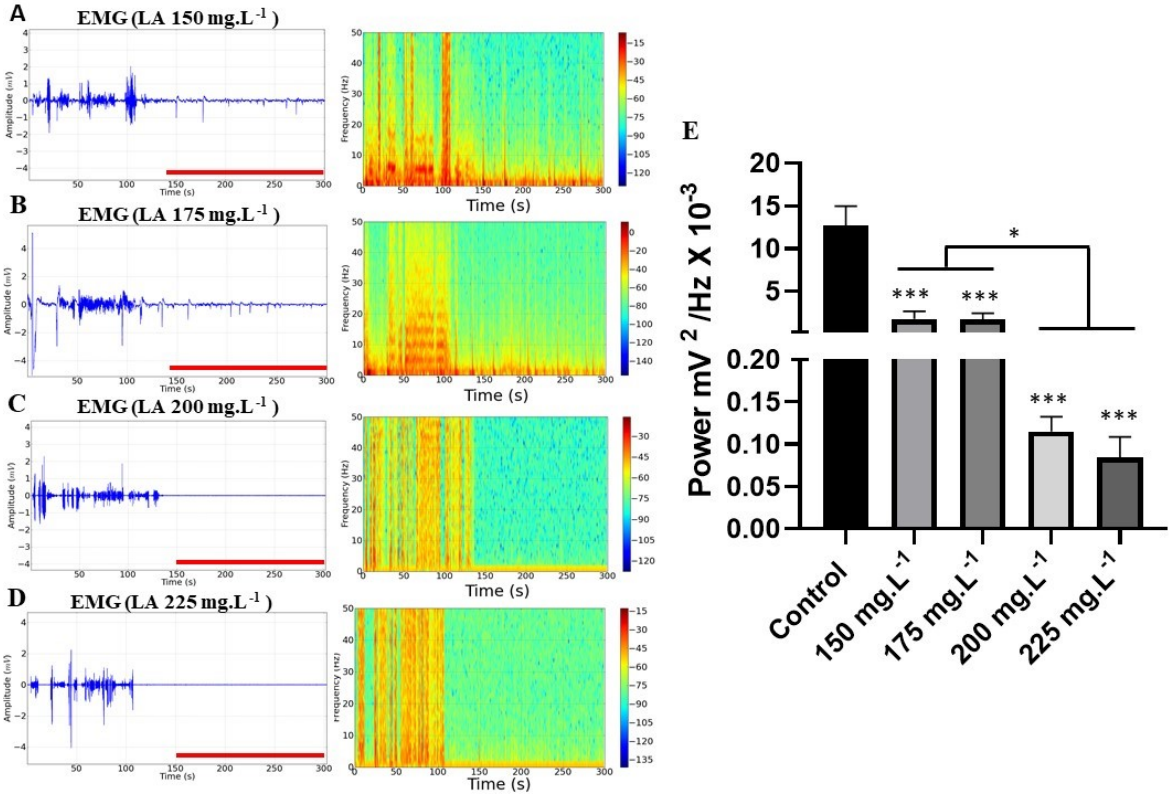
Figure 3 – Dorsal muscle contraction activity in resting juveniles of tambaqui, Colossoma macropomum. Electromyographic tracing (EMG) of 60 s (A); 5-s EMG amplification demonstrating the tracing pattern (B); and spectrogram of frequency showing the power distribution in frequencies of up to 50 Hz (C). The colorimetric scale shows the intensity of the signal (power) over time and across different frequencies.



Fonte: Elaborada pelo autor do trabalho.

During the immersion baths at 150 and 175 mg/L LA, a gradual decrease in muscle contraction activity was observed, however, after 150 s, no full muscle relaxation was observed, which can be observed by the irregular tracing patterns shown in Figure 4A and B (left). However, the concentrations were sufficient to induce a loss of posture (as presented in Figure 1 A). The spectrograms of frequency (Figure 4A and B, right) indicate energy levels in line with low-intensity muscle contraction over the last 150 s of the record.

Figure 4 – Dorsal muscle contraction activity in juveniles of tambaqui, Colossoma macropomum subjected to immersion baths with lidocaine (LA) at 150 mg/L (A, left) and the respective spectrogram of frequency (A, right); electromyographic tracing (EMG) during immersion at 175 mg/L LA (B, left) and the respective spectrogram of frequency (B, right). EMG at 200 mg/L LA (C, left) and the respective spectrogram of frequency (C, right); EMG during immersion at 225 mg/L LA (D, left) and the respective spectrogram of frequency (D, right). EMG recordings were performed for 5 min. Red lines indicate intervals with decreased muscle activity mostly from the second halves of the records and forward. Mean muscle contraction power over the last 150 s of the 5-min recordings (E) (ANOVA followed by Tukey's test, ***P <0.0001; *P < 0.05, n = 9). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).



Fonte: Elaborada pelo autor do trabalho.

