UNIVERSIDADE FEDERAL DE PERNAMBUCO
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA APLICADA À SAÚDE
LABORATÓRIO DE IMUNOPATOLOGIA KEIZO ASAMI

PRISCILA RAFAELA LEÃO SOARES

EFEITO AGUDO E CRÔNICO DO LUFENURON SOBRE OS PARÂMETROS BIOLÓGICOS DE TAMBAQUI (*Colossoma macropomum*).

RECIFE
2016

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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Biologia Aplicada à Saúde do Laboratório de Imunopatologia Keizo Asami pela Universidade Federal de Pernambuco, como requisito parcial para a obtenção do título de Mestre em Biologia Aplicada à Saúde.

Orientador:

Professor Doutor Pabyton Gonçalves Cadena

Coorientador:

Professor Doutor Luiz Bezerra de Carvalho Junior

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Dedico

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me respondeu e deu novas forças ao meu
coração”.
Salmos 138 – 4.

RESUMO

O lufenuron é um inseticida benzoilureia e uma substância tóxica que interfere na síntese de quitina nos insetos. Apesar do lufenuron ser um inseticida amplamente utilizado na agricultura e aquicultura, estudos relacionados aos possíveis efeitos tóxicos em diferentes grupos de animais são escassos na literatura. Este trabalho teve por objetivo avaliar os efeitos tóxicos agudo e crônico do pesticida benzoilureia (lufenuron) sobre os parâmetros biológicos de *Colossoma macropomum* (Tambaqui). Para realização do teste agudo, juvenis de tambaqui foram divididos em um grupo controle e cinco grupos experimentais com exposição de 0,1 a 0,9 mg/L de lufenuron por 96 h, com replicata (n = 120). Os animais também foram submetidos a teste de toxicidade crônica por quatro meses em concentrações de 0,1 e 0,3 mg/L de lufenuron, e também um grupo controle (n = 60). A última concentração corresponde a 50% da CL_{50} 96 h (0,58 mg/L) determinada no teste agudo. A presença de hemorragias foi observada nos olhos (hifema), nas nadadeiras e nos opérculos dos peixes expostos a 0,7 e 0,9 mg/L de lufenuron no teste agudo. Análises histológicas mostraram alterações na morfologia das brânquias dos peixes submetidos ao teste de toxicidade aguda, como aneurisma lamelar e congestionamento de sangue nas lamelas. No teste crônico, a análise de glicose no sangue e os parâmetros morfométricos não apresentaram diferenças significativas pelo teste de Tukey ($p > 0,05$). Em geral, *C. macropomum* exibiram comportamentos associados ao estresse quando exposto ao lufenuron em ambos os testes. O lufenuron promoveu danos na retina dos peixes como na capacidade de responder aos estímulos nas células fotorreceptoras e ON-bipolares no teste agudo. Deste modo, o lufenuron apresentou vários efeitos tóxicos em relação aos parâmetros biológicos em *C. macropomum*. Esta preocupação com o uso e descarte do lufenuron indica a exigência de ações ambientais, com a mitigação, para prevenir a bioacumulação e/ou biomagnificação.

PALAVRAS-CHAVE: Inseticida, benzoilureia, peixe amazônico, comportamento animal, eletrorretinograma.

ABSTRACT

Lufenuron is a benzoylurea insecticide and a toxic substance that interfere in chitin synthesis in insects. Although lufenuron is an insecticide widely used in agriculture and aquaculture, rare are studies described in the literature that relates to possible toxic effects on different groups of animals. This work aimed to evaluate acute and chronic toxic effects of benzoylurea pesticide (lufenuron) on biological parameters of *Colossoma macropomum* (Tambaqui). To do the acute test, Tambaqui juveniles were divided into a control group and five experimental groups with exposure to from 0.1 to 0.9 mg/L of lufenuron for 96 h, with replicate (n = 120). Animals were also submitted to chronic toxicity test for four months in concentrations of 0.1 and 0.3 mg/L of lufenuron, also a control group was done (n = 60). This last concentration corresponded to 50% of LC_{50} 96 h (0.58 mg/L) determined in the acute test. The presence of hemorrhages was observed in eyes (hyphema), fins and operculum of fish exposed to 0.7 and 0.9 mg/L of lufenuron in acute test. Histological analysis showed changes in the morphology of fish gills submitted to acute toxicity test, as lamellar aneurysm and blood congestion inside lamellae. In chronic test, blood glucose analysis and morphometric parameters showed no significant differences by Tukey test ($p > 0.05$). In general, *C. macropomum* exhibit behaviors associated with stress when exposed to lufenuron in both tests. Lufenuron promoted damage in fish retina as in ability to respond to stimuli in photoreceptors and in ON-bipolar cells in acute test. Thus, lufenuron showed several toxic effects in relation to biological parameters in *C. macropomum.* This concern about the use and discard of lufenuron indicates the requirement of environmental actions, as mitigation, to prevent bioaccumulation and/or biomagnification.

KEYWORDS: Insecticide, benzoylurea, amazonian fish, animal behavior, electroretinogram.

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LISTA DE ABREVIATURAS E SIGLAS

NL	Nadar Lento	*SS*	Slow Swimming
NR	Nadar Rápido	*FS*	Fast Swimming
NJ	Nadar em Grupo	*GS*	Group in Swimming
ES	Emergir e Submergir	*ES*	Emerge and Submerge
FI	Ficar imóvel	*SM*	Staying Motionless
PR	Perseguir	*CH*	Chase
FR	Fugir	*EE*	Escape
AF	Ataque Frontal	*FA*	Frontal Attack
AL	Ataque Lateral	*LA*	Lateral Attack
CO	Comer	*EA*	Eat
FO	Forragear	*FO*	Forage
RA	Respiração Aérea	*AB*	Aerial Breath
SA	Saltar	*JU*	Jump
NE	Natação Errática	*ER*	Erratic Swimming
DO	Deitado	*LD*	Lying Down
NS	Natação Superficial	*SW*	Surface Swimming
CP	Coprofagia	*CP*	Coprophagy
PVA	Pendurado Verticalmente na Água	*HVW*	Hanging Vertically in the Water
ERG	Eletrorretinograma	*ERG*	Electroretinogram

SUMÁRIO

1. Introdução

O Brasil se apresenta como uma potência na produção de commodities e atualmente é o maior consumidor de agrotóxicos do mundo. O consumo total de agrotóxicos no Brasil é equivalente a 5,2 Kg por habitante (INCA, 2015). Dentre os agrotóxicos utilizados no Brasil destaca-se o lufenuron (SANTOS et al., 2011). A nomenclatura da IUPAC desta substância é (RS)-1-[2,5-dicloro-4-(1,1,2,3,3,3-hexafluoropropoxil) fenil]-3-(2,6-difluorobenzoil) ureia (AHIRE; ARORA; MUKHERJEE, 2008). Este é um inseticida benzoilureia, assim como diflubenzuron e flucycloxuron (ZAIDI; SOLTANI, 2011), que interfere no desenvolvimento larval por inibição ou bloqueio da síntese de quitina, principal constituinte do exoesqueleto dos insetos (VÁZQUEZ et al., 2014).

Este inseticida é utilizado no controle de insetos em geral de culturas como algodão milho, beterraba, batatas, uvas, frutas cítricas e plantas ornamentais (FAO, 2008). O lufenuron também é utilizado no combate do ectoparasita *Argulus* sp. em *Cyprinus carpio* (carpa), contudo não foram observados qualquer efeito negativo nesta espécie de peixe na concentração de 0,1 mg por litro de água por Mayer et al. (2013).

Apesar do lufenuron ser um inseticida bastante utilizado na agricultura e piscicultura, estudos relacionados aos possíveis efeitos tóxicos em diferentes grupos de animais são escassos na literatura. Os pesticidas, como herbicidas e inseticidas podem acumular no ecossistema aquático e exercer efeitos tóxicos nos organismos aquáticos (GHASEMZADEH; SINAEI; BOLOUKI, 2015). Isto pode ocorrer pelo fato de muitos pesticidas não serem espécie-específico. Além disso, estes pesticidas podem ser persistentes no ambiente e em corpos ‘água, visto que áreas agrícolas são encontradas próximas a rios e lagos. De acordo com a *European Food Safety Authority*, o lufenuron foi caracterizado como muito tóxico para organismos aquáticos (EFSA, 2008). Em geral, pesticidas do grupo benzoilureia apresentam efeitos tóxicos em crustáceos, peixes, insetos aquáticos, nematodas e anelídeos como também zooplâncton (VÁZQUEZ et al., 2014).

Os pesticidas podem contaminar o meio aquático através do escoamento após aplicação do agrotóxico, principalmente causados por fortes chuvas e irrigação intensa. Adicionalmente, lavagens de equipamentos e recipientes utilizados, derramamentos acidentais e deriva após pulverização aérea, podem contaminar corpos d’água próximos (NOVELLI et al., 2016).

Pelo fato dos peixes estarem em diversos ecossistemas aquáticos e serem bastante sensíveis a presença de produtos químicos na água, estes podem ser utilizados como bioindicadores. Sua posição no topo da teia trófica aquática quando comparados a outros bioindicadores, fornecem uma visão integrada de todo o ambiente aquático. Consequentemente, tornando-os mais vulneráveis quando o agente tóxico atinge a teia trófica por biomagnificação, representando assim, um risco para saúde humana (NEUMANN-LEITÃO e EL-DEIR, 2009; ABDEL-MONEIM; AL-KAHTANI; ELMENSHAWY, 2012) já que estes animais são utilizados como alimento. Tendo em vista, que o uso de inseticidas pode provocar efeitos tóxicos nos peixes e humanos, podendo resultar em altos riscos de intoxicação (MAGELLAN et al., 2014).

Diante da premissa apresentada acima, é necessário o desenvolvimento de modelos animais para estudar o efeito do lufenuron e entender como este afeta os sistemas biológicos. Como alternativa a espécie *Colossoma macropomum* (Tambaqui) tem sido utilizada em estudos como bioindicadores da qualidade ambiental e pode apresenta-se como uma boa alternativa para os estudos ecotoxicológicos. O tambaqui é um peixe oriundo da bacia amazônica de grande importância econômica (MATSUO; WOOD; VAL, 2005; SALAZAR-LUGO et al., 2011) e se destaca como a espécie mais amplamente cultivada no Brasil (MPA, 2011) inclusive sendo utilizada no Estado de Pernambuco.

O tambaqui é utilizado em estudos de toxicidade com bipiridina como o Dicloreto de paraquate (SALAZAR-LUGO et al., 2011) e herbicida não-seletivo à base de glifosato (BRAZ-MOTA et al., 2015). Não foram encontrados estudos relacionados com o efeito do benzoilureia com um agente tóxico em *C. macropomum*. Diante disto, estudos utilizando o tambaqui são necessários devido à sua ampla criação comercial no Brasil bem como em outros países da América do Sul. O presente trabalho teve por objetivo avaliar os efeitos tóxicos agudo e crônico do pesticida benzoilureia (lufenuron) sobre parâmetros biológicos de *Colossoma macropomum* (Tambaqui). Adicionalmente, as informações obtidas poderão ser utilizadas como parâmetro comparativo para futuros estudos na área de toxicologia ambiental e também para informar aos órgãos reguladores os efeitos desta substância na espécie estudada.

2. Revisão de Literatura

2.1. Lufenuron

O lufenuron, assim como diflubenzuron, teflubenzuron, hexaflumurom e o novaluron (ZOTTI et al., 2012; BOWEN; KARD, 2012) são inseticidas comerciais, usados principalmente na agricultura. O lufenuron também vem sendo empregado na piscicultura como meio de tratamento de ectoparasitas (MAYER et al., 2013). Estes inseticidas compreendem o grupo das benzoilureias, um grupo importante de pesticidas com atividade herbicida ou inseticida (GIL-GARCIA et al., 2001) utilizados a partir do início anos 1970 (MATSUMURA, 2010), que são frequentemente utilizados no controle de pragas em frutas (ZHOU et al., 2009).

As benzoilureias possuem intensa atividade inseticida, boa atividade biológica, vasta seletividade e baixa resistência entre os insetos (MATSUMURA, 2010; ZHOU et al., 2009). Inseticidas do tipo benzoilureias também interferem na atividade do sistema endócrino, regulando o crescimento, reprodução e metamorfose dos insetos em muitas espécies de pragas que causam prejuízos na agricultura, impedindo assim o processo de muda (YANG et al., 2014). Seu mecanismo de ação está ligado a inibição da síntese de quitina, sendo esta um componente importante do exoesqueleto dos insetos (YANG et al., 2015; GANGISHETTI et al., 2009), impedindo a polimerização e deposição da quitina, provocando a morte do inseto alvo (ZHOU et al., 2009).

Entretanto, podem apresentar riscos de contaminação aquática e a produtos naturais, devido a sua persistência no ambiente (YANG et al., 2015). Sendo estes também tóxicos para vários organismos, dentre estes os organismos aquáticos (EFSA, 2008).

Na agricultura o lufenuron é usado para o controle de pragas e doenças que podem causar redução na produção. Destaca-se seu uso em cultivos de vegetais como tomates (MALHAT et al., 2012; BLETSOU et al., 2013), uvas (PAYÁ, et al., 2013), algodão (CZEPAK et al., 2005), feijões verdes, ervilhas, pimenta (BLETSOU et al., 2013), coco (FONTES; FERREIRA; SIQUEIRA, 2002; SILVA et al., 2008), algodão, milho, beterraba e também em plantas ornamentais (FAO, 2008).

O lufenuron se destaca entre os inseticidas reguladores de crescimento de insetos porque impede a formação e reduz a viabilidade dos ovos (KHAJEPOUR; IZADI; ASARI, 2012; MANSUR et al., 2010; MOREIRA et al. 2007). Este interfere na formação

da endocutícula, na redução da procutícula e também na organização da cutícula dos insetos (DEAN et al., 1998; GANGISHETTI et al., 2009).

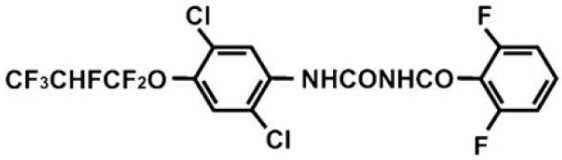
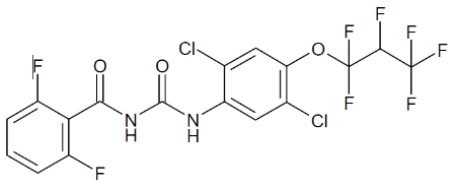
O contato com o lufenuron se dá por via oral seguida de ingestão e dessa forma ele é utilizado no controle de pragas de insetos mastigadores e sugadores, como também de ácaros fitófagos demonstrando assim a sua eficácia no combate a vários representantes das ordens de insetos. Dentre estes destacam-se: cupins, baratas jovens, pulgas, besouros, moscas das frutas; larvas de mariposas e entre outros (FONTES; FERREIRA; SIQUEIRA, 2002; BOWEN; KARD, 2012; BLETSOU et al., 2013; AHIRE; ARORA; MUKHERJEE, 2008; CHANG; CHO; LI, 2012; EL-SHEIKH, 2015).

Podemos destacar o estudo de Mansur et al. (2010), que analisaram o efeito do lufenuron sobre a oogênese de *Rhodnius prolixus*, conhecido como barbeiro no Brasil. Este inseto é o transmissor *Trypanosoma cruzi*, o agente etiológico da doença de Chagas. O lufenuron em concentração de 7,5 e 15 µg/fêmea foi injetado na cavidade do metatórax. Foi observada a redução no número dos ovos e na sua viabilidade, além de modificações na forma e na cor. No número e tamanho dos oócitos e redução da incorporação de N-acetilglicosamina na quitina nos ovários.

Segundo a Agência Nacional de Vigilância Sanitária (ANVISA, 2003), a classificação toxicológica do lufenuron é classe III – Medianamente tóxico. Dentre os critérios para a classificação toxicológica de lufenuron, destaca-se: I - as formulações líquidas e sólidas que apresentam DL_{50} oral, para ratos, superior a 200 mg/kg e até 2.000 mg/kg e 50 mg/kg e até 500 mg/kg, respectivamente. II - As formulações líquidas e sólidas que apresentam DL_{50} dérmica, para ratos, superior a 400 mg/kg e até 4.000 mg/kg e 100 mg/kg e até 1.000 mg/kg, respectivamente. III - as formulações que não apresentam opacidade na córnea e aquelas que apresentam irritação reversível dentro de 72 h nas mucosas oculares dos animais testados, dentre outros critérios de avaliação de acordo com a ANVISA.

As propriedades físico-químicas do lufenuron, estão descritas na Tabela 1. Trata-se de um composto estável e persistente em produtos de origem vegetal (EFSA, 2008). Por ser um inseticida de longa duração o lufenuron é utilizado em produtos de armazenamento, entretanto esta característica pode aumentar o risco de contaminação aquática (AHIRE; ARORA; MUKHERJEE, 2008). O lufenuron é persistente no meio ambiente e pode-se sofrer acumulação no solo (EFSA, 2008).

Tabela 1: Propriedades Físico-Químicas do Lufenuron.

Nome comum	Lufenuron
Nome comercial do produto	Match® 50 EC (50 g/L)
Estrutura Química	 (SANTOS et al., 2012)
Fórmula Estrutural	 (FAO, 2008)
Fórmula Molecular	$C_{17}H_8Cl_2F_8N_2O_3$
Nomenclatura (IUPAC)	(RS)-1-[2,5-dicloro-4-(1,1,2,3,3,3-hexafluoropropoxil)fenil]-3-(2,6- difluorobenzoil) ureia (AHIRE; ARORA; MUKHERJEE, 2008)
Peso molecular	511.2 g/mol (EFSA, 2008)
pKa	10.18 ± 0.05 (ácido) (FAO, 2008)
Ponto de fusão	168.7 para 169.4°C (FAO, 2008)
Solubilidade em água	pH 5: 54 µg/L (25 °C) pH 7: 46 µg/L (25 °C) pH 9: 64 µg/L (25 °C)
Solubilidade em solventes orgânicos	Todos em g/L a 25ºC: acetona 460 diclorometano 84 acetato de etila 330 hexano 0,10 metanol 52 octanol 8,2 tolueno 66

Os dados para meia-vida da degradação do lufenuron a 25 ºC, em pH 5,0 e 7,0 é de 30 dias e em pH 9,0, aproximadamente 21,3 dias que corresponde a 512 horas (FAO, 2008). Em condições aeróbicas e solos biologicamente ativos a meia-vida da degradação do lufenuron é de 13 a 20 dias (MAYER et al., 2013).

Quanto a sua toxicologia em mamíferos, por via oral o lufenuron é absorvido pelo trato gastrointestinal e bioacumula no tecido adiposo e com biodisponibilidade sistêmica de 70%. O lufenuron é lentamente excretado pelas fezes e não possui potencial mutagênico e carcinogênico (EFSA, 2008).

Em ratos o lufenuron apresentou baixa toxicidade aguda por via oral e dérmica, sendo a DL_{50} superior a 2000 mg/kg, e por inalação a CL_{50} foi superior a 2,3 mg/L/4h. Mostrou-se ligeiramente irritante para o olho e em contato com a pele pôde causar sensibilização. Em estudo de administração oral por 90 dias, foram observadas alterações no peso e convulsões tônico-clônicas, o nível de efeito adverso não observado de 10 mg/kg/dia. Em cães em um estudo de um ano foram observadas convulsões e morte em doses maiores ou igual a 30 mg/kg/dia. Diante deste fato, observasse que a exposição prolongada por ingestão oral pode apresentar efeitos graves para a saúde (EFSA, 2008).

Estudos de toxicidade aguda e crônica de lufenuron em peixes não publicados na literatura científica são descritos na FAO (2008). Os valores estabelecidos para dose letal de lufenuron em peixes de água doce a 96 h de exposição estática, para *Oncorhynchus mykiss* (truta arco-íris) teve a CL_{50} superior a 73 mg/L, *Lepomis macrochirus* (bluegill*)* com a CL_{50} superior a 29 mg/L, *Cyprinus carpio* (carpa) teve a CL_{50} superior 63 mg/L e *Ictalurus punctatus* (bagre) com a CL_{50} superior a 45 mg/L.

Em toxicidade crônica, *Oncorhynchus mykiss* (truta arco-íris) exposto a 21 dias em dose de 0,0020, 0,0043, 0,0090, 0,018, 0,069 mg/L, obteve menor concentração letal superior 0,069 mg/L, não foi observado efeito letal ou subletal nas doses testadas. O teste avaliando o ciclo de vida completo de *Pimephales promelas* nas doses 0,0025, 0,0050, 0,010, 0,020 e 0,040 mg/L, a concentração de efeito não observado foi de 0,02 mg/L sendo esse valor baseado na eclosão dos ovos e sobrevivência da geração F1 (FAO, 2008).

Apesar do lufenuron ser um inseticida que age inibindo o crescimento dos insetos, tem se visto seu uso em diversas áreas. Na piscicultura, como meio de tratamento de ectoparasitas em peixes (MAYER et al., 2013; Patente US20150125509A1); na medicina veterinária, no controle de pulgas em cães e gatos (WISMER; MEANS, 2012) e no tratamento de dermatofitose em gatos (RAMADINHA et al., 2010; MANCIANTI;

DABIZZI; NARDONI, 2009), como também, seu efeito nematicida (LAZNIK; TRDAN, 2014) e parasiticida (BREIJO et al., 2011).

O trabalho de Mayer et al. (2013), relataram o uso de lufenuron como tratamento para ectoparasita (*Argulus sp*) em *Cyprinus carpio*. *Argulus sp*., conhecido como piolho de peixe, é um crustáceo parasita que possui um exoesqueleto de quitina. O lufenuron é um inseticida benzoilureia que inibi a síntese de quitina e assim o desenvolvimento do ectoparasita. O tratamento consistiu de 0,1 mg/L de lufenuron na água, colocado uma vez por semana durante cinco semanas. Foi verificada a eficiência na redução da população dos ectoparasitas, porém nesse artigo os autores não relataram os possíveis efeitos tóxicos nos peixes.

A patente US20150125509A1 descreveu a utilização da alimentação com lufenuron para o controle de piolhos do mar (*Lepeophtheirus salmonis*, *Caligus elongatus*, *Caligus rogercresseyi*) que infesta peixes, especialmente salmões. O tratamento consiste em uma dose diária de 1 a 30 mg de lufenuron/kg de biomassa de peixe por um período de 3 a 14 dias. A quantidade total do lufenuron durante o período deve ser de 7 a 350 mg/kg de biomassa de peixe. O tratamento possui um efeito mais prolongado contra o piolho do mar, por um período de 150 dias, este consiste em eliminar, reduzir ou prevenir infestações de piolhos do mar.

Na medicina veterinária o lufenuron é usado para o controle de pulgas em cães e gatos impedindo o desenvolvimento dos ovos e no tratamento de dermatofitose. Nos cães e gatos, o lufenuron é armazenado no tecido adiposo e lentamente liberado na circulação. Este não é metabolizado e excretado pela bile sendo assim eliminado pelas fezes (WISMER; MEANS, 2012)

Em outro estudo, Ramadinha et al. (2010), avaliaram o uso do lufenuron no tratamento de dermatofitose causada por *Microsporum canis* em gatos. O tratamento consistiu em 46 gatos, com 120mg/kg de lufenuron em quatro doses com intervalo de 21 dias. Os autores obtiveram resultados satisfatórios para o tratamento com o lufenuron, apenas um gato não apresentou melhora.

Como efeito nematicida, Laznik e Trdan (2013), observaram a influência de oito inseticidas dentre estes o lufenuron em nematóides entomopatogênicos (Steinernematidae e Heterorhabditidae) em diferentes temperaturas (15, 20, 25 ºC) em condição de laboratório. Os nematóides entomopatogênicos são importantes agentes de controle biológico de insetos, tendo em vista que eles podem entrar em contato com diversos produtos químicos, como inseticidas. O lufenuron e abamectin foram os únicos

inseticidas, dentre os oito testados, que apresentaram diferenças significativas na taxa de mortalidade em relação ao controle.

Breijo et al. (2011), verificaram o uso exclusivo de lufenuron ou em combinação com albendazol como fármacos terapêuticos para a equinococose cística larval - hidatidose (*Echinococcus granulosus).* Trata-se de uma doença comum a homens e animais. O lufenuron foi injetado subcutaneamente em ratos infectados por esta bactéria. Como resultados obtidos, o lufenuron em combinação com albendazol promoveu um efeito parasiticida ao reduzir em 30 a 40% o crescimento do cisto devido a alterações ultraestruturais na parede do mesmo.

2.1. *Colossoma macropomum* (Tambaqui)

Colossoma macropomum (Cuvier, 1818) está classificado na ordem Characiformes, família Characidae e subfamília Serrasalminae. É uma espécie nativa da América do Sul, das bacias dos rios Amazonas e Orinoco (DAIRIKI; SILVA, 2011; SALAZAR-LUGO et al., 2011).

