ESCOLA DE CIÊNCIAS DA SAÚDE E DA VIDA
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E EVOLUÇÃO DA BIODIVERSIDADE
DOUTORADO EM ECOLOGIA E EVOLUÇÃO DA BIODIVERSIDADE

BRUNA DUTRA DE CASTRO

EFEITOS ADVERSOS DE HERBICIDAS À BASE DE GLIFOSATO E DE ÁCIDO DICLOROFENOXIACÉTICO (2,4D) EM DIFERENTES ESTÁGIOS DE DESENVOLVIMENTO PÓS-ECLOSÃO DE PEIXES ANUAIS

Porto Alegre
2023

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

ESCOLA DE CIÊNCIAS DA SAÚDE E DA VIDA

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E EVOLUÇÃO DA BIODIVERSIDADE

EFEITOS ADVERSOS DE HERBICIDAS À BASE DE GLIFOSATO E DE ÁCIDO DICLOROFENOXIACÉTICO (2,4D) EM DIFERENTES ESTÁGIOS DE DESENVOLVIMENTO PÓS-ECLOSÃO DE PEIXES ANUAIS

Bruna Dutra de Castro

TESE DE DOUTORADO

Pontifícia Universidade Católica do Rio Grande do Sul
Pró-Reitoria de Pesquisa e Pós-Graduação
Av. Ipiranga, 6681 – Prédio 1 – Térreo
Porto Alegre – RS – Brasil
Fone: (51) 3320 3513
E-mail: propesq@pucrs.br
Site: www.pucrs.br

Porto Alegre – RS – Brasil
2023

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

ESCOLA DE CIÊNCIAS DA SAÚDE E DA VIDA

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E EVOLUÇÃO DA BIODIVERSIDADE

EFEITOS ADVERSOS DE HERBICIDAS À BASE DE GLIFOSATO E DE ÁCIDO DICLOROFENOXIACÉTICO (2,4D) EM DIFERENTES ESTÁGIOS DE DESENVOLVIMENTO PÓS-ECLOSÃO DE PEIXES ANUAIS

Bruna Dutra de Castro

Orientadora: Dra. Guendalina Turcato Oliveira

TESE DE DOUTORADO

Porto Alegre – RS – Brasil

2023

AGRADECIMENTOS

Meus agradecimentos em especial a minha família e meu namorado que sempre me apoiaram e estiveram ao meu lado em todos os momentos da minha trajetória. Também, gostaria de agradecer imensamente a minha orientadora Drª. Guendalina Turcato Oliveira por toda atenção, dedicação, ensinamentos e confiança durante a minha longa caminhada na PUCRS vinculada ao Laboratório de Fisiologia da Conservação, desde a iniciação científica na graduação até hoje na pós-graduação.

Durante esse período, tive o privilégio de trabalhar com pessoas incríveis que contribuíram no desenvolvimento deste trabalho com os peixes anuais. Portanto, quero agradecer ao Dr. Luis Esteban Krause Lanés, a todos meus amigos e colegas do Laboratório de Fisiologia da Conservação - PUCRS, a parceria com os pesquisadores da UNISINOS, FURG e Instituto Pró-Pampa.

Por fim, agradeço a Pontifícia Universidade Católica do Rio Grande do Sul, o Programa de Pós-graduação em Ecologia e Evolução da Biodiversidade e o Centro de Modelos Biológicos Experimentais (CeMBE-PUCRS), por proporcionarem meios e suporte técnico para o desenvolvimento da pesquisa, e aprimoramento da minha formação. E, também agradeço a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão da bolsa de pesquisa.

RESUMO

Efeitos adversos de herbicidas à base de Glifosato e de Ácido Diclorofenoxiacético (2,4d) em diferentes estágios de desenvolvimento pós-eclosão de peixes anuais

Os ambientes aquáticos são constantemente expostos e suscetíveis a entrada de agroquímicos. Estes são aplicados em culturas para o controle e eliminação de pragas, visando o aumento da produtividade. Entretanto, quando presentes no meio ambiente podem alcançar ecossistemas aquáticos e prejudicar espécies não-alvo. Os agrotóxicos mais utilizados no Brasil são os herbicidas, destacando-se os à base de glifosato e o ácido 2,4-Diclorofenoxiacético (2,4-D), que são aplicados em diferentes culturas e podem apresentar risco aos habitats ocupados por peixes anuais que estão próximos a estas plantações. Cabe ressaltar que estes animais apresentam uma distribuição restrita, ocupando brejos, poças e lagoas rasas formadas pelas chuvas, mas que secam anualmente no período de estiagem. Além disso, são considerados modelos potenciais para estudos em laboratório, visto que apresentam um ciclo de vida curto e rápido que está intimamente relacionado ao local que vivem. Para isso, requerem características específicas para sobreviver como um crescimento acelerado, um intenso processo reprodutivo e grande deposição de ovos como uma estratégia para manutenção da população em ambiente natural.

Dessa forma, buscamos avaliar o efeito dos herbicidas Roundup Original© (princípio ativo glifosato nas concentrações de 65 µg/L, 130 µg/L e 260 µg/L) e Dez© (princípio ativo ácido 2,4-Diclorofenoxiacético (2,4-D) nas concentrações de 4 µg/L, 15 µg/L e 30 µg/L) em dois estágios de desenvolvimento (adulto-jovem e senil) e em ambos os sexos do peixe anual *Cynopoecilus* sp. Analisamos marcadores de balanço oxidativo e de metabolismo intermediário em animais coletados em campo e transportados até o laboratório para as experimentações. Estes foram medidos, aclimatados por 10 dias e após, expostos a cada um dos herbicidas por sete dias, em todas as etapas os peixes foram alimentados diariamente com ração comercial. Parâmetros abióticos (pH, O2 dissolvido, sólidos totais dissolvidos, condutividade de temperatura da água) foram controlados. Após a exposição, os animais foram medidos e crioeutanasiados (nitrogênio líquido) para as análises dos marcadores de balanço oxidativo e de metabolismo intermediário. Para as análises de balanço oxidativo foram determinados os níveis de Lipoperoxidação (TBARS) e Proteínas Totais (PT), além da quantificação

da atividade das enzimas antioxidantes Superóxido Dismutase (SOD) e Catalase (CAT), e da enzima de biotransformação Glutationa S-Transferase (GST). Já para o metabolismo foram realizadas a determinação dos níveis de Glicogênio (GG), Proteínas Totais (PT) e Ácido Úrico (AU) dos animais. Todos os resultados obtidos foram analisados no programa SPSS Statistics com os devidos testes estatísticos. Os resultados estão organizados em três artigos.

O primeiro artigo intitulado "*Development stage-dependent oxidative stress responses to the exposure to Roundup Original© in a neotropical annual killifish"*, publicado na Revista *Environmental Toxicology and Pharmacology*, onde são apresentados os resultados obtidos com a exposição ao herbicida Roundup Original© de machos adultos-jovens e senis. Neste capítulo, observamos que a capacidade do sistema antioxidante de machos de *Cynopoecilus* sp. é eficiente nas duas fases do desenvolvimento biológico analisadas impedindo a ocorrência de mortalidade. Apesar disto, os senis apresentaram níveis 2 vezes maiores de peroxidação lipídica estando estes associados à manutenção dos níveis de atividade das enzimas antioxidantes (SOD e CAT). Cabe ressaltar que os indivíduos senis apresentaram uma diminuição da atividade da glutationa S-transferase o que pode afetar negativamente estes indivíduos possivelmente reduzindo sua capacidade reprodutiva e/ou seu ciclo de vida. Neste capítulo também mostramos que os marcadores fisiológicos estabelecidos, bem como a espécie, parecem promissores para serem usados em estudos futuros de senescência e toxicologia.

O segundo artigo intitulado "*Toxic effect of a Glifosate-Based herbicide associated with different developmental stages in neotropical annual killifish females"*, está submetido a Revista *Environmental Toxicology and Pharmacology*. Neste capítulo nós avaliamos os efeitos do herbicida Roundup Original© (princípio ativo glifosato: 65, 130 e 260µg/L) sobre o balanço oxidativo e marcadores do metabolismo intermediário em diferentes estágios de desenvolvimento pós-eclosão (adulto-jovem e senil) em fêmeas de *Cynopoecilus* sp. Esses marcadores incluíram lipoperoxidação (TBARS), superóxido dismutase (SOD), catalase (CAT), glutationa S-transferase (GST), relação SOD/CAT, proteínas totais (PT), glicogênio (GG) e ácido úrico (AU). Fêmeas senis apresentaram aumento de atividade da CAT e GST, uma diminuição da relação SOD/CAT, combinado com um aumento de PT e AU quando comparados aos valores do grupo controle. A análise de variância de duas-vias mostrou uma diferença significativa entre faixas etárias e grupos para vários biomarcadores sugerindo uma

maior sensibilidade das fêmeas ao glifosato. Novamente, o impacto deste herbicida parece ser mais pronunciado em animais senis.

Por fim, no terceiro artigo intitulado "Análise do efeito do composto químico DEZ© (2,4-diclorofenoxiacético) sobre o ciclo de vida do peixe anual neotropical", a ser submetido a Revista *Chemosphere*, aborda sobre a exposição de machos e fêmeas nos dois estágios de desenvolvimento expostos ao Dez© (4 µg/L, 15 µg/L e 30 µg/L). Foram realizadas análises em parâmetros de estresse oxidativo e metabolismo intermediário após a exposição ao herbicida de fêmeas e machos da mesma espécie. Nossos resultados mostraram que o estágio de desenvolvimento de maior vulnerabilidade tanto para machos como para fêmeas foi na fase senil, principalmente, em relação aos parâmetros oxidativos. As concentrações mais elevadas de 15 e 30µg/L foram as mais prejudiciais a espécie determinando níveis elevados de TBARS, indicando dano oxidativo. Além disso, as fêmeas de *Cynopoecilus* sp. parecem ser mais suscetíveis ao princípio ativo 2,4D do que os machos, visto que foram observados um maior número de alterações nos marcadores bioquímicos tanto de metabolismo como de balanço oxidativo.

Mesmo não sendo considerada uma espécie-alvo para estes agrotóxicos, *Cynopoecilus* sp. é impactada com a exposição a concentrações ambientalmente relevantes destes herbicidas. Outro aspecto a se destacar, é que tanto o glifosato como o 2,4D estão presentes em diferentes formulações comerciais no Brasil, sendo estes os agrotóxicos mais vendidos em nosso país. Dessa forma, sugerindo maiores restrições no seu uso principalmente, em locais próximos aos ocupados por peixes anuais, visto que muitas espécies se encontram em algum grau de ameaça. Tanto o conjunto de biomarcadores escolhido como a espécie se mostraram adequados e promissores para o desenvolvimento de ensaios toxicológicos de caráter ontogenético, evidenciando uma sensibilidade diferenciada, especialmente para os senis; tais aspectos devem ser considerados em estudos de impacto de xenobióticos sobre estes grupos.

Palavras-chave: Agrotóxicos; Ambiente aquático; *Cynopoecilus* sp.; Balanço oxidativo; Metabolismo.

ABSTRACT

Adverse effects of herbicides based on glyphosate and dichlorophenoxyacetic acid (2,4d) on different stages of post-hatching development of annual killifish

Aquatic environments are constantly exposed and susceptible to the entry of agrochemicals. These are applied to crops to control and eliminate pests, aiming at increasing productivity. However, when present in the environment, they can reach aquatic ecosystems and harm non-target species. The most widely used pesticides in Brazil are herbicides, especially those based on glyphosate and 2,4-Dichlorophenoxyacetic acid (2,4-D), which are applied to different crops and may pose a risk to habitats occupied by annual fish. that are close to these plantations. It should be noted that these animals have a restricted distribution, occupying marshes, puddles and shallow lakes formed by the rains, but which dry up annually during the dry season. In addition, they are considered potential models for laboratory studies, since they have a short and fast life cycle that is closely related to the place they live. For this, they require specific characteristics to survive, such as accelerated growth, an intense reproductive process and large egg laying as a strategy for maintaining the population in a natural environment.

Thus, we sought to evaluate the effect of the herbicides Roundup Original© (active ingredient glyphosate at concentrations of 65 µg/L, 130 µg/L and 260 µg/L) and Dez© (active ingredient 2,4-Dichlorophenoxyacetic acid (2,4 -D) at concentrations of 4 µg/L, 15 µg/L and 30 µg/L) in two developmental stages (young-adult and senile) and in both sexes of the annual killifish *Cynopoecilus* sp. We analyzed markers of oxidative balance and intermediary metabolism in animals collected in the field and transported to the laboratory for experimentation. These were measured, acclimatized for 10 days and after, exposed to each of the herbicides for seven days, in all stages the fish were fed daily with commercial feed. Abiotic parameters (pH, dissolved O2, total dissolved solids, water temperature conductivity) were controlled. After exposure, the animals were measured and cryoeuthanized (liquid nitrogen) for analyzes of markers of oxidative balance and intermediary metabolism. For the analyzes of oxidative balance, the levels of Lipoperoxidation (TBARS) and Total Proteins (PT) were determined, in addition to the quantification of the activity of the antioxidant enzymes Superoxide

Dismutase (SOD) and Catalase (CAT), and of the biotransformation enzyme Glutathione S-Transferase (GST). As for the metabolism, the determination of the levels of Glycogen (GG), Total Proteins (PT) and Uric Acid (AU) of the animals were carried out. All results obtained were analyzed using the SPSS Statistics program with the appropriate statistical tests. The results are organized into three articles.

The first article entitled *"Development stage-dependent oxidative stress responses to the exposure to Roundup Original© in a neotropical annual killifish"*, published in the journal Environmental Toxicology and Pharmacology, where the results obtained with exposure to the herbicide Roundup Original© of males are presented young and senile adults. In this chapter, we observed that the capacity of the antioxidant system of males of *Cynopoecilus* sp. it is efficient in the two phases of biological development analyzed, preventing the occurrence of mortality. Despite this, the senile showed 2 times higher levels of lipid peroxidation, which are associated with the maintenance of activity levels of antioxidant enzymes (SOD and CAT). It should be noted that senile individuals showed a decrease in glutathione S-transferase activity, which may negatively affect these individuals, possibly reducing their reproductive capacity and/or their life cycle. In this chapter we also show that the established physiological markers, as well as the species, seem promising to be used in future studies of senescence and toxicology.

The second article entitled *"Toxic effect of a Glifosate-Based herbicide associated with different developmental stages in neotropical annual killifish females"*, is submitted to Revista Environmental Toxicology and Pharmacology. In this chapter we evaluated the effects of Roundup Original© herbicide (active ingredient glyphosate: 65, 130 and 260µg/L) on the oxidative balance and intermediary metabolism markers at different stages of post-hatching development (young-adult and senile) in females from *Cynopoecilus* sp. These markers included lipoperoxidation (TBARS), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), SOD/CAT ratio, total protein (PT), glycogen (GG) and uric acid (AU). Senile females showed increased CAT and GST activity, a decrease in the SOD/CAT ratio, combined with an increase in PT and UA when compared to control group values. Two-way analysis of variance showed a significant difference between age groups and groups for several biomarkers suggesting a greater sensitivity of females to glyphosate. Again, the impact of this herbicide appears to be more pronounced in senile animals.

Finally, in the third article entitled *"Analysis of the effect of the chemical compound DEZ© (2,4-Dichlorophenoxyacetic) on the life cycle of neotropical annual killifish"*, to be submitted to Chemosphere Magazine, addresses the exposure of males and females in two developmental stages exposed to Dez© (4 µg/L, 15 µg/L and 30 µg/L). Analyzes were performed on parameters of oxidative stress and intermediary metabolism after exposure to the herbicide in females and males of the same species. Our results showed that the most vulnerable stage of development for both males and females was in the senile phase, mainly in relation to oxidative parameters. The highest concentrations of 15 and 30µg/L were the most harmful to the species, determining high levels of TBARS, indicating oxidative damage. Furthermore, females of *Cynopoecilus* sp. seem to be more susceptible to the 2,4D active principle than males, since a greater number of alterations in biochemical markers of both metabolism and oxidative balance were observed.

Even though it is not considered a target species for these pesticides, *Cynopoecilus* sp. is impacted by exposure to environmentally relevant concentrations of these herbicides. Another aspect to be highlighted is that both glyphosate and 2,4D are present in different commercial formulations in Brazil, which are the most sold pesticides in our country. Thus, suggesting greater restrictions on its use, mainly in places close to those occupied by annual fish, since many species are in some degree of threat. Both the set of biomarkers chosen and the species proved to be adequate and promising for the development of ontogenetic toxicological tests, showing a differentiated sensitivity, especially for the senile; such aspects must be considered in studies of the impact of xenobiotics on these groups.

Keywords: Pesticides; Aquatic environment; *Cynopoecilus* sp.; Oxidative balance; Metabolism.

RELAÇÃO DE FIGURAS

SUMÁRIO

APRESENTAÇÃO

O presente trabalho intitulado "Toxicidade de Herbicidas à base de Glifosato e de Ácido Diclorofenoxiacético em diferentes estágios de desenvolvimento pós-eclosão de Peixes Anuais", foi elaborado como parte das exigências necessárias para a obtenção do título de Doutor pelo Programa de Pós-Graduação em Ecologia e Evolução da Biodiversidade (PPG-EEB) da Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS).

ESTRUTURA DA TESE

A tese está estruturada em **"Apresentação"** mostrando a estrutura da tese e o marco teórico, **"Capítulo I, II e III"** em forma de artigo científico, e finaliza com as **"Considerações Finais da Tese"**. O Marco Teórico aborda sobre os agrotóxicos na agricultura, ecotoxicologia aquática, metabolismo e estresse oxidativo, e peixes anuais.

Os capítulos estão redigidos em forma de artigo científico conforme as regras das revistas. No **Capítulo I e II** foi realizado o estudo com a exposição de peixes anuais, machos e fêmeas, de diferentes faixas etárias ao herbicida Roundup Original©. Destaco que o artigo do Capítulo I já está publicado na revista "*Environmental Toxicology and Pharmacology"*, a qual é Qualis A2 na plataforma Capes na área de Biodiversidade, onde também foi submetido o artigo do Capítulo II. E, o **Capítulo III** aborda sobre a exposição da mesma espécie de peixe anual, de ambos os sexos e diferentes idades, ao herbicida Dez©. Este último será submetido a revista *"Chemosphere"*, que está classificada na plataforma Capes/Qualis A1 na área de Biodiversidade.

MARCO TEÓRICO

Agrotóxicos e Ecotoxicologia Aquática

A Revolução Verde iniciou na década de 1940 e ficou conhecida por abordar um conjunto de inovações tecnológicas na agricultura para aprimorar a produção agrícola e melhorar a produção de alimentos. Para as constantes transformações na agricultura, o desenvolvimento de substâncias químicas, como agrotóxicos, fertilizantes químicos, fungicidas, herbicidas entre outros foram utilizados com frequência no meio para o controle de pragas nas lavouras. Entretanto, foi observado que essa revolução a longo prazo não trouxe apenas melhorias, mas consequências prejudiciais em relação ao uso de elementos químicos perigosos no meio ambiente, destacando a contaminação de solos e a perda da biodiversidade natural (MICHAEL C. NEWMAN, 2015).

Desde então, a agricultura vem se expandindo com o incentivo do aumento da população que necessita de uma maior oferta de alimento e, com isso, têm ocasionado um acréscimo na produção de substâncias químicas, como por exemplo agrotóxicos, pela indústria a fim de que os agricultores utilizem esses químicos para aumentar o cultivo nas plantações (BOMBARDI, 2017). De acordo com ROCHA; GRISOLIA (2018) o mercado de agrotóxicos do Brasil é o maior do mundo, sendo os herbicidas responsáveis por 45% do total destes agrotóxicos. Cabe destacar que, segundo dados de 2021 do IBAMA, dentre os herbicidas mais utilizados no Brasil aqueles a base de Glifosato e 2,4D estão ocupando os primeiros lugares do ranking de agrotóxicos mais vendidos (IBAMA, 2021).

O glifosato pertence ao grupo químico das glicinas (Fig. 1) e suas formulações contém como ingredientes básicos: sal de isopropilamina (IPA) do ácido Nfosfonometilglicina, um surfactante e a água. Sabe-se que a solubilidade do glifosato em água é grande (10,000 - 15,700 mg/L a 25ºC e pH 7), e dependendo do tipo de solo e matéria orgânica sua meia-vida é de 30 a 90 dias (RODRIGUES & ALMEIDA, 2005). Giesy et al. (2000) relatam que, a meia vida do glifosato e AMPA (ácido aminometilfosfônico) em ambientes aquáticos varia de 7 a 14 dias. Além disso, o glifosato é o princípio ativo do herbicida comercial Roundup Original©. Esse herbicida é indicado para culturas de algodão, arroz, café, cana-de-açúcar, soja e milho. É um herbicida bastante requisitado na agricultura por apresentar na sua composição uma substância surfactante POEA (polioxietileno amina) que facilita a penetração em plantas

(MARTINS-GOMES et al. 2022). Contudo, estudos já evidenciaram que este surfactante pode provocar um efeito tóxico intensificado se entrar em contato com organismos no meio ambiente, chegando a ser duas a três vezes mais tóxico que o próprio glifosato (GIESY et al. 2000). Este herbicida é classificado por ser de ação sistêmica e não seletivo, representando um perigo no meio ambiente por poder ser transportado para outros locais, assim como as formulações comerciais do Ácido Diclorofenoxiacético (2,4D) (ISLAM et al. 2018).

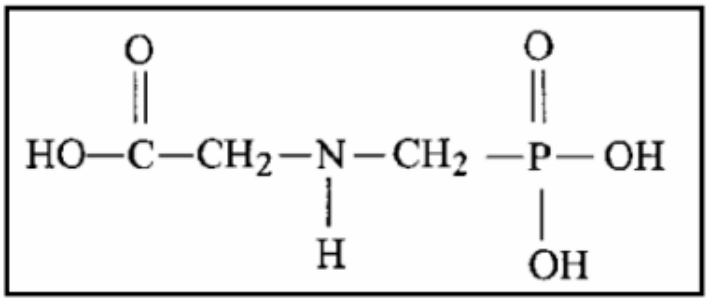


Fig. 1 Estrutura química do glifosato, adaptado de Rodrigues & Almeida (2005), extraído de Persch (2015).

O 2,4D pertence ao grupo químico dos ácidos ariloxialcanóicos (Fig.2) e os ingredientes incluídos em suas formulações são: ésteres, ácidos e vários sais (RED, 2005). É amplamente utilizado em culturas de arroz, cana-de-açúcar, milho, soja e pastagens (THIEL et al. 2020), sua meia vida em águas superficiais é de 4,5 a 7 dias (NETO et al, 2012) e solubilidade em água é de 44,558 mg L^{-1} a 25°C (Gervais et al. 2008; Peterson et al. 2016). Além disso, o 2,4D é o ingrediente ativo do composto comercial Dez©. Esse apresenta eficácia na sua aplicação para o controle de ervas daninhas, sendo um produto de escolha por muitos agricultores. Sabe-se que tal herbicida pode representar uma ameaça para espécies não-alvo (THIEL et al. 2020).

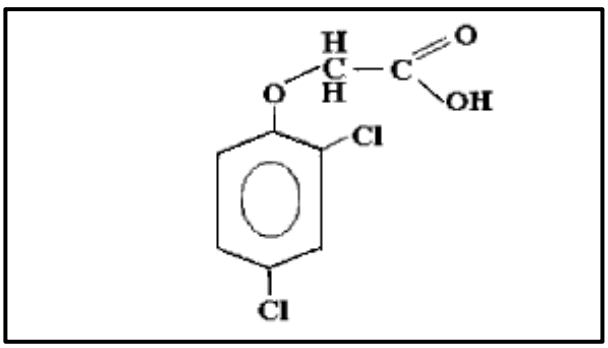


Fig. 2 Estrutura química do 2,4D, extraído de Campos et al. (2004).

Dessa forma, com a ampla utilização de agrotóxicos na agricultura sabe-se que o destino dos poluentes aplicados pode dispersar e uma porcentagem desses químicos pode alcançar corpos d'água e contaminar diversos sistemas aquáticos (riachos, banhados, lagos etc.), resultando na modificação e poluição desses locais (PERSCH et al. 2018; VOLCAN; GONÇALVES; LANÉS, 2011). Portanto, além do despejo direto desses produtos contaminantes no ambiente aquático, também podem ser transportados através de outras vias, destacando-se as rotas de Lixiviação e Escoamento Superficial (QUEIROZ et al. 2011). Segundo QUEIROZ et al. (2011), o processo de lixiviação ocorre através do transporte de moléculas do agrotóxico para camadas mais profundas do solo, alcançando e contaminando massas subterrâneas de água. Já no escoamento superficial ocorre o transporte do agroquímico acumulado às partículas do solo erodido, assim contribuindo para a contaminação das águas superficiais (QUEIROZ et al. 2011). Sendo assim, o impacto da contaminação desses ambientes aquáticos pode ser muitas vezes irreversível e gerar efeitos bastante tóxicos, prejudicando a biota (HWANG et al. 2009; PERSCH et al. 2018). São alguns efeitos encontrados em estudos que foram provocados pela exposição a diferentes agrotóxicos: alterações de comportamento e em reservas energéticas, desajustes na reprodução, redução de massa corporal entre outros (DA SILVA; BORGES-MARTINS; OLIVEIRA, 2021; GAAIED et al. 2020; THIEL et al. 2020; ZEBRAL et al. 2018c). Dessa forma, é considerado um grande desafio da ecotoxicologia aquática em predizer esses efeitos que podem acontecer no ambiente, buscando evitar a possível mortalidade de diversos organismos vivos.

Para esse estudo, a escolha dos herbicidas com princípios ativos glifosato e 2,4D está relacionada a ampla utilização desses agrotóxicos na agricultura da região sul do Brasil, principalmente nas áreas de cultivo de arroz irrigado. Nessas áreas, o arroz passa a maior parte do seu cultivo submerso, sendo que nesses locais é o habitat de muitas espécies que podem ser impactadas (GIESY; DOBSON; SOLOMON, 2000; PERSCH et al. 2017). Além disso, sabe-se que Resolução do Conselho Nacional do Meio Ambiente (CONAMA) n° 357/2005 ordena sobre a classificação de corpos d'água, determinando como valor máximo permitido para água doce de Classe I a concentração de 65µg/L (Glifosato) e 4,0 µg/L (2,4D). Portanto, as concentrações utilizadas nesse estudo foram baseadas a partir das concentrações permitidas.

Biomarcadores: Metabolismo e Estresse Oxidativo

A fisiologia da conservação emprega conceitos e conhecimentos fisiológicos buscando compreender como a biodiversidade responde as mudanças e estressores ambientais (COOKE et al. 2013). Sendo assim, essa disciplina científica possibilita ferramentas que permitem a caracterização dos organismos frente às alterações no ambiente as quais estão expostos, estando inserido nesse contexto o estabelecimento de biomarcadores de metabolismo e estresse oxidativo. Os biomarcadores (ou marcadores biológicos) são determinados como indicadores do estado fisiológico do organismo a exposição a estressores presentes no ambiente. Dentre os biomarcadores, pode-se destacar a intensidade da atividade de marcadores do metabolismo intermediário, do estresse oxidativo e de biotransformação (BRAGHIROLLI; OLIVEIRA; OLIVEIRA, 2016; PERSCH et al. 2017).

As EROs compreendem um grupo de moléculas derivadas do oxigênio molecular, que são formadas por reações de redução-oxidação (Redox) (SIES; JONES, 2020). Além disso, apresentam importante função nos processos fisiológicos do ciclo de vida do animal (ex.: defesa do sistema imunológico) (APEL; HIRT, 2004; DOWLING; SIMMONS, 2009; VASCONCELOS et al., 2007). Dentre as EROs está o Peróxido de Hidrogênio (H_2O_2), Ânion Radical Superóxido (O2•-) e Radical Hidroxila (OH-). O Ânion Radical Superóxido (O_2•-) é produzido continuamente em vários processos celulares e tem grande importância para as células de defesa (BARREIROS; DAVID; DAVID, 2006; SIES; JONES, 2020; SIES, 1991; VASCONCELOS et al., 2007). O Peróxido de Hidrogênio (H_2O_2) é gerado pela dismutação do O_2•- catalisada por enzimas, que se encontram no peroxissomo. É reconhecido como o principal EROs na regulação redox de atividades biológicas (BARREIROS; DAVID; DAVID, 2006; SIES; JONES, 2020; SIES, 1991; VASCONCELOS et al., 2007). O H_2O_2, apesar de não ser um radical livre, por não ter um elétron desemparelhado na sua última camada eletrônica como tem o O_2•- e o OH•, é uma espécie com alto potencial reativo. Por participar da reação de geração do radical OH• que tem ação deletéria potencial, uma vez que esse se constitui no mais reativo dos radicais livres, pois pode alterar qualquer estrutura celular que se encontre próxima. Diferente dos radicais livres, o H_2O_2 tem vida longa e é capaz de atravessar as membranas celulares apresentando-se potencialmente tóxico para as células. O Radical Hidroxila (OH-) é um iniciador da peroxidação lipídica

(BARREIROS; DAVID; DAVID, 2006; SIES; JONES, 2020; SIES, 1991; VASCONCELOS et al., 2007).

Quando o animal é exposto a ambientes contaminados pode ocasionar um aumento na produção de EROs e, como resposta a esse aumento, o sistema antioxidante pode atuar minimizando os efeitos prejudiciais no organismo (ISAKSSON, 2010; PERSCH et al., 2017). Contudo, essa exposição ao ambiente contaminado pode provocar efeitos no sistema antioxidante dos animais, podendo ocorrer a baixa atividade da enzima ou até mesmo a inibição destas em diversos tecidos (FERREIRA et al., 2010; TONI et al., 2013). Dessa forma, ocorre o desequilíbrio do sistema antioxidante e a produção excessiva de EROs, conduzindo a efeitos deletérios no organismo e podendo gerar um dano oxidativo, também chamado de estresse oxidativo (SIES, 1993). Esse dano provocado pela produção exacerbada de EROs pode afetar vários tipos de moléculas biológicas em busca de estabilidade através de reações químicas (SIES, 1993), induzindo a um quadro de risco em relação a sobrevivência do animal (APEL; HIRT, 2004; COSTANTINI, 2014; SIES, 2018). Segundo SIES (1993) o estresse oxidativo pode estar envolvido em processos como mutagênese, danos a membrana, peroxidação lipídica, oxidação de proteínas entre outros mecanismos (SIES, 1993).

Frente a isso, o estresse oxidativo indica uma alteração da Homeostase Redox, que está responsável pelo estado fisiológico estável ou de equilíbrio do organismo (URSINI; MAIORINO; FORMAN, 2016). Dessa forma, quando um indivíduo é apresentado a desafios oxidativos que são eventos potencialmente prejudiciais que desafiam a homeostase redox, o sistema biológico reage para eliminar esse desafio com o intuito de evitar danos e resultando na alteração estável desta homeostase (SIES; JONES, 2020; URSINI; MAIORINO; FORMAN, 2016). Além disso, segundo Sies (2019) o estresse oxidativo pode ser classificado conforme as escalas de intensidade que variam de Euestresse Oxidativo (respostas benéficas) a Distresse Oxidativo (respostas deletérias). Dessa forma, entende-se que Euestresse Oxidativo (níveis baixos) é quando não há rompimento da homeostase ou formação de EROs em níveis que o sistema antioxidante é capaz de agir e essas EROs atuam como importantes sinalizadores durante a sobrecarga; e Distresse Oxidativo (níveis elevados) é quando há uma interrupção da sinalização redox e/ou dano oxidativo (SIES, 2019).

Para combater as EROs e manter o equilíbrio redox, os organismos apresentam o sistema antioxidante que é dividido em enzimático e não-enzimático. O sistema enzimático pode ser representado pelas enzimas, como: Superóxido dismutase (SOD), Catalase (CAT) e a Glutationa Peroxidase. A SOD catalisa a dismutação do ânion radical superóxido (O_2 •-) convertendo em peróxido de hidrogênio (H2O2) e oxigênio (O_2) (APEL; HIRT, 2004; CONSTANTINI, 2014; VASCONCELOS et al., 2007). A CAT é responsável por catalisar a conversão de H_2O_2 em O_2 e H_2O (APEL; HIRT, 2004; BARBOSA et al., 2010; CONSTANTINI, 2014; VASCONCELOS et al., 2007). Já a Glutationa peroxidase é a enzima responsável pela metabolização de peróxidos orgânicos e inorgânicos, apresentando um baixo Km para o H_2O_2. Sua atividade depende da glutationa reduzida, que é oxidada em glutationa oxidada (APEL; HIRT, 2004; BARBOSA et al., 2010; CONSTANTINI, 2014; VASCONCELOS et al., 2007). Estas enzimas constituem a primeira linha de defesa do sistema antioxidante (Persch et al., 2017).

Já o sistema antioxidante não-enzimático é constituído por moléculas de baixo peso molecular e inclui, por exemplo: as vitaminas (ex.: A, C e E), os minerais (ex.: zinco, cobre, selênio e magnésio), a glutationa (GSH) e o ácido úrico (BARBOSA et al., 2010; HERMES-LIMA, 2004). A vitamina A (β-caroteno) tem o papel na proteção contra a oxidação de lipídeos e DNA; a vitamina C (ácido ascórbico) é sintetizada endogenamente e é um potente antioxidante e eliminador de radicais livres; a vitamina E (α-tocoferol) converte O_2• e H_2O_2 em formas menos reativas e desempenha um papel importante na proteção dos ácidos graxos; os minerais podem atuar como cofatores das enzimas antioxidantes; como também, a GSH que também tem atividade antioxidante não enzimática e pode reagir com vários radicais e exerce funções importantes na célula como cofator da família da GPx (BARBOSA et al., 2010; CONSTANTINI, 2014; HERMES-LIMA, 2004; MACHADO et al. 2018). Além desses, o ácido úrico que pode ter propriedades antioxidantes no organismo, através do metabolismo do nitrogênio. De acordo com HERMES-LIMA (2004) o ácido úrico é produzido pela oxidação da xantina catalisada pela xantina oxidase ou xantina desidrogenase. Esse antioxidante não-enzimático procede do metabolismo das purinas e atua neutralizando as EROs (MACHADO et al. 2018; TAKASI et al. 2017).

Dentro deste contexto de biomarcadores, destaca-se as enzimas de biotransformação que têm sido amplamente estudadas, com especial relevância a

Glutationa S-transferase (GST) de ampla distribuição entre diferentes organismos vivos. As GSTs atuam em rotas de excreção de substâncias endo e xenobióticas e, protegem as células contra toxicidade química e estresse, mantendo a homeostase celular. Esta enzima pertence à uma família multifuncional de enzimas que atuam catalisando a conjugação da molécula de glutationa a várias outras moléculas. As GSTs desempenham vários papéis fisiológicos, tais como sequestro e transporte de compostos hidrofóbicos endógenos, os quais incluem hormônios esteróides, heme, bilirubinas, ácidos da bile e seus metabólitos, além de metabolizarem produtos da lipoperoxidação (CNUBBEN et al. 2001; PINHEIRO; OLIVEIRA, 2016). O processo de biotransformação de metabólitos tem como objetivo tornar a molécula mais polar, aumentar o tamanho e o peso molecular para ser mais facilmente excretada.

Dessa forma, quando os animais são expostos aos agrotóxicos podem apresentar respostas ou estratégias metabólicas e oxidativas diferentes, estando até mesmo relacionada e de acordo com a fase do ciclo de vida da espécie, como uma forma de adaptação ao ambiente contaminado (FANTA et al., 2003; PERSCH et al., 2018). O efeito a exposição pode ser indireto sobre os parâmetros fisiológicos e bioquímicos do organismo, afetando níveis de armazenamento de energia na tentativa de se adaptar ao estresse induzido, são alguns exemplos: glicogênio (polímero de glicose utilizado com fonte de energia), lipídeos totais (importante fonte de energia e controle da homeostasia do organismo), triglicerídeos (hidrolisados em vários tecidos como fígado, rins e músculo) e proteínas totais (desempenham o papel vital na fisiologia das células) (PERSCH et al., 2018). Contudo, essa resposta de adaptação compensatória ao estresse da exposição pode provocar o catabolismo de metabólitos para garantir a funcionalidade dos tecidos para essas situações (CERICATO et al., 2009; MENEZES et al., 2015). Sendo assim, ocorrendo o consumo de metabólitos como uma fonte de energia para manter o equilíbrio do organismo e, também, o aumento de reservas destes em outros tecidos (PERSCH et al., 2018). Sendo assim, os animais têm como efeito dessa exposição o investimento e utilização de suas reservas como uma forma de adaptação ao meio e, com isso, podendo resultar em mudanças em outros processos, por exemplo: inibição do crescimento, aceleração da metamorfose, comprometimento do desenvolvimento, da reprodução, do comportamento e até mesmo da sobrevivência (COLTRO et al., 2017; PERSCH et al., 2018).

Além disso, como efeito a essa exposição aos agrotóxicos pode haver alterações em parâmetros oxidativos que estão envolvidos em processos fisiológicos do animal como na produção de espécies reativas de oxigênio (EROs), que têm grande importância no sistema biológico do animal, e na intensidade da atividade de enzimas do balanço oxidativo e de biotransformação (BRAGHIROLLI; OLIVEIRA; OLIVEIRA, 2016; DANTZER et al., 2014; PERSCH et al., 2017).

De acordo com Fanta et al. (2003) o estudo de parâmetros fisiológicos em peixes expostos a concentrações subletais de agrotóxicos se faz de extrema importância, visto que o período de permanência do poluente no ambiente pode não acarretar a mortalidade imediata de organismos não-alvo e sim problemas em outros processos. Frente a isso, diversos estudos vêm sendo desenvolvidos com peixes de água doce expostos a diferentes herbicidas para a determinação de marcadores metabólicos e de estresse oxidativo. Estudo de PERSCH et al. (2017, 2018) avaliaram o efeito de diferentes concentrações dos herbicidas, Roundup, Primoleo e Facet, em alevinos adultos e jovens *Rhamdia quelen* para a determinação de marcadores metabólicos (proteínas totais, lipídeos totais, triglicerídeos e glicogênio) e de estresse oxidativo (Lipoperoxidação (TBARS), Superóxido Dismutase (SOD), Catalase (CAT)). Os resultados mostraram que a exposição aos agrotóxicos testados provocou alterações na composição bioquímica dos tecidos da espécie, principalmente ao tecido branquial que tem o papel importante nas trocas gasosas e no equilíbrio osmótico. Em relação aos marcadores de estresse oxidativo, o sistema antioxidante atuou de forma eficiente na defesa do organismo quando exposto ao Roundup e Primoleo, porém a exposição ao Facet provocou danos significativos nos tecidos destacando as brânquias, o rim e o tecido muscular (PERSCH et al., 2017, 2018).

Também, PANETTO et al. (2019) realizaram um estudo com *Danio rerio* exposto ao herbicida Roundup® para verificar os efeitos no metabolismo energético (proteína, glicose, glicogênio, triglicerídeos) e na atividade enzimática (alanina aminotransferase (ALT), aspartato aminotransferase (AST) e Hexoquinase) do *zebrafish*. Os resultados com a exposição mostraram que o herbicida pode induzir problemas na embriogênese relacionada à incapacidade da bexiga natatória de inflar, sendo este dispositivo importante na flutuação. Nenhum efeito significativo foi observado sobre os parâmetros metabólicos e as atividades enzimáticas ALT e AST, contudo a atividade da Hexoquinase foi afetada pela exposição (PANETTO et al., 2019).

E, estudo de THIEL et al. (2020) avaliaram o efeito do herbicida 2,4-D em *Zebrafish* (*Danio rerio*) sobre o metabolismo energético mitocondrial, o comportamento exploratório e o status oxidativo para a determinação dos níveis de Lipoperoxidação (TBARS) e atividade das enzimas Catalase (CAT) e Superóxido dismutase (SOD). Os resultados mostraram que a exposição ao 2,4-D, nas concentrações encontradas no ambiente, provocou alterações no metabolismo mitocondrial e estresse oxidativo em *Zebrafish*, prejudicando até mesmo o comportamento inato. Nesse estudo ocorreu um aumento da atividade da CAT e reduziu a razão SOD/CAT; além disso, os animais aumentaram a distância total percorrida e os cruzamentos entre diferentes zonas. Sendo assim, os resultados deste estudo sugeriram que o 2,4-D presente no ambiente pode induzir efeitos prejudiciais na capacidade dos organismos não alvos (THIEL et al., 2020).

Bioindicador: Peixes Anuais

O estabelecimento de bioindicadores (ou indicadores biológicos) permite avaliar a qualidade do ambiente. De acordo com VAN GESTEL & VAN BRUMMELEN (1996) os indicadores biológicos, são considerados organismos vivos que indicam e fornecem informações sobre as condições e alterações que ocorrem no meio. Dessa forma, para uma melhor representatividade do sistema enfatiza-se a importância na padronização de organismos autóctones na pesquisa (PERSCH et al. 2017). Sendo assim, a partir da padronização dessas espécies consideradas bioindicadoras é possível compreender as mudanças fisiológicas no ciclo de vida desses animais no meio ambiente (BRAGHIROLLI; OLIVEIRA; OLIVEIRA, 2016).

A ordem Cyprinodontiformes é representada por duas famílias, Nothobranchiidae e Rivulidae, as quais estão intimamente relacionadas e apresentam características fisiológicas e ecológicas recíprocas (LANÉS, 2011). Esses animais habitam ambientes aquáticos temporários (Fig. 3) que têm como característica secarem sazonalmente, ou seja, as áreas se formam em épocas de chuva e dessecam na estação de seca (COSTA, 1998; LANÉS, 2011). Tais ambientes temporários faz com que esses animais tenham um ciclo de vida curto e rápido (Fig. 4), sendo assim, estimulam mecanismos de adaptação específicos para que consigam sobreviver no meio em que vivem, como: rápido desenvolvimento, maturação sexual, intenso processo reprodutivo e eficiente

produção e deposição de ovos no substrato (BEROIS et al. 2012; LAUFER et al. 2009). Segundo POLACIK; PODRABSKY (2015) tal característica de deposição dos ovos pode ser interpretada como uma estratégia de sobrevivência da população para dar continuidade a espécie em ambiente natural. Assim como, outra característica relevante é o mecanismo de diapausa dos embriões (fases de desenvolvimento embrionário: DI, DII e DIII) que está intimamente relacionada as condições do ambiente (BEROIS et al. 2012; PODRABSKY; HAND, 1999; WOURMS, 1972). Esse mecanismo de diapausa é considerado uma adaptação dos peixes anuais que permite a manutenção destas populações em ambientes que secam completamente. Na diapausa I ocorre no início do desenvolvimento e está associada à dispersão e a reagregação dos blastômeros. Na diapausa II ocorre a formação do eixo embrionário, o estabelecimento das fundações do sistema nervoso central e o surgimento de baixos batimentos cardíacos (BEROIS et al., 2012; WOURMS, 1972). E, por fim, a diapausa III que acontece antes da eclosão, quando o embrião está bem desenvolvido eclodindo apenas quando as condições do ambiente apresentarem-se favoráveis (BEROIS et al., 2012; PODRABSKY; HAND, 1999).

Além disso, por esses animais apresentarem tais características e, principalmente, o ciclo de vida curto e rápido são reconhecidos como potenciais modelos biológicos na pesquisa, pois permitem o desenvolvimento de estudos que avaliem aspectos funcionais ao longo de estágios específicos do ciclo de vida. Também, possibilitam estudos sobre o processo de envelhecimento destes organismos através da deterioração relacionada a idade (BEROIS et al. 2012; LUCAS-SÁNCHEZ et al. 2014), tendo em vista que o desenvolvimento de pesquisa com vertebrados sobre os processos de envelhecimento é limitado pela falta de modelos que apresentem ciclo de vida curto (GENADE et al. 2005). E, cabe ressaltar que assim como outras espécies de peixes, os peixes anuais apresentarem semelhanças como a oviparidade, transparência de ovos e embriões, capacidade de estocagem de ovos e fácil manutenção em laboratório (BEROIS et al. 2012).

Dessa forma, a espécie escolhida para a pesquisa é a *Cynopoecilus* sp. (Fig. 5) que pertence à família Rivulidae, endêmica no território brasileiro (LOUREIRO et al. 2018). Ocorre em locais que podem coincidir com áreas de intensa atividade agrícola, principalmente o cultivo de arroz irrigado (AMARANTE JR et al. 2002). Também, de

acordo com KASTER GARCEZ et al. (2020) os peixes anuais são um dos grupos mais ameaçados, pois abrangem populações pequenas e isoladas.

Frente a isto, o objeto geral desse estudo propõe avaliar o efeito dos herbicidas Roundup Original© (princípio ativo glifosato) e Dez© (princípio ativo 2,4D) em diferentes estágios de desenvolvimento (adulto-jovem e senil) de machos e fêmeas de *Cynopoecilus* sp. sobre marcadores de metabolismo intermediário e balanço oxidativo.

Fig. 3 Ambiente aquático temporário, onde foi coletada a espécie em estudo. Dunas Altas, Município de Palmares do Sul, Rio Grande do Sul/Brasil. Fonte: Castro, B.D.

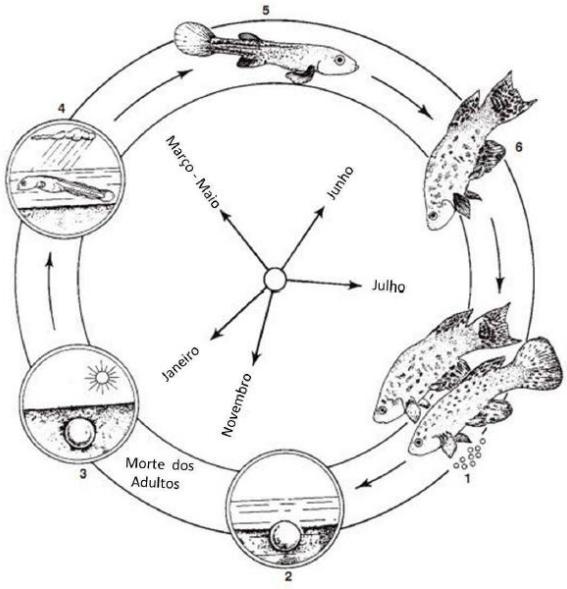


Fig. 4 Ilustração esquemática do ciclo de vida do peixe anual no Sul, baseado no ciclo de vida da espécie *Austrofundulus myersi* Dahl, 1958. Fonte: Modificado por Lanés (2011) de Wourms (1972).

Fig. 5 Peixe anual *Cynopoecilus* sp. Fonte: Castro, B.D.

CAPÍTULO 1

DEVELOPMENT STAGE-DEPENDENT OXIDATIVE STRESS RESPONSES TO THE EXPOSURE TO ROUNDUP ORIGINAL© IN A NEOTROPICAL ANNUAL KILLIFISH

Environmental Toxicology and Pharmacology 95 (2022) 103976

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Development stage-dependent oxidative stress responses to the exposure to roundup original© in a neotropical annual killifish

Bruna Dutra De Castro [a,1], Luis Esteban Krause Lanés [b,2], Robson Souza Godoy [c,3], Leonardo Maltchik [d,4], Guendalina Turcato Oliveira [a,*,5]

[a] PUCRS, Pontifical Catholic University of Rio Grande do Sul, School of Health and Life Sciences, Conservation Physiology Laboratory, Conservation Physiology Laboratory, Porto Alegre, RS, Brazil
[b] Pro-Pampa Institute, Pelotas, RS, Brazil
[c] UNISINOS, Universidade do Vale do Rio dos Sinos, Centro de Ciências, Programa de Pós-Graduação de Biologia, Laboratório de Ecologia e Conservação de Ecossistemas Aquáticos, São Leopoldo, RS, Brazil
[d] FURG, Federal University of Rio Grande, Rectory, Pro-Rectory of Research and Graduate Studies, University of Rio Grande Foundation, Rio Grande, RS, Brazil

ARTICLE INFO

Editor: Silvia Franzellitti

Keywords:
Cynopoecilus sp.
Life cycle
Glyphosate
Oxidative balance
Senescence
Toxicology

ABSTRACT

Herbicides are the most commonly applied pesticides in Brazil, specifically those based on glyphosate, and are used for different crops, near the habitats of annual killifish. Annual killifish presents a short life cycle with generally restricted geographic distribution. In this context, we evaluated the effect of the Roundup Original© (65, 130 and 260 μg. L^{-1} of glyphosate) herbicide on different development stages (adult-young and senile) of the annual killifish (*Cynopoecilus* sp.). We quantified the oxidative balance markers (superoxide dismutase, catalase, glutathione S-transferase, lipid peroxidation levels, and total proteins). We observed that the senile individuals presented 2-fold higher lipid peroxidation levels associated with the maintenance of superoxide dismutase and catalase activity levels even after exposure to the herbicide. However, senile subjects were negatively impacted by the exposure to formulations containing glyphosate, and this was related to a loss of glutathione S-transferase activity. Our research demonstrated that the established physiological markers and this species look promising for toxicology studies.

1. Introduction

Aquatic ecosystems are constantly exposed and vulnerable to chemicals (López-Valcárcel and Parra Del Arco, 2021). According to Lima et al. (2018), agricultural areas contribute to diffuse pollution in these water bodies. The use of chemical substances (e.g., fertilizers, pesticides) in intensive agriculture allows the transport and entry of different forms into the water, which may occur through drainage groundwater, runoff, leaching, and spray drift (Cosgrove et al., 2019; Lima et al., 2018; Moraes et al., 2020).

According to Rocha & Grisolia (2018), Brazil is considered the largest pesticide market globally, specifically the glyphosate-based herbicides that have increased their global use (Bridi et al., 2017; Riaño et al., 2020). Roundup® is a commercial formulation containing glyphosate as an active ingredient. This herbicide is classified as non-selective with a systemic action of the chemical group of substituted glycines (Persch et al., 2017). In addition, it uses a POEA (polyoxyethylene amine) surfactant to facilitate penetration into plants and promotes a more significant toxic effect in organisms. This herbicide is widely used to cultivate corn, soybeans and irrigated rice (Giesy et al., 2000; Persch et al., 2017). The concentration and occurrence of glyphosate in surface waters it depends on the climate, proximity to

* Correspondence to: Conservation Physiology Laboratory, Department of Morphophysiological Sciences, Faculty of Health and Life Sciences, Pontifical Catholic University of Rio Grande do Sul, Ipiranga Avenue, 6681 Pd. 12, Block C, class 270, 90619–900 Porto Alegre, RS, Brazil.
E-mail address: guendato@pucrs.br (G.T. Oliveira).
[1] https://orcid.org/0000–0001-7418–1164
[2] https://orcid.org/0000–0001-5295–3733
[3] https://orcid.org/0000–0001-5757–9146
[4] https://orcid.org/0000–0002-5321–7524
[5] https://orcid.org/0000–0001-7929–917X

https://doi.org/10.1016/j.etap.2022.103976
Received 2 June 2022; Received in revised form 13 August 2022; Accepted 8 September 2022
Available online 12 September 2022

agricultural regions and frequency of use and dose used (Coupe et al., 2011). Studies in different locations in South America show that the active ingredient glyphosate has been found in surface and groundwater in concentrations ranging from 0.001 to 2.02 mg/L (Avigliano And Schenone, 2015; Freire et al., 2012; Okada et al., 2018; Peruzzo et al., 2008).

Glyphosate residues in water bodies can affect several aquatic organisms, such as crustaceans, fish and amphibians (Bridi et al., 2017; Gill et al., 2018; Panetto et al., 2019). In commercial formulations glyphosate is not present alone, but in combination with other additives (i.e., surfactants and co-formulants) that are added to enhance herbicide adhesion to the leaf surface and transport through the waxy cuticle membrane to the site of action (Parlapiano et al., 2021). Studies have shown that these glyphosate-based herbicides (GBH) are usually more toxic to animals than the active ingredient alone, and in addition, they confer different toxicity to these formulations when compared to each other (Tsui And Chu, 2003; Contaro-jara et al., 2009; Cuhra et al., 2013; Parlapiano et al., 2021).

Studies have been developed with freshwater fish exposed to commercial formulations of the herbicide glyphosate to verify the effect of exposure on metabolic markers and oxidative stress, as well as to assess the sensitivity of these organisms. Such research has shown that exposure to glyphosate can affect and cause significant tissue damage in *Rhandia quelen* and goldfish (Lushchak et al., 2009; Persch et al., 2017, 2018), induce oxidative stress even at sublethal doses in *Clarias gariepinus* (Nwonumara and okogwu, 2020) and to induce problems in the embryogenesis of *Danio rerio*, related to the inability of the swim bladder to inflate (Panetto et al., 2019). In addition, other studies were carried out with annual killifish species, *Nothobranchius neumanni* and *Austrolebias nigrofasciatus*, for short-term toxicity tests. *Nothobranchius neumanni* showed to have more sensitivity to the herbicide glyphosate compared to other classical fish models (Kafula et al., 2022) and *A. nigrofasciatus* showed reduced fertility, superior embryonic thermal tolerance, and alteration in embryonic diapause, indicating that this herbicide has negative results in fish reproduction, embryonic development and thermal tolerance (Zebral et al., 2018).

Fish species are considered organisms that indicate environmental degradation and can be used as biomarkers of aquatic ecosystem quality since they are susceptible to changes and environmental stressors (Albinati et al., 2009; Fausch et al., 1990; Panetto et al., 2019). They have great potential as a model organism in toxicological research to verify the impacts of using pesticides in agriculture, such as exposing the annual killifish (*Nothobranchius furzeri)* to 3,4-DCA in combination with temperature variation (Philippe et al., 2019). In ecotoxicological research, using autochthonous organisms adapted to the local environment is also relevant, as they provide results more compatible with the reality (Albinati et al., 2009; Persch et al., 2017; Zebral et al., 2018). Moreover, the annual killifish (*Nothobranchius furzeri*) is considered a model organism in ecotoxicology for toxicity tests (Philippe et al., 2017, 2018).

Cynopoecilus sp. is an annual killifish of the Rivulidae family and is endemic to southern Brazil. These organisms have a short post-hatch and a rapid life cycle, which is associated with inhabiting temporary wetlands that dry out seasonally (Cellerino et al., 2016; Costa, 1998; Loureiro et al., 2018). In southern Brazil, the area of occurrence of some species of annual killifish generally coincides with intensive agricultural activity, especially the cultivation of irrigated rice (Amarante et al., 2002; Volcan et al., 2015). In addition, the life cycle characteristics of this species, as well as its distribution, allow its use as a biological model for laboratory studies, including those on aging and toxicology (Volcan et al., 2015).

The physiology evaluation of aquatic organisms exposed to pesticides in agricultural areas close to their occurrence is crucial (Persch et al., 2018). Physiological tools can be used to characterize biological diversity and its ecological implications, as well as provide knowledge of the responsiveness of these organisms to environmental changes and stressors (Cooke et al., 2013). These tools (e.g., oxidative balance analysis) are widely used in studies of pesticide exposure, as shown by Ferreira et al. (2010); Menezes et al. (2015); Persch et al., (2017); 2018); Thiel et al. (2020). Pesticides may influence oxidative homeostasis on the reactive oxygen species (ROS) production, alteration in scavenging enzyme system, and lipid peroxidation, promoting oxidative stress. Furthermore, the aging process generally induces the exacerbated production of ROS, potentially leading to an oxidative stress increment (Sule et al., 2022).

Recently, Castro et al. (2021) demonstrated the first seasonal characterization of this species regarding the variation in oxidative stress biomarkers under natural conditions, representing a baseline. These authors observed that males and females used different physiological strategies for redox balance during their life cycles. Their oxidative balance was influenced by their reproductive period and environmental variables (Castro et al., 2021). We used males to avoid the possible influence of hormonal variations typical of females on the oxidative balance.

The present study evaluated the effect of three concentrations of Roundup Original® herbicide on the male specimens of *Cynopoecilus* sp. at different development stages (adult-young and senile). We determined the oxidative balance markers of these individuals. Our study was the first to analyze stage-dependent oxidative stress responses in Neotropical annual killifish exposed to a pesticide. We expected males at both development stages to increase energy expenditure in response to the stress caused by exposure to pesticides, especially at higher concentrations, impacting the antioxidant and biotransformation system. Furthermore, in senile males, a more remarkable change in the parameters of oxidative stress was expected, together with the aging process of the animals, which exhibits an increased risk of mortality related to the progressive decline in fitness and vital functions.

2. Materials and methods

2.1. Ethics approval

The procedures performed were approved by the Chico Mendes Institute for Biodiversity Conservation (ICMBio n°68290–8) and the Ethics Committee for the Use of Animals (CEUA: 9400 e 8271).

2.2. Model Organism and Acclimatization

Annual killifish present in southern Brazil has had its dynamic populations monitored over the last decade (Keppeler et al., 2013, 2015; Lanés et al., 2012; Lanés et al., 2015; 2016). Lanés et al., (2014); (2016) detailed the population dynamics of *Cynopoecilus* species under natural conditions. The species inhabits temporary pools that maintain water for a maximum of 8 months (6–8 months), shows a gradual increase in body size and decrease in density throughout the cycle, and there is no overlap in generations. The lifespan of *Cynopoecilus* occurs during the period of low temperatures, and the post larvae hatch synchronously in austral fall and early winter (May to July). The killifish mortality increases as the water temperature and habitat desiccation increase in late austral Spring (Lanés et al., 2016).

We sampled this species in a natural temporary pool of Dunas Altas, Palmares do Sul municipality, situated in the coastal plain of Rio Grande do Sul, southern Brazil (S 30°23′32.5″; W 050°19′32.4″). This area was selected for its history of non-use of pesticides since it is used for extensive livestock farming on natural grasslands and silviculture (*Pinus* spp.). Killifishes were collected by active sampling using a D-shaped hand net. In each sampling location, we measured the physical-chemical parameters of the water [temperature, oxidation-redox potential (ORP), total dissolved solids (TDS), salinity, pH, and conductivity] in the natural habitat using a portable multiparameter device (Table 1).

For the downstream analyses, only male specimens at different development stages were collected in the different months over one

Table 1
Abiotic parameters of the collection site water used in this study.

Sampling Campaigns	Temperature (°C)	pH	Conductivity (Ms/cm)	ORP (mV)	Salinity	TDS (ppm)
June	18.12	4.83	0.00	72.57	0.16	192.33
November	25.07	5.04	0.00	27.27	0.27	255.17

year: early life cycle (June, adult-young individuals) and the end of the life cycle (November, senile individuals). We obtained approximately eighty individuals in this study. These animals were acclimatized for 10 days in a controlled environment [temperature: 20 ± 1 °C; light/dark photoperiod: 12 h:12 h; constant aeration (PAPA, CLIVIO, MONTAGNE 2015); pH: 6.4–7.0; ammonia: 0.0–1.0; nitrite: 0.0–0.25]. They were fed ad libitum with *Artemia salina* every day (once a day).

2.3. Toxicological assay

The commercial formulation of Roundup Original® (active ingredient glyphosate) was used in the form typically introduced to the natural environment. The experimentation protocol was the same for both stages of development (adult-young and senile). We exposed 60 individuals to three ecologically relevant concentrations of Roundup Original® (65, 130 and 260 μg/L) for seven days after the 10 days of acclimation. The remaining animals (20) constituted the control group and were maintained under acclimation conditions without exposure. Each experimental group consisted of a quadruplicate, and the glyphosate concentration in the aquariums was obtained from a stock solution (1 g a.i./L) prepared from Roundup® Original.

The Environmental Chemistry Laboratory (PUCRS) performed the chemical quantification of glyphosate in the mother solution using the methodology proposed by Marques et al. (2009) [fluoride (F-), chloride (Cl-), nitrate (NO3-), phosphate (PO4–3), and sulphate (SO4–2) anions were determined simultaneously with glyphosate]. A 1.2 g/L concentration of glyphosate was found in this quantification, which remained stable over the five consecutive days. The concentration of glyphosate in the aquarium water was not quantified, being only nominal. Our exposure time respected the half-life reported for glyphosate in the literature (from 7 to 70 days: Giesy et al., 2000).

The concentrations were based on those allowed in water for human consumption after simplified treatment (Class I: maximum value of 65 μg/L) and advanced treatment (Class II: maximum value of 280 μg/L) as provided in the Brazilian legislation by the Resolution of the National Council for the Environment (n° 357/2005; CONSELHO NACIONAL DO MEIO AMBIENTE-CONAMA, 2005). The highest concentrations were established from the regulated, being twice and four times the concentration of 65 μg/L. As well as in glyphosate concentrations found in Brazilian surface waters, ranging from 100 μg/L to 700 μg/L (Peruzzo et al., 2008; Queiroz et al., 2011); in surface waters close to agricultural areas, these ranged from 360 μg/L to 2160 μg/L (Iguchi, 2012; Rodrigues and Almeida, 1998). The abiotic parameters mentioned remained under control. We fed the animals that remained ad libitum with *Artemia salina* every day (once a day).

After the seven-day exposure period, all individuals were euthanized in liquid nitrogen, measured (total length) with a digital calliper (accuracy of 0.01 cm) and weighed (body mass) with an analytical scale (accuracy of 0.001 g). The total length (TL) refers to the distance between the maxilla's anterior end and the caudal fin's end. All biological materials were labeled and stored in a freezer (−80 °C) for downstream analyses of the oxidative balance markers.

2.4. Homogenized preparation

The whole specimens were homogenized in phosphate buffer solution (20 mM, pH 7.4) plus 1 mM PSMF and 140 mM potassium chloride in a volume of seven times the body mass (1 g: 7 ml) for the pools (with 2–3 animals). We used an Ultra-Turrax® (IKA-WERK) and ice bath (0–4 °C). These pools were centrifuged (SORVALL RC-5B Refrigerated Centrifuge) under refrigeration (4°C) at 3600 rpm for 10 min. We used the supernatant for the enzymatic assay (SOD, CAT and GST) and the measurement of lipoperoxidation (LPO) and total proteins (PT). Samples were determined in triplicate (SOD, CAT and GST) and quadruplicate (LPO ans PT) for each biomarker.

2.5. Biomarker analysis

The homogenate supernatant was used for the total proteins (TP) analysis. We performed the quantification using a commercial kit of total proteins by BioTécnica. The assay includes a reaction of the proteins in the sample with copper ions in an alkaline medium. The product of this reaction results in a violet color which is detected at an absorbance of 550 nm. The total protein values were used to normalize the oxidative stress parameters quantified.

We determined the lipoperoxidation (LPO) levels by measuring the damage of membrane lipids and detecting thiobarbituric acid reactive substances (TBARS). We added 300 μL of trichloroacetic acid (TCA), 200 μL of thiobarbituric acid (TBA), 100 μL of Milli-Q water, and 100 μL of the homogenate to microtubes, according to the method described by Buege & Aust (1978). The microtubes were heated to 100 °C for 15 min and cooled for 10 min. Subsequently, to extract the product from this solution, 600 μL of n-butyl alcohol was added to the mixture, stirred in a vortex, and centrifuged at 3600 rpm for 10 min (Lima and Abdalla, 2001). The concentration of TBARS was measured using the supernatant in spectrophotometry at a wavelength of 535 nm. We expressed the results in nmoles de TBA. mg of protein^{-1}.

Superoxide dismutase (SOD) activity was determined in a reaction medium consisting of glycine-NaOH (50 mM, pH 11) and epinephrine (1 mM) (Boveris and Cadenas, 1982). The method inhibits the reaction between the superoxide radical and epinephrine, and a colored product (adrenochrome) is formed with the oxidation of epinephrine. The samples were centrifuged at 3000 rpm for 3 min (4 °C) to perform this procedure. We obtained three curves in 40 μL (960 μL of SOD buffer + 40 μL of sample), 30 μL (970 μL of SOD buffer + 30 μL of sample), and 20 μL volumes (980 μL of SOD buffer + 20 μL of sample), in which we aimed for a total volume of 1000 μL plus 17 μL of epinephrine. Readings were obtained by spectrophotometry at 480 nm. The results were expressed in units of SOD. mg of TP^{-1}. min^{-1}, where a unit of SOD (U) corresponded to the amount of enzyme that inhibits epinephrine oxidation by 50%.

We determined the catalase activity (CAT) with enzymatic kinetic spectrophotometry, according to the method described by Boveris & Chance (1973), which consists of evaluating the decay of hydrogen peroxide (H2O2). Initially, samples were centrifuged at 10000 rpm for 5 min (4 °C). The reaction medium comprised 955 μL of CAT buffer, 10 μL of sample and 35 μL of H2O2 (total volume 1000 μL). We detected the CAT activity at 240 nm, and the results were expressed in nmoles H_2O_2 min^{-1}.mg of protein^{-1}.

The activity of glutathione S-transferase (GST) was quantified following the method of conjugating 1-chloro 2,4 dinitrobenzene (CDNB) and reducing glutathione (GSH) as described by Boyland & Chasseaud (1969). We centrifuged the samples at 3000 rpm for 3 min, and the supernatant was used for reading in spectrophotometry at 340 nm. The results were expressed in nmoles of conjugate CDNB. min^{-1}.mg of protein^{-1}.

2.6. Statistical analysis

Statistical analysis was performed using SPSS Statistics for Windows, version 26.0. We performed the Shapiro-Wilk's test to assess normality, and homogeneity was performed using the Levine's test. We used a two-way ANOVA to determine the effects of age (adult-young and senile) and herbicide concentration to which the animals were exposed, in addition to the interaction between these factors (age and concentration) on the biomarkers. For the parametric data ($p > 0.05$), we used the one-way analysis of variance with Dunnett's posthoc test to compare the groups exposed to the herbicide with the control group at each age (young-adult and senile). Moreover, the Kruskal-Walli's test was used for non-parametric data ($p < 0.05$), followed by the Dunn test to verify the difference between the experimental groups (0, 65, 130, and 260 μg/L) of each age. These values were considered significant when $p < 0.05$. All results were expressed as mean ± standard error.

3. Results

After the two-way ANOVA, no interaction ($p > 0.05$) was observed between age and herbicide concentration for any analyzed biomarkers. However, this analysis (two-way ANOVA) showed an effect of age (young-adult and senile) on SOD, CAT, and TBARS levels. The levels of TBARS were about 2 times higher in senile control animals than in young adults, and this difference remained after exposure to different concentrations of the herbicide. In addition, when we compared the activity of antioxidant enzymes between young and senile adults, we observed a decrease in SOD and an increase in CAT in all experimental groups (Table 2). We observed a significant effect on the CAT and GST activity levels, total protein, TBARS levels, and SOD/CAT ratio when considering the herbicide concentrations (0, 65, 130, and 260 μg/L).

In young adults, exposure to Roundup® did not significantly alter the total protein (Fig. 1-A) and TBARS (Fig. 1-B) levels. Although the results showed a decrease in SOD (Fig. 1-C) and GST (Fig. 1-F) activity at higher herbicide concentrations (130 μg/L and 260 μg/L), these levels of activity were not different from those of the control group ($p = 0.216$ and $p = 0.148$, respectively). We also observed that for the CAT activity, where the levels did not differ from the control group ($F= 2.329$; $p = 0.131$) (Fig. 1-D) but showed an increasing trend in the higher concentration of the herbicide (260 μg/L). The decrease in SOD and increase in CAT at the highest concentration of the herbicide contributed to a significant decrease ($F = 2.741$; $P = 0.043$) in the SOD/CAT ratio compared to the control group (Fig. 1-E). Table 3 shows the full-length data before and after herbicide exposure. We observed a significant difference between the initial and final total lengths in the control groups, 130 μg/L and 260 μg/ Table 3). There was no significant difference in body mass when comparing the initial and final values in each experimental group (Table 4). When comparing the total length and body mass values after the exposure period to the control group, we did not observe significant differences in these parameters ($F= 0.315$ and $p = 0.815$, $F= 0.662$ and $p = 0.581$, respectively). Data referring to senile exposure showed no changes in TBARS levels (Fig. 2-B). There were no significant differences in the activity of SOD (Fig. 2-C), CAT (Fig. 2-D) and the SOD/CAT ratio (Fig. 2E), although there was a tendency for levels to decrease in the groups exposed to Roundup®. Moreover, we observed a significant increase in the total protein levels (Fig. 2-A) at a concentration of 130 μg/L and a decrease in GST activity ($F = 17.727$; $P = 0.01$). This difference was significant at higher concentrations (130 μg/L and 260 μg/L) compared to the control group (Fig. 2-F). Regarding total length and body mass before and after exposure (Tables 3–4), only the control group showed an increased body mass. However, when we compared the body mass of senile animals after the exposure period to the control group, we observed a significant difference ($F= 4.502$ and $p = 0.011$), suggesting an apparent decrease in the body mass gain of the animals exposed to 65 μg/L.

Table 2
Physiological markers results (mean ± standard error) of adult-young and senile annual killifish (Cynopoecilus sp.) exposed to different Roundup Original© concentrations. Total Protein (TP), Thiobarbituric Acid Reactive Substances (TBARS), Glutathione S-transferase (GST), Catalase (CAT), Superoxide dismutase (SOD), and SOD/CAT ratio. The asterisk (*) represents the difference between age groups ($p < 0.05$).

Development stage	Physiological Marker	Roundup Original©			
		Control	65 ug/L	130 ug/L	260 ug/L
Adult-young | Senile	*TP*	10.42 ± 1.25 | 11.77 ± 0.90	9.90 ± 2.61 | 11.58 ± 1.10	13.30 ± 0.59 | 15.64 ± 0.98	14.06 ± 0.98 | 14.00 ± 0.91
	*TBARS**	9.58 ± 1.95 | 18.67 ± 0.47	8.59 ± 2.14 | 20.73 ± 2.25	4.43 ± 1.59 | 15.98 ± 0.97	6.05 ± 1.31 | 16.15 ± 1.04
	*SOD**	14.28 ± 1.86 | 6.36 ± 0.95	16.62 ± 5.37 | 8.56 ± 0.76	10.42 ± 0.62 | 7.29 ± 0.66	9.62 ± 0.79 | 7.57 ± 0.54
	*CAT**	6.24 ± 0.51 | 13.10 ± 1.24	9.48 ± 1.67 | 13.75 ± 1.52	5.98 ± 1.06 | 9.87 ± 0.52	8.69 ± 1.24 | 10.78 ± 1.15
	SOD/CAT	2.37 ± 0.47 | 2.18 ± 0.42	1.67 ± 0.24 | 1.63 ± 0.24	1.89 ± 0.29 | 1.38 ± 0.13	1.15 ± 0.12 | 1.43 ± 0.15
	GST	0.40 ± 0.06 | 0.46 ± 0.01	0.45 ± 0.14 | 0.50 ± 0.02	0.28 ± 0.03 | 0.32 ± 0.02	0.30 ± 0.03 | 0.37 ± 0.01

4. Discussion

When comparing the biomarkers of adult-young and senile animals, we observed that lipid peroxidation levels are about 2 times higher in senile animals, indicating a natural increase in oxidative stress levels that may be associated with an aging functional loss. These results were reinforced by the lack of interaction between age (young-adult and senile) and herbicide concentration found in the ANOVA two-way test. Organisms that allocate a high energy load to life-history traits (growth and reproduction) present a decline in the antioxidant defenses throughout life, related to their survival rates (Monaghan et al., 2009; Metcalfe and Alonso-Alvarez 2010). Annual killifish have a concise post-hatch life cycle with rapid growth, early maturation and prolonged reproduction (Cellerino et al., 2016; Costa, 1998; Loureiro et al., 2018). We observed a difference between the TBARS values of senile and young adults, which increased after exposure to different herbicide concentrations and reached values from 2.4 to 3.6 times higher in the senile. Such a response profile suggests that oxidative damage (lipoperoxidation) can be intensified by exposure to ecologically relevant concentrations of this herbicide, possibly decreasing reproductive capacity and shortening the animals' lifespan.

Exposure to pesticides is considered a challenge for oxidative balance (Redox Homeostasis), which can initiate a response and direct cellular homeostasis to the oxidative threshold (Ursini et al., 2016). This exposure can cause changes in the oxidative parameters involved in physiological and pathological processes, such as an increase in the production of reactive oxygen species (ROS) (Dantzer et al., 2014; Ursini et al., 2016). However, a redox feedback reaction is considered an adaptive response to decrease and restore the organism's homeostasis (Ursini et al., 2016). The activity intensity of the oxidative balance enzymes and biotransformation act to minimize these harmful effects (Dantzer et al., 2014; Isaksson, 2010; Persch et al., 2018).

Our results demonstrated an efficient antioxidant system capacity of adult-young males of *Cynopoecilus* sp. even after exposure to ecologically

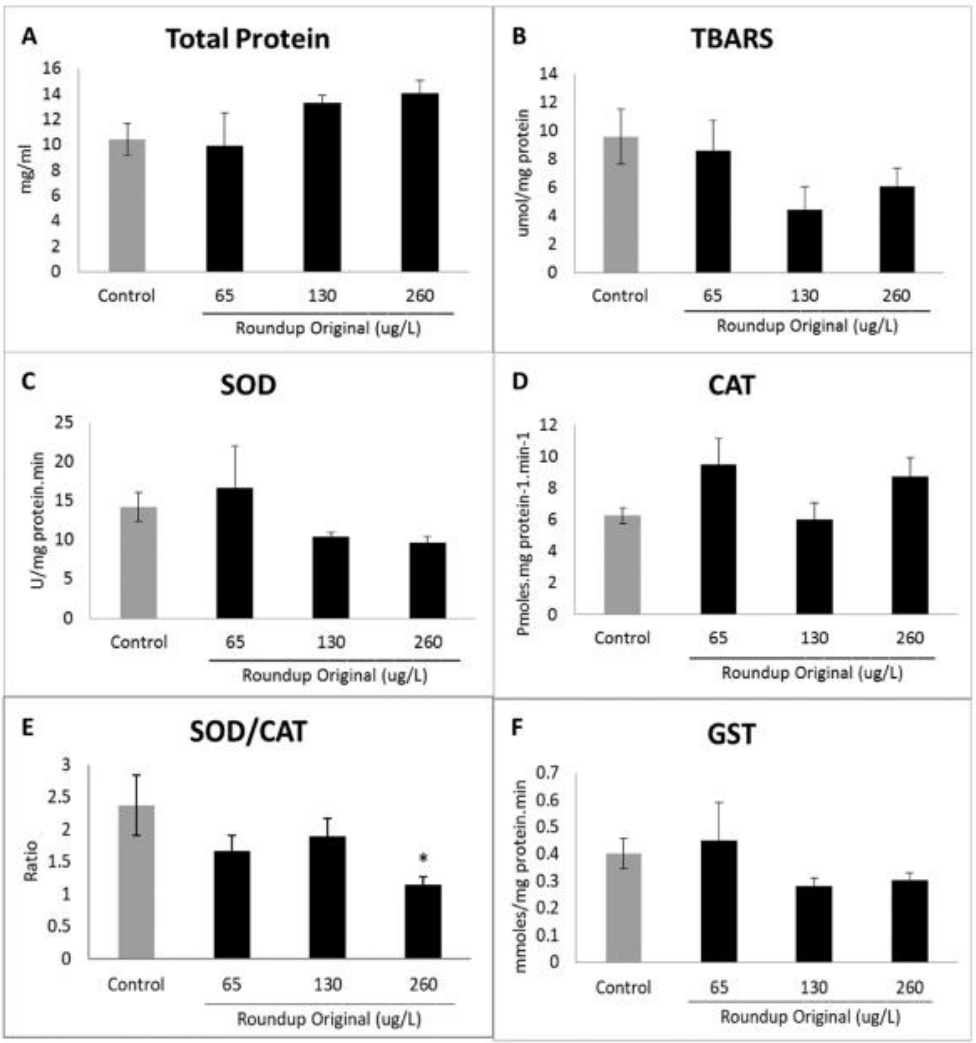


Fig. 1. The effects of Roundup Original (65, 130 and 260 μg/L) on oxidative balance parameters in adult-young of annual killifish (*Cynopoecilus* sp.). (A) Total Protein (TP), (B) thiobarbituric acid reactive substances (TBARS), (C) Superoxide dismutase (SOD), (D) Catalase (CAT), (E) SOD/CAT ratio and (F) Glutathione S-transferase (GST). Bars represent means ± standard error. (*) Significantly different from control group ($p < 0.05$).

relevant concentrations of glyphosate in the commercial formulation (Roundup Original®). Such results may be closely related to a more robust antioxidant system due to the short life cycle of this species (about six to seven months of life), as suggested by Castro et al. (2021) for *Cynopoecilus fulgens*. These animals live in ephemeral environments with widely varying environmental conditions and need to use strategies to withstand these natural challenges to their life cycle (Berois et al., 2012).

Furthermore, our results agreed with the Godoy et al. (2020) study. They evaluated the seasonal variation and environmental links of different oxidative stress markers in the Neotropical annual killifish species, *Austrolebias minuano*. The authors suggested that annual killifish have a well-developed oxidative stress control system determined by an efficient antioxidant system for most of their life cycle, especially in males (Godoy et al., 2019). A recent work by Castro et al. (2021) demonstrated that the antioxidant system of *C. fulgens* in different post-hatch development stages is highly efficient, indicating the ability to sustain oxidative homeostasis until the end of the life cycle (senile) (castro et al., 2021). Persch et al., (2017, 2018) showed that juveniles and newly matured adults of *Rhamdia quelen* exposed to herbicides (atrazine, glyphosate and quinclorac) have different metabolic and oxidative strategies, which were related to their life cycle phase and the exposure to xenobiotic.

According to Glusczak et al. (2011), the determination of lipoperoxidation (LPO) includes quantifying TBARS levels, used as an oxidative damage marker in fish. In the experimental circumstances of this work, no significant changes in TBARS levels were observed in both development stages (adult-young and senile). Such a response profile reinforced the hypothesis of a highly efficient antioxidant system in annual killifish. Persch et al. (2018) also observed a lack of increase in the TBARS levels in different organs (gills, liver and muscle) of juveniles and adults of *R. quelen* exposed for seven days to the herbicide Roundup® (18, 36, 72 and 144 μg/ L of glyphosate). The exposure of *R. quelen* to Roundup® was associated with an increase in CAT activity

Table 3
Initial and final total length (mm) values (mean ± standard error) of adult-young and senile annual killifish (Cynopoecilus sp.) exposed to different Roundup Original© concentrations. The independent samples test was performed in each experimental group (Control, 65 μg/L, 130 μg/L and 260 μg/L) to compare the animals' measurements. The asterisk (*) represents the difference between the initial and final total length ($p < 0.05$).

Development Stage	Group	Initial Total Length	Final Total Length	df	F	p
Adult-young	Control*	22.55 ± 0.82	25.74 ± 0.21	6	2.63	0.01
	65 μg/L	23.69 ± 1.09	24.91 ± 1.43	4	0.09	0.53
	130 μg/L*	22.11 ± 0.43	25.92 ± 0.68	6	0.47	0.00
	260 μg/L*	22.93 ± 0.46	26.51 ± 0.69	6	0.11	0.01
Senile	Control	23.75 ± 1.02	25.50 ± 0.38	4	4.20	0.18
	65 μg/L	22.63 ± 0.65	25.15 ± 1.35	4	1.27	0.17
	130 μg/L	24.70 ± 0.32	26.49 ± 0.68	6	1.81	0.06
	260 μg/L	24.62 ± 0.82	27.23 ± 0.68	6	0.13	0.05

Table 4
Initial and final body mass (g) results (mean ± standard error) of adult-young and senile annual killifish (Cynopoecilus sp.) exposed to different Roundup Original© concentrations. The independent samples test was performed in each experimental group (Control, 65 μg/L, 130 μg/L and 260 μg/L) to compare the animals' measurements. The asterisk (*) represents the difference between the initial and final body mass ($p < 0.05$).

Development Stage	Group	Initial Body Mass	Final Body Mass	df	F	p
Adult-young	Control	0.18 ± 0.02	0.22 ± 0.01	6	1.38	0.19
	65 μg/L	0.39 ± 0.06	0.23 ± 0.02	4	5.54	0.08
	130 μg/L	0.21 ± 0.03	0.19 ± 0.02	6	3.41	0.66
	260 μg/L	0.26 ± 0.04	0.22 ± 0.01	6	32.26	0.31
Senile	Control*	0.16 ± 0.01	0.24 ± 0.01	4	0.00	0.02
	65 μg/L	0.18 ± 0.01	0.16 ± 0.02	4	6.61	0.60
	130 μg/L	0.17 ± 0.01	0.19 ± 0.01	6	0.03	0.16
	260 μg/L	0.19 ± 0.02	0.22 ± 0.02	6	0.01	0.21

which may have been sufficient to contain the increase in lipid peroxidation (Persch et al., 2017).

Therefore, we suggest that adult-young seem to be able to modulate the antioxidant system to the point that they do not undergo oxidative stress even when exposed to ecologically relevant concentrations of glyphosate.This hypothesis can be evidenced by the SOD/CAT ratio, which showed a significant difference at the highest concentration of pesticide and was determined by a decrease in SOD and an increase in CAT. According to ZOCCHE et al. (2014), the high activity of SOD and CAT is usually observed in organisms exposed to environmental pollutants. This response may be related to the joint action of these enzymes in the first line of defense against oxidative stress (Atli and Canli, 2007). However, according to Pinho et al. (2006), the ratio between the activity of SOD/CAT antioxidant enzymes may be more relevant in evaluating the antioxidant profile than the absolute levels of these individual enzymes.

A recent study by Thiel et al. (2020) with *Danio rerio* showed that the increase in CAT reduced the SOD/CAT ratio, indicating that exposure to the herbicide 2,4-dichlorophenoxyacetic acid increased the antioxidant response against H_2O_2 and prevented lipid peroxidation. Our data did not significantly differ in individual SOD and CAT. However, we also observed a significant reduction in the SOD/CAT ratio in adult-young, suggesting that these enzymes' joint action prevents lipid peroxidation, especially at the highest exposure concentration (260 μg/L).

These adult-young animals might be in the process of greater energy allocation for the development period, in which males invest in somatic growth and the process of sexual maturation (Castro et al., 2021) to achieve greater body size and mass and attract a reproductive partner (Passos et al., 2013, 2014). When we compared the morphological data of young adults, an increase in total length and body mass was observed in all experimental groups, regardless of the stress caused by herbicide exposure. The same pattern was not observed in the senile animals, where the males exposed to the herbicide did not show a gain of body mass compared to the control group. The exposure effect, mainly of senile killifish, can determine a deviation of a significant part of the energy reserves to maintain homeostasis to the detriment of other functions such as reproduction species. Future studies to clarify the impact of this herbicide on the reproductive activity of these animals should be carried out.

Furthermore, a significant decrease in GST activity levels at the highest concentrations of Roundup Original® (130 μg/L and 260 μg/L) was observed in senile males compared to the control group. The defense system of the body can simultaneously activate the action of GST, which is a biotransformation enzyme that catalyzes the conjugation of the glutathione molecule to endo and xenobiotics, such as organic hydroperoxides formed mainly in the lipoperoxidation process (Cnubben et al., 2001; Papadopoulos et al., 2004; Pinheiro and Oliveira, 2016). This loss of GST activity in exposed animals may be associated with the higher TBARS levels observed in this group compared to young adults. We suggest that this decrease in GST activity may represent a loss of functional capacity of the biotransformation process, which may increase the residence time and decrease the elimination of this herbicide from the organism. We cannot rule out a loss of GST enzymatic activity due to the depletion of the glutathione reserve. The oxidized glutathione/reduced glutathione pair maintains the cell's redox environment and promotes the reactivation of antioxidant enzymes. Such a response with decreased GST activity can accelerate the mortality of these animals, further shortening their post-hatch life cycle.

Therefore, our data suggest that senile males of this species are more susceptible to the xenobiotic than adult-young, especially at higher pesticide concentrations (130 and 260 μg/L of active ingredient). Castro et al. (2021) showed that the GST activity in male *C. fulgens*, collected in the natural environment, decreased throughout the life cycle, showing a lower enzyme activity in the senescence phase. The authors suggested that GST plays a vital role during sexual maturation, acting in the biotransformation of sex hormones secreted at this stage and showing a subsequent decrease in its activity at the end of the life cycle.

Perhaps herbicide exposure in the natural environment can intensify this loss of GST activity, accelerating the loss of reproductive capacity and death in these animals. Fertility and reproductive parameters of annual killifish are positively related to body size. Development and rapid growth exhibited by annual killifish do not compromise fecundity (Polačik et al., 2014). These are iteroparous and income breeders' fishes, which use energy acquired directly for continuous reproduction (Polacik and Podrabsky, 2015). Our results suggest that oxidative balance alterations in larger and senile individuals due to Roundup exposure may reduce egg bank stocks in wild populations. The exposure to Roundup showed adverse effects on the annual killifish, *Austrolebias nigrofasciatus*, one of the effects being reduced fertility (Zebral et al., 2018). There was a reduction in Zebrafish egg production when exposed to 10 mg/L of glyphosate (Uren Webster et al., 2014).

Regarding senescence, when comparing the results for adult-young and seniles in each biomarker, we observed that senile males have higher TBARS levels (from 20 to 15 μmoles/mg of protein) than adult-

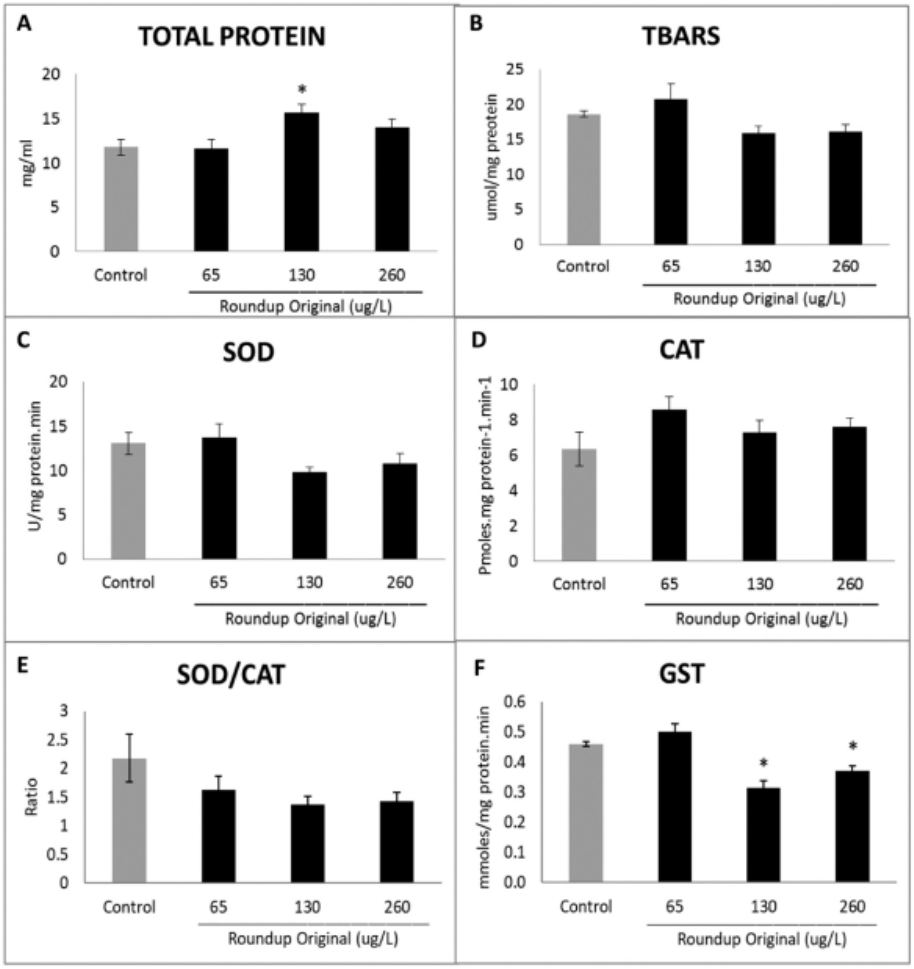


Fig. 2. The effects of Roundup Original (65, 130 and 260 μg/L) on oxidative balance parameters in seniles of annual killifish (*Cynopoecilus* sp.). (A) Total Protein (TP), (B) thiobarbituric acid reactive substances (TBARS), (C) Superoxide dismutase (SOD), (D) Catalase (CAT), (E) SOD/CAT ratio and (F) Glutathione S-transferase (GST). Bars represent means ± standard error. (*) Significantly different from control group ($p < 0.05$).

young (from 10 to 4 μmol/mg of protein) in all experimental groups (control, 65, 130 and 260 μg/L of glyphosate). With this comparison, possible deterioration of the physiological integrity of this species in the senescence phase can be suggested, which is related to the mitochondrial dysfunctions that occur with aging, inducing an increase in the production of ROS (Blażek et al., 2017; Dong et al., 2017). Our results indicate that senile males seem to be more vulnerable and sensitive to the tested toxicity than adult-young, given the difference in TBARS levels between these stages.

5. Conclusion

Regardless of age and development stages, males of *Cynopoecilus* sp. exposed to ecologically relevant concentrations of Roundup managed to modulate the antioxidant enzymes (SOD and CAT) to the point of not showing lipid peroxidation and mortality. However, our results show that senile males of this annual killifish seem to be more vulnerable to commercial glyphosate formulation than adult-young, especially regarding the biotransformation system translated by decreased GST activity. These animals live in temporary wetlands, which determines a short post-hatch life cycle, with males remaining reproductive until senescence (Lanés et al., 2014). In addition, the area of occurrence of some species of annual killifish generally coincides with areas where there is a significant advance in agricultural activity (Amarante et al., 2002; Volcan et al., 2015). The negative impact on the biotransformation system and the oxidative balance must be considered when establishing conservation proposals for these animals. These animals and the established markers proved to be sensitive and seem promising for longitudinal senescence studies and toxicology.

Funding

This work was partially funded by National Council for Scientific and Technological Development (CNPq) through a master's scholarship to B.

D.C.

CRediT authorship contribution statement

B.D.C, L.E.K.L., G.T.O. conceived the study; L.E.K.L., R.S.G., L.M. collected the animals, B.D.C. performed the biochemical analyzes and produced the results; B.D.C, L.E.K.L., G.T.O. analyzed and produced the data.; B.D.C., G.T.O. wrote the paper. All Authors revised the paper.

Declaration of Competing Interest

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

Acknowledgments

We thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for granting B.D.C a scholarship and the Pontifical Catholic University of Rio Grande do Sul (PUCRS) for all the technical support. To the Center for Experimental Biological Models (CeMBE) for supplying some materials and to the Environmental Chemistry Laboratory and Dr. Marçal Pires for quantifying glyphosate. We thank Sarah H. D. dos Santos for the careful review of the English in this work.

Declaration of Competing interest

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

References

Albinati, A.C.L., et al., 2009. Biomarcadores histológicos - toxicidade crônica pelo Roundup em piauçu (*Leporinus macrocephalus*). Arq. Bras. De. Med. Vet. e Zootec. v. 61 (n. 3), 621–627. https://doi.org/10.1590/S0102-09352009000300015.

Amarante, O.P., et al., 2002. Glifosato: propriedades, toxicidade, usos e legislação. Quím. Nova v. 25 (n. 4), 589–593. https://doi.org/10.1590/S0100-40422002000400014.

Atli, G., Canli, M., 2007. Enzymatic responses to metal exposures in a freshwater fish *Oreochromis niloticus*. Comp. Biochem. Physiol. - C. Toxicol. Pharmacol. v. 145 (n. 2), 282–287. https://doi.org/10.1016/j.cbpc.2006.12.012.

Avigliano, E., Schenone, N.F., 2015. Human health risk assessment and environmental distribution of trace elements, glyphosate, fecal coliform and total coliform in Atlantic Rainforest mountain rivers (South America). Microchem. J. 122, 149–158. https://doi.org/10.1016/j.microc.2015.05.004.

Berois, N., et al., 2012. Annual fish: Developmental adaptations for an extreme environment. Wiley Interdiscip. Rev.: Dev. Biol. v. 1 (n. 4), 595–602. https://doi.org/10.1002/wdev.39.

Blažek, R., et al., 2017. Repeated intraspecific divergence in life span and aging of African annual fishes along an aridity gradient. Evolution v. 71 (n. 2), 386–402. https://doi.org/10.1111/evo.13127.

Boveris, A., Cadenas, E., 1982. Production of superoxide radicals and hydrogen peroxide in mitochondria. In: Oberley, L.W. (Ed.), Superoxide dismutase. Flórida: Boca Raton, v. 2p. CRC Press, pp. 15–30.

Boveris, A., Chance, B., 1973. The mitochondrial generation of hydrogen peroxide. Gen. Prop. Eff. Hyperb. Oxyg. Biochem. J. v. 134 (n. 3), 707–716. https://doi.org/10.1042/bj1340707.

Boyland, E., Chasseaud, L.F., 1969. The role of glutathione and glutathione s-transferases in mercapturic acid biosynthesis. Adv. Enzymol. Relat. Areas Mol. Biol. v. 32, 173–219. https://doi.org/10.1002/9780470122778.ch5.

Bridi, D., et al., 2017. Glyphosate and Roundup® alter morphology and behavior in zebrafish. Toxicology v. 392 (n. October), 32–39. https://doi.org/10.1016/j.tox.2017.10.007.

Buege, J.A., Aust, S.D., 1978. Micro Lipid Peroxidation. Methods Enzym 302–310. https://doi.org/10.1016/s0076-6879(78)52032-6.

Castro, B.D., et al., 2021. Biomarkers of oxidative stress in the post-embryonic characterization of the neotropical annual killifish. Biogerontology v. 0. https://doi.org/10.1007/s10522-021-09931-0.

Cellerino, A., Valenzano, D.R., Reichard, M., 2016. From the bush to the bench: the annual Nothobranchius fishes as a new model system in biology. Biol. Rev. v. 91 (n. 2), 511–533. https://doi.org/10.1111/brv.12183.

Cnubben, N.H.P., et al., 2001. The interplay of glutathione related processes in antioxidant defense. Environ. Toxicol. Pharmacol. v. 10, 141–152. https://doi.org/10.1016/s1382-6689(01)00077-1.

Conselho nacional, D.O., 2005. CONSELHO NACIONAL DO MEIO AMBIENTE-CONAMA. RESOLUÇÃO N° 357 DE MARÇO DE 2005 MINISTÉRIO DO MEIO AMBIENTE. DE 17 DE MARÇO DE 2005.

Contaro-jara, V., Klingelmann, E., Wiegand, C., 2009. Bioaccumulation of glyphosate and its formulation Roundup Ultra in *Lumbriculus variegatus* and its effects on biotransformation and antioxidant enzymes. Environ. Pollut. 157, 57–63. https://doi.org/10.1016/j.envpol.2008.07.027.

Cooke, S.J., et al., 2013. What is conservation physiology? Perspectives on an increasingly integrated and essential science. Conserv. Physiol. v. 1 (n. 1), 1–23. https://doi.org/10.1093/conphys/cot001.

Cosgrove, S., Jefferson, B., Jarvis, P., 2019. Pesticide removal from drinking water sources by adsorption: a review. Environ. Technol. Rev. v. 8 (n. 1), 1–24. https://doi.org/10.1080/21622515.2019.1593514.

Costa, W.J.E.M., 1998. Phylogeny and classification of revulidae revisited: origin and evolution of annualism and miniaturization in rivulid fishes (Cyprinodontiformes: Aplocheiloidei). J. Comp. Biol. v. 3 (n. 1), 1–62.

Coupe, R.H., Kalkhoff, S.J., Capel, P.D., Gregoire, C., 2011. Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins. Pest Manag. Sci. 68 (1), 16–30. https://doi.org/10.1002/ps.2212.

Cuhra, M., Traavik, T., Bohn, T., 2013. Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in *Daphnia magna*. Ecotoxicology 22 (2), 251–262. https://doi.org/10.1007/s10646-012-1021-1.

Dantzer, B., et al., 2014. Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species. Conserv. Physiol. v. 2 (n. 1), 1–18. https://doi.org/10.1093/conphys/cou023.

Dong, Y., et al., 2017. Aging asymmetry: systematic survey of changes in age-related biomarkers in the annual fish *Nothobranchius guentheri*. Fish. Physiol. Biochem. v. 43 (n. 2), 309–319. https://doi.org/10.1007/s10695-016-0288-1.

Fausch, K.D., et al., 1990. Fish communities as indicators of environmental degradation. Am. Fish. Soc. Symp. v. 8 (n. July), 123–144.

Ferreira, D., et al., 2010. Assessment of oxidative stress in *Rhamdia quelen* exposed to agrichemicals. Chemosphere v. 79 (n. 9), 914–921. https://doi.org/10.1016/j.chemosphere.2010.03.024.

Freire, R., Schneider, R.M., Freitas, F.H., De, Bonifácio, C.M., Tavares, C.R.G., 2012. Monitoring of toxic chemical in the basin of Maringá stream. Acta Sci. Technol. 34, 295–302. https://doi.org/10.4025/actascitechnol.v34i3.10302.

Giesy, J.P., Dobson, S., Solomon, K.R., 2000. Ecotoxicological Risk Assess. Roundup® Herbic. 35–120. https://doi.org/10.1007/978-1-4612-1156-3_2.

Gill, J.P.K., et al., 2018. Glyphosate toxicity for animals. Environ. Chem. Lett. v. 16 (n. 2), 401–426. https://doi.org/10.1007/s10311-017-0689-0.

Glusczak, L., et al., 2011. Acute exposure to glyphosate herbicide affects oxidative parameters in piava (*Leporinus obtusidens*). Arch. Environ. Contam. Toxicol. v. 61 (n. 4), 624–630. https://doi.org/10.1007/s00244-011-9652-4.

Godoy, R.S., et al., 2019. Oxidative stress resistance in a short-lived Neotropical annual killifish. Biogerontology v. 0123456789. https://doi.org/10.1007/s10522-019-09855-w.

Godoy, R.S., et al., 2020. Oxidative stress resistance in a short-lived Neotropical annual killifish. Biogerontology v. 21 (n. 2), 217–229. https://doi.org/10.1007/s10522-019-09855-w.

Iguchi, E.Y., 2012. Alter. comportamentais em *Geophagus Bras.* submetidos à ação do Herbic. glifosato. Botucatu - SP.

Isaksson, C., 2010. Pollution and its impact on wild animals: a meta-analysis on oxidative stress. EcoHealth v. 7 (n. 3), 342–350. https://doi.org/10.1007/s10393-010-0345-7.

Kafula, Y.A., Philippe, C., Pinceel, T., Munishi, L.K., Moyo, F., Vanschoenwinkel, B., Brendonck, L., Thoré, E.S.J., 2022. Pesticide sensitivity of *Nothobranchius neumanni*, a temporary pond predator with a non-generic life-history. Chemosphere 291. https://doi.org/10.1016/j.chemosphere.2021.132823.

Keppeler, F.W., et al., 2013. The diet of *Cynopoecilus fulgens* Costa, (Cyprinodontiformes: Rivulidae) in Southern Brazil wetlands. Ital. J. Zool. v. 80 (n. May), 291–302. https://doi.org/10.1080/11250003.2012.744107.

Keppeler, F.W., et al., 2015. The morphology-diet relationship and its role in the coexistence of two species of annual fishes. Ecol. Freshw. Fish. v. 24 (n. 1), 77–90. https://doi.org/10.1111/eff.12127.

Lanés, L.E.K., et al., 2015. Effects of an artificial and annual opening of a natural sandbar on the fish community in a coastal lagoon system: a case study in Lagoa do Peixe floodplains, southern Brazil. J. Appl. Ichthyol. v. 31 (n. 2), 321–327. https://doi.org/10.1111/jai.12687.

Lanés, L.E.K., et al., 2016. Seasonal dynamics in community structure, abundance, body size and sex ratio in two species of Neotropical annual fishes. J. Fish. Biol. v. 89 (n. 5), 2345–2364. https://doi.org/10.1111/jfb.13122.

Lanés, L.E.K., Keppeler, F.W., Maltchik, L., 2012. Abundance, sex-ratio, length-weight relation, and condition factor of non-annual killifish *Atlantirivulus riograndensis* (Actinopterygii: Cyprinodontiformes: Rivulidae) in Lagoa do Peixe National Park, a Ramsar site of Southern Brazil. Acta Ichthyol. Et. Piscat. v. 42 (n. 3), 247–252. https://doi.org/10.3750/AIP2011.42.3.09.

Lanés, L.E.K., Keppeler, F.W., Maltchik, L., 2014. Abundance variations and life history traits of two sympatric species of Neotropical annual fish (Cyprinodontiformes: Rivulidae) in temporary ponds of southern Brazil. J. Nat. Hist. v. 48 (n. 31–32), 1971–1988. https://doi.org/10.1080/00222933.2013.862577.

Lima, É.S., Abdalla, D.S.P., 2001. Peroxidação lipídica: mecanismos e avaliação em amostras biológicas. Rev. Bras. De. Ciências Farm. v. 37 (n. 3), 293–303.

Lima, L.B.D.D.E., et al., 2018. Use of biomarkers to evaluate the ecological risk of xenobiotics associated with agriculture. Environ. Pollut. v. 237, 611–624. https://doi.org/10.1016/j.envpol.2018.02.011.

López-Valcárcel, M.E., Parra, G., Del Arco, A., 2021. Environmental disturbance history undermines population responses to cope with anthropogenic and environmental

stressors. Chemosphere v. 262. https://doi.org/10.1016/j.chemosphere.2020.128373.
Loureiro, M., et al., 2018. Review of the family Rivulidae (Cyprinodontiformes, Aplocheiloidei) and a molecular and morphological phylogeny of the annual fish genus *Austrolebias* Costa 1998. Neotrop. Ichthyol. v. 16 (n. 3), 1–20 https://doi.org/10.1590/1982-0224-20180007.
Lushchak, O.V., Kubrak, O.I., Storey, J.M., Storey, K.B., Lushchak, V.I., 2009. Low toxic herbicide Roundup induces mild oxidative stress in goldfish tissues. Chemosphere 76 (7), 932–937. https://doi.org/10.1016/j.chemosphere.2009.04.045.
Marques, M.N., Passos, E.A., da Silva, M.T.S., Correia, F.O., Santos, A.M.O., Gomes, S.S., Alves, J.P.H., 2009. Determination of glyphosate in water samples by IC. J. Chromatogr. Sci. v. 47, 822–824. https://doi.org/10.1093/chromsci/47.9.822 n. 9.
Menezes, C., et al., 2015. Commercial formulation containing 2,4-D affects biochemical parameters and morphological indices of silver catfish exposed for 90 days. Fish. Physiol. Biochem. v. 41 (n. 2), 323–330. https://doi.org/10.1007/s10695-014-9985-9.
Moraes, J.S., et al., 2020. Zebrafish (*Danio rerio*) ability to activate ABCC transporters after exposure to glyphosate and its formulation Roundup Transorb®. Chemosphere v. 248. https://doi.org/10.1016/j.chemosphere.2020.125959.
Nwonumara, G.N., Okogwu, O.I., 2020. Oxidative and biochemical responses as indicators of chemical stress in Clarias gariepinus (Burchell, 1822) juvenile exposed to glyphosate. Sci. Afr. 8 https://doi.org/10.1016/j.sciaf.2020.e00373.
Okada, E., Pérez, D., Gerónimo, E., Aparicio, D.E., Massone, V., Costa, J.L, H., 2018. Nonpoint source pollution of glyphosate and AMPA in a rural basin from the southeast Pampas, Argentina. Environ. Sci. Pollut. Res. 25, 15120–15132. https://doi.org/10.1007/s11356-018-1734-7.
Panetto, O.S., et al., 2019. The effects of Roundup® in embryo developent and energy metabolism of the zebrafish (*Danio rerio*). Comp. Biochem. Physiol. Part - C: Toxicol. Pharmacol. v. 222 (n. April), 74–81. https://doi.org/10.1016/j.cbpc.2019.04.007.
Papa Ng, Clivio Ga, Montagne J, 2015. Husbandry, Reproduction, and Embryo Culture of Annual Fishes under Laboratory Conditions. In: Berois, N., García, G., Sá, R.O. (Eds.), Annual Fishes: Life History Strategy, Diversity, and Evolution. CRC Press, pp. 99–108. https://doi.org/10.1201/b19016-9.
Papadopoulos, A.I., et al., 2004. Glutathione S-transferase in the insect *Apis mellifera macedonica*: Kinetic characteristics and effect of stress on the expression of GST isoenzymes in the adult worker bee. Comp. Biochem. Physiol. - C. Toxicol. Pharmacol. v. 139 (n. 1–3), 93–97. https://doi.org/10.1016/j.cca.2004.09.010.
Parlapiano, I., Biandolino, F., Grattagliano, A., Ruscito, A., Libralato, G., Prato, E., 2021. Effects of commercial formulations of glyphosate on marine crustaceans and implications for risk assessment under temperature changes. Ecotoxicol. Environ. Saf. 213, 112068 https://doi.org/10.1016/j.ecoenv.2021.112068.
Passos, C., et al., 2013. Intra- and intersexual selection on male body size in the annual killifish *Austrolebias charrua*. Behav. Process. v. 96, 20–26. https://doi.org/10.1016/j.beproc.2013.01.008.
Passos, C., et al., 2014. Seasonal variation in female mate choice and operational sex ratio in wild populations of an annual fish, *Austrolebias reicherti*. PLoS ONE v. 9 (n. 7). https://doi.org/10.1371/journal.pone.0101649.
Persch, T.S.P., et al., 2017. Changes in intermediate metabolism and oxidative balance parameters in sexually matured three-barbeled catfishes exposed to herbicides from rice crops (Roundup®, Primoleo® and Facet®). Environ. Toxicol. Pharmacol. v. 58 (n. August), 170–179, 2018. https://doi.org/10.1016/j.etap.2018.01.004.
Persch, T.S.P., et al., 2017. Metabolic parameters and oxidative balance in juvenile *Rhamdia quelen* exposed to rice paddy herbicides: Roundup®, Primoleo®, and Facet®. Chemosphere v. 174, 98–109. https://doi.org/10.1016/j.chemosphere.2017.01.092.
Peruzzo, P.J., Porta, A.A., Ronco, A.E., 2008. Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in north pampasic region of Argentina. Environ. Pollut. v. 156 (n. 1), 61–66. https://doi.org/10.1016/j.envpol.2008.01.015.
Philippe, C., et al., 2017. Acute and chronic sensitivity to copper of a promising ecotoxicological model species, the annual killifish *Nothobranchius furzeri*. Ecotoxicol. Environ. Saf. v. 144 (n. January), 26–35, 2017. https://doi.org/10.1016/j.ecoenv.2017.05.047.
Philippe, C., et al., 2018. Protocol for acute and chronic ecotoxicity testing of the turquoise Killifish *Nothobranchius furzeri*. J. Vis. Exp. v. (n. 134), 1–7, 2018. https://doi.org/10.3791/57308.
Philippe, C., et al., 2019. Interactive effects of 3,4-DCA and temperature on the annual killifish *Nothobranchius furzeri*. Aquat. Toxicol. v. 212 (n. May), 146–153, 2019. https://doi.org/10.1016/j.aquatox.2019.05.009.
Pinheiro, L.C., Oliveira, G.T., 2016. Oxidative status profile in different tissues of *Parastacus brasiliensis promatensis* (Crustacea, Decapoda, Parastacidae) over a seasonal cycle. J. Exp. Zool. Part A: Ecol. Genet. Physiol. v. 325 (n. 5), 318–328. https://doi.org/10.1002/jez.2019.
Pinho, R.A., et al., 2006. Imbalance in SOD/CAT activities in rat skeletal muscles submitted to treadmill training exercise. Cell Biol. Int. v. 30 (n. 10), 848–853. https://doi.org/10.1016/j.cellbi.2006.03.011.
Polacik, M., Podrabsky, J.E., 2015. Tempor. Environ. Extrem. Fishes: Ecol., Evol., Physiol. Teleosts Extrem. Environ. 1–29. https://doi.org/10.1007/978-3-319-13362-1_10.
Polačik, M., et al., 2014. Alternative intrapopulation life-history strategies and their trade-offs in an African annual fish. J. Evolut. Biol. v. 27 (n. 5), 854–865. https://doi.org/10.1111/jeb.12359.
Queiroz, G.M.P., et al., 2011. Transporte de Glifosato pelo Escoamento Superficial e por Lixiviação em um solo agrícola. Quím. Nova v. 34 (n. 2), 190–195. https://doi.org/10.1590/S0100-40422011000200004.
Riaño, C., et al., 2020. Effect of glyphosate (Roundup Active®) on liver of tadpoles of the colombian endemic frog *Dendropsophus molitor* (amphibia: Anura). Chemosphere v. 250. https://doi.org/10.1016/j.chemosphere.2020.126287.
Rocha, G.M., Grisolia, C.K., 2018. Why pesticides with mutagenic, carcinogenic and reproductive risks are registered in Brazil. Dev. World Bioeth. v. 19 (n. 3), 148–154. https://doi.org/10.1111/dewb.12211.
Rodrigues, N.R., Almeida, F.S., 1998. Guia de Herbicidas, 4th ed. IAPAR, Londrina.
Sule, R.O., Condon, L., Gomes, A.V., 2022. A common feature of pesticides: oxidative stress - the role of oxidative stress in pesticide-induced toxicity. Oxid. Med. Cell. Longev. v., 2022. https://doi.org/10.1155/2022/5563759.
Thiel, N.A., et al., 2020. exposure to the herbicide 2,4-dichlorophenoxyacetic acid impairs mitochondrial function, oxidative status, and behavior in adult zebrafish. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-020-10497-6.
Tsui, M.T., Chu, L.M., 2003. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. Chemosphere 52, 1189–1197. https://doi.org/10.1016/S0045-6535(03)00306-0.
Uren Webster, T.M., et al., 2014. Effects of glyphosate and its formulation, Roundup, on reproduction in zebrafish (*Danio rerio*). Environ. Sci. Technol. v. 48 (n. 2), 1271–1279. https://doi.org/10.1021/es404258h.
Ursini, F., Maiorino, M., Forman, H.J., 2016. Redox homeostasis: the Golden Mean of healthy living. Redox Biol. v. 8, 205–215. https://doi.org/10.1016/j.redox.2016.01.010.
Volcan, M.V., et al., 2015. Annual Fishes (Rivulidae) from Southern Brazil: A Broad-scale Assessment of their Diversity and Conservation. Annual Fishes: Life History Strategy, Diversity, and Evolution. CRC Press,, Boca Raton, pp. 185–204. https://doi.org/10.1201/b19016-14.
Zebral, Y.D., et al., 2018. A glyphosate-based herbicide reduces fertility, embryonic upper thermal tolerance and alters embryonic diapause of the threatened annual fish *Austrolebias nigrofasciatus*. Chemosphere v. 196, 260–269. https://doi.org/10.1016/j.chemosphere.2017.12.196.
Zocche, J.J., et al., 2014. Heavy-metal content and oxidative damage in *Hypsiboas faber*: The impact of coal-mining pollutants on amphibians. Arch. Environ. Contam. Toxicol. v. 66 (n. 1), 69–77. https://doi.org/10.1007/s00244-013-9949-6.

CAPÍTULO 2

TOXIC EFFECT OF A GLIFOSATE-BASED HERBICIDE ASSOCIATED WITH DIFFERENT DEVELOPMENTAL STAGES IN NEOTROPICAL ANNUAL KILLIFISH FEMALES

RESEARCH ARTICLE

TOXIC EFFECT OF A GLIFOSATE-BASED HERBICIDE ASSOCIATED WITH DIFFERENT DEVELOPMENTAL STAGES IN NEOTROPICAL ANNUAL KILLIFISH FEMALES

BRUNA DUTRA DE CASTRO[1], LUIS ESTEBAN KRAUSE LANÉS[2], ROBSON SOUZA GODOY[3], GUSTAVO KASPER CUBAS[1], AMANDA DABDAB BRANDELLI[1], LEONARDO MALTCHIK[4], GUENDALINA TURCATO OLIVEIRA[1]*

[1] PUCRS, School of Health and Life Sciences, Conservation Physiology Laboratory, Conservation Physiology Laboratory, Porto Alegre, RS, Brazil

[2] Pro-Pampa Institute, Pelotas, RS, Brazil

[3] UNISINOS University, Sciences Center, Biology Program, Laboratory of Ecology and Conservation of Aquatic Ecosystems, São Leopoldo, RS, Brazil

[4]FURG, Federal University of Rio Grande, Rectory, Pro-Rectory of Research and Graduate Studies, University of Rio Grande Foundation, Rio Grande, RS, Brazil

B.D. de Castro: https://orcid.org/0000-0001-7418-1164

L.E.K. Lanés: https://orcid.org/0000-0001-5295-3733

R.S. Godoy: https://orcid.org/0000-0001-5757-9146

L. Maltchik: https://orcid.org/0000-0002-5321-7524

G.K. Cubas: https://orcid.org/0000-0001-9781-688X

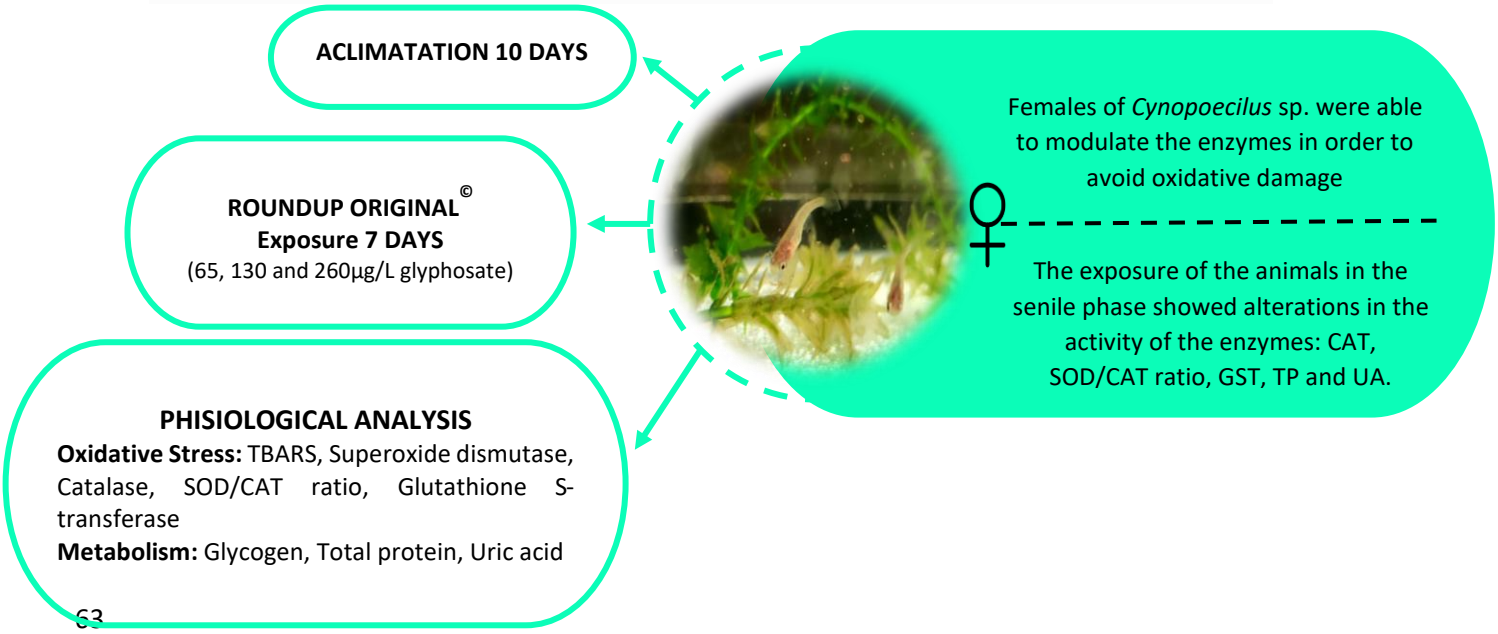
A.D. Brandelli: https://orcid.org/0000-0003-0815-8008

G.T. Oliveira: https://orcid.org/0000-0001-7929-917X

***Corresponding Author:** Guendalina Turcato Oliveira

Conservation Physiology Laboratory, Department of Morphophysiological Sciences, Faculty of Health and Life Sciences, Pontifical Catholic University of Rio Grande do Sul, Ipiranga Avenue, 6681 Pd. 12, Block C, class 270, 90619-900, Porto Alegre, RS, Brasil. ***E-mail addresses:*** guendato@pucrs.br (G.T. Oliveira).

Graphical abstract



Highlights

- Exposure of females to Roundup Original© herbicide does not cause lipoperoxidation.
- Glyphosate-based herbicide exposure modulated oxidative balance markers.
- Herbicide exposure caused changes in metabolism of the young adults (Total Protein and Uric Acid).
- Exposure to the herbicide caused changes in energy markers in Senile (Glycogen and TP).
- Senile females were more vulnerable to a glyphosate-based herbicide.

Abstract

Glyphosate is Brazil's most widely used pesticide and is applied to different crops that may be close to temporary wetlands, posing a threat to populations of annual killifish that live in these locations. We evaluated the effects of Roundup Original© herbicide (active ingredient glyphosate: 65, 130 and 260 µg/L) on the oxidative balance and intermediary metabolism markers in different stages of post-hatching development (young adult and senile) in female of *Cynopoecilus* sp. These markers included lipoperoxidation (TBARS), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase, SOD/CAT ratio, total proteins (TP), glycogen (GG), and uric acid (UA). Senile females showed an increase in CAT and GST and a decrease in the SOD/CAT ratio; combined with an increase in TP and UA when compared to the control group. The two-way analysis showed a significant difference between age groups and groups for several biomarkers suggesting a greater sensitivity to females to glyphosate.

Keywords

Cynopoecilus sp.; Temporary wetlands; Roundup Original©; Oxidative and biochemical markers; defense system.

1. Introduction

Glyphosate is considered the best-selling herbicide in the world, and one of its commercial formulations, Roundup® herbicide, is widely used in agriculture to control the production of weeds (ORTIZ-ORDOÑEZ et al. 2011). However, such commercial formulations contain other substances considered inert. Roundup® consists of a POEA (polyoxyethylene amine) non-ionic surfactant substance to enhance the efficiency of the active ingredient, i.e., glyphosate, by facilitating absorption in plants (HUED; OBERHOFER; DE LOS ÁNGELES BISTONI, 2012). In addition, it is a broad-spectrum, post-emergent, systemic, and non-selective herbicide that is applied in one location and transported to other distant locations (GILL et al. 2018).

With the continuous use of glyphosate in agriculture, motivated by increased productivity, crops became more resistant to the agrochemical, resulting in unlimited and excessive use by farmers, triggering an impact on the environment and threatening various non-target organisms (MESSAAD & Al ZAILAIE 2017; GILL et al. 2018). Once present in the environment, it can reduce the number of species and environmental quality, modify food chains, and even alter the energy flow (PANETTO et al. 2019; PÉREZ et al. 2011). According to GILL et al. (2018), glyphosate toxicity can be observed in unicellular and even multicellular organisms found in soil and water, including aquatic vertebrates such as fish. Fish are of great importance in the food chain of aquatic ecosystems, maintaining the natural balance (GILL et al. 2018). These organisms indicate environmental degradation as they are sensitive to its changes (FAUSCH et al. 1990). Several studies have shown the effects of glyphosate exposure in these aquatic organisms, including damage to the development of embryos, larval and adult stages (AMES et al. 2022; BRIDI et al. 2017; LE DU-CARRÉE et al. 2021). SÁNCHEZ et al. (2021) conducted a study with *Jenynsia multidentata*, a species that naturally inhabits agricultural areas in southern Brazil and Argentina, showed that Roundup® formulations are harmful and that they affect essential behaviors for the survival of fish.

Some annual killifish species (e.g., *Cynopoecilus* sp.) inhabit temporary wetlands in southern Brazil, which sometimes are close to intense agricultural activities, such as irrigated rice cultivation (COSTA, 1998; LOUREIRO et al. 2018; VOLCAN et al. 2015). Due to their restricted distribution, these animals may be at constant risk in their habitat. A recent study with an African annual killifish (*Nothobranchius neumanni*) demonstrated that although these were resistant to environmental stress, their sensitivity

to pollutants was like that of classical fish models (KAFULA et al. (2022). Therefore, it is imperative to study the physiology and response capacity of organisms exposed to pesticide residues in the environment (COOKE et al. 2013). Annual killifish are targeted as biological models in research, as they have a short and fast life cycle, which is a critical characteristic for cultivation and toxicological tests in the laboratory (POLAČIK; BLAŽEK; REICHARD, 2016; ZEBRAL et al. 2018).

We evaluated the effect of three concentrations of the Roundup Original® herbicide on the oxidative balance and intermediary metabolism in different age groups (young adult and senile) of *Cynopoecilus* sp. We expect that females would present more intense responses to the evaluated markers in relation to another study with males of *Cynopoecilus* sp (CASTRO et al. 2022). In addition, we believe that senile females will present more pronounced changes in oxidative balance and energy expenditure when exposed to the Roundup Original® herbicide, especially at the highest concentration tested.

2. Materials and methods

2.1 Ethics

The Chico Mendes Institute for Biodiversity Conservation (ICMBio n°68290-8) and the Ethics in Animal Use Committee (CEUA: 9400 and 8271) approved the experimental protocol.

2.2 Fish and experimental conditions

We used approximately 80 young adult and senile females of *Cynopoecilus* sp., collected in natural pools of Dunas Altas, Municipality of Palmares do Sul, Rio Grande do Sul/Brasil (S 30°23’32.5” W 050°19’32.4”). Samplings were carried out in june and november 2021 in an area with no history of pesticide usage within or around the site. We recorded the physical-chemical parameters of the water using a portable multiparameter device, which included temperature, redox-oxidation potential (ORP), total dissolved solids (TDS), salinity, pH, and conductivity (Table 1).

In the Center for Experimental Biological Models (CeMBE, PUCRS) laboratory, all animals were measured in terms of total length (TL) with a digital calliper (precision of 0.01cm) and body mass with an analytical balance (precision of 0.001 g). Total length measurement included the distance between the maxilla’s anterior and caudal fin’s ends. We then distributed them into aquariums, and the acclimation period lasted for 10 days,

after which the Roundup Original© exposure period began. The animals were separated into the control group (C) with dechlorinated water only, without adding the herbicide, and three concentration groups (65 µg/L [R1]; 130 µg/L [R2]; 260 µg/L [R3] – See Fig. 1). All groups were maintained for seven days, including the control group, given the half-life reported for glyphosate in the literature (GIESY; DOBSON; SOLOMON, 2000).

These herbicide concentrations were based on those allowed in water for human consumption after simplified treatment (Class I: maximum value of 65µg/L) and after advanced treatment (Class II: maximum value of 280µg/L) as established by the Brazilian legislation (Resolution of the National Council for the Environment [CONAMA]: no. 357/2005). We determined the highest concentrations from those found in Brazilian surface waters, ranging from 100µg/L to 700µg/L (QUEIROZ et al. 2011). To contaminate the water in the aquariums, a mother solution of the herbicide containing 1g/L of glyphosate was prepared. Furthermore, we quantified the concentration of gyphosate in mother solution in the Laboratory of Environmental Analytical Chemistry (PUCRS) using the methodology proposed by Marques et al. (2009). This concentration was 1253 mg/L and showed no significant decay.

The animals were kept in aquariums with constant aeration, controlled room temperature (20±1°C) and photoperiod of 12h:12h (light/dark) throughout the experiment. We monitored and recorded the water parameters (pH, ammonia, and nitrite), and all animals were fed *ad libitum* with *Artemia salina*. Their measurements (TL and body mass) were evaluat ed again at the end of the experiment (acclimation (10 days) and exposure periods (7 days)), after which animals were euthanized in liquid nitrogen, identified in individualized flasks, and stored in a -80ºC freezer for subsequent biochemical analyses. These procedures followed what was described by Dutra et al. (2022).

2.3 Enzyme assay and oxidative damage

The homogenates using whole specimens in a phosphate buffer solution (20mM, pH 7.4, plus 1mM PSMF and 140mM potassium chloride) with a volume of seven times the body mass obtained (1g: 7 ml) and created pools containing two animals. During this process, we used the Ultra-Turrax® (IKA-WERK) in an ice bath (0-4°C). Afterwards, each pool was centrifuged (SORVALL RC-5B Refrigerated Superseed Centrifuge) under refrigeration (4ºC) at 3600 rpm for 10 minutes. The supernatant was

used for biochemical analyses determined in duplicate and/or triplicate. The total proteins in the supernatant were quantified using a commercial kit and used to standardize the oxidative balance data.

Lipoperoxidation (LPO) by detecting thiobarbituric acid reactive substances (TBARS). First, we added the following to microtubes, as described by BUEGE; AUST (1978): 300 µL of trichloroacetic acid (TCA), 200 µL of thiobarbituric acid (TBA), 100 µL of Milli-Q water and 100 µL of the homogenate. These microtubes were heated to 100°C for 15 minutes and cooled down for another 10 minutes. Finally, we added 600 µL of n-butyl alcohol to the mixture, stirred them with a Vortex and centrifuged at 3600 rpm for 10 minutes to extract the product from this solution (LIMA; ABDALLA, 2001). TBARS was detected at 535 nm, and the result was expressed in nmoles of TBA.mg protein^{-1}.

Superoxide dismutase (SOD) was determined in a reaction medium consisting of glycine-NaOH (50mM, pH 11) and epinephrine (1mM)(BOVERIS; CADENAS, 1982), with the inhibition of the reaction between the superoxide radical and epinephrine and obtaining the formation of a colored product (adrenochrome). We centrifuged the samples at 3000 rpm for 3 minutes (4°C). Three curves were obtained at 40 µL, 30 µL and 20 µL, with a total volume of 1000µL and another 17µL of epinephrine. SOD was detected at 480 nm, and the result was expressed in SOD units.min-1.mg of PT-1 (one SOD unit = the amount of enzyme inhibited by 50% of the oxidation rate of the detector used [epinephrine]).

Catalase (CAT) according to the method described by (BOVERIS; CHANCE, 1973) with the decay of hydrogen peroxide (H_2O_2). The samples were centrifuged at 10000 rpm for 5 minutes (4°C), followed by verifying the CAT activity with enzymatic kinetics at 240nm. The result was expressed as nmoles H_2O_2 min-1.mg protein^{-1}.

Glutathione S-transferase (GST) was quantified following the method described by (BOYLAND; CHASSEAUD, 1969) with the conjugation of 1-chloro 2,4 dinitrobenzene (CDNB) and reduced glutathione (GSH). The method consists of centrifuging the samples at 3000 rpm for 3 minutes and using the supernatant for analysis in spectrophotometry at 340 nm. The result was expressed in nmoles of CDNB conjugate.min-1.mg protein^{-1}.

2.4 Metabolic determination

For the metabolic analyses, the homogenate e prepared using the entire animal to measure total proteins (TP), uric acid (AU) and glycogen (GG) from the extraction method of VAN HANDEL (1965). We added 2mL of 30% KOH to the entire animal at 100°C for approximately four hours. After this stage, we measured TP with a BioTécnica kit (a technique based on the reaction of proteins in the sample with copper ions in an alkaline medium and detected at an absorbance of 550 nm). We also assessed UA with the commercial kit from BioTécnica, which is based on catalyzing the oxidation of uric acid in the sample; its reactions produce a compound with a pink color that is determined at 505 nm.

We added 2 drops of sodium sulphate (Na_2SO_4) to each tube, with the rest of the homogenate, and vortexed. Next, 5 mL of absolute ethyl alcohol was added to each tube and vortexed again for subsequent centrifugation at 3000 rpm for 10 minutes. We then discarded the supernatant, and two more washing processes were performed with 2 mL of distilled water and 5 mL of absolute ethyl alcohol, following the same process of agitation, centrifugation, and disposal of the supernatant. In the last step, the precipitate was resuspended in 0.3 mL of distilled water and vortexed, followed by acid hydrolysis (with 0.1 mL HCl in 0.1 mL of sample suspension and heated at 100°C for one hour) and neutralization with 0.1 mL of sodium carbonate (Na_2CO_3). We used the commercial glucose kit from BioTécnica to quantify the glucose released by glycogen hydrolysis, based on the oxidized glucose method, and determined at 505 nm.

2.5 Statistical analysis

We estimated the sstatistical analyses with SPSS Statistics for Windows version 26.0. Shapiro-Wilk and Kolmogorov-Smirnov tests were performed to assess normality, followed by the Levene homogeneity test to define the complementary test. For parametric data ($p>0.05$), the one-way analysis of variance (ANOVA one-way) was used, with Tukey posthoc (when Levene's test indicated $p>0.05$) and ANOVA-Welch with Games-Howell (when the Levene test indicated $p<0.05$). We compared the exposure groups to the control group in each biomarker. When the data was non-parametric ($p<0.05$), we used the Kruskal-Wallis and Dunn tests to indicate the difference between the experimental groups. In addition, a two-way analysis of variance was used, with age group (young adult x senile) and treatment groups (Control, 65 µg/L,

130 µg/L, and 260 µg/L) as fixed factors. Significant values ($p<0.05$) were considered, and all results were expressed as mean ± standard error.

We compared the total length (TL) and body mass (BM), including the initial (before exposure) and final (after exposure), with the Independent Sample Test (T-Test) for each group (Control, 65 µg/L, 130 µg/L, and 260 µg/L).

3. Results

3.1 Oxidative balance

The results of oxidative balance demonstrated that the exposure of young adult females to Roundup Original© herbicide did not present significant differences ($p>0.05$) in the markers and the three concentrations compared to the control group (Fig. 2). However, we observed that CAT decreased in the group exposed to 130 µg/L of glyphosate when compared to the 65 µg/L group, while the SOD/CAT ratio was reduced by 65 µg/L when compared to the 130 and 260 µg/L groups (Fig. 2D and E). The results of senile females showed a significant difference in the following markers: CAT, SOD/CAT ratio, and GST (Fig. 3D, E, and F, respectively). CAT activity was three times higher in the groups exposed to 65 and 130 in relation to the control group ($F = 6.998$; $p = 0.013$) (Fig. 3D). We also observed a decrease of the same intensity (3 times) in the SOD/CAT ratio at concentrations of 65µg/L and 130 µg/L compared to the control group ($F=6.844$; $p=0.013$) (Fig. 3E). There was an increase of about 100% in the activity of the biotransformation enzyme (GST) at a concentration of 130 and 260µg/L compared to the control group ($F=5,738$; $p=0.022$) (Fig. 3F). The total proteins contained in the supernatant remained constant in both age groups (Fig.2A and 3A).

Data referring to the two-way analysis of variance (ages and groups) showed a significant difference between ages in the following markers: TBARS ($F=84.464$; $p=0.000$), GST ($F=4.922$; $p=0.041$), SOD ($F=42.563$; $p=0.000$), CAT ($F=16.058$; $p=0.001$) and SOD/CAT ratio ($F=46.993$; $p=0.000$) (Table 2). We can see that TBARS levels, and SOD and CAT activity are lower in senile individuals and remain lower even after exposure to different concentrations. The SOD/CAT ratio, on the other hand, behaves in the opposite way, it is higher and remains higher in the elderly compared to young adults. As for GST, this is lower in senile females (control and 65) and after exposure to 130 and 260 µg/L of glyphosate, we observed similar levels of activity between ages (Table 2).

299
300
301 3.2 Intermediary metabolism
302 We observed significant differences in total protein and uric acid in young adult
303 females exposed to Roundup Original© (Fig. 4B and C). There was an increase (4 times)
304 in TP levels at the concentration of 65 and 130 µg/L (F=3.055; p=0.006), and an increase
305 (3.5 times) in UA levels (F=8.854) at 130µg/L (p=0.008) and 260µg/L (p=0.005)
306 compared to the control group (Fig. 4B and C). A significant decrease (2 times) in GG
307 levels was observed in senile females when we compared the 130µg/L with the 260
308 µg/L groups (Fig. 4D). However, for PT levels, we observed a 300% increase in the 260
309 µg/L group when compared to the control group (F=25.965; p=0.000) (Fig. 4E).
310 The two-way analysis of variance showed a significant difference between the age
311 groups in glycogen (F=47,164; p=0,000), where glycogen values were always lower in
312 senile females (Table 3).

313 3.3 Body Measurements

314 In tables 4 and 5 we can see the results obtained for the total length and body mass
315 of the animals at the beginning of the experiment and at the end of the experimental
316 period (17 days). We observed growth in total length only in senile animals even when
317 exposed, the increase ranged from 22.4% (Control) to 14% (glyphosate 260µg/L); in
318 young adults there were no significant variations in any of the groups. As for body mass,
319 we verified a significant increase in all groups and ages. However, the groups exposed
320 to the highest herbicide concentrations (130 and 260 µg/L of glyphosate) both in young
321 and senile adults showed the lowest increase in body mass.
322
323 **4. Discussion**

324 We evaluated three concentrations of a commercial formulation of glyphosate,
325 Roundup Original©, in females of *Cynopoecilus* sp. and observed a significant alteration
326 in the activities of the enzymes when senile females were exposed to the herbicide, a
327 process also observed in males of the same species (CASTRO et al. 2022). Such
328 responses may suggest that, despite the efficient modulation capacity of the enzymes to
329 avoid oxidative damage in lipids, both sexes of *Cynopoecilus* sp. showed sensitivity to
330 pesticide exposure in the senile stage, leading to or which leads to the detriment of

reproduction. The reproductive period of annual fish is intense and occurs daily from sexual maturity to death (Gonçalves et al. 2011; Godoy et al. 2020).

Considering that annual killifish have great resilience in nature, as they live in ephemeral environments and need to seek quick strategies to face the challenges related to their short life cycle (BEROIS et al. 2012), it can be suggested that they present a high resistance to pesticides. However, we cannot rule out that such responses may affect and restrict other essential mechanisms of their development, such as growth and reproduction, as already suggested for tadpoles and fish (DE LIMA COLTRO et al. 2017; PERSCH et al. 2018). We observed that females in both age groups did not show significant changes in TBARS levels, a marker of oxidative damage (GLUSCZAK et al. 2011). This response profile corroborates a study by CASTRO et al. (2022), which showed that males of the same species had an efficient antioxidant system when exposed to the same herbicide. The enzymes remained active in the body's defense and balance process, which justifies the control of TBARS levels. We cannot rule out the occurrence of oxidative damage to proteins and other molecules (proteins, DNA, RNA).

According to ZOCCHE et al. (2014) and ATLI; CANLI (2007), SOD and CAT enzymes represent the first defense against exposure to pollutants. the absence of oxidative damage, especially in senile females, can be associat with significant changes in some markers, such as CAT and SOD/CAT ratio (Fig. 3). The exposure of *Cynopoecilus* sp. to Roundup Original© induced an increase in CAT activity and a reduction in the SOD/CAT ratio at concentrations of 65 µg/L and 130 µg/L. Thiel et al. (2020) observed an increase in CAT and a reduction in the SOD/CAT ratio in *zebrafish* exposed to another herbicide (2,4-D). The role of CAT is highly significant for the antioxidant system by preventing the increase in lipid peroxidation and converting H_2O_2 into O2 and H_2O (PERSCH et al. 2018). Thus, our results indicate that glyphosate exposure increased the antioxidant response against H_2O_2, which probably plays a role in preventing the production of the •OH radical and preventing lipid peroxidation.

SOD is responsible for catalyzing the dismutation of the superoxide radical anion (O2•-), converting it into hydrogen peroxide (H_2O_2) and oxygen (O_2) (APEL; HIRT, 2004; COSTANTINI, 2014). A study conducted for Thiel et al. (2020) with *Danio rerio* exposed to the 2,4D herbicide showed responses like those found for senile females in our study, i.e., maintenance of SOD activity combined with an increase in CAT activity and a decrease in the SOD/CAT ratio. Ayanda et al. (2021) did not observe an increase

in SOD activity after two weeks of exposure to concentrations between 26.5 and 106 µg/L of glyphosate in *Clarias gariepinus*, only after 28 days of exposure did the authors observe an increase in SOD activity.

Ballesteros et al. (2009) point out that the response of the activity of antioxidant enzymes is quite variable, can increase activity of these enzymes or decrease or be maintained depending on the intensity and duration of the stressor and the susceptibility of the exposed species. Persch et al. (2018) studying three-barbered catfishes, showed the liver and kidneys were the organs most affected by exposure to herbicides, and that catalase was the main enzyme involved in the detoxification of atrazine, quinclorac and glyphosate. The response profile found to *Cynopoecilus* sp. reinforces the hypothesis that CAT plays a fundamental role in this time exposition and age of females (senile) to contain oxidative damage to lipids.

Our study also showed an increase in GST at a concentration of 130 and 260 µg/L of glyphosate for senile females. This result showed the opposite of that Castro et al. (2022) found in senile males of the same species, which had a decrease in activity at the highest concentrations of glyphosate. In addition, a study with *Cynopoecilus fulgens* demonstrated a decrease in GST activity at the end of the life cycle in senile females collected in the natural environment (CASTRO et al. 2021). Therefore, the increase in GST activity in senile females observed in present work may indicate the presence of herbicide in the environment, as well as an ability of these animals to activate the biotransformation system even at the end of the species' life cycle.

GST metabolize carcinogens, environmental pollutants, drugs, and a broad spectrum of other xenobiotics. This enzyme promotes the conjugation of the of electrophilic compounds with glutathione, facilitating the transport and excretion of these compounds (CNUBBEN et al. 2001; PAPADOPOULOS et al. 2004). In addition, GST participates in the defense against oxidative stress because it can metabolize harmful compounds such as degradation of lipid peroxidation avoiding oxidative damage, which helps to explain the lack of increase in TBARS levels verified in this work.

Furthermore, pesticide exposure can affect other physiological and biochemical parameters, such as altering the energy storage levels to adapt to induced stress (PERSCH et al. 2018). In our study, exposure *Cynopoecilus* sp. to different concentrations of Roundup Original© led to the maintenance of glycogen (young and senile adults) and an increased in total proteins (young and senile adults) (Fig. 4). Such

responses suggest that the exposure in both age groups may be related to either or both a decrease in protein catabolism and an increase in protein synthesis. PERSCH et al. (2018) also demonstrated an increase in total protein levels and a decrease in glycogen and triglyceride reserves in the muscle tissue of fish exposed to Facet®. Uric acid, on the other hand, seems to act as a non-enzymatic antioxidant in young adults, since its levels are elevated at all concentrations of the herbicide. In tadpoles exposed to glyphosate, studies by authors have found similar results for uric acid levels, also suggesting an antioxidant role for this molecule. (WILKENS et al. 2019; DA SILVA et al. 2021).

Exposure to the Roundup Original© herbicide can cause changes in oxidative, biochemical, and physiological parameters, as demonstrated in this study with females, but without causing oxidative damage. Therefore, our results of oxidative balance in females corroborate other studies showing that annual killifish collected in a natural environment have an efficient antioxidant system (CASTRO et al. 2021; GODOY et al. 2019) and that, like males, they can avoid oxidative damage in lipids even when exposed to Roundup Original© herbicide (CASTRO et al. 2022). However, both sexes at the end of their life cycles seem more susceptible to glyphosate, suggesting that this characteristic is related to the aging process of these animals (DONG et al. 2017).

Conclusion

We conclude that females of *Cynopoecilus* sp. have an efficient defense system in both age groups and can modulate their enzymes to avoid oxidative damage in lipids. Furthermore, this response pattern agrees with our previous study (CASTRO et al., 2022) using males of the same species exposed glyphosate. Senile males and females seem more sensitive to herbicide exposure than young adults. However, we cannot rule out that exposures for longer periods of time and/or higher concentrations may generate more pronounced effects on the antioxidant and biotransformation system, further shortening the lifespan of these animals and compromising their reproductive success.

Acknowledgments

We thank the Coordination for the Improvement of Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) the doctoral fellow ship to BDC fellowship and

the Pontifical Catholic University of Rio Grande do Sul (PUCRS) for all the technical support. We also thank the Center for Experimental Biological Models (CeMBE) for supplying some materials.

References

AMES, J. et al. Effects of glyphosate on *zebrafish*: a systematic review and meta-analysis. **Ecotoxicology.** Springer, 1 out. 2022. https://doi.org/10.1007/s10646-022-02581-z

APEL, K.; HIRT, H. REACTIVE OXYGEN SPECIES: Metabolism, Oxidative Stress, and Signal Transduction. **Annual Review of Plant Biology**, v. 55, n. 1, p. 373–399, 2004. https://10.1146/annurev.arplant.55.031903.141701

ATLI, G.; CANLI, M. Enzymatic responses to metal exposures in a freshwater fish *Oreochromis niloticus*. **Comparative Biochemistry and Physiology - C Toxicology and Pharmacology**, v. 145, n. 2, p. 282–287, 2007. https://10.1016/j.cbpc.2006.12.012

BALLESTEROS, M.L., WUNDERLIN, D.A., BISTONI, M.A. Oxidative stress responses in different organs of *Jenynsia multidentata* exposed to endosulfan. Ecotoxicol. Environ. Saf. v.72, n.1, p. 199–205, 2009. http://dx.doi.org/10.1016/j.ecoenv.2008.01.008.

BEROIS, N. et al. Annual fish: Developmental adaptations for an extreme environment. **Wiley Interdisciplinary Reviews: Developmental Biology**, v. 1, n. 4, p. 595–602, 2012. https://10.1002/wdev.39

BOVERIS, A.; CADENAS, E. Production of superoxide radicals and hydrogen peroxide in mitochondria. Em: OBERLEY, L. W. (Ed.). **Superoxide dismutase**. Flórida: Boca Raton: CRC Press, 1982. v. 2p. 15–30.

BOVERIS, A.; CHANCE, B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. **The Biochemical journal**, v. 134, n. 3, p. 707–716, 1973. https://doi.org/10.1042/bj1340707

BOYLAND, E.; CHASSEAUD, L. F. The Role of Glutathione and Glutathione S-transferases in Mercapturic Acid Biosynthesis. **Advances in enzymology and related areas of molecular biology**, v. 32, p. 173–219, 1969. https://doi.org/10.1002/9780470122778.ch5

463 BRIDI, D. et al. Glyphosate and Roundup® alter morphology and behavior in *zebrafish*.
464 **Toxicology**, v. 392, n. October, p. 32–39, 2017.
465 https://doi.org/10.1016/j.tox.2017.10.007

466 BUEGE, J. A.; AUST, S. D. Microsomal Lipid Peroxidation. **Methods Enzym**, p. 302–
467 310, 1978. https://doi.org/10.1016/s0076-6879(78)52032-6

468 CASTRO, B. D. et al. Biomarkers of oxidative stress in the post-embryonic
469 characterization of the neotropical annual killifish. **Biogerontology**, v. 0, 2021.
470 https://doi.org/10.1007/s10522-021-09931-0

471 CASTRO, B. D. et al. Development stage-dependent oxidative stress responses to the
472 exposure to roundup original© in a neotropical annual killifish. **Environmental**
473 **Toxicology and Pharmacology**, v. 95, p. 103976, out. 2022.
474 https://doi.org/10.1016/j.etap.2022.103976

475 CNUBBEN, N. H. P. et al. The interplay of glutathione related processes in antioxidant
476 defense. **Environ. Toxicol. Pharmacol.**, v. 10, p. 141–152, 2001.
477 https://doi.org/10.1016/s1382-6689(01)00077-1

478 COOKE, S. J. et al. What is conservation physiology? Perspectives on an increasingly
479 integrated and essential science. **Conservation Physiology**, v. 1, n. 1, p. 1–23, 2013.
480 https://doi.org/10.1093/conphys/cot001

481 COSTA, W. J. E. M. Phylogeny and Classification of Revulidae Revisited: Origin and
482 Evolution of Annualism and Miniaturization in Rivulid Fishes (Cyprinodontiformes:
483 Aplocheiloidei). **Journal of Comparative Biology**, v. 3, n. 1, p. 1–62, 1998.

484 COSTANTINI, D. Oxidative Stress and Hormesis in Evolutionary Ecology and
485 Physiology. [s.l.] Springer Heidelberg New York Dordrecht London, 2014.
486 https://doi.org/ 10.1007/978-3-642-54663-1_3

487 DA SILVA, P.R., BORGES-MARTINS, M., OLIVEIRA, G.T. *Melanophryniscus*
488 *admirabilis* tadpoles' responses to sulfentrazone and glyphosate-based herbicides: an
489 approach on metabolism and antioxidant defenses. **Environ Sci Pollut Res**. v.28,
490 p.4156–4172. 2021 https://doi.org/10.1007/s11356-020-10654-x

491 DE LIMA COLTRO, M. et al. Influence of the Herbicide Facