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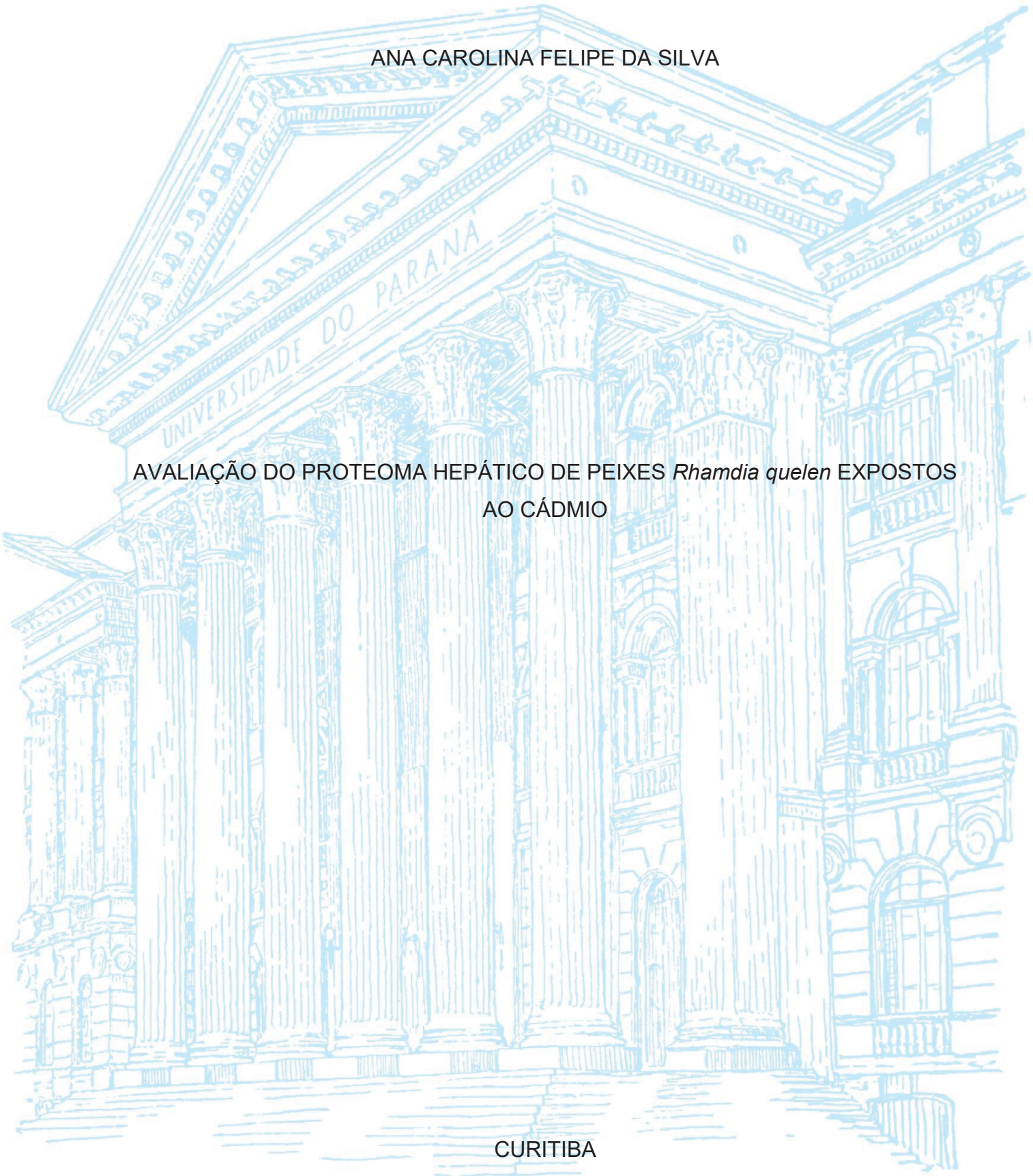

AVALIAÇÃO DO PROTEOMA HEPÁTICO DE PEIXES *Rhamdia quelen* EXPOSTOS AO CÁDMIO

CURITIBA

2024

ANA CAROLINA FELIPE DA SILVA

AVALIAÇÃO DO PROTEOMA HEPÁTICO DE PEIXES *Rhamdia quelen* EXPOSTOS AO CÁDMIO

Dissertação apresentada como requisito parcial à obtenção do título de Mestre em Farmacologia, no Programa de Pós-Graduação em Farmacologia, Setor de Ciências Biológicas, da Universidade Federal do Paraná.

Orientadora: Profa. Dra. Helena Cristina da Silva de Assis

Co-orientadora: Dra. Letícia da Silva Pereira

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação FARMACOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **ANA CAROLINA FELIPE DA SILVA** intitulada: **AVALIAÇÃO DO PROTEOMA HEPÁTICO DE PEIXES Rhamdia quelen EXPOSTOS AO CÁDMIO**, sob orientação da Profa. Dra. HELENA CRISTINA DA SILVA DE ASSIS, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestra está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 27 de Setembro de 2024.

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01/10/2024 09:45:03.0
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It's not that I don't feel the pain

It's just I'm not afraid of hurting anymore

Last Hope - Paramore

RESUMO

A urbanização, industrialização e expansão populacional, associadas a atividades antrópicas, têm causado graves problemas ambientais, como a poluição de ambientes aquáticos com contaminantes potencialmente tóxicos e persistentes. Entre esses contaminantes, o cádmio (Cd) se destaca por sua capacidade de bioacumulação e biomagnificação, representando riscos à saúde humana e ao ecossistema. Por ser um metal não essencial, o Cd pode ser tóxico mesmo em baixas concentrações. Este metal é utilizado em diversas indústrias, como mineração, produção de fertilizantes, pilhas e baterias; por isso, frequentemente atinge corpos d'água e contamina a fauna local. No Brasil, a resolução 357/2005 do Conselho Nacional do Meio Ambiente (CONAMA) estabelece limites permitidos de Cd na água doce entre 1 e 10 µg/L. Embora muitos estudos tenham demonstrado os efeitos tóxicos do Cd em peixes, inclusive no fígado — principal órgão do metabolismo corporal —, há ainda lacunas de conhecimento acerca da diversidade de espécies e resultados contraditórios relatados pelos autores. Nesse contexto, a análise proteômica surge como uma ferramenta que avalia as proteínas diferencialmente expressas indicativas de alterações metabólicas associadas à hepatotoxicidade da exposição ao Cd. Desta forma, o presente estudo avaliou os impactos do Cd no proteoma hepático de peixes machos da espécie *Rhamdia quelen* expostos a concentrações de 0 (controle), 0,1, 1, 10 e 100 µg/L por via hídrica durante 15 dias. A análise proteômica foi padronizada com o banco de dados UniProtKB, mais adequado para espécies nativas que ainda não estão bem anotadas. Em relação às proteínas, foram identificadas 117 proteínas diferencialmente expressas, principalmente nos peixes expostos a 10 µg/L, limite permitido pelo CONAMA. Essas proteínas estão envolvidas em vias metabólicas cruciais para o funcionamento do organismo, incluindo metabolismo de carboidratos (Pklr, Gpia, Gapdh, Ugp2a), produção de energia (Sdha, Fh, Atp5pb, Atp5po), resposta imune (Tom1l2, Kars1), detoxificação (Cyp2y3, Cyp8b1.1, Ugt1ab), e síntese/degradação proteica (Rpl11, Rps24, Psma8, Psmb5). Essas alterações nas vias incluem tanto o aumento como a diminuição da expressão de algumas proteínas, que corroboram outros efeitos danosos para o fígado, como o aumento de estresse oxidativo, principalmente no grupo exposto a 10 µg/L. Esses resultados, portanto, fornecem informações cruciais sobre os mecanismos de toxicidade do Cd no fígado de peixes e indicam a necessidade de reconsiderar os limites permitidos para o Cd na legislação ambiental brasileira.

Palavras-chave: Metal. Jundiá. Proteômica. Hepatotoxicidade. Ecotoxicologia.

ABSTRACT

Urbanization, industrialization, and populational expansion, combined with anthropogenic activities, have led to critical environmental problems, such as the pollution of aquatic environments with potentially toxic and persistent contaminants. Among these contaminants, cadmium (Cd) is notable for its capacity to bioaccumulate and biomagnify, posing significant risks to human health and the ecosystem. As a non-essential metal, Cd can be toxic even at low concentrations. This metal is used in several industries, such as mining, fertilizer production, and batteries; frequently reaching water bodies and contaminating the local fauna. In Brazil, resolution 357/2005 of the National Environmental Council (CONAMA) establishes permissible limits for Cd in freshwater between 1 and 10 µg/L. Although many studies have demonstrated the toxic effects of Cd in fish, including in the liver — the main organ for body metabolism — there are still knowledge gaps about species diversity and contradictory results reported by the authors. In this context, proteomic analysis emerges as a tool that evaluates differentially expressed proteins that may indicate metabolic alterations linked to hepatotoxicity from Cd exposure. The present study evaluated the impacts of Cd on the liver proteome of male *Rhamdia quelen* fish exposed to concentrations of 0 (control), 0.1, 1, 10, and 100 µg/L for 15 days. The proteomic analysis was conducted using the UniProtKB database, which is more suitable for native species with limited annotation. Regarding proteins, 117 differentially expressed proteins were identified, primarily in fish exposed to 10 µg/L, the limit allowed by CONAMA. These proteins are involved in crucial metabolic pathways, including carbohydrate metabolism (Pklr, Gpia, Gapdh, Ugp2a), energy production (Sdha, Fh, Atp5pb, Atp5po), immune response (Tom1l2, Kars1), detoxification response (Cyp2y3, Cyp8b1.1, Ugt1ab), and protein synthesis/degradation (Rpl11, Rps24, Psma8, Psmb5). The observed alterations in these pathways, including both upregulation and downregulation of the proteins, corroborate other evidence of liver damage, such as increased oxidative stress, mainly in the group exposed to 10 µg/L. Therefore, these findings provide valuable insight into the mechanisms of Cd toxicity in fish liver and indicate the need to reconsider the permissible limits for Cd in Brazilian environmental regulations.

Keywords: Metal. Jundiá. Proteomics. Hepatotoxicity. Ecotoxicology.

APRESENTAÇÃO

Esta dissertação é apresentada em formato alternativo, de acordo com as normas do Programa de Pós-Graduação em Farmacologia da UFPR. Este trabalho está composto por uma introdução e objetivos. Na sequência, é apresentado o artigo científico, resultante deste período de mestrado, organizado conforme as normas da revista pela qual foi submetida e com suas respectivas referências. Há, também, a sessão de material suplementar submetido em conjunto com o artigo. Por fim, são apresentadas as considerações finais do trabalho, seguida pelas referências utilizadas nestas seções fora do artigo científico.

LISTA DE FIGURAS E TABELAS

ARTIGO CIENTÍFICO

LISTA DE ABREVIATURAS OU SIGLAS

ABC - *Ammonium bicarbonate*

ACN - Acetonitrila

ANOVA - *Analysis of variance*

ATP - Adenosina trifosfato

Cd - Cádmio

Cd^{2+} - Íon cádmio

$CdCl_2$ - Cloreto de cádmio

$CdSO_4$ - Sulfato de cádmio

CEUA - Comitê de Ética no Uso de Animais

CONAMA - Conselho Nacional do Meio Ambiente

Cu - Cobre

CYP450 - Citocromo P450

DEP - *Differentially expressed protein*

DMSO - Dimetil sulfóxido

DNA - *Deoxyribonucleic acid*

DTT - Dithiothreitol

EPA - *Environmental Protection Agency*

FC - *Fold change*

Fe - Ferro

Fe^{2+} - Ferroso

GO - *Gene ontology*

GSEA - *Gene set enrichment analysis*

HCl - Ácido clorídrico

Hg - Mercúrio

ICP-OES - *Inductively Coupled Plasma Optical Emission Spectroscopy*

KEGG - *Kyoto Encyclopedia of Genes and Genomes*

LFQ - *Label-free quantification*

mRNA - *Messenger RNA*

MS - *Mass spectrometry*

MT - Metalotioneína

NADH - *Reduced nicotinamide adenine dinucleotide*

NADPH - *Nicotinamide adenine dinucleotide phosphate*

NCBI - *National Center for Biotechnology Information*

Ni-Cd - Níquel-cádmio

NSI - *Nanospray ionization*

NUDIX - *Nucleoside diphosphates linked to moiety-X*

OMS - Organização Mundial da Saúde

Pb - Chumbo

PVC - Cloreto de polivinila

RNA - *Ribonucleic acid*

ROS - *Reactive Oxygen Species*

SDS-PAGE - *Polyacrylamide gel electrophoresis with sodium dodecyl sulfate*

TCA - *Tricarboxylic acid*

TFA - *Trifluoroacetic acid*

tRNA - *Transfer RNA*

UniProtKB - *Universal Protein Knowledgebase*

Zn - Zinco

βME - β-mercaptoetanol

SUMÁRIO

1. INTRODUÇÃO

A expansão populacional tem impulsionado a demanda por recursos que assegurem qualidade de vida. Somada a processos antropogênicos de industrialização e urbanização, são gerados extensos níveis de degradação ambiental, resultando na contaminação de ambientes aquáticos. A água, além de essencial para o consumo humano, é um recurso natural amplamente utilizado em diversas atividades, como agropecuária, pesca, geração de energia, processos industriais e lazer (GARCIAS; SANCHES, 2009; EFFENDI, 2016). Corpos d'água como rios, lagos, mares e reservatórios são, muitas vezes, destinos finais de diversos processos naturais e industriais, sofrendo impactos devido ao despejo de efluentes potencialmente tóxicos e capazes de contaminar esse ambiente (CAROLIN et al., 2017), resultando na redução tanto da qualidade como da disponibilidade deste recurso.

Os contaminantes ambientais são substâncias químicas, sintéticas ou naturais, capazes de causar danos à saúde humana, e ao ecossistema (SAUVÉ; DESROSIERS, 2014; MITRA et al., 2022). Dentro dessa classe, metais como mercúrio (Hg), chumbo (Pb) e cádmio (Cd), se destacam. Esses elementos são classificados como não essenciais, pois não estão presentes na dieta e nem possuem função biológica conhecida; e, portanto, podem apresentar respostas de toxicidade desde baixas concentrações (MÉNDEZ-ARMENTA; RÍOS, 2007; ZARCO-FERNÁNDEZ et al., 2016). Além disso, tais metais podem possuir afinidade por íons e proteínas que determinam a alta capacidade de se acumularem na água, em sedimentos, e em tecidos biológicos, aumentando o risco de bioacumulação em organismos aquáticos expostos a estes contaminantes (ASAGBA; ERIYAMREMU; IGBERAESE, 2008; MOISEENKO; GASHKINA, 2016).

Posteriormente a bioacumulação, pode ocorrer o fenômeno de biomagnificação, no qual a concentração de metais aumenta no organismo progressivamente à medida que se avança nos níveis tróficos da cadeia alimentar (ZHU; CHAN, 2012). Tal efeito ocorre porque os metais não sofrem biodegradação (CASTRO-GONZÁLEZ; MÉNDEZ-ARMENTA, 2008; REZANIA et al., 2016).

1.1 Cádmio (Cd)

Descoberto em 1817, o Cd é um metal não essencial conhecido por sua alta toxicidade e potencial carcinogênico (BERTIN; AVERBECK, 2006). Sua forma iônica (Cd^{2+}) forma compostos hidrossolúveis, como o cloreto de cádmio ($CdCl_2$) e o sulfato de cádmio ($CdSO_4$). Este metal é encontrado na natureza associado a erupções vulcânicas, incêndios florestais, e minérios de zinco (Zn), cobre (Cu) e Pb (ZULFIQAR et al., 2022); mas também é liberado por meio de atividades antrópicas como a mineração, queima de combustíveis fósseis, fabricação de pigmentos e estabilizadores de cloreto de polivinila (PVC), fertilizantes fosfatados, além do descarte – muitas vezes incorreto – de pilhas e baterias Ni-Cd e de ligas metálicas (GUMPU et al., 2015; JINDAL; VERMA, 2015; MITRA et al., 2022). A exposição dos seres humanos ao Cd vem principalmente pela ingestão oral de alimentos e água contaminados, e inalação e exposição à fumaça do cigarro e tabaco (VLACHOU et al., 2021; ALBELTAGY et al., 2022). Somado a sua baixa taxa de excreção, resultando em uma meia-vida de até 30 anos (WEN; WANG, 2024), o Cd tem sido associado à diversos tipos de câncer, além de distúrbios ósseos e imunossupressão em humanos (BRIFFA; SINAGRA; BLUNDELL, 2020; CHARKIEWICZ et al., 2023).

Devido às diversas fontes de emissão de Cd, este poluente pode atingir o ambiente aquático por meio de chuvas, uso de fertilizantes e irrigação de áreas agrícolas, percolação do solo, derrames acidentais e carreamento do ar (ARIAS et al., 2007; ASLAM; OKAL; WASEEM, 2023). Dessa forma, águas residuais não tratadas percorrem o solo e lençóis freáticos, atingindo, mais uma vez, os corpos d’água e os organismos que lá habitam. Embora estudos indiquem que o Cd é encontrado naturalmente em concentrações menores que 1 µg/L, consideradas seguras (GARCÍA-REYES; ORTEGA-BARRALES; MOLINA-DÍAZ, 2006; FAROON et al., 2012; KUBIER; HAMER; PICHLER, 2019), algumas regiões do mundo possuem registros de concentrações que excedem, significativamente, os limites estabelecidos por legislações locais. No Egito, por exemplo, foram relatadas concentrações variando entre de 6 e 62 µg/L no Egito (MANSOUR; SIDKY, 2002), enquanto na França os valores foram de 10 a 12 µg/L (BEN SALEM et al., 2014). No Brasil, autores relatam concentrações de 1,8 a 813 µg/L (RIETZLER; FONSECA; LOPES, 2001; CAVALCANTI et al., 2014; LIMA et al., 2015; VIANA et al., 2018).

A Organização Mundial da Saúde (OMS) e a Agência de Proteção Ambiental Americana (EPA, do inglês *Environmental Protection Agency*) estabelecem como seguros limites de 3 a 5 µg/L de Cd na água potável (BRIFFA; SINAGRA; BLUNDELL, 2020). Em relação ao Brasil, a Resolução nº 357 de 2005 do Conselho Nacional do Meio Ambiente (CONAMA), estabelece que os níveis máximos permitidos de Cd variam de acordo com as classes de água doce destinadas ao abastecimento humano. Para as classes de água doce 1 e 2, que passam por tratamento simplificado e/ou convencional, respectivamente, o limite estabelecido é de até 1 µg/L. Já para a água doce de classe 3, que exige tratamento avançado, o limite é de 10 µg/L (CONAMA, 2005). Também, na sua Resolução nº 430 de 2011, que dispõe sobre efluentes lançados na água sem tratamento prévio, o limite máximo permitido é de 200 µg/L (CONAMA, 2011). É importante notar que esta segunda resolução é crucial para estabelecer limites de concentração do metal em diferentes compostos. À vista disso, o Cd se destaca como um dos metais mais persistentes no meio ambiente, apresentando grande potencial de bioacumulação em organismos aquáticos (BERTIN; AVERBECK, 2006; ZHU; CHAN, 2012; GENCHI et al., 2020).

Diante desse cenário, diversos trabalhos – incluindo estudos de nosso laboratório – buscaram avaliar os efeitos desse metal em peixes, por serem organismos constantemente expostos e suscetíveis à contaminação do seu habitat (PEREIRA et al., 2016; VICENTINI et al., 2022). Já se sabe que a exposição ao Cd provoca alterações tanto a nível molecular, na expressão de genes e proteínas, bem como alterações histopatológicas e bioquímicas, afetando principalmente vias de ação antioxidante e de biotransformação (ESPINOZA; WILLIAMS; GALLAGHER, 2012; LIU et al., 2022).

1.2 Danos hepáticos provocados pelo Cd em peixes

Apesar de não possuir função biológica conhecida, as propriedades químicas do Cd permitem a competição com outros cátions divalentes, como cálcio e zinco, alterando a homeostase celular (GENCHI et al., 2020). De acordo com Lee e colaboradores (2023), a toxicidade do Cd está diretamente relacionada à indução do estresse oxidativo, que resultam em danos de membrana, alteração na expressão gênica, ativação de respostas imunes, desencadeamento de mecanismos apoptóticos, e geração adicional de espécies reativas de oxigênio (ROS, do inglês

Reactive Oxygen Species), culminando em morte celular. Além do mais, estudos mostram que a mitocôndria, organela fundamental no metabolismo energético, é uma das principais estruturas celulares afetadas pelo Cd. Danos mitocondriais comprometem a fosforilação oxidativa e síntese de ATP, molécula chave para energia celular (LI; XIE, 2018; LU et al., 2020); algo que pode ser detectado em análises de ciências ômicas, como descrito em breve.

Ao ser absorvido, o Cd liga-se a proteínas plasmáticas e é distribuído aos tecidos, mais especificamente àqueles que possuem alta taxa de produção de metalotioneína (MT), como o tecido hepático – órgão fundamental no metabolismo (ZHU; CHAN, 2012; NORDBERG et al., 2018). As MT são proteínas que contêm grande número de resíduos de cisteína, e por isso se caracterizam por grande afinidade ao Cd, possuindo a principal função de armazenamento e desintoxicação de metais, na tentativa de proteger os tecidos de eventuais danos (BAKIU et al., 2022).

Em peixes, efeitos adversos associados à desregulação endócrina; distúrbios de desenvolvimento e crescimento; e alterações de osmorregulação são comumente observados (DAS; KAR; PATRA, 2023). Inclusive, já foi demonstrado na literatura que o Cd é capaz de induzir hepatotoxicidade através da produção exacerbada de ROS, da interferência na funcionalidade de enzimas do sistema citocromo P450, ativação de vias apoptóticas, bem como na modulação do metabolismo proteico e de carboidratos em diferentes espécies de peixes (DORTS et al., 2011; PRETTO et al., 2014; YANG et al., 2016; CHEN; CHAN, 2018; SOUZA-ARROYO et al., 2022).

No entanto, o mecanismo de toxicidade promovido pelo Cd permanece indefinido. Parte disso se deve porque os estudos avaliam diferentes concentrações de Cd, em diferentes espécies de peixes, e em diferentes tempos de exposição (OKORIE et al., 2014; MIN et al., 2021). Dessa forma, é fundamental que novos estudos sejam feitos e que explorem, através de uma metodologia padronizada, os efeitos do Cd no fígado, a fim de estabelecer as vias metabólicas mais afetadas por esse xenobiótico. Outrossim, também surge a necessidade de considerar dados anteriores e estudá-los sob uma visão mais detalhada.

1.3 Modelo animal – *Rhamdia quelen*

O peixe *Rhamdia quelen* (Quoy and Gaimard, 1824), também conhecido como jundiá, é uma espécie de bagre de água doce, pertencente à ordem Siluriformes e

nativa da América do Sul até o México (GOMES et al., 2000) (FIGURA 1). Esta espécie possui alta relevância gastronômica e comercial no Brasil, devido à alta capacidade de adaptação a diferentes temperaturas, fácil produção, e sabor agradável (BARCELLOS et al., 2001). Por ser uma espécie facilmente encontrada e sensível a alterações ambientais, principalmente àquelas condizentes com o Brasil, se vê grande potencial científico para estudos em ecotoxicologia.

O *R. quelen* tem sido um modelo animal para avaliação de diferentes contaminantes ambientais, dentre eles os agrotóxicos (MELA et al., 2013b; BERNARDI et al., 2022; BANAEE et al., 2023), fármacos (GUILOSKI et al., 2017a, 2017b; RIBAS et al., 2017; KITAMURA et al., 2023), e metais (MELA et al., 2013a; PRETTO et al., 2010, 2011, 2014; PEREIRA et al., 2016; VICENTINI et al., 2022, 2024). Por exemplo, o estudo de Pereira e colaboradores (2016) avaliou o efeito do Cd no tecido hepático de peixes *Rhamdia quelen*. Nele, foram encontradas alterações significativas nas análises bioquímicas, histopatológicas e de genotoxicidade, principalmente relacionadas às enzimas antioxidantes. Entretanto, há ainda uma lacuna em relação a outras proteínas e vias metabólicas que possam estar envolvidas no mecanismo de toxicidade do Cd; o que pode ser feito pela análise proteômica, que permite uma visão mais detalhada dos efeitos da exposição no tecido avaliado.

FIGURA 1. Exemplar de *Rhamdia quelen*.
FONTE: Fernandes, 2018.

1.4 Análise proteômica

A proteômica é uma ferramenta que estuda, em larga-escala, conjuntos de proteínas e suas modificações pós-traducionais de um tecido ou célula em determinada condição, fornecendo informações a nível mecanístico (RODRIGUES et al., 2012; LIANG; MARTYNIUK; SIMMONS, 2020). Essa abordagem vem sendo amplamente empregada para elucidar principais alvos moleculares e mecanismos

fisiológicos da ação das substâncias, devido ao seu importante papel de investigação de alterações na expressão proteica (GAMAGE et al., 2022).

Segundo Steen e Mann (2004), a técnica envolve o uso de amostras biológicas – como cultivos celulares ou tecidos específicos – que passam por um protocolo de extração e digestão de proteínas. Essas amostras são, então, inseridas em equipamentos de cromatografia acoplada a espectrometria de massas para separação e ionização de sequências peptídicas. Este processo gera espectros de massa armazenados em arquivos digitais que, quando analisados com ferramentas de bioinformática e comparados a bancos de dados, possibilitam a identificação das proteínas, bem como de suas funções e envolvimentos em processos metabólicos (FIGURA 2).

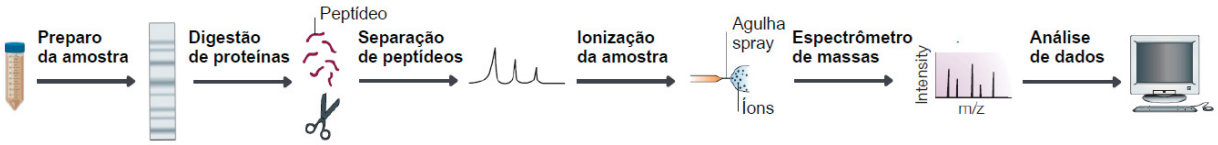

FIGURA 2. Etapas para um estudo de proteômica.
FONTE: Adaptado de Steen e Mann, 2004.

Recentemente, a proteômica tem recebido destaque em estudos de ecotoxicologia, por conta do potencial de busca e caracterização de biomarcadores relacionados a diferentes classes de contaminantes (DORTS et al., 2011; BELLGARD, 2013; GANDAR et al., 2017; LÓPEZ-PEDROUSO et al., 2020). Um estudo de nosso laboratório, realizado por Ribas e colaboradores (2017), demonstrou que a análise proteômica permitiu a visualização de diversas alterações no sistema imunológico de peixes *R. quelen* expostos ao diclofenaco. Vicentini, Simmons e Silva de Assis (2024) relataram alterações em diversas proteínas em *R. quelen* exposto a mudanças de temperatura. Outros estudos da literatura investigaram os efeitos do Cd nas brânquias, fígado e rins de diferentes espécies aquáticas, também encontrando diversas vias metabólicas que podem estar relacionadas, desde vias apoptóticas, até migração e viabilidade celular, sistema antioxidante e metabolismo energético (COSTA et al., 2010; DORTS et al., 2011, 2014; LING et al., 2012).

Embora seja bastante promissora, a proteômica em peixes ainda se mostra bastante limitada. Isso se justifica tanto pela concentração de Cd utilizada nos estudos, como pela diversidade de espécies investigadas (ZHU; CHAN, 2012), baixo número de estudos com espécies nativas, e à dificuldade em estabelecer bancos de dados

para cada espécie. Ao não termos um padrão de resposta, muitos estudos se limitam a modelos experimentais melhores anotados na literatura, como o *Danio rerio* (zebrafish).

2. OBJETIVOS

2.1 Objetivo geral

Avaliar, através da proteômica, os potenciais efeitos de diferentes concentrações de cádmio no tecido hepático de peixes machos *Rhamdia quelen* por exposição hídrica.

2.2 Objetivos específicos

- Analisar e filtrar os dados da espectrometria de massas, referente às proteínas detectadas no fígado dos animais expostos às diferentes concentrações de Cd;
- Analisar as proteínas diferencialmente expressas em cada grupo de exposição ao Cd;
- Identificar as funções moleculares, processos biológicos e vias metabólicas em que as proteínas diferencialmente expressas estejam envolvidas;
- Avaliar se há diferenças no perfil de proteínas alteradas entre os grupos expostos às diferentes concentrações de Cd;
- Discutir as implicações das alterações observadas para o organismo avaliado.

ARTIGO CIENTÍFICO

Submetido

Exposure to cadmium alters metabolic pathways in the hepatic proteome of a Neotropical catfish

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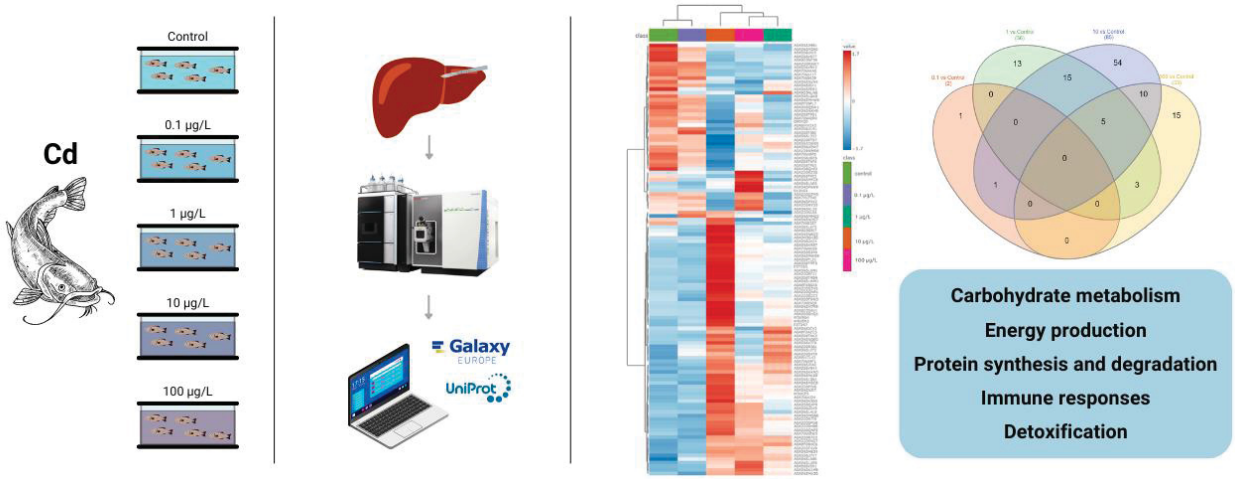
Graphical abstract



ABSTRACT

Cadmium (Cd) is a non-essential metal known for its toxic effects. Its potential to biomagnify and bioaccumulate has been raising concerns about harmful effects on aquatic environments and human beings. However, Cd toxicity effects seem to be variable across species, and its mechanism of toxicity remains poorly understood. This study aimed to evaluate the liver proteome of male *Rhamdia quelen* fish exposed to environmentally relevant concentrations of Cd. For this, fish were allocated in 60 L tanks and exposed to different Cd concentrations: 0.1; 1; 10 and 100 µg/L, in laboratory conditions. After 15 days, fish were anesthetized with benzocaine and after euthanasia the liver was collected for proteomic analysis. It was defined that UniProtKB is the best database for the proteomic analysis of *R. quelen*. From the initial 1,366, 117 differentially expressed proteins were statistically significant, most of them belonging to the group of animals exposed to 10 µg/L. These differentially expressed proteins were mainly related to metabolic pathways concerning energy production (Sdha, Fh, Atp5pb, Atp5po), carbohydrate metabolism (Pklr, Gpia, Gapdh, Ugp2a), protein synthesis and degradation (Rpl11, Rps24, Psma8, Psmb5), immune responses (Tom1l2), and detoxification (Ugt1ab, Cyp8b1.1 and Cyp2y3). Nonetheless, the concentration of 10 µg/L brings the higher major biological relevance, given that this concentration is found in freshwater ecosystems, and can affect several metabolic processes. This study provides crucial information about the mechanism of toxicity of Cd in the *R. quelen* catfish liver, in addition to suggesting a protocol standardization for proteomic analysis in this species.

Keywords: Metal; *Rhamdia quelen*; proteomics; hepatotoxicity; ecotoxicology.

Highlights:

- Cadmium (Cd) can induce stress in the liver of a Neotropical catfish.
- The UniProtKB database is better for proteomic analysis of *Rhamdia quelen*.
- Differentially expressed proteins (DEPs) were observed after Cd exposure.
- DEPs were mainly related to energy production and protein metabolism.
- The findings are relevant for current legislation about Cd limit levels.

3. Introduction

Cadmium (Cd) is a non-essential metal with highly toxic effects on different biological systems (Pereira et al., 2016). Besides its natural presence in the environment, Cd release occurs due to anthropogenic activities, such as mining and fossil fuel combustion, and industrial products, such as Ni-Cd batteries, paint pigments, cigarette smoke, PVC plastics, and chemical fertilizers (Albeltagy et al., 2022; Aslam, Okal and Waseem, 2023). Because of urbanization, climate change, and incorrect disposal of these products, Cd reaches the aquatic environment and affects the living beings in this ecosystem. Added to this, due to the potential of this metal to bioaccumulate and biomagnify, many toxic effects are observed even at exposure to low concentrations, not only in aquatic species but also in humans (Mashhadikhan et al., 2022; Das, Kar and Patra, 2023). Researchers indicate that Cd is one of the most toxic metals found in effluents, due to its physicochemical properties and half-life of up to 30 years, emphasizing that toxic responses can occur both acutely and chronically (Mezynska and Brzóska, 2018; Khan et al., 2022; Wen and Wang, 2024).

The main effects of Cd toxicity in humans include carcinogenesis, renal failure, bone disorders, and immunosuppression (Bertin and Averbeck, 2006; Carolin et al., 2017; Briffa, Sinagra and Blundell, 2020). In fishes, the literature showed the disruption of several systems, such as endocrine, osmoregulation, and development; corroborating gene, tissue, and organ toxicity (Kim, Jee and Kang, 2004; Vicentini et al., 2022; Das, Kar and Patra, 2023). However, instead of merely inducing oxidative stress, substantial evidence in the literature suggests that Cd-induced toxicity affects metabolic pathways such as carbohydrate metabolism, energy production, and protein synthesis and degradation (Paul and Small, 2019; Lu et al., 2020; Souza-Arroyo et al., 2022). In addition, several shreds of evidence suggest that Cd toxic responses are not the same through different species of fish (Tilton, Foran and Benson, 2003; Okorie et al., 2014; Min et al., 2021).

The liver is one of the primary target tissues for xenobiotics metabolism and Cd storage, due to its ability to synthesize metallothionein (MT), a protein that stores transition metals (Liu et al., 2022). Once absorbed from water — either through gills or skin — or ingested, Cd accumulates in the organism as Cd-MT complexes, since it has no metabolic function. After that, as a divalent cation, Cd competes with other divalent ions, such as calcium, magnesium, and zinc, binding to numerous receptors and

enabling antagonistic responses of cellular homeostasis (Lee et al., 2023). Continued exposure to Cd leads to liver overload, promoting apoptosis and necrosis, genotoxic responses, reactive oxygen species (ROS) production, depletion of the antioxidant system, disruption of cellular processes and tissue damage when different organisms are exposed to Cd (Matović et al., 2015; Genchi et al., 2020; Charkiewicz et al., 2023).

In Brazil, the 357/2005 resolution of the National Environment Council (CONAMA) accepts the concentrations of Cd, ranging from 1 to 10 µg/L, in different classes of freshwater (CONAMA, 2005). However, this resolution is outdated, and it is known that these concentrations might promote toxic responses that can reach humans through the food chain (Lee, Choi and Kim, 2022; Liu et al., 2022). In ecotoxicology studies, the Neotropical catfish *Rhamdia quelen* (Teleostei: Siluriformes), a native species from South America, has been extensively used in research due to its commercial relevance, ease of production, and sensitivity to environmental contamination (Barcellos et al., 2001; Vicentini, Simmons and Silva de Assis, 2024). Although biomarker studies have demonstrated toxic responses of Cd in this animal model (Pereira et al., 2016; Vicentini et al., 2022), the metabolic pathways that lead to these responses remain a gap in understanding this metal's toxic mechanism of action. Further research is needed to elucidate the main metabolic pathways affected by this metal.

Proteomics is one of the many -omics approaches that provide detailed comprehension of the toxicity of different components (Dorts et al., 2011). It is known as a tool that measures the total complement of proteins in a given condition, providing information at a mechanistic level and capturing post-translational modifications of proteins, as those proteins directly guide higher-level phenotypic effects (Liang et al., 2018, 2020). More specifically, label-free quantification (LFQ) proteomics is preferable for toxicology studies and biomarker discovery whereas it enables the identification of an unlimited number of proteins in complex matrices such as biological tissues (Yao, McShane and Castillo, 2013; Rozanova et al., 2021). Despite promising, proteomics analysis in native fish species is still poor, due to the low number of species researched. This limitation results in database scarcity and restricts the studies to more established models, such as zebrafish (*Danio rerio*).

This study aimed to establish a method for proteomic analysis in *R. quelen* and evaluate the hepatic proteome after exposure to different Cd concentrations to better understand the Cd mechanism of toxicity.

4. Material and Methods

4.1 Water analysis

During exposure, water samples were collected for Cd concentration analysis through inductively coupled plasma optical emission spectroscopy (ICP-OES), and considering 1 µg/L as the minimum limit of Cd detection (Pereira et al., 2016).

4.2 Animal model and cadmium exposure

Seventy-five adult males of *R. quelen* (14.45 ± 0.30 cm; 25.46 ± 1.69 g) were acquired from local pisciculture (Toledo, Paraná, Brazil), and acclimatized for 30 days at the Environmental Toxicology Laboratory (Curitiba, Paraná, Brazil). Animals were allocated in 60 L tanks at 5 fishes per tank, with filtered and aerated water, a controlled temperature of 26 ± 2º C, and a photoperiod of 12 h. Daily feeding (35% crude protein, Primor, Brazil) was provided. The Ethics Committee approved all animal-use procedures at the Federal University of Paraná (CEUA/BIO-UFPR no. 912).

After acclimatization, each group (n = 15) was exposed, for 15 days, to the following Cd concentrations: control; 0.1; 1; 10, and 100 µg/L. These concentrations were obtained from a stock solution of 1 g/L of cadmium chloride ($CdCl_2$, Sigma-Aldrich). During the exposure period, one-third of the tank water was renewed daily, and more Cd solution was added to maintain Cd concentrations (Pereira et al., 2016). At the end of the exposure period, fish were anesthetized with benzocaine (0.1 µg/L) for 1 minute, followed by euthanasia through a medullary section. Three livers of each group were collected randomly for total protein extraction and proteomic analysis, and the remaining samples were utilized for biochemical biomarkers (Pereira et al., 2016).

4.3 Protein extraction and digestion

Approximately 10 mg of each liver samples wet weight were collected for protein extraction with lysis buffer (SDS 4%; DTT 0.1 M; Tris HCl 0.1 M; pH 7.5) at a 1:5 (m/v) proportion. Homogenization occurred in three cycles of 2 minutes in a Minilys® device, in medium speed, followed by 1 hour in an ultrasonic bath to break DNA strands. Then, samples were centrifuged at 16000 x g for 5 minutes, and the supernatant was collected into a fresh microtube. The total protein concentration was measured in a Nanodrop® device by light absorbance at 280 nm. The sample buffer (SDS 2%; Tris

HCl 80 mM pH 7.5; glycerol 12%; βME 5%; bromofenol blue 0.005%) was added into each sample (40 µg/µL), which was subsequently heated at 95 ºC for 5 minutes.

The samples were run in a 13% SDS-PAGE until they entered the resolving phase of the gel and stained with Coomassie Blue 0.1%. Each lane representing a sample was cut from the gel, placed in a microtube, and bleached in 25 mmol/L ammonium bicarbonate (ABC) solution in 50% ethanol. Then, the gel was dehydrated with ethanol 100% and dried in a centrifugal vacuum evaporator (SpeedVac, ThermoFisher) with a pressure of 10 Pa. Dried samples were reduced with DTT (10 mmol/L) for 1 hour at 56 ºC, alkylated with iodacetamide solution (55 mmol/L) for 45 minutes at 25 ºC, and washed with ABC solution (50 mmol/L).

Following washing, dehydration, and drying, the trypsin solution (12.5 ng/µL, diluted in ABC solution) was added to the samples and kept at 4 ºC for 20 minutes. The excess trypsin was removed, and samples were kept in a digestion buffer (ABC, 50 mmol/L) for 16 to 18 hours at 37 °C. The peptides were extracted with two steps of trifluoroacetic acid (TFA) 3% and acetonitrile (ACN) 30%, followed by two extractions with ACN 100%. The supernatant was dried in SpeedVac for 3 hours at 40°C, and purified by StageTips-C18.

4.4 Nanoliquid chromatography-tandem mass spectrometry (LC-MS/MS) assay

Samples were submitted to an Easy-nLC 1000 liquid chromatograph coupled to hybrid mass spectrometry LTQ Orbitrap XL ETD, from Thermo Scientific. Each run was performed in triplicate. Peptide separation was done with a C18 column (1.9 µm particle diameter x 75 µm capillary internal diameter and 300 mm capillary length). The mobile phase A consisted of 0.1% formic acid and 5% dimethyl sulfoxide (DMSO) in water, while phase B was formic acid 0.1% and 5% DMSO in acetonitrile. Peptides eluted at a flow rate of 250 nL/min, with a linear gradient from 5 to 40% of phase B in 120 min. MS analysis was performed in positive ion mode of 2.7 kV and 100 µA, with an ionization source of nanospray (NSI). The full scan range was m/z of 300 - 2,000 with a resolution of 60,000. The 10 most intense peaks were selected for fragmentation, and the dynamic exclusion was 90 s.

4.5 Database selection

Samples mass spectra were analyzed using Galaxy's MaxQuant version 2.0.3.0 and MaxQuant desktop version 2.2.0.0 (for instrumental parameters, please consult

Table S1 of the supplementary material). For protein identification, the Siluriformes order (taxon ID: 7995) proteome database was obtained from NCBI and UniProtKB, accessed in December 2023, with 755,739 entries and 200,460 entries, respectively. A two-way ANOVA followed by Bonferroni post-hoc was performed for these parameters (number of identified proteins and % of identified MS2 as dependent variables; database and platforms as independent variables) in GraphPad Prism 8.0.1, and the most significant platform and database were included for further analysis.

4.6 Proteomics analysis

Protein relative quantification was done with LFQ intensity. Data were filtered considering only proteins with 2 or more unique peptides; removing potential contaminants, and proteins identified only by site or reversed. Metaboanalyst version 6.0 (Ewald et al., 2024) was used for data analysis, with a t-test and unpaired data. Proteins were considered differentially altered when p-value ≤ 0.05 and fold change (FC) higher than 1.5. These statistically significant proteins had their identification code uploaded to the BLAST function at the selected database, for searching their zebrafish (*D. rerio*) ortholog gene symbols. Then, gene symbols were uploaded for gene ontology (GO) analysis, and biological process and molecular function terms were identified through Panther Classification System 19.0 (Mi et al., 2013). After GO, data were uploaded to ShinyGO 0.80 (Ge, Jung and Yao, 2020) for gene set enrichment analysis (GSEA). Their pathways were identified by the Kyoto Encyclopedia of Genes and Genomes, KEGG (Kanehisa et al., 2021). Missing information was identified through literature research.

5. Results and Discussion

Cd exposure impacts fish tissues through multiple mechanisms (Liu et al., 2022). However, the effects on the liver must be better understood due to different study designs such as exposure duration, concentrations of the contaminant, and species model. In this innovative study, we report the effects of different concentrations of Cd, mainly related to those detected environmentally, on the proteome liver of *R. quelen* fish exposed for 15 days. This section details the definition of the optimal database for such studies, the primary findings linked to the number of proteins, the common metabolic pathways identified; and a comparison with the literature.

5.1 Water analysis of Cd concentration

Water samples from control and 0.1 µg/L groups didn't detect Cd levels above the detection limit (< 1 µg/L), while the groups 1, 10, and 100 µg/L had consistent Cd concentrations compared to those firstly established in each tank, with a standard deviation of ± 10% (Pereira et al., 2016).

5.2 Selected database and platform for data analysis

Two-way ANOVA (Fig 1) showed no significant difference between MaxQuant's desktop and Galaxy versions platforms. The Galaxy platform is an open-source and free-to-use service with public clouds that don't require previous installation or high-performance computers (Afgan et al., 2018). This service allows faster processing, reproducibility, and collaborative analysis with bioinformatic tools like MaxQuant (Pinter et al., 2022). Therefore, it was decided to continue with Galaxy.

However, the UniProtKB database had a higher percentage of identified mass spectra ($F(1, 1) = 2318872$; $P = 0.0004$) and a higher number of identified proteins ($F(1, 1) = 358.9$; $P = 0.0336$), even after filtering contaminants and proteins identified only by site or reversed (Fig 1). This finding can be correlated to the heterogeneity of species and the scarcity of information in non-model organism databases (López-Pedrouso et al., 2020), demanding a higher taxonomic level with enough identified proteins in our study. Besides, the database must have precise and detailed information, and UniProtKB has more accurate and detailed annotations (Bateman et al., 2023). UniProtKB is widely used in ecotoxicology omics studies (Dorts et al., 2011; Azevedo et al., 2020; Yuan, Zheng and Gu, 2021). The NCBI database has the contribution of different repositories, including those from UniProtKB, whose information can be redundant and conflicting. For this reason, the following results were obtained through the Galaxy platform and UniProtKB database (taxon ID: 7995; 200,460 entries).

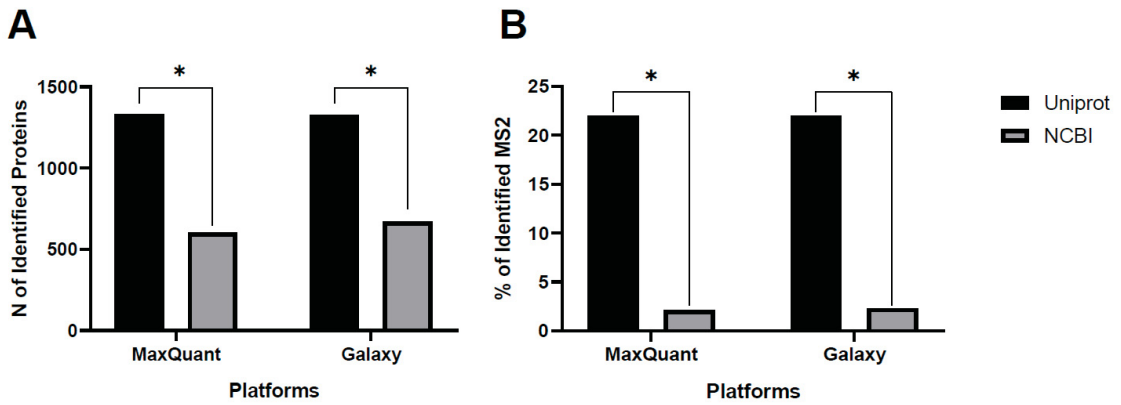


Fig. 1. Analysis of the most suitable database and platform for proteomic analysis of *R. quelen* (Siluriformes). Number of proteins (A) and percentage of MS2 (B) identified in MaxQuant and Galaxy platforms, comparing Uniprot and NCBI databases. (*) denotes statistical significance ($p \leq 0.05$), Two-way ANOVA followed by Bonferroni post-hoc.

5.3 Differentially expressed proteins (DEPs)

A total of 1,366 proteins were identified initially, reduced to 760 when filters were applied, ending in 117 significantly differentially expressed compared to the control group after the t-test (Table S2). It is possible to visualize by the Venn diagram that the groups exposed to 1 and 10 µg/L presented more DEPs (Fig 2). The FC values showed that one protein was up- and another one down-regulated in animals exposed to 0.1 µg/L Cd; 22 proteins were up-regulated and 14 down-regulated in 1 µg/L; 47 up- and 38 down-regulated in 10 µg/L, and, finally, 24 up- and nine down-regulated proteins in 100 µg/L (supplementary material tables S3 - S6). 32 proteins were altered concomitantly in more than 1 group, mainly between groups 1 and 10 µg/L; 5 proteins were changed concomitantly in 1, 10, and 100 µg/L. The heatmap in Fig 3 summarizes how group 10 presented more proteins up- and down-regulated than the other groups.

The key point that must be considered is that the literature describes bioconcentration in the liver as not only time-dependent, but also concentration-dependent and species-dependent (Zhu, Li and Zheng, 2018; Maria et al., 2022). A study by Pereira et al. (2016) demonstrated that *R. quelen* exposed to different Cd concentrations for 15 days resulted in histopathological changes, suggesting tissue damage, and liver bioconcentration starting at 10 µg/L, the concentration in which we found more DEPs. Paul and Small (2021) reported bioconcentration in the liver after exposing *Ictalurus punctatus* to 6 µg/L $CdCl_2$ for 3 and 6 months; Lacave et al. (2020) reported bioconcentration in the liver of *D. rerio* after 21 days of exposure at 10 µg/L. These findings suggest that lower concentrations of Cd, even those environmentally

relevant, accumulate in the liver of different species, increasing its vulnerability to toxic responses.

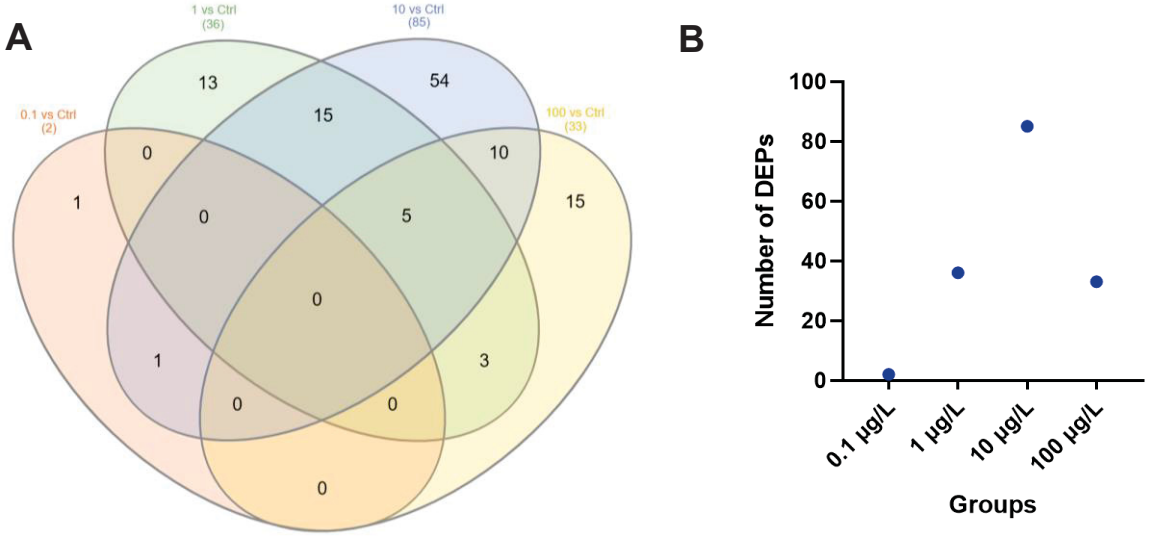


Fig. 2. (A) Venn diagram of the liver proteome of *R. quelen* males exposed for 15 days to different cadmium concentrations (0.1, 1, 10, and 100 µg/L), with the number of differentially expressed proteins (DEPs) in each group when compared to the control group; and (B) representative curve of the number of DEPs in each group when compared to the control group.

Another relevant aspect is that Cd liver toxicity appears to have a non-linear response. The number of DEPs changes directly proportionally as the concentration increases up to 10 µg/L, and inversely proportional subsequently, which may be related to lower toxicity responses at 100 µg/L. Renieri et al. (2017) evaluated Cd toxicity in *D. rerio* for 30 days, along a broad spectrum of exposures: 0, 5, 25, 75, 100, and 1000 µg/L. Their findings showed that the higher concentration had the lowest mortality rate, suggesting a hormesis phenomenon (Calabrese, 2005). This phenomenon indicates that exposure to higher doses of Cd could be less harmful due to the adaptive responses (Sandbichler and Höckner, 2016; Renieri et al., 2017). In any case, it is important to highlight that these are laboratory studies with controlled conditions, unlike field studies in which animals are simultaneously exposed to a mixture of contaminants. Therefore, while this adaptive effect can be observed, it does not invalidate the caution that must be taken with the concentrations of contaminants released into the aquatic environment.

5.4 Metabolic pathways affected due to Cd concentrations

Among the GO analysis of the groups in Panther Classification System, the main molecular functions found included binding and catalytic activity; while the biological processes involved were mainly cellular and metabolic (Fig S1). In a more detailed approach, statistical analysis revealed different patterns of DEPs detected in each group. For example, the group exposed to the lowest concentration of Cd (0.1 µg/L) had two significantly altered proteins: decreased expression of glycine N-methyltransferase (Gnmt), and increased expression of target of myb1-like 2 membrane-trafficking protein (Tom1l2). Gnmt acts as a regulator of DNA methylation and might be correlated with detoxification enzymes (Fang et al., 2010); because of that, this decreased activity can lead to inefficient cellular processes, such as DNA damage and inefficient detoxification. Regarding the Tom1l2, Taylor et al. (2023) indicated that this protein is involved in inflammatory processes and the immune response in *Salmo salar*; thus, the up-regulation of this protein is potentially related to an enhanced immune response to counteract Cd exposure.

According to hierarchical clustering in the heatmap (Fig 3), the groups exposed to 1 and 100 µg/L of Cd demonstrated statistical proximity. Alterations in biological processes, particularly cellular organization, were highlighted in these groups. Notably, lamin-B2 (Lmnb2) and histone H2A (H2az2a), proteins associated with heterochromatin formation and nuclear integrity, were up-regulated, suggesting an attempt to maintain nuclear integrity and a defense mechanism for chromatin condensation to prevent the transcription of proteins that are possibly linked to Cd-induced stress (Hashimoto, Majumdar and Tsuji, 2017). Moreover, significant changes in cytoskeleton activity, essential for cellular integrity, shape, and motility, were observed (Dorts et al., 2014). Specifically, the up-regulation of spectrin beta chain (Sptbn1) and septin (Septin6), proteins related to cytoskeleton organization, were detected at 100 µg/L. In a similar manner, Chen et al. (2022) reported up-regulation of cytoskeleton proteins of *Gobiocypris rarus* larvae, indicating that Cd exposure can disrupt cell organization. In addition, the decreased expression of vacuolar protein sorting-associated protein 29 (Vps29) and sodium bicarbonate transporter-like protein 11 (Slc4a11), proteins involved in endocytic recycling, intracellular and transmembrane transport, suggests a link to Cd accumulation within the cell, leading to imbalances in membrane integrity, transcription, and metabolite transport (Haydon and Cobbett, 2007; Yao et al., 2022).

Two other proteins must be considered: RNA helicase (Ddx3xa) and lysine-tRNA ligase (Kars1). Ddx3xa is part of a group of RNA helicases that play an important role in regulating genes involved in gonadal differentiation and development in teleost fishes (Tian et al., 2017). In this context, exposure to 1 µg/L of Cd led to the up-regulation of Ddx3xa, suggesting an advanced stage of gonadal maturation that could be correlated with liver bioconcentration of this metal (Kime et al., 1996; Vicentini et al., 2022). Kars1, on the other hand, belongs to a recently identified family of aminoacyl-tRNA synthetases, involved in triggering or silencing immune responses and apoptosis, beyond regulating protein translation (Guo and Schimmel, 2013). In this study, Kars1 was significantly down-regulated at 10 and 100 µg/L (Tables S5-S6). This suggests that, under Cd-induced stress, the reduced expression of Kars1 in the liver may represent an attempt to balance the immune response with detoxification, without triggering apoptosis in healthy cells. However, it could also indicate an immune system compromised by Cd. Although few studies on zebrafish have explored the neurological effects of aminoacyl-tRNA synthetases (Waldron et al., 2017; Giong and Lee, 2022), there is still a lack of evidence that Cd has the potential to cause these effects, requiring further research.

The cytochrome P450 (CYP450) superfamily is one of the most important biomarkers of liver metabolism, as they give a warning of xenobiotics in the aquatic environment (Al-Arabi and Goksøyr, 2002; Savassi et al., 2020). Previous studies have shown decreased expression of different enzymes of the CYP450 family in zebrafish exposed to Cd, reducing the detoxification capacity (Yang et al., 2016; Chen and Chan, 2018). Our observations are consistent with those reported, because cytochrome P450, family 2, subfamily Y, polypeptide 3 (Cyp2y3) was deeply down-regulated not only at 1 µg/L but also at 10 µg/L, while cytochrome P450, family 8, subfamily B, polypeptide 1 (Cyp8b1.1) was up-regulated only at 10 µg/L, reinforcing the metabolic imbalance caused by Cd exposure.

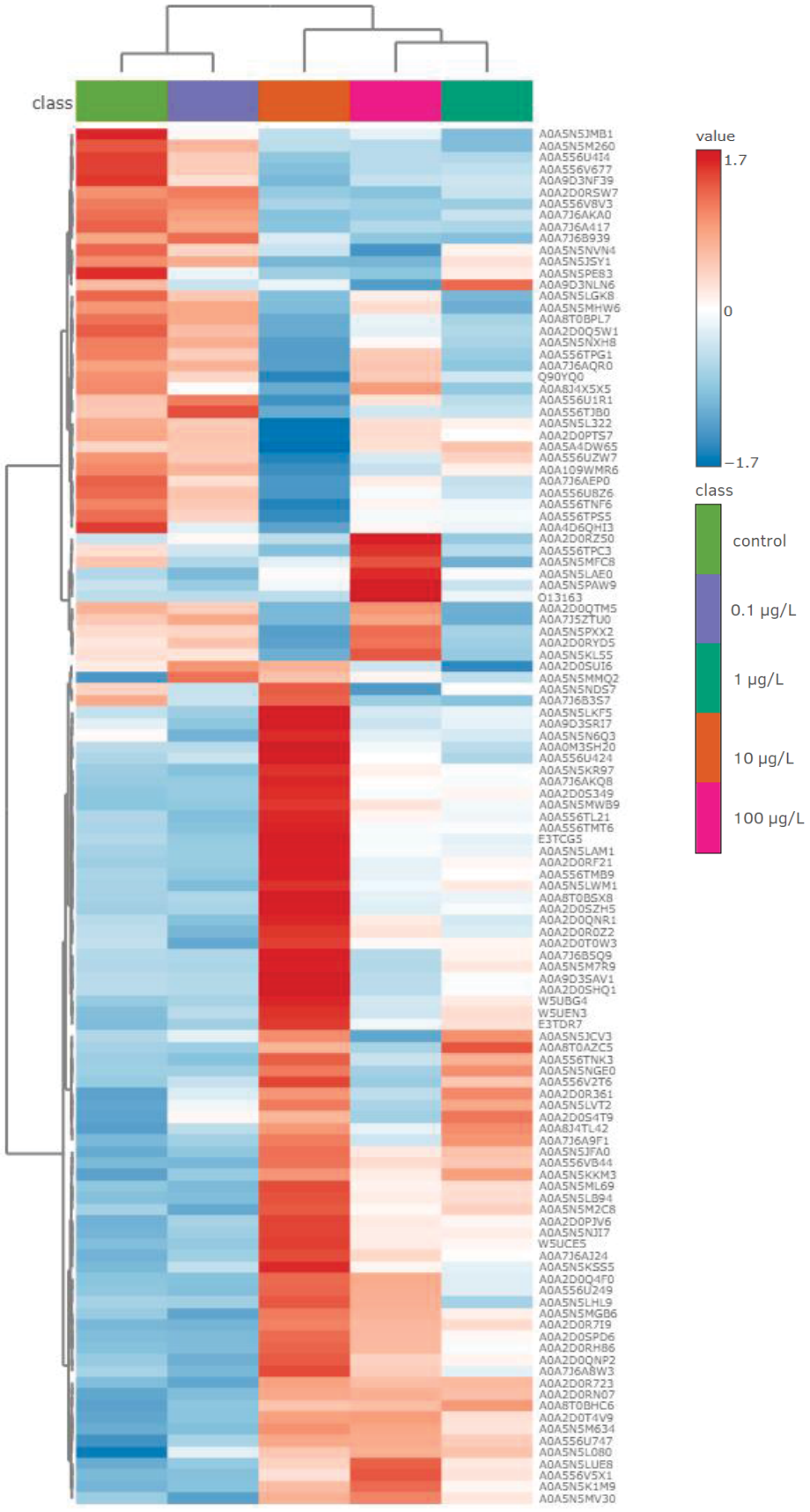


Fig. 3. Heatmap created by MetaboAnalyst representing intensity changes of DEPs in the liver of *R. quelen* exposed for 15 days to different cadmium concentrations (0.1, 1, 10 and 100 µg/L).

Focusing on the group with major expression alteration, 10 µg/L, solute carrier organic anion transporter (Slco1d1), an important transporter for xenobiotics and steroid hormones in the fish liver (Popovic et al., 2013), also showed increased expression. This could be linked with enhanced transport of Cd into the cells, promoting its toxicity and leading to liver injury, as proposed by Thévenod (2010) for mammalian cells. Another possibility is that Cd could interact with Fe^{2+}, inhibiting its absorption in intestinal cells and, consequently, disrupting Fe homeostasis, similar to the observations of Kwong and Niyogi (2009) in rainbow trout (*Oncorhynchus mykiss*).

Additionally, glyceraldehyde-3-phosphate dehydrogenase (Gapdh), a well-known enzyme from gene expression studies, also exhibited increased expression at 10 µg/L. While Gapdh primarily plays a role in glucose metabolism (Paul and Small, 2021); it also has significant immunomodulatory capacities, as described by Cho et al. (2008) in response to viral and bacterial infections in the marine teleost *Oplegnathus fasciatus*. The literature indicates that oxidative stress can also modulate Gapdh expression, which may be an indirect effect of Cd toxicity (Shekh et al., 2017). Thus, the up-regulation of Gapdh in our model is an expected result. In terms of the immunological response, Tom1l2, the same protein expressed at 0.1 µg/L, was also up-regulated at 10 µg/L, reinforcing its role in mitigating Cd adverse effects.

Proteins with transcriptional and post-transcriptional roles were also identified. Argonaute-2 (Ago2), a member of the argonaute family, was first characterized in the rare minnow, *Gobiocypris rarus*, by Su et al. (2009). This protein regulates translation and miRNA processing in antiviral and antibacterial defense. To the best of our knowledge, its role in other pathways, such as Cd detoxification, remains unclear. Because of that, we propose that the increased expression of Ago2 at 10 µg/L may be linked to a post-transcriptional response, though further investigation is needed to confirm this. Another up-regulated protein particularly involved in gene regulation is the nudix hydrolase 21 (Nudt21). Nudt21 is part of the NUDIX family, associated with numerous metabolic pathways, including mRNA processing and the removal of toxic metabolites (Bhandari et al., 2023). This finding suggests that Nudt21 expression is correlated to detoxification mechanisms, but these effects in fish require further study.

Among the DEPs identified as being concomitantly altered in animals exposed to 1, 10, and 100 µg/L Cd, 17β-hydroxysteroid dehydrogenase 10 (Hsd17b10) is the most up-regulated protein detected. This enzyme is crucial in the steroidogenesis pathway from a common precursor, cholesterol, oxidizing fatty acids (Steckelbroeck et

al., 2003). Hsd17b10 was detected in the liver of *Paralichthys olivaceus*, indicating its involvement in metabolic processes, including the catabolism of sex hormones (Zou et al., 2020). Hemoglobin α-adult 1 (Hbaa1) was down-regulated in 1 and 10 µg/L. Hbaa1 is expressed not only in erythrocytes and seems to have a crucial role in oxygen transport and reducing ROS production through the Fe^{3+} state stability (Butcher et al., 2014; Farhadi et al., 2023), therefore having importance in energy production (Dangre, Manning and Brouwer, 2010). Given these proteins' expression are mainly altered at 1 and 10 µg/L, Cd must disrupt liver function at initial concentrations due to oxidative stress, and promote adaptive responses afterward. Another important protein detected was aspartyl-tRNA synthetase (Dars1), essential for amino acid metabolism, which was deeply down-regulated in these three groups. This protein is related to motor impairment in the brain (Ma et al., 2020) and fatty acid metabolism in the liver (Klugmann et al., 2022). Poynton et al. (2011) characterized that the most disrupted KEGG metabolic pathways of *Daphnia magna* exposed to Cd involved transcriptional responses and aminoacyl-tRNA biosynthesis, highlighting its needful role in cellular homeostasis.

5.5 GSEA reveals the main metabolic pathways affected by Cd

The GSEA revealed 10 main biological pathways that had DEPs: starch and sucrose metabolism, proteasome, pyruvate metabolism, tricarboxylic acid cycle (TCA cycle), carbon metabolism, glycolysis/gluconeogenesis, biosynthesis of amino acids, oxidative phosphorylation, ribosome, and metabolic pathways (Figure 4, Table S7).

The literature has already demonstrated that Cd disrupts hepatic carbohydrate metabolism in fish (Soengas et al., 1996; Annabi, Said and Messaoudi, 2013). In our study, the GSEA revealed 4 proteins up- and 5 down-regulated that have a relationship with carbohydrate metabolism (Table S7). At 1 µg/L, glycogen [starch] synthase (Gys1) and hydroxyacylglutathione hydrolase (Hagh), components of gluconeogenesis and pyruvate metabolism, respectively, were decreased. At 10 µg/L, Hagh was also decreased, but now with alpha-1,4-glucan phosphorylase (Pygb), and triosephosphate isomerase B (Tpi1b); while glucose-6-phosphate isomerase (Gpia), UTP-glucose-1-phosphate uridylyltransferase (Ugp2a), and Gapdh had increased expression. All these enzymes are involved in gluconeogenesis and glycolysis. Finally, at 100 µg/L, Ugp2a, from gluconeogenesis, and pyruvate kinase (Pklr), from pyruvate metabolism, were increased.

Although the altered proteins varied depending on the concentration of Cd that fish were exposed to, an imbalance of carbohydrate metabolism suggests adaptive responses to a greater metabolic demand on glucose production and energy expenditure (Zheng et al., 2023). In fact, Paul and Small (2019) evaluated the effects of Cd on the early-life stages of *Ictalurus punctatus* and discussed that Gapdh is a rate-limiting step of the production of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) through glycolysis and pentose phosphate pathway. As we observed, the elevated expression of Gapdh indicates not only inflammatory responses, as previously mentioned, but also greater energy demand for fish development due to oxidative stress, suggesting a Cd detoxification response in the catfish liver (Cuypers et al., 2010; Pretto et al., 2014).

Carbohydrate metabolism, TCA cycle, and oxidative phosphorylation have interconnected biochemical reactions, acting directly or indirectly in energy production. Moreover, research reveals that mitochondria is the main organelle affected by Cd exposure (Dorts et al., 2014; Go et al., 2014). Succinate dehydrogenase (Sdha), fumarate hydratase (Fh), and malate dehydrogenase (Mdh2), enzymes of the TCA cycle (Raimundo, Baysal and Shadel, 2011), were mostly up-regulated after Cd exposure. TCA cycle also seems to be involved in the immune response by promoting phagocytosis (Choi, Son and Baek, 2021; Yang et al., 2021). Therefore, increased Sdha, Fh, and Mdh2 expression at 10 µg/L suggests susceptibility to infection by a compromised immune system and a higher demand for cellular energy for different metabolic processes. Another process involved in energy production is beta-oxidation, which takes place in contexts of high-energy demand and depleted glycogen storage in different nutritious diets (Zhu et al., 2016). As we mentioned earlier, whereas carbohydrate metabolism is probably disrupted, beta-oxidation must be an alternative pathway. In this context, one interesting finding is the increased expression of the medium-chain specific acyl-CoA dehydrogenase (Acadm), a beta-oxidation enzyme, pointing to the necessity of energy production.

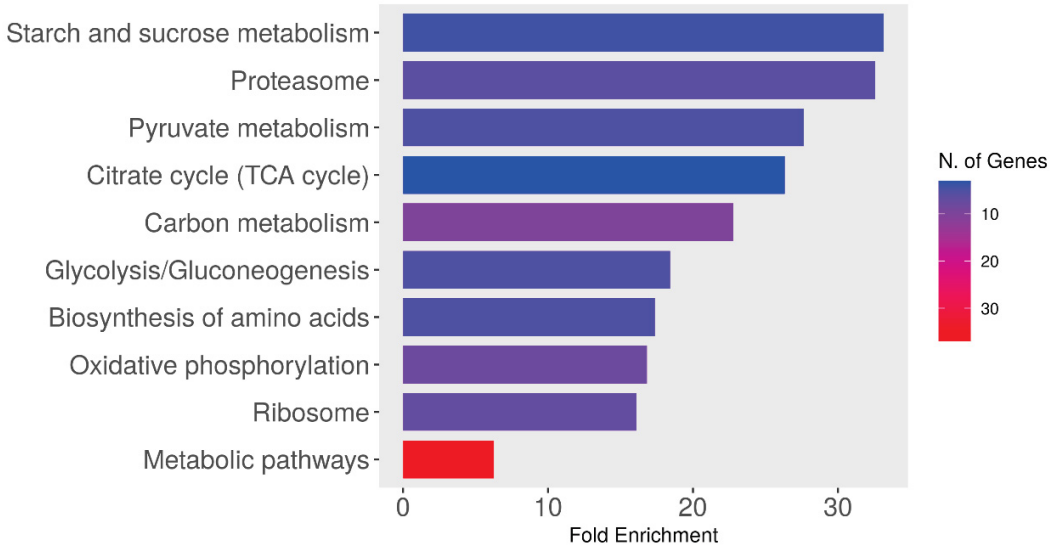


Fig. 4. Main altered metabolic pathways revealed by the GSEA, through ShinyGO platform, in the catfish liver exposed to Cd, mainly 10 µg/L.

Oxidative phosphorylation is the main cellular process involved in energy production. This pathway comprises five complexes with electron transfer roles, creating an energy gradient that drives the synthesis of ATP. Our study showed elevated expression levels of NADH-ubiquinone oxidoreductase (Ndufs1), Sdha, cytochrome c and c-1 (Cyc and Cyc1), as well as ATP synthase subunits O, B, and alpha (Atp5po, Atp5pb, Atp5fa1) after exposition to Cd. This finding suggests an increased energy demand and expenditure in the liver of *R. quelen* exposed to Cd, mainly 10 µg/L. Similarly, Li and Xie (2018) observed that *Silurus meridionalis* increased the energetic cost of maintaining life after Cd stress. However, this increased demand is also correlated with the production of ROS, due to the transfer of electrons to oxygen or water molecules, leading to the formation of superoxide radical, hydrogen peroxide, or hydroxyl radical (Wang et al., 2004; Lu et al., 2020).

Energy production is directly related to the general metabolism of the organism, which includes how it is going to fight against environmental contamination. Several proteomic investigations have concluded that Cd induces oxidative stress (Costa et al., 2010; Lu et al., 2012); however, the exact mechanism of Cd-induced stress is not fully understood (Go et al., 2014). Contrary to the literature, our results didn't show altered levels of expression of the proteins from the antioxidant system. Nevertheless, regarding detoxification processes, we detected glucuronosyltransferase (Ugt1ab) down-regulated at 10 µg/L. Ugt1ab has an important ecotoxicological relevance given its activity in phase II of xenobiotic metabolism by adding hydrophilic groups to facilitate

excretion (Gaworecki, Rice and Van Den Hurk, 2004). Thus, decreased expression of Ugt1ab implies inefficient detoxification responses in fish exposed to Cd, leading to cellular damage.

For these cellular responses to occur, fundamental processes like protein synthesis and degradation must work properly. Zhang et al. (2017) describe ribosomal proteins as basic components for protein synthesis and ribosome assembly itself. In our study, significant changes were observed in this pathway within the catfish liver exposed to 10 µg/L. Large ribosomal protein subunits Rpl11, Rpl23, Rpl30, and Rpl32, along with small ribosomal protein subunits Rps3a and Rps24, were down-regulated; while only the large ribosomal protein subunit L31 (Rpl31) was up. This likely reflects reduced translational processes and disrupted protein synthesis. Similarly, Ma et al. (2019) and Vicentini, Simmons, and Silva de Assis (2024) also found decreased expression of ribosomal proteins in the liver of grass carp (*Ctenopharyngodon idellus*) exposed to enrofloxacin and *R. quelen* exposed to temperature stress, respectively, underscoring the importance of ribosome function in ecotoxicology studies. When it comes to the degradation of misfolded or damaged proteins, proteasome is one of the main systems for this function. We found that proteasome subunits alpha Psma1 and Psma4, and Proteasome subunit beta Psmb5 were down-regulated, while proteasome subunit alpha Psma8 was up. Our data align with previous studies on *Cottus gobio* exposed to sublethal concentrations of Cd (Dorts et al., 2011) and zebrafish hepatic cell line treated with Cd (Chen and Chan, 2018), concluding that Cd is capable of interfering with protein degradation by directly altering the expression of proteasome proteins, potentially leading to cellular damage.

6. Conclusion

Proteomics, a highly sensitive technique, enables the identification of protein patterns that can be used as potential biomarkers for specific contaminants in ecotoxicology. This study established a method for proteomic analysis of *R. quelen* catfish and highlights the biological effects of environmentally relevant Cd concentrations in this species. Our primary findings reveal significant metabolic alterations in fish exposed to 1 and 10 µg/L, the same permitted by Brazilian freshwater regulations. The data indicate that Cd-induced stress elevates immune responses and energy demand through pathways involving carbohydrate metabolism, the TCA cycle,

oxidative phosphorylation, detoxification processes, and protein synthesis and degradation. These results suggest that certain metabolic pathways mitigate adverse effects of Cd on the catfish liver. This is a concern for both aquatic fauna and human population, emphasizing the need to review on the permissible Cd levels in freshwater, particularly those intended for consumption.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution

Ana Carolina Felipe da Silva: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. Letícia da Silva Pereira: Writing – review & editing, Visualization, Supervision, Methodology. Rodrigo Soares Caldeira Brant: Writing – review & editing, Methodology. Maiara Vicentini: Writing – review & editing, Visualization, Supervision. Helena Cristina Silva de Assis: Writing – original draft, Supervision, Funding acquisition, Conceptualization.

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8. Supplementary Material

Table S1: Parameters for MaxQuant and Galaxy analysis.

Parameter version	2.0.3.0 and 2.2.0.0
Date of writing	2024 January, 8
Include contaminants	True
Use normalized ratios for occupancy	True
Minimum peptide length	7
Minimum score of unmodified proteins	0
Minimum score of modified proteins	40
Minimum unique peptides	0
Minimum peptides	1
Modifications included in protein quantification	Oxidation (M); Acetyl (Protein N-term)
Stabilize large LFQ ratios	True
Separate LFQ in parameter groups	False
Require MS/MS for LFQ comparisons	True
Calculate peak properties	False
Maximum peptide mass (Da)	4600
Minimum peptide length for unspecific search	8
Maximum peptide length for unspecific search	25
Evaluate variant peptides separately	True
MS/MS tol. (FTMS)	20 ppm
Top MS/MS peaks per Da interval. (FTMS)	12
Da interval. (FTMS)	100
MS/MS deisotoping (FTMS)	True
MS/MS deisotoping tolerance (FTMS)	7
MS/MS deisotoping tolerance unit (FTMS)	Ppm
MS/MS higher charges (FTMS)	True
MS/MS water loss (FTMS)	True
MS/MS ammonia loss (FTMS)	True
MS/MS dependent losses (FTMS)	True
MS/MS recalibration (FTMS)	False

Table S2: Significantly differentially expressed proteins (DEPs) information of *Rhamdia quelen* exposed to different concentrations of Cd for 15 days.

Uniprot Entry	Peptides	Unique peptides	Sequence coverage [%]	Unique sequence coverage [%]	Mol. weight [kDa]	Score	id
A0A5N5MMQ2	3	3	8.3	8.3	56.601	13.831	1011
A0A5N5N6Q3	5	5	22	22	33.341	13.597	1063
A0A7J5ZTU0	2	2	23.3	23.3	11.015	3.1771	838
A0A8T0AZC5	3	3	3.2	3.2	158.04	16.658	1300
A0A556VB44	2	2	9.5	9.5	27.655	11.738	773
A0A5N5KL55	4	4	22.4	22.4	15.849	23.903	898
A0A7J6A9F1	2	2	20.7	20.7	15.215	11.115	1211
A0A2D0QTM5	3	3	37	37	14.343	19.309	190
A0A5N5LVT2	2	2	9.2	9.2	28.2	8.2379	983
A0A5N5MHW6	3	3	11.7	11.7	34.159	4.9567	1022
A0A5N5L080	2	2	8.2	8.2	64.881	8.1107	920
A0A5N5NGE0	9	9	34.3	34.3	35.217	33.805	1079
A0A7J6AKQ8	4	4	4.4	4.4	66.52	3.7596	1221
W5UEN3	21	21	43.7	43.7	61.173	195.04	1122
A0A5N5LGK8	6	5	28.2	24.2	46.971	24.292	946
A0A5N5KKM3	3	3	22.9	22.9	28.564	15.898	897
W5UBG4	9	6	22.3	14.9	55.079	44.029	1359
A0A556V8V3	11	4	24.4	10.3	59.135	13.935	761
A0A2D0Q5W1	4	3	17.2	12.6	27.184	23.615	130
A0A556TMB9	4	4	6.2	6.2	103.27	11.957	518
A0A5N5NJI7	6	6	30	30	27.809	18.477	1082
A0A5N5M260	3	3	25.3	25.3	20.438	5.4007	422
A0A8T0BHC6	4	4	21.3	21.3	37.469	14.823	1310

A0A5N5M634	22	22	13.5	13.5	274.11	42.528	1001
A0A2D0RN07	10	10	3.2	3.2	532.78	17.849	280
A0A7J6A417	7	7	13.3	13.3	56.25	22.972	1204
A0A7J6B3S7	8	8	32.2	32.2	25.524	14.362	1244
A0A7J6B939	5	3	18.5	9.9	43.844	23.121	1108
A0A556TNK3	13	2	22.5	5.5	55.141	5.7266	532
A0A5N5JFA0	4	4	11.6	11.6	72.479	11.081	813
A0A5N5MFC8	6	4	9.2	6.6	84.54	16.656	1009
A0A5N5JMB1	9	9	33.8	33.8	30.116	33.139	781
A0A2D0S4T9	4	4	12.1	12.1	51.323	11.45	340
A0A2D0R723	2	2	9.6	9.6	22.499	3.8346	231
A0A2D0SUI6	5	5	9.2	9.2	66.204	12.633	410
A0A5N5JCV3	2	2	4.4	4.4	48.577	4.4487	806
E3TDR7	4	4	26.5	26.5	27.009	12.286	1352
A0A2D0R361	7	6	9.9	8.4	77.713	14.288	218
A0A5N5KR97	5	3	13.6	8.1	52.267	13.549	96
A0A2D0SHQ1	2	2	4.6	4.6	53.705	6.0705	376
A0A556TPS5	8	5	40.3	26.1	24.644	24.739	544
A0A5N5LHL9	2	2	4.6	4.6	75.677	7.1679	953
A0A9D3SAV1	6	6	10.4	10.4	97.894	11.348	1341
A0A5N5PE83	2	2	3.6	3.6	60.013	1.9604	1125
A0A556U424	2	2	9.2	9.2	20.631	3.5375	644
A0A556V2T6	2	2	10.4	10.4	25.424	2.9754	182
A0A8T0BPL7	11	2	42.7	12.5	26.518	8.3381	1312
A0A556TPC3	5	3	10.1	6.1	50.078	18.076	539
A0A5N5M7R9	3	3	1.8	1.8	173.23	4.7655	1005
A0A2D0PTS7	4	4	34.6	34.6	20.939	16.247	94

A0A2D0RYD5	3	3	12.6	12.6	45.185	21.543	318
A0A5A4DW65	6	6	15.2	15.2	65.052	15.327	155
A0A2D0R7I9	14	2	19	2.6	109.74	9.7606	226
A0A556TMT6	22	2	8.7	0.9	333.26	6.0289	521
A0A556V677	3	3	3.5	3.5	120.96	10.241	744
A0A556TJB0	40	2	17	0.5	273.24	11.111	495
A0A2D0RSW7	8	3	16.5	8.9	65.625	20.23	300
A0A9D3NF39	3	3	4.9	4.9	99.32	14.384	1323
A0A5N5LWM1	6	2	15.7	4.5	50.533	10.532	980
A0A7J6B5Q9	3	2	3.8	2.3	78.41	5.8569	1247
W5UCE5	5	5	13.6	13.6	46.079	16.955	1233
A0A7J6AQR0	4	2	13.2	10.4	49.381	16.558	1230
A0A556U8Z6	8	3	14.6	7.6	72.527	40.986	668
A0A5N5LB94	4	4	8.6	8.6	83.413	9.5242	940
A0A5N5L322	6	6	34.2	34.2	18.61	22.202	927
A0A109WMR6	3	2	12.5	8.7	29.579	20.276	51
A0A556TNF6	6	6	5.3	5.3	107.61	11.388	531
A0A5N5LAM1	7	7	36.3	36.3	28.1	24.999	121
A0A556TL21	16	2	36.9	9.4	56.919	15.248	510
A0A5N5PXX2	5	2	39.4	14.4	11.527	16.881	462
A0A556UZW7	3	3	13.3	13.3	37.467	7.6118	692
A0A2D0R0Z2	5	5	26.6	26.6	32.924	22.006	208
A0A556U249	8	5	13.7	10.6	61.535	88.15	629
A0A5N5NXH8	4	4	17.4	17.4	26.817	11.231	1103
A0A5N5KSS5	3	3	9.2	9.2	57.159	4.5253	81
A0A5N5ML69	11	2	32.8	5.4	57.472	84.712	1033
A0A7J6A8W3	6	6	25.1	25.1	30.31	17.314	1210

A0A8J4TL42	2	2	2.8	2.8	123.68	25.458	1258
A0A556U1R1	4	4	6.6	6.6	82.808	23.459	627
A0A2D0SZH5	19	19	37.2	37.2	63.537	175.96	430
A0A7J6AJ24	3	3	9	9	65.541	11.238	403
A0A0M3SH20	8	8	34.6	34.6	36.143	35.002	43
A0A5N5MGB6	8	8	15.7	15.7	85.385	26.732	263
A0A9D3SRI7	9	2	30.3	5.4	33.3	51.483	1346
A0A556U4I4	6	3	27.2	14.3	32.307	5.4355	646
A0A2D0S349	5	2	11.3	5	79.443	21.173	334
A0A2D0PJV6	15	15	22.2	22.2	106.08	89.381	67
A0A2D0RH86	3	3	6.9	6.9	49.862	11.806	267
A0A556TPG1	8	8	37.1	37.1	29.2	39.249	540
E3TCG5	17	2	47.1	7.5	35.889	105.02	1311
A0A8J4X5X5	3	3	1.3	1.3	265.21	16.823	1283
A0A2D0RF21	4	2	7	3.5	59.144	3.3236	259
A0A7J6AKA0	4	3	15.4	12.8	34.393	8.3942	1219
A0A2D0QNR1	6	6	31.4	31.4	30.253	32.021	176
Q90YQ0	4	4	29.8	29.8	15.131	22.998	29
A0A2D0SPD6	19	19	12.3	12.3	270.92	76.5	399
A0A7J6AEP0	7	7	43.3	43.3	29.376	31.3	367
A0A5N5M2C8	11	3	31.2	9.7	62.849	29.62	990
A0A5N5JSY1	8	8	24.6	24.6	51.164	54.068	832
A0A4D6QHI3	6	3	19.5	11.4	36.416	21.267	476
A0A5N5LKF5	13	13	52.7	52.7	35.565	56.825	959
A0A2D0T0W3	4	4	13.7	13.7	34.517	14.595	434
A0A5N5PAW9	12	2	42.5	11.2	25.521	25.413	1119
A0A2D0Q4F0	3	3	2.8	2.8	138.39	5.355	123

A0A5N5LAE0	14	8	50	31.4	28.394	92.809	937
A0A5N5NVN4	5	2	13.3	5	68.844	14.13	1096
A0A5N5MWB9	12	3	22.5	7.7	46.181	38.523	1048
A0A556V5X1	4	3	32.3	25	13.195	4.2306	246
A0A5N5K1M9	17	3	40.5	8.6	57.079	44.609	865
A0A556U747	5	2	25.3	11.4	26.187	4.7515	653
A0A2D0T4V9	4	2	2.4	1.3	208.74	13.038	450
O13163	3	3	16.2	16.2	16.052	6.563	26
A0A8T0BSX8	6	5	7.7	6.4	112.51	17.61	1314
A0A2D0QNP2	14	2	26.2	3.7	60.095	7.9309	174
A0A5N5NDS7	13	6	24.2	13.6	73.243	24.421	1067
A0A5N5MV30	7	4	3.8	2.1	267.14	9.3259	297
A0A9D3NLN6	11	2	15.3	3.9	117.95	13.942	1324

Table S3: DEPs of *Rhamdia quelen* liver exposed to 0.1 µg/L of Cd for 15 days.

Uniprot Entry	Uniprot ID_DANRE	Gene Symbol	Protein Name	Length	log2(FC)	raw.pval	DEP (Up or Down)
A0A5N5MMQ2	R4GEE3	*tom1l2*	TOM1-like protein 2 (Target of myb1-like 2 membrane-trafficking protein)	524	2.6225	0.0119	Up
A0A5N5N6Q3	Q6P607	*gnmt*	Glycine N-methyltransferase (EC 2.1.1.20)	294	-0.7499	0.0238	Down

Table S4: DEPs of *Rhamdia quelen* liver exposed to 1 µg/L of Cd for 15 days.

Uniprot Entry	Uniprot ID_DANRE	Gene Symbol	Protein Name	Length	log2(FC)	raw.pval	DEP (Up or Down)
A0A7J5ZTU0	Q6P6E0	*atp5l*	ATP synthase subunit	103	-2.5492	0.0000	Down
A0A8T0AZC5	F1QE38	*ptpra*	Receptor-type tyrosine-protein phosphatase alpha (EC 3.1.3.48)	831	2.3270	0.0001	Up
A0A556VB44	Q24JV3	*rpl31*	Large ribosomal subunit protein eL31 (60S ribosomal protein L31)	123	2.4503	0.0003	Up
A0A5N5KL55	Q6ZM17	*hbaa1*	Alpha globin-like (Novel protein similar to zebrafish hemoglobin alpha-adult 1 (Hbaa1)) (Si:ch211-5k11.8) (Si:xx-by187g17.1)	143	-1.1947	0.0005	Down
A0A7J6A9F1	F1R0Z8	*pfdn4*	Prefoldin subunit 4	135	2.4595	0.0006	Up
A0A2D0QTM5	Q1LUT0	*nutf2*	Nuclear transport factor 2 (NTF-2)	127	-2.6410	0.0014	Down
A0A5N5LVT2	Q6DEJ9	*dpm1*	Dolichol-phosphate mannosyltransferase subunit 1 (EC 2.4.1.83)	250	2.5619	0.0020	Up
A0A5N5MHW6	Q6P963	*hagh*	Hydroxyacylglutathione hydrolase, mitochondrial (EC 3.1.2.6) (Glyoxalase II) (Glx II)	303	-2.5809	0.0027	Down
A0A5N5L080	E9QJN3	*acot11a*	Acyl-CoA thioesterase 11a (Acyl-coenzyme A thioesterase 11 isoform X1)	579	2.6454	0.0034	Up
A0A5N5NGE0	Q7ZUS9	*etfa*	Electron transfer flavoprotein subunit alpha (Alpha-ETF)	333	0.7720	0.0042	Up
A0A7J6AKQ8	B3DFN3	*lmnb2*	Lamin-B2	583	2.8521	0.0047	Up
W5UEN3	Q803B0	*hspd1*	60 kDa heat shock protein, mitochondrial (EC 5.6.1.7) (60 kDa chaperonin) (Chaperonin 60) (Heat shock protein 60)	575	0.6164	0.0056	Up
A0A5N5LGK8	Q803T1	*dcn*	Decorin (Bone proteoglycan II)	373	-2.8784	0.0063	Down
A0A5N5KKM3	A8E7N8	*psmb4*	Proteasome subunit beta	256	2.6245	0.0093	Up
W5UBG4	A9LDD9	*shmt2*	glycine hydroxymethyltransferase (EC 2.1.2.1)	492	0.6366	0.0107	Up
A0A556V8V3	A0AUR8	*dars1*	Aspartate--tRNA ligase, cytoplasmic (EC 6.1.1.12) (Aspartyl-tRNA synthetase)	531	-2.6695	0.0109	Down
A0A2D0Q5W1	Q7ZUP0	*anp32a*	Acidic leucine-rich nuclear phosphoprotein 32 family member A	254	-1.7657	0.0120	Down
A0A556TMB9	Q6DI22	*hadh*	3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35)	309	0.8853	0.0133	Up

A0A5N5NJI7	Q5XJJ3	*atp5pb*	ATP synthase subunit b	252	0.6941	0.0162	Up
A0A5N5M260	Q7ZV68	*vps29*	Vacuolar protein sorting-associated protein 29 (Vesicle protein sorting 29)	182	-1.7190	0.0186	Down
A0A8T0BHC6	Q1RLT1	*gmpr2*	GMP reductase (GMPR) (EC 1.7.1.7) (Guanosine 5'-monophosphate oxidoreductase) (Guanosine monophosphate reductase)	348	1.5298	0.0193	Up
A0A5N5M634	A0A8M2B4I2	*prpf8*	Pre-mRNA-processing factor 8 (Pre-mRNA-processing-splicing factor 8)	2342	0.7771	0.0234	Up
A0A2D0RN07	Q2LEK1	*dync1h1*	Cytoplasmic dynein 1 heavy chain 1 (Dynein cytoplasmic 1 heavy chain 1)	4643	0.9144	0.0238	Up
A0A7J6A417	Q4VBR9	*cyp2y3*	Cytochrome P450 precursor (EC 1.14.14.1) (Zgc:110692)	491	-1.2491	0.0267	Down
A0A7J6B3S7	A2AWE1	*prdx1*	thioredoxin-dependent peroxiredoxin (EC 1.11.1.24)	199	-0.9883	0.0290	Down
A0A7J6B939	Q4V947	*map2k1*	Dual specificity mitogen-activated protein kinase kinase 1 (EC 2.7.12.2) (ERK activator kinase 1) (MAPK/ERK kinase 1)	395	-1.8197	0.0298	Down
A0A556TNK3	A0A8M1NXI0	*pdia3*	Protein disulfide-isomerase (EC 5.3.4.1)	494	0.7599	0.0307	Up
A0A5N5JFA0	Q7ZVF3	*sdha*	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial (EC 1.3.5.1) (Flavoprotein subunit of complex II) (Fp)	661	1.4952	0.0364	Up
A0A5N5MFC8	Q7SYB8	*gys1*	Glycogen [starch] synthase (EC 2.4.1.11)	700	-0.8816	0.0383	Down
A0A5N5JMB1	Q6PBY1	*rps3a*	Small ribosomal subunit protein eS1 (40S ribosomal protein S3a)	267	-0.8665	0.0383	Down
A0A2D0S4T9	A0A8M3AK10	*u2af2b*	Splicing factor U2AF subunit (U2 snRNP auxiliary factor large subunit)	456	1.4355	0.0387	Up
A0A2D0R723	Q6DRD1	*atp5po*	ATP synthase subunit O, mitochondrial (Oligomycin sensitivity conferral protein)	209	0.7461	0.0388	Up
A0A2D0SUI6	Q5RKM6	*farsb*	phenylalanine--tRNA ligase (EC 6.1.1.20)	590	-0.5966	0.0389	Down
A0A5N5JCV3	Q7ZVG7	*fgg*	Fgg protein (Fibrinogen gamma chain) (Fibrinogen, gamma polypeptide)	431	1.5748	0.0404	Up
E3TDR7	Q5XJS8	*hsd17b10*	3-hydroxyacyl-CoA dehydrogenase type-2 (Hsd17b10 protein) (Hydroxysteroid (17-beta) dehydrogenase 10)	260	1.0746	0.0431	Up
A0A2D0R361	B0S6P6	*ddx3xa*	RNA helicase (EC 3.6.4.13)	709	1.6321	0.0478	Up

Table S5: DEPs of *Rhamdia quelen* liver exposed to 10 µg/L of Cd for 15 days.

Uniprot Entry	Uniprot ID_DANRE	Gene Symbol	Protein Name	Length	log2(FC)	raw.pval	DEP (Up or Down)
A0A5N5MMQ2	R4GEE3	*tom1l2*	TOM1-like protein 2 (Target of myb1-like 2 membrane-trafficking protein)	524	2.8043	0.0007	Up
A0A7J5ZTU0	Q6P6E0	*atp5l*	ATP synthase subunit	103	-2.4389	0.0001	Down
A0A556VB44	Q24JV3	*rpl31*	Large ribosomal subunit protein eL31 (60S ribosomal protein L31)	123	2.6283	0.0021	Up
A0A5N5KL55	Q6ZM17	*hbaa1*	Alpha globin-like (Novel protein similar to zebrafish hemoglobin alpha-adult 1 (Hbaa1)) (Si:ch211-5k11.8) (Si:xx-by187g17.1)	143	-2.1337	0.0001	Down
A0A2D0QTM5	Q1LUT0	*nutf2*	Nuclear transport factor 2 (NTF-2)	127	-2.5280	0.0012	Down
A0A5N5LVT2	Q6DEJ9	*dpm1*	Dolichol-phosphate mannosyltransferase subunit 1 (EC 2.4.1.83)	250	2.9140	0.0022	Up
A0A5N5MHW6	Q6P963	*hagh*	Hydroxyacylglutathione hydrolase, mitochondrial (EC 3.1.2.6) (Glyoxalase II) (Glx II)	303	-2.4663	0.0021	Down
A0A5N5LGK8	Q803T1	*dcn*	Decorin (Bone proteoglycan II)	373	-2.7650	0.0060	Down
W5UBG4	A9LDD9	*shmt2*	glycine hydroxymethyltransferase (EC 2.1.2.1)	492	0.9563	0.0027	Up
A0A556V8V3	A0AUR8	*dars1*	Aspartate--tRNA ligase, cytoplasmic (EC 6.1.1.12) (Aspartyl-tRNA synthetase)	531	-2.5531	0.0093	Down
A0A2D0Q5W1	Q7ZUP0	*anp32a*	Acidic leucine-rich nuclear phosphoprotein 32 family member A	254	-2.5312	0.0012	Down
A0A5N5NJI7	Q5XJJ3	*atp5pb*	ATP synthase subunit b	252	0.9038	0.0098	Up
A0A5N5M260	Q7ZV68	*vps29*	Vacuolar protein sorting-associated protein 29 (Vesicle protein sorting 29)	182	-1.4008	0.0475	Down
A0A5N5M634	A0A8M2B4I2	*prpf8*	Pre-mRNA-processing factor 8 (Pre-mRNA-processing-splicing factor 8)	2342	0.8047	0.0190	Up
A0A2D0RN07	Q2LEK1	*dync1h1*	Cytoplasmic dynein 1 heavy chain 1 (Dynein cytoplasmic 1 heavy chain 1)	4643	0.7240	0.0419	Up
A0A7J6A417	Q4VBR9	*cyp2y3*	Cytochrome P450 precursor (EC 1.14.14.1) (Zgc:110692)	491	-1.8498	0.0211	Down
A0A556TNK3	A0A8M1NXI0	*pdia3*	Protein disulfide-isomerase (EC 5.3.4.1)	494	0.6936	0.0493	Up
A0A5N5JFA0	Q7ZVF3	*sdha*	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial (EC 1.3.5.1) (Flavoprotein subunit of complex II) (Fp)	661	1.5465	0.0461	Up

A0A5N5JMB1	Q6PBY1	*rps3a*	Small ribosomal subunit protein eS1 (40S ribosomal protein S3a)	267	-0.9740	0.0128	Down
A0A5N5JCV3	Q7ZVG7	*fgg*	Fgg protein (Fibrinogen gamma chain) (Fibrinogen, gamma polypeptide)	431	1.3479	0.0153	Up
E3TDR7	Q5XJS8	*hsd17b10*	3-hydroxyacyl-CoA dehydrogenase type-2 (Hsd17b10 protein) (Hydroxysteroid (17-beta) dehydrogenase 10)	260	1.4102	0.0113	Up
A0A5N5KR97	Q4V966	*septin7a*	Septin	443	0.6083	0.0000	Up
A0A2D0SHQ1	F1RC64	*gss*	Glutathione synthetase (GSH-S) (EC 6.3.2.3)	475	2.5908	0.0000	Up
A0A556TPS5	Q7ZUG6	*rpl30*	Large ribosomal subunit protein eL30 (60S ribosomal protein L30)	117	-1.7756	0.0000	Down
A0A5N5LHL9	A0A8M9PU70	*slco1d1*	Solute carrier organic anion transporter family member	689	2.6682	0.0001	Up
A0A9D3SAV1	K4I874	*ago2*	Protein argonaute-2 (Argonaute2) (EC 3.1.26.n2) (Eukaryotic translation initiation factor 2C 2) (eIF-2C 2) (eIF2C 2) (Protein slicer)	873	2.4602	0.0001	Up
A0A5N5PE83	F1R1P2	*ugt1ab*	glucuronosyltransferase (EC 2.4.1.17)	536	-2.3604	0.0001	Down
A0A556U424	Q8QFU2	*gpia*	Glucose-6-phosphate isomerase (EC 5.3.1.9)	553	2.6445	0.0001	Up
A0A556V2T6	Q7T3C6	*nudt21*	Cleavage and polyadenylation specificity factor subunit 5 (Nudix hydrolase 21)	228	2.4901	0.0002	Up
A0A8T0BPL7	Q90XG0	*tpi1b*	Triosephosphate isomerase B (TIM-B) (EC 5.3.1.1) (Methylglyoxal synthase B) (EC 4.2.3.3) (Triose-phosphate isomerase B)	248	-2.4984	0.0002	Down
A0A556TPC3	Q6YBS2	*cap1*	Adenylyl cyclase-associated protein	463	-2.2926	0.0003	Down
A0A5N5M7R9	E7F8G5	*arhgap35a*	Rho GTPase-activating protein 35	1536	2.5828	0.0005	Up
A0A2D0PTS7	Q6IQI6	*rpl11*	Large ribosomal subunit protein uL5 (60S ribosomal protein L11)	178	-2.4781	0.0006	Down
A0A2D0RYD5	F1QPF7	*cth*	Cystathionine gamma-lyase (EC 4.4.1.1) (Cysteine-protein sulfhydrase) (Gamma-cystathionase)	404	-2.3606	0.0008	Down
A0A5A4DW65	A5WUM0	*prkaa1*	Acetyl-CoA carboxylase kinase (EC 2.7.11.1) (EC 2.7.11.31) (Hydroxymethylglutaryl-CoA reductase kinase)	573	-2.5122	0.0015	Down
A0A2D0R7I9	Q9DEU1	*atp1a1b*	Sodium/potassium-transporting ATPase subunit alpha	1025	2.7101	0.0021	Up
A0A556TMT6	A4IG19	*pygb*	Alpha-1,4 glucan phosphorylase (EC 2.4.1.1)	843	1.1169	0.0022	Up
A0A556V677	A0A8M1NRH1	*slc4a11*	Sodium bicarbonate transporter-like protein 11	846	-2.5048	0.0023	Down

A0A556TJB0	E7F5V3	*fasn*	Fatty acid synthase (EC 1.1.1.100) (EC 1.3.1.39) (EC 2.3.1.38) (EC 2.3.1.39) (EC 2.3.1.41) (EC 2.3.1.85) (EC 3.1.2.14) (EC 4.2.1.59)	2511	-2.4394	0.0025	Down
A0A2D0RSW7	Q6DHE6	*kars1*	Lysine--tRNA ligase (EC 6.1.1.6) (Lysyl-tRNA synthetase)	602	-2.4769	0.0027	Down
A0A9D3NF39	A0A8M9P3G8	*acsl6*	Arachidonate--CoA ligase (EC 6.2.1.15) (EC 6.2.1.3)	674	-2.5616	0.0030	Down
A0A5N5LWM1	A2AR68	*gdi1*	Rab GDP dissociation inhibitor	447	0.9041	0.0032	Up
A0A7J6B5Q9	A0A2R8QJF6	*unm_hu7910*	Aspartyl/asparaginyl beta-hydroxylase isoform X2 (Un-named hu7910)	600	2.7306	0.0034	Up
W5UCE5	A2CG95	*acadm*	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial (EC 1.3.8.7)	426	1.0604	0.0038	Up
A0A7J6AQR0	Q7ZTR9	*lipf*	Lipase	396	-2.4562	0.0047	Down
A0A556U8Z6	Q6NXA6	*adh5*	S-(hydroxymethyl)glutathione dehydrogenase (EC 1.1.1.284)	376	-2.9295	0.0051	Down
A0A5N5LB94	A5D6S3	*mmut*	Methylmalonyl-CoA mutase, mitochondrial (EC 5.4.99.2) (Methylmalonyl-CoA isomerase)	757	2.6509	0.0054	Up
A0A5N5L322	Q24JV1	*rpl32*	60S ribosomal protein L32	135	-2.5439	0.0070	Down
A0A109WMR6	F4ZGF2	*comta*	Catechol O-methyltransferase A (EC 2.1.1.6)	256	-1.6970	0.0085	Down
A0A556TNF6	I3ISZ9	*hrob*	Si:dkey-260c8.8 (Uncharacterized protein C17orf53 homolog)	597	-1.6284	0.0091	Down
A0A5N5LAM1	Q6P0I2	*psma8*	Proteasome subunit alpha type	251	0.5967	0.0094	Up
A0A556TL21	B8JMZ1	*ugp2a*	UTP--glucose-1-phosphate uridylyltransferase (EC 2.7.7.9)	507	0.8860	0.0098	Up
A0A5N5PXX2	Q6IQM2	*cyc*	Cytochrome c	104	-1.9440	0.0118	Down
A0A556UZW7	Q6PC14	*rpl23*	Large ribosomal subunit protein uL14 (60S ribosomal protein L23)	140	-1.7168	0.0128	Down
A0A2D0R0Z2	Q6ZM50	*capza1b*	F-actin-capping protein subunit alpha	286	0.6066	0.0129	Up
A0A556U249	Q3B7R0	*cyc1*	Cytochrome c-1	307	0.7462	0.0130	Up
A0A5N5NXH8	Q6DGX8	*psma1*	Proteasome subunit alpha type	262	-2.5999	0.0130	Down
A0A5N5KSS5	Q6NWY2	*oxsr1b*	Serine/threonine-protein kinase OSR1 (EC 2.7.11.1) (Oxidative stress-responsive 1 protein)	520	1.8353	0.0131	Up
A0A5N5ML69	Q7SX99	*fh*	Fumarate hydratase, mitochondrial (Fumarase) (EC 4.2.1.2)	509	0.6212	0.0134	Up
A0A7J6A8W3	Q7T3H9	*hyi*	Putative hydroxypyruvate isomerase (EC 5.3.1.22)	276	0.5993	0.0166	Up

A0A8J4TL42	F1QFW2	*trim2a*	Tripartite motif-containing protein 2 (EC 2.3.2.27) (E3 ubiquitin-protein ligase TRIM2) (RING-type E3 ubiquitin transferase TRIM2)	744	1.6628	0.0179	Up
A0A556U1R1	Q566U2	*nsdhl*	NAD(P) dependent steroid dehydrogenase-like (Nsdhl protein) (Sterol-4-alpha-carboxylate 3-dehydrogenase, decarboxylating (EC 1.1.1.170))	345	-1.7527	0.0180	Down
A0A2D0SZH5	Q08BA1	*atp5fa1*	ATP synthase subunit alpha	551	0.6304	0.0181	Up
A0A7J6AJ24	A0A8M9QKL4	*picalma*	Phosphatidylinositol-binding clathrin assembly protein isoform X17	630	2.0132	0.0187	Up
A0A0M3SH20	Q9I9E9	*eif2s1b*	Eukaryotic translation initiation factor 2 subunit 1 (Eukaryotic translation initiation factor 2 subunit alpha)	315	0.6276	0.0192	Up
A0A5N5MGB6	F1QGH7	*ctnnb1*	Catenin beta-1 (Beta-catenin)	780	0.6652	0.0247	Up
A0A9D3SRI7	Q6P3J9	*adka*	Adenosine kinase (AK) (EC 2.7.1.20) (Adenosine 5'-phosphotransferase)	359	0.6763	0.0260	Up
A0A556U4I4	A4QP55	*acmsd*	2-amino-3-carboxymuconate-6-semialdehyde decarboxylase (EC 4.1.1.45) (Picolinate carboxylase)	336	-2.1581	0.0260	Down
A0A2D0S349	Q5RKM2	*ndufs1*	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	731	1.2138	0.0262	Up
A0A2D0PJV6	A0A8M2BCH0	*ap1b1*	AP complex subunit beta	963	0.7667	0.0305	Up
A0A2D0RH86	A0A8M2BL09	*septin6*	Septin	432	1.6214	0.0323	Up
A0A556TPG1	Q6IQD0	*psmb5*	Proteasome subunit beta	269	-2.7528	0.0342	Down
E3TCG5	Q5XJ10	*gapdh*	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (EC 1.2.1.12) (Peptidyl-cysteine S-nitrosylase GAPDH) (EC 2.6.99.-)	333	0.6361	0.0342	Up
A0A8J4X5X5	E9QFU5	*myo6b*	Unconventional myosin-VI (Unconventional myosin-6)	1252	-1.6105	0.0344	Down
A0A2D0RF21	Q6AZW0	*cyp8b1.1*	Cytochrome P450, family 8, subfamily B, polypeptide 1 (EC 1.14.18.8) (Zgc:92406)	510	2.8901	0.0351	Up
A0A7J6AKA0	Q6DG85	*uox*	Uricase (EC 1.7.3.3) (Urate oxidase)	298	-2.0845	0.0391	Down
A0A2D0QNR1	Q8AWD0	*vdac2*	Voltage-dependent anion-selective channel protein 2	283	0.6282	0.0403	Up
Q90YQ0	Q642J7	*rps24*	40S ribosomal protein S24	131	-1.2768	0.0406	Down
A0A2D0SPD6	A0A8M2B1Z7	*tln1*	Talin-1 isoform X4	2541	0.9187	0.0421	Up
A0A7J6AEP0	Q7ZV77	*psma4*	Proteasome subunit alpha type	261	-1.2483	0.0423	Down

A0A5N5M2C8	Q6DRE5	*nop56*	Nucleolar protein 56 (Nucleolar protein 5A)	548	0.6975	0.0424	Up
A0A5N5JSY1	Q66I24	*ass1*	Argininosuccinate synthase (EC 6.3.4.5) (Citrulline--aspartate ligase)	414	-1.4652	0.0430	Down
A0A4D6QHI3	Q9PVK4	*ldhba*	L-lactate dehydrogenase B-A chain (LDH-B-A) (EC 1.1.1.27)	334	-1.7447	0.0441	Down
A0A5N5LKF5	Q7T334	*mdh2*	Malate dehydrogenase (EC 1.1.1.37)	337	0.6041	0.0459	Up
A0A2D0T0W3	Q6NYA1	*hnrnpabb*	Heterogeneous nuclear ribonucleoprotein A/B (Zgc:77052)	309	0.6984	0.0474	Up

Table S6: DEPs of *Rhamdia quelen* liver exposed to 100 µg/L of Cd for 15 days.

Uniprot Entry	Uniprot ID_DANRE	Gene Symbol	Protein Name	Length	log2(FC)	raw.pval	DEP (Up or Down)
A0A5N5KL55	Q6ZM17	*hbaa1*	Alpha globin-like (Novel protein similar to zebrafish hemoglobin alpha-adult 1 (Hbaa1)) (Si:ch211-5k11.8) (Si:xx-by187g17.1)	143	0.5876	0.0204	Up
A0A556V8V3	A0AUR8	*dars1*	Aspartate--tRNA ligase, cytoplasmic (EC 6.1.1.12) (Aspartyl-tRNA synthetase)	531	-2.6363	0.0084	Down
A0A5N5M634	A0A8M2B4I2	*prpf8*	Pre-mRNA-processing factor 8 (Pre-mRNA-processing-splicing factor 8)	2342	0.9771	0.0081	Up
A0A2D0RN07	Q2LEK1	*dync1h1*	Cytoplasmic dynein 1 heavy chain 1 (Dynein cytoplasmic 1 heavy chain 1)	4643	0.9198	0.0144	Up
A0A7J6B3S7	A2AWE1	*prdx1*	thioredoxin-dependent peroxiredoxin (EC 1.11.1.24)	199	-0.8780	0.0266	Down
A0A7J6B939	Q4V947	*map2k1*	Dual specificity mitogen-activated protein kinase kinase 1 (EC 2.7.12.2) (ERK activator kinase 1) (MAPK/ERK kinase 1)	395	-1.7263	0.0350	Down
A0A2D0R723	Q6DRD1	*atp5po*	ATP synthase subunit O, mitochondrial (Oligomycin sensitivity conferral protein)	209	0.6539	0.0362	Up
E3TDR7	Q5XJS8	*hsd17b10*	3-hydroxyacyl-CoA dehydrogenase type-2 (Hsd17b10 protein) (Hydroxysteroid (17-beta) dehydrogenase 10)	260	0.7646	0.0386	Up
A0A5N5LHL9	A0A8M9PU70	*slco1d1*	Solute carrier organic anion transporter family member	689	2.5935	0.0006	Up
A0A5N5LUE8	A0A8M9PT53	*flna*	Filamin-A isoform X1	2552	1.1742	0.0055	Up
A0A5N5PE83	F1R1P2	*ugt1ab*	glucuronosyltransferase (EC 2.4.1.17)	536	-2.4444	0.0001	Down
A0A556V677	A0A8M1NRH1	*slc4a11*	Sodium bicarbonate transporter-like protein 11	846	-1.7644	0.0273	Down

A0A2D0RSW7	Q6DHE6	*kars1*	Lysine--tRNA ligase (EC 6.1.1.6) (Lysyl-tRNA synthetase)	602	-2.5608	0.0025	Down
A0A556U249	Q3B7R0	*cyc1*	Cytochrome c-1	307	0.7771	0.0015	Up
A0A2D0RZ50	Q6NZZ2	*arpc4*	Actin-related protein 2/3 complex subunit 4	168	0.6441	0.0192	Up
A0A5N5MGB6	F1QGH7	*ctnnb1*	Catenin beta-1 (Beta-catenin)	780	0.7319	0.0477	Up
A0A2D0RH86	A0A8M2BL09	*septin6*	Septin	432	1.4943	0.0342	Up
A0A2D0SPD6	A0A8M2B1Z7	*tln1*	Talin-1 isoform X4	2541	0.8853	0.0044	Up
A0A5N5PAW9	A0A8N7UVW5	*si:dkey-23a13.8*	Histone H2B	161	0.7116	0.0001	Up
A0A2D0Q4F0	A0A8M1RJV2	*tpp2*	Tripeptidyl-peptidase 2 (EC 3.4.14.10) (Tripeptidyl aminopeptidase)	1249	2.5839	0.0005	Up
A0A5N5LAE0	E7EXD7	*LOC570115*	Histone H2A	128	0.7684	0.0007	Up
A0A5N5NVN4	Q66I16	*etfdh*	Electron transfer flavoprotein-ubiquinone oxidoreductase (ETF-QO) (EC 1.5.5.1)	617	-2.6912	0.0009	Down
A0A5N5MWB9	Q05AL9	*krt94*	Uncharacterized protein LOC768289 (Zgc:153629)	431	0.6157	0.0035	Up
A0A556V5X1	Q71PD7	*h2az2a*	Histone H2A.V (H2A.F/Z)	128	2.0245	0.0087	Up
A0A5N5K1M9	B8JMZ1	*ugp2a*	UTP--glucose-1-phosphate uridylyltransferase (EC 2.7.7.9)	507	1.0119	0.0088	Up
A0A556U747	Q6DRF3	*psmb1*	Proteasome subunit beta type-1 (Proteasome beta-subunit C5)	237	0.6582	0.0096	Up
A0A2D0T4V9	F1QBC0	*plxnb2a.1*	Plexin b2a (Plexin-B2 isoform X1)	1864	0.5871	0.0114	Up
O13163	Q90486	*ba1*	Hemoglobin subunit beta-1 (Beta-1-globin) (Beta-A1-globin) (Hemoglobin beta-1 chain)	148	1.2029	0.0223	Up
A0A8T0BSX8	Q7SXK3	*pklr*	Pyruvate kinase (EC 2.7.1.40)	538	0.8642	0.0226	Up
A0A2D0QNP2	Q6NZ29	*glud1a*	Glutamate dehydrogenase 1, mitochondrial (EC 1.4.1.3)	544	0.6214	0.0364	Up

A0A5N5NDS7	A8KBY8	*anxa6*	Annexin	667	-1.2625	0.0367	Down
A0A5N5MV30	A0A8M3BDW6	*sptbn1*	Spectrin beta chain	2363	1.6480	0.0422	Up
A0A9D3NLN6	Q7SX99	*fh*	Fumarate hydratase, mitochondrial (Fumarase) (EC 4.2.1.2)	509	-3.8526	0.0461	Down

Figure S1. Molecular function (A) and biological process (B) identified through Panther Classification System, of altered proteins in the catfish *Rhamdia quelen* exposed to different Cd concentrations for 15 days.

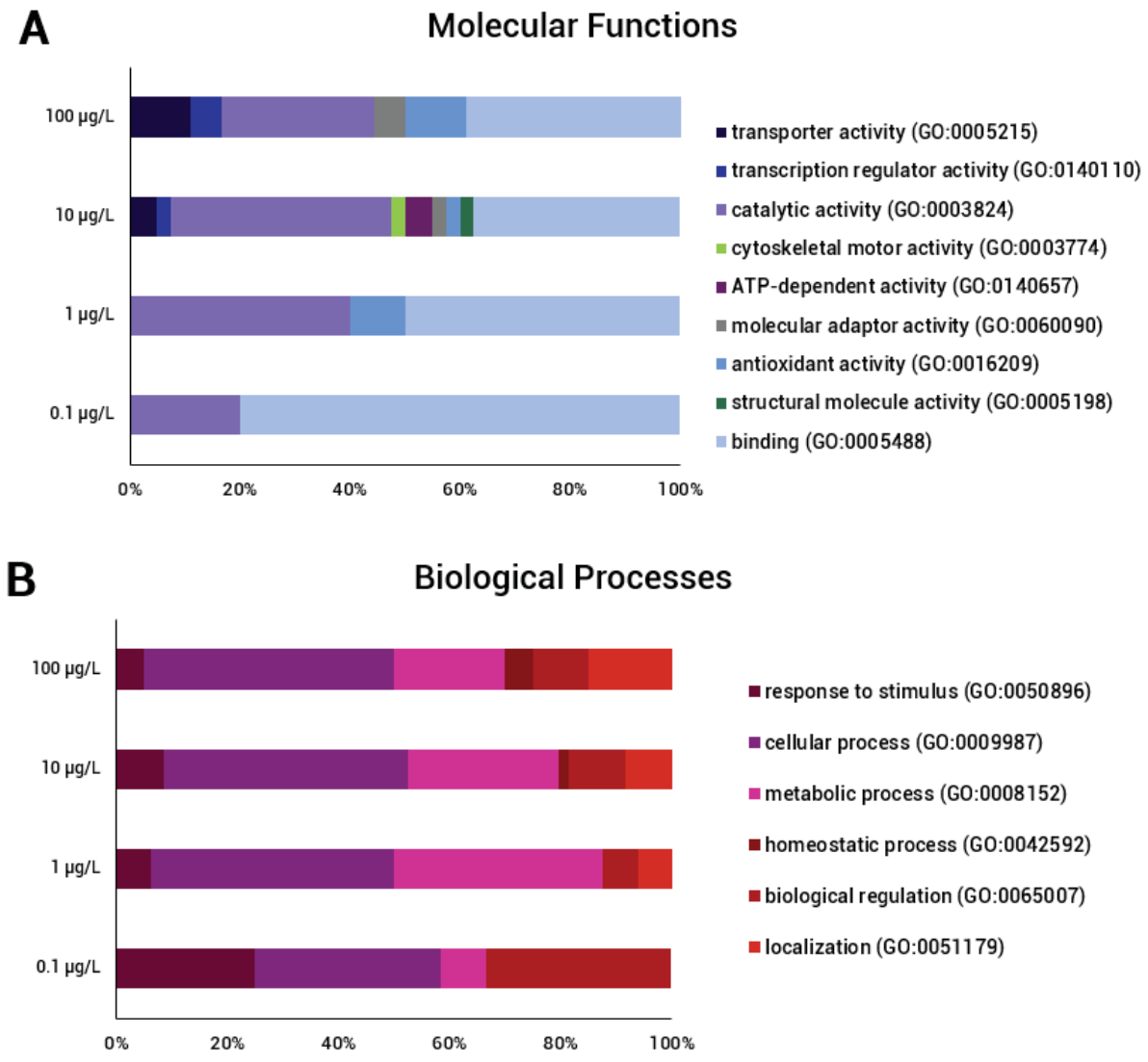

Table S7: Liver proteins of *Rhamdia quelen* exposed to different Cd concentrations detected in the Gene Set Enrichment Analysis (GSEA).

					[CdCl2] µg/L	
Pathway	**KEGG ID**	**Fold Enrichment**	**Protein symbol**	**Protein name**	**Up**	**Down**
Starch and sucrose metabolism	dre00500	33.17	*Gpia*	Glucose-6-phosphate isomerase	10	-
			Gys1	Glycogen [starch] synthase	-	1
			Pygb	Alpha-1,4 glucan phosphorylase	-	10
			Ugp2a	UTP--glucose-1-phosphate uridylyltransferase	10, 100	-
Proeasome	dre03050	32.57	*Psmb5*	Proteasome subunit beta	-	10
			Psma4	Proteasome subunit alpha type	-	10
			Psma8	Proteasome subunit alpha type	10	-
			Psma1	Proteasome subunit alpha type	-	10
			Psmb1	Proteasome subunit beta type-1 (Proteasome beta-subunit C5)	100	-
			Psmb4	Proteasome subunit beta	1	-
Pyruvate metabolism	dre00620	27.64	*Pklr*	Pyruvate kinase	100	-
			Adh5	S-(hydroxymethyl)glutathione dehydrogenase	-	10
			Hagh	Hydroxyacylglutathione hydrolase, mitochondrial	-	1, 10
			Fh	Fumarate hydratase	10	100
			Mdh2	Malate dehydrogenase	10	-
Tricarboxylic acid cycle (TCA cycle)	dre00020	26.34	*Sdha*	Succinate dehydrogenase	1, 10	-
			Fh	Fumarate hydratase	10	100
			Mdh2	Malate dehydrogenase	10	-
Carbon metabolism	dre01200	22.79	*Shmt2*	Glycine hydroxymethyltransferase	1, 10	-
			Pklr	Pyruvate kinase	100	-
			Adh5	S-(hydroxymethyl)glutathione dehydrogenase	-	10

			Gpia	Glucose-6-phosphate isomerase	10	-
			Glud1a	Glutamate dehydrogenase 1	100	-
			Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	10	-
			Sdha	Succinate dehydrogenase	1, 10	-
			Fh	Fumarate hydratase	10	100
			Mdh2	Malate dehydrogenase	10	-
			Tpi1b	Triosephosphate isomerase B	-	10
Glycolysis/Gluco-neogenesis	dre00010	18.43	*Pklr*	Pyruvate kinase	100	-
			Adh5	S-(hydroxymethyl)glutathione dehydrogenase	-	10
			Gpia	Glucose-6-phosphate isomerase	10	-
			Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	10	-
			Tpi1b	Triosephosphate isomerase B	-	10
Biosynthesis of amino acids	dre01230	17.36	*Shmt2*	Glycine hydroxymethyltransferase	1, 10	-
			Pklr	Pyruvate kinase	100	-
			Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	10	-
			Cth	Cystathionine gamma-lyase	-	10
			Tpi1b	Triosephosphate isomerase	-	10
Oxidative phosphorylation	dre00190	16.82	*Atp5l*	ATP synthase subunit L	-	1, 10
			Atp5po	ATP synthase subunit O	1, 100	-
			Sdha	Succinate dehydrogenase	1, 10	-
			Cyc	Cytochrome c	-	10
			Atp5pb	ATP synthase subunit b	1, 10	-
			Ndufs1	NADH-ubiquinone oxidoreductase	10	-
			Atp5fa1	ATP synthase subunit alpha 1	10	-
			Cyc1	Cytochrome c-1	10, 100	-
Ribosome	dre03010	16.08	*Rpl30*	Large ribosomal subunit protein eL30	-	10

			Rpl23	Large ribosomal subunit protein uL14	-	10
			Rps3a	Small ribosomal subunit protein eS1	-	1, 10
			Rps24	40S ribosomal protein S24	-	10
			Rpl11	Large ribosomal subunit protein uL5	-	10
			Rpl32	Large ribosomal subunit protein eL32	-	10
			Rpl31	Large ribosomal subunit protein eL31	1, 10	-
Metabolic pathways	dre01100	6.27	*Shmt2*	Glycine hydroxymethyltransferase	1, 10	-
			Ugt1ab	Glucuronosyltransferase	-	10, 100
			Pklr	Pyruvate kinase	100	-
			Adh5	S-(hydroxymethyl)glutathione dehydrogenase	-	10
			Gpia	Glucose-6-phosphate isomerase	10	-
			Glud1a	Glutamate dehydrogenase 1	100	-
			Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	10	-
			Cth	Cystathionine gamma-lyase	-	10
			Atp5l	ATP synthase subunit L	-	1, 10
			Atp5po	ATP synthase subunit O	1, 100	-
			Hagh	Hydroxyacylglutathione hydrolase	-	1, 10
			Adka	Adenosine kinase	10	-
			Hyi	Putative hydroxypyruvate isomerase	10	-
			Sdha	Succinate dehydrogenase	1, 10	-
			Fh	Fumarate hydratase	10	100
			Gys1	Glycogen [starch] synthase	-	1
			Pygb	Alpha-1,4 glucan phosphorylase	-	10
			Gnmt	Glycine N-methyltransferase	-	0.1
			Acadm	Medium-chain specific acyl-CoA dehydrogenase,	10	-
			Mdh2	Malate dehydrogenase	10	-

Cyc	Cytochrome c	-	10
Uox	Uricase	-	10
Hadh	3-hydroxyacyl-CoA dehydrogenase	1	-
Dpm1	Dolichol-phosphate mannosyltransferase subunit 1	1, 10	-
Atp5pb	ATP synthase subunit b	1, 10	-
Hsd17b10	Hydroxysteroid (17-beta) dehydrogenase 10	1, 10, 100	-
Gss	Glutathione synthetase	10	-
Ndufs1	NADH-ubiquinone oxidoreductase	10	-
Nsdhl	NAD(P) dependent steroid dehydrogenase-like	-	10
Atp5fa1	ATP synthase subunit alpha 1	10	-
Ugp2a	UTP--glucose-1-phosphate uridylyltransferase	10, 100	-
Acmsd	2-amino-3-carboxymuconate-6-semialdehyde decarboxylase	-	10
Fasn	Fatty acid synthase	-	10
Tpi1b	Triosephosphate isomerase B	-	10
Comta	Catechol O-methyltransferase A	-	10
Cyc1	Cytochrome c-1	10, 100	-
Gmpr2	Guanosine 5'-monophosphate oxidoreductase	1	-

9. CONSIDERAÇÕES FINAIS

O presente estudo se propôs a avaliar o proteoma hepático de peixes *R. quelen*, fornecendo um mapeamento geral das potenciais alterações metabólicas induzidas pela exposição ao Cd. A partir da análise e filtragem de dados provenientes da espectrometria de massas, foi possível identificar as proteínas detectadas pelo equipamento, bem como suas funções, através do banco de dados do UniProtKB e ferramentas de GO. No total, foram identificadas 117 proteínas diferencialmente expressas, sendo que o grupo de animais expostos a 10 µg/L havia o maior número de alterações. Entre as proteínas estatisticamente significativas, destacam-se três vias metabólicas: metabolismo de carboidratos, produção de energia e síntese e degradação de proteínas.

Este estudo é particularmente relevante para análise proteômica de espécies nativas, sendo o primeiro estudo que avalia toxicidade do Cd em *R. quelen* por essa abordagem. Porém, algumas limitações devem ser mencionadas. Primeiro, o estudo foi conduzido apenas com machos, que são mais presentes na piscicultura, já que possuem maior desenvolvimento muscular e, portanto, são mais consumidos pela população. Mesmo assim, ressalta-se a necessidade de investigar esses efeitos também em fêmeas para compreender possíveis diferenças sexuais na resposta à toxicidade em relação ao meio ambiente. Segundo, as proteínas identificadas refletem o estresse induzido por Cd somente no décimo quinto dia de exposição, com concentrações definidas previamente. Estudos com outros delineamentos experimentais podem revelar padrões distintos de proteínas identificadas, ou variações nos efeitos dessas concentrações dependendo do tempo de exposição. A técnica de proteômica oferece sensibilidade suficiente para detectar alterações em baixas concentrações e em diferentes intervalos de tempo, contribuindo para um melhor entendimento da toxicidade do Cd.

Para melhor entender as funções das proteínas e seus envolvimentos em vias metabólicas, utilizou-se o banco de dados de *Danio rerio*, por ser um organismo modelo em toxicologia e ter seu banco de dados bem anotado. Assim, são necessárias pesquisas adicionais que para sequenciar o genoma e estabelecer um banco de dados próprio da espécie em questão. Outro aspecto relevante é que, apesar do fígado ser um alvo de toxicidade do Cd, outros órgãos podem ser afetados, sendo importante avaliar estes impactos em outros tecidos, como rim. Ademais, mesmo que

o Cd passe por processos de biotransformação, é importante compreender seus efeitos para considerar outras possibilidades, como sinergismo de contaminantes, que podem prejudicar espécies do ambiente aquático.

Em conjunto, nossos dados indicam que o estresse induzido pelo Cd, principalmente em condições ambientalmente relevantes, é capaz de aumentar respostas imunes e demanda energética, além de comprometer processos de síntese e degradação de proteínas. Esses achados sugerem que certas vias metabólicas atenuam ou agravam os efeitos adversos do Cd no fígado do *R. quelen*, enfatizando a necessidade de revisar as legislações vigentes sobre os níveis permitidos deste metal em águas doces.

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