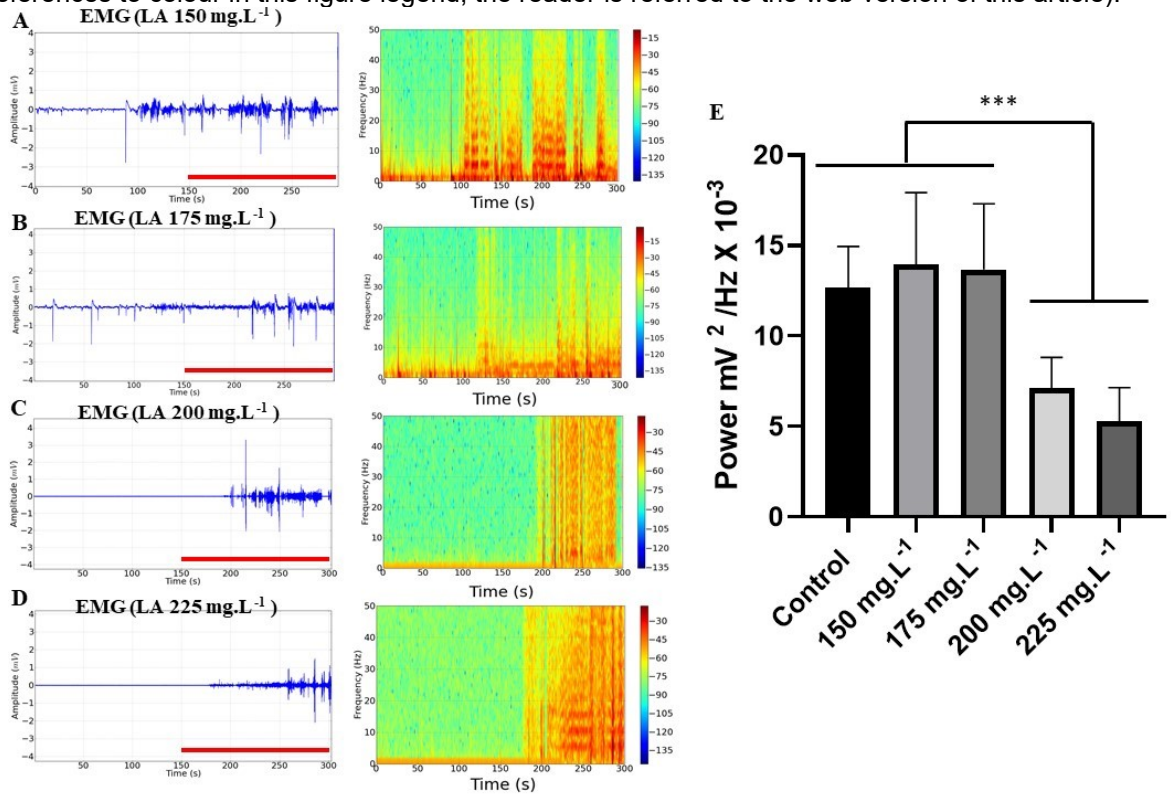
Exposure to 200 and 225 mg/L LA led to full relaxation of the dorsal muscle after 150 s. Myorelaxation is shown in Figure 4C and D (left), which after 150 s presented an isoelectric trace. Such a pattern overlaps with the spectrograms of frequencies attained (Figure 4C and D, right). Although these concentrations caused intense muscular relaxation, they occurred at the expense of a longer posture reflex recovery (Figure 1B).

The average power of muscle contraction during LA exposure is presented in Figure 4E. Normal muscle contraction of the controls had an average of 12.6 ± 2.2 mV2/ Hz x 10^{-3}, which was higher than those mean values of groups exposed to 150 mg/L and 175 mg/L LA (1.7 ± 0.8 mV2/ Hz x 10^{-3} and 1.6 ± 0.7 mV2/ Hz x 10^{-3}, respectively). Exposure to 200 mg/L and 225 mg/L LA resulted in respective averages

of 0.1 ± 0.01 mV2/ Hz x 10^{-3} and 0.08 ± 0.02 mV2/ Hz x 10^{-3}, which in turn were lower (F (4.40) = 195.8; *P* < 0.0001) relative to the controls and those of animals exposed to 150 and 175 mg/L LA.

To assess the recovery of muscle activity, records were acquired after the immersion baths. A clear tracing pattern of muscle activity resumption could be observed in fish exposed to 150 mg/L and 175 mg/ L LA after 100 and 120 s in a washout, however, muscle activity was not fully restored by the end of the recording interval (5 min) (Figure 5A and B, left), which could also be confirmed in the spectrograms (Figure 5A and B, right).

Figure 5 – Dorsal muscle contraction activity in juveniles of tambaqui, Colossoma macropomum during recovery after immersion baths with lidocaine (LA) at 150 mg/L (A, left) and the respective spectrogram of frequency (A, right); electromyographic tracing (EMG) during recovery from exposure to 175 mg/L LA (B, left) and the respective spectrogram of frequency (B, right). EMG in recovery after exposure to 200 mg/L LA (C, left) and the respective spectrogram of frequency (C, right); EMG during recovery from 225 mg/L LA exposure (D, left) and the respective spectrogram of frequency (D, right). EMG recordings were performed for 5 min. Red lines indicate the resumption of muscle activity mostly from the second halves of the records and forward. Mean muscle contraction power over the last 150 s of the 5-min recordings (E) (ANOVA followed by Tukey's test, ***P < 0.0001; *P < 0.05, n = 9). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).



Fonte: Elaborada pelo autor do trabalho.