A ordem Characiformes corresponde a um dos maiores grupos de peixes de água doce em todo o mundo, com aproximadamente 1800 espécies. Esta ordem é composta por três famílias africanas, 14 famílias neotropicais e uma família transatlântica (MIRANDE, 2010). Muitos representantes dessa ordem são importantes economicamente e ecologicamente. Algumas espécies desempenham funções essenciais dentro do ecossistema aquático, como fluxo de energia e a ciclagem de material (OLIVEIRA et al., 2011).

Characidae compreende a maior e mais diversificada família entre os peixes neotropicais, com cerca de 1200 espécies (MIRANDE, 2010), que correspondem a 58% das espécies da ordem Characiformes (OLIVEIRA et al., 2011). São peixes que vivem em diferentes habitats, e apresentam variedade no formato de seus corpos. Predominam em águas lênticas e em regiões de baixas latitudes, alguns representantes desta família são conhecidos como tetras (DIAS; FIALHO, 2009; MIRANDE, 2009).

Colossoma é um dos gêneros endêmicos mais abundantes da Bacia Amazônica, de grande importância econômica para as famílias humanas que vivem ao longo de seu curso (GOULDING; CARVALHO, 1982; AFFONSO et al., 2002; MARCON; FILHO, 1999). As espécies do gênero *Colossoma* são conhecidos vulgarmente por gamitama, cachama e cachama negra (SOUZA, 2009).

Colossoma macropomum (Fig. 1) destaca-se como o segundo maior peixe de escama do rio Amazonas, ficando atrás do pirarucu (*Arapaima gigas*), podendo alcançar um metro de comprimento e 30 kg de peso corporal em seu habitat natural (GOULDING; CARVALHO, 1982). Na fase juvenil, seus alimentos são ricos em fibras e sua dieta muda de acordo com as estações. Durante o período chuvoso o consumo flutua entre sementes e frutas, na temporada de seca ocorre a ingestão de zooplâncton e arroz selvagem. *C. macropomum* é caracterizado como peixe onívoro-oportunista e gregário com bom crescimento (ALMEIDA; LUNDSTEDT; MORAES, 2006; ARIDE et al., 2006; ARAÚJO et al., 2004; RODRIGUES, 2014).

Figura 1: *Colossoma macropomum* (Fonte: Própria autora).

Seu ciclo de vida é de aproximadamente 15 anos, caracterizando-o como um peixe longevo, com comportamento migratório complexo sazonal para fins reprodutivos e de forrageamento (MARCON; FILHO, 1999). São espécies de alta fecundidade com desova total e ovos semipelágicos, sua maturidade sexual é alcançada entre o terceiro e quarto ano de vida (CHAGAS; VAL, 2003).

Tambaquis são tolerantes a variações de pH entre 4,0 e 8,0, demonstrando ausência de perturbação do equilíbrio iônico (MARCON; FILHO, 1999). Em seu ambiente natural os tambaquis são encontrados em águas escuras (pH 3,8 - 4,9) e barrenta

(pH 6,2 - 7,2), nestas últimas águas em maior ocorrência, em menor ou inexistente em águas claras (pH 4,5 - 7,8) (DAIRIKI; SILVA, 2011). Sua predominância corresponde a sua resistência a águas com pH ácido. Quando a espécie é submetida a exposição de águas alcalinas por um longo período de tempo, isto pode ocasionar mudanças nos parâmetros fisiológicos na redução do crescimento e hematológicos como diminuição do hematócrito, concentração de hemoglobina e células vermelhas no sangue do animal (ARIDE; ROUBACH; VAL, 2007).

O tambaqui habita lagoas abertas, muitas vezes sujeito a condições temporárias de hipóxia (ou até a anóxia), entretanto é considerada uma espécie tolerante a hipóxia (~ 1 mg/L). Essa característica confere certa facilidade na captura do oxigênio, devido ao aumento de volume dos lábios inferiores para forma um funil que pode direcionar a água da superfície para dentro da boca e sobre as brânquias, visto que, em condição de hipóxia ambiental, o animal realiza a respiração na superfície aquática. Essa estratégia contribui em até 30% do teor de oxigênio captado e distribuído por meio do sangue para os tecidos (AFFONSO et al., 2002; SUNDIN et al., 2000; DAIRIKI; SILVA, 2011).

A floresta inundada é o principal habitat do tambaqui, e este ocorre em todos os tipos de águas amazônicas (ARIDE et al., 2006). Ele exibe alta correlação genotípica e plasticidade fenotípica, o que lhe permite viver nos ambientes muito heterogêneos da Amazônia (ALMEIDA; LUNDSTEDT; MORAES, 2006). No momento das migrações realizadas pelos tambaquis as gônadas estão bem desenvolvidas (GOULDING; CARVALHO, 1982). Os menores indivíduos encontrados com gônadas desenvolvidas possuíam 56 cm de comprimento. Em cativeiro, a reprodução de tambaqui depende da indução hormonal e fertilização artificial (VARELA JUNIOR, 2011).

Colossoma macropomum possui uma tonalidade característica, quando adulto apresenta manchas escuras irregulares ventrais e caudais, na região dorsal exibe coloração esverdeada, contudo a intensidade de suas cores é influenciada pelas características da água no qual se encontra, como transparência e cor (GOULDING; CARVALHO, 1982; BORGES, 2013). Estes peixes possuem a capacidade de modificar sua coloração de acordo com o ambiente, este fato é visto como um processo adaptativo essencial para sua sobrevivência. Tais como a predação e a captura do alimento.

Esta espécie possui boca pequena e lábios carnudos pouco adaptados para alimentação piscívora. Possui dentes molariformes com as margens afiadas usados para triturar e mandíbulas potentes, adaptadas para alimentos rígidos e também apresentam dentes faringeanos poucos desenvolvidos. Seu estomago é bem definido e elástico, seu

intestino corresponde 2 a 2,5 vezes o tamanho de seu corpo, sendo este um atributo favorável para uma maior retenção dos nutrientes necessários para o desenvolvimento do peixe (GOULDING; CARVALHO, 1982; ROTTA, 2003; RODRIGUES, 2014).

Cada seção do trato gastrintestinal do tambaqui possui um perfil de enzimas. Para os peixes que eram alimentados com alto nível de proteínas (35%) observaram a predominância e aumento de proteases no estômago. O tambaqui destaca-se pela alteração de seu perfil enzimático, que pode ser modificado de acordo com o alimento ingerido. Tal fato esclarece a aceitação do animal a diversos tipos de alimentos com diferentes composições. Outra particularidade da espécie são os cecos pilóricos, os principais produtores de amilase, podendo chegar até 75 cecos em seu trato gastrointestinal (DAIRIKI; SILVA, 2011).

A bexiga natatória do tambaqui é dividida em duas câmaras, anterior e posterior. Elas têm como função estabilizar o peixe em posição diagonal. Esse tipo de postura é exibido quando o peixe está se alimentando próximo a superfície (DAIRIKI; SILVA, 2011).

Suas brânquias são compostas por vários rastros branquiais alongados, típicos de peixes planctófagos, alongados que permite a filtração e retenção de partículas e ou alimento, como na captura do zooplâncton. O primeiro arco branquial compreende cerca de 85-100 rastros branquiais (GOULDING; CARVALHO, 1982; VIDAL et al., 1998; DAIRIKI; SILVA, 2011).

Os tambaquis são encontrados em águas com temperatura entre 25 °C e 34 °C (DAIRIKI; SILVA, 2011). São considerados como peixes de forma arredondada de importância econômica na piscicultura brasileira. São mais cultivados no Norte, Centro-Oeste e Nordeste, devido aos fatores climáticos das regiões e receptividade do mercado (RODRIGUES, 2014).

O tambaqui corresponde a 40% do comércio de peixe na Amazônia (ALMEIDA; LUNDSTEDT; MORAES, 2006). Destaca-se como o terceiro peixe mais cultivado na aquicultura brasileira (SANTOS et al., 2013). Dentre as características peculiares que contribuem economicamente para sua produção na aquicultura, destaca-se: a fácil adaptação ao cativeiro, hábito alimentar onívoro com tendência à herbivoria, hábito filtrador e frugívoro, a fácil aceitação de rações artificiais, crescimento rápido, bom ganho de peso e rusticidade, a boa aceitação no mercado e a adequação às técnicas de reprodução artificial e a alta tolerância a alterações físico-químicas da água (SOUZA et al., 2014; MENDONÇA et al., 2012; SANTOS et al., 2013; ARIDE; ROUBACH; VAL, 2007).

Outro fato que também contribui para sua aceitação no mercado é o sabor de sua carne (DAIRIKI; SILVA, 2011).

No Brasil em 2011, a produção da aquicultura continental (água doce) foi de 544.490,0 toneladas, correspondendo 86% do total da produção nacional da pesca continental e marinha. Neste mesmo ano a produção de tambaqui foi de 111.084,1 toneladas correspondente a pesca continental nacional. Destacando-se dentre as espécies mais cultivadas em 2011 (MPA, 2011).

A utilização de *Colossoma macropomum* como bioindicador da qualidade ambiental têm sido relatadas em diversos trabalhos como Affonso et al. (2002), Ferreira da Costa et al. (2004), Matsuo; Wood; Val (2005), Assis et al. (2007, 2010), Duarte; Honda; Val (2010), Rico et al. (2011), Salazar-Lugo et al. (2009, 2011), na avaliação da espécie aos efeitos de potenciais poluentes.

Dentre estes, Affonso et al. (2002), relataram as mudanças nos parâmetros hematológicos e nos níveis metabólicos em tambaqui expostos a hipóxia e sulfeto de hidrogênio (H_2S) *in vivo* e *in vitro*. Tendo em vista que o H_2S é produzido em condições naturais e frequentemente está associado com a poluição da água, a hipóxia no meio aquático também é ocasionada por fatores naturais e antropogênicos (AFFONSO et al., 2002; OBA; MARIANO; SANTOS, 2009). O estudo concluiu que os resultados encontrados para a resposta fisiológica induzida pelo o sulfeto em tambaqui está associado à sua alta tolerância à hipóxia ambiental.

Assis et al. (2007), verificaram a atividade de acetilcolinestarase (AChE) no cérebro de tambaqui sobre a ação de diclorvós, um inseticida organofosforado conhecido como inibidor de AChE, e como controle negativo deltametrina um inibidor de canais de potássio e sódio. Não foi observado diferença significativa entre as atividades da AChE na presença e ausência de deltametrina. Entretanto, mesmo em concentrações baixas o diclorvós inibiu a AChE extraída de tambaqui. Por fim, o trabalho ressalta que a AChE cerebral de tambaqui pode ser proposta como uma ferramenta para monitoramento do ambiente aquático.

Por fim, Salazar-Lugo et al. (2011) relataram a exposição de *C. macropomum* a 10 mg/L de herbicida paraquate em diferentes temperaturas (18, 29 e 35 °C), durante 21 dias. Os autores observaram que a temperatura influenciou na incidência e gravidade das alterações histológicas nas brânquias, fígado e rins em tambaqui, demostrando que os parâmetros histológicos em tambaqui são importantes para monitoramento ambiental.

3. Objetivos

3.1. Objetivo Geral

O presente trabalho teve como objetivo estudar o efeito agudo e crônico do lufenuron nos parâmetros biológicos de Tambaqui (*Colossoma macropomum)*.

3.2. Objetivos Específicos

- Determinar a concentração letal (CL_{50} 24 e 96 h) do lufenuron para *Colossoma macropomum*;
- Avaliar o efeito do lufenuron em teste de toxicidade aguda e crônica sobre a sobrevivência;
- Analisar o efeito do lufenuron sobre os parâmetros morfométricos em *Colossoma macropomum* no teste de toxicidade crônica;
- Entender como o lufenuron afeta as brânquias da espécie em relação às células e tecidos;
- Determinar o efeito crônico do lufenuron sobre a concentração de glicose no sangue;
- Estudar o efeito do lufenuron sobre o comportamento do animal;
- Avaliar a retina por eletrorretinograma fotópico, as células fotorreceptoras e ON-bipolares nos peixes submetidos ao teste de toxicidade aguda e crônica.

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Effect of acute and chronic toxicity of benzoylurea pesticide on the biological parameters of *Colossoma macropomum*

Abstract

Lufenuron is a benzoylurea insecticide and a toxic substance that interfere in chitin synthesis in insects. Although lufenuron is an insecticide widely used in agriculture and aquaculture, rare are studies described in the literature that relates to possible toxic effects on different groups of animals. This work aimed to evaluate acute and chronic toxic effects of benzoylurea pesticide (lufenuron) on biological parameters of *Colossoma macropomum* (Tambaqui). To do the acute test, Tambaqui juveniles were divided into a control group and five experimental groups with exposure to from 0.1 to 0.9 mg/L of lufenuron for 96 h, with replicate (n = 120). Animals were also submitted to chronic toxicity test for four months in concentrations of 0.1 and 0.3 mg/L of lufenuron, also a control group was done (n = 60). This last concentration corresponded to 50% of LC_{50} 96 h (0.58 mg/L) determined in the acute test. The presence of hemorrhages was observed in eyes (hyphema), fins and operculum of fish exposed to 0.7 and 0.9 mg/L of lufenuron in acute test. Histological analysis showed changes in the morphology of fish gills submitted to acute toxicity test, as lamellar aneurysm and blood congestion inside lamellae. In chronic test, blood glucose analysis and morphometric parameters showed no significant differences by Tukey test ($p > 0.05$). In general, *C. macropomum* exhibit behaviors associated with stress when exposed to lufenuron in both tests. Lufenuron promoted damage in fish retina as in ability to respond to stimuli in photoreceptors and in ON-bipolar cells in acute test. Thus, lufenuron showed several toxic effects in relation to biological parameters in *C. macropomum*. This concern about the use and

discard of lufenuron indicates the requirement of environmental actions, as mitigation, to prevent bioaccumulation and/or biomagnification.

Keywords

Insecticide; lufenuron; amazonian fish; animal behavior; electroretinogram.

1. Introduction

Brazil produces many commodities and is currently the largest consumer of agrotoxics in the world. The total consumption of pesticides in Brazil is equivalent to the consumption of 11,5 lbs per Brazilian inhabitant (INCA, 2015). Among agrotoxics used in Brazil there is lufenuron (Santos et al., 2011). The IUPAC nomenclature of this substance is (RS)-1-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy) phenyl]-3-(2,6-difluorobenzoyl) urea (Arihe et al., 2008). This is a benzoylurea insecticide, as well as diflubenzuron and flucycloxuron (Zaidi and Soltani, 2011), that interferes on the larval development by inhibiting or blocking the synthesis of chitin, the main constituent of insects' exoskeleton (Vázquez et al., 2014).

This insecticide is used against insects in general in crops of cotton, maize, sugar beet, potatoes, grapes, citrus and ornamentals (FAO, 2008). Lufenuron also is used against ectoparasites *Argulus* sp. in *Cyprinus carpio* (carp), no adverse effect was observed in this fish species in concentration of 0.1 mg per liter of water by Mayer et al. (2013).

Despite lufenuron be an insecticide widely used in agriculture and pisciculture, studies that describes toxic effects on different groups of animals are rare in the literature. Pesticides, as herbicides and insecticides, can accumulate in aquatic ecosystems and exert toxic effects on aquatic organisms (Ghasemzadeh et al., 2015). This can occur because many pesticides are not specie-specific. Also, these pesticides can be persistent in the environment and water bodies once agricultural areas are found near rivers and lakes. According to the European Food Safety Authority (EFSA, 2008), lufenuron was characterized as very toxic to aquatic organisms. In general, the benzoylurea group pesticides exhibit toxic effects in crustaceans, fish, aquatic insects, nematodes, annelids and zooplankton (Vázquez et al., 2014).

Pesticides can contaminate the aquatic environment through runoff after application, mainly caused by heavy rains and intense irrigation. Additionally, to wash equipment and containers used can promote accidental spills and aerosols that can contaminate nearby water bodies (Novelli et al., 2016).

Fish can be used as bioindicators in various aquatic ecosystems because they are quite sensitive to the presence of chemicals in water. Its position is at the top of the aquatic trophic web when compared to other biomarkers, provides an integrated view of the entire aquatic environment. Consequently, they are more vulnerable when the toxic agent reaches the trophic web by biomagnification. It represents a risk to human health as these animals are used as food (Abdel-Moneim et al., 2012). Then, there is a high risk of poisoning after eating these animals considering that the use of insecticides can cause toxic effects in fish and humans (Magellan et al., 2014).

In this way, the development of animal models is necessary to study the effect of lufenuron and understand how it affects biological systems. Moreover, *Colossoma macropomum* (Tambaqui) has been used in studies as bioindicator of environmental quality and is a good alternative to the ecotoxicological studies. Tambaqui is an endemic fish in the Amazon basin with great economic importance (Salazar-Lugo et al., 2011) and stands out as one of most cultivated species in Brazil (MPA, 2011).

Tambaqui has been used in toxicity studies with bipyridine as paraquat dichloride (Salazar-Lugo et al., 2011) and non-selective glyphosate-based herbicide (Braz-Mota et al., 2015). As far it concerned, there was no studies found related to the effect of benzoylurea as a toxic agent in *C. macropomum*. Therefore, studies using Tambaqui are necessary due to its wide use in commercial breeding in Brazil as well as other South America countries. This study aimed to evaluate acute and chronic toxic effects of benzoylurea pesticide (lufenuron) on biological parameters of *C.*

macropomum (Tambaqui). Additionally, the information obtained may be used as a comparative parameter for future studies in environmental toxicology and also to report to regulatory agencies the effects of this substance in the studied species.

2. Materials and methods

2.1. Management

Juvenile *Colossoma macropomum* (Tambaqui) were obtained by local fish farm (*Estação de Aquicultura Continental Professor Johei Koike – Universidade Federal Rural de Pernambuco - UFRPE*) and housed in aquariums at the *Laboratório de Ecofisiologia e Comportamento Animal - LECA* of the same institution (*UFRPE*). The protocols used in this study were approved by Ethics Committee in the use of animals of the same University, protocol number 140/2014.

The experiments were performed with a photoperiod of 12:12 light/dark. The water was treated with sodium thiosulfate to reduce the levels of chlorine and sodium hydroxide to adjust the pH to 6.8. The average temperature of water in aquariums was 26 ± 1 °C. During the acclimatization period, fish were fed once a day with extruded commercial fish feed (40% crude protein). Tambaqui were kept in aquariums with a final volume of 18 L, with continuous aeration (11 mg/L of DO) and a density of 1.8 animal/L.

2.2. Acute Toxicity Test

Preliminary tests were performed to determine the experimental concentrations. It was considerate lufenuron concentration used in the treatment of ectoparasites in fish (0.1 mg/L) used by Mayer et al. (2013). To determinate LC_{50} 24 and 96 h, feeding was suspended for 48 h before the experiments. Assays were performed in a static system. Juvenile Tambaqui (120 animals, 3.04 g ± 0.97 and 5.85

cm ± 0.55) were divided into a control group and five experimental groups (n = 10 to each treatment) with 0.1, 0.3, 0.5, 0.7 and 0.9 mg/L of lufenuron diluted in aquarium water. Animals were exposed to lufenuron for 96 h. and the experiment was done twice (replicate). Mortality was daily verified after exposure. The collected data were analyzed using the Probit method with Biostat Pro Software 5.9.8 Version. Parameters evaluated were: histology, behavioral studies and electroretinogram.

2.3. Chronic toxicity test

Chronic toxicity of lufenuron in *C. macropomum* was evaluated for four months. It was used semi-static system with complete washing of aquarium once a week and insecticide replacement. The experiment was done twice (replicate). Concentrations used were 0.1 and 0.3 mg/L of lufenuron and a control group; 0.3 mg/L corresponded to 50% of the LC_{50} 96 h previously determined in acute toxicity test. During the chronic toxicity test, animals were fed once a day with 100 mg of fish feed per fish. Ten fish were used for each treatment. The parameters evaluated were weight and length measurement, analysis of blood glucose levels, behavioral studies and electroretinogram.

2.4. Collect of experimental data

2.4.1. Weight and length measurement

The verification of weight (g) and length (nose to tail) (cm) were measured each 20 days exclusively in the chronic toxicity test.

2.4.2. Analysis of blood glucose levels

After the chronic toxicity test, fish were fasted for 14 h before the blood collection. It was sampled with a syringe from the caudal vein according to Zang et al. (2013). Ten animals from each experimental group were used for measurement of

glucose levels. Blood glucose was measured using a handheld glucometer (G-Tech Free 1, SD Biosensor inc. South Korea).

2.4.3. Histology

For histological analyses of fish submitted to acute test, the gills were prepared for inclusion being fixed in 10% formaldehyde for 24 h. Then, they were dehydrated by increasing ethanol concentrations (80-100%) for 15 minutes and embedded into historesin. Blocks were cut with a Minot microtome (LEICA RM 2035). Sections were submitted to staining techniques by toluidine blue for morphological tissue analysis. Histological images were examined with photomicroscope Leica®. Images were captured and scanned by LAS Leica Image software.

2.4.4. Behavioral studies

Initially, it was made an ethogram with behavioral patterns for *C. macropomum* using *ad libitum* method. Behavioral observations were recorded once a day in acute test for 96 h. and twice a week in chronic test for four months from a fixed point (a distance of 1.5 m from the aquarium) to not influence the fish behavior. Animal's activities were recorded using instantaneous scan sampling method (Altmann, 1974). Activities were classified into four categories: Moving, Interaction Agonistic, Maintenance and Behaviors Associated with Stress. Time for fish observation was 30-40 min for acute and chronic toxicity tests. It was determined one min of observation followed by one min of interval for each aquarium. During chronic toxicity test, feeding was administered 10 min before the observations.

2.4.5. Electroretinogram – ERG

We have developed the technique of photopic ERG on eye of *C. macropomum in vivo* aiming to evaluate retinal activity. ERGs full-field by light flashes were performed in three animals for each experimental group of acute test that survived after

96 h. In acute test, the number of animals to conduct the photopic exam was standardized according to the amount of fish that survived in the group submitted to 0.7 mg/L of lufenuron. The exam in the chronic test was performed at the end of the experiment, in four fish per treatment. In the chronic test, we had more fish to obtain the ERGs.

Animals were anesthetized by placing them in 30 mg/L of eugenol solution. Fish were considered anesthetized when did not respond to mechanical stimuli on the fins. To maintain anesthetize standard, fish were placed in lateral recumbency into a recipient with anesthetic solution at the mouth level. Also, water with anesthetic was administered orally through the catheter to both: assist breathing and help to anesthesia maintenance. After stabilization and animal placement, the eye to be examined was anesthetized topically with proxymetacaine 0.5% (w/v) and lubricated with methylcellulose 2%.

ERGs were recorded from three monopolar electrodes: (1) reference, introduced in the dorsal subcutaneous area (head); (2) active DTL contact the center of the cornea; and (3) ground wire (local) placed under in the caudal fin. In the exam room with light (approximately 30 cd/m^2), five visual stimulations of 3 cd/m^2 (0.7 Hz) were used for photopic measurements. The system used was Neuropack 2 MEB-7102A/k of Nihon Kohdem.

To data collection, it was considered amplitude in microvolts (μV) and implicit time in milliseconds (ms); 500 ms. were analyzed. It was considered the following conventions: the a-wave amplitude, which consists in the interval between the baseline and the negative pick of a-wave. Implicit time of a-wave, consists in the interval between the stimulus and the appearance of a-wave. The b-wave amplitude, consists in

the interval between the a-wave and the peak b-wave. Implicit time of the b-wave, consists in the interval between the stimulus and the appearance of the b-wave.

2.5. Statistical analyses

All data were presented by mean ± SD. The results were analyzed by *one way* ANOVA. When the difference was significant, means were compared by Tukey test with $p < 0.05$. Statistical analyses were performed using the Origin Pro Academic 2015 (Origin Lab. Northampton, MA USA).

3. Results

3.1. Acute Toxicity Test

After 96 h of exposure to lufenuron, the following rates of mortality were found: 100% mortality to animals exposed to 0.9 mg/L of lufenuron, 85% and 10% for animals exposed to 0.7 and 0.5 mg/L of lufenuron, respectively. For the other groups mortality was not observed. The results of lethal concentration of 24/96 h in *Colossoma macropomum* exposed to lufenuron are shown in Table 1.

[Table 1]

It was observed an increase of opercular beat and swimming near the water slide in fish exposed to concentrations equal or higher than 0.5 mg/L of luferunon. In fish submitted to 0.7 mg/L (Fig. 1A - 1D) and 0.9 mg/L of lufenuron (Fig. 1E - 1H) it was observed hemorrhages in: eye (hyphema), operculum, pectoral, dorsal, anal and caudal fins. These fish also exhibited loss of scales and redness in their bodies.

[Figure 1]

In the control group histological analysis in gills of *C. macropomum* showed the standard structure of the gill arches found in teleost fish, with the presence of mucus, pavement and pillar cells in primary and secondary lamellae. The gills of

animals submitted to acute lufenuron concentration of 0.1 mg/L showed morphological changes in gill tissue, characterized by lamellar epithelium lifting, blood congestion in secondary lamellae, lamellar fusion and lamellar aneurysm. These types of changes also were observed in fish exposed to 0.3 mg/L of lufenuron. Fish from this group also presented: blood congestion in primary lamellae and necrosis. For fish exposed to 0.5 mg/L of lufenuron, it was verified the same signals observed in group submitted to 0.3 mg/L of lufenuron. Besides, for animals submitted to a concentration of 0.7 mg/L of lufenuron it was observed necrosis, lamellar epithelium lifting and blood congestion in the primary lamellae (Fig. 2). All animals exposed to 0.9 mg/L of lufenuron did not survive after 96 h of experiment.

[Figure 2]

3.2. Chronic toxicity test

At the end of exposure in the chronic toxicity test (four months), mortality was observed in animal exposed to 0.1 mg/L (5%) and 0.3 mg/L (5%) of lufenuron. In the control group no mortality was recorded. For all groups (control, 0.1 and 0.3 mg/L) weight and length measurements showed no significant difference ($p > 0.05$) (Fig. 3). Additionally, blood glucose levels after 14 h of fasting were: 116.8 ± 14 mg/dL, 122 ± 31.5 mg/dL and 121.1 ± 17.6 mg/dL, to control and for groups of fish exposed to 0.1 mg/L and 0.3 mg/L pf lufenuron, respectively; no significant difference was shown by Tukey test ($p > 0.05$).

[Figure 3]

3.3.1. Behavioral studies in the acute toxicity test

After behavioral observations of *C. macropomum* by the *ad libitum* method, the ethogram was developed. Behaviors were grouped into categories: Moving, Agonistic Interaction, Maintenance and Behaviors Associated with Stress (Table 2).

[Table 2]

The results of behavioral observations of *C. macropomum* submitted to acute toxicity test are represented in Figure 4A. In Moving category, *Slow Swimming* was the behavioral event most observed for all groups. However, only groups exposed to 0.7 mg/L (43.7% ± 19.2) and 0.9 mg/L (22% ± 31.1) of lufenuron showed significant differences when compared to control (75.3% ± 9.3) by Tukey test ($p < 0.05$). In this same category, *Fast Swimming, Staying Motionless* and *Emerge and Submerge* showed no significant differences ($p > 0.05$) in the experimental groups when compared with the control.

Regarding to Agonistic Interaction and Maintenance categories were exhibited the following behaviors for all groups: *Chase*, *Escape*, *Frontal Attack*, *Lateral Attack* and *Forage*; except for group of animals exposed to 0.9 mg/L of lufenuron as it was observed 100% mortality at the first 24 h the acute toxicity test for this group. However, none of behavioral events mentioned above showed significant differences ($p > 0.05$) when compared to control.

To behaviors associated with stress (*Aerial Breath*, *Jump*, *Surface Swimming*, *Coprophagy* and *Hanging Vertically in the Water*), it was not observed significant difference ($p > 0.05$) when data from all experimental groups were compared to data from control group. For behaviors *Erratic Swimming* (ER) and *Lying Down* (LD) only data from animals exposed to 0.9 mg/L of lufenuron showed significant difference ($p < 0.05$) when compared with control group that showed no behavioral event for this behavior. Data were 14.1% ± 19.9 for ER and 0.8% ± 1.1 for LD, for fish exposed to 0.9 mg/L of lufenuron.

3.3.2. Behavioral studies in the chronic toxicity test

Regarding the chronic toxicity test, in the Moving category *Slow Swimming* was the most observed behavior in control and experimental groups (from 74.8% ± 7 to 78.7% ± 9.2). However, no significant difference ($p > 0.05$) was observed when experimental groups were compared to control. Besides, data from experimental groups for *Fast Swimming*, *Group in Swimming* and *Emerge and Submerge* behaviors did not exhibit significant differences ($p > 0.05$) when compared to control group. For *Staying Motionless* behavior, the higher frequency observed was in group exposed to 0.3 mg/L of lufenuron (7.4% ± 9.4), only this group showed significant differences ($p < 0.05$) in relation the control (3.2% ± 3.7) (Fig. 4B).

In the Agonistic Interaction category (*Chase*, *Escape*, *Frontal Attack* and *Lateral Attack* behaviors), the only behavior with significant difference ($p < 0.05$) compared with control (5.9% ± 2.8) was *Lateral Attack*. This was observed in fish exposed to 0.3 mg/L of lufenuron (and 4.2% ± 2.1). For the category Maintenance, in data from *Eat* behavior no significant difference ($p > 0.05$) was observed in both experimental groups compared to control. Data from *Forage* showed significant difference ($p < 0.05$) for groups exposed to 0.1 mg/L (1.4% ± 1.1) and 0.3 mg/L (1.4% ± 1.5) of lufenuron, when compared to control (0.6% ± 0.9).

When data from Behaviors Associated with Stress were analyzed, data from *Jump* and *Coprophagy* behaviors showed no significant differences ($p > 0.05$) when compared to control. *Erratic Swimming* was only exhibited by group exposed to 0.3 mg/L of lufenuron (0.02% ± 0.1). *Surface Swimming* behavior was observed only in groups exposed to 0.1 mg/L (0.04% ± 0.15) and 0.3 mg/L (0.8% ± 2.6) of lufenuron with no significant difference ($p > 0.05$) compared to the control group.

[Figure 4]

3.4. Electroretinograms – ERGs in fish from acute and chronic toxicity test

Standard ERG photopic of Tambaqui (control group) and ERG photopic of groups exposed to lufenuron are shown in Figure 5. The means obtained in photopic exam, implicit time and amplitude of the waves of groups are presented in Table 3. Regarding to results of chronic toxicity test, data from experimental groups showed no significant difference ($p > 0.05$) in implicit time and amplitude of the waves when compared to data from control group. On the other hand, for the acute toxicity test, the implicit time of a-waves for the group exposed to 0.7 mg/L of lufenuron were 11 times higher than data from control group. For b-waves, all experimental groups showed significant difference ($p < 0.05$) when compared to control. It was an increase between 8 and 11 times on the implicit time to animal respond the stimuli on the experimental treatments. In amplitude of the b-waves the group exposed to 0.1 mg/L of lufenuron showed significant difference ($p < 0.05$) when compared to control, in this case there was a decrease of 3 times in the amplitude of the b-wave, in the activation of ON-bipolar cells.

[Figure 5]

[Table 3]

4. Discussion

In all experimental groups of juvenile *C. macropomum* submitted to lufenuron sub-lethal effects were observed in evaluated parameters. To Tambaqui LC_{50} 24 h of lufenuron was 0.61 mg/L and to 96 h was 0.58 mg/L. Similar experiments are related in the literature. It is related LC_{50} 96h of lufenuron in experiments conducted in other fish species. As for *Oncorhynchus mykiss* (rainbow trout) ($LC_{50} > 73$ mg/L), *Lepomis macrochirus* (bluegill sunfish) ($LC_{50} > 29$ mg/L), *Cyprinus carpio* (carp) ($LC_{50} > 63$ mg/L) and *Ictalurus punctatus* (catfish) ($LC_{50} > 45$ mg/L) (FAO, 2008). It was observed

that juveniles *C. macropomum* had higher sensitivity to lufenuron when compared with species described above. However, FAO (2008) data do not specify information as age or weight of fish's species studied. Then, a further discussion is difficult to be done. Maybe, fish species described by FAO (2008) were studied at adult age. Because of this, higher concentration of lufenuron could be used to determine LC_{50}.

Among the histological damage in gills, lamellar aneurysm and blood congestion in lamellae occurred by rupture of the pillar cells. It allows expansion of the vascular space and accumulation of blood cells. Thus, there is an increase of hemorrhages risk and it possibly affects the vascular system (Ramírez-Duarte et al., 2008; Barja-Fernández et al., 2013).

In this study it was observed hemorrhages in eyes (hyphema), fins and operculum. Also, redness in fish bodies exposed to 0.7 and 0.9 mg/L of lufenuron was observed in the acute toxicity test. Thereby, vascular problems related by Ramírez-Duarte et al. (2008) were observed in this work. Hemorrhages in *C. macropomum* occurred in various parts of fish body probably caused by physiological response after contacting with lufenuron solution.

It was observed histological changes in gills of *C. macropomum* when exposed to lufenuron acutely. Danabas et al. (2015) reported that the presence of pollutants and/or toxic agents can cause histological changes in gills. These organs are essential for gaseous exchange, osmoregulation, acid-base balance, excretion of nitrogen compounds and tasting, but they are the first to suffer the effects of contaminants and/or stressors due to the fact that they are in direct contact with water (Abdel-Moneim et al., 2012; Murussi et al., 2016). In this way, we can infer that lufenuron acted as a toxic agent for *C. macropomum.*

Among histological results, gills of *C. macropomum* exposed to lufenuron exhibited lamellar epithelium lifting, lamellar aneurysm, lamellar fusion, necrosis and blood congestion in primary and secondary lamellae. Branchial changes as lamellar epithelium lifting and lamellar fusion can be understood as a defense mechanism because it could result in increased water/blood barrier. This increase promotes a delay to toxic substances enter into the bloodstream (Ramírez-Duarte et al., 2008; Abalaka et al., 2015).

These changes can be characterized as adaptive strategies in order to maintain the physiological functions, due to the contact of gills with toxic agents. However, it could result in secondary effects as respiratory and ionic disturbances that can compromise structural integrity of gills (Braz-Mota et al., 2015). These same authors analyzed short-term exposure to the Roundup® - a non-selective glyphosate-based herbicide - in *C. macropomum*. Fish were exposed to concentrations of 10 and 15 mg/L glyphosate, corresponding to 50% and 75% of LC_{50} 96 h. Among the changes observed in gills structure, the most common in fish exposed to Roundup® 75% was lamellar fusion, necrosis, filament epithelium lifting and aneurysm.

Ghasemzadeh et al. (2015) verified exposure to diazinon (organophosphate insecticide) in concentration between 10 and 30 µg/L during 96 h in *Scatophagus argus* fish species. Changes in the gills of fish exposed were observed such as lamellar fusion and filament epithelium lifting. Tabassum et al. (2016) examined acute effects of pendimethalin (herbicide) in *Channa punctate* fish at sub-lethal concentrations (0.5 and 0.8 ppb). They observed histopathological change in gills, as fusion of secondary lamellae, necrosis, epithelial lifting and blood congestion in the vascular axis of primary filaments, and other alterations. Thus, we can deduce that fish submitted to pesticide

exposure as lufenuron, glyphosate, diazinon and pendimethalin show damages in gills since the products get in touch with the organ related to fish breath.

In relation to the chronic toxicity test, low mortality rates (5 %) were found in groups exposed to 0.1 and 0.3 mg/L of lufenuron, which corresponds to the concentrations used for treatment of ectoparasites in fish and 50% LC_{50} 96 h lufenuron juvenile *C. macropomum*, respectively. After 21 study days in *Oncorhynchus mykiss* (rainbow trout) exposed to concentrations from 0.0020 to 0.069 mg/L of lufenuron, at 0.069 mg/L, no mortality or sub-lethal effect was observed (FAO, 2008). This difference in mortality data from FAO (2008) and from our results can be explained by physiological differences in studied fish species and the concentration of lufenuron used. Our study exposed to Tambaqui about 4 times more lufenuron than the concentration exposed to rainbow trout cited by FAO (2008).

Data from weight and length showed no significant differences ($p > 0.05$) in chronic toxicity test in this study. So, concentrations of 0.1 and 0.3 mg/L of lufenuron did not interfere on morphometric parameters of juveniles Tambaqui for 4 months. Similar results were reported for Zaidi and Soltani (2011). They verified the effect of the chronic toxicity of insecticides diflubenzuron (16 and 78 ng/L) and flucycloxuron (35 ng/L and 1.9 µg/L) in females of *Gambusia affinis*. These insecticides are chitin synthesis inhibitors, as lufenuron. After 28 days of exposure in Zaidi and Soltani (2011) experiment, fish showed no significant difference ($p > 0.05$) for weight and length when exposed to insecticides.

The determination of blood glucose levels in fish is very important to evaluate fish health considering that elevated blood glucose levels may be related to various stressors (Moraes et al., 2009). The results of blood glucose levels analysis of Tambaqui submitted to chronic toxicity test showed no significant difference ($p > 0.05$) between

control (116.8 ± 14 mg/dL) and experimental groups in this work. It can be indicative of fish adaptation to pesticide exposure or that lufenuron do not interferes on glucose metabolism in the experimental conditions assayed. The same results were found by Moraes et al. (2009) studying *Leporinus obtusidens* fish exposed to clomazone herbicide for 90 days.

Commonly, it has been demonstrated that observation of behavioral parameters is an advantageous method for environmental monitoring and is closely related to the physiological and ecological processes (Scott and Sloman, 2004). This method is characterized as non-invasive, inexpensive and easy to obtain for large amount of data.

In relation to the Maintenance category in the chronic test, the presence of lufenuron caused an increase search for food (*Forage*) in groups exposed to 0.1 and 0.3 mg/L of lufenuron ($p < 0.05$). Scherer et al. (1997) demonstrated that changes in *Forage* behavior are sensitive and ecologically significant, can be considered indicators of environmental changes. Probably by the fact that Tambaqui fish were continually exposed to a stressor, an environmental change, *Forage* behavior was more observed.

The decrease in the frequency of *Lateral Attack* behavior by the group exposed to 0.3 mg/L of lufenuron in the chronic toxicity test, collaborates with the statement of Scott and Sloman (2004): exposure to toxins can promote social imbalance in fish and may increase or decrease agonistic behaviors.

Fish exposed to 0.7 and 0.9 mg/L of lufenuron acutely showed a lower frequency of *Slow Swimming* events ($p < 0.05$). Also, it was observed that fish submitted to 0.9 mg/L of lufenuron showed more frequently *Erratic Swimming* and *Lying Down* behaviors when compared to control group ($p < 0.05$). It was observed a toxic effect of lufenuron that may interfere on energetic consumption of fish because the frequency of observation of *Slow Swimming* behavior decreased and *Lying Down*

increased. Regarding chronic toxicity test, frequency of *Staying Motionless* behavioral event was only observed in 0.3 mg/L group ($p < 0.05$) this can collaborate with the discussion above.

Studies have identified behavioral changes in the presence of contaminated environments and assigned it as an indicator parameter of animal health. One example is the study of Sarikaya et al. (2004) that investigated effect of fenitrothion (insecticide with neurotoxic effects) in *Corydoras paleatus*. These authors observed behavioral changes, such as loss of equilibrium, erratic swimming, hanging vertically in the water, motionlessness temporary and lying down. These behaviors were aggravated when concentration of fenitrothion was increased. As behavioral events of erratic swimming, lying down and motionlessness temporary were observed in this work it can be said that fish were intoxicated by lufenuron. As cited above, in this study behavioral events have changed due to environmental conditions.

This study describes behavioral changes as pesticide effects on Tambaqui. Other effects are described in literature as effects of pesticides on fish. Miron et al. (2005), analyzed the effects of clomazone, quinclorac and metsulfuron-methyl herbicides in *Rhamdia quelen*, during 96 h. At doses of 10 and 20 mg/L of clomazone, fish showed erratic swimming. At the concentrations of 375 and 400 mg/L of quinclorac fish did not feed and showed loss of equilibrium and lethargic behavior. For fish exposed to metsulfuron-methyl, they observed abnormal burst swimming reactions at all tested concentrations.

Köprücü et al. (2006), studied acute toxicity of organophosphorus pesticide (diazinon) in different concentrations (from 1 to 64 mg/L) and its effects on behavior of fingerling European catfish (*Silurus glanis L.*). Changes in behavior were observed in the highest diazinon concentration, as loss of equilibrium, erratic swimming, rapid gill

movement, hanging vertically in the water, and staying motionless on the aquarium bottom. Melo et al. (2015), observed short-term toxicity of rotenone (pesticide) in *Danio rerio* embryos and juveniles of *Poecilia reticulata*. In embryos of *D. rerio*, rotenone affected the behavior of loss of equilibrium and lying on the bottom of the microplate. In juvenile *P. reticulata* among observed behavioral changes were: paralysis, loss of equilibrium and erratic swimming. Similar behaviors as reported by Miron et al. (2005), Köprücü et al. (2006) and Melo et al. (2015) were observed in this work as erratic swimming, staying motionless and lying on the bottom. This behavioral events reported here were statically significative ($p < 0.05$) when compared with control group.

Electroretinogram (ERG) is a non-invasive electrophysiology method used to evaluate retinal activity, especially the function of photoreceptors (Hughes et al., 1998; Makhankov et al., 2004). It measures light-induced changes of electrical activity of the eye in response to light (Makhankov et al., 2004). The *in vivo* ERG response consist initially of a-waves (negative) originated from photoreceptors followed by b-waves (positive) related to the activity of ON-bipolar cells that represent the layers of retinal neurons (Hughes et al., 1998; Makhankov et al., 2004). Diseases and injuries in the visual system can affect the electrophysiologic potentials (Komáromy et al., 2002). In this work, the experimental groups showed significant differences ($p < 0.05$) at the implicit time in the a-waves and b-waves when compared to control in the acute toxicity test. It can be understood as delayed response (beginning of the wave record) to the stimulus (flashes of light). Probably lufenuron promoted damage to the retina of *C. macropomum*. The damage might be in the capacity to respond to stimuli in the photoreceptors and ON-bipolar cells. As b-wave amplitude decreased in the

experimental group exposed to 0.1 mg/L of lufenuron ($p < 0.05$). Probably this pesticide interfered on ON-bipolar cell activation.

Considering that the vision is a fundamental sensory system for fish interaction with the environment and is related to a wide variety of behaviors, such as feeding, foraging, reproduction and defense mechanism (Pereira et al., 2016), damage in the retina by the presence of lufenuron in water can influence the survival of *C. macropomum*.

For fish submitted to chronic toxicity test, no significant differences ($p > 0.05$) were observed in the photopic exam, even at similar concentrations to those found in acute test. This fact can be explained by the process called neurogenesis (Sabbah et al., 2012) in the fish retina and/or biochemical compensatory mechanisms (Montalbán-Soler et al., 2012). Future experiments will elucidate these possibilities. Contrary to mammals and other vertebrates, fish eyes grow throughout life, potentially allowing the optimization of the visual system to changes in visual demands of the environment.

5. Conclusions

Effects of lufenuron were studied on the biological parameters of *Colossoma macropomum* performing acute and chronic toxicity tests. It was observed that the mortality rate and toxic effects, as presence of external hemorrhages, were more intense in groups of fish exposed acutely to 0.7 and 0.9 mg/L of lufenuron. Histological analyses of *C. macropomum* gills exposed acutely to the pesticide indicated that they can be used as water quality indicator. In both tests, *C. macropomum* exhibit behaviors associated with stress when exposed to lufenuron. Moreover, the ERGs analyses were indicative that lufenuron promoted retina damage of *C. macropomum* when acute test was done. These damages include changes to the capacity to respond to stimuli in the

photoreceptor and ON-bipolar cells and in activation of ON-bipolar cells. Despite, lufenuron is used as pesticide against arthropods, our results showed a series of toxic effects on fish, a vertebrate animal. This concern about the use and discard of lufenuron indicates the requirement of environmental actions, as mitigation, to prevent bioaccumulation and/or biomagnification.

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Figure 1. *Colossoma macropomum* exposed to 0.7 and 0.9 mg/L of lufenuron. Group of fish exposed to 0.7 mg/L of lufenuron: (A) hemorrhages in dorsal, anal and caudal fins. (B and C) hemorrhages in eye (hyphema) and redness in their bodies. (D) hemorrhages in eye (hyphema) and in operculum. Group of fish exposed to 0.9 mg/L of lufenuron: (E) hemorrhages in pectoral, dorsal and anal fins, in operculum and redness in their bodies. (F and G) hemorrhages in eyes and loss of scales. (H) hemorrhages in caudal fins.

Figure 2. Longitudinal cut of gills *Colossoma macropomum* in acute toxicity test. Control group – A (scale bar 20 μm): primary lamellae (Pl), secondary lamellae (Sl), mucus cells (Mc), pillar cells (dotted arrow) and pavement cells (arrow). Fish exposed to 0.1 mg/L of lufenuron - B (scale bar 20 μm) and C (scale bar 100 μm): lamellar epithelium lifting (*), blood congestion (Bc), lamellar fusion (arrow) and lamellar aneurysm (La). Fish exposed to 0.3 mg/L of lufenuron - D (scale bar 20 μm) and E (scale bar 100 μm): lamellar epithelium lifting (*), lamellar aneurysm (La), lamellar fusion (arrow), blood congestion (Bc) in primary lamellae and necrosis (dotted arrow). Fish exposed to 0.5 mg/L of lufenuron - F and G (scale bar 100 μm, respectively): lamellar aneurysm (La), blood congestion (Bc) in primary lamellae and lamellar fusion (arrow). Fish exposed to 0.7 mg/L of lufenuron - H (scale bar 20 μm) and I (scale bar 100 μm): blood congestion (Bc) in primary lamellae, lamellar epithelium lifting (*) and necrosis (dotted arrow). Toluidine Blue.

Figure 3. Length (cm) and weight (g) of *Colossoma macropomum* exposed to lufenuron in chronic toxicity test.

Figure 4. Behavioral events *Colossoma macropomum* submitted to acute (A) and chronic (B) toxicity test of lufenuron. Events are: SS - *Slow Swimming*; FS - *Fast Swimming*; GS - *Group in Swimming*; ES - *Emerge and Submerge*; SM - *Staying Motionless*; CH - *Chases*; EE - *Escape*; FA - *Frontal Attack*; LA - *Lateral Attack*; EA - *Eat*; FO - *Forage*; AB - *Aerial Breath*; JU - *Jump*; ER - *Erratic Swimming*; LD - *Lying Down*; SW - *Surface Swimming*; CP - *Coprophagy*; HVW - *Hanging Vertically in the Water*. ***** Significant difference when compared to control group ($p < 0.05$).

Figure 5. Electroretinogram photopic of *Colossoma macropomum in vivo*. Implicit time and amplitude of the a-waves and b-waves are shown. Acute toxicity test: Control (standard), 0.1 mg/L, 0.3 mg/L, 0.5 mg/L and 0.7 mg/L.

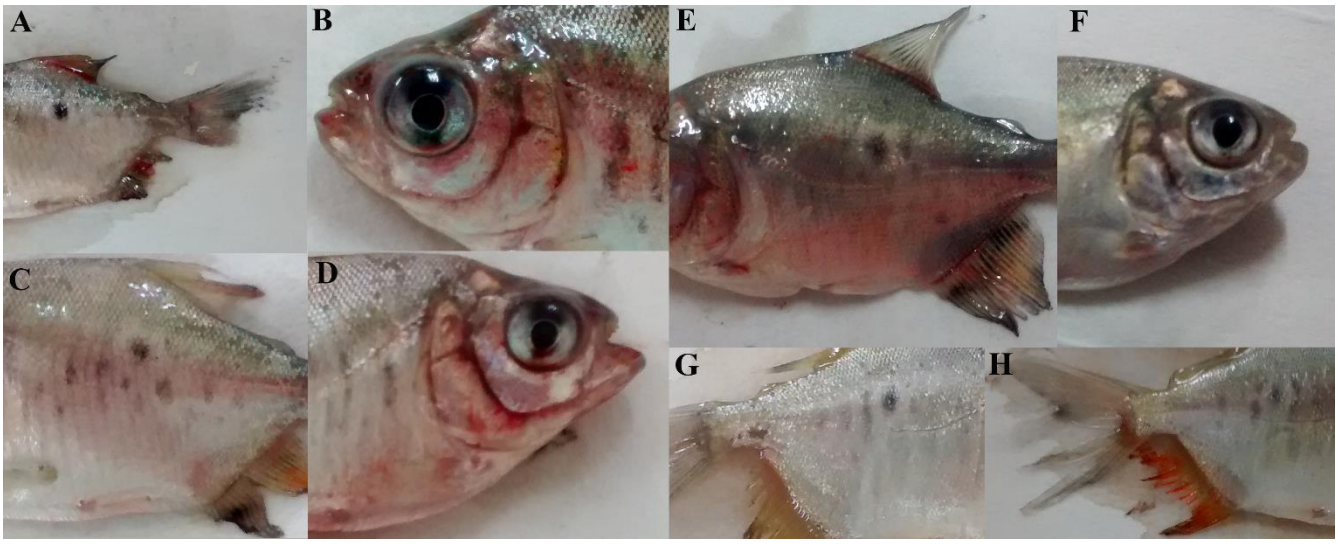


Figure 1

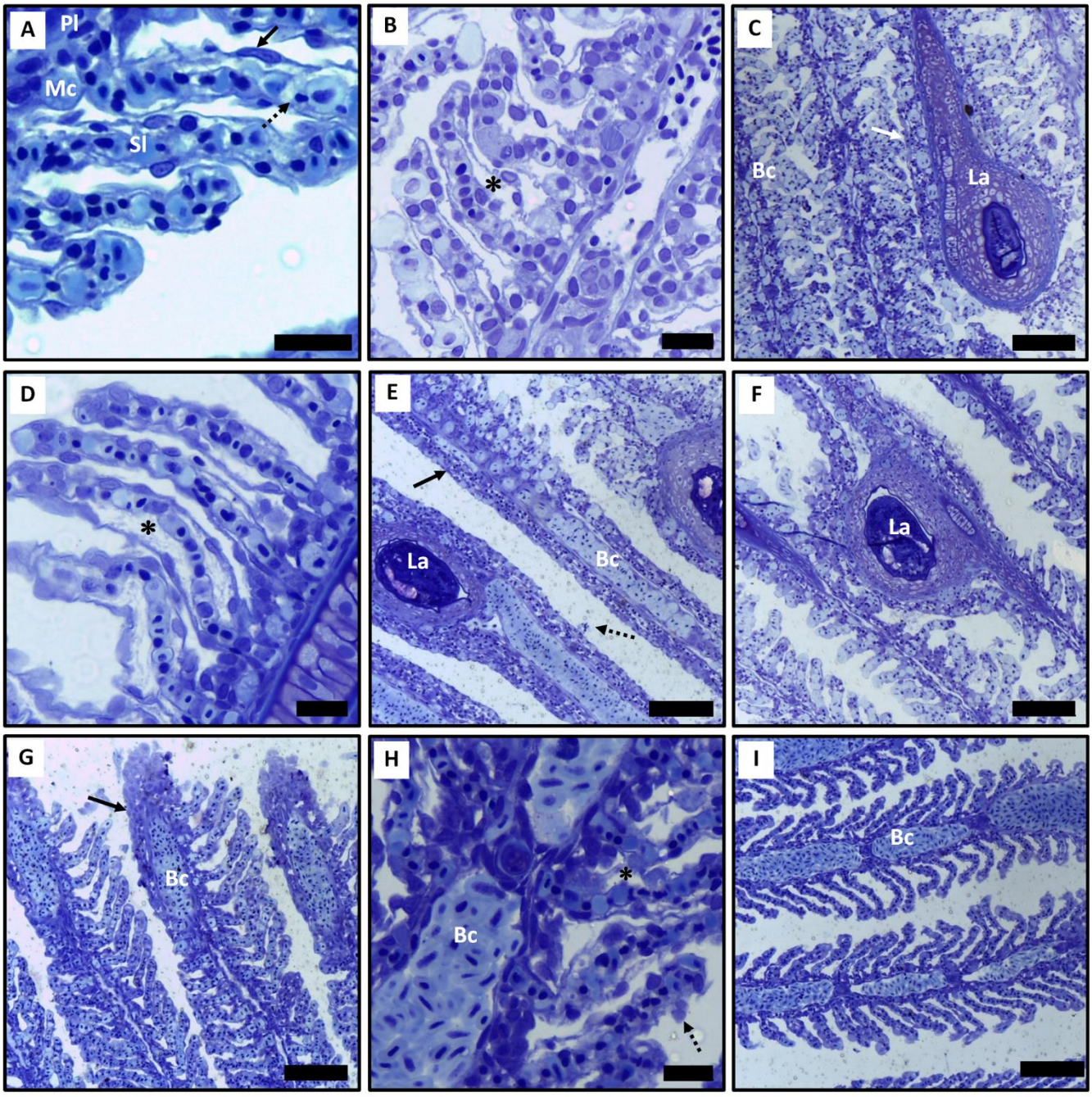
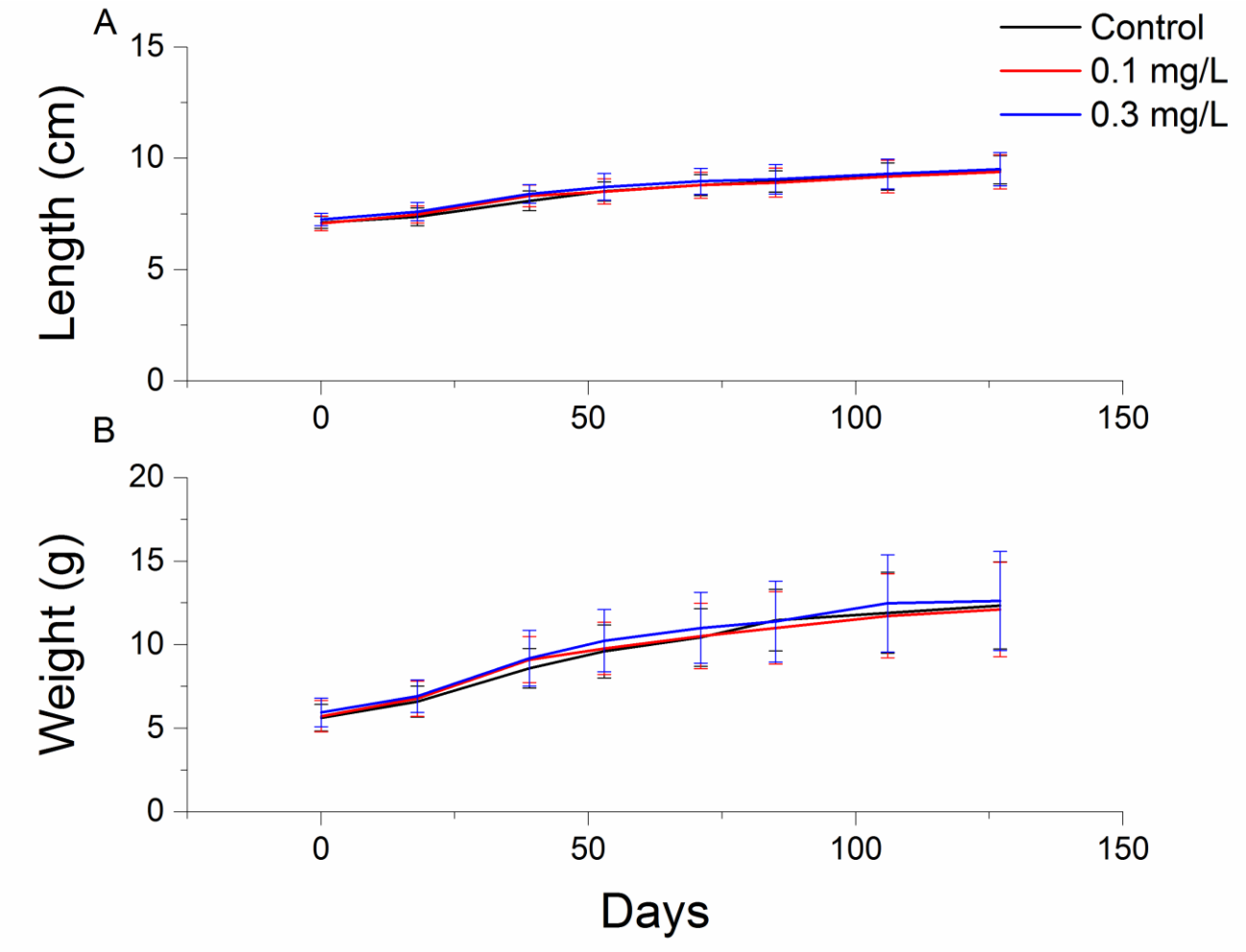


Figure 2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5
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0
50
100
150
Control
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B
Weight (g)
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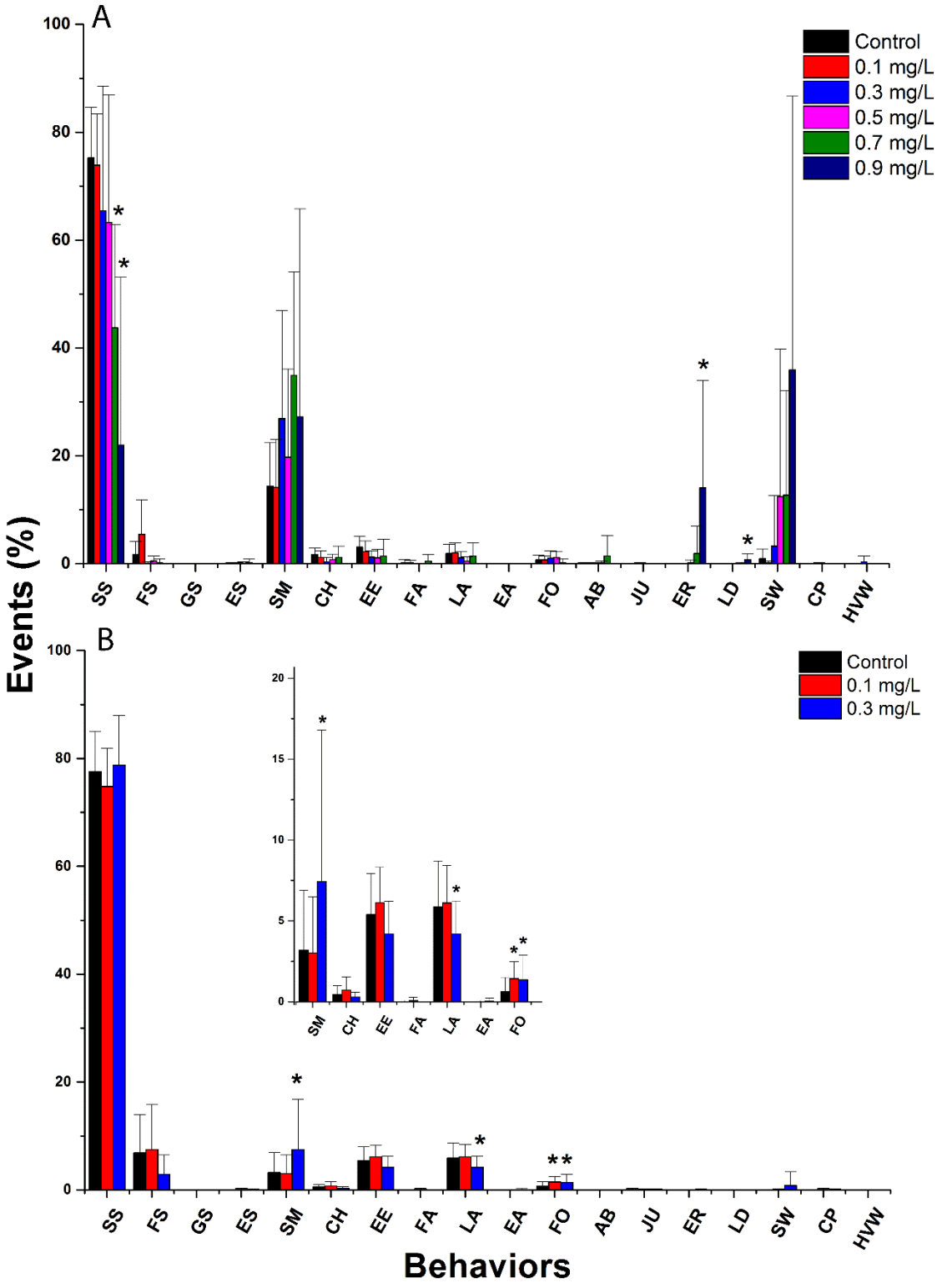
15
10
5
0
0
50
100
150
Days

Figure 3


A
B
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0.1 mg/L
0.3 mg/L
0.5 mg/L
0.7 mg/L
0.9 mg/L
Events (%)
Behaviors
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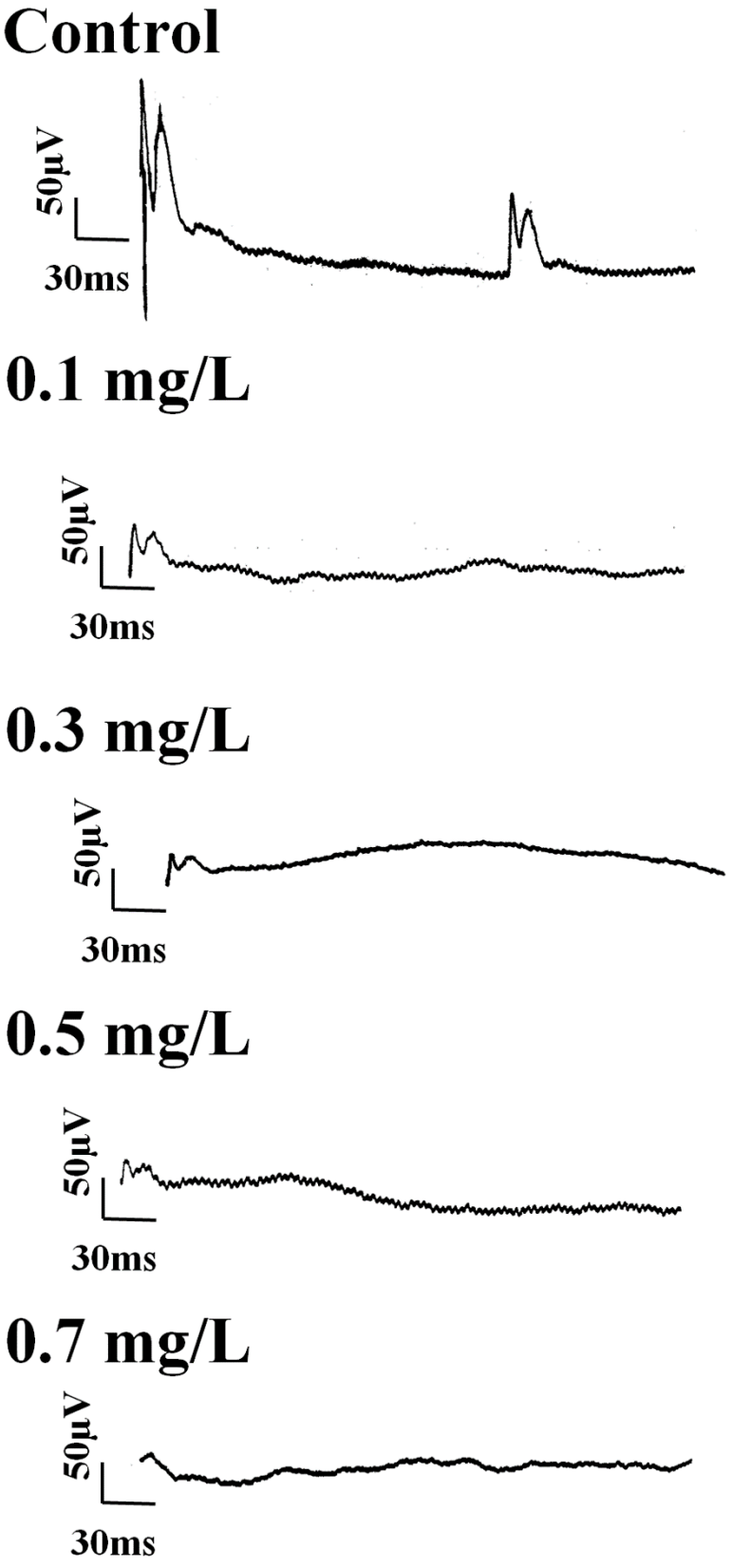
FS
GS
ES
SM
CH
EE
FA
LA
EA
FO
AB
JU
ER
LD
SW
CP
HVW

Figure 4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30ms
0.1 mg/L
50µV
30ms
0.3 mg/L
50µV
30ms
0.5 mg/L
50µV
30ms
0.7 mg/L
50µV
30ms

Figure 5

Table 1. Lethal concentration (24/96 h) of lufenuron in *Colossoma macropomum.*

Treatments	Parameters							
	24 h		D.F.	χ^2	96 h		D.F.	χ^2
	$LC_{50\ (CI\ 95\%)}$	$LC_{90\ (CI\ 95\%)}$			$LC_{50\ (CI\ 95\%)}$	$LC_{90\ (CI\ 95\%)}$		
Lufenuron	$0.61_{(0.41-0.81)}$	0.82	3	$2.81x10^{37}$	$0.58_{(0.46-0.71)}$	0.78	3	$3.03x10^{33}$

LC_{50} or $LC_{90\ (IC95\%)}$ – lethal concentration and confidence limits at 95%.
D.F. – degrees of freedom. χ^2– Chi-square value. N = 120.

Table 2. Ethogram Behavioral of *Colossoma macropomum.*

Category	*Behavior*	*Code*	*Description*
Moving	Slow Swimming	SS	Slow swimming in any direction
	Fast Swimming	FS	Fast swimming in any direction
	Group in Swimming	GS	Directed displacement of various animals (school of fish), slow or fast.
	Emerge and Submerge	ES	Emerge to water surface, fast aerial breath, and submerge
	Staying Motionless	SM	Do not present displacement by 5 s at least.
Agonistic Interaction	Chase	CH	Fast swimming towards the opponent, doing or not physical contact.
	Escape	EE	Move away from opponent's chase or attack.
	Frontal Attack	FA	Mutual attack with the open mouth position done faced the other fish.
	Lateral Attack	LA	Attack biting the side of the opponent's body.
Maintenance	Eat	EA	Food swallow.
	Forage	FO	Active search for food.
Behaviors Associated with Stress	Aerial Breath	AB	Aerial breath for long 5 s.
	Jump	JU	Jump over the water surface.
	Erratic Swimming	ER	Spiral displacement.
	Lying Down	LD	Immobility in the aquarium bottom for 5 s at least, with one side of the body on the aquarium bottom.
	Surface Swimming	SW	Displacement near to surface.
	Coprophagy	CP	Eat feces.
	Hanging Vertically in the Water	HVW	Vertical position head up near the water slide for 5 s at least.

Table 3. Results of photopic exam, implicit time and amplitude of the waves in *Colossoma macropomum* groups exposed to lufenuron in acute and chronic toxicity tests.

Tests	Groups	Implicit time (ms)		Amplitude (μV)	
		a-wave	b-wave	a-wave	b-wave
Acute toxicity	Control	5.6 ± 0.69	13.2 ± 2.75	13.81 ± 12.12	90.43 ± 3.26
	0.1 mg/L	22.4 ± 15.3	113.07 ± 19.42*****	18.03 ± 2.4	28.2 ± 10.76*****
	0.3 mg/L	30.2 ± 11.36	132 ± 16.09*****	22.18 ± 25.51	52.87 ± 14.47
	0.5 mg/L	24.2 ± 1.93	110.07 ± 16.41*****	23.93 ± 5.69	59.97 ± 13.12
	0.7 mg/L	63 ± 34.38*	150.67 ± 21.78*****	19.83 ± 3.27	50.4 ± 26.07
Chronic toxicity	Control	7.25 ± 0.5	14 ± 0.8	11.5 ± 17.1	11.6 ± 5.1
	0.1 mg/L	6.25 ± 0.5	11.98 ± 0.78	8.64 ± 5.77	8.69 ± 4.45
	0.3 mg/L	7.75 ± 0.96	12 ± 1.41	15.94 ± 23.99	4.64 ± 1.24

***** Significant difference when compared to control group ($p < 0.05$).

5. Conclusões

O presente trabalho teve como objetivo estudar o efeito agudo e crônico do lufenuron nos parâmetros biológicos de *Colossoma macropomum*, diante disto podemos concluir que:

- A CL_{50} 24 e 96 h de lufenuron para *C. macropomum* foi 0,61 e 0,58 mg/L, respectivamente.
- Como era de se esperar no teste de toxicidade aguda, a taxa de mortalidade foi maior para os peixes submetidos as maiores concentrações de lufenuron. Por sua vez, o teste crônico apresentou 5% nas taxas de mortalidade para 0,1 e 0,3 mg/L de lufenuron;
- Nas concentrações 0,7 e 0,9 mg/L de lufenuron, os efeitos tóxicos foram mais acentuados no teste agudo, ocorreram hemorragias externas;
- De acordo com os resultados obtidos no teste de toxicidade crônica, não foram observados diferenças significativas ($p>0,05$) para peso, comprimento e concentração de glicose no sangue dos peixes expostos ao lufenuron;
- As brânquias de *C. macropomum*, podem servir como indicador da qualidade da água, visto que apresentaram alterações histológicas quando submetidas ao teste de toxicidade aguda do lufenuron;
- *C. macropomum* exibiu comportamentos associados ao estresse quando exposto ao lufenuron;
- Os resultados dos ERGs são indicativos de que o lufenuron promoveu danos na retina de *C. macropomum* potencialmente quanto a capacidade de responder aos estímulos nas células fotorreceptoras e ON-bipolares e na ativação das células ON-bipolares no teste de toxicidade aguda. Indica que o lufenuron promoveu danos na retina dos peixes, quanto a capacidade de responder aos estímulos. Entretanto, não foram observados efeitos nos peixes submetidos ao teste crônico;
- Reconhecido como pesticida com claros efeitos negativos em artrópodes, nossos resultados mostraram uma série de efeitos tóxicos em peixe, um vertebrado. Isto alerta os órgãos fiscalizadores para a criação de medidas mitigadoras que controlem o uso e o descarte deste tipo de substância.

6. Anexos

Anexo 1 – Licença da Comissão de Ética no Uso de Animais para a realização desta dissertação.

UFRPE

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Número da licença	140/2014
Número do processo	23082.023391/2014
Data de emissão da licença	03 de Novembro de 2014
Título do Projeto	Efeito agudo e crônico de lufenuron sobre os parâmetros biológicos de tambaqui (Colossoma macropomum).
Finalidade (Ensino, Pesquisa, Extensão)	Pesquisa
Responsável pela execução do projeto	Pabyton Gonçalves Cadena
Colaboradores	Maria Adélia Borstelmann de Oliveira; Priscila Rafaela Leão Soares; André Lucas Corrêa de Andrade; Danilo Martim Fonseca de Oliveira; Luiz Bezerra de Carvalho Júnior; Franklin Maglliano Cunha.
Tipo de animal e quantidade total autorizada	Peixe ; total de 180 animais.

Profª. Dra. Marlóyne José Afonso Accioly Lins Amorim
(Presidente da CEUA-UFRPE)

Anexo 2 – Trabalhos publicados em anais de eventos

ANDRADE, A. L. C.; SILVA, S. C. B. L.; SOARES, P. R. L.; SILVA, M. C. G.; SANTOS, T. P.; CADENA, P. G. Effect of salinity on heart rate of *Pomacea lineata* neonates as a noninvasive method for animal physiology study. In: 50th Annual Congress of the Brazilian Society of Physiology (SBFis), Águas de Lindóia. SBFIS 2015.

OLIVEIRA, D. M. F.; SOARES, P. R. L.; ANDRADE, A. L. C.; WANDERLEY-TEXEIRA, V.; NOGUEIRA, A. J.; CUNHA, F. M.; CADENA, P. G. Temperature effect on behavioral analysis and histochemistry of the gills and liver of *Astronotus ocellatus*. In: 1st PanAmerican Congress of Physiological Science, Foz do Iguassu, 2014.

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SANTOS, T. P.; OLIVEIRA, E. G. S.; SOARES, P. R. L.; SILVA, S. C. B. L.; ANDRADE, A. L. C.; SILVA, M. C. G.; CADENA, M. R. S.; CADENA, P. G. Thyroxine effect as endocrine disruptor in behavior of tambaqui (*Colossoma macropomum*). In: 50th Annual Congress of the Brazilian Society of Physiology (SBFis), Águas de Lindóia, 2015.

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SOARES, P. R. L.; ANDRADE, A. L. C.; OLIVEIRA, D. M. F.; SILVA, M. C. G.; WANDERLEY-TEXEIRA, V.; CUNHA, F. M.; CADENA, P. G. Hypoxia effect on behavior and morphology of gills in *Corydoras schwartzi*. In: 1st PanAmerican Congress of Physiological Science, Foz do Iguassu, 2014.

SOUZA, E. Q.; OLIVEIRA, E. G. S.; ANDRADE, A. L. C.; SILVA, S. C. B. L.; SANTOS, T. P.; SOARES, P. R. L.; SILVA, M. C. G.; CADENA, M. R. S.; CADENA, P. G. Effects of estradiol and bisphenol as endocrine disruptors on the aggressive behavior

of *Betta splendens*. In: 50th Annual Congress of the Brazilian Society of Physiology (SBFis), Águas de Lindóia, 2015.

SOUZA, E. Q.; OLIVEIRA, E. G. S.; ANDRADE, A. L. C.; SILVA, S. C. B. L.; SANTOS, T. P.; SOARES, P. R. L.; CADENA, P. G. Bisfenol A como interferente endócrino e seus efeitos em *Betta splendens*. In: XV Jornada de Ensino, Pesquisa e Extensão, Recife 2015.

AQUATIC TOXICOLOGY

AUTHOR INFORMATION PACK

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ISSN: 0166-445X

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AUDIENCE

Environmental Toxicologists, Marine Biologists, Ecotoxicologists, Biochemical Toxicologists, Conservationists.

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