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RESPOSTAS METABÓLICAS E ENZIMÁTICAS EM JUNDIÁS *Rhamdia quelen* (HEPTAPTERIDAE) E PIAVAS *Leporinus obtusidens* (ANOSTOMIDAE) EXPOSTOS A HERBICIDAS UTILIZADOS NA CULTURA DO ARROZ IRRIGADO

TESE DE DOUTORADO

Denise dos Santos Miron

Santa Maria, RS, Brasil
2009

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por

Denise dos Santos Miron

Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do grau de **Doutor em Bioquímica Toxicológica**

Orientadora: Profa. Dra. Vera Maria Morsch
Co-orientadora: Profa. Dra. Vânia Lucia Loro

Santa Maria, RS, Brasil
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Universidade Federal de Santa Maria
Centro de Ciências Naturais e Exatas
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica

A Comissão Examinadora, abaixo assinada, aprova a Tese de Doutorado

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elaborada por
Denise dos Santos Miron

como requisito parcial para a obtenção do grau de
Doutora em Bioquímica Toxicológica

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Santa Maria, 15 de janeiro de 2009.

DEDICATÓRIA

Dedico este trabalho ...

... à minha filha, amor da minha vida *Antonella Miron Nissola*.

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A Deus, por estar sempre junto a mim... nas decisões e nas conquistas. Por isso, meu mais sincero agradecimento, o meu muito obrigado!

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RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica
Universidade Federal de Santa Maria

RESPOSTAS METABÓLICAS E ENZIMÁTICAS EM JUNDIÁS, *Rhamdia quelen* (HEPTAPTERIDAE) E PIAVAS *Leporinus obtusidens* (ANOSTOMIDAE) EXPOSTOS A HERBICIDAS UTILIZADOS NA CULTURA DO ARROZ

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Data e local de defesa: Santa Maria, 15 de janeiro de 2009.

Neste estudo, investigaram-se os efeitos toxicológicos da exposição de peixes aos herbicidas COLOCAR CLASSE clomazone (isoxazolidinonas- Gamit®), quinclorac (quinolinas- Facet®) e metasulfuron metil (sulfoniluréia- Ally®), utilizados na lavoura de arroz. Primeiramente, foram realizados experimentos com jundiás (*Rhamdia quelen*) para estabelecer uma concentração média letal em 96 horas (CL_{50}-96h) de exposição aos herbicidas e determinar a atividade da AChE cerebral e muscular nesses peixes. Em experimentos adicionais, jundiás foram expostos por 45 dias em águas retiradas do cultivo do arroz após a aplicação dos herbicidas. Foram determinados parâmetros de sobrevivência, crescimento e metabólicos (glicose, lactato, glicogênio e proteína) nestes peixes. Piavas (*Leporinus obtusidens*) foram submetidas à exposição (96 e 192 h) do clomazone na concentração utilizada na lavoura de arroz (0,5 mg/L) e, em seguida a testes de recuperação (192 h) em água livre deste herbicida. Foram avaliados parâmetros enzimáticos (AChE e CAT), formação de TBARS, carbonilação de proteínas e alguns metabólicos (glicogênio, lactato, glicose e proteína) em diferentes tecidos. Os resultados demonstraram que os valores de CL_{50}-96h para os jundiás nos herbicidas foram: 7,32 mg/L para o clomazone, 395 mg/L para o quinclorac e para o metasulfuron metil este valor não foi obtido, pois os peixes sobreviveram em concentração máxima de 1200 mg/L deste herbicida. Nossos resultados revelaram que o clomazone é um potente inibidor da atividade da AChE em jundiás, mostrando uma inibição de até 83% em cérebro e de 89% em músculo. No entanto, quinclorac e metasulfuron metil causaram aumento da AChE cerebral (98 e 179%, respectivamente) e diminuição dessa atividade no músculo (88 e 56%, respectivamente). Depois de 45 dias de exposição aos herbicidas, os jundiás expostos ao quinclorac demonstraram diminuição de 4% nos índices de sobrevivência. E, em água com clomazone e com quinclorac ocorreu redução no crescimento dos peixes. No tecido hepático dos jundiás expostos ao clomazone e quinclorac houve aumento de glicogênio, redução nos níveis de lactato. No tecido muscular verificou-se um aumento do lactato. Em piavas expostas ao clomazone (96 e 192 h) houve diminuição na atividade da AChE, em cérebro e em coração dos peixes. A atividade da AChE muscular diminuiu depois de 192 h de exposição. Em relação à recuperação da atividade da AChE em piavas, observou-se que a inibição persistiu após 192 h em água livre de herbicida em cérebro, músculo e olho. Os níveis de TBARS apresentaram-se aumentados em cérebro de piavas nos períodos de exposição, enquanto em fígado e em músculo foi observado o aumento depois de 192 h de exposição. Em relação ao período de recuperação, verificou-se

que os níveis de TBARS não retornaram aos valores iniciais, com exceção do fígado. A exposição de piavas resultou num aumento na formação de carbonilação proteíca em fígado, que não foi recuperado. No fígado, a diminuição da atividade da CAT está relacionada com o aumento de TBARS hepático, demonstrando que este herbicida induz dano oxidativo. Além disso, no presente estudo os níveis de glicogênio em fígado e em rins de piavas expostas ao clomazone mostraram um aumento, enquanto os valores de lactato, glicose e de proteína obtido para esses tecidos diminuíram. No tecido muscular de piavas expostas por 96 h ao clomazone foi demonstrado um aumento do glicogênio que após o período de recuperação retornou aos valores do controle. Nesse mesmo tecido, essas piavas apresentaram um aumento de lactato, a diminuição de glicose e de proteína, sendo que o lactato não foi recuperado. Estes resultados indicam que após a exposição de piavas ao clomazone (96 e 192 h) algumas mudanças enzimáticas e metabólicas não podem ser recuperadas, mesmo submetendo os peixes ao tratamento em água livre de herbicida (192 h). Contudo, os resultados obtidos no presente estudo demonstraram que a exposição de peixes a herbicidas utilizados na lavoura de arroz irrigado afeta o sistema colinérgico, os parâmetros metabólicos e oxidativos causando uma condição de estresse oxidativo. Portanto, os parâmetros avaliados em jundiás e piavas podem ser recomendados para o monitoramento de contaminação da água por clomazone, quinclorac e metasulfuron metil.

Palavras-chave: herbicidas, clomazone, quinclorac, metasulfuron metil, jundiá, piavas, CL_{50}, metabolismo e enzimas.

ABSTRACT

Doctoral Thesis
Pos-Graduate Program in Toxicological Biochemistry
Universidade Federal de Santa Maria

METABOLIC AND ENZYMATIC RESPONSES ON SILVER CATFISH *Rhamdia Quelen* (HEPTAPTERIDAE) AND PIAVAS *Leporinus Obtusidens* (ANOSTOMIDAE) EXPOSURE TO HERBICIDES UTILIZED IN THE RICE FIELDS

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The effects of the toxic exposure of fish to the herbicides clomazone (isoxazolidinone-Gamit®), quinclorac (quinoline-Facet®) and metsulfuron methyl (sulfonylurea-Ally®), used in rice fields were investigated. Experiments were carried out with silver catfish (*Rhamdia quelen*) to establish an average lethal concentration in 96 hours (LC_{50} – 96 h) of exposure to herbicides as well as to determine brain and muscle AChE activity in this specie. In additional experiments, silver catfish were placed into water, for 45 days, taken from the rice cultivation after the application of herbicides. Survival parameters as well as growth and metabolic (glucose, lactate, glycogen and protein) were determined in this species. Piava (*Leporinus obtusidens*) were exposed (96 and 192 h) to clomazone in the concentration used for rice culture (0.5 mg/L) and recovery tests (192 h) in water free of herbicide were performed. Enzymatic (AChE and CAT), formation of TBARS, protein carbonylation and some metabolic parameters (glycogen, lactate, glucose and protein) were evaluated in different tissues. Results showed that LC_{50}-96 h values for silver catfish were 7.32 mg/L for clomazone, 395 mg/L for quinclorac and no values were obtained for metsulfuron methyl, the fish survived in maximum concentration of 1200 mg/L. Our findings showed that clomazone is a potent inhibitor of AChE activity in silver catfish, showing an inhibition up to 83% in brain and 89% in muscle. However, quinclorac and metsulfuron methyl caused an increase of the brain AChE activity (98 and 179%, respectively) and a decrease in the muscle AChE activity (88 and 56% respectively). After 45 days of quinclorac exposure, silver catfish showed a decrease of 4% in the survival rates. In water with clomazone and quinclorac a reduction in the growth of fish was observed. In the liver tissue of silver catfish exposed to clomazone and quinclorac a glycogen increase and a reduction in the glucose and lactate levels were observed

indicating liver gluconeogenesis. In the muscle tissue, there was a decrease in the mus glycogen, with a lactate increase. There was a decrease in the AChE activity in brain and heart of piava exposed to clomazone (96 and 192 h). AChE activity decreased in muscle after 192 h of exposure. Regarding the recovery of the AChE activity in piava**,** the inhibition persisted after 192 h in water free of herbicide in brain, muscle and eye. TBARS levels were increased in the brain of piava during the exposure periods, whereas an increase in liver and muscle was observed after 192 h of exposure. TBARS levels did not return to controls values during the recovery period, except for liver. The exposure of piava resulted in an increase in the formation of protein carbonyl in liver, which was not recovered. In liver, the reduction in the CAT activity is related to the increase in liver TBARS, showing that this herbicide causes oxidative damage. Furthermore, in this study the glycogen levels in kidneys of piava exposed to clomazone showed an increase whereas lactate, glucose and protein values to these tissues were decreased. In muscle tissue of piava exposed to clomazone (96 h) was demonstrate a glycogen increased that after recovery returned to the values of control. Muscle tissue of piavas also showed lactate increased, a decreased glucose and protein, however lactate levels not were recovered. These results demonstrate that after exposing piava to clomazone (96 and 192 h) some metabolic and enzymatic changes could not be recovered, even exposing the fish to water free of herbicide (192 h). The results of this study showed that the exposure of fish to herbicides used in rice culture affects the cholinergic system, as well as the metabolic and oxidative parameters causing a condition of oxidative stress. Therefore, the parameters evaluated in piava and silver catfish may be recommended for the monitoring of water contamination by clomazone, quinclorac and metsulfuron methyl.

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LISTA DE ABREVIATURAS

ACh: acetilcolina

AChE: acetilcolinesterase

ALS: alanina aminotransferase

ATP: adenosina trifosfato

BuChE: butirilcolinesterase

CAT: catalase

CL_{50}: concentração letal média

DNA: ácido desoxirribonucléico

EROs : espécies reativas de oxigênio

GPx: glutationa peroxidase

LPO: peroxidação lipídica

SOD: superóxido dismutase

TBARS: substâncias reativas ao ácido tiobarbitúrico

RNA: ácido ribonucléico

SUMÁRIO

1. INTRODUÇÃO

Na atividade agrícola é indispensável à adoção de procedimentos de manejo para obter-se o aumento da produtividade. O controle de plantas daninhas na lavoura permite um maior rendimento da área plantada e geralmente é realizado através de agroquímicos (IRGA, 2001). O uso de herbicidas requer a aplicação cuidadosa das quantidades adequada bem como, exige cuidados quanto á época indicada para o agroquímico. Cuidados relativos a estes fatores melhoram a qualidade nas colheitas e, minimizam a possibilidade de intoxicações (humanas, de animais domésticos, etc.). A quantidade de herbicidas nos recursos hídricos varia entre regiões e depende da dosagem aplicada, das características químicas do produto e das condições ambientais durante a aplicação (HUBER et al*.,* 2000). Dependendo das características físico-químicas, o resíduo do químico pode se ligar ao material particulado em suspensão, se depositar no sedimento do fundo ou ser absorvido por organismos, podendo então ser detoxificado ou acumulado. Além disso, pode ser transportado no sistema aquático por difusão nas correntes de água ou nos organismos aquáticos, ou até mesmo retornar à atmosfera por volatilização, evidenciando uma interação contínua dos químicos entre sedimento e água, influenciada pelo movimento da água, turbulência e temperatura (NIMMO, 1985).

Alguns poluentes em ambientes aquáticos podem afetar a saúde e a sobrevivência dos peixes (DEZFULI et al., 2003). Entretanto, há poucos trabalhos relacionados á toxicidade de herbicidas utilizados em cultura de arroz irrigado, e poucos dados relacionados à curva dose-efeito dos herbicidas com as espécies nativas. Os herbicidas clomazone (isoxazolidinona), quinclorac (quinolina) e metasulfuron metil (sulfoniluréia) são registrados no Brasil para o controle de plantas daninhas em diferentes culturas, como em lavouras arrozeiras, nas concentrações

finais de 0,7; 0,003 e 0,75 mg/L, respectivamente (RODRIGUES & ALMEIDA, 1998, 2005). E, uma vez na água, o resíduo do agroquímico pode ser absorvido por organismos, podendo então bioacumular-se ou sofrer detoxificação. Com relação aos herbicidas de amplo uso em culturas de arroz irrigado no Rio Grande do Sul (RS), o clomazone se apresenta como o menos tóxico para peixes quando comparado ao quinclorac e ao metasulfuron metil (JONSSON et al., 1998). Testes de toxicidade em peixes, feitos a partir da concentração média letal (CL_{50}) permitem avaliar o potencial deletério que algumas substâncias químicas podem exercer sobre organismos aquáticos, em condições controladas de laboratório (RAND & PETROCELLI, 1985). No peixe tetrarisca-negra (*Hyphessobrycon scholzei*) o valor da CL_{50}-96 h para o clomazone foi equivalente a 27,67 mg/L, enquanto a dose comercial normalmente utilizada na lavoura é de 0,4 a 0,7 mg/L e, não houve evidências de alterações significativas nos níveis de proteínas totais e de macro e microelementos nos tecidos (JONSSON et al., 1998). No entanto, RESGALLA JUNIOR et al. (2002) demonstraram que a exposição do clomazone sobre carpa comum (*Cyprinus carpio*) oferece altos riscos ambientais, pois a diferença entre a CL_{50}-96 h (13,94 mg/L) e a concentração recomendada pelo fabricante estão próximas, indicando maior potencial de risco no uso desse produto.

Vários parâmetros enzimáticos podem ser utilizados para avaliar a toxicidade de herbicidas (FERNÁNDEZ-VEJA et al., 2002). Um destes é a medida da atividade da acetilcolinesterase (AChE, EC 3.1.1.7), é uma enzima importante para o sistema colinérgico. A medida da atividade da AChE tem sido utilizada como um indicador de toxicidade causado pela exposição de compostos como organofosforados e carbamatos em peixes (SANCHO et al., 2000). É descrito na literatura que a atividade da AChE em peixes pode ser afetada também, por pesticidas de outras classes e por

esse motivo vem sendo amplamente utilizada como um indicador de efeitos tóxicos (FERNÁNDEZ-VEGA et al., 2002; DUTTA & ARENDS, 2003; MIRON et al., 2005).

Algumas alterações fisiológicas devido à exposição de herbicidas também podem ser analisadas através do estudo de parâmetros metabólicos em peixes (DAS & MUKHERJEE, 2003). As mudanças no metabolismo de carboidratos de peixes expostos a diferentes agrotóxicos, podem servir de bons indicadores secundários de toxicidade (BEGUM, 2004). De acordo, foi constatado que a exposição de carpa comum ao lindane (100 mg/L por 72 h) e de catfish indiano (*Heteropneustes fossilis*) ao malation (8 mg/L por 96 h) aumenta significativamente o nível de glicose no plasma e causa uma redução concomitante de glicogênio em músculo e em fígado (GLUTH & HANKE, 1985; LAL et al., 1986). A exposição de jundiás ao herbicida 2,4-D (ácido diclorofenoxiacético) (600 e 700 mg/L por 96 h) também causa alterações no metabolismo de carboidratos, como aumento nos níveis de lactato muscular, favorecendo o metabolismo anaeróbico (CATTANEO et al., 2008). SAHIB et al. (1984) e FERNÁNDEZ-VEJA et al. (2002) sugerem que peixes expostos a contaminantes podem alterar o conteúdo de proteína (hepática e muscular) indicando uma resposta adaptativa fisiológica para compensar a condição de estresse ao tóxico.

Além disso, os poluentes podem provocar estresse oxidativo e levar a um aumento na produção de espécies reativas de oxigênio (EROs) em organismos aquáticos (AHMAD et al., 2000; SEVGILER et al., 2004). Uma reação típica induzida por EROs envolve a peroxidação de ácidos graxos poliinsaturados (LPO) que tem sido observada em várias espécies de peixes. Estes apresentam vários sistemas antioxidantes, tais como, as enzimas catalase (CAT), superóxido dismutase (SOD) e a glutationa peroxidase (GPx) (RADI et al., 1985). Assim, mudanças no conteúdo dos antioxidantes podem também demonstrar a presença de contaminantes (AHMAD et al., 2000).

Portanto, os peixes podem apresentar várias respostas à exposição de agentes tóxicos, como herbicidas. Segundo ORUÇ & ÜNER (1999), mudanças bioquímicas em peixes são influenciadas de acordo com a espécie exposta ao herbicida, pelo tempo e também pela concentração de exposição. As respostas são demonstradas através de testes de CL_{50} (RAND & PETROCELLI, 1985), alterações nos níveis de vários parâmetros enzimáticos, fisiológicos e metabólicos (FERNÁNDEZ-VEJA et al., 2002). Segundo BEGUM (2004) algumas das alterações causadas pela exposição a herbicidas podem ser revertidas. Considerando o exposto acima, torna-se importante os estudos relacionados às respostas dos efeitos tóxicos em peixes submetidos a um tratamento de recuperação e de detoxificação em água sem adição de herbicida. Dessa forma é importante estabelecer subsídios para auxiliar no cultivo de peixes nativos, como o jundiá (*Rhamdia quelen*) e a piava (*Leporinus obtusidens*) já que são espécies promissoras no sistema de rizipiscicultura devido à facilidade de cultivo e aceitação comercial. Além disso, o conhecimento dos efeitos causado pela exposição ao clomazone, quinclorac e ao metasulfuron metil proporcionará um maior embasamento no que diz respeito a aspectos relacionados a respostas metabólicas, enzimáticas e, conseqüentemente de estresse oxidativo nas espécies estudadas.

1.1 Objetivos

1.2 Objetivo Geral

O objetivo do presente trabalho foi avaliar os efeitos toxicológicos da exposição de jundiás e piavas a três tipos de herbicidas utilizados na lavoura de arroz: clomazone (isoxazolidinona- Gamit®), quinclorac (quinolina- Facet®) e metasulfuron metil (sulfoniluréia- Ally®).

1.3 Objetivos Específicos

- Determinar a CL_{50}-96 h para jundiás expostos aos herbicidas clomazone, quinclorac e metasulfuron metil;

- Investigar os efeitos dos herbicidas sobre a atividade da enzima acetilcolinesterase (AChE) em cérebro e em músculo de jundiás;

- Investigar os efeitos da água usada no cultivo do arroz após a aplicação do clomazone, quinclorac e metasulfuron metil sobre a sobrevivência, crescimento e parâmetros metabólicos (glicose, lactato, glicogênio e proteína) de jundiás;

- Verificar os efeitos causados pela exposição ao clomazone na concentração usada na lavoura de arroz (0,5 mg/L), sobre AChE, TBARS, carbonilação de proteínas e atividade da catalase (CAT) de piavas;

- Investigar os efeitos da exposição a 0,5 mg/L de clomazone sobre alguns parâmetros metabólicos (glicogênio, lactato, glicose e proteínas totais) em fígado, músculo e rim de piavas;

- Analisar se após a exposição do clomazone (96 e 192 h) as possíveis mudanças (enzimáticas e metabólicas) podem ser recuperadas a níveis normais, submetendo os as piavas ao tratamento em água livre de herbicida (192 h);

- Verificar se os parâmetros avaliados em jundiás e em piavas poderão ser recomendados para o monitoramento de contaminação da água por herbicidas utilizados nas lavouras de arroz irrigado.

2. REVISÃO DE LITERATURA

2.1. Contaminação Ambiental

No Brasil são cultivados anualmente 1,3 milhões de hectares com arroz irrigado dos quais cerca de 950 mil (73%) estão no Rio Grande do Sul (RS) (AGRIANUAL, 2000). O arroz (*Oryza sativa L*.) é um dos cereais mais cultivados do mundo e, considerado a base da alimentação de boa parte da população mundial. O controle de plantas daninhas na cultura de arroz irrigado permite um maior rendimento da área plantada e geralmente é realizado através do uso de agroquímicos, principalmente herbicidas (IRGA, 2001). O sistema pré-geminado de arroz caracteriza-se pela drenagem da área irrigada, efetuada após o período de semeadura, podendo desencadear graves problemas ambientais com a liberação de herbicidas que estão em suspensão na água de irrigação. Esta água liberada no meio ambiente pode afetar vários organismos, principalmente os aquáticos, como os peixes. Estes podem absorver através das brânquias e superfície do corpo essas substâncias tóxicas (SEIM et al., 1997). Salienta-se que o uso sem controle de herbicidas em culturas irrigadas, como as lavouras de arroz, pode afetar diretamente a vida dos organismos expostos aos mesmos, ou indiretamente quando seus habitats são alterados, afetando, por exemplo, a qualidade da água, que pode ser influenciada pelas interações entre os seus componentes químicos e os componentes dos herbicidas. Com isso, muitos parâmetros físicos e químicos, tais como: temperatura, pH, oxigênio dissolvido, dureza e turbidez podem sofrer alterações. Mudanças nestes parâmetros, que influenciam as condições biológicas do ecossistema aquático, podem alterar a produtividade do cultivo, bem como as respostas fisiológicas dos peixes (ARANA, 1997).

2.2. Rizipiscicultura

O Brasil destaca-se pelo uso de vários herbicidas, destes diversos são indicados para uso nas culturas de arroz irrigado (IRGA, 2001). A lavoura arrozeira irrigada tem sido alvo de estudos voltados para os efeitos nocivos causados sobre a qualidade da água. Porém, a procura por parte de técnicos e produtores de alternativas para a redução de custos da lavoura arrozeira está motivando a combinação do sistema peixe e arroz irrigado, ou seja, a rizipiscicultura. Esta proporciona a possibilidade de manejo reduzindo o uso de maquinária, conservando o meio ambiente e, ainda aumentando a renda do produtor atravéz da venda do peixe (EMATER, 1999). Na rizipiscicultura podem-se reduzir os custos da lavoura, pois são utilizadas algumas espécies de peixes que preparam o solo para os próximos cultivos do arroz irrigado, reciclam a matéria orgânica e consomem sementes de espécies invasoras no solo, contribuindo também para a redução de larvas de insetos e caramujos (EMATER, 2001). A partir do estudo dos efeitos de herbicidas em peixes associados às lavouras pode-se minizar a possibilidade de contaminação da água e dos organismos pelos produtos químicos. Assim, torna-se importante determinar os efeitos tóxicos em peixes de interesse comercial como os jundiás (*Rhamdia quelen*) e piavas (*Leporinus obtusidens*), os quais poderiam ser utilizado no consórcio arroz-piscicultura.

2.3. Jundiá (*Ramdia quelen*)

O jundiá, *Rhamdia quelen* (Quoy & Gaimard, 1824; família Heptapteridae; ordem Siluriforme) (Figura 1) é uma espécie de teleósteo nativa da região Sul da América do Sul (GOMES et al., 2000). Sua coloração varia de marrom-avermelhado claro a cinza ardósia. No Brasil, está presente na região da Depressão Central do Rio Grande do Sul, entre outros locais (GUEDES, 1980). Na Argentina, é conhecido também como bagre, bagre negro, bagre sapo e bagre sul-americano (GOMES et al., 2000). Esta

espécie tem preferência de viver em águas calmas, escondendo-se embaixo de troncos e pedras. Têm hábitos noturnos e são considerados onívoros, alimentando-se de invertebrados (crustáceos, insetos), restos vegetais e detritos orgânicos. Porém, possui uma clara tendência a carnivoria (GUEDES, 1980; GOMES et al., 2000; BALDISSEROTTO & RADUNZ NETO, 2004). Este peixe é considerado rústico, pois suporta o intenso frio da região Sul do Brasil durante o inverno, bem como tem seu crescimento potencializado durante o verão (BARCELLOS et al., 2003; SOSO et al., 2007). Os alevinos são capazes de sobreviver a diversas alterações na água como salinidade, pH, dureza, oxigênio e amônia (GOMES et al., 2000; MIRON et al., 2008). De acordo com BARCELLOS et al. (2004), o jundiá quando cultivado a uma densidade de 2 a 4 peixes/m^2, pode alcançar 600 a 800 g em 8 meses de cultivo. As fêmeas apresentam aproximadamente 66,5 cm e tem um tempo de vida de 21 anos; os machos, atingem 52 cm e tem um tempo de vida de 11 anos. Devido às características de sobrevivência desta espécie, ela se torna bastante favorável para as práticas de aqüicultura que são bem freqüentes na região Sul do Brasil, principalmente no Rio Grande do Sul (RS) (BARCELLOS et al., 2003; LAZZARI et al., 2006). Além disso, esses peixes possuem uma carne de sabor agradável que é bem aceita pelos consumidores (LAZZARI et al., 2006). Sendo assim, esta espécie apresenta grande importância comercial e possui boas características para o cultivo em nossa região.

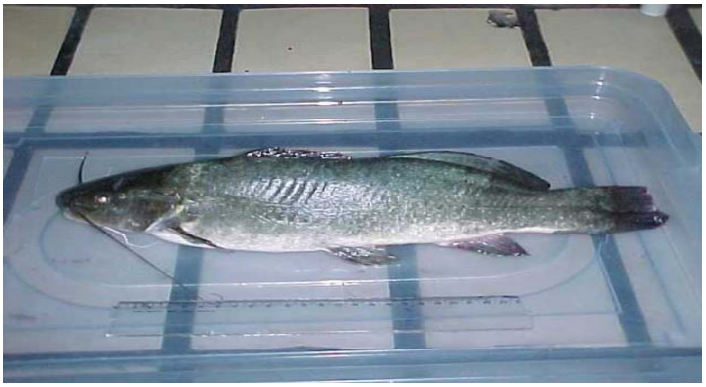

FIGURA 1- Exemplar de Jundiá (*Rhamdia quelen*).

2.4. Piava (*Leporinus obtusidens*)

A piava, *Leporinus obtusidens* (Valenciennes, 1836; família Anostomidae; ordem Characiforme) (Figura 2) é uma espécie de teleósteo nativa da região Sul do Brasil e bem aceita pelos consumidores. Além disso, as espécies do gênero *Leporinus* são recomendadas para o policultivo e consorcionamento, inclusive para rizipiscicultura (ANDRIAN et al., 1994; BALDISSEROTTO et al., 2005). Podem ser encontradas nas Bacias do São Francisco, do Paraná e do Uruguai (ZANIBONI FILHO & SCHULZ, 2003). No baixo Rio Uruguai, é uma espécie de elevada importância comercial e recreativa. A alimentação é diversificada em juvenis e adultos, destacando-se, as sementes, organismos aquáticos, crustáceos e moluscos (FRACALOSSI et al., 2002). Em geral, as espécies do gênero *Leporinus* aceitam prontamente a ração e o alimento preparado desde as primeiras fases de vida. O período reprodutivo se concentra nos meses de dezembro e janeiro, apresentando desova total. São encontrados em ambientes de água limpa.

FIGURA 2- Exemplar de Piava (*Leporinus obtusidens*)

2.5. Herbicida Clomazone

O clomazone (2- [(2-clorobenzil)]-4,4-dimetil-1,2-oxazolidin-3-ona), pertencente ao grupo químico das isoxazolidinona, é encontrado na forma comercial de concentrado emulsionável 36 ou 50% (GAMIT®) e apresenta a estrutura abaixo (Figura 3).

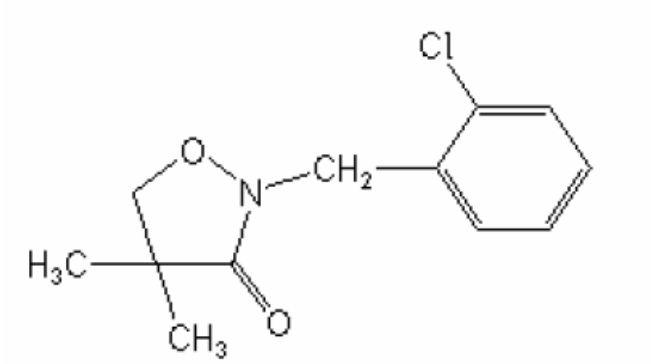

FIGURA 3- Estrutura Química do Clomazone (Senseman, 2007).

É um herbicida seletivo, utilizado em pré ou pós-emergência inicial no controle de várias espécies daninhas na cultura do arroz irrigado no sul do Brasil (ANDRES & MACHADO, 2004) e também nas culturas de soja, mandioca, cana-de-açúcar e algodão. Este produto possui grande solubilidade em água (1100 mg/L) e meia vida de 28 a 84 dias, dependendo do tipo de solo e do nível de matéria orgânica (COLBY et al., 1989).

Herbicidas desse grupo atuam de maneira geral na síntese dos pigmentos carotenóides e apresentam o mecanismo de ação abaixo (Figura 4). O bloqueio da síntese desses pigmentos é o fenômeno responsável pelo surgimento do sintoma característico de “albinismo”. O uso do clomazone nas plantas inibe a biossíntese de carotenóides e a folhagem produzida é totalmente branca após o tratamento, isto pode ser chamado de "crescimento albino” (RODRIGUES & ALMEIDA, 1998). O crescimento cessa e aparecem necroses. Este herbicida não inibe diretamente a biossíntese de clorofila. A ocorrência da perda de clorofila resulta da destruição desta pela luz (fotooxidação), ou talvez devido à falta de carotenóides indiretamente causando a alteração da biossíntese normal de clorofila e do desenvolvimento do cloroplasto (BRAMLEY & PALLET, 1993). Um papel importante dos carotenóides é o de proteger a clorofila da fotooxidação. Normalmente a energia da forma reativa de clorofila é dissipada através dos carotenóides. Quando os carotenóides não estão

presentes, estas clorofilas iniciam reações de degradação. No entanto, os herbicidas que inibem a biossíntese de carotenóides não afetam os carotenóides pré-existentes. Portanto os tecidos formados antes do tratamento não mostram os sintomas albinos típicos (OLIVEIRA, 2001).

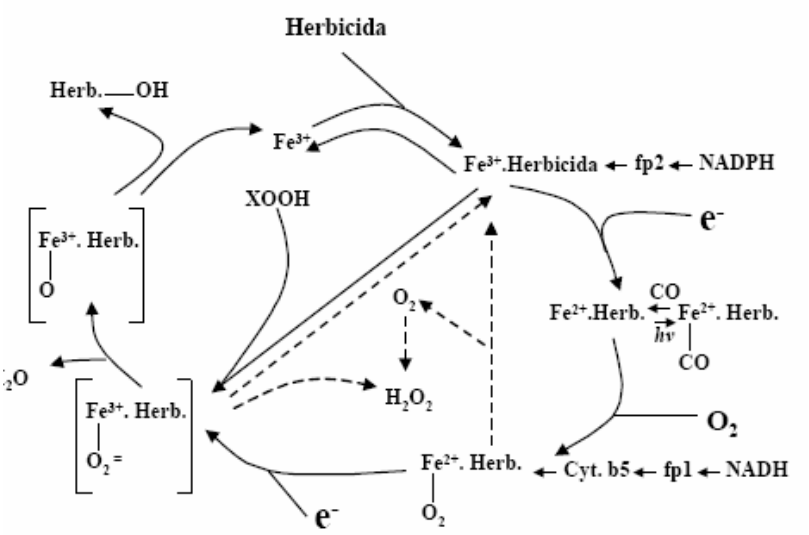

FIGURA 4- Mecanismo de Ação do Clomazone em Plantas (Oliveira, 2001).

2.6. Herbicida Quinclorac

O quinclorac (3, 7-dicloroquinolina-8- ácido carboxílico), um herbicida pertencente ao grupo químico das quinolinas, é encontrado na forma comercial de concentrado emulsionável 50% (FACET®) e apresenta a estrutura abaixo (Figura 5).

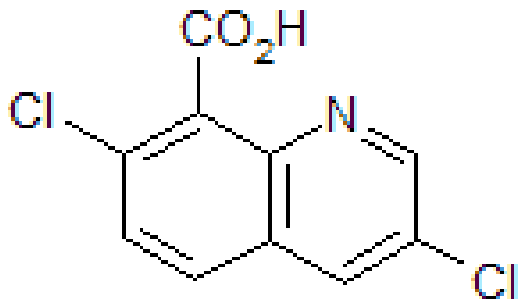

FIGURA 5- Estrutura Química do Quinclorac (Senseman, 2007).

Dentre os herbicidas seletivos utilizados na lavoura de arroz, o quinclorac, mimetizador de auxina, reúne flexibilidade na aplicação (pré e pós-emergência), eficiência de controle de *Echinochloa* spp., *Digitaria* spp. e *Setaria* spp., baixa toxicidade ao homem e aos animais (GROSSMANN & KWIATKOWSKI, 2000). Este produto possui solubilidade na água de 0,065 mg/L e meia vida de 21 dias na água ou solo (ZANELLA et al., 2002). A ação inicial envolve o metabolismo de ácidos nucléicos e a plasticidade da parede celular. Acredita-se que o herbicida quinclorac possa causar a acidificação da parede celular através do estímulo da atividade da bomba de prótons da ATPase, ligada à membrana celular. A redução no pH induz à elongação celular pelo aumento da atividade de certas enzimas responsáveis pelo afrouxamento celular. Baixas concentrações estimulam a RNA polimerase, resultando em aumentos subseqüentes de RNA, DNA e biossíntese de proteínas. Aumentos anormais nesses processos levam à síntese de auxinas e giberilinas, as quais promoverão divisão e alongamento celular acelerado e desordenado nas partes novas da planta, ativando seu metabolismo e levando ao seu esgotamento. Por outro lado, em concentrações mais altas, esses herbicidas inibem a divisão celular e o crescimento, geralmente nas regiões meristemáticas, que acumulam tanto assimilados provenientes da fotossíntese quanto o herbicida transportado pelo floema. Esses herbicidas estimulam a liberação de etileno que, em alguns casos, pode produzir sintomas característicos de epinastia associados à exposição a esses herbicidas (AHRENS, 1994).

2.7. Herbicida Metasulfuron Metil

O metasulfuron metil (methyl2-[[[(4-metoxi-6-methyl-1,3,5-triazine-2-) amino]carbonyl]amino] sulfonyl-benzoate-), pertencente ao grupo químico das

sulfoniluréia, é encontrado na forma comercial de concentrado emulsionável 50% (ALLY®) e apresenta a estrutura abaixo (Figura 6).

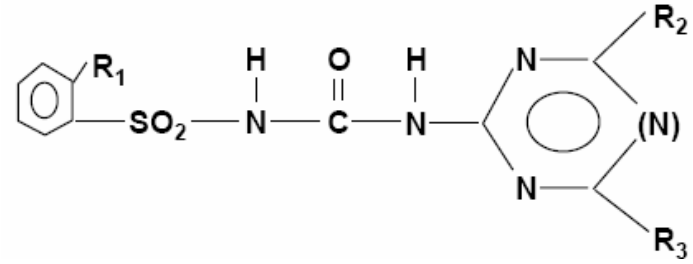

FIGURA 6- Estrutura Química do Metasulfuron Metil (Senseman, 2007).

Este composto é amplamente utilizado em lavouras de arroz no Sul do Brasil contra gramíneas invasoras dessas culturas (ZANELLA et al., 2002). Metasulfuron metil é uma nova classe de herbicida caracterizado pelo controle das plantas daninhas em lavouras a baixas concentrações (2-75 g/ha) (ARUFE et al., 2004). Segundo ZANELLA et al. (2002), o metasulfuron metil possui solubilidade na água de 9,5 mg/L e meia vida de 30 dias no solo ou, até de 30 a 120 dias (TREZZI et al., 2001). Segundo OLIVEIRA (2001), os herbicidas inibidores da ALS (alanina aminotransferase) foram responsáveis por 17% do mercado mundial de herbicidas em 1994, mais do que qualquer outro grupo isoladamente. Uma grande variedade de culturas é sensível às doses recomendadas de sulfuniluréias. Quando culturas são plantadas em rotação com herbicidas desse grupo, a ocorrência de danos depende da quantidade de herbicida persistente na estação seguinte, o que, por sua vez, é influenciada pelo pH do solo, umidade e temperatura. A mesma cultura pode responder de maneira diferente a um mesmo nível de resíduos de sulfuniluréias dependendo de vários fatores ambientais e do solo. O mecanismo de ação desse herbicida é a inibição da ALS, que é a enzima chave na rota de biossíntese de aminoácidos valina, leucina e isoleucina (Figura 7). Após a absorção, esse herbicida é rapidamente translocado para áreas de crescimento ativo (meristemas, ápices), onde o crescimento é inibido em plantas suscetíveis. As plantas acabam morrendo

devido à incapacidade de produzir alguns aminoácidos essenciais de que necessita (DURNER et al., 1991).

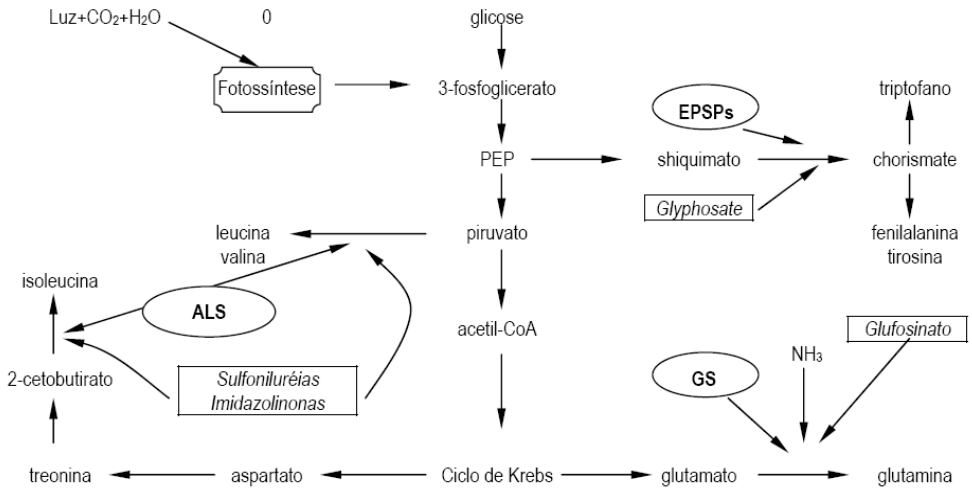

FIGURA 7- Mecanismo de Ação do Metasulfuron Metil em Plantas (Oliveira, 2001).

2.8. Concentração Letal Média (CL_{50})

A CL_{50}-96h é a concentração capaz de causar a mortalidade ou letalidade de 50% dos indivíduos até 96 horas após o início do teste de toxicidade. Estes testes são recomendados para estudos preliminares a fim de estabelecer estimativas de toxicidade das substâncias (SOLOMON, 1997). Os mesmos indicam que existe uma variação entre os diferentes agroquímicos quanto a sua toxicidade (expressa pelos valores de CL_{50}), bem como quanto ao índice de segurança (estimado pela divisão da CL_{50} pela concentração provável utilizada na lavoura). Assim, quanto maior o valor do índice, menor é o risco desse produto de causar efeito letal sobre os organismos (RESGALLA JÚNIOR, 2002). Segundo SOLOMON (1997), índices de segurança superiores a 20 demonstram produtos com menor risco de impacto ambiental. Assim, o uso de CL_{50} para testes de toxicidade permite avaliar o potencial deletério que

alguns químicos exercem sobre os organismos, sob condições controladas de laboratório (RAND & PETROCELLI, 1985). A classe toxicológica de determinado produto também se baseia na integração de suas características fisicoquímicas, envolvendo sua persistência ou degradação (meia-vida, constantes de hidrólise, fotólise e outros) e a sua capacidade de bioconcentração.

É importante que na avaliação da toxicidade dos agroquímicos sejam utilizadas espécie bioindicadoras, como os peixes, que ocupam o topo da cadeia alimentar. Estes testes permitem a identificação dos produtos químicos que apresentam menores riscos de impacto ambiental e toxicidade, além de reconhecer aqueles que poderiam ser utilizados nas lavouras de arroz consorciado com peixes. O efeito tóxico pode variar conforme a espécie testada, de acordo com o tipo de agroquímico e tempo de exposição. Por exemplo, avaliou-se a toxicidade aguda do clomazone determinando a CL_{50}-96 h para os alevinos de tetrarisca-negra (*Hyphessobrycon scholzei*) onde o valor foi equivalente a 27,67 mg/L, enquanto em truta arco-íris (*Salmo gairdineri*) foi de 19 mg/L (JONSSON et al., 1998; VECIL et al., 2002). Em juvenis de carpa comum (*Cyprinus carpio*) a CL_{50}-96 h foi de 19,52 mg/L a exposição do clomazone; 6,65 mg/L ao quinclorac e 26 mg/L para a exposição ao metasulfuron metil (RESGALLA JÚNIOR et al., 2002). Estudos de toxicidade da carpa comum exposta ao cypremetrin demonstram valores para CL_{50}-96 h de 0,004 a 0,0022 mg/L e após 48 h de exposição o valor da CL_{50} é de 0,006 mg/L (DAVID et al., 2004). Contudo, trabalhos recentes indicam que não há uma relação constante entre concentração letal e a concentração segura para a sobrevivência ou crescimento. Ela varia de espécie para espécie, podendo ser em torno de 3% da concentração letal para catfish de canal (*Ictalurus punctatus*) e 12% para *Pimephales promelas* (TOMASSO, 1994). Contudo, os valores de CL_{50}- 96h são muito superiores às

concentrações estabelecidas para efeitos metabólicos ou sub-letais (RESGALLA JÚNIOR, 2002).

2.9. Parâmetros de Crescimento

Xenobióticos encontrados no ambiente podem gerar toxicidade às populações expostas e causar alterações no crescimento dos organismos. Os testes de toxicidade são baseados na sobrevivência, crescimento e na reprodução de peixes (SOSO et al., 2007). NIEVES-PUIGDOLLER et al. (2007) demonstraram que em *Atlantic salmon* smolts expostos a níveis subletais do herbicida atrazina (0,1 mg/L) apresentam distúrbios na osmorregulação, na alimentação e no crescimento. ALVAREZ et al. (2005) mostraram que a exposição a 0,004 e 0,008 mg/L de atrazine por 96h reduz o crescimento de larvas de Red drum (*Sciaenops ocellatus*). Em adição, estudos tem evidenciado uma diminuição na população de rotíferos (*Brachionus* sp.) alimentados com microalgas expostas ao triazine; demonstrando que a população pode acumular poluente através da cadeia alimentar (RIOBOON et al., 2007).

2.10 A Atividade da Acetilcolinesterase

As colinesterases são enzimas importantes na neurotransmissão colinérgica central e periférica, além de exercerem funções como a detoxificação de xenobióticos (ROEX et al., 2003). De acordo com suas propriedades catalíticas e especificidade a substratos, sensibilidade a inibidores e distribuição tecidual, as colinesterases podem ser classificadas em dois tipos principais: acetilcolinesterase (AChE; E.C. 3.1.1.7) e butirilcolinesterase ou pseudocolinesterase (BuChE; E.C. 3.1.1.8) (MASSOULIÉ et al., 1993). A AChE é distribuída por todo o corpo e hidrolisa preferencialmente ésteres com grupamento acetil, como a acetilcolina (ACh) (DUTTA & ARENDS, 2003). A

enzima AChE é responsável por degradar o neurotransmissor ACh, regulando seus níveis no sistema nervoso (central e periférico), nas junções neuromusculares e nas fendas sinápticas (Figura 8). MENDEL & RUDNEY (1943), demonstraram que carpas (*Cyprinus carpio)* possuem apenas AChE no cérebro. Outros trabalhos também relataram AChE cerebral em várias espécies de peixes (GAAL, et al., 1980; KOZLOVSKAYA et al., 1993; CHUIKO, 2000). A atividade da AChE cerebral pode ser afetada por fatores como: a temperatura ambiental, a espécie, o ciclo reprodutivo, o sexo e a idade (YI et al., 2007). Assim, esta enzima tem sido utilizada como um indicador para diagnosticar o efeito da exposição de compostos como carbamatos e organofosforados (CHUIKO, 2000; SANCHO et al., 2000; FERNÁNDEZ-VEJA et al., 2002). Estes pesticidas, ao inibirem a atividade da AChE induzem o acúmulo do neurotransmissor ACh, nas sinapses colinérgicas (central e periféricas) e junções neuromusculares, levando a uma super-estimulação das células-alvo. Como conseqüência, várias alterações motoras, como distúrbios na locomoção, no equilíbrio, tremores, convulsões e até morte dos peixes (SAGLIO & TRIJASSE, 1998; BRETAUD et al., 2000; SANCHO et al., 2000; FERNÁNDEZ-VEGA et al., 2002; ROEX et al., 2003). Em geral, os peixes intoxicados com inseticidas anticolinesterásicos mostram sinais de paralisia muscular, hiperatividade e perda de equilíbrio (CERÓN et al., 1996). O carbofuran é um inseticida, nematicida e acaricida utilizado mundialmente e está incluído no grupo dos carbamatos. Este é considerado um inibidor da AChE e muito tóxico para peixes e mamíferos (BRETAUD et al., 2000; ALVES et al., 2002). Além disso, estudos têm verificado que outras classes de pesticidas podem também causar alterações na atividade da AChE em peixes (BRETAUD et al., 2000, DUTTA & ARENDS, 2003; MIRON et al., 2005; GLUSCZAK et al., 2006; MORAES et al., 2007). DUTTA & ARENDS (2003) observaram inibição da atividade da AChE cerebral em *Lepomis macrochirus* expostos ao organoclorado

endosulfan. Assim, a medida da atividade da enzima acetilcolinesterase (AChE) pode ser utilizada como um parâmetro indicador para avaliar a toxicidade de pesticidas em peixes.

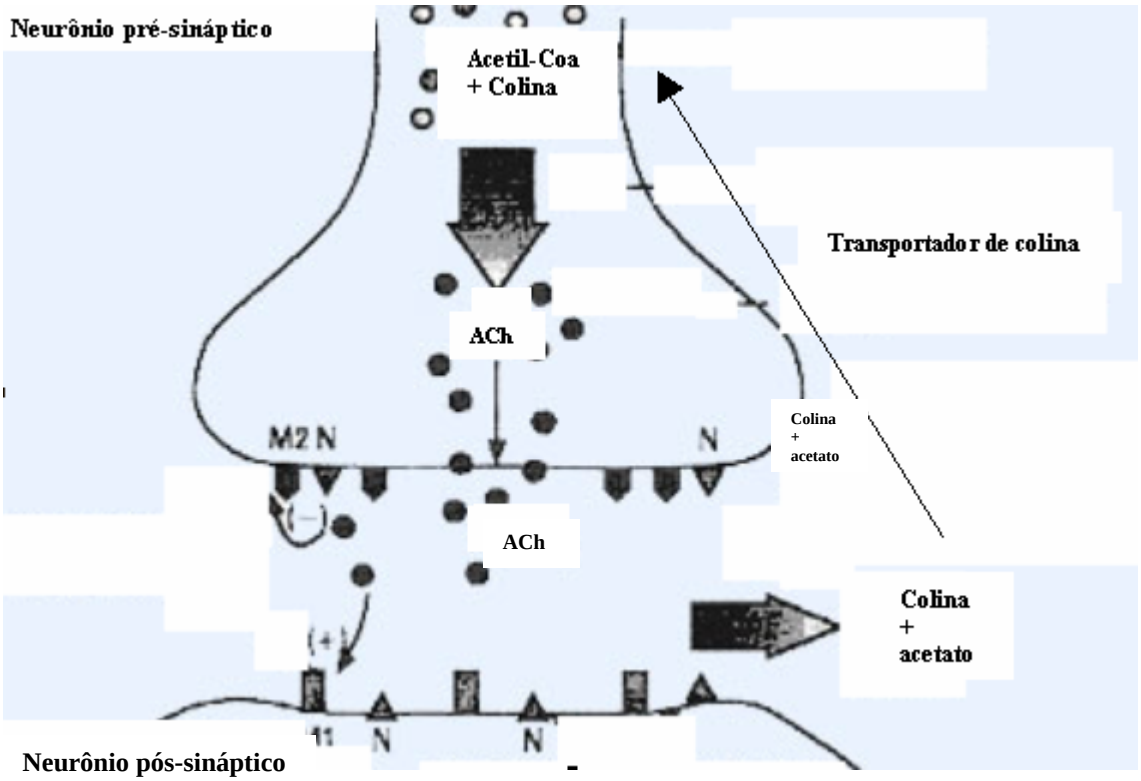

FIGURA 8- Sinapse colinérgica (Adaptado de Soreq e Seidman, 2001).

2.11. Intermediários Metabólicos

Diversas mudanças que ocorrem no metabolismo de peixes devido à exposição a herbicidas estão sendo utilizadas para identificar o estresse gerado pela contaminação ambiental devido à toxicidade do químico (JYOTHI & NARAYAN, 1999; DAS & MUKHERJEE, 2003; BEGUM, 2004). Recentemente, tem sido demonstrado que durante a exposição aos produtos químicos, a síntese e a degradação de diferentes metabólitos podem ser ativadas diferentemente para suprir as necessidades de energia dos peixes (AGUIAR, et al. 2004; SCOTT & SLOMAN, 2004; GLUSCZAK et al., 2007). Assim, os níveis de glicose, glicogênio, lactato e proteína em diferentes tecidos de peixes expostos a herbicidas tem sido estudados

por vários autores (JYOTHI & NARAYAN, 1999; ORUÇ & ÜNER, 1999; SANCHO et al., 2000; DAS & MUKHERJEE, 2003; BARCELLOS et al., 2004; CRESTANI et al., 2006; GLUSCZAK et al., 2006,2007).

O fígado é considerado um órgão essencial nos processos de acumulação, biotranformação e principalmente desintoxicação de toxinas químicas (SUAREZ & MOMMSEN, 1987; PEIXOTO et al., 2006). Além disso, este desempenha papel integrador entre os mecanismos energéticos do organismo, ajudando a regular as taxas de glicose no sangue, estocando-a na forma de glicogênio. E, se o nível de glicose no sangue diminui, este converte o glicogênio em glicose e devolve-o ao sangue para que seja distribuído a todos os órgãos ou tecidos do organismo que estejam necessitando. O glicogênio existe principalmente no fígado, mas este é também presente no músculo. A exposição de jundiás ao herbicida clomazone aumenta os níveis de glicogênio no fígado e diminui no músculo, demonstrando que os peixes usam esta fonte de energia para compensar a situação de estresse à exposição (CRESTANI et al., 2006). Assim, os peixes afetados por estresse ambiental podem ter alterado os níveis de glicogênio, degradando-o, e ocasionando grandes perdas dessa reserva de glicose. Isto reflete um mecanismo de compensação fisiológica em resposta a exposição á químicos como agrotóxicos (BIDINOTTO et al., 1997; JYOTHI & NARAYAN, 1999; ORUÇ & ÜNER, 1999; BEGUM, 2004).

Além dos níveis de glicose e glicogênio as alterações metabólicas em peixes expostos a herbicidas podem ser analisadas também nos níveis de lactato e proteína. Através da gliconeogênese podem-se gerar moléculas de glicose a partir de outras moléculas orgânicas como piruvato, lactato, glicerol e alguns aminoácidos (RAHAMI & ABDOLLAHI, 2007). Então, ocasionalmente os peixes podem usar outros precursores como o lactato, como uma fonte de energia (BEGUM &

VIJAYARAGHAVAN, 1999). Muitos pesticidas induzem condições de hipóxia tecidual nos peixes, favorecendo o metabolismo anaeróbico para obtenção de energia (KNOX et al., 1980; ORUÇ & ÜNER, 1999). Portanto, o aumento nos níveis de lactato hepático, muscular e plasmático representa uma resposta rápida à necessidade de energia através do metabolismo anaeróbico e, já foi observado em vários estudos (BEGUM & VIJAYARAGHAVAN, 1999; CRESTANI et al., 2006; GLUSCZAK et al., 2006; FONSECA et al., 2008). No geral, todas mudanças indicam desordens metabólicas e são usadas como indicadores da toxicidade de herbicidas. Além disso, uma grande parte do organismo dos peixes é formada por proteínas e estas também podem ser utilizadas como o combustível preferido para fornecer a demanda de energia durante uma situação de estresse (PEIXOTO et al., 2006). Nesse sentido, foi observado que a exposição ao carbofuran causa uma diminuição no conteúdo protéico hepático e muscular de *Clarias batrachus* (linn) (BEGUM, 2004). Assim, o catabolismo de proteínas pode também indicar uma adaptação fisiológica dos peixes, para compensar a condição de toxicidade gerada pela exposição ao produto químico (SANCHO et al., 1998; VEJA et al., 2002).

2.12 Estresse Oxidativo e Defesa Antioxidante

As reações de oxidações são essenciais no metabolismo normal dos organismos aeróbicos. Nas células aeróbicas, espécies reativas de oxigênio (EROs) são produzidas durante o metabolismo oxidativo produzindo radicais livres, os quais são produtos da redução parcial do oxigênio (BOVERIS & BERMÚDEZ, 1996). Estes podem causar danos em componentes estruturais e funcionais das células, levando a modificação ou perdas das funções celulares (VAL et al., 1996). Porém, essa produção é equilibrada com uma produção equivalente de antioxidantes, que visa neutralizar os efeitos deletérios dos Eros (AHMAD et al., 2000). As enzimas catalase

(CAT; EC, 1.11.1.6), superóxido dismutase (SOD) e Glutationa peroxidase (GPx) são responsáveis pela proteção celular contra as EROs e, assim formam parte do sistema antioxidante celular. Estas mostram maior atividade no fígado, órgão de captação/transformação enzimática de EROs (LEMAIRE et al., 1994). As alterações dessas enzimas em peixes podem ser usadas como indicadores de exposição aos poluentes aquáticos (AHMAD et al., 2000; LI et al., 2003, MORAES et al., 2007). Atualmente, várias classes de poluentes, entre eles os herbicidas, podem aumentar a produção de EROs em diversos organismos aquáticos, como os peixes, ocasionando assim uma situação de estresse oxidativo (WINSTON, 1991; AHMAD et al., 2000; ÜNER et al., 2005). Com o aumento excessivo das EROs intracelulares, a capacidade de atuação das defesas antioxidantes é prejudicada, ocorrendo um desequilíbrio entre pró-oxidantes e antioxidantes (enzimático ou não enzimático). Conseqüentemente, devido sobrecarga do mecanismo antioxidante, ocorre estresse oxidativo (ÜNER et al., 2006). Em uma situação de estresse oxidativo pode ocorrer ainda a peroxidação lipídica (LPO) que é observada pelo aumento de malondialdeído (MDA) e a carbonização de proteínas que indica possíveis danos causados as proteínas (PARVEZ & RAISUDDIN, 2005). A LPO e o aumento da carbonilação de proteínas induzida por poluentes ambientais são algumas das principais causas de doenças que ocorrem em peixes (LI et al., 2003; PARVEZ & RAISUDDIN, 2005). Segundo, RADI et al. (1985), a formação de LPO induzida por poluentes tem sido observada em espécies de peixes que apresentam vários sistemas antioxidantes, entre eles as enzimas CAT e SOD. Assim, mudanças no conteúdo desses parâmetros podem também refletir a presença de tóxicos (AHMAD et al., 2000).

2.13. Capacidade de Recuperação

As alternativas para minimizar o impacto dos herbicidas são várias. Primeiramente é recomendada a utilização das doses mínimas na aplicação, o que geralmente propicia uma redução de 30-50% em relação à dose máxima do herbicida, mas pode chegar até 85% no caso do glifosato (IRGA, 2001). Também se sugere manter estática a lâmina de água por um período mínimo de duas semanas após a aplicação, o que pode proporcionar uma redução de 97% do clomazone aplicado (NOLDIN, 2001), bem como construir adequadamente as taipas-ronda para evitar extravasamento (MARCHESAN et al., 2007). Deste modo, estima-se que a água da lavoura que for liberada para os rios e lagos apresentará uma quantidade menor do agroquímico no ambiente. No entanto, é imprescindível obter informações sobre os efeitos desses produtos químicos durante a exposição e sucessivamente sobre um período de recuperação em água livre de produto químico, uma vez que as alterações em peixes expostos a determinados químicos podem ser reversíveis, ou menos prolongadas quando comparado a outros químicos (DEMBELÉ et al., 1999).

No entanto, o tempo de recuperação dos peixes depende de vários fatores, tais como: o tipo, a concentração e o tempo de exposição ao pesticida e a espécie testada (SANCHO et al, 2000; MIRON et al., 2005). O conteúdo de proteínas e o glicogênio hepático, em peixes após a exposição ao carbofuran permanece diminuídos em após três dias em água sem o inseticida. SANCHO et al. (1998) observaram que a maioria das alterações metabólicas não persiste após o período de recuperação de 8 dias. Estas informações são muito importantes para obter medidas do potencial tóxico dos pesticidas, os quais quando aplicados em excesso ou de maneira errônea, podem afetar o ecossistema aquático e exercer efeitos adversos em outros organismos associados.

3. RESULTADOS E DISCUSSÃO

Os resultados e discussão deste trabalho serão apresentados em dois artigos científicos e dois manuscritos, distribuídos em quatro capítulos, como segue:

Capitulo I: Efeitos dos herbicidas clomazone, quinclorac e metasulfuron metil sobre atividade da acetilcolinesterase de jundiás (*Rhamdia quelen*) (Heptapteridae).

Artigo I: Effects of the herbicides clomazone, quinclorac, and metsulfuron methyl on acetylcholinesterase activity in the silver catfish (*Rhamdia quelen*) (Heptapteridae).

Capítulo II: Sobrevivência, crescimento e parâmetros metabólicos de jundiás (*Rhamdia quelen*) expostos a herbicidas usados no arroz.

Manuscrito I: Survival, growth and metabolic parameters of silver catfish (*Rhamdia quelen*) exposed to herbicides used in rice fields.

Capítulo III: Efeitos bioquímicos do herbicida clomazone sobre piavas (*Leporinus obtusidens*).

Artigo II: Biochemical effects of clomazone herbicide on piava (*Leporinus obtusidens*).

Capítulo IV: Formulação comercial contendo clomazone afeta alguns parâmetros metabólicos de piavas (*Leporinus obtusidens*).

Manuscrito II: Commercial formulation containing clomazone affects some metabolic parameters in piava (*Leporinus obtusidens*).

CAPÍTULO I

ARTIGO I:

Effects of the herbicides clomazone, quinclorac, and metsulfuron methyl on acetylcholinesterase activity in the silver catfish (*Rhamdia quelen*) (Heptapteridae)

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Effects of the herbicides clomazone, quinclorac, and metsulfuron methyl on acetylcholinesterase activity in the silver catfish (*Rhamdia quelen*) (Heptapteridae)

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Abstract

Fingerlings of the silver catfish (*Rhamdia quelen*) were exposed to three herbicides widely used in rice culture in south Brazil: clomazone, quinclorac, and metsulfuron methyl. LC_{50} was determined and acetylcholinesterase (AChE) activity was evaluated in brain and muscle tissue of fish exposed to different herbicide concentrations after 96 h (short term). The LC_{50} value (nominal concentration) was 7.32 mg/L for clomazone and 395 mg/L for quinclorac, but was not obtained for metsulfuron-methyl since all fingerlings survived the highest concentration of 1200 mg/L. Brain and muscle AChE activity in unexposed fish were 17.9 and 9.08 μmol/min/g protein, respectively. Clomazone significantly inhibited AChE activity in both tissues, achieving maximal inhibition of about 83% in brain and 89% in muscle tissue. In contrast, quinclorac and metsulfuron methyl caused increases in enzyme activity in the brain (98 and 179%, respectively) and inhibitions in muscle tissue (88 and 56%, respectively). This study demonstrated short-term effects of exposure to environmentally relevant concentrations of rice field herbicides on AChE activity in brain and muscle tissue of silver catfish.

Keywords: Herbicides; Acetylcholinesterase; Brain; Muscle; Fish; Silver catfish (*Rhamdia quelen*)

1. Introduction

Herbicide contamination of surface waters derived from agricultural practices is a problem of worldwide importance. In fish, previous studies on herbicides and pesticides have focused on the effects of contaminant exposure on the activity of acetylcholinesterase (AChE) (Chuiko, 2000; Bretaud et al., 2000; Dutta and Arends, 2003). The measurement of this enzyme, present in the cholinergic synapses and motor end plates, has been used by different authors to monitor carbamate and organophosphate effects in insects and vertebrates including fish (Chuiko, 2000; De La Torre et al., 2002; Fernández-Vega et al., 2002). Dutta and Arends (2003) showed reduced AChE activity in tissue of fish that were exposed to the organochlorine endosulfan. Assays of AChE (EC 3.1.1.7) as a biomarker in different tissues provide sensible methods for detecting water contamination by many pesticides or herbicides (Sancho et al., 2000). Disturbances in AChE activity can also affect locomotion and equilibrium in exposed organisms and may impair feeding, escape, and reproductive behavior (Saglio and Trijasse, 1998; Bretaud et al., 2000). Carbofuran and atrazine produce adverse behavioral changes in goldfish after a short-term exposure to sublethal concentrations (Saglio and Trijasse, 1998).

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Alterations in swimming have been found in fish exposed to diuron (Bretaud et al., 2000).

Clomazone (isooxazolidinone), quinclorac (quinoline), and metsulfuron methyl (sulfonylurea) are herbicides extensively used in agriculture, especially in paddy rice fields (Rodrigues and Almeida, 1998; Jonsson et al., 1999). Sulfonylurea, quinolines, and isooxazolidinones are the most potent herbicides known today and are soluble in water (Ware, 2003); they are widely used in rice fields in southern Brazil, with activity against Poaceae (Jonsson et al., 1998). Water solubility of clomazone is 1100 mg/L and its half-lives in water and soil are less than 30 days and between 30 and 135 days, respectively. Water solubility of quinclorac is 0.065 mg/L and its half-life in water is 21 days. The half-life of metsulfuron methyl in the soil is 30 days and its water solubility is 9.5 mg/L (Barceló and Hennion, 2002; Ware, 2003). Aquatic contamination by these products may occur in and around agricultural areas and may adversely affect aquatic fauna (Jonsson et al., 1999). However, little attention has been given to the possible occurrence of short-term sublethal toxicity of herbicides to nontarget organisms such as fish (De La Torre et al., 2002).

Silver catfish, *Rhamdia quelen* (Siluriformes, Heptapteridae), is a native freshwater fish of southern Brazil; it can survive cold winters and grows in the summer. In aquaculture systems, silver catfish can reach 600–800 g of body weight in 8 months (Barcellos et al., 2004). No information is available on changes in AChE activity in response to short-term exposure to herbicides in silver catfish. Thus, the purpose of this study was to verify the relation between the lethal concentration (LC_{50}) of herbicides used in rice culture and the AChE enzyme activity in brain and muscle tissue of silver catfish as a possible early biomarker of exposure to these herbicides.

2. Materials and methods

2.1. Materials

Acetylthiocholine, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents used in the experiments were of the highest analytical grade (Aldrich). Herbicides were obtained commercially as follows: clomazone (2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone) (Gamit; 36% purity), quinclorac (3,7-dichloroquinoline-8-carboxylic acid) (Facet; 50% purity), and metsulfuron methyl (methyl2-[[[(4-metoxi-6-methyl-1,3,5-triazine-2-) amino]carbonil] amino] sulfonylbenzoate-sulfonylurea) (Ally; 50% purity). The sources of herbicides were: FMC (EUA), BASF, and Dupont do Brasil, respectively.

2.2. Animals

Silver catfish fingerlings (*R. quelen*) of both sexes were obtained from fish culture Bela Vista near Santa Maria, Rio Grande do Sul state, Brazil. The fish weight at the time of sampling was 2.8 ± 0.2 g and the fish length was 6 ± 1.0 cm. Fish were acclimated to laboratory conditions for 15 days. They were kept in tanks (250 L) under a natural photoperiod (12 h light–12 h dark). The water was constantly aerated in a static system. Physicochemical characteristics of water were as follows: temperature 21 ± 2 °C, pH 7.2 ± 0.03, dissolved oxygen 7.2 ± 1.0 mg/L, nonionized ammonia 7 ± 0.1 μg/L, nitrite 0.05 ± 0.01 mg/L, hardness 20 ± 0.5 mg/L $CaCO_3$, and alkalinity 45 ± 1.1 mg/L $CaCO_3$. Fish were fed ad libitum three times a day (08:30, 12:00, and 17:30 h) with commercial fish food (42% crude protein, Supra, Brazil). Feces and pellet residues were removed daily by suction.

2.3. Exposures

After acclimation, fish were transferred to boxes (40 L) with controlled aeration and temperature. Groups of 10 fish per plastic box (three replicates) were exposed for 96 h to each herbicide concentration: clomazone 1, 5, 10, 20, and 50 mg/L; quinclorac 100, 200, 300, 375, and 400 mg/L; and metsulfuron methyl 200, 400, 600, 800, and 1200 mg/L. For each herbicide concentration test, a set of 10 fish/box (in triplicate) was used as a control (same conditions without herbicide) and sampled at each time. Herbicides were added to the water only at the beginning of the experiment. Water quality did not change throughout the experimental period. Feces and pellet residues were removed daily by suction. During the experimental period, the water parameters were as follows: temperature 21 ± 0.5 °C, pH 7.32 ± 0.07, dissolved oxygen 7.2 ± 1.0 mg/L, nonionized ammonia 6.5 ± 0.1 μg/L, nitrite 0.05 ± 0.01 mg/L, hardness 22 ± 1.0 mg/L $CaCO_3$, and alkalinity 44 ± 2.0 mg/L $CaCO_3$. Mortality from each concentration of herbicide was recorded for estimation of LC_{50}. During the experiment, activity (normal, erratic swimming, lethargy) and feeding behavior (feeding or not) were observed, registered, and compared to control.

2.4. Sampling and enzyme assay

At the end of the exposure period (96 h), six fish from each box were killed and placed on ice. Brain and muscle tissues were removed, frozen in liquid nitrogen, and then stored at –20 °C until the AChE assay. For the determination of enzyme activity,

herbicide concentrations bracketing the LC_{50} (except for metsulfuron methyl, because the LC_{50} for this species was not determined) were used: clomazone (0, 5, 10, and 20 mg/L), quinclorac (0, 100, 375, and 400 mg/L), and metsulfuron methyl (0, 400, 800 and 1200 mg/L). All

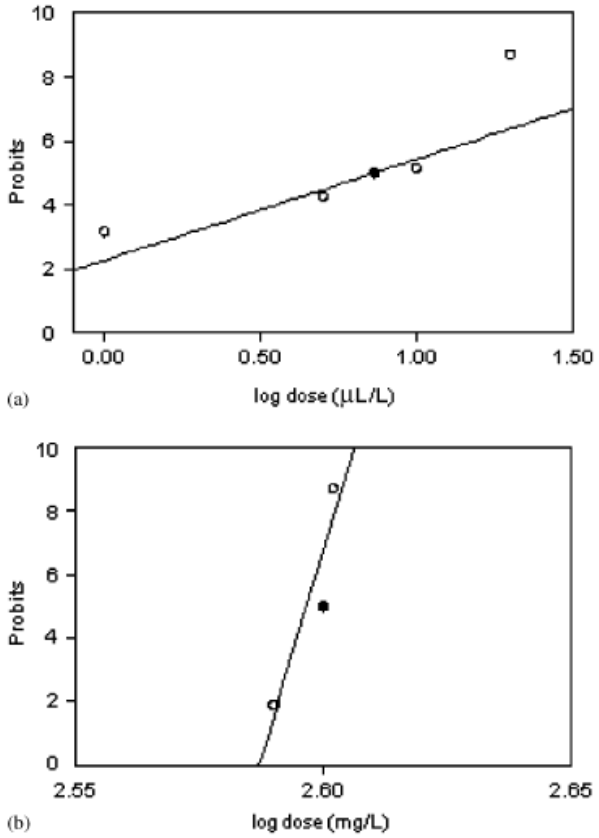

Fig. 1. Mortality of silver catfish exposed for 96 h to clomazone (a) and quinclorac (b). Open circles indicate LC_{50}, and close circles indicate log-doses tested.

enzyme tests were made in duplicate ($n = 3$). AChE activity was assayed as described by Ellman et al. (1961) and modified by Villescas et al. (1981) using acetylthiocholine (0.8 mM) as the substrate and DTNB as the chromogen. Absorbance of 2 mL of reaction medium containing 0.1 M potassium phosphate buffer, pH 7.5, 1 mM DTNB measured acetylthiocholine hydrolysis at 25 °C and 412 nm using a Hitachi (U-2001) spectrophotometer, with 5–10 µg of protein concentration for the muscle and brain. Protein was determined by the Coomassie blue method (Bradford, 1976) using bovine serum albumin as standard. Enzyme activity was expressed as µmol of acetylthiocholine (AcSCh) hydrolyzed per min per gram of protein.

2.5. Statistical analysis

The mean LC_{50} for 96 h was calculated using probit analysis as described by Finney (1971). The AChE activity data were analyzed using one-way analysis of variance followed by Tukey–Kramer test and expressed as mean ± standard error ($n = 3$). The differences were considered to be significant at a probability level of $P < 0.01$ between treatments and controls.

3. Results

3.1. LC_{50} value and behavior observations

The LC_{50} for clomazone was 7.32 mg/L (confidence interval: 5.68–9.03) (Fig. 1a). Silver catfish fingerlings exposed to the highest doses of clomazone (20 and 50 mg/L) did not feed. Swimming activity was normal at the lowest clomazone doses (1 and 5 mg/L), but higher concentrations provoked erratic swimming (Table 1). For quinclorac the LC_{50} was 395 mg/L (confidence

Table 1
Lethal concentrations (96 h) and effect of different concentrations of clomazone, quinclorac, and metsulfuron methyl on feeding behavior, activity, and brain and muscle AChE activity in silver catfish fingerlings

Herbicide	LC_{50} (mg/L)	Feeding behavior	Activity	Brain AChE activity (% of control)	Muscle AChE activity (% of control)
Clomazone	7.32				
5 mg/L		Feeding	Normal	20	22
10 mg/L		Feeding	Erratic swimming	17	11
20 mg/L		Not feeding	Erratic swimming	32	11
Quinclorac	395.0				
100 mg/L		Feeding	Lethargic	198	18
375 mg/L		Not feeding	Lethargic	186	19
400 mg/L		Not feeding	Lethargic	197	12
Metsulfuron methyl	Not stablished				
400 mg/L		Feeding	Hyperactivity	195	44
800 mg/L		Feeding	Hyperactivity	245	53
1200 mg/L		Feeding	Hyperactivity	279	49

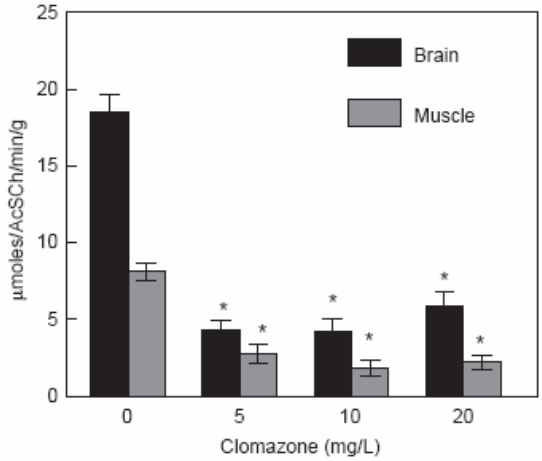

Fig. 2. Effects on the AChE activity after 96-h exposure to 0 (control), 5, 10, and 20 mg/L of clomazone in the brain and muscle. AChE activity (µmol/AcSCh)/min/g protein) is expressed as mean±SE ($n = 3$). Significant difference from control is indicated: $^{*}P < 0.001$.

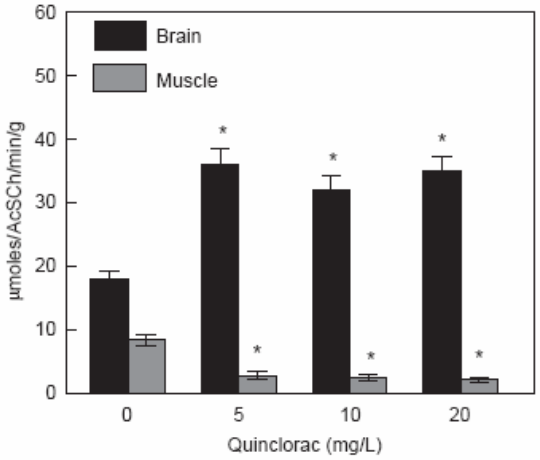

Fig. 3. Effects on the AChE activity after 96 h exposure to 0 (control), 100, 375, and 400 mg/L of quinclorac in the brain and muscle. AChE activity (µmol/AcSCh)/min/g protein) is expressed as mean±SE ($n = 3$). Significant difference from control is indicated: $^{*}P < 0.001$.

interval: 394 to 395.9 mg/L) (Fig. 1b). Animals exposed to the highest quinclorac concentrations (375 and 400 mg/L) did not feed and those exposed to the lower tested concentrations (100 mg/L) showed loss of equilibrium and lethargic behavior (Table 1). For metsulfuron methyl, LC_{50} was not obtained, since all fingerlings survived even at the highest concentration used (1200 mg/L). Fingerlings showed normal feeding behavior but abnormal burst swimming reactions at all concentrations tested (Table 1).

3.2. *Effects of herbicides clomazone, quinclorac, and metsulfuron methyl on brain and muscle AChE activity*

Brain of unexposed control fish showed higher specific AChE activity than muscle (17.9 against 9.08 µmol/min/g protein, respectively). After clomazone exposure, AChE activity significantly decreased ($P < 0.05$) at all concentrations tested (Fig. 2) in both tissues. Clomazone inhibition reached maxima of 83% in the brain and 89% in the muscle tissue. Conversely, exposure to all quinclorac and metsulfuron methyl concentrations tested significantly increased enzyme activity in brain (86–98% for quinclorac and 95–179% for metsulfuron methyl as compared to control values). A different result was obtained for muscle tissue where AChE activity reduced from 81% to 88% for quinclorac and from 47% to 56% for metsulfuron methyl as compared to control values (Figs. 3 and 4).

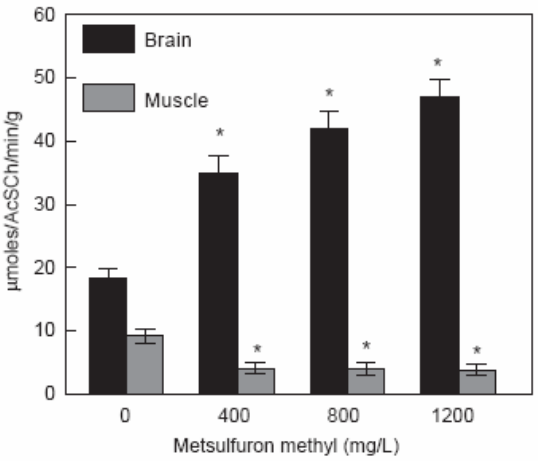

Fig. 4. Effects on the AChE activity after 96-h exposure to 0 (control), 400, 800, and 1200 mg/L of metsulfuron methyl in the brain and muscle. AChE activity (µmol/AcSCh)/min/g protein) is expressed as mean±SE ($n = 3$). Significant difference from control is indicated: $^{*}P < 0.001$.

4. Discussion

In southern Brazil the herbicides clomazone, metsulfuron methyl, and quinclorac are applied to rice culture by spraying, yielding final concentrations in the rice field of 0.7, 0.003, and 0.75 mg/L, respectively (Rodrigues and Almeida, 1998). As clomazone LC_{50} for silver catfish is 7.32 mg/L, the recommended concentration in the field is within an order of magnitude of the LC_{50}, which indicates that the use of this herbicide could be harmful for this species. The 96-h LC_{50} values for clomazone for rainbow trout and bluegill are 19 and 34 mg/L (Vencil et al., 2002), and that for *Hyphessobrycon scholzei*, it is 27.3 mg/L (Jonsson et al., 1998), indicating that silver catfish are very sensitive to this

herbicide. On the other hand, LC_{50} of metsulfuron methyl for rainbow trout is 150 mg/L (Vencil et al., 2002), while in the current study doses of 1200 mg/L did not provoke any mortality of silver catfish fingerlings. The recommended application rate of metsulfuron methyl is much lower than the concentrations used in this experiment, and therefore this herbicide appears safe for silver catfish.

AChE activity is frequently used as a biomarker of herbicide and pesticide toxicity. The activity of this enzyme is extremely important for many physiological functions, such as prey location, predator evasion, and orientation toward food. When AChE activity decreases, ACh is not broken and accumulates within synapses which therefore cannot function in a normal way (Dutta and Arends, 2003).

The results of the present study showed that, in unexposed fish brain, AChE-specific activity was twofold higher than that in muscle tissue. Higher brain AChE activity compared to that of muscle was also observed in channel catfish (*Ictalurus punctatus*) (Straus and Chambers, 1995). However, in juvenile goldfish (*Carassius auratus*) AchE-specific activity was higher in the skeletal muscle than in the brain (Bretaud et al., 2000). Our study showed that for all herbicides tested, there was a decrease in AChE activity in muscle tissue. In the same way, muscle AChE activity in European eels (*Anguilla anguilla*) exposed to 0.22 mg/L of thiobencarb herbicide was depressed 35% in a 96-h test, with eels also showing tremors, lethargy, and erratic swimming (Fernández-Vega et al., 2002). Exposure to 50 μg/L of carbofuran for 48 h inhibited 23% of the AChE activity in skeletal muscle of goldfish (Bretaud et al., 2000). Changes in muscle AChE activity observed in silver catfish are reflected in movement disturbances as shown in Table 1.

Our results obtained from silver catfish exposed to clomazone revealed that this herbicide is a potent brain AChE inhibitor (68–83%). Brain AChE inhibition was also observed in European eels exposed to diazinon (0.042 mg/L, inhibition higher than 75%) (Cerón et al., 1996), and in *Lepomis macrochirus* exposed to endosulfan (0.001 mg/L for 96 h, inhibition of 16%) (Dutta and Arends, 2003). Furthermore, carbamate herbicides (Fernández-Vega et al., 2002) and sulfonylurea (Bretaud et al., 2000) herbicides were observed to produce declines in brain AChE activity. In addition, erratic swimming and convulsions have been observed by Fernández-Vega et al. (2002). However, in our experiment both sulfonylurea and quinoline herbicides enhanced brain AChE activity. Perhaps this contradictory result is a response to an injurious toxicological situation. Animals could be compensating stress by enhancing AChE activity.

Cholinesterase inhibition in brain and muscle produces adverse effects in movement because AChE participates in neuronal and neuromuscular transmissions (Fernández-Vega et al., 1999, 2002). The activation observed in AChE activity after exposure to metsulfuron methyl and quinclorac could represent an increase in the hydrolysis of the neurotransmitter acetylcholine, with a consequent decreased activation of nicotinic and muscarinic receptors. On the other hand, AChE inhibition observed with clomazone leads to an accumulation of acetylcholine, causing overstimulation of the receptors. Thus, activation or inhibition of AChE can influence the process of cholinergic neurotransmission and promote undesirable effects. Changes in AChE activity observed in this study help to explain behavior alterations such as erratic or lethargic swimming induced by herbicides.

5. Conclusion

This study showed that herbicides used in rice culture may affect fish behavior. The most dangerous is clomazone since its concentrations in rice fields are only 1/10 that of the LC_{50}. AChE activity may be an early biomarker of toxicity as shown for quinclorac and metsulfuron methyl.

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CAPÍTULO II

MANUSCRITO I:

Survival, growth and metabolic parameters of silver catfish (*Rhamdia quelen*) exposed to herbicides used in rice fields.

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(Em fase de Redação)

Survival, growth and metabolic parameters of silver catfish (*Rhamdia quelen*) exposed to herbicides used in rice fields

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Abstract

The objective of the present study was to investigate in silver catfish (*Rhamdia quelen*) the effects of water drained from rice fields 21 days after receiving application of the commercial formulation of the herbicides clomazone (0.5 mg L^{-1}), quinclorac (0.375 mg L^{-1}) and metsulfuron methyl (0.002 mg L^{-1}). The parameters analysed after of exposure to herbicides were: growth specific (15, 30 and 45 days); biomass and survival (45 days); some metabolic parameters (15 and 45 days). Metsulfuron methyl was not detected in the water seven days after the application in the rice field, while quinclorac and clomazone were detected even after 14 and 28 days. The fish exposed to quinclorac exhibited 96 % survival, while those exposed to the other treatments presented 100% survival. The exposure to clomazone and quinclorac showed in the fish significantly lower weight, length and specific growth rate after 45 days, while significantly lower biomass was observed only in those exposed to quinclorac. Clomazone and quinclorac resulted in higher glycogen levels in the liver, but lower glucose and lactate in the fish as compared to control. Fish exposed to metsulfuron methyl also showed the same responses, but only after 15 days exposure. Lower glycogen and glucose and higher lactate levels were detected in exposure to all herbicides after 45 days. The results obtained conclude that water drained from rice fields that received an application of clomazone and quinclorac may affect normal growth and metabolism of fish, while metsulfuron methyl appears to be safe. Apparently silver catfish showed a mechanism glycogen storage in the hepatic tissue as strategies to support the increased metabolic demand due to detoxification of the herbicides. In the muscle it seems to occur a stressful condition with increased lactate levels, indicating clear energy degradation.

Keywords: clomazone; quinclorac; metsulfuron methyl; rice-fish culture; metabolic response

1. Introduction

The pollution of natural waters is one of the most critical environmental issues. Several hundreds pesticides of different chemical structure are used worldwide in agriculture. These pesticides are considered to be essential for agricultural development, but some of them can provoke serious environmental contamination, principally in water (Zanella et al., 2000). Rice is one of the major cereal crops and is grown predominantly in the tropics and subtropics. This culture has been expanded largely by mechanization of farming, higher yielding varieties of rice, high levels of fertilizer, and widespread use of herbicides (Fernando, 1993). The practice of fish-culture in rice fields has had a checkered history dating back to 2000 years in China. Fish culture as an integrated and concurrent activity with rice culture in the same field is important for the rational utilization of limited land resources, as well as a sustainable source of fish protein, additional income and employment generation (Jamu and Costa-Pierce, 1995). Due to their widespread distribution and toxic nature, herbicides may have a serious impact on the aquatic environment, and can also affect aquaculture species indirectly through their toxicity to phytoplankton. The EEC Directive 80/778 concerning water quality for human consumption established a maximum concentration of 0.1 μgL^{-1} for each individual herbicide. The sum of total residual toxicants cannot exceed 0.5 μgL^{-1} (Aguillar et al., 1997). Clomazone (isooxazolidinone), quinclorac (quinoline) and metsulfuron methyl (sulfonylurea) are post-emergence herbicides widely used in paddy rice fields in Southern Brazil, with activity against weeds and aquatic gramineae (Jonsson et al., 1998). Clomazone is currently used for weed control in the cultivation of soybeans, cotton, rice, sugar cane, corn, tobacco, and various vegetable crops (Liu et al, 1996). This herbicide possesses a residual effect lasting up to 120 days and is highly soluble in water (1.100 mg L^{-1}), with a consequent potential for groundwater contamination (Rodrigues and Almeida, 1998). Quinclorac is used in post-emergence application to control some broad leaves weeds in rice and turfelds (Zanella et al., 2002). Metsulfuron methyl is an herbicide characterized by weed control at very low use rates (2-75 g/ha- converter para µg/L) (Arufe et al., 2004).

Contamination of the environment by herbicides may cause several behavioral and disorders in freshwater ecosystems. Chemical when applied in excess, due great distribution and toxic nature can impact the aquatic ecosystem and exert adverse effect in organisms associates (Bowmer, 1987). Herbicides may slow down reflexes,

swimming, feeding, survival, growth, and metabolic system of fish (Hussein et al., 1996; Oruç and Uner, 1999; Scott et al., 2004; Miron et al., 2005). Alterations caused in fish by the toxic effects of herbicides can be recognized also with measurements of proteins and carbohydrates (Begum, 2004).

The silver catfish, *Rhamdia quelen* (Quoy and Gaimard, 1824; Heptapteridae), occurs from southern Mexico to central Argentina, and was chosen for this experiment due to its ecological importance. The culture of this fish is also interesting because it presents a good growth rate, high fertilization rate and hatchery and is acceptable on the consumer market (Piaia et al., 1999). Studies were made to determine the best water conditions to improve culture of this species (Zaions and Baldisserotto, 2000; Gomes et al., 2001; Baldisserotto and Radunz Neto, 2004; Baldisserotto and Gomes, 2005). Thus, the aim of this study was to determine the effect of water from rice fields with herbicides in silver catfish growth, survival and some metabolic parameters. The understanding of these effects can indicate if the water removed from the rice fields and discharged into the rivers could impair fish life.

2. Material and methods

2.1 *Herbicides and rice culture*

A permanent flooded irrigation (10 cm water depth) of the rice crop was started 15 days after emergence of the plants. The following herbicides were applied separately to the rice crops: 0.5 mg L^{-1} clomazone (2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isooxazolidinone, Gamit, 50 % purity), 0.375 mg L^{-1} quinclorac (3, 7-dichloroquinoline-8-carboxylic acid, Facet, 50% purity) and 0.002 mg L^{-1} metsulfuron methyl (methyl2-[[[(4-metoxi-6-methyl-1,3,5-triazine-2-)amino]carbonyl]amino] sulfonyl-benzoate – sulfonylurea, Ally, 50 % purity). The water for the control group received the same treatment, but no herbicide was applied to the rice culture. Each treatment had three replicates.

2.2. *Fish*

Silver catfish juveniles (weight, 3.15 ± 0.03 g; length, 7.06 ± 0.05 cm) were obtained from a local commercial fish culture and transported to the Fish Physiology Laboratory, Universidade Federal of Santa Maria, Santa Maria, southern Brazil. Fish were acclimated in 250 L tanks at a stocking density of 400 fish.m^{-3} for 15 days. After acclimation, the water of these tanks was replaced for water removed from rice culture

ponds 21 days after herbicide application. The water used for this experiment was removed from the rice culture only 21 days after herbicide application because at this period farmers start draining the water with the herbicides into nearby rivers. Each 250 L tank contained a small funnel coated with a plastic mesh and positioned on the bottom. This funnel was connected to a tube and discharged water into a filter made of acrylic wool over the surface of the box. Continuous aeration with an air pump (20 W) promoted water circulation through this system, which was used to reduce water turbidity. Silver catfish were exposed for 45 days to water drained from rice culture. Each fish tank received the water from a different replicate of the rice culture, and consequently, there were three replicates for each treatment. Fish were fed three times a day (08:30, 12:00, and 17:30 h) with commercial feed (28 % CP, Supra, Brazil) (5 % total biomass). Feces and pellet residues were removed at three days intervals by suction, and around 20% water was replaced by water collected from the rice culture at the same day. Water parameters were as follows: temperature 21 ± 1 ^{o}C, pH 7.2 ± 0.02 units, dissolved oxygen 7.4 ± 0.03 mg L^{-1}, non-ionized ammonia 0.7 ± 0.01 μl L^{-1}, alkalinity 45 ± 1.4 mg L^{-1} $CaCO_3$ and hardness 20 ± 0.5 mg L^{-1} $CaCO_3$. Photoperiod was 12 h light – 12 h dark, with luminosity of 0.6 lux (measured with a LI-COR photometer model LI-185B), since dark environments reduce stress of silver catfish (Piaia et al., 1999).

2.3 *Fish samples*

Samples of 15 juveniles were collected from each replicate at 15, 30, and 45 days after the beginning of the experiment to measure weight and length. Specific growth rate (SGR) was calculated for each collect by the method of Jørgensen and Jobling (1993). At day 45 all surviving fish were collected to determine survival and biomass (individual mean weight x number of surviving fish).

2.4 *Metabolic evaluation*

The sampled fish at 15 and 45 days were killed by punching the spinal cord. Liver and muscle were excised and immediately frozen in liquid nitrogen and then stored at –20 °C for metabolic assay**.** For determination 50-100 mg of these tissues were dissolved in an equal volume of 20 % TCA using a Potter-Elvehjem homogenizer. The acid homogenate was centrifuged at 3000*g* for 10 min and supernatant was used for the determinations of glucose and lactate. Glucose was estimated by the method of DuBoie (1956) and lactate by the method of Harrower and Brown (1972). Protein was

determined by the method of Lowry et al. (1951) after alkaline digestion of the tissues with KOH. Tissue glycogen was determined after ethanol isolation followed by acid hydrolysis (Bidinotto and Moraes, 1997).

2.5. *Water evaluation*

Through the experimental period, water parameters were analyzed daily. The pH was measured with a pHmeter Oakton, total ammonia nitrogen (NH_3 + NH_4^+) and non-ionized ammonia nitrogen (N-NH_3) was determined by the method as described by Boyd and Tucker (1992) Temperature and dissolved oxygen were determined with an YSI oxygen meter (model Y5512), alkalinity using a kit from Alfa Tecnoquímica (Florianópolis, Brazil) and water hardness by the EDTA titrimetric method. The herbicide concentration in the water was measured by high-performance liquid chromatography (HPLC) according to Zanella et al. (2002).

2.6. *Statistical analysis*

The experimental design was fully randomized, with three replicates per treatment. All data are expressed as mean ± standard deviation (SD), with the level of significance at $P < 0.05$. Weight, length, specific growth rate, total biomass, and metabolic parameters were analyzed by one-way ANOVA and the Tukey-Kramer test. Survival was analyzed by the chi-square test (Instat Program version 2.05).

3. Results

Waters collected from the rice fields that received application of clomazone, quinclorac and metsulfuron methyl showed similar water quality parameters compared to control water: temperature (22.0 ± 0.6 ºC), dissolved oxygen (6.5 ± 0.1 mg L^{-1}), pH (7.3 ± 0.2 units), total ammonia (0.8 ± 0.2 mg L^{-1}), total alkalinity (87 ± 0.1 mg L^{-1} $CaCO_3$), and water hardness (75 ± 0.1 mg L^{-1} $CaCO_3$). Herbicides concentration in the water from the rice fields decreased as time went by. The decrease in metsulfuron methyl concentration was gradual and after 14 days of application it was not detected in the water. Quinclorac and clomazone presented the highest concentration decrease between 7 and 14 days after application, and were not detected after 21 and 60 days, respectively (Table 1).

After 45 days exposure to herbicides the fish exposed to quinclorac presented 4% mortality. After exposed to clomazone was showed significantly lower weight,

length and SGR in fish after 15 and 45 days as compared to the control group. Fish exposed to quinclorac presented lower weight, length and biomass than the control group after 45 days, while those exposed to metsulfuron methyl showed higher weight and length 30 days, but at 45 days these values were not significantly different from the control group (Table 2).

In tissue of hepatic of silver catfish exposed to clomazone, quinclorac and metasulfuron methyl was observed glycogen increased and lactate decreased after 15 days, but after 45 days glycogen hepatic increased only in fish exposed to clomazone and quinclorac herbicide. (Figure 1, 2 and Table 3). The levels of muscle glycogen increased after 45 days in fish exposed to all herbicides tested (Table 3). After 45 days muscle glucose levels decrease and lactate levels were significantly higher in fish exposed to all herbicides used compared to control fish (Figure 2). Hepatic and muscle protein content was not affected significantly by the treatments (Table 3).

4. Discussion

Water parameters were maintained at optimum levels for silver catfish culture (Baldisserotto and Radunz Neto, 2004). Environmental pollutants such as herbicides are harmful to aquatic organisms and a great deal of research has been conducted to understand the effects of toxicants on the survival. In the water of rice used in this studied with addition of clomazone and metsulfuron methyl exhibit 100% survival level. Silver catfish was more sensitive to quinclorac because noted influence in proportion of survival that decreased 4 % as compared to control. In according Miron et al. (2004), this result suggests that concentration of quinclorac applicator in culture rice can have affect in fish, such as behavior or survival.

The lethal concentration (LC_{50}-96 h) of the studied herbicides for silver catfish is well above the initial concentration applied to the rice fields (mg L^{-1}): 7.32 for clomazone and 390 to 400 for quinclorac and to metsulfuron methyl it was not obtained, since all silver catfish survived even to 1.200 (Miron et al., 2004). In addition, in the present study the water collected from the rice fields 21 days after application showed quinclorac and metsulfuron methyl levels below detection limit, and clomazone concentration was 0.003 mg L^{-1}. These results are in agreement with Zanella et al. (2000) which demonstrated that analysis of water from the experimental rice field show that clomazone is persistent at 28 days after application the concentration was still the limit normally for environmental waters (0.001 to 0.003 mg

L^{-1}). In constrast the quinclorac its half-life in water is 21 days and metsulfuron methyl is 30 days in the soil (Barceló and Hennion, 2002).

Exposure to clomazone and quinclorac (but not metsulfuron methyl) impaired growth of silver catfish. Miron et al. (2004) observed decreased feeding in silver catfish exposed to clomazone (20 and 50 mg L^{-1}), quinclorac (390 and 400 mg L^{-1}), but not in those exposed to metsulfuron methyl (up to 1.200 mg L^{-1}). Some xenobiotic that in environment can cause damage at population affect growth. Alvarez et al., (2005) showed that Red drum larvae (*Sciaenops ocellatus*) exposed to atrazine for 96 h (40 and 80 mg L^{-1}) reduced growth rate and demonstrated an elevated rate of energy utilization in total metabolic rate that could reduce the population by as much as 24% of fish larvae. Nieves-Puigdoller et al. (2007) demonstrated sublethal levels of the herbicide atrazine (0.1 mg L^{-1}) in laboratory conditions alter normal physiological functions of Atlantic salmon smolts, cause osmoregulatory disturbance, reduction in food intake and growth of the fish. Decreased food consumption and increased lethargy were observed in Nile tilapia (*Oreochromis niloticus*) and catfish (*Chrysichthyes auratus*) after exposure to 3 and 6 mg L^{-1} atrazine (Hussein et al., 1996).

The present findings demonstrate clearly a disrupted metabolism in silver catfish exposed to the herbicides. In fish the site of glycogen synthesis and storage is the liver, which is also the metabolic center for detoxification and a source of glucose (Suarez and Mommsen, 1987; Moon, 1998). In the hepatic tissue our results showed that fish maintained in water from the rice fields that received clomazone and quinclorac presented higher glycogen, but lower lactate levels as compared to control fish. Silver catfish exposed to metsulfuron methyl also showed these responses in hepatic tissue, but only after 15 days, and values returned to control levels after this period. We suggest that this is a strategy of the liver in response to an increased metabolic demand for detoxification. Cattaneo et al. (2008), observed that silver catfish exposed to 700 mg L^{-1} at 2, 4-D for 96h also decreased hepatic glucose levels.

Silver catfish showed muscle metabolic changes when exposed to waters originating from the rice fields treated with all herbicides. These changes may be due to utilization of carbohydrates for energy production as a response to stress toxicity, such hypoxia condition or hyperexcitability that the fish shows after exposure herbicides (Gimeno et. al., 1995; Oruç and Üner, 1999). These metabolic disorders could also be due to a fermentative strategy, with glycogen mobilization to muscle lactate elevation indicated energy depletion by herbicide exposure. Some authors

usually describe this strategy for other fishes exposed to herbicides (Verma et al., 1983; Gill et al., 1991; Jyothi and Narayan, 1999; Aguiar, 2000). In agreement with Ferrando and Andreu-Moliner (1991), who reported a decrease muscle glycogen level in eels (*Anguilla anguilla*), after sublethal exposure to lindane. Studies have shown that the stress caused by herbicides is frequently followed by depletion modification of muscle and this is one of the many consequences of oxidative stress in fish (Oruç and Üner, 1999; Sayeed et al., 2003).

5. Conclusion

The results obtained in the present study allow concluding that exposure of silver catfish to water from rice fields that received the recommended concentration of clomazone and quinclorac for rice culture may affect its growth and metabolism even if this species is exposed to the water 21 days after herbicide application. The exposed fish showed a mechanism of mobilizing energy from glucose and storage glycogen in the hepatic tissue, due to the increased metabolic demand of the detoxification. In addition, the glycogen degradation and increased lactate levels in the muscle of fish exposed to the studied herbicides indicate clear energy degradation in response to this stressful condition. In addition, metsulfuron methyl appears safer for silver catfish than clomazone and quinclorac. The studied parameters indicate that silver catfish can be a bioindicator for herbicides contamination.

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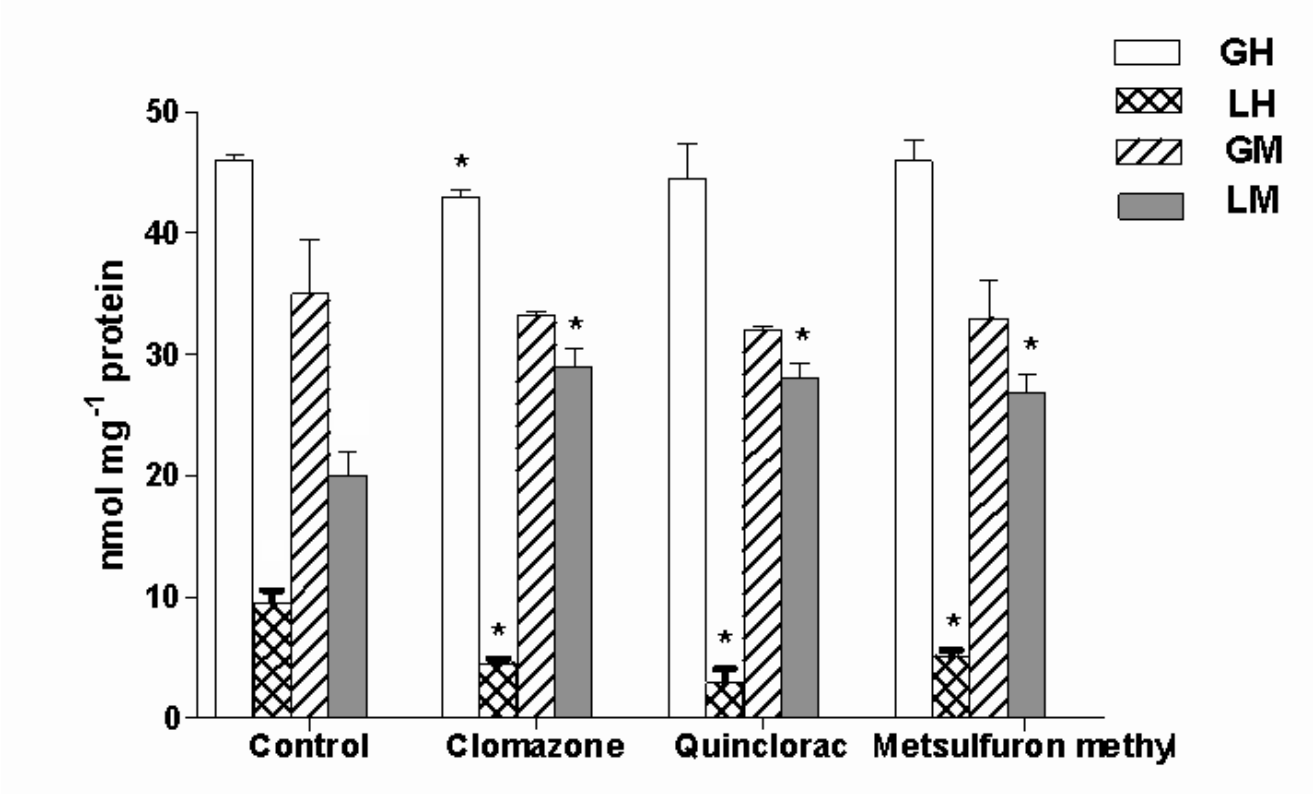
Figure caption

Figure 1. Glucose and lactate (nmol mg^{-1} protein) levels in the liver and muscle of silver catfish (*Rhamdia quelen*) exposed 15 days at water from rice fields applied to the rice fields. Seen as (GH) Hepatic glucose, (LH) Hepatic lactate, (GM) Muscle glucose and (LM) Muscle lactate. Results are the means ± SD of ten fish per group. (*) Indicates significant difference from control group (P<0.05).

Figure 2. Glucose and lactate (nmol mg^{-1} protein) levels in the liver and muscle of silver catfish (*Rhamdia quelen*) exposed 45 days at water from rice fields applied to the rice fields. Seen as (GH) Hepatic glucose, (LH) Hepatic lactate, (GM) Muscle glucose and (LM) Muscle lactate. Results are the means ± SD of ten fish per group. (*) Indicates significant difference from control group (P<0.05).

Fig 1.


GH
LH
GM
LM
nmol mg-1 protein
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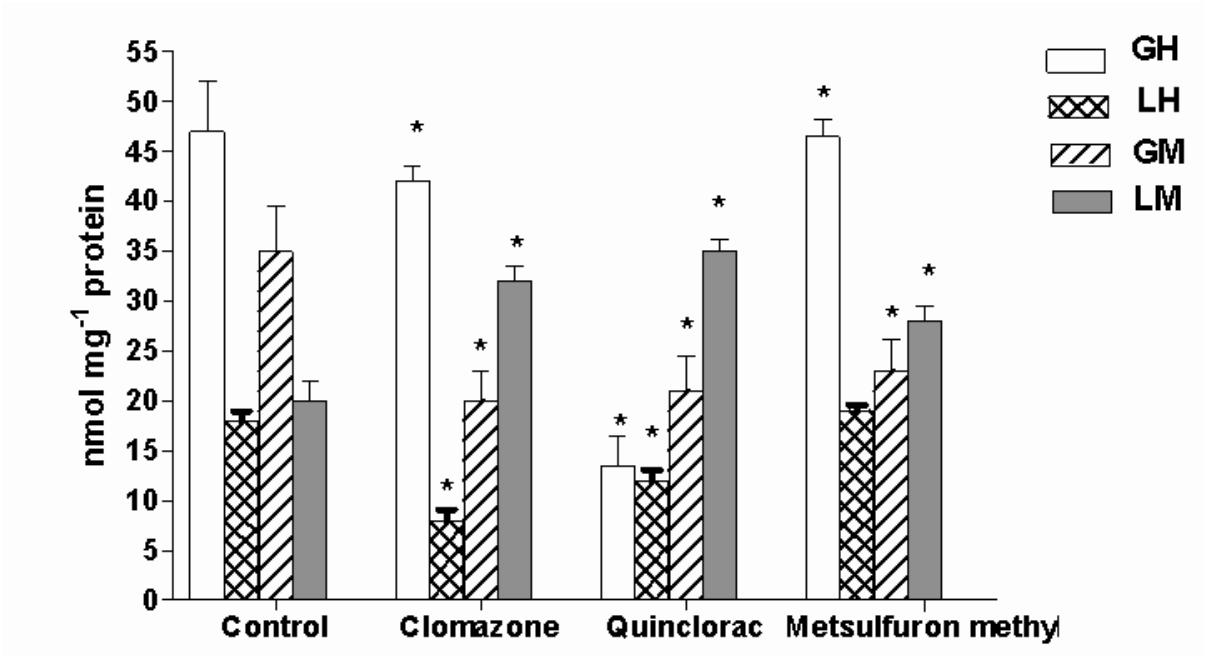
Control
Clomazone
Quinclorac
Metsulfuron methyl
*

Fig 2.


GH
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Control
Clomazone
Quinclorac
Metsulfuron methyl

Table 1. Concentration of herbicides applied to the rice fields as a function of time.

Days	Herbicides (mg L^{-1})		
	Clomazone	Quinclorac	Metsulfuron-methyl
0 (nominal)	0.500	0.375	0.002
1	0.390 (0.342-0.430)	0.131(0.099-0.146)	0.0013
7	0.199 (0.081-0.323)	0.072 (0.040-0.126)	0.0014
14	0.006 (0.005-0.008)	0.003 (0.000-0.0047)	nd
21	0.003 (0.002-0.004)	nd	nd
28	0.001 (0.001-0.002)	nd	nd
60	nd	nd	nd

Metsulfuron methyl, detection limit: 0.0005 mg L^{-1}; nd: non detectable.

Table 2. Weight, length, specific growth rate, biomass and survival of silver catfish (*Rhamdia quelen*) exposed to water from rice fields applied to the rice fields.

	Herbicide (mg L^{-1})			
	Control	Clomazone 0.5	Quinclorac 0.375	Metsulfuron-methyl 0.002
Weight (g)				
Initial	3.15 ± 0.03	3.15 ± 0.03	3.15 ± 0.03	3.15 ± 0.03
15 days	4.77 ± 0.73	4.23 ± 0.17*	4.57 ± 0.99	4.96 ± 0.49
30 days	7.09 ± 1.05	7.05 ± 0.76	6.96 ± 1.11	7.19 ± 1.18*
45 days	12.38 ± 1.52	10.55 ± 3.22*	9.89 ± 1.02*	12.80 ± 3.22
Length (cm)				
Initial	7.06 ± 0.05	7.06 ± 0.05	7.06 ± 0.05	7.06 ± 0.05
15 days	7.70 ± 0.46	7.50 ± 0.06*	7.59 ± 0.21	7.72 ± 0.26
30 days	9.02 ± 0.28	9.07 ± 0.24	9.02 ± 0.46	9.70 ± 0.59*
45 days	10.75 ± 0.31	10.09 ± 0.76*	10.00 ± 0.29*	10.57 ± 0.10
Specific growth rate (%/day)				
15 days	2.77 ± 1.03	1.96 ± 0.17*	2.48 ± 1.14*	3.03 ± 0.64
30 days	1.32 ± 1.01	1.70 ± 0.46	1.40 ± 0.52	1.23± 0.59
45 days	1.23 ± 1.95	0.83± 0.30*	0.78 ± 0.41*	1.28± 0.29*
Biomass (g)				
45 days	1070 ± 69.38	1040 ±131.76	996 ± 53.00*	1060 ± 289.73
Survival (%)				
45 days	100	100	96*	100

Values are means ± SD (n=15). (*) Indicate significant difference from control group ($P \leq 0.05$)

Table 3. Glycogen (nmol/ mg protein), protein (mg/ mg tissue) in liver and muscle tissues of silver catfish (*Rhamdia quelen*) at water from rice fields that received application of herbicides applied to the rice fields.

	Herbicide (mg L^{-1})			
	Control	Clomazone	Quinclorac	Metsulfuron methyl
		0.5	0.375	0.002
	Liver			
15 days				
Glycogen	72.04 ± 0.17	101.65 ± 0.34*	87.40 ± 0.11*	92.54 ± 0.34*
Protein	107 ± 0.003	112 ± 0.003	104 ± 0.003	109 ± 0.004
45 days				
Glycogen	70.57 ± 0.17	99.89 ± 0.34*	79.11± 0.34*	75.77 ± 0.11
Protein	110 ± 0.003	115 ± 0.003	100.1 ± 0.003	120 ± 0.004
	Muscle			
15 days				
Glycogen	5.27 ± 0.1	5.07 ± 0.1	5.04 ± 0.12	5.26 ± 0.15
Protein	74.1 ± 0.003	76 ± 0.003	74.4 ± 0.004	74 ± 0.005
45 days				
Glycogen	0.75 ± 0.1	0.4 ± 0.1*	0.48 ± 0.12*	0.4 ± 0.15*
Protein	72 ± 0.003	72 ± 0.003	75 ± 0.004	71 ± 0.005

Values are means ±SD (*n*= 15). (*) Indicate significant difference from group ($P \leq 0.01$)

CAPÍTULO III

ARTIGO II:

Biochemical effects of clomazone herbicide on piava (*Leporinus obtusidens*).

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Biochemical effects of clomazone herbicide on piava (*Leporinus obtusidens*)

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ABSTRACT

This study aims to verify the effects of the clomazone concentration used in rice fields on acetylcholinesterase (AChE), thiobarbituric acid reactive substances (TBARS), protein carbonyl and catalase activity in tissues of piava (*Leporinus obtusidens*). LC_{50}-96 h was 5.0 mg L^{-1} and the fish were exposed to 1/10 of LC_{50}-96 h: 0.5 mg L^{-1} of clomazone for 96 and 192 h. The same parameters were also assayed after a recovery period of 192 h in clean water. AChE activity was reduced only in the brain and heart of fish exposed for 96 h. AChE activity was decreased in the brain, muscle and heart tissues after 192 h of exposure. After 192 h of recovery period, AChE activity remained diminished in brain and muscle and showed a decrease in eye. However, after 192 h of recovery, AChE activity in heart was recovered. Fish showed increased TBARS levels in brain at all experimental periods. TBARS levels decreased in liver and muscle tissues after 192 h of exposure. The increase in muscle TBARS persisted in fish transferred to clean water. Protein carbonyl in the liver was increased in all periods studied including the recovery period. Catalase activity was reduced during all periods. The present study demonstrates the occurrence of disorders in AChE, TBARS, protein carbonyl and catalase activity in piava. The results also show changes in fish after exposure to an environmentally relevant concentration of clomazone. Most effects observed persisted after the recovery period. Thus, these parameters may be used to monitor clomazone toxicity in fish.

1. Introduction

In southern Brazil, the herbicide based clomazone (2-(2-chlorophenyl) methyl-4,4-dimethyl-3-isoxazolidinone) is extensively used in paddy rice fields to control agricultural pests (Jonsson et al., 1998). Although it is highly effective, it is toxic to non-target organisms, such as fish (Crestani et al., 2006). This herbicide causes groundwater contamination due to its water solubility (1100 mg L^{-1}) (Colby et al., 1989). Clomazone residues can last for up to 130 d in agricultural water and were detected in 90% of water samples collected from courses near rice cultivation regions (Zanella et al., 2002).

Many environmental impacts on fish caused by toxicants have demonstrated the effects of organic pesticides on several species. Pesticides cause changes in physiological and metabolic functions of the fish organism such as neurotransmission and functions of the immunity system (Gill et al., 1991; Aldegunde et al., 1999). One of these changes is the depletion of energy metabolism, because the intoxicated organisms spend more energy increasing the activity of several enzymatic systems to mitigate toxic effects (Dethloff et al., 1999). The most common indicator of neurological dysfunction is cholinesterase (ChE) activity, which is frequently used as an indirect measure of acetylcholinesterase (AChE; EC 3.1.1.7) activity. AChE is responsible for degrading the neurotransmitter acetylcholine for end cholinergic neural transmission. Organophosphate pesticides previously shown to inhibit brain AChE activity in fish include diazinon, malathion and folidol (Beauvais et al., 2000; Brewer et al., 2001; Aguiar et al., 2004). Several carbamate pesticides, including carbofuran, diuron, nicosulfuron (Bretaudt et al., 2000), and thiobencarb have also been shown to inhibit brain AChE activity (Sancho et al., 2000; Fernández-Vega et al., 2002). Similar results have been observed with other pesticides such as endosulfan (Dutta and Arends, 2003) and clomazone (Miron et al., 2005; Crestani et al., 2007). Pesticides may induce oxidative stress, leading to the generation of free radicals and causing lipid peroxidation (LPO) (Kehrer, 1993; Sevgiler et al., 2004). Fish are able to uptake and retain different xenobiotics in water via active or passive processes. Therefore, parameters measured in fish may be used to monitor the potential of contamination by pesticides to environmental and could be used to investigate biological effects such as enzymatic disturbances. The piava (*Leporinus obtusidens*) is a widely cultivated native freshwater fish from Southern Brazil with great commercial importance (Andrian et al., 1994; Glusczak et al., 2006). Thus, this fish was used in order to enhance the scarce information available in the literature on

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clomazone toxicity in native fish species. This study aims to assess some parameters of toxicity in piava and to find potential toxicity indicators for clomazone exposure.

2. Materials and methods

2.1. Chemicals

The herbicide clomazone (2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone) used in this study was obtained commercially from the FMC Corporation (Gamit; 36% purity, Philadelphia, EUA) and dissolved in water. Acetylthiocholine (ATC), 5,5′dithio-bis(2-nitrobenzoic acid) (DTNB), bovine serum albumin, Triton X-100, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), 2-thiobarbituric acid (TBA), and sodium dodecyl sulfate (SDS) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Fish

Piava fingerlings (*L. obtusidens*) of both sexes were obtained from the Santa Maria Federal University (UFSM) fish farm (RS, Brazil). Fish (weight, 8.0 ± 1.0 g; length, 6.0 ± 1.0 cm) were acclimated to laboratory conditions for 15 d, in tanks (250 L). They were kept in continuously aerated water in a static system and with a natural photoperiod (12 h light–12 h dark). Water parameters were measured every day and were as follow: temperature 23 ± 2.0 °C, pH 7.7 ± 0.2 units, dissolved oxygen 6.5 ± 1.0 mg L^{-1}, non-ionized ammonia 0.007 ± 0.01 mg L^{-1}, nitrite 0.03 ± 0.01 mg L^{-1}, alkalinity 66 ± 1.3 mg L^{-1} $CaCO_3$ and hardness 20 ± 1.4 mg L^{-1} $CaCO_3$. All water parameters were determined according to Boyd and Tucker (1992). During the experimental period, fish were fed ad libitum two times a day (8:30 and 17:30 h) with commercial fish pellets (42% crude protein, Supra, Brazil). Fecal remains and food residues were removed by suction every other day.

2.3. Experimental design

Previous experiments carried out in our laboratory established 7.32 mg L^{-1} (nominal concentration) as the LC_{50} 96 h for clomazone (Miron et al., 2005). The clomazone concentration usually recommended in rice fields is of 0.5 to 1.0 mg L^{-1} (Rodrigues and Almeida, 1998). The concentration chose (0.5 mg L^{-1}) was in accordance with the calculated concentration of clomazone used in rice fields. After the acclimation period, groups of 10 fish were transferred to glass boxes (45 L) with controlled aeration and temperature. Stock solutions were prepared by dissolving clomazone in water. This solution was added to the experimental boxes. Piavas 10 fish per box (triplicate) were exposed to 0.0 (control) and 0.5 mg L^{-1} clomazone for 96 h. In a second experiment, piavas (10 fish/box in triplicate) were exposed to 0.0 (control) and 0.5 mg L^{-1} clomazone for 192 h and, subsequently, 15 fish were removed and transferred to clean water. The herbicide concentration was monitored every 2 d by high-performance liquid chromatography (HPLC) (Zanella et al., 2002) to verify values in the experimental boxes. Clomazone concentration in the water after 48 h was approximately 90% of the initial concentration (data not shown). The water in the boxes was renewed every 48 h to maintain the concentration of clomazone constant during the period of exposure. Water quality parameters during the treatment period were the same as those for the acclimation period.

2.4. Sampling

After the experimental period, fish were killed by punching the spinal cord behind the opercula and were sampled. Brain, white muscle, eye, heart and liver samples were rapidly removed, washed in 150 mM saline solution, dried with filter paper, packed in Teflon tubes and kept at −4 °C for analyses. AChE activity, thiobarbituric acid reactive substances (TBARS), protein carbonyl and catalase activity were measured in this study.

2.5. Acetylcholinesterase assay

Tissue samples were weighed and homogenized in a Potter-Elvejhem glass/Teflon homogenizer with 150 mM NaCl. The homogenates were centrifuged for 15 min at 3000*g* at 5 °C and the supernatant was used as the enzyme source. Acetylcholinesterase (AChE; EC 3.1.1.7) activity was measured as described by Ellman et al. (1961) and modified by Miron et al. (2005). Aliquots of supernatant (50, 50, 100 and 200 μL) (brain, eye, muscle and heart, respectively) were incubated at 25 °C for 2 min with 0.1 M phosphate buffer pH 7.5, 1 mM and DTNB as chromogen. After 2 min, the reaction was initiated by the addition of acetylthiocholine (0.08 M) as substrate. The final volume was 2.0 mL. Absorbances were determined at 412 nm during 2 min. Enzyme activity was expressed as μmol of acetylthiocholine (AcSCh) hydrolyzed per minute per mg of protein.

2.6. TBARS levels

Peroxides produced can be quantified by a TBARS assay. This is performed by a malondialdehyde (MDA) reaction with 2-thiobarbituric acid (TBA), which is optically measured. Liver, muscle and brain homogenates (100–400 μL) were added to 8.1% sodium dodecyl sulfate (SDS), 2.5 M acetic acid (pH 3.4), 0.8% thiobarbituric acid and the final volume was adjusted to 2.0 mL. The reaction mixture was placed in a microcentrifuge tube and incubated for 90 min at 95 °C. After cooling, it was centrifuged at 5.000*g* for 10 min and optical density was determined at 532 nm. TBARS levels are expressed as nmol MDA per mg of protein according to Ohkawa et al. (1979).

2.7. Protein carbonyl assay

The liver tissue was homogenized in 10 volumes (w v^{-1}) of 10 mM Tris–HCl buffer pH 7.4 using a glass homogenizer. The protein carbonyl content was determined by the method described by Yan et al. (1995), with some modifications. Briefly, homogenates were diluted to 0.7–0.8 mg mL^{-1} of protein in each sample, and 1 mL aliquots were mixed with 0.2 mL of 2,4-dinitrophenylhidrazine (10 mM DNPH) or 0.2 mL HCl 2 M. After incubation at room temperature for 1 h in a dark room, 0.5 mL of denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, containing SDS 3%), 2.0 mL of heptane (99.5%) and 2.0 mL of ethanol (99.8%) were added sequentially, mixed with vortex agitation for 40 s and centrifuged for 15 min. Next, the protein isolated from the interface was washed two times with 1 mL of ethyl acetate/ethanol 1:1 (v v^{-1}), and suspended in 1 mL of denaturing buffer. Each DNPH sample was read at 370 nm in a Femto Scan spectrophotometer against the corresponding sample (blank), and total carbonylation was calculated using a molar extinction coefficient of 22 000 M^{-1} cm^{-1}.

2.8. Catalase assay

Catalase (CAT; EC 1.11.1.6) activity was assayed by ultraviolet spectrophotometry (Nelson and Kiesow, 1972). Samples of liver were homogenized in a Potter-Elvejhem glass/Teflon homogenizer with 20 mM potassium phosphate buffer, pH 7.4 (with 0.1% Triton X-100 and 150 mM NaCl) (1:20 dilution), centrifuged at 10 000*g* for 10 min at 4 °C. Briefly, the assay mixture consisted of 2.0 mL potassium phosphate buffer (50 mM, pH 7.0), 0.05 mL H_2O_2 (0.3 M) and 0.05 mL homogenate. Changes in H_2O_2 absorbance in 60 s were

measured at 240 nm. Catalase activity was calculated in terms of μmol mg^{-1} protein min^{-1}.

2.9. Protein determination

Protein levels for oxidative stress parameters were spectrophotometrically estimated by the method of Bradford (1976), using bovine serum albumin as standard.

2.10. Statistical procedures

Statistical analyses were performed using a two-way analysis of variance (ANOVA) to determinate concentration and time effects. Means were compared by the Tukey test and expressed as mean ± standard deviation. A P value < 0.05 was considered to be significant.

3. Results

AChE activity in different tissues of *L. obtusidens* exposed to clomazone (96 and 192 h) and submitted to a recovery period (192 h) is shown in Fig. 1. AChE activity in the brain decreased significantly ($P < 0.05$) after both experimental periods when compared to the control group for this tissue. In general, the brain showed higher AChE activity than the other tissues followed by muscle, eye and heart. In the muscle, a decrease in activity was observed only after 192 h of exposure, while in the eye it was observed only in the recovery period. A reduction of AChE activity was observed in the heart, after clomazone exposure (96 and 192 h). In relation to the recovery period (192 h), all tissues, except the heart showed a reduction in AChE activity.

The fish exposed to clomazone showed enhanced TBARS levels, particularly in the brain (Fig. 2). An increase of TBARS in brain tissue was observed at all periods of exposure (99% – 96 h and 65% – 192 h; $P < 0.05$) and also after the recovery period (27%; $P < 0.05$) when compared with the control group. In the liver, TBARS levels were significantly ($P < 0.05$) increased after 192 h of exposure but returned to the control value after the same period of recovery. Muscle tissue showed increased TBARS levels after 192 h of exposure and recovery. Similar to the results obtained for brain TBARS, liver protein carbonyl (Fig. 3) contents increased at all the time studied including recovery period. The increase of protein carbonyl observed was 68.8% at 96 h and 81.3% at 192 h. The increase in protein carbonyl continued after the recovery period (20.8%, $P < 0.05$). Catalase activity in liver tissue showed a significant inhibition after clomazone exposure (Fig. 4). The decrease observed was 29.1% and 24.2% after 96 and 192 h, respectively. A similar decrease (21.9%) was observed in liver catalase activity after the recovery period.

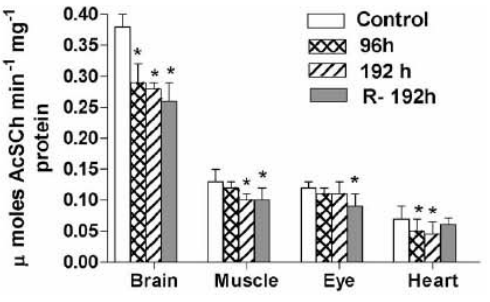

Fig. 1. AChE activity (μmol AcSCh $min^{-1} mg^{-1}$ protein) in brain, muscle, eye and heart of *Leporinus obtusidens* after exposure to 0.5 mg L^{-1} of clomazone (96 h and 192 h) and recovery (R – 192 h). Data are reported as mean ± SD ($n = 15$). *, Indicates significant difference from control ($P < 0.05$).

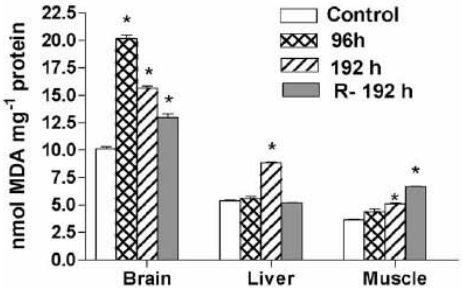

Fig. 2. TBARS levels (nmol MDA mg^{-1} protein) in brain, liver and muscle of *Leporinus obtusidens* after exposure to 0.5 mg L^{-1} of clomazone (96 h and 192 h) and recovery (R – 192 h). Data represent the mean ± SD ($n = 15$). * Indicates difference between groups and control values ($P < 0.05$).

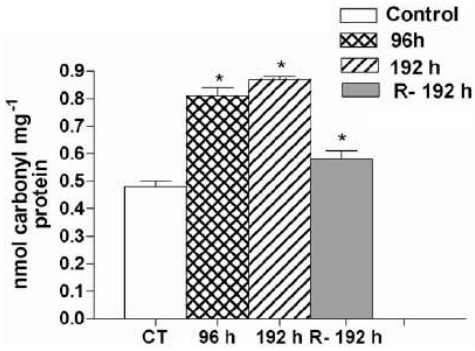

Fig. 3. Protein carbonyl (nmol carbonyl mg^{-1} protein) in liver of *Leporinus obtusidens* after exposure to 0.5 mg L^{-1} of clomazone (96 h and 192 h) and recovery (R – 192 h). Data are reported as mean ± SD ($n = 15$). * Indicates significant difference from control ($P < 0.05$).

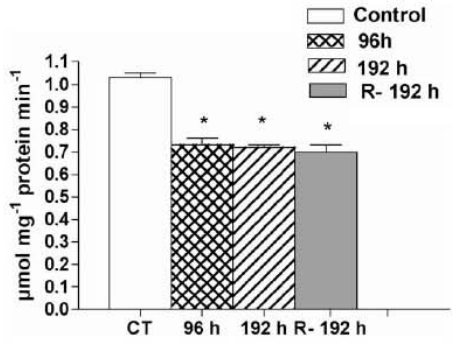

Fig. 4. Catalase activity (μmol mg^{-1} protein min^{-1}) in liver of *Leporinus obtusidens* after exposure to 0.5 mg L^{-1} of clomazone (96 h and 192 h) and recovery (R – 192 h). Data are reported as mean ± SD ($n = 15$). * Indicates significant difference from control ($P < 0.05$).

4. Discussion

Measurements of acetylcholinesterase (AChE) in different fish tissues are routinely used as a biomarker of exposure to different contaminants such as herbicides. Low concentrations of these compounds can inhibit AChE activity, which leads to an accumulation of acetylcholine at central cholinergic synapses and at vertebrate neuromuscular junctions (Miron et al., 2005; Crestani et al., 2007). The results of the present study show that, in the control group, brain AChE activity was higher than in muscle, eye and heart. Higher brain AChE activity compared to that of muscle was also observed in piava (*L. obtusidens*) exposed to glyphosate (Glusczak et al., 2006) and in silver catfish (*Rhamdia quelen*) exposed to clomazone (Miron et al., 2005; Crestani et al., 2007). However, in goldfish (*Carassius auratus*), AChE activity was higher in the skeletal muscle than in the brain (Bretaudt et al., 2000). Our study showed that exposure of fish to clomazone concentrations used in rice fields significantly decreased brain AChE activity at all periods tested. After 192 h of exposure, AChE levels showed a reduction in all tissues tested when compared with the control group ($P<0.05$). In relation to the recovery period (192 h), AChE activity remained inhibited, except in the heart. These results suggest that different tissues and times of exposure may influence the amount of reduction of AChE activity as well as the recovery. Crestani et al. (2007) observed inhibition in brain and muscle AChE activity of silver catfish at all periods of exposure (12, 24, 48, 96, and 192 h) to clomazone (0.5 mg L^{-1}). However, different from our results, brain and skeletal muscle AChE activity were recovered after 96 h and 192 h, respectively, in clean water.

In this study, the level of AChE activity in the eye was significantly reduced after 192 h of recovery, this result may suggest the presence of herbicide in fish tissue. Sancho et al. (2000), reported decreased eye AChE activity in eels (*Anguilla anguilla*) after exposure to 0.22 mg L^{-1} of thiobencarb herbicide for 96 h and after 1 week of recovery the same inhibitory effect was observed. The present results for AChE activity in the whole eye may be connected to the high-nerve activity in this tissue and also to the amount of acetylcholine present in the optic nerve (Harlin, 1991; Sancho et al., 2000). In this study, an inhibition of AChE activity was demonstrated in heart tissue of fish after 192 h of clomazone exposure. These results reveal that the heart tissue may be a sensitive biomarker for AChE activity after clomazone exposure. In heart tissue, inhibition of AChE was not observed after 192 h of recovery. As a consequence, these alterations can cause an overall decline in neural and muscular control (Dutta and Arends, 2003). More commonly, AChE activities in the brain are employed for these purposes. In fact, the duration of exposure and the time required for recovery, as well as the type of pesticide and fish tissues considered, can also affect AChE activity.

Lipid peroxidation (LPO) is one of the principal processes induced by oxidative stress from pollutants such as herbicides and has been observed in several fish species (Ahmad et al., 2004). Variations in the activities of antioxidant enzymes have been proposed as indicators of pollutant-mediated oxidative stress (Ahmad et al., 2000; Sayeed et al., 2003). The present study demonstrated that herbicide exposure at rice field concentration altered TBARS levels in fish tissues. Fish exposed to clomazone presented increased TBARS levels in brain, liver and muscle tissues. Similarly, Uner et al. (2006) observed high-TBARS levels in muscle of *Cyprinus carpio* exposed to diazinon. In the liver, we observed that TBARS levels increased only after 192 h of exposure to clomazone and returned to the control value after the same period of recovery. Crestani et al. (2007) also observed increased TBARS levels in the brain and liver of silver catfish after clomazone exposure. Elevation of lipid peroxidation in study suggests participation of free radical induced oxidative cell injury mediated by clomazone toxicity. The reversible TBARS levels in liver tissue may indicate an attempt to adapt and compensate for herbicide induced oxidative stress (Doyotte et al., 1997). The results indicate also that fish exposition to clomazone causes significant changes in TBARS production in all tissues and the effect varies depending on the tissue considered.

A variety of studies have associated an increase in TBARS with AChE inhibition as an indication of pesticide-mediated oxidative stress (Uner et al., 2005, 2006; Oruç and Usta, 2007). Similar to these studies, our findings also demonstrated an increase in TBARS levels and AChE inhibition, for both brain and muscle tissue after 192 h exposure and recovery period. Taken together, these results suggest that accumulation of free radicals in tissue of piava could induce cholinergic changes leading to AChE inhibition. In fact, some authors consider that AChE inhibition induces cholinergic hyperactivity, initiating the accumulation of free radicals and leading to lipid peroxidation, which in turn may lead to cell injury. This may be a result of an effective antioxidant system that is operating as an adaptive response of fish (Yang et al., 1996; Uner et al., 2006; Oruç and Usta, 2007).

Protein is one of the main targets for the elucidation of effects of pesticides in several species. Recently, it was shown that pesticides can induce oxidative modification of proteins and this is also one of the many consequences of oxidative stress in fish (Sayeed et al., 2003). The present findings demonstrate the role of oxidative stress and free radical formation in these effects. The highly reactive hydroxyl radical (OH^-), which is one of the reactive oxygen species generated in the process leading to oxidative stress, is considered to be responsible for the formation of carbonyl groups in proteins (Oliver, 1987). Exposure of fish to clomazone in this study resulted in an increase of liver protein carbonyl. 48 h of exposure to various pesticides (deltamethrin, endosulfan and paraquat) also caused a significant increase in the protein carbonyl content in liver, kidney and gill of freshwater fish *Channa punctata* (Bloch) (Parvez and Raisuddin, 2005). Assay of carbonyl groups in proteins provides a convenient technique for detecting and quantifying oxidative modifications of protein (Levine et al., 1990).

Exposure of fish to clomazone resulted in the reduction of the antioxidant enzyme catalase in the liver tissue of piava at all experimental periods. Crestani et al. (2007) also showed a reduction in catalase activity in the liver of silver catfish exposed to clomazone (0.5 or 1.0 mg L^{-1}) after 12, 24, 96 h and 192 h. In a number of other studies using different fish species, similar results were also achieved. In the liver of freshwater fish *C. punctatus* (Bloch) evaluated after 24 h of treatment with endosulfan, lipid peroxidation was elevated and CAT was decreased (Pandey et al., 2001). This demonstrates that herbicides induce peroxidative damage in the liver causing the alteration of levels of antioxidants. Deltamethin exposure also caused a decrease in CAT activity in liver, kidney and gill tissues of *C. punctatus* (Sayeed et al., 2003). This decline in CAT activity could be due to excessive production of O_2 as indicated by Brainy et al. (1996). The results indicate that piava resist to oxidative stress through others antioxidant mechanisms, preventing an increase in lipid peroxidation during exposure to the herbicide clomazone.

5. Conclusions

In summary, the present work demonstrated that the clomazone concentrations used in agricultural fields cause changes in oxidative stress parameters in piava (*L. obtusidens*). It is evident that, from an eco-physiological point of view, the use of this herbicide in agriculture and aquaculture must be carefully evaluated. In conclusion, the health of this fish species may be affected by the presence of clomazone in the water and alterations of the parameters analysed could be used to monitor toxicity of this herbicide in *L. obtusidens*.

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CAPÍTULO IV
MANUSCRITO II:

Commercial formulation containing clomazone affects some metabolic parameters in piava (*Leporinus obtusidens*)

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(Em fase de redação)

Commercial formulation containing clomazone affects some metabolic parameters in piava (*Leporinus obtusidens*)

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Abstract

This study aimed to assess the effects of a commercial formula containing clomazone (0.5 mg L^{-1}), at the concentration used in paddy rice fields, on metabolic parameters in piava (*Leporinus obtusidens*). The effects of this herbicide in piava was tested at 96 and 192 h of exposure and after a recovery of 192 h in clean water. Liver glycogen levels increased at all periods tested. In muscle glycogen levels was reduced only at 96 h of exposure. Glucose and protein levels showed a decrease in all tissues tested at both periods of exposure, but only in the muscle this decrease persists after the recovery period. Muscle ammonia levels decreased at all periods tested. Lactate content decreased in liver and kidney tissues at all exposure periods and increased in the muscle. Our results demonstrated a preference for the anaerobic pathway of energy production specially on the muscle tissue, in piavas exposed to clomazone. Considering the alterations observed in the parameters studied, this fish species could be a good bioindicator of clomazone toxicity on water in areas located near agricultural fields.

Keywords: Piava (*Leporinus obtusidens*); Herbicide; Clomazone; Metabolism; Exposure; Recovery

1. Introduction

Environmental contamination by chemicals used in agricultural activities has become a problem of worldwide importance (Bretaud et al., 2000; Oruç et al., 2004). Although pesticides are recognized as an economical way to control pests, their indiscriminate use can be toxic to other species in the environment, including fish (Oruç and Üner, 1999; Rao, 2006). The presence of pesticide in water can cause several alterations in metabolic parameters of fish, which may indicate an adaptation of the organism or may affect fish health (Begum, 2004).

Clomazone (isooxazolidinone) is a highly effective herbicide that is extensively used for crop protection in rice fields (Jonsson et al., 1998). Primel et al. (2005) reported that the analysis of samples from farms in the Southern Region of Brazil demonstrated it to be the most used herbicide in irrigated rice fields in this region. Due to fish sensitivity to contaminated water, changes in their metabolism frequently indicate responses to a stress situation. Recently, investigations demonstrated that during exposure to chemicals, the synthesis and degradation of metabolic can be induced differently to meet fish energy needs (Aguiar, et al., 2004; Scott and Sloman, 2004; Glusczak et al., 2007). Glycogen is mainly stored in the liver or in the hepatopancreas and its content varies among species. It has been reported that carbohydrates stored in liver and muscles are the first used in response to a stress condition (Vijayavel et al., 2006). According to Crestani et al. (2006), findings in silver catfish (*Rhamdia quelen*) exposed to clomazone suggested that glycogen was stored in liver and muscular glycogen consumed demonstrating that the fish used this energy source to compensate a stress situation.

The fish liver seems to be the central organ involved in the metabolism and, together with the kidney, mostly responsible for glucose production. However, in the occasional event of glucose unavailability, fish may use other precursors, such as lactate, as a source of energy (Begum and Vijayaraghavan, 1999). These changes indicate metabolic disorders and have been used as indicators of herbicide toxicity (Fonseca et al., 2008). Moreover, as a large part of the fish organism is made up of proteins, proteins may be the preferred fuel to supply the energy demand (Bhavan and Geraldine, 1997; Peixoto et al., 2006). In this way, the exposure to carbofuran caused a decrease in protein in the liver and muscle of *Clarias batrachus* (linn) (Begum, 2004). A reduction in protein content indicates a physiological adaptability of fish, possibly to compensate the stress generated by pesticide exposure (Vega et al., 2002).

Piava (*Leporinus obtusidens*), found in Southern Brazil, was chosen for this toxicity test due to its ecological and commercial importance. Several studies have been carried out to determine metabolic changes caused by herbicide contamination in fish species (Begum, 2004; Crestani et al., 2006; Fonseca et al., 2008), but no studies on clomazone toxicity have been performed for this native species. Thus, the purpose of this study was to verify if an environmentally relevant concentration of a commercial clomazone formula alter some metabolic parameters of *L. obtusidens*, in order to determine if this species could be used as a bioindicator to determine herbicide toxicity in fish exposed to contaminated water.

2. Material and methods

2.1. Fish

Piavas (*Leporinus obtusidens*) of both sexes with an average (weight of 8.0 ± 1.0 g; length of 6.0 ± 1.0 cm) were obtained from the Santa Maria Federal University (UFSM) fish farm (RS, Brazil). The fish were acclimated to laboratory conditions for 15 days, in tanks (250 L), in continuously aerated water in a static system and with a natural photoperiod (12h light – 12h dark). All water parameters were checked every day and determined according to Boyd and Tucker (1992) as follow: temperature 23 ± 2.0 ^{o}C, pH 7.7 ± 0.2 units, dissolved oxygen 6.5 ± 1.0 mg L^{-1}, non-ionized ammonia 0.007 ± 0.01 mg L^{-1}, nitrite 0.03 ± 0.01 mg L^{-1}, alkalinity 66 ± 1.3 mg L^{-1} $CaCO_3$ and hardness 20 ± 1.4 mg L^{-1} $CaCO_3$. During the experimental period, fish were fed ad libitum two times a day (8:30 and 17:30 h) with commercial fish pellets (42% crude protein, Supra, Brazil). Fecal remains and food residues were removed by suction every other day.

2.2. Experimental design

Clomazone herbicide used in this study was of commercial source (Gamit - 50% purity) obtained from FMC Corporation (Philadelphia, USA). The concentration chose of 0.5 mg L^{-1} was in accordance with the environmentally relevant concentration clomazone used in rice fields (Rodrigues and Almeida, 1998). After the acclimation period, groups of 10 fish were transferred to glass boxes (45 L) and were controlled the water parameters. The stock solutions were prepared by dissolving clomazone in water and added to the experimental boxes. In each box 10 fish (in triplicate) were exposed for 96 h: 0.0 (control)

and 0.5 mg L^{-1} with clomazone. In a second experiment, 10 fish by box (in triplicate) were exposed to 0.0 (control) and 0.5 mg L^{-1} clomazone for 192 h subsequently, 15 fish were removed and transferred to clean water during 192 h. The concentration clomazone in the experimental boxes was monitored every two days by high-performance liquid chromatography (HPLC) (Zanella et al., 2002). Clomazone concentration in the water after 48 h was approximately 90 % of the initial concentration (data not shown). The water in the boxes was renewed every 48 h to maintain the concentration of clomazone constant during the period of exposure. Water quality parameters during the treatment period were the same as those for the acclimation period.

2.3. Analytical procedures

After exposure (96 and 192 h) and recovery period (192 h), fish was killed by punching the spinal cord behind the opercula. Samples of liver, muscle and kidney tissue were rapidly removed, washed in 150 mM saline solution, dried with filter paper, packed in Teflon tubes and kept at -4°C for analyses of metabolic parameters. Glycogen was determined according to Bidinotto et al., 1998. Protein analysis was determinate according to Lowry et al., 1951. For lactate and glucose determination, tissues samples were homogenized with 10 % trichloroacetic acid using a motor-driven teflon pestle and centrifuged at 1000 x *g* for 10 min. Deproteinated supernatant was used for lactate determination by Harrower and Brown, 1972. Glucose measured according to Duboie et al. (1956) and total ammonia was determined according to Boyd and Tucker, 1992.

2.4. Statistical procedures

Statistical analyses were performed using a one-way analysis of variance (ANOVA). Means were compared by Tukey test and expressed as mean ± standard deviation (n = 15). The minimum significance level used was 95 % (P<0.05).

3. Results

As seen in Figure 1, was observed for all periods tested that the liver glycogen concentration increase and reduction in hepatic lactate levels significantly. Fish exhibited a decrease in protein and glucose levels in this tissue after both periods of exposure (96 and 192 h), which returned to control values after 192 h of recovery (Table 1).

Muscle tissue showed different clomazone-induced responses (Figure 2 and Table 1). Glycogen levels increased only after 96 h of exposure and this increase did not occur at the other periods tested. The fish presented a significant increase in muscle lactate levels for all periods tested, including the recovery period. After the exposure periods, fish showed a reduction in muscle protein and glucose levels as seen in Table 1.

The influence of clomazone on biochemical changes in the kidney is shown in Figure 3 and Table 1. Glycogen increased after of exposure periods. A significant reduction in the lactate level was observed at all periods tested, including the recovery, while glucose and protein decreased only at 96 and 192 h of exposure. Total ammonia did not change at any experimental period (Table 1).

4. Discussion

This study clearly suggests a metabolic disruption in *L. obtusidens* exposed to a commercial formula of clomazone. The increase in liver glycogen during exposure and persisting after a recovery period in clean water shows the storage of an energy reserve. On the contrary, the lactate level was found to be decrease, indicating liver gluconeogenesis. In addition, hepatic glucose was reduced during exposure to clomazone. Our results concerning liver metabolites are in accordance with studies in which herbicide exposure resulted in glycogen synthesis and storage in the fish liver (Crestani et al., 2006). According to other authors (Suarez and Mommsen, 1987; Peixoto et al., 2006), the fish liver has been shown to be the main organ of accumulation, biotransformation and detoxification of chemicals.

As fish white muscle constitutes more than 50 % of body mass, fish can use the anaerobic process to obtain energy stores, such as glycogen (Knox et al., 1980). In the present study, muscle glycogen was only decreased at 96 h of exposure and returned to control values after the recovery period. Similar responses were observed in *Cyprinus carpio* and *R*. *quelen* where muscle glycogen was reduced after exposure to 2,4- D herbicide (Oruç and Üner, 1999; Cattaneo et al., 2008). In fact, fish species show

different metabolic responses to toxicity-induced stress and glycogen metabolism could be specific to the tissue, species and time of exposure considered. Based on our results, muscle glucose presented a reduction at 96 h of exposure to clomazone similar to that observed for the glycogen level. This may indicate that in a period of stress generated by herbicide toxicity, a rapid degradation of tissue glycogen is accompanied by the use of glucose as a way of mobilizing energy.

These results show that clomazone induced metabolic disorders and a clear response against energy depletion. The decrease in muscle glycogen and glucose observed with a concurrent increase in lactate indicate a response against herbicide toxicity. Similar findings was presented by Glusczak et al. (2007), in *L. obtusidens* exposed to glyphosate herbicide and corroborate with the premise that metabolic parameters in this fish species may be indicators of herbicide contamination. These results are also in agreement with Begum and Vijayaraghavan (1999), who reported an increase in lactate as a measure of anaerobic metabolism, which could indicate a condition of stress in fish. Likewise, our studies demonstrated increased lactate levels as a product of glycolysis, suggesting that exposure to clomazone caused a situation of stress and demanded a great deal of energy from fish tissues.

The fish kidney is another important organ used to evaluate response to clomazone. In this study, kidney glycogen levels showed an increase similar to that obtained for liver glycogen levels. Both tissues are important for maintaining the body water balance and toxic chemicals can temporarily or permanently disrupt their functions (Miller, 2002; Oruç and Usta, 2006). In the present study, a reduction in kidney glycogen after a recovery period in clean water probably indicates a compensatory response to an herbicide-induced situation of stress. Thus, based on the present results, it can be suggested that the glycogen stored in the kidney can be used to compensate or detoxify in a situation of stress induced by herbicide exposure. Moreover, as the kidney receives the post brachial blood flow, it is important in the detoxification or elimination of contaminants in fish and can be a good site to evaluate toxicity in fish (Gallagher and Di Giulio, 1992; Sancho et al., 1997; Üner et al., 2005).

5. Conclusion

It can be concluded that the metabolism was disrupted in piava (*Leporinus obtusidens*) after exposure to a commercial formula of clomazone and that the effects

observed in fish depend of the exposure time considered. In addition, muscle tissue showed a preference for the anaerobic pathway of energy production. There were some similar patterns among tissues, as well as some differences, which were generally consistent with differences in organ functions. The clomazone concentration used in this study caused some metabolic disorders that persisted after a recovery period in clean water. Therefore, this study with *L*. *obtusidens* provides information on the adverse effects of clomazone and supports the theory that this fish species could be an early bioindicator of toxicity in fish in areas located near agricultural fields.

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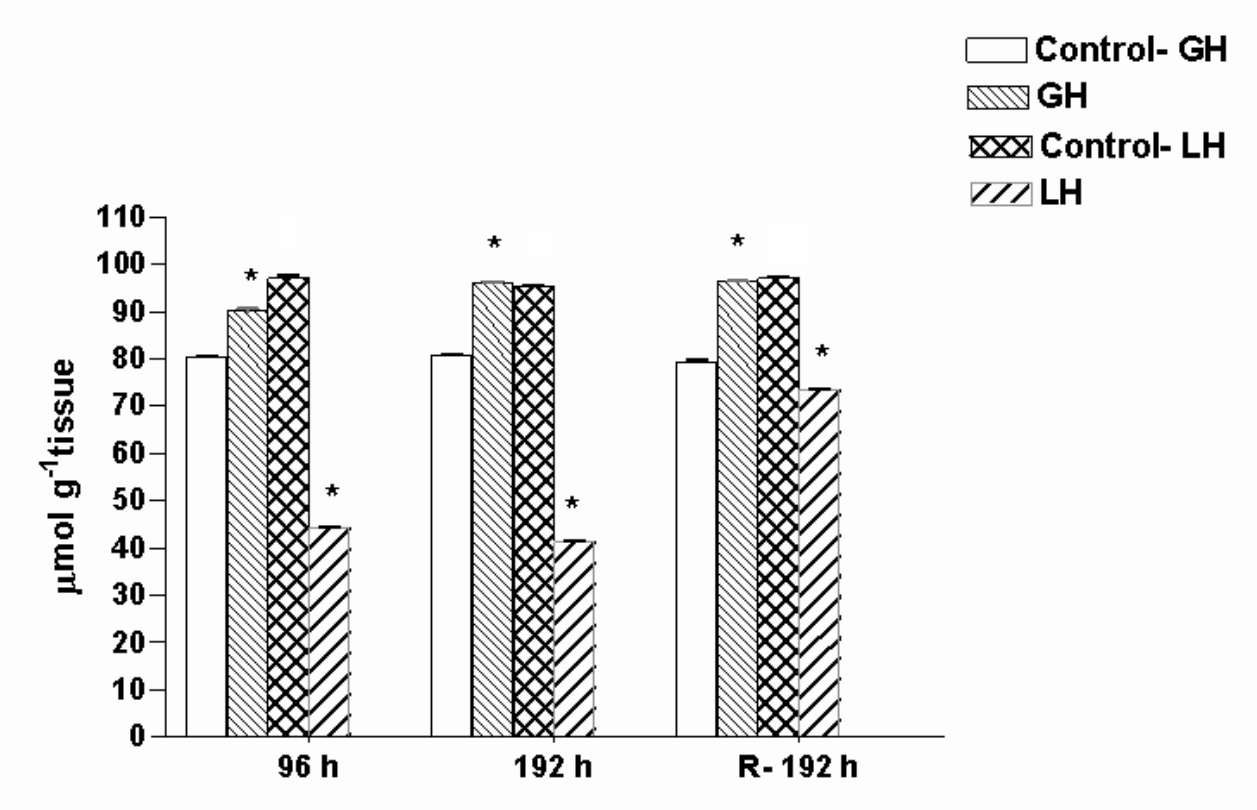
FIGURE CAPTIONS

Figure 1. Glycogen and lactate (µmol g^{-1} tissue) levels in liver of *Leporinus obtusidens* after exposure to 0.5 mg L^{-1} of clomazone (96 and 192 h) and recovery (R- 192 h). Seen as (Control- GH) Glycogen hepatic control, (GH) Glycogen hepatic, (Control- LH) Lactate hepatic control and (LH) Lactate hepatic. Date are means ±SD (*n*=15); * Indicate a significant difference at *P*< 0.05.

Figure 2. Glycogen and lactate (µmol g^{-1} tissue) levels in muscle of *Leporinus obtusidens* after exposure to 0.5 mg L^{-1} of clomazone (96 and 192 h) and recovery (R- 192 h). Seen as (Control- GM) Glycogen muscle control, (GM) Glycogen muscle, (Control- LM) Lactate muscle control and (LM) Lactate muscle. Date are means ±SD (*n*=15); * Indicate a significant difference at *P*< 0.05.

Figure 3. Glycogen and lactate (µmol g^{-1} tissue) levels in kidney of *Leporinus obtusidens* after exposure to 0.5 mg L^{-1} of clomazone (96 and 192 h) and recovery (R- 192 h). Seen as (Control- GK) Glycogen kidney control, (GK) Glycogen kidney, (Control- LK) Lactate kidney control and (LK) Lactate kidney. Date are means ±SD (*n*=15); * Indicate a significant difference at *P*< 0.05.

Fig 1.


Control- GH
GH
Control- LH
LH
µmol g-1tissue
110
100
90
80
70
60
50
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30
20
10
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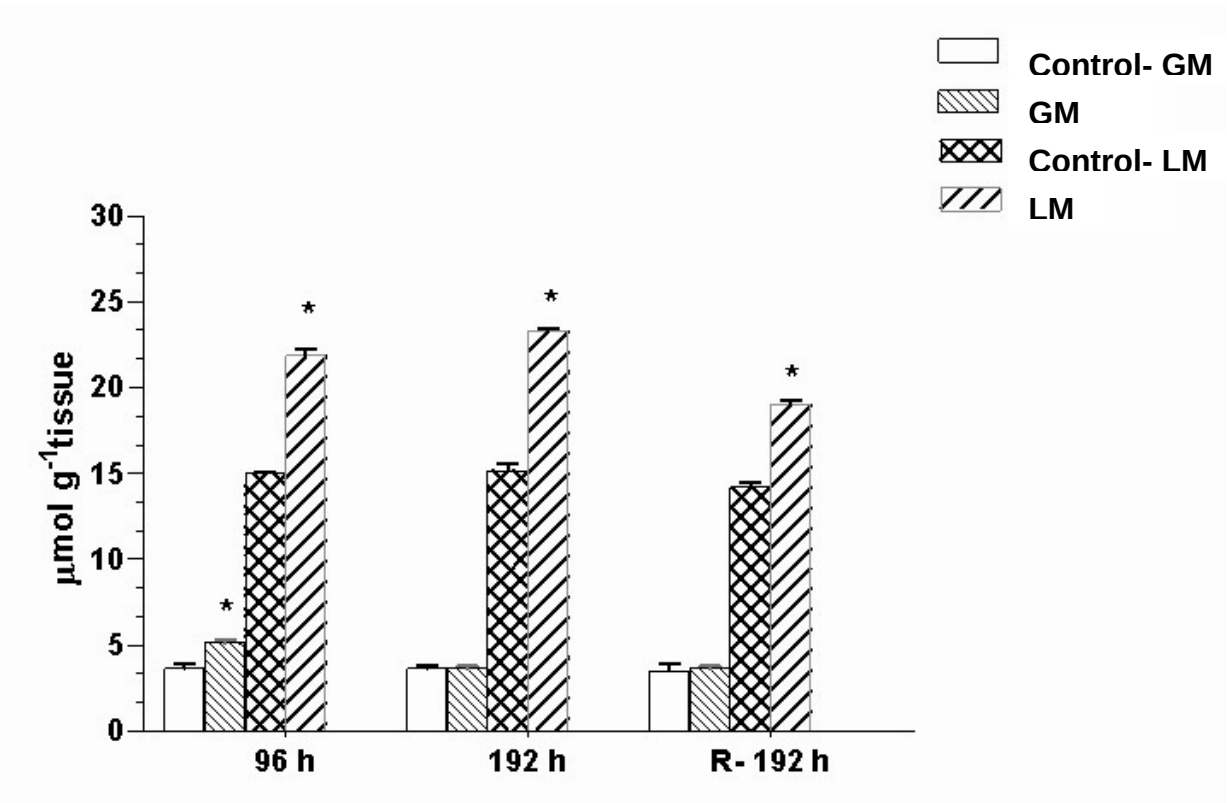
*
*
*
96 h
192 h
R- 192 h

Fig 2.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GM
Control- LM
LM
µmol g-1tissue
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25
20
15
10
5
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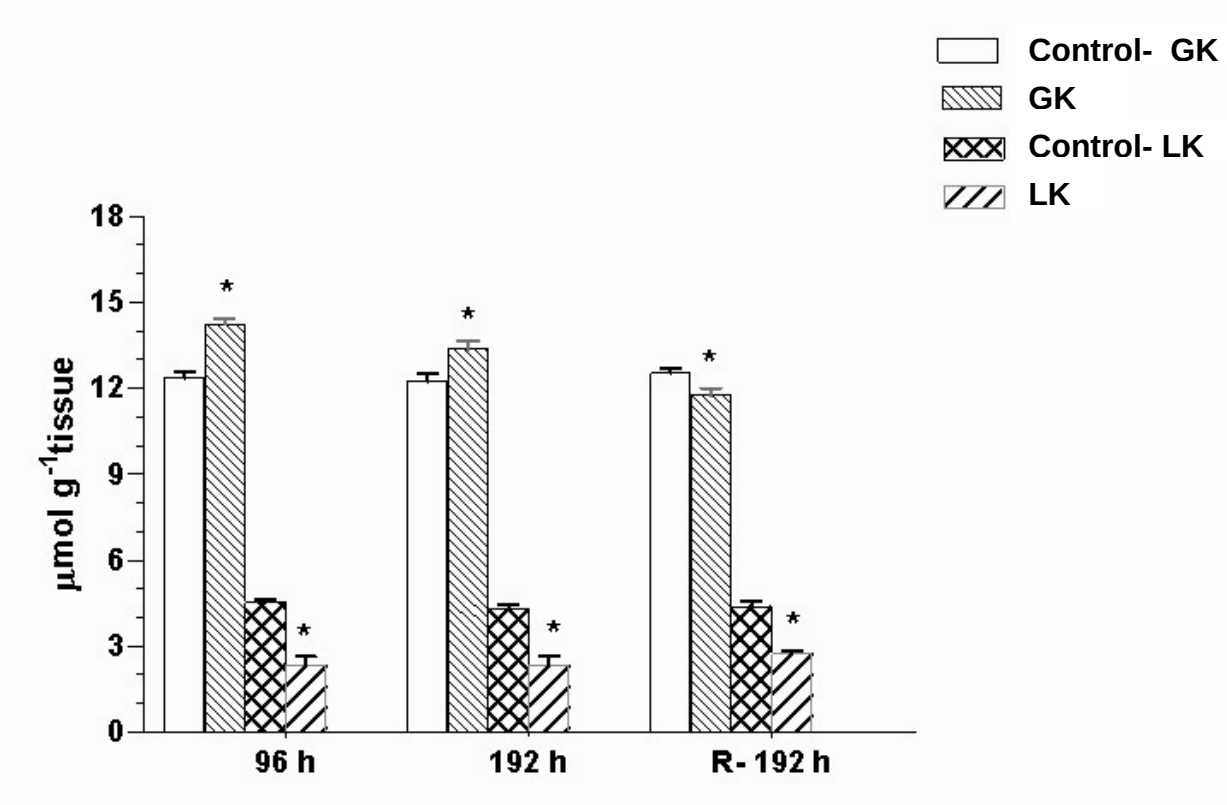
96 h
192 h
R- 192 h

Fig 3.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GK
Control- LK
LK
µmol g-1tissue
18
15
12
9
6
3
0
*
96 h
192 h
R- 192 h

Table 1

Changes in liver, muscle and kidney of *Leporinus obtusidens* exposed to clomazone (96 and 192 h) and recovery period (192 h).

	Exposure (h)		Recovery (h)
	96	192	192
Liver			
Glucose			
Control	18.04±0.44	18.08±0.35	17.80±0.40
0.5 mg L^{-1}	14.32±0.56*	14.27±0.48*	17.83±0.49
Protein			
Control	67.98±6.75	68.79±1.35	69.04±6.33
0.5 mg L^{-1}	52.28±3.65*	51.41±4.43*	68.26±4.47
Muscle			
Glucose			
Control	1.43±0.07	1.42±0.02	1.41±0.02
0.5 mg L^{-1}	1.16±0.09*	1.01±0.08*	1.37±0.03*
Protein			
Control	172.17±1.53	173.27±1.24	173.48±0.78
0.5 mg L^{-1}	166.73±1.64*	148.20±0.75*	146.98±4.93*
Ammonia			
Control	0.16±0.006	0.18±0.002	0.14±0.005
0.5 mg L^{-1}	0.14±0.005*	0.14±0.001*	0.14±0.007
Kidney			
Glucose			
Control	2.91±0.07	2.92±0.05	2.92±0.02
0.5 mg L^{-1}	1.80±0.22*	1.59±0.13*	2.88±0.07
Protein			
Control	173.02±7.05	174.05±4.24	173.50±2.78
0.5 mg L^{-1}	133.25±6.21*	137.35±6.48*	173.84±6.53
Ammonia			
Control	0.07±0.01	0.07±0.008	0.07±0.01
0.5 mg L^{-1}	0.06±0.009	0.07±0.01	0.069±0.007

Glucose (μmol/g tissue); *Protein* (mg/g tissue) and *Ammonia* (μg/g tissue). Date are means ± SD (n = 15). * Indicate a significant difference at P<0.05 compared to control group.

4. DISCUSSÃO

Neste estudo, os herbicidas clomazone, metasulfuron metil e quinclorac usados na cultura da lavoura de arroz, induziram grandes variações de toxicidade, em termos da CL_{50}-96 h no peixe jundiá (*Rhamdia quelen*). Estes após a exposição ao clomazone e ao quinclorac, apresentaram CL_{50} de 7,32 mg/L e 395 mg/L, respectivamente. Entretanto, o Clomazone, para truta arco-íris (*Salmo gairdineri*) e bluegil apresenta CL_{50}-96 h de 19 e 34 mg/L, respectivamente (VENCIL et al., 2002) e para o tetrarisca-negra (*Hyphessobrycon scholzei*) é de 27,3 mg/L (JONSSON et al., 1998). Segundo, RESGALLA JÚNIOR (2002), o herbicida quinclorac (*Cyprinus carpio*) apresenta CL_{50}-96 h de 6,65 mg/L para carpa comum. Para o metasulfuron metil, a máxima concentração testada (1200 mg/L) para jundiás não causou mortalidade e, conseqüentemente parece menos prejudicial para este peixe. Diferente, para truta arco-íris foi encontrado o valor de CL_{50}-96h de 150 mg/L (VENCIL et al., 2002).

A medida da AChE em diferentes tecidos de peixes vem sendo estudada como um possível biomarcador para monitorar a exposição aos poluentes tais como os herbicidas (FERNANDEZ-VEGA et al., 2002; DUTTA & ARENDS, 2003). Na literatura existem relatos que a exposição de peixes aos herbicidas thiobencarb e diazinon causa 75% de inibição da AChE no cérebro de enguias européias (*Anguilla anguilla*) (CÉRON et al., 1996; FERNÁNDEZ-VEGA et al., 2002). No músculo, a atividade da AChE encontra-se aumentada (65%) em piavas (MORAES et al., 2007) e inibida (45%) em jundiás expostos ao clomazone (CRESTANI et al., 2007). Nesse estudo nós observamos que, no grupo controle

dos peixes (jundiás e piavas) expostos a todos herbicidas testados, a atividade da AChE cerebral foi mais elevada do que nos demais tecidos analisados. Resultados semelhantes foram observados em outros estudos com jundiás expostos ao clomazone (CRESTANI et al., 2007) e com piavas expostas ao glifosato (GLUSCZAK et al., 2006). Entretanto, em goldfish (*Carassius auratus*), a atividade da AChE muscular do grupo controle foi mais elevada do que no cérebro (BRETAUD et al., 2000).

Nossos resultados revelaram que o clomazone é um potente inibidor da atividade da AChE cerebral. Em jundiás expostos às concentrações testadas deste herbicida, durante 96 h, a atividade AChE em cérebro foi inibida em 83%. E, a exposição de piavas a 0,5 mg/L causou uma diminuição da AChE de (%) nos períodos de exposição (96h e 192h) e de recuperação (192 h). Trabalhos de CERÓN et al. (1996) e DUTTA & ARENDS (2003) também observaram a diminuição da AChE cerebral em enguias européias (0,042 mg/L de diazinon) e em *Lepomis macrochirus* (0,001 mg/L de endosulfan). Com relação a AChE muscular nos jundiás expostos (96 h) ao metsulfuron-metil e ao quinclorac foi observado um aumento da atividade da AChE. Esse efeito foi igualmente obtido em outras espécies, tais como enguias européias expostas a 22 mg/L do thiobencarb (FERNÁNDEZ-VEGA et al., 2002) e em goldfish expostos a 0,050 mg/L do carbofuran (BRETAUD, 2000). Além disso, nestes estudos foram identificadas alterações, tais como, tremores, letargia e a natação errática. Porém, em piavas nossos resultados demonstraram uma redução da AChE muscular somente após 192h de exposição, a qual persistiu no período de recuperação. A ativação ou a inibição de AChE podem influenciar a concentração da acetilcolina e, conseqüentemente causar alterações no processo de neurotransmissão colinérgica, podendo promover efeitos no comportamento dos peixes. Em nossos testes de CL_{50}-96h, os jundiás expostos aos diferentes herbicidas apresentaram mudanças comportamentais, tais como a diminuição da alimentação e nado errático nas concentrações do clomazone de 20 e 50 mg/L. Nas concentrações do

quinclorac de 390 e 400 mg/L, além de diminuir a ingestão de alimentos, os peixes apresentaram letargia. Porém, os peixes expostos ao metasulfuron metil mostraram o comportamento de alimentação normal e comportamento hiperativo. Esses dados indicam que a atividade da AChE parece ser importante para a localização da presa, fuga do predador (natação) e também para a orientação da alimentação em peixes (DUTTA & ARENDS, 2003).

Em piavas, expostas ao clomazone, o nível da atividade da AChE de olhos foi reduzido somente durante o período de recuperação, sugerindo a presença de herbicida nesse tecido. SANCHO et al. (2000), relataram uma diminuição da atividade da AChE em olho de enguias européias após 96 h de exposição ao thiobencarb (0.22 mg/L) e após 1 semana da recuperação o mesmo efeito inibitório foi observado. Os resultados para a atividade da AChE em olho podem estar relacionados à alta atividade do nervo óptico e à quantidade de acetilcolina (HARLIN, 1991; SANCHO et al., 2000). Uma inibição da atividade da AChE foi demonstrada também em coração de piavas após 96h de exposição ao clomazone. Porém, em relação à recuperação da atividade da AChE estudada em piavas expostas ao clomazone, observou-se que esta continuou inibida após 192 h em água isenta de herbicida em cérebro, músculo e olho. No coração a atividade não foi recuperada. Estes resultados revelaram que a atividade da AChE em coração também pode ser um biomarcador sensível para avaliar a toxicidade do clomazone em piavas. Em outros estudos a recuperação da AChE cerebral e muscular foi demonstrada em jundiás expostos a este herbicida (CRESTANI et al., 2007). Assim, levando em consideração os resultados obtidos por SANCHO et al. (2000) sugere-se que a atividade da AChE pode ser reversível e é influenciada por vários fatores, entre eles: a classe química do herbicida, a espécie exposta e o tecido analisado. A medida da atividade da AChE é considerada um indicador de toxicidade para herbicidas em peixes.

Os resíduos dos herbicidas usados nas lavouras de arroz irrigado podem causar interação desses com a água e, assim evidenciar as alterações no meio ambiente (NIMMO, 1985). Porém, segundo BALDISSEROTTO & RADUNZ NETO (2004) os parâmetros de qualidade de água foram mantidos em níveis aceitáveis

para a cultura de peixes durante todas as exposições realizadas neste estudo. No entanto, nosso trabalho demonstrou que jundiás expostos em águas coletadas de lavouras de arroz irrigado (após 21 dias da aplicação de herbicidas) sofreram efeitos na sobrevivência e crescimento. No aspecto da sobrevivência, este peixe mostrou ser mais sensível a exposição na água da lavoura com quinclorac, pois se observou uma diminuição de 4% na sobrevivência. Assim, este resultado demonstrou que a concentração de quinclorac na cultura de arroz irrigado pode afetar os índices de sobrevivência desses peixes.

No presente estudo foi observado que o tempo de degradação dos herbicidas aplicados na cultura de arroz irrigado variou entre 7-28 dias nas águas amostradas. Sendo que, o clomazone permaneceu detectável até 28 dias nas águas de arroz, o quinclorac até 14 dias e o metasulfuron metil foi detectado somente até 7 dias após a aplicação. De acordo, BARCELÓ & HENION (2003) demonstraram que o clomazone possui grande solubilidade na água (1100 mg/L) e pode permanecer detectável até 30 dias. Similarmente, LAVY (1998) demonstrou resíduos do quinclorac até 36 dias após a aplicação no estado de Arkansas (EUA). Outros herbicidas que permanecem por um período longo de tempo em águas de uso no arroz irrigado foram imazetapir (30 dias), quinclorac e bentazon (21 dias) (MARCOLIN et al., 2003). Por outro lado, propanil e metsulfuron metil foram detectados somente entre 7 a 10 dias após sua aplicação (MACHADO et al., 2003; REIMCHE et al., 2008). Considerando os dados obtidos pode-se inferir que o herbicida clomazone é potencialmente mais tóxico, pois sua persistência na água é maior. De acordo com dados obtidos em áreas próximas as lavouras de arroz no Rio Grande do Sul, este herbicida foi detectado em 90% das amostras coletadas. A exposição de jundiás tanto em água com clomazone como com quinclorac resultou em redução no crescimento em nossos testes. ALVAREZ et al. (2005) demostraram que larvas de Red drum (*Sciaenops ocellatus*) expostos ao atrazine durante 96 h (40 e 80 $\mu g\ l^{-1}$) tiveram uma redução na taxa de crescimento, diminuindo a população de larvas desses peixes em aproximadamente 24%. NIEVES-PUIGDOLLER et al. (2007) demonstraram que o herbicida atrazine altera funções fisiológicas dos Atlantic salmon smolts, causando

distúrbios que reduzem a ingestão de alimentos e, assim diminuindo o crescimento dos peixes. Consumo diminuído de alimento e letargia aumentada foram observados na tilapia do Nilo (*Oreochromis niloticus*) e em catfish (*Chrysichthyes auratus*) após a exposição a 0,3 e 0,6 mg/L ao atrazine (HUSSEIN et al., 1996).

Neste estudo foram também observadas alterações no metabolismo de jundiás expostos a águas coletadas da lavoura de arroz. O fígado, além de ser considerado o centro metabólico para a desintoxicação é uma rica fonte de glicose para os peixes (SUÁREZ & MOMMSEN, 1987; MOON, 1998). No tecido hepático nossos resultados mostraram que os jundiás mantidos por 45 dias na água com clomazone e quinclorac apresentaram um aumento de glicogênio, e redução nos níveis da glicose e de lactato, indicando uma gliconeogênese hepática aumentada. Já os jundiás expostos ao metasulfuron metil mostraram as mesmas respostas hepáticas, mas somente em 15 dias de exposição retornando aos níveis dos controles no final do período experimental. Resultados semelhantes foram observados em nossos experimentos com piavas expostas ao clomazone durante 96 e 192h e durante o período de recuperação. Nossos resultados estão de acordo com outros estudos (SUÁREZ & MOMMSEN, 1987; PEIXOTO et al., 2006; CRESTANI et al., 2006; CATTANEO et al. (2008). Portanto, sugere-se que o aumento de glicogênio no tecido hepático em jundiás e piavas tenha sido uma estratégia de armazenamento de energia devido o aumento da demanda metabólica para o processo de detoxificação desses herbicidas, uma vez que o fígado de peixes é o principal órgão de acumulação, de biotransformação e de desintoxicação dos produtos químicos.

Uma vez que o músculo dos peixes constitui mais de 50% da massa do corpo, os peixes podem usar o processo anaeróbico para obter energia, através da oxidação do glicogênio (KNOX et al., 1980). No tecido muscular de jundiás expostos às águas do arroz contendo clomazone, quinclorac e metasulfuron metil verificou-se uma diminuição do glicogênio muscular, com aumento concomitante do lactato neste tecido. Semelhantemente, nossos resultados monstram uma diminuição do glicogênio muscular e da glicose seguido do aumento de lactato,

em piavas expostas ao clomazone (96 h), porém o glicogênio retornou aos valores do controle após o período da recuperação. Esses resultados podem indicar uma situação de estresse, com hipóxia tecidual gerado pela toxicidade do herbicida. Consequentemente, uma rápida degradação do glicogênio acompanhada do uso de glicose pode ser uma maneira de mobilizar a energia necessária para tal condição de estresse. Estas mudanças sugerem que a utilização de carboidratos para a produção energética, possa ser uma resposta à toxicidade induzida pela exposição ao herbicidas (GIMENO et al., 1995; ORUÇ & ÜNER, 1999; VIJAYAVEL et al., 2006).

Resultados similares referentes ao tecido muscular foram apresentados por GLUSCZAK et al., (2007), em piavas expostas ao glifosato. FERRANDO & ANDREU-MOLINER (1991), também relataram uma diminuição do glicogênio muscular em enguias européias (*Anguila Anguila*), após a exposição ao lindane. ORUÇ & ÜNER (1999) e CATTANEO et al. (2008) observaram em *Cyprinus carpio* e *R. quelen* a diminuição de glicogênio do músculo após a exposição ao 2,4–D. Em relação ao aumento de lactato, estes resultados estão de acordo comos observados por BEGUM & VIJAYARAGHAVAN (1999), e esta resposta parece ser uma medida do metabolismo anaeróbico, indicando uma condição do esforço dos peixes. Do mesmo modo, nossos estudos demonstraram níveis aumentados do lactato muscular, sugerindo que a exposição ao clomazone causa uma situação de estresse, e também ocorre hipóxia tecidual com aumento da demanda energética.

O rim dos peixes é um outro órgão importante que pode ser usado para avaliar a toxicidade de herbicidas. Este órgão recebe a circulação sanguínea branquial do corpo nos peixes e, assim é importante na desintoxicação, na eliminação dos contaminadores. Portanto, no rim pode ser avaliado a toxicidade de produtos químicos em peixes (GALLAGHER & DI GIULIO, 1992; SANCHO et al., 1997; ÜNER et al., 2005). No presente estudo, os níveis do glicogênio em rins de piavas expostas ao clomazone foram aumentados similar ao obtido para o fígado. Entretanto, no período de recuperação ocorreu a redução nos níveis de glicogênio. Esta resposta parece indicar uma compensação a uma situação do

estresse induzido pelo clomazone. Então, os tecidos hepático e renal analisados no presente estudo demonstraram ser importantes para manter o balanço de água do corpo e produtos químicos tóxicos que podem temporariamente ou permanentemente interromper suas funções (MILLER, 2002; ÜNER et al., 2006). De fato, as espécies de peixes mostram diferentes respostas metabólicas ao estresse induzido por contaminantes e o metabolismo do glicogênio pode ser especifico considerando a espécie, o tecido e o tempo de exposição do peixe.

As variações nas atividades de enzimas antioxidantes também podem ser indicadores do estresse oxidativo causado por poluentes (AHMAD et al., 2000; SAYEED et al., 2003). A peroxidação lipídica (formação de TBARS) é um dos principais processos induzidos pelo estresse oxidativo decorrentes de herbicidas, em peixes (AHMAD et al., 2004). Em nosso estudo, os níveis de TBARS estavam aumentados no tecido do cérebro de piavas depois de 96 e 192 h de exposição. Em fígado e músculo também foi observado este aumento, mas somente depois de 192 h de exposição. Em relação ao período de recuperação, verificou-se que em todos os tecidos os níveis de TBARS retornaram aos valores iniciais, com exceção do fígado. De acordo, CRESTANI et al. (2007) também observaram níveis de TBARS aumentados em cérebro e fígado de jundiás após a exposição ao clomazone. Similarmente, ÜNER et al. (2006) observaram elevados níveis de TBARS em músculo do *Cyprinus carpio* exposto ao diazinon. A elevação do LPO observada no estudo sugere uma situação de estresse oxidativo, a qual pode induzir a formação de radicais livres mediada pela exposição ao clomazone. A recuperação dos níveis de TBARS pode indicar um mecanismo compensatório através do sistema antioxidante, e uma tentativa de adaptar-se a condição de estresse induzido pelo herbicida. Estudos recentes associam um aumento dos níveis de TBARS com a inibição da AChE indicando estresse oxidativo induzido pelos pesticidas (SEVGILER et al., 2005; ÜNER et al., 2006; ÜNER et al 2007; ORUÇ & USTA, 2007). Similar a estes estudos, nossos resultados demonstraram um aumento em níveis de TBARS e uma inibição da AChE para o cérebro e o músculo de piavas depois de 192 h de exposição e de recuperação. Estes resultados sugerem que radicais livres em tecidos de piavas poderia induzir

mudanças na hiperatividade colinérgica, a qual pode levar a inibição da AChE. Alguns autores consideram que a inibição da AChE induz peroxidação lipídica que por sua vez pode levar ao dano celular. Este pode ser um resultado de um sistema antioxidante eficaz que funciona como uma resposta adaptável dos peixes (YANG et al., 1996; ÜNER et al., 2006; ORUÇ & USTA, 2007).

Proteínas são um dos alvos para a elucidação dos efeitos de pesticidas em diversas espécies. Recentemente, mostrou-se que estes podem induzir modificações oxidativas em proteínas sendo isso, também, uma de muitas conseqüências do estresse oxidativo nos peixes (SAYEED et al., 2003). Sabe-se que o radical hidroxila (OH^-) é altamente reativo, ou seja, é uma espécie reativa de oxigênio que é formado em uma situação de estresse oxidativo e é considerado responsável pela formação de grupos carbonilados nas proteínas (OLIVER, 1987). Portanto, o ensaio de grupos carbonil em proteínas constitui-se em uma técnica adequada para quantificar modificações oxidativas em proteínas. Neste estudo, a exposição de piavas ao clomazone resultou num aumento na carbonilação de proteína no fígado, demonstrando uma condição de estresse oxidativo. Vários pesticidas (deltamethrin, endosulfan e paraquat) também causaram aumento de proteínas carboniladas em tecidos hepáticos, renais e branquiais de *Channa punctata* (Bloch) (PARVEZ &RAISUDDIN, 2005).

No fígado, a diminuição observada na atividade da enzima catalase (CAT) em piavas expostas ao clomazone demonstra que este herbicida induz dano oxidativo no fígado causando alterações nos sistemas dos antioxidantes. PANDEY et al. (2001) observaram resultados similares quando expôs *C*. *punctatus* (Bloch) ao endosulfan. CRESTANI et al. (2007) mostraram que o clomazone (0,5 ou 1,0 mg/ L) causa redução da CAT em fígado de jundiás. A exposição de deltamethin causou uma diminuição na atividade da CAT em fígado, rins e brânquias de *C*. *punctatus* (SAYEED et al., 2003). Este declínio na atividade da CAT poderia ser devido à produção excessiva de O_2 como indicado por BRAINY et al. (1996). Os resultados do presente estudo indicam que piavas resistem ao estresse oxidativo durante a exposição ao clomazone.

5. CONCLUSÕES

-A CL_{50}-96h estimada para jundiás expostos ao herbicida clomazone e quinclorac foi equivalente a 7,32 (I.C. de 5,68 a 9,03 mg/L) e 395 mg/L (I.C. de 394 a 395,9 mg/L), respectivamente. Para jundiás expostos ao metasulfuron metil, a CL_{50}-96h não foi obtida, pois os peixes sobreviveram a máxima concentração testada (1200 mg/L). Assim, o clomazone apresenta maior potencial de risco de contaminação para jundiás e, o quinclorac e o metasulfuron metil apresentam menor risco.

- Os herbicidas estudados neste trabalho afetaram a atividade da AChE em cérebro e músculo de jundiás. O clomazone mostrou-se um potente inibidor em ambos tecidos. Assim, pode-se inferir que a medida da atividade da AChE em cérebro e músculo pode ser um biomarcador primário da toxicidade para a exposição ao quinclorac e metasulfuron metil.

- Depois de 45 dias de exposição aos herbicidas, os jundiás foram mais sensíveis à sobrevivência quando expostos a água da lavoura com quinclorac. O clomazone e o quinclorac foram prejudiciais ao crescimento dos peixes. Os herbicidas afetaram também os parâmetros metabólicos dos jundiás, que utilizaram uma estratégia de armazenamento energético através das reservas de glicogênio hepático para compensar a demanda do processo de detoxificação destes produtos químicos.

- Nossos estudos com piavas revelaram que o clomazone inibe aproximadamente 50% da atividade da AChE cerebral, mesmo com uma concentração aproximada a utilizada nas lavouras. A elevação dos níveis de TBARS em cérebro, fígado e músculo de piavas sugerem uma situação de estresse oxidativo induzindo a formação de radicais livres mediada pela exposição ao clomazone. No fígado a formação de proteínas carboniladas mostrou uma condição de estresse oxidativo. A diminuição hepática da atividade da enzima CAT demonstra que o clomazone induz dano oxidativo no fígado e causa alterações no sistema antioxidante.

- As piavas demonstraram alterações metabólicas semelhantes aos jundiás expostos ao clomazone. A soma dos resultados obtidos permite concluir que o fígado estoca glicogênio para suprir a demanda energética frente à intoxicação. O tecido muscular demonstrou mobilização de energia através de uma estratégia anaeróbica, onde glicogênio e glicose são oxidados até lactato. O tecido muscular responde a uma hipóxia tecidual gerada pela exposição de piavas ao clomazone.

- Após período de recuperação das piavas em água livre de clomazone, a atividade da AChE permaneceu diminuída em cérebro, músculo e olho. A elevação dos níveis de TBARS em cérebro e em músculo, bem como a formação de proteínas carboniladas e a atividade da CAT em fígado não foram recuperadas. A exposição ao clomazone também causou alterações metabólicas que não foram recuperadas, tais como, aumento do glicogênio hepático e do lactato muscular. Portanto, após a exposição de piavas ao clomazone algumas mudanças enzimáticas e metabólicas não podem ser recuperadas, mesmo submetendo os peixes ao tratamento em água livre de herbicida por 192 h.

- Os parâmetros avaliados em jundiás e piavas podem ser recomendados para o monitoramento de contaminação de águas por clomazone, quinclorac e metasulfuron metil, considerando as condições laboratoriais deste estudo.

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