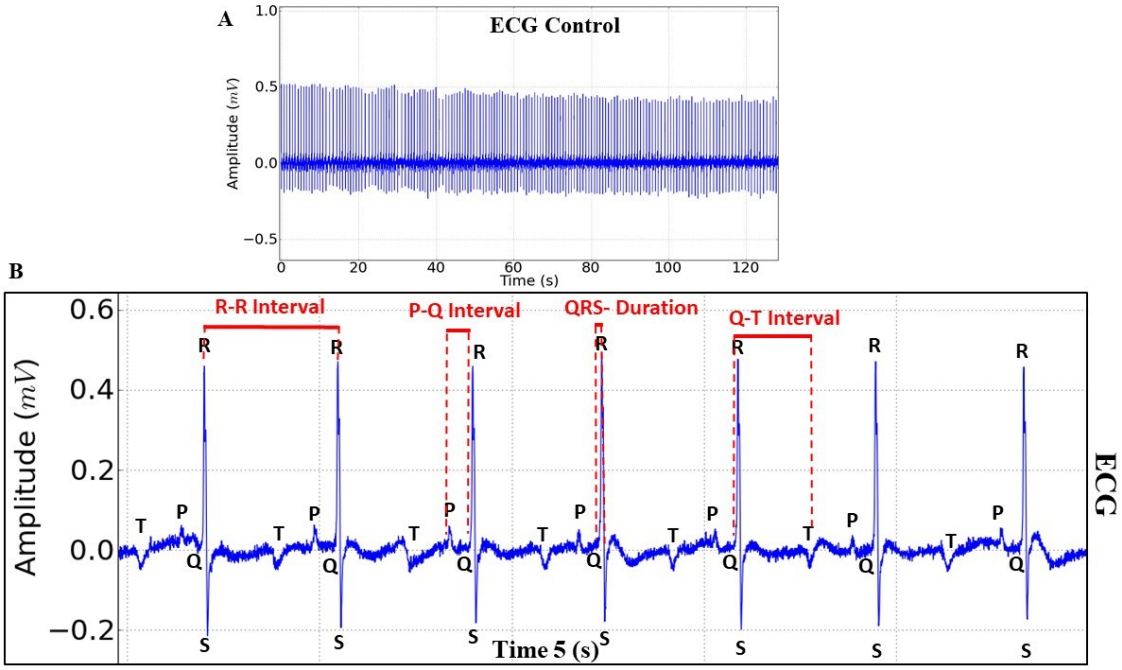
For the 200 and 225 mg/L LA-exposed groups, the recordings showed more intense myorelaxation and longer recovery (Figure 5C and D, left). The muscle response in fish exposed to 225 mg/L LA showed a gradual increase (Figure 5D, left) whereas, initially, the spectrogram presented lower energy levels with increased activity in the second half of the record (Figure 5C and D, right).

The mean power for muscle contraction of the control group during recovery was 12.6 ± 2.2 mV2/ Hz x 10^{-3} showing no differences against those of the 150 mg/L LA (13.9 ± 3.9 mV2/ Hz x 10^{-3}) and 175 mg/L LA (13.6 ± 3.6 mV2/ Hz x 10^{-3}) exposed fish. Fish at 200 mg/L and 225 mg/L LA had average powers of 7.1 ± 1.6 mV2/ Hz x 10^{-3} and 5.2 ± 1.8 mV2/ Hz x 10^{-3}, respectively, which were lower than the power in the controls and fish submitted to 150 mg/L and 175 mg/L LA ($F(4, 40) = 18.04$, $P < 0.0001$) (Figure 5E).

2.3.3 CARDIAC ACTIVITY OF TAMBAQUI C. MACROPOMUM DURING IMMERSION BATH WITH LIDOCAINE

The cardiac activity in the controls showed a mean frequency of 89 ± 4 bpm, at amplitudes around 0.5 mV (Figure 6A; 7E). In 5-s snapshots, all cardiac waves and complexes could be observed in sinus rhythm, with atrial activity represented by P waves, ventricular activity by QRS complexes, and ventricular repolarization by T waves (Figure 6B).

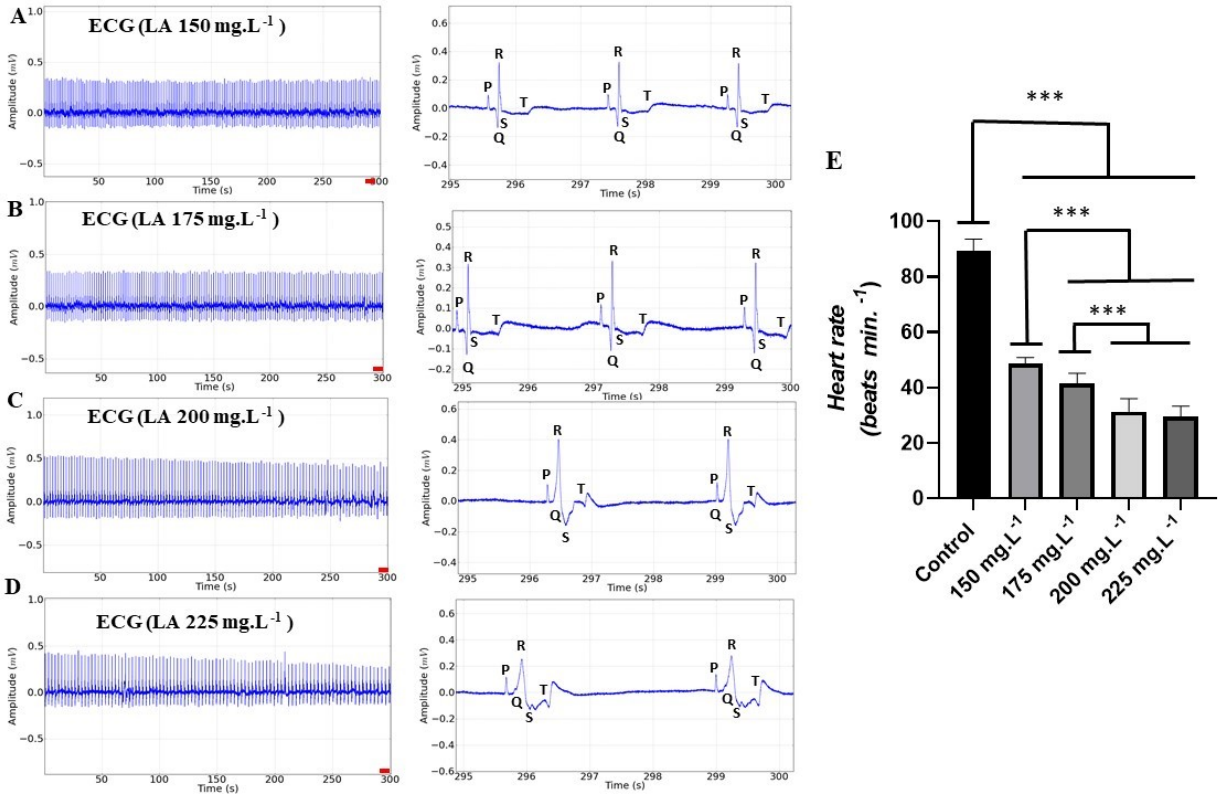
Figure 6 – The cardiac activity of Colossoma macropomum (A) and amplification of the record in a 5-s snapshot showing sinus rhythm in which RR, PQ, and QT intervals (s) and QRS complex duration (s) are indicated in red colour (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).



Fonte: Elaborada pelo autor do trabalho.

During exposure to LA at 150 mg/L and 175 mg/L, the ECG showed sinus bradycardia (Figure 7A and B), and the heart rate decreased 45.51% in the 150 mg/L LA exposed group and 53.73% in the 175 mg/L LA exposed fish, compared to the controls (Figure 7E).

Figure 7 – Cardiac activity of Colossoma macropomum juveniles during immersion bath with 150 mg/L lidocaine (LA) (A, left). Amplification of the record in the last 5 s (295–300 s), showing cardiac waves and complex (A, right); the response of fish exposed to 175 mg/L LA (B, left) also with 5-s amplification (295–300 s) demonstrating the pattern of waves and cardiac complex (B, right). Cardiac activity at 200 mg/L LA (C, left); amplification of the record in the last 5 s (295–300 s), showing cardiac waves and complex (C, right); the response of fish exposed to 225 mg/L LA (D, left) also with 5-s amplification (295–300 s) demonstrating the pattern of waves and cardiac complex (D, right). Mean values for heart rate in beats per minute during exposure to lidocaine at 150, 175, 200, and 225 mg/L (E) (ANOVA followed by Tukey's test ***P < 0.0001; **P < 0.001, n = 9).



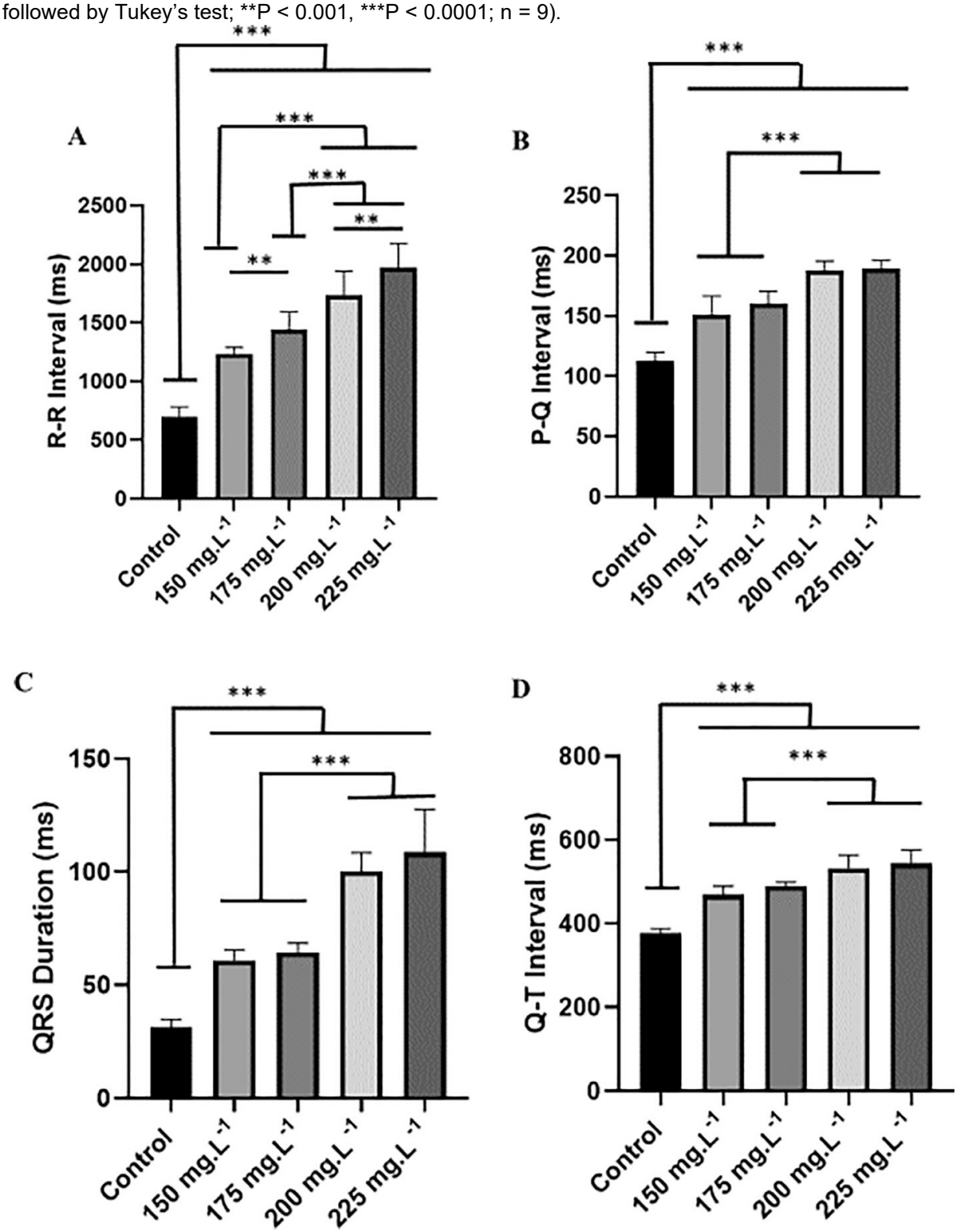
Fonte: Elaborada pelo autor do trabalho.

Bradycardia was dose-dependent as fish were submitted to higher concentrations of LA (200 and 225 mg/L LA) (Figure 7C and D), showing more intense bradycardia relative to the controls (64.92% and 66.90%, respectively). Alteration in the T wave could also be observed (Figure 7C and D, right).

The heart rate was significantly affected by lidocaine. The controls had an average of 89 ± 4 bpm, which was higher than that of the group treated with 150 mg/L LA (48 ± 2 bpm). Fish exposed to 175 mg/L presented a heart rate average of 41 ± 3 bpm, which was lower than that of the group treated with 150 mg/L LA. The average heart rates for animals exposed to 200 mg/L and 225 mg/L were 31 ± 4 bpm and 29 ± 3 bpm, respectively, being much lower than those of the controls and 150 mg/L LA and 175 mg/L LA exposed fish ($F_{(4, 40)} = 361.16$; $P < 0.0001$) (Figure 7E).

The mean RR interval for the control group was 694.6 ± 87.9 ms, which was shorter compared to the other groups. The 150 mg/L LA treated group had a mean RR interval of 1235 ± 56.01 ms, shorter than that of the 175 mg/L LA exposed fish (1442 ± 150.4 ms). The 200 mg/L LA group had a mean interval of 1736 ± 205.1 ms, i.e., longer than those of the controls, 150 and 175 mg/L LA exposed groups. The mean RR interval for the 225 mg/L LA exposed fish was 1968 ± 208 ms and was longer than any other group ($F(4.40) = 90.90$; $P < 0.0001$) (Figure 8A).

Figure 8 – Mean values for RR (A), PQ Intervals (B), QRS complex duration (C), and QT interval (D) in Colossoma macropomum during exposure to lidocaine at 150, 175, 200, and 225 mg/L (ANOVA followed by Tukey's test; **P < 0.001, ***P < 0.0001; n = 9).



Fonte: Elaborada pelo autor do trabalho.

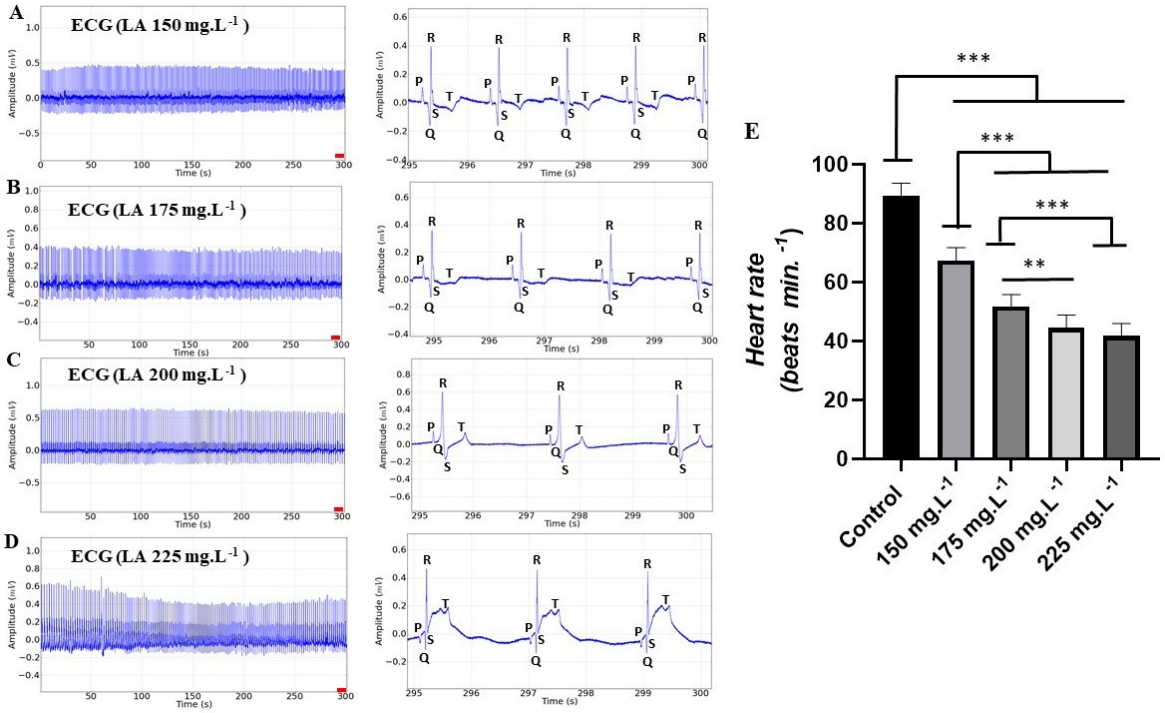
The mean PQ interval for the control group was 112.8 ± 7.01 ms, being the shortest interval among the groups. The 150 and 175 mg/L LA exposed fish had respective means of 150.9 ± 15.6 ms and 160.3 ± 10.4 ms, showing shorter intervals relative to those of fish exposed to 200 and 225 mg/L LA, which had mean PQ intervals of 187.8 ± 7.7 ms and 189.6 ± 6.6 ms, respectively ($F_{(4,40)} = 87.73$; $P < 0.0001$) (Figure 8B).

The mean duration of the QRS complex for the control group during induction was 31.1 ± 3.6 ms, which was shorter compared to the other groups. The 150 mg/L LA treated group had a mean QRS complex duration of 60.7 ± 4.6 ms and did not differ from that of fish exposed to 175 mg/L LA (64.4 ± 4.0 ms). Fish exposed to 200 and 225 mg/L LA had mean intervals of 100.2 ± 8.2 ms and 108.7 ± 18.9 ms, respectively, which were longer than all other groups ($F_{(4.40)} = 93.72$; $P < 0.0001$) (Figure 8C).

For the control group, the mean QT interval during induction was 37.7 ± 9.8 ms and was shorter than those of the other groups. Fish treated with 150 and 175 mg/L LA showed respective means of 469.1 ± 20.3 ms and 490 ± 8.9 ms, which were shorter intervals than those of fish exposed to 200 mg/L and 225 mg/L LA (532.2 ± 31.3 ms and 544.5 ± 32.03 ms, respectively) ($F_{(4.40)} = 75.88$; $P < 0.0001$) (Figure 8D).

During recovery from exposure to 150 and 175 mg/L LA, there was reversibility of the ECG changes (Figure 9A and B). Heart rate increased by 27.71% for the 150 mg/L LA treated group and 20.18% for the 175 mg/L LA treated fish within 5 min during washout, which indicates resumption of cardiac activity, albeit rather slow-paced (Figure 9A and B, right).

Figure 9 – Cardiac activity in Colossoma macropomum during recovery after immersion bath with 150 mg/L lidocaine (LA) (A, left). Amplification of the record in the last 5 s (295–300 s), showing cardiac waves and complex (A, right). Fish in recovery after immersion bath with 175 mg/L LA (B, left). A 5-s snapshot (295–300 s) is also presented demonstrating the electrocardiogram waves and complex (A, right). Cardiac activity in recovering fish exposed to 200 mg/L LA (C, left). Amplification of the record in the last 5 s (295–300 s) demonstrating the waves and cardiac complex (C, right). Tracings of fish recovering from 225 mg/L LA exposure (D, left). Amplification of 5 s (295–300 s) demonstrating the electrocardiogram waves and complex (D, right). Mean heart rate in beats per minute (E) during recovery after exposure to LA at 150, 175, 200, and 225 mg/L (ANOVA followed by Tukey's test; ***P < 0.0001; **P < 0.001; *P < 0.01; n = 9).



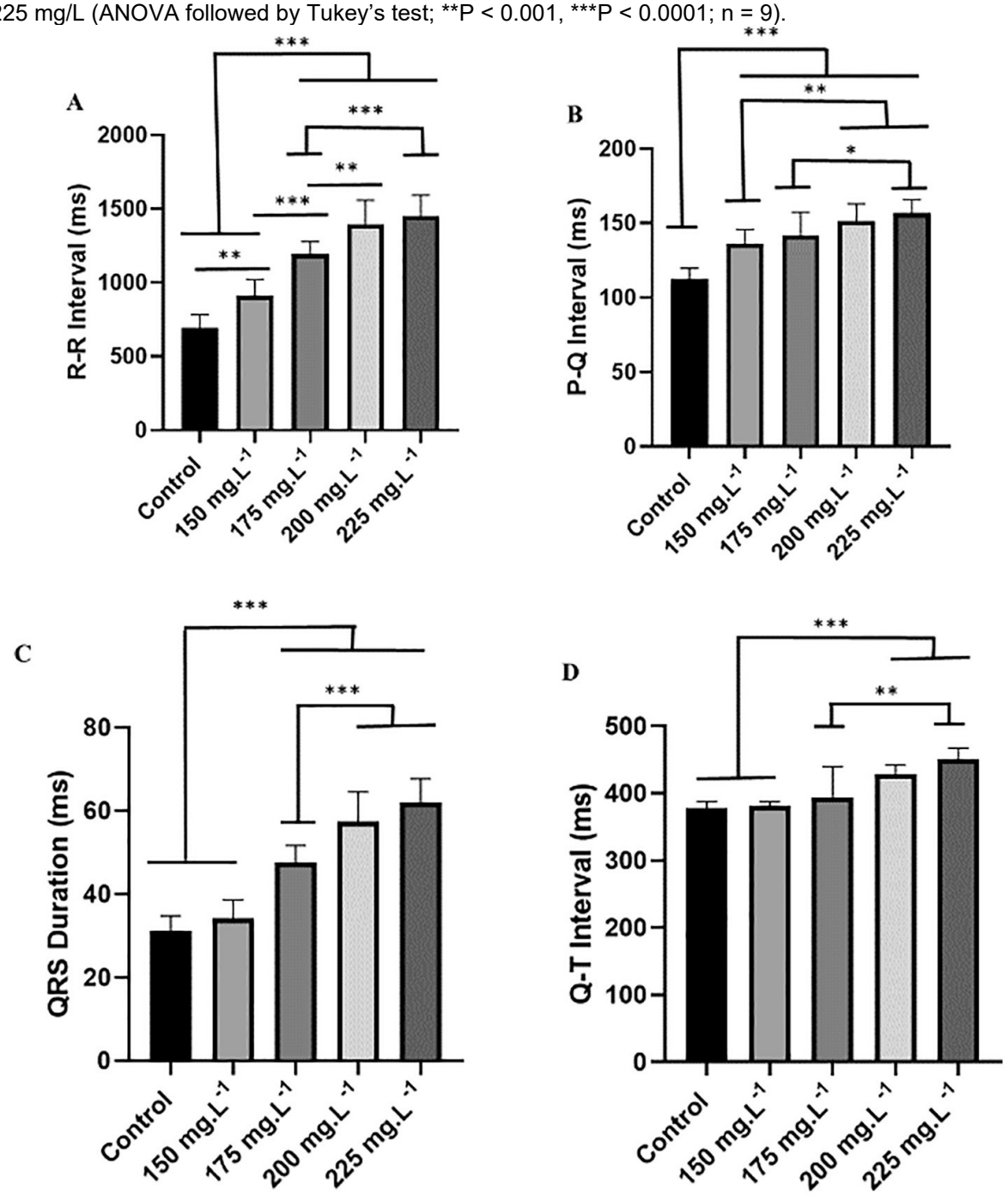
Fonte: Elaborada pelo autor do trabalho.

Fish recovering from exposure to 200 and 225 mg/L LA (Fig. 8 C and D), showed a partial resumption of cardiac function around 29.50% and 29.24%, respectively. However, an altered T wave was observed for the 225 mg/L LA exposed fish (Figure 9D, right).

The recovery of heart rate was dose-dependent. The higher the concentration, the longer it took for the reversing of the bradycardia. The controls had a mean frequency of 89 ± 4 bpm, higher than the other treated groups. Fish subjected to 150 mg/L LA had a mean frequency of 67 ± 4 bpm, which was significantly higher than the frequency of fish submitted to 175 mg/L LA (51 ± 4 bpm). Fish submitted to 200 and 225 mg/L LA had respective means of 44 ± 4 bpm and 41 ± 4 bpm which were lower than those of the controls and fish exposed to 150 and 175 mg/L LA ($F(4.40) = 192.3$; $P < 0.0001$) (Figure 9E).

The mean RR interval during recovery for the control group was 694.6 ± 87.9 ms, which was shorter compared to the other groups. Fish exposed to 150 mg/L LA had a mean RR interval of 910.9 ± 83.7 ms and showed a shorter interval compared to fish exposed to 175 mg/L LA (1194 ± 80 ms). The 200 mg/L LA treated group had a mean interval of 139.4 ± 166.2 ms, which was longer than those intervals observed in the controls, and fish exposed to 150 mg/L and 175 mg/L LA. The mean RR interval for the 225 mg/L LA group was 1448 ± 143.8 ms and was longer than those of the controls, and fish exposed to 150, 175, and 200 mg/L LA ($F(4.40) = 61.90$; $P < 0.0001$) (Figure 10A).

Figure 10 – Mean values for RR (A), PQ intervals (B), duration of the QRS complex (C), and QT interval (D) during recovery of Colossoma macropomum after exposure to lidocaine (LA) at 150, 175, 200, and 225 mg/L (ANOVA followed by Tukey's test; **P < 0.001, ***P < 0.0001; n = 9).



Fonte: Elaborada pelo autor do trabalho.

The PQ interval during recovery in the controls was 112.8 ± 7.01 ms, which was shorter than the other groups. The 150 and 175 mg/L LA exposed fish had respective means of 136.2 ± 9.6 ms and 141.9 ± 15.5 ms. The 150 mg/L LA treated group showed a shorter interval compared to groups exposed to 200 and 225 mg/L LA (151.5 ± 11.6 ms and 156.8 ± 8.9 ms, respectively). The 175 mg/L LA treated group had a shorter

PQ interval compared to fish exposed to 225 mg/L LA ($F(4, 40) = 21.92$; $P < 0.0001$) (Figure 10B).

2.4 Discussion

There is still rather limited knowledge on the use of anesthetics in fish due to poor validation of processes and analyses of the safety, and efficacy of sedation and anesthesia. Additional studies which lend credence to the broad use of suitable synthetic and natural anesthetics are needed to manipulate live fish. Lidocaine elicited prompt loss of the righting reflex and reduced aversive behavior compared to tricaine methane sulfonate (MS-222), which is widely used for handling and euthanasia of fish (Collymore et al., 2016). Similar to our observations, lidocaine did not result in behavioral adverse effects in trout (*Oncorhynchus mykiss)* receiving intramuscular infiltration (Chatigny et al., 2018). Moreover, lidocaine was more appropriate compared to MS-222, or 2-phenoxyethanol, in association with propofol in the anesthesia of juvenile zebrafish *(Danio rerio*) (Owen and Kelsh, 2021).

In this study, the loss of reflex was achieved in a shorter time at higher doses; and showed similarity in the reduction of the dorsal muscle power at 200 and 225 mg/L LA. However, at lower doses, significant muscle activity was still present as per the EMG attained. The establishment of effective doses for fish anesthesia is difficult and varies according to water quality, fish species, size, and overall experimental conditions (Ross and Ross, 2008).

Presumably, the lingering excitatory activity in the dorsal muscle in lower doses without full muscle relaxation contributed to the rapid recovery of the muscle reflex in these cases. Contrastingly, fish exposed to higher doses (200 and 225 mg/L LA) had an intense muscular relaxation and there was a steady and gradual resumption of muscle contraction power, albeit more slowly at the highest dose. This property of lidocaine fits well with the expected features of a sedative because the deepening of anesthesia was dependent on the concentration of the anesthetic, which allowed for the characterization of superficial and deep anesthetic planes.

However, one should bear in mind that the sole assessment of behavior such as the registration of latencies to body immobilization or muscle relaxation is not

enough to state that the anesthetic has led to analgesia or loss of sensation, which not only are interesting but sought after effects (Ross and Ross, 2008; Barbas et al., 2017).

Lidocaine is a voltage-dependent sodium channel blocker drug used as a fast-acting local anesthetic. In the case of fish, it is used for general anesthesia. Lidocaine has also a central cholinergic contribution, decreasing the activity of cerebral acetylcholinesterase (AChE) in zebrafish after systemic administration through immersion baths (Abreu et al., 2019). Increasing doses of lidocaine rapidly induce the loss of muscle tonus and make recovery more gradual. Regarding the drug potency, muscle contraction recoveries at 200 and 225 mg/L LA were much similar, demonstrating the efficiency of lidocaine in immersion baths for systemic sedation and deep anesthesia. Yet, at lower doses (150 and 175 mg/L LA) muscle activity was still preserved.

Regarding cardiac responses, the control group had a mean heart rate similar to those found in previous studies (Florindo et al., 2004; Gilmour et al., 2005; Armelin et al., 2016; Barbas et al., 2017), with ECG characteristics showing normal electrical activity, morphology, amplitude, and polarity of P and T waves; also with normal RR, PQ, and QT intervals and QRS complex duration. In fish exposed to 150 and 175 mg/L LA, the QRS complex became predominantly positive, increasing and presenting a slight widening of the amplitudes, marking the anesthetic induction. At these doses, lidocaine increased the RR interval, but ventricular repolarization occurred within normal limits, in agreement with the postulates of Newman (2000) and Hanci et al. (2013).

Nonetheless, lidocaine at 200 and 225 mg/L, caused a reduction in the amplitude and widening of the QRS complex and prolongation of the QT segment >0.5 ms with inversion of T wave polarity. It explains the widening in the QT and RR intervals in these groups, evidencing a state in which lidocaine delayed ventricular repolarization. The prolongation of the QRS complex duration results from the drug-induced blockade of Na+ channels, along with the impairment of Ca^{2+} influx and K^{+} efflux, which ultimately lead to increased cardiac complex duration as the dose is incremented (Holstege et al., 2006; Lionte et al., 2012).

The increased PQ interval in all groups is related to the synaptic blocking effect of the drug, delaying the transmission of impulses through the atrioventricular pathway. In agreement with this finding, Rosen et al. (1976) and Echt et al. (1989), reported a

reduction in the action potential duration of Purkinje fibers from dogs as lidocaine doses increased.

The marked bradycardia observed in fish exposed to lidocaine is due to the depressant effect on the heart. Studies have shown the capacity of lidocaine to reduce cardiac activity in the rat heart due to the inhibition of the atrioventricular node and atrial activity. In addition, lidocaine was able to decrease the resting diastolic potential, the maximum depolarization rate, and the dose-dependent amplitude of the action potential in an isolated rabbit Purkinje fiber ventricular muscle model (Komai and Rusy, 1981; Moller and Corvino, 1988; Hamilton et al., 2004). Finally, studies with zebrafish larvae demonstrated the bradycardic effect of lidocaine found from the increase in RR intervals, showing a reduced heart rate; this being possibly caused by an increased vagal activity (Vargas, 2017).

As the concentration of lidocaine increases, the depressant effect on cardiac activity is greater and may lead to severe bradycardia with QT prolongation. This effect has already been demonstrated for other anesthetics used on fish, like MS-222 in zebrafish (Huang et al., 2010; Popovic et al., 2012) and propofol in *Acipenser oxyrinchus* (Fleming et al., 2003), which had significant heart rate depressant potential.

The recovery process after anesthesia was gradually reversible, being delayed according to the concentration used, with a slow and variable return as a common irregular response that has already been reported in fish submitted to anesthetic baths (Mylonas et al., 2005; Ross and Ross, 2008; Barbas et al., 2017). The gradual reversibility of cardiac activity, irrespective of the lidocaine dose, demonstrated the safety of the drug, without important deleterious changes to the length, morphology, amplitude, and polarity of the waves and electrocardiological intervals. Overall, the recovery of the RR and QT intervals was observed, with the gradual narrowing of the QRS complex, also modulated by the concentration used. It is in line with Marking and Meyer (1985) and Aydın and Orhan (2020), whose suggestion for an ideal recovery time threshold from anesthesia should be in <300 s.

However, the change in the form of the T wave, with an increase in amplitude and duration, usually accompanied by QT prolongation was observed at 225 mg/L LA. However, such alterations were transient and reversible.

In conclusion, this study adds to the body of knowledge on fish anesthesia and reinforces that anesthesia in immersion baths with lidocaine hydrochloride can be

safely used in tambaqui (*C. macropomum*) as an alternative to other anesthetics, especially those in which important side effects have been documented.

Author statement

Details of each author with their contribution in this paper: Luana Rodrigues Vieira - Project administration; Yago Luiz Gonçalves Pereira - Data acuration; Laura Andrade Diniz - Data acuration; Chirlene Pinheiro Nascimento - Supervision; Alex Luiz Mendes da Silva - Formal analysis; Julianne Elba Cunha Azevedo - Helped supervise the project; Vanessa J´oia de Mello - Critical revision of the article; Nilton Akio Muto - Drafting the article; Luis Andr´e Luz Barbas - Data analysis and interpretation; Mois´es Hamoy - Final approval of the version to be published.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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3 CONCLUSÕES INTEGRADORAS

Em conclusão, este estudo contribui para o conhecimento sobre anestesia de peixes e reforça que a anestesia em banhos de imersão com cloridrato de lidocaína pode ser usada com segurança em tambaqui (*C. macropomum*) como alternativa a outros anestésicos, especialmente aqueles nos quais importantes efeitos colaterais foram documentados.

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