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PARÂMETROS BIOQUÍMICOS EM JUNDIÁS EXPOSTOS AO ZINCO

DISSERTAÇÃO DE MESTRADO

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PARÂMETROS BIOQUÍMICOS EM JUNDIÁS EXPOSTOS AO ZINCO

Jossiele Wesz Leitemperger

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

Orientadora: Prof[a]. Dr[a]. Vania Lucia Loro

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Bioquímica Toxicológica

A Comissão Examinadora, abaixo assinada, aprova a
Dissertação de Mestrado

PARÂMETROS BIOQUÍMICOS EM JUNDIÁS EXPOSTOS AO ZINCO

elaborada por
Jossiele Wesz Leitemperger

como requisito parcial para obtenção do grau de
Mestre em Ciências Biológicas: Bioquímica Toxicológica

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Santa Maria, 05 de junho de 2014.

Dedico este trabalho aos meus pais,
Jomar e Santa
a minha irmã
Jossane,
ao meu namorado,
Luis Eduardo
que sempre me apoiaram,
me deram força e
acreditaram em mim

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RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica
Universidade Federal de Santa Maria, RS, Brasil
PARÂMETROS BIOQUÍMICOS EM JUNDIÁS EXPOSTOS AO ZINCO
Autora: Jossiele Wesz Leitemperger
Orientadora: Vania Lucia Loro
Data e Local da Defesa: Santa Maria, 05 de junho de 2014.

A contaminação do ambiente aquático ocorre como consequência das atividades industriais, agrícolas e antropogênicas. Ecossistemas aquáticos são frequentemente contaminados com metais como cobre e zinco, que são essenciais em baixas concentrações, mas tóxicos em concentrações elevadas. Sendo assim, o objetivo deste estudo foi estabelecer a concentração letal média (CL_{50}) em 96 horas do zinco para jundiá (*Rhamdia quelen*) e avaliar os possíveis efeitos sobre parâmetros bioquímicos destes peixes expostos a 0,0 (controle), 1,0 e 5,0 mg/L de zinco por 96h. Os parâmetros analisados foram substâncias reativas ao ácido tiobarbitúrico (TBARS), proteína carbonil, acetilcolinesterase (AChE), superóxido dismutase (SOD), catalase (CAT), glutationa S-transferase (GST), tióis não-proteicos (NPSH) e Na^+/K^+-ATPase. O valor da CL_{50} encontrado foi de 8,07 mg/L. Os resultados da exposição subletal mostraram que os níveis de TBARS diminuíram no fígado e no cérebro em todas concentrações de Zn testadas. Nas brânquias, houve um aumento dos níveis de TBARS na concentração de 1,0 mg/L e uma diminuição na 5,0 mg/L, enquanto que no músculo não foram observadas alterações. A exposição ao zinco diminuiu a carbonilação de proteínas no fígado de jundiás expostos a 5,0 mg/L e nas brânquias nas duas concentrações testadas. No cérebro não houve alterações na proteína carbonil e no músculo, houve um aumento nas duas concentrações. No fígado, a atividade da SOD aumentou em ambas as concentrações de zinco testadas. A atividade da CAT no fígado não teve alteração em nenhuma das concentrações. A atividade da GST diminuiu no fígado e aumentou no cérebro para ambas as concentrações utilizadas. Nas brânquias, o aumento da GST ocorreu na concentração de 1,0 mg/L e no músculo dos peixes expostos a 5,0 mg/L. Os níveis de NPSH não mostraram alterações no fígado e nem nas brânquias. No cérebro houve um aumento nos níveis de NPSH na concentração de 5,0 mg/L, enquanto que no músculo diminuiu nas duas concentrações. A atividade da AChE em cérebro diminuiu nas duas concentrações de zinco, enquanto que no músculo não houve alteração na atividade desta enzima. A atividade da Na^+/K^+-ATPase foi inibida em brânquias e intestino em todas as concentrações usadas. Com estes resultados, podemos concluir que o zinco alterou os parâmetros bioquímicos dos jundiás e que a inibição da AChE cerebral e da Na^+/K^+-ATPase no intestino e brânquias podem ser utilizadas como biomarcadores de águas contaminadas por zinco.

Palavras-chave: Concentração letal média. Estresse oxidativo. Jundiás. Metal. Na^+/K^+-ATPase.

ABSTRACT

Dissertation of Master's Degree
Post-Graduat Program in Biological Sciences: Toxicological Biochemistry
Federal University of Santa Maria, RS, Brazil
BIOCHEMICAL PARAMETERS IN SILVER CATFISH EXPOSED TO ZINC
Author: Jossiele Wesz Leitemperger
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Date and place of the defense: Santa Maria, June, 05th, 2014.

The contamination of the aquatic environment occurs as a consequence of industrial, agricultural and anthropogenic activities. Aquatic ecosystems are often contaminated with metals such as copper and zinc, which are essential in low concentrations but toxic at high concentrations. Thus, the aim of this study was to establish the mean lethal concentration (LC_{50}) within 96 hours of zinc for silver catfish (*Rhamdia quelen*) and to evaluate the possible effects on biochemical parameters of fish exposed to 0.0 (control), 1.0 and 5.0 mg/L zinc for 96h. The parameters analyzed were reactive substances to thiobarbituric acid (TBARS), protein carbonyl, acetylcholinesterase (AChE), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), non-protein thiols (NPSH) and Na^+/K^+-ATPase. The LC_{50} value found was 8.07 mg/L. The results of sublethal exposure showed that TBARS levels decreased in liver and brain at every tested Zn concentration. In gills, there was an increase on TBARS levels at 1.0 mg/L and a decrease at 5.0 mg/L, while no changes in muscle were observed. Exposure to zinc decreased protein carbonyls in liver of silver catfish exposed to 5.0 mg/L and gills in every tested concentration. In brain there were no changes in protein carbonyl and in muscle there was an increase at both concentrations. In liver, the SOD activity increased at both zinc concentrations. CAT activity in liver did not change at any concentration. The GST activity decreased in liver and brain increased at both concentrations used. In gills, GST increased in fish exposed to 1.0 mg/L and in muscle at 5.0 mg/L. NPSH levels showed no changes in liver either in gills. In brain there was an increase on NPSH levels at 5.0 mg/L, while the muscle declined at both concentrations. The AChE activity in brain decreased at both zinc concentrations, whereas there was no change in muscle activity for this enzyme. The Na^+/K^+-ATPase activity was inhibited in gill and intestine in every used concentration. We may conclude that zinc altered biochemical parameters of silver catfish and also the inhibition of brain AChE and Na^+/K^+-ATPase activity in intestine and gills may be used as biomarkers of waters contaminated by zinc.

Keywords: Mediam lethal concentration. Oxidative stress. Silver catfish. Metal. Na^+/K^+-ATPase activity.

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MANUSCRITO

LISTA DE ABREVIAÇÕES

AChE: Acetilcolinesterase

ADP: Adenosina Difosfato

ATP: Adenosina Trifosfato

CAT: Catalase

CL_{50}: Concentração Letal Média

CDNB: 1-cloro-2,4-dinitrobenzeno

DNA: Ácido Desoxirribonucleico

DNPH: 2,4 – dinitrofenilhidrazina

CONAMA: Conselho Nacional do Meio Ambiente

DTNB: Ácido 5,5' – ditio-bis-2-nitrobenzóico

EROs (ROS): Espécies Reativas ao Oxigênio

GPx: Glutationa Peroxidase

GSH: Glutationa Reduzida

GST: Glutationa S-Transferase

H_2O_2: Peróxido de Hidrogênio

LPO: Peroxidação Lipídica

MDA: Malondialdeído

NPSH: Tióis Não-Proteicos

$O_2^{\cdot -}$: Ânion Superóxido

OH: Radical Hidroxila

SDS: Lauril Sulfato de Sódio ou Duodecil Sulfato de Sódio

SOD: Superóxido Dismutase

TBA: Ácido 2-tiobarbitúrico

TBARS: Substâncias Reativas ao Ácido Tiobarbitúrico

TCA: Ácido Tricloroacético

SUMÁRIO

1. INTRODUÇÃO

Poluição ambiental é toda a ação de contaminar água, solo e ar, causando efeito negativo no seu equilíbrio e baixando o potencial de crescimento do ecossistema. No Brasil, foi criado em 1982 o Conselho Nacional do Meio Ambiente (CONAMA), pela Lei 6.938/81, sendo o órgão responsável pelas deliberações de toda a política nacional do meio ambiente. Ele existe para assessorar, estudar e propor ao Governo, as linhas de direção que devem tomar as políticas governamentais para a exploração e preservação do meio ambiente e recursos naturais. Uma de suas principais competências é estabelecer normas, critérios e padrões relativos ao controle e à manutenção da qualidade do meio ambiente, com vistas ao uso racional dos recursos ambientais, principalmente os hídricos. O CONAMA classificou como III aquelas águas que podem ser destinadas ao abastecimento para consumo humano, após tratamento convencional ou avançado; à irrigação de culturas arbóreas, cerealíferas e forrageiras; à pesca amadora; à recreação de contato secundário; e dessedentação de animais.

Levando em consideração que o rápido desenvolvimento da indústria e da agricultura resultou em um aumento na poluição dos rios, lagos e reservatórios com produtos químicos orgânicos, agrotóxicos e metais pesados, que são considerados muito perigosos para invertebrados, peixes e humanos (ULUTURHAN e KUCUKSEZGIN, 2007), o CONAMA estabeleceu valores padrão para a quantidade máxima destes poluentes em águas. Frequentemente quantidades significativas de águas residuais contendo metais pesados são descarregadas nos rios. Nestes sistemas aquáticos geralmente são encontrados altos níveis de mercúrio, cromo, chumbo, cádmio, zinco e níquel (ZHOU et al., 2007). Estes metais podem ser fortemente acumulados na água, em sedimentos e em cadeias alimentares aquáticas, resultando em efeitos subletais ou a morte de populações de peixes locais (McGEER et al., 2000; XU et al., 2004). As principais causas do aumento da presença destes contaminantes são as descargas geradas por atividades industriais e atividades relacionadas ao uso de fertilizantes na agricultura (ROMANI et al., 2003; DAUTREMEPUITS et al., 2004). Assim, as águas contaminadas podem atingir os sistemas de água doce e ambientes marinhos prejudicando os ecossistemas que ali vivem.

Metais pesados como o cobre e o zinco são essenciais para o metabolismo dos peixes, enquanto que outros, como o mercúrio, o chumbo e o cádmio não possuem papel biológico conhecido (CANLI e ATLI, 2003). O zinco é um elemento essencial que atua como componente estrutural e tem propriedades indispensáveis à vida (BENGARI e PATIL, 1986).

Ele tem um importante papel no metabolismo celular, atuando como cofator de uma série de reações enzimáticas. No entanto, a absorção de zinco pode levar a efeitos tóxicos. A absorção de metais pode ocorrer através das brânquias, do trato gastrintestinal e revestimento epitelial, entre outros órgãos (SCHLENK e BENSO, 2001; ATLI e CANLI, 2007). Os peixes tendem a desenvolver várias respostas bioquímicas e fisiológicas em respostas ao xenobióticos que entram no seu organismo. Estas respostas podem resultar na adaptação do organismo ao poluente ou induzir efeitos negativos na sobrevivência e condições de saúde do peixe (DE SMET e BLUST, 2001). O equilíbrio entre as defesas antioxidantes e a geração de espécies reativas ao oxigênio (EROs) é fundamental para a homeostase do animal. Quando há um desequilíbrio entre pró-oxidantes e defesa antioxidante, uma situação de estresse oxidativo ocorre levando a uma produção excessiva de EROs que pode causar danos aos lipídeos, carboidratos e ácidos nucleicos (DE SMET e BLUST, 2001).

As EROs tais como o radical ânion superóxido ($O_2^{\cdot-}$), peróxido de hidrogênio (H_2O_2) e radical hidroxila ($^{\cdot}OH$) podem reagir com macromoléculas biológicas e produzir a peroxidação lipídica (LPO), danos ao DNA e oxidação de proteínas, resultando no estresse oxidativo (LUSHCHAK, 2011; LORO et al., 2012) (Fig. 1).

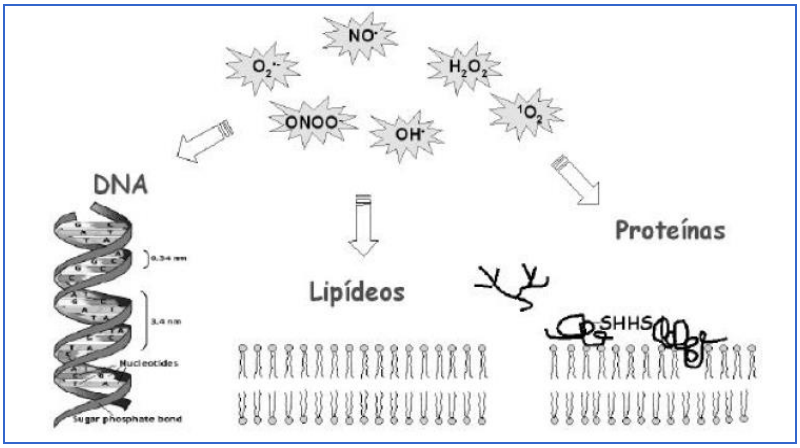

Figura 1. Dano oxidativo a macromoléculas biológicas (adaptado de TORRES, 2003).

O dano aos lipídeos induz o fenômeno conhecido como peroxidação lipídica (LPO). É o resultado da atuação de radicais livres sobre as membranas biológicas, que são ricas em ácidos graxos poliinsaturados. Um dos mais conhecidos produtos da LPO é o malondialdeído (MDA), o qual é produto final da degradação não enzimática de ácidos graxos poliinsaturados e é ensaiado com o ácido tiobarbitúrico e expresso em substâncias reativas ao ácido tiobarbitúrico (TBARS) (LUSHCHAK e BAGNYUKONA, 2006). Altos níveis de MDA elevam a formação

de lipoperóxidos e indicam um aumento da LPO. As EROs também podem causar prejuízo às proteínas. O dano à estrutura proteica pode ocasionar em clivagens das ligações peptídicas, modificações nos resíduos dos aminoácidos, oxidações dos grupos sulfidrila, formação de proteína carbonil, entre outras (LUSHCHAK e BAGNYUKONA, 2006).

Apesar do perigo das EROs, as células possuem mecanismos de defesa para neutralizar os efeitos prejudiciais dos radicais livres (SIES, 1993). O sistema de defesa antioxidante tem sido muito estudado, pois protege os sistemas biológicos contra os efeitos deletérios dos processos ou reações que levam à oxidação das biomoléculas ou das estruturas celulares (Fig. 2). As defesas antioxidantes englobam componentes de natureza enzimática e não enzimática. As principais enzimas são a superóxido dismutase (SOD), a catalase (CAT) e a glutationa peroxidase (GPx) e o sistema de defesa antioxidante não enzimático é representado, principalmente, pelos níveis de ácido ascórbico, tióis não-proteicos (NPSH) e glutationa reduzida (GSH) (CAMPANA et al., 2003; FAROMBI et al., 2007; GIODA et al., 2007).

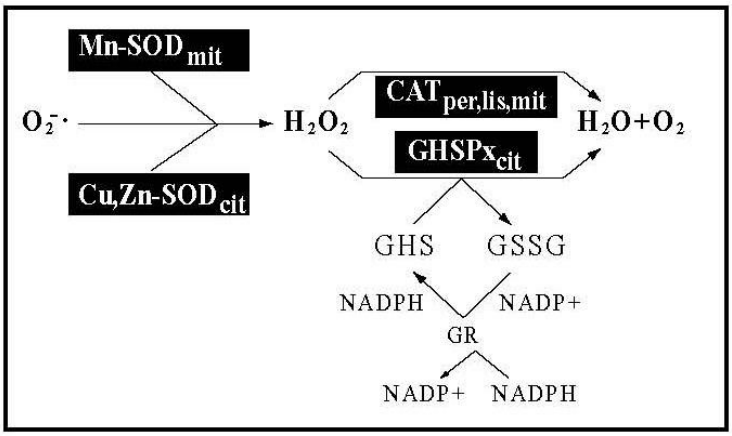

Figura 2. Enzimas antioxidantes

A SOD é uma das primeiras enzimas a atuar na linha de defesa, sendo responsável por catalisar a conversão do ânion superóxido em peróxido de hidrogênio. O peróxido de hidrogênio é degradado em água e oxigênio molecular via catalase. A CAT é uma enzima tetramérica, consistindo de quatro subunidades idênticas, que contém um único grupo ferroprotoporfirina. Já a glutationa S-transferase (GST) age em processos de biotransformação e detoxificação de xenobióticos, é composta por 3 famílias de enzimas (citosólica, mitocondrial e microssomal), que atuam contra radicais livres gerados por poluentes inorgânicos, tais como metais (MEYER et al., 2003; DAUTREMEPUITS et al., 2004; FAROMBI et al., 2007).

Os NPSH são importantes no sistema de defesa antioxidante não enzimático, que protege os tecidos de peixes contra danos oxidativos ao neutralizar os radicais livres e outros tipos de EROS. A glutationa reduzida (GSH) é o tiol não-proteico mais abundante presente nas células animais. Age como principal antioxidante não-enzimático nas células e está envolvida em numerosos processos que são essenciais para as funções biológicas normais, como a síntese de DNA e proteínas. A GSH também protege as células de substâncias tóxicas através da conjugação de metabólitos, incluindo xenobióticos e produtos da peroxidação lipídica, resultando em um intermediário menos tóxico e assim, reduzindo as injúrias nas células. A conjugação da GSH com estes metabólitos é catalisada pela GST.

Outro parâmetro utilizado para avaliar a intoxicação por metais é a medida da atividade da enzima acetilcolinesterase (AChE). As colinesterases são enzimas que desempenham importantes papéis na neurotransmissão colinérgica central e periférica, hidrolisando ésteres de colina. Alterações na atividade da AChE em resposta à exposição de peixes a xenobióticos podem afetar a locomoção e o equilíbrio (LIONETTO et al., 2003; ROMANI et al., 2003; GIODA et al., 2013).

Para avaliar a toxicidade de metais como o zinco, têm-se sugerido que a avaliação da atividade de ATPase pode ser utilizada como um sinal de alerta. ATPases são enzimas ligadas à membrana responsáveis pelo transporte de íons e, assim, ajudam na regulação do volume celular e da pressão osmótica (MONSERRAT et al., 2007; ATLI e CANLI, 2010). O mecanismo chave da toxicidade de metais tem sido relatado como sendo uma disfunção osmorregulatória associada com a inibição da ATPase em tecidos como brânquias e intestino (McGEER e WOOD, 1998). Dados da literatura demonstraram que há variações consideráveis (inibição e estimulação) nas atividades da ATPase em peixes expostos a vários metais, incluindo cádmio, cobre, zinco e chumbo (ATLI e CANLI, 2007). Mudanças nas atividades têm sido utilizadas com sucesso como biomarcadores de exposição a contaminantes metálicos (FATIMA e AHMAD, 2005; SANCHEZ et al., 2005).

O jundiá é encontrado no sudeste do México ao centro da Argentina (Figura 3). É um peixe de água doce, omnívoro com tendência piscívora preferindo crustáceos, insetos, restos de vegetais e detritos orgânicos (GOMIERO; SOUZA; BRAGA, 2007). Possui hábito noturno e vive em lagos e poços fundos dos rios, preferindo ambientes de águas mais calmas com fundo de areia e lama, junto às margens e vegetação (BALDISSEROTTO; GOMES, 2010, cap. 11, p.302).

Figura 3. Exemplar de *Rhamdia quelen*

Esta espécie tem despertado grande interesse no sul do Brasil devido as suas características como resistência ao manejo e crescimento rápido mesmo nos meses de inverno. Além disso, o jundiá é um peixe que apresenta excelente aceitação pelo mercado consumidor, tanto para pesca quanto para a alimentação, sendo uma espécie com excelentes características para o processamento (BARCELLOS et al., 2001; 2003; 2004). É também uma das espécies nativas mais estudadas em termos de ação de poluentes.

Estudos apontam que o zinco afeta parâmetros bioquímicos em várias espécies de peixes (GIODA et al., 2007; 2013; LORO et al., 2012). Com isso, os órgãos dos peixes se tornam o principal alvo de pesquisa na busca de identificar como o peixe exposto está reagindo para eliminar ou se adaptar ao metal.

Considerando que quantidade máxima de zinco em águas é de 5,0 mg/L (CONAMA, 2005), é relevante conhecer os efeitos que este metal pode causar em peixes nativos como os jundiás. Assim, com base nos resultados deste estudo, estabelecer biomarcadores para estudos futuros de biomonitoramento de ambientes aquáticos contaminados com este metal.

2. OBJETIVOS

2.1 Objetivo geral

Avaliar os efeitos do zinco sobre parâmetros de estresse oxidativo em jundiá (*Rhamdia quelen*).

2.2 Objetivos específicos

- Estabelecer o valor da concentração letal média CL_{50} (96h) do zinco para jundiá;
- Investigar os efeitos do zinco sobre a atividade colinérgica;
- Investigar a possível alteração do perfil oxidativo e a resposta das enzimas antioxidantes após exposição ao zinco;
- Avaliar o efeito do zinco sobre a atividade da Na^+/K^+-ATPase em brânquias e intestino.

3. MANUSCRITO

Jundiás (*Rhamdia quelen*) expostos ao zinco: concentração letal média e parâmetros oxidantes e antioxidantes

Silver catfish (*Rhamdia quelen*) exposed to zinc: lethal concentration, oxidative and antioxidants parameters

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ABSTRACT

Contamination of aquatic ecosystems by metals has caused various biochemical changes in aquatic organisms, and fish are recognized as indicators of environmental quality. Silver catfish (*Rhamdia quelen*) were exposed to six concentrations of zinc (Zn): 1.0; 2.5; 5.0; 7.5; 10.0 and 12.5 mg/L for 96h to determine mean lethal concentration (LC_{50}). The value obtained was 8.07 mg/L. In a second experiment, fish were exposed to concentrations of 0.0 (control); 1.0 or 5.0 mg/L of Zn for 96h and after this period the liver, gills, brain, muscle and intestine were collected for biochemical analysis. Lipid peroxidation as indicated by thiobarbituric acid-reactive substance (TBARS) decreased in liver and brain for all Zn concentrations tested, while in gills the TBARS levels increased at 1.0 mg/L and declined at 5.0 mg/L. Zn increased protein carbonyl in muscle of silver catfish and decreased in the other tissues. The enzyme superoxide dismutase (SOD) increased in both groups of exposure, however catalase (CAT) activity did not change. Glutathione S-transferase (GST) decreased in liver and increased in the gills (1.0 mg/L), muscle (5.0 mg/L) and brain (1.0 and 5.0 mg/L). Non-protein thiols changed only in brain and muscle. Zn exposure inhibited brain acetylcholinesterase activity (AChE) at both concentrations tested, but did not change in muscle. Exposure to Zn inhibited the activity of Na^+/K^+-ATPase in gills and intestine at both concentrations tested. Our results demonstrate that Zn alter biochemical parameters in silver catfish. Note were thy inhibition of AChE and the Na^+/K^+-ATPase which may be used as biomarkers of waterborne Zn toxicity.

Keywords: aquatic contamination, fish, metal, Na^+/K^+-ATPase

1. Introduction

The aquatic environment receives numerous compounds, such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pesticides and metals (Martínez – Álvarez et al. 2005). Most of these substances are potent oxidants and may generate cellular imbalance and oxidative stress in aquatic organisms (Winston, 1991). Metals may be strongly accumulated in sediments and aquatic food chains, resulting in sublethal effects or death of local fish (McGeer et al. 2000; Almeida et al. 2002; Xu et al. 2004). Zinc is a transition metal which participates in the modulation of regulatory proteins and cellular activities (Oteiza and Mackenzie, 2005). Is known that zinc is an integral part of more than 200 isolated enzymes from different species (Valle, 1988). Zinc ions specifically bind to many membrane receptors, transporters and channels, modulating their activities (Huang, 1997). Recently, the role of zinc as an important signaling molecule has emerged. Zinc has been found to modulate cellular signal reception, second messenger metabolism, protein kinases, and phosphatase activities as well as regulate the DNA binding of transcription factors (MacDonald, 2000; Beyersmann, 2002; Tamir et al. 2004; Hershfinkel et al. 2007). However, zinc absorption could lead to toxic effects (Atli and Canli, 2007). Products made of zinc may enter the aquatic ecosystem indirectly through its use in agriculture. Moreover, it also might reach in aquatic ecosystems through soil erosion by wind, drifting in the act of spraying pesticides, run off from rain and groundwater contamination.

Zinc exposure may promote an increase of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radicals, and hydroxyl radicals, leading to impairment of normal oxidative metabolism and finally to oxidative stress (Lushchak, 2011). A number of biochemical parameters can be used to evaluate the oxidative stress state generated by metal exposure. These include lipid peroxidation (LPO), formation of protein carbonyls, as well as enzymes which act as defensive mechanisms in fish tissues (Campana et al. 2003; Farombi et al. 2007). The antioxidant system include various enzymes such as superoxide dismutases (SOD), which catalyze the dismutation of superoxide radical to hydrogen peroxide and catalase (CAT), which act to degrade hydrogen peroxide. The glutathione S-transferase (GST) exhibits important detoxifying activities against lipid hydroperoxides generated by inorganic pollutants such as metals (Meyer et al. 2003; Dautremepuits et al. 2004; Farombi et al. 2007). The non-protein thiols (NPSH) levels protect fish tissues against oxidative damage by neutralizing free radicals and other types of ROS. Another parameter used to assess metal poisoning is the activity of the enzyme acetylcholinesterase (AChE). Cholinesterases are

enzymes which play important roles in the central and peripheral cholinergic neurotransmission, hydrolyzing choline esters. Changes on AChE activity in response to exposure of fish to xenobiotics may affect locomotion and balance (Lionetto et al. 2003; Romani et al. 2003). Na^+/K^+-ATPase is a membrane bound enzyme which is important in osmoregulatory tissues such as gill and intestine. This enzyme has important functions such as ion transport, maintenance of the electrochemical gradient and regulation of cell volume. Inhibition of Na^+/K^+-ATPase by xenobiotics may produce adverse effects in the organisms, being an important index for tolerable levels of a large group of environmental contaminants (Atli and Canli, 2007). Xenobiotic may also alter Na^+/K^+-ATPase activity by disrupting energy-producing metabolic pathways or interacting directly with the enzyme (Alam and Frankel, 2006). Fish are able to uptake and retain different xenobiotics dissolved in water via active or passive processes and may be used to detect and evidence pollutants released into their environment. The interest of understanding the physiological mechanisms associated with fish response to environmental stress has been growing. Studying the biological responses to environmental chemicals through the use of biomarkers provides means to understand environmental levels of pollutants in biological terms, and most important, it may be used for the assessment of environmental quality in specific situations.

Fish are widely used for assessment of the quality of aquatic environments, and some fish species serve as good bioindicators of environmental pollution. The silver catfish, *Rhamdia quelen*, is an endemic fish species of South America and is the primary native species raised in southern Brazil. It is a commercially relevant species for fisheries in South Brazil, due to its high-quality meat (Baldisserotto, 2009). However, the toxicity of metals to this species has seldom been studied. The aim of the present study was evaluate the effect of sublethal concentrations of Zn on oxidative damage in various tissues of the silver catfish and test possible specific tissue responses.

2. Materials and Methods

2.1. Chemicals

Malondialdehyde (MDA), 2-thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS) and 2,4-dinitrophenylhydrazine (DNPH) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical reagent grade and purchased from Merck (Rio de Janeiro, Brazil).

2.2. Animals

Male and female silver catfish (8.3 ± 1.2 g and 10.0 ± 1.8 cm) were obtained from a fish farm (Santa Maria, Rio Grande do Sul, Brazil). Fish were acclimated to laboratory conditions for 10 days in 250 L tanks prior to experiments. They were kept in tap water continuously aerated with a static system and with a natural photoperiod (12h light/12h dark). Water conditions were: temperature 24.5 ± 1.3^{o}C, pH 7.5 ± 0.2 units, dissolved oxygen 8.65 ± 0.8 mg/L, non ionized ammonia 0.6 ± 0.04 μg/L, nitrite 0.04 ± 0.01 mg/L, hardness 27.5 ± 2.4 mg/L $CaCO_3$ and alkalinity 39.5 ± 3.0 mg/L $CaCO_3$. The experimental protocol was approved by the Committee on Ethics and Animal Welfare of Federal University of Santa Maria – RS – Brazil under the number: 117/2013.

2.3. Experimental design

2.3.1. Lethal concentration (LC_{50})96 hours determination

After the acclimation period, groups of ten fish were transferred to 40 L boxes for LC_{50} determination. Physico-chemical characteristics of LC_{50} experimental water were as follows: temperature 24.3 ± 1.0 ºC, pH 7.4 ± 0.3 units, dissolved oxygen 7.85 ± 0.6 mg/L, non ionized ammonia 0.8 ± 0.01 μg/L, nitrite 0.05 ± 0.02 mg/L, hardness 27.8 ± 2.7 mg/L $CaCO_3$ and alkalinity 38 ± 2.5 mg/L $CaCO_3$. Nominal concentrations of zinc ($ZnSO_4$. $7H_2O$) were 1.0, 2.5, 5.0, 7.5, 10.0 and 12.5 mg/L. Mortality from each concentration was recorded to the estimation of LC_{50} 96h.

2.3.2. Exposure to sublethal Zn concentrations

Groups of eight fish per box were exposed to different Zn concentrations (0.0, 1.0 or 5.0 mg/L) for 96 h. The fish were not fed during the experimental period. Physico-chemical characteristics of water were as follows: temperature 25.5 ± 1.0 ^{o}C, pH 7.6 ± 0.1 units, dissolved oxygen 8.7± 0.5 mg/L, non ionized ammonia 0.8 ± 0.01 μg/L, nitrite 0.07 ± 0.01 mg/L, hardness 27.6 ± 3.0 mg/L $CaCO_3$ and alkalinity 41 ± 2.5 mg/L $CaCO_3$. Water quality did not change throughout the experimental period. Metal concentration was measured daily by atomic absorption and adjusted when necessary (Table 1).

2.4. Biochemical Parameters

2.4.1. Sample preparation

At the end of the exposure period, eight fish of each group were sampled, the fish were anaesthetized with benzocaine hydrochloride (12 mg/L) and euthanized by punching the spinal cord behind the opercula. Liver, gills, brain, intestine and muscle samples were quickly removed, washed with 150 mM saline solution, packed in teflon tubes and kept at -80°C for the assays of oxidant and antioxidant parameters. Tissue samples were prepared through homogenization (1:5, w/v) in buffer containing Tris-HCl (100mM, pH 7.75), EDTA (2 mM) and $MgCl_2$ (5mM). The homogenates were centrifuged at 10,000 x g for 10 min.

2.4.2. TBARS assay

Lipid peroxidation was estimated by TBARS production, performed by malondialdehyde (MDA) reaction with 2-thiobarbituric acid (TBA), which was spectrophotometrically measured. In liver, gills, brain and muscle homogenates (100-400 µL) were added TCA 10% and 0.67% thiobarbituric acid, totaling a final volume of 1.0 mL. The reaction mixture was placed in a micro-centrifuge tube and incubated for 30 min at 95 ºC and optical density was measured in a spectrophotometer at 532 nm. TBARS levels were expressed as nmol MDA/mg protein according to Buege and Aust (1978).

2.4.3. Protein carbonyl assay

Protein carbonyl content was assessed by the method described by Yan et al. (1995). Briefly, soluble protein (1.0 mL) was reacted with 10 mM DNPH in 2N hydrochloric acid. After incubation at room temperature for one hour in dark, 0.5 mL of denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, containing SDS 3.0%), 2.0 mL of heptane (99.5%) and 2.0 mL of ethanol (99.8%) were added sequentially, vortexed for 40s and centrifuged for 15 min. Then, the isolated protein from the interface was washed twice by resuspension in ethanol/ethyl acetate (1:1), and suspended in 1.0 mL of denaturing buffer and the carbonyl content was measured spectrophotometrically at 370 nm. Assay was performed in duplicate and two blank tubes incubated with 2N HCl without DNPH was included for each sample. The total carbonylation was calculated using a molar extinction coefficient of 22.000 M/cm and expressed as nmol carbonyl/mg protein.

2.4.4. Superoxide dismutase (SOD) activity

SOD activity in liver was performed based on inhibition of the radical superoxide reaction with adrenalin as described by Misra and Fridovich (1972). In this method, SOD present in the sample competes with the detection system for radical superoxide. A unit of SOD is defined as the amount of enzyme which inhibits by 50% the speed of oxidation of adrenaline. SOD activity is determined by measuring the speed of adrenochrome formation, observed at 480 nm, in a reaction medium containing glycine-NaOH (50 mM, pH 10) and adrenaline (1 mM). The activity was expressed as UI SOD/ mg protein.

2.4.5. Catalase (CAT) activity

Catalase activity in liver was assayed by ultraviolet spectrophotometry (Nelson and Kiesow, 1972). 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. Change of H_2O_2 absorbance in 60 s was measured at 240 nm. Catalase activity was expressed in µmol/ min /mg protein.

2.4.6. Glutathione S-transferase (GST) activity

GST activity was measured according to Habig et al. (1974) using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate in liver, gills, brain and muscle. The formation of S-2, 4-dinitrophenyl glutathione was monitored by the increase in absorbance at 340 nm against blank. The activity was expressed as µmol GS-DNB/ min/mg protein.

2.4.7. Non-protein thiols (NPSH)

NPSH was determined in liver, gills, brain and muscle by the method of Ellman (1959). To determine the NPSH levels of tissues an aliquot (400 µL) of supernatant was added in a phosphate buffer 0.5 mM (pH 6.8), 10mM 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), cysteine 0.5 mM. The reaction color was measured at 412 nm. NPSH levels were expressed as µmol/mg protein.

2.4.8. Acetylcholinesterase (AChE) activity

AChE activity was measured based in the method described by Ellman et al. (1961). Aliquots of supernatant (50–100 µL for brain and muscle, respectively) were pre-incubated at 30°C for 2 min with 0.1 M phosphate buffer, pH 7.5, 1 mM DTNB as chromogen. After 2 min, the reaction was initiated by the addition of acethylthiocholine (1 mM) as substrate for the reaction mixture. The final volume was 2.0 mL. Absorbances were determined at 412 nm

during 2 min. Enzyme activity was expressed as mol of acethylthiocholine (ASCh) hydrolyzed/mg protein/min.

2.4.9. Na^+/K^+-ATPase activity

Gill and intestine Na^+/K^+- ATPase activities were assayed using a modification of the method described by Bianchini and Castilho (1999). Na^+/K^+-ATPase activity was determined as the difference between phosphate liberated from ATP in the presence of K^+ (medium A) and in the absence of K^+ with 1 mM of ouabain (medium B). For each assay, 20 µL of the homogenate fraction was added and mixed to 2.0 mL of assay media containing the following final concentrations. The medium A was: 77 mM NaCl, 19 mM KCl, 6 mM $MgCl_2$, 3 mM ATP, and buffer Tris-HCl 0.1 M at pH 7.6. The medium B was: 96 mM NaCl, 6 mM $MgCl_2$, 3 mM ATP, 1 mM ouabain, and buffer Tris-HCl 0.1M at pH 7.6. The reaction started with the addition of the homogenate and was incubated at 30°C for 30 min. The reaction was stopped by adding 0.2 mL of trichloroacetic acid (20%) to the reaction medium. Phosphate concentration in the reaction medium was determined using a modification of the method of Fiske and Subbarow (1925). Enzyme specific activity was expressed as µmols Pi/mg protein/hour.

2.4.10. Protein determination

Protein was determined by the Comassie blue method the Bradford (1976) using bovine serum albumin as standard. Absorbance of samples was measured at 595 nm.

2.5. Statistical analysis

The mean lethal concentration (LC_{50}) for 96h was calculated using the Spearman-Karber test. Biochemical parameters were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test and expressed as mean ± standard error of mean (SEM). Differences were considered to be significant at a probability level of $P < 0.05$.

3. Results

The zinc concentrations in water (mg/L) are showed in Table 1. Measurements of waterborne zinc levels during the experimental periods showed variations of less than 10%. The LC_{50} (96h) obtained for zinc was 8.07mg/L (6.64 – 9.8 mg/L) (Figure 1).

After 96h of exposure, TBARS levels decreased in liver for every concentrations tested compared to control and between zinc exposed groups. In brain, TBARS levels decreased only in relation to the control group. In gills, there was an increase on TBARS levels at 1.0 mg/L. And reduction at 5.0 mg/L compared to control group and 1.0 mg/L. The muscle of silver catfish did not show any variation on TBARS levels after exposure to zinc (Table 2).

Protein carbonyl was reduced in the liver only in fish exposed to 5 mg/L of zinc as compared to control and concentration of 1.0 mg/L. In gills, the decrease occurred for both tested concentrations in relation to control and tested concentrations. Differently in muscle results, there were increase in protein carbonyl in fish exposed to 1.0 and 5.0 mg/L of zinc compared to the control group. In brain, no changes were recorded in protein carbonyl for all concentrations tested (Table 2).

The SOD activity increased in liver of fish exposed to zinc at both tested concentrations compared to the control group (Figure 2A). CAT activity in liver did not have variation after exposure (Figure 2B). GST activity decreased in the liver at both tested concentrations compared to the control group. On the contrary, brain GST activity increased for every tested concentration as compared to the control. Gill showed increase of GST only in the concentration 1.0 mg/L compared to the control group. Muscle GST activity increased at the highest concentration in relation to the control group and 1.0 mg/L of zinc (Table 2).

The NPSH levels in liver and gill did not show any changes for every exposed group to zinc. In brain, there increased the NPSH levels in concentrations of 5.0 mg/L compared to the control group and 1.0 mg/L of zinc. Muscle showed a different response to NPSH levels, exhibiting inhibition of in both tested concentrations compared to the control group (Table 2).

AChE activity in the brain decreased at both tested concentrations compared to the control group (Figure 3A). In muscle, the activity this enzyme did not change after zinc exposure at any tested concentration (Figure 3B).

The Na^+/K^+-ATPase activity showed an inhibition in the intestine and gills at both tested concentrations as compared to the control group (Figure 4A and 4B, respectively).

4. Discussion

The zinc LC_{50} (96h) value for silver catfish is 8.07 mg/L. Others species are more resistant than silver catfish. Gioda et al. (2007) obtained an LC_{50} for piava exposed to zinc of

23.6 mg/L. Ebrahimpour et al. (2010) found a LC_{50} (96h) in soft water of 13.7 mg/L for the species *Capoeta fusca.* Santos et al. (2012) found a LC_{50} (96h) for *Astyanax* aff. *bimaculatus* the value of 10 mg/L of zinc. These values found including the present study to LC_{50} are above of value allowed by the Brazilian legislation (5 mg/L) according to CONAMA - Brazilian National Council of the Environment (CONAMA, 2005).

The present study showed that zinc exposure below LC_{50} concentrations altered TBARS levels in silver catfish tissues. Fish exposed to metal showed decreased TBARS levels in the liver and brain for all zinc tested concentrations. Liver showed SOD increased levels and for brain the combined activity of GST and increased levels of NPSH could explain the reduced of TBARS levels for these tissues. The gills showed different response for concentration of 1.0 mg/L TBARS levels, increasing in the highest concentration and decreasing in 5.0 mg/L. Gioda et al. (2007) also observed a decrease in TBARS levels in brain of *Leporinus obtusidens* (piava) exposed to concentrations of 2.3 and 4.6 mg/L of zinc during 30 days. The same authors also observed an increase in liver TBARS of piava exposed to 4.6 mg/L of zinc for 45 days and observed an increase in the levels of TBARS in muscle of piava exposed to zinc in concentrations of 2.3 and 4.6 mg/L during 30 and 45 days. Loro et al. (2012) observed an increase in TBARS levels in gills, liver and muscle of *Fundulus heteroclitus* exposed to zinc at 0.5 mg/L. In the present study the fish exposed showed the lower LC_{50} than others compared, however, exhibited major antioxidant response against lipid peroxidation, because TBARS levels was increased only in gills in concentration of 1.0 mg/L.

Zinc exposure causes decreased in liver protein carbonyl in the highest concentration and gills decreased for every tested concentration. In muscle, on the contrary an increase in protein carbonyl levels were recorded. Muscle showed GST increase only in concentration of 5 mg/L. This fact was helping the capacity of detoxification of muscle tissue. However, antioxidant defenses were unable to prevent protein damage. The increased of protein carbonyl observed in muscle of *R. quelen* could be compared with Loro et al. (2012) the zinc exposure (0.5 mg/L) caused increase in this parameter in gill, liver and muscle of *F. heteroclitus* after 96h of exposure. The present response pointed the differences concerning fish species out, metal concentrations and specific tissue response.

The enzymatic antioxidant defense system proved to be compromised after zinc exposure. The SOD activity in liver increased in both zinc tested concentrations. The present result evidence the important role of SOD to protect fish tissues against zinc toxicity. Similar results were obtained by Vieira et al. (2011) in goldfish exposed to 1mM of Mn. Loro et al. (2012) also observed increase in SOD activity in liver the *F. heteroclitus* exposed to 0.5 mg/L

of zinc in water with increased salinity. Increased SOD activity combined with the lack of response of CAT, could be responsible for accumulation of hydrogen peroxide (H_2O_2), and then, the excess of H_2O_2 may react forming hydroxyl radicals, which linked to proteins causing damage. The protein damage probably affected enzyme activity and may be confirmed by the increase of protein carbonyl in the muscle tissue of the silver catfish.

The GST constitutes a complex family of proteins which play roles in both normal cellular metabolism and in the detoxification of a wide variety of xenobiotic compounds. They are predominantly cytosolic defense systems responsible for protecting cellular components against various toxic effects and oxidative stress (Sen and Semiz, 2007). In present study, observed a decrease of GST in liver the *R. quelen* in both tested Zn concentrations. Loro et al. (2012) found similar response after exposure of *F. heteroclitus* to 0.5 mg/L Zn in fresh water. Atli and Canli (2010) also observed decreased in liver GST of *Oreochormis nilotis* after acute copper exposure. However, in the same study they observed an increase in hepatic GST of *O. niloticus* on acute and chronic exposure to zinc. The GST activity in brain increased in fish exposed to 1.0 and 5.0 mg/L of zinc. Crupkin and Menone (2013) also observed an increased in the GST activity in brain the *Australoheros facetus* (Cichlidae, Pisces) exposed to 1.53 mg/L of cadmium. In gills, GST activity increased only in a concentration of 1.0 mg/L of zinc. Similar results were observed for Crupkin and Menone (2013) in fish exposed to cadmium in concentrations of 0.31 and 1.53 mg/L. Loro et al. (2012) observed an increased in the GST activity in muscle of the *F. heteroclitus* in water with different salinities. In present study observed an increase in GST activity in muscle of silver catfish exposed to 5.0 mg/L of zinc. The different response observed for GST activity evidence the different role of GST in tissues of *R. quelen* exposed to zinc. In relation to non-enzymatic antioxidants, NPSH levels failed to combat the damage caused by zinc, once showed no changes in the liver and gill, and showed a decrease in muscle. In the brain, there was an increase in the levels of NPSH in the highest concentration. The NPSH was measured indirectly by GSH. The liver is a major site for GSH synthesis, but GSH is transported to other tissues after being synthesized in the liver (Deneke and Fanburg, 1989). The lack of response from NPSH levels in the liver could be due to action of other antioxidants such as SOD. The GSH produced could be used for example to GPx activity, considering that Lipid peroxidation and protein damage were prevented in this organ. Loro et al. (2012) observed a decrease in GSH levels in muscle of *F. heteroclitus* expose to zinc in fresh water. However, found an increase in the levels of GSH in fish exposed to different salinities. Pandey et al.

(2008) found decrease levels of GSH in gill of *Channa punctata* Bloch exposed to multiple trace metals.

Zinc exposure decreased brain AChE activity in both tested concentrations. Similar results were obtained by Pretto et al. (2010) who observed a decreased in AChE activity in brain of silver catfish exposed to cadmium for 14 days. Gioda et al. (2013) also found decreased in AChE activity for brain of piava exposed to zinc 2.3 mg/L for 45 days. Inhibition of AChE may result in excessive stimulation of cholinergic nerves, resulting in behavioral alterations such as tremors, convulsions, and erratic or lethargic swimming. The decrease of AChE activity could be explained by the fact that metals may bind to functional groups of protein, such as imidazole, sulfhydryl groups as AChE or carboxyl (Lima et al. 2013). Once the enzyme is bound to some of these functional groups, its catalytic activity could be compromised, leading to the loss of enzyme function. The AChE response exhibited by brain in the present study is the most common for poisoning or metals such as cadmium, zinc or copper. On the other hand, the exposure of fish to metals may cause an increase (Romani et al. 2003) or no change in enzyme activity (Senger et al. 2006). Zinc poisoning frequently interfere with calcium-mediated neurotransmitter released at neuromuscular junctions. In the brain, zinc could inhibits enzymes such as Mg^{2+}-ATPase or Na^{+}/K^{+}-ATPase causing metabolic effects and disrupting neurotransmitter uptake. In fact, was observed decreased on gills and intestine Na^{+}/K^{+}-ATPase. The similar inhibition, on the brain could be the causes of AChE changes reported here.

Fish gill and intestine are the first target organs to interact with the environment and also have key roles in osmoregulation, which makes them vital to fish organs. The ATPase responses may be used as an early signal of osmoregulatory damage and acid-based regulatory systems in organs, such as gill and intestine. The results of this study showed that the activity of Na^{+}/K^{+}-ATPase was inhibited in gill and intestine in silver catfish for both zinc tested concentrations. Shaw et al. (2012) also observed inhibition of Na^{+}/K^{+}-ATPase in gills of rainbow trout exposed to 20µg/L of $CuSO_4$, but not in the activity of Na^{+}/K^{+}-ATPase in intestine. Baysoy et al. (2013) observed inhibition of Na^{+}/K^{+}-ATPase in gills the *O. niloticus* exposed to 1 µg of chromium in water with 2 ppt salinity as compared to the saline control. Decreases in enzyme activity may be due to membrane damage or deterioration of ion homeostasis. Metals may cause increased epithelial permeability and inhibit active ion uptake, subsequently reducing Na^{+}/K^{+}-ATPase activity and decreasing the number of active chloride cells. This results in a disturbance of normal osmoregulatory function (Atli and Canli, 2007). The decrease in enzyme activity could be associated with the high affinity of metals to –SH

groups on the enzyme molecule, membrane disruption or disturbance of ion homeostasis as showed in the present study.

In conclusion, exposure to zinc in sublethal concentrations altered the oxidant and antioxidant profile in silver catfish (*R. quelen*). These changes may impair the ability of tissues to deal with xenobiotics. From our results, we highlight the inhibition of Na^+/K^+-ATPase activity caused by Zn in gill and intestine of *R. quelen,* indicating changes in ionoregulation. The present study confirm the hypothesis that brain AChE and Na^+/K^+-ATPase activities could be used as a biomarker for zinc contaminated water.

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References

Alam, M., Frankel, T., 2006. Gill ATPase of silver perch, *Bidyanus bidyanus* (Mitchell), and golden perch, *Macquaria ambigua* (Richardson): effects of environmental salt and ammonia. Aquaculture. 251, 118-133.

Almeida, J.A., Diniz, Y.S., Marquez, S.F.G., Faine, L.A., Ribas, B.O., Burneiko, R.C., Novelli, E.L.B., 2002. The use of the oxidative stress as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to in vivo cadmium contamination. Environ. Int. 27, 673-679.

Atli, G., Canli, M., 2010. Response of antioxidant system of freshwater fish *Oreochromis niloticus* to acute and chronic metal (Cd, Cu, Cr, Zn, Fe) exposures. Ecotoxicol. Environ. Saf. 73, 1884-1889.

Atli, G., Canli, M., 2007. Enzymatic responses to metal exposures in a freshwater fish *Oreochromis niloticus*. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 145, 282-287.

Baldisserotto, B., 2009. Piscicultura continental no Rio Grande do Sul: situação atual, problemas e perspectivas para o futuro. Ciência Rural. 39, 291-299.

Baysoy, E., Atli, G., Canli, M., 2013. The effects of salinity and salinity + metal (chromium and lead) exposure on ATPase activity in the gill and intestine of tilapia *Oreochromis niloticus*. Arch. Environ. Contam. Toxicol. 64, 291-300.

Beyersmann, D., 2002. Homeostasis and cellular functions of zinc. Materialwiss. Werkstofftech. 33, 764-769.

Bianchini, A., Castilho, P.C., 1999. Effect of zinc exposure on oxygen consumption and gill Na^+K^+-ATPase of the Estuarine Crab *Chasmagnathus granulate* Dana, 1951 (Decapoda-Grapsidae). Bull. Environ. Contam. Toxicol. 62, 63-69.

Bradford, M.M.A., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.

Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. Methods Enzymol. 52, 302-309.

Campana, O., Sarasquete, C., Blasco, J., 2003. Effecton on ALA-D activity, metallothionein levels, and lipid peroxidation in blood, kidney, and liver of the toadfish *Halobatrachus didactylus*. Ecotoxicol. Environ. Saf. 55, 116-125.

CONAMA (Conselho Nacional do Meio Ambiente), 2005. Resolução CONAMA nº357 de 17/03/05. http://www.mma.gov.br/port/conama/legiabre.cfm?codlegi=459. Access in: 22.01.2014.

Crupkin, A.C., Menone, M.L., 2013. Changes in the activities of glutathione-S-transferases, glutathione reductase and catalase after exposure to different concentrations of cadmium in *Australoheros facetus* (Cichlidae, Pisces). Ecotoxicol. Environ. Contam. 8, 21-25.

Dautremepuits, C., Paris-Palacios, S., Betoulle, S., Vernet, G., 2004. Modulation in hepatic and head kidney parameters of carp (*Cyprinus carpio L.*) induced by copper and chitosan. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 137, 325-333.

Deneke, S.M., Fanburg, B.L., 1989. Regulation of cellular glutathione. Am. J. Physiol. 257, 163-173.

Ebrahimpour, M., Alipour, H., Rakhshah, S., 2010. Influence of water hardness on acute toxicity of copper and zinc on fish. Toxicol. Ind. Health. 26, 361-365.

Ellman, G.L., Courtney, K.D., Andres Jr V., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95.

Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. 82, 70–77.

Farombi, E.O., Adelowo, O.A., Ajimoko, Y.R., 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environment pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogum River. Int. J. Environ. Res. Public Health. 4, 158-165.

Fiske, C.H., Subbarow, Y., 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66, 375-400.

Gioda, C.R., Loro, V.L., Pretto, A., Salbego, J., Dressler, V., Flores, E.M., 2013. Sublethal zinc and copper exposure affect acetylcholinesterase activity and accumulation in different tissues of *Leporinus obtusidens*. Bull. Environ. Contam. Toxicol. 90, 12-16.

Gioda, C.R., Lissner, L.A., Pretto, A., da Rocha, J.B., Schetinger, M.R., Neto, J.R., Morsch, V.M., Loro, V.L., 2007. Exposure to sublethal concentrations of Zn (II) and Cu (II) changes biochemical parameters in *Leporinus obtusidens*. Chemosphere. 69, 170-175.

Habig, W.H., Pabst, M.J., Jacoby, W.B., 1974. Glutathione S-transferase, the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130-7139.

Hershfinkel, M., Silverman, W.F., Sekler, I., 2007. The zinc sensing receptor, a link between zinc and cell signaling. Mol. Med. 13, 331-336.

Huang, E.P., 1997. Metal ions and synaptic transmission: think zinc. Proc. Natl. Acad. Sci. U.S.A. 94, 13386-13387.

Lima, D., Roque, G.M., Almeida, E.A., 2013. In vitro and in vivo inhibition of acetylcholinesterase and carboxylesterase by metals in zebrafish (*Danio rerio*). Mar. Environ. Res. 91, 45-51.

Lionetto, M.G., Caricato, R., Giordano, M.E., Pascariello, M.F., Marinosci, L., Schettino, T., 2003. Integrated use of biomarkers (acetylcholinesterase and antioxidant enzymes activities) in *Mytilus galloprovincialis* and *Mullus barbatus* in an Italian coastal marine area. Mar. Pollut. Bull. 46, 324-330.

Loro, V.L., Jorge, M.B., Silva, K.R., Wood, C.M., 2012. Oxidative stress parameters and antioxidant response to sublethal waterborne zinc in euryhaline teleost Fundulus heteroclitus: protective effects of salinity. Aquat. Toxicol. 110-111, 187-193.

Lushchak, V., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol. 101, 13–30.

MacDonald, R.S., 2000. The role of zinc in growth and cell proliferation. J. Nutr. 130, 1500s-1508s.

Martínez-Álvarez, R.M., Morales, A.E., Sanz, A., 2005. Antioxidant defenses in fish: biotic and abiotic factors. Rev. Fish Biol. Fish. 15, 75 – 88.

McGeer, J.C., Szebedinszky, C., McDonald, D.G., Wood, C.M., 2000. Effect of chronic sublethal exposure to waterborne Cu, Cd, or Zn in Rainbow Trout 1: Ionoregulatory disturbance and metabolic costs. Aquat. Toxicol. 50(3), 231-243.

Meyer, J.N., Smith, J.D., Winston, G.W., Di Giulio, R.T., 2003. Antioxidant defenses in killifish (*Fundulus heteroclitus*) exposed to contaminated sediments and model pro-oxidants: short-term and heritable responses. Aquat. Toxicol. 65, 377-395.

Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247, 3170-3175.

Nelson, D.P., Kiesow, L.A., 1972. Enthalphy of decomposition of hydrogen peroxide by catalase at 25°C (with molar extinction coefficients of H_2O_2 solution in the UV). Anal. Biochem. 49, 474-478.

Oteiza, P.I., Mackenzie, G.G., 2005. Zinc, oxidant-triggered cell signaling, and human health. Mol. Aspects Med. 26, 245-255.

Pandey, S., Parvez, S., Ansari, R.A., Ali, M., Kaur, M., Hayat, F., Ahmad, F., Raisuddin, S., 2008. Effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of a freshwater fish, *Channa punctata* Bloch. Chem. Biol. Interact. 174, 183-192.

Pretto, A., Loro, V.L., Morsch, V.M., Moraes, B.S., Menezes, C., Clasen, B., Hoehne, L., Dressler, V., 2010. Acetylcholinesterase activity, lipid peroxidation, and bioaccumulation in silver catfish (*Rhamdia quelen*) exposed to cadmium. Arch. Environ. Contam. Toxicol. 58, 1008-1014.

Romani, R., Antognelli, C., Baldracchini, F., De Santis, A., Isani, G., Giovannini, E., Rosi, G., 2003. Increased acetylcholinesterase activities in specimens of *Sparus auratus* exposed to sublethal copper concentrations. Chem. Biol. Interact. 145, 321-329.

Santos, D.C.M., Matta, S.L.P., Oliveira, J.A., Santos, J.A.D., 2012. Histological alterations in gills of *Astyanax aff. bimaculatus* caused by acute exposition to zinc. Exp. Toxicol. Pathol. 64, 861-866.

Sen, A., Semiz, A., 2007. Effects of metal and detergents on biotransformation and detoxification enzymes of leaping mullet (*Liza saliens*). Ecotoxicol. Environ. Saf. 68, 405-411.

Senger, M.R., Rosemberg, D.B., Rico, E.P., Arizi, M.B., Dias, R.D., Bogo, M.R., Bonan, C.D., 2006. In vitro effect of zinc and cadmium on acetylcholinesterase and ectonucleotidase activities in zebrafish (*Danio rerio*) brain. Toxicol. In Vitro. 20, 954-958.

Shaw, B.J., Al-Bairuty, G., Handy, R.D., 2012. Effects of waterborne copper nanoparticles and copper sulphate on rainbow trout, (*Oncorhynchus mykiss*): Physiology and accumulation. Aquat. Toxicol. 116-117, 90-101.

Tamir, H.A., Sharir, H., Schwartz, B., Hershfinkel, M., 2004. Extracellular zinc triggers ERK-dependent activation of Na+/H+ exchange in colonocytes mediated by the zinc-sensing receptor. J. Biol. Chem. 279, 51804-51816.

Vallee, B.L., 1988. Zinc: biochemistry, physiology, toxicology and clinical pathology. Biofactors. 1, 31-36.

Vieira, M.C., Torronteras, F.C., Canalejo, A., 2012. Acute toxicity of manganese in goldfish *Carassius auratus* is associated with oxidative stress and organ specific antioxidant responses. Ecotoxicol. Environ. Saf. 78, 212-217.

Winston, G.W., 1991. Oxidants and antioxidants in aquatic animals. Comp. Biochem. Physiol. C. 100, 173-176.

Xu, Y.J., Liu, X.Z., Ma, A.J., 2004. Current research on toxicity effect and molecular mechanism of heavy metals on fish. Mar. Sci. 28(10), 67-70.

Yan, L.J., Traber, M.G., Packer, L., 1995. Spectrophotometric method for determination of carbonyls in oxidatively modified apolipoprotein B of human low-density lipoproteins. Anal. Biochem. 228, 349–351.

Tables

Table 1. Waterborne Zn levels through the experimental period.

Day	0.0 mg/L	1.0 mg/L	5.0 mg/L
1	< 0.004	1.10±0.002	5.01±0.05
3	< 0.003	0.98±0.004	4.85±0.07
5	<0.004	0.95±0.002	4.82±0.06

Table 2. TBARS, protein carbonyl, glutathione S-transferase and non-protein thiols in liver, gills, brain and muscle of silver catfish after exposure to different Zn levels for 96h.

TBARS	Liver	Gills	Brain	Muscle
0.0 mg/L	4.40 ± 0.03^{c}	2.35 ± 0.04^{b}	5.29 ± 0.04^{b}	1.66 ± 0.04^{a}
1.0 mg/L	3.40 ± 0.03^{b}	2.83 ± 0.07^{c}	4.27 ± 0.05^{a}	1.62 ± 0.10^{a}
5.0 mg/L	2.73 ± 0.09^{a}	1.62 ± 0.01^{a}	4.30 ± 0.11^{a}	1.69 ± 0.12^{a}
Protein carbonyl				
0.0 mg/L	5.50 ± 0.19^{b}	7.07 ± 0.19^{c}	6.30 ± 0.19^{a}	5.25 ± 0.05^{a}
1.0 mg/L	$5.36\pm0,11^{b}$	5.12 ± 0.05^{b}	6.53 ± 0.20^{a}	9.21 ± 0.06^{b}
5.0 mg/L	$3.89\pm0,22^{a}$	4.50 ± 0.17^{a}	6.19 ± 0.11^{a}	9.52 ± 0.28^{b}
GST				
0.0 mg/L	0.39 ± 0.003^{b}	0.26 ± 0.017^{a}	0.17 ± 0.010^{a}	0.15 ± 0.002^{a}
1.0 mg/L	0.25 ± 0.009^{a}	0.34 ± 0.014^{b}	0.24 ± 0.013^{b}	0.14 ± 0.004^{a}
5.0 mg/L	0.26 ± 0.013^{a}	0.24 ± 0.010^{a}	0.21 ± 0.012^{b}	0.17 ± 0.004^{b}
NPSH				
0.0 mg/L	0.40 ± 0.005^{a}	0.61 ± 0.050^{a}	0.56 ± 0.015^{a}	0.17 ± 0.005^{b}
1.0 mg/L	0.41 ± 0.018^{a}	0.52 ± 0.010^{a}	0.56 ± 0.020^{a}	0.10 ± 0.004^{a}
5.0 mg/L	0.39 ± 0.004^{a}	0.53 ± 0.006^{a}	0.63 ± 0.010^{b}	0.12 ± 0.010^{a}

Values are means ± SEM, n = 8 fish/group. TBARS is expressed as nmol MDA/mg protein. Protein carbonyl is expressed as nmol carbonyl/mg protein. GST is expressed as µmol GS-DNB/min/mg protein. NPSH is expressed as µmol NPSH/mg protein. Different letters indicated significant differences among the groups. $P < 0.05$

Figures

Figure 1. Mortality rate in silver catfish exposed to different Zn levels for 96h.

Figure 2. SOD (A) and CAT (B) activities in liver of silver catfish after exposure to different Zn levels for 96h. Data are reported as mean ± SEM (n=8, duplicate). Different letters indicated significant differences among groups. $P < 0.05$

Figure 3. AChE activity in brain (A) and muscle (B) of silver catfish exposed to different Zn levels for 96h. Data are reported as mean ± SEM (n=8, duplicate). Different letters indicated significant differences among groups. $P < 0.05$

Figure 4. Na^+/K^+-ATPase activity in intestine (A) and gills (B) of silver catfish after exposure to different Zn levels for 96h. Data are reported as mean ± SEM (n=8, duplicate). Different letters indicated significant differences among groups. $P < 0.05$

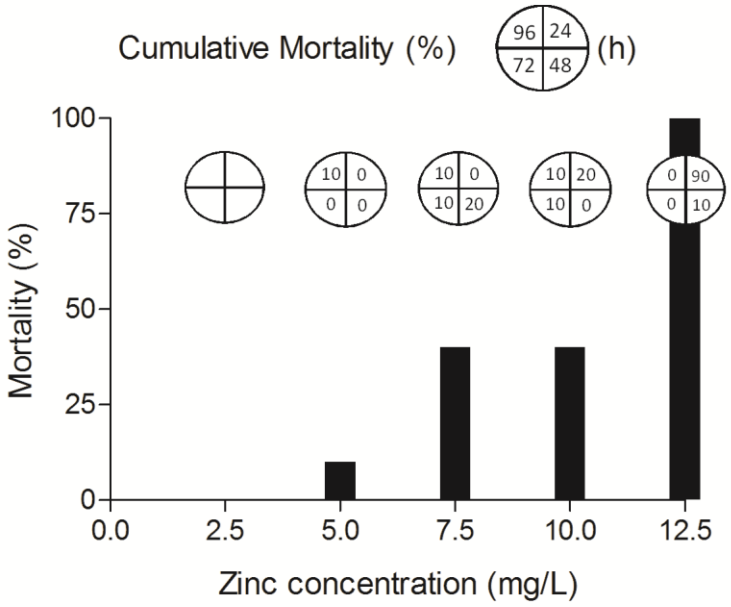

Figure 1.

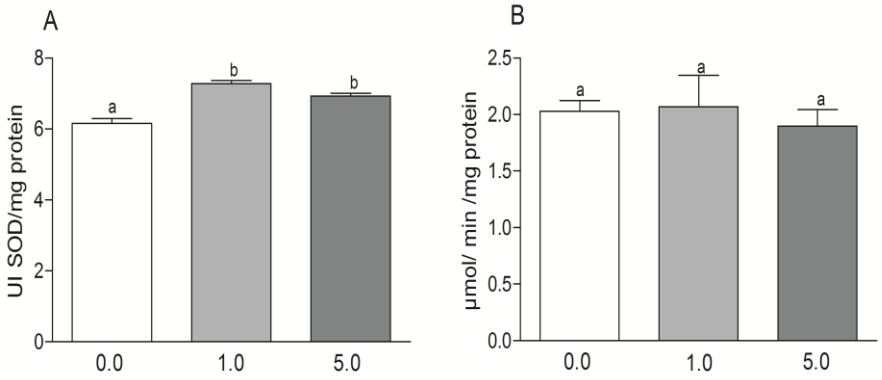

Figure 2

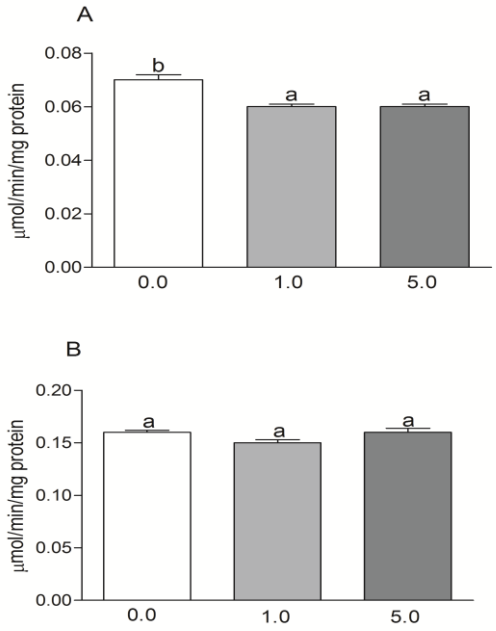

Figure 3

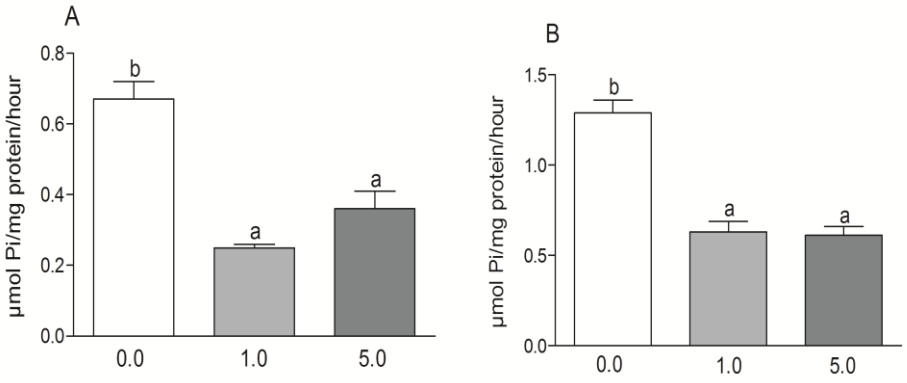

Figure 4

4. CONCLUSÕES

- A concentração letal média do zinco em 96 horas CL_{50} (96h) para *R. quelen* é de 8,07 mg/L.

- A exposição ao zinco inibiu a acetilcolinesterase no cérebro. A inibição da AChE pode resultar na estimulação excessiva dos nervos colinérgicos. Esta diminuição pode ser explicada pelo fato de que metais como o zinco, podem ligar-se aos grupos funcionais da proteína. Uma vez que a enzima é ligada a um destes grupos funcionais, a sua atividade catalítica pode ser comprometida.

- O zinco alterou o perfil oxidante do jundiá de forma diferente entre os tecidos estudados. No fígado, não houve estresse oxidativo, pois houve ação das defesas enzimáticas. A falta de resposta a partir de níveis de NPSH no fígado pode ser devido à ação de outros antioxidantes, tais como SOD. Contudo, no músculo a resposta foi diferente, onde foi possível notar que houve dano às proteínas e que as defesas antioxidantes não foram suficientes para neutralizar o dano. Nas brânquias, o dano lipídico ocorreu na concentração menor, o que pode estar relacionado com aumento da GST nesta concentração. No cérebro, as defesas antioxidantes foram suficientes para reduzir o dano oxidativo.

- O zinco alterou o equilíbrio osmorregulatório nas brânquias e intestino dos jundiás. Os metais podem causar um aumento da permeabilidade epitelial e inibir a absorção de íons, reduzindo a Na^+/K^+-ATPase e perturbando a função osmorregulatória normal.

REFERÊNCIAS

ATLI, G.; CANLI, M. Response of antioxidant system of freshwater fish *Oreochromis niloticus* to acute and chronic metal (Cd, Cu, Cr, Zn, Fe) exposures. **Ecotoxicology and Environmental Safety**, v.73, p.1884-1889, 2010.

ATLI, G.; CANLI, M. Enzymatic responses to metal exposures in a freshwater fish *Oreochromis niloticus*. **Comparative Biochemistry and Physiology, Part C**, v.145, p.282-287, 2007.

BALDISSEROTTO, B.; GOMES, L.C. **Espécies nativas para piscicultura no Brasil.** Santa Maria: Editora da UFSM, 2ªEd, 2010, p.302.

BARCELLOS, L. J. G. et al. Nursery rearing of jundiá, *Rhamdia quelen* (Quoy & Gaimard) in cages: cage type, stocking density and stress response to confinement. **Aquaculture**, v.232, p.383-394, 2004.

BARCELLOS, L. J. G. et al. Hematological and biochemical characteristics of male jundiá (*Rhamdia quelen Quoy & Gaimard Pimelodidae*): changes after acute stress. **Aquatic Research**, v. 34, p. 1465-1469, 2003.

BARCELLOS, L. J. G. et al. Steroid Profiles in Cultured Female Jundiá, the Siluridae *Rhamdia quelen* (Quoy & Gaimard Pisces Teleostei), during First Reproductive Cycle. **General and Comparative Endocrinology**, v. 121, p. 325-332, 2001.

BENGARI, K.V.; PATIL, H.S. Respiration, liver glycogen and bioaccumulation in *Labeo rohita* exposed to zinc. **Indian Journal Comparative Animal Physiological,** v.4, p.79-84, 1986.

BRASIL. Conselho Nacional do Meio Ambiente. Resolução nº 357: http://www.mma.gov.br/port/conama/ (Acesso em: 14.05.14).

CAMPANA, O. et al. Effecton on ALA-D activity, metallothionein levels, and lipid peroxidation in blood, kidney, and liver of the toadfish *Halobatrachus didactylus.* **Ecotoxicology and Environmental Safety,** v.55, p.116-125, 2003.

CANLI, M., ATLI, G. Natural occurrence of metallothionein-like proteins in the liver of fish *Oreochromis niloticus* and effects of cadmium, lead, copper, zinc and iron exposures on their profiles. **Bulletin of Environmental Contamination and Toxicology**, v.70, p.619-627, 2003.

DAUTREMEPUITS, C. et al. Modulation in hepatic and head kidney parameters of carp (*Cyprinus carpio*) induced by copper and chitosan. **Comparative Biochemistry Physiology C Toxicological Pharmacological**, v.137, p. 325-333, 2004.

DE SMET, H.; BLUST, R. Stress responses and changes in protein metabolism in carp *Cyprinus carpio* during cadmium exposure. **Ecotoxicology and Environmental Safety**, v.48, p.255-262, 2001.

FAROMBI, E.O. et al. Biomarkers of oxidative stress and heavy metal levels as indicators of environment pollution in African catfish (*Clarias gariepinus*) from Nigeria Ogum River. **International Journal Environmental Research Public Health**, v.4, p.158-165, 2007.

FATIMA, R.A., AHMAD, M. Certain antioxidant enzymes of *Allium* cepa as biomarkers for the detection of toxic heavy metals in wastewater. **Science of the Total Environment**, v.346, p.256-273, 2005.

GIODA, C.R. et al. Sublethal zinc and copper exposure affect acetylcholinesterase activity and accumulation in different tissues of *Leporinus obtusidens*. **Bulletin of Environmental Contamination and Toxicology**, v.90, p.12-16, 2013.

GIODA, C.R. et al. Exposure to sublethal concentrations of Zn (II) and Cu (II) changes biochemical parameters in *Leporinus obtusidens*. **Chemosphere**, v.69, p. 170-175, 2007.

GOMIERO, L.M.; SOUZA, U.P.; BRAGA, F.M.S. Reprodução e alimentação de *Rhamdia quelen* (Quoy & Gaimard, 1824) em rios do Núcleo Santa Virgínia, Parque Estadual da Serra do Mar, São Paulo, SP. **Biota Neotropica**, v.7, p.127-133, 2007.

LIONETTO, M.G. et al. Integrated use of biomarkers (acetylcholinesterase and antioxidant enzymes activities) in *Mytilus galloprovincialis* and *Mullus barbatus* in an Italian coastal marine area. **Marine Pollution Bulletin,** v.46, p. 324-330, 2003.

LORO, V.L. et al. Oxidative stress parameters and antioxidant response to sublethal waterborne zinc in euryhaline teleost *Fundulus heteroclitus*: protective effects of salinity. **Aquatic Toxicology**, v.110-111, p.187-193, 2012.

LUSHCHAK, V. Environmentally induced oxidative stress in aquatic animals. **Aquatic Toxicology**, v.101, p.13–30, 2011.

LUSHCHAK, V.; BAGNYUKONA, T.V. Effects of different environmental oxygen levels on free radical processes in fish. **Comparative Biochemistry and Physiology, Part B**, v.44, p.283-289, 2006.

McGEER, J.C. et al., Effect of chronic sublethal exposure to waterborne Cu, Cd, or Zn in *Rainbow Trout* 1: Ionoregulatory disturbance and metabolic costs. **Aquatic Toxicology**, v. 50 (3), p.231-243, 2000.

McGEER, J.C.; WOOD, C.M. Protective effects of water Cl^- on physiological responses to waterborne silver in rainbow trout. **Canadian Journal of Fisheries and Aquatic Science**, v. 55, p.2447-2454, 1998.

MEYER, J.N. et al. Antioxidant defenses in killifish (*Fundulus heteroclitus*) exposed to contaminated sediments and model pro-oxidants: short-term and heritable responses. **Aquatic Toxicology,** v.65, p. 377-395, 2003.

MONSERRAT, J.M. et al. Pollution biomarkers in estuarine animals: critical review and new perspectives. **Comparative Biochemistry and Physiology**, v.146, p.221-234, 2007.

ROMANI, R., et al. Increased acetylcholinesterase activities in specimens of *Sparus auratus* exposed to sublethal copper concentrations. **Chemico - Biological Interactions,** v.145, p.321-329, 2003.

SANCHEZ, W. et al. Cooper-induced oxidative stress in three-spined stickleback: relationship with hepatic metal levels. **Environmental Toxicology Pharmacology,** v.19, p.177-183, 2005.

SCHLENK, D.; BENSON, W.H. Target organ toxicity in marine and freshwater Teleosts. Taylor and Francis, London, UK, p.372, 2001.

SIES, H. Strategies of antioxidants defenses. **European Journal of Biochemistry**, v.215, p.213-219, 1993.

TORRES, B.B. **Nutrição e esporte uma abordagem bioquímica.** Departamento de Bioquímica, Instituto de Química, USP, 2003.

ULUTURHAN, E.; KUCUKSEZGIN, F. Heavy metal contaminants in Red Pandora (*Pagellus erythrinus*) tissues from the Eastern Aegean Sea, Turkey. Water Research, v.41, p.1185-1192, 2007.

XU, Y.J. et al. Current research on toxicity effect and molecular mechanism of heavy metals on fish. **Marine Science,** v.28(10), p. 67-70, 2004.

ZHOU, Q. et al. Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. Analytica Chemica Acta, v.14, p.135-150, 2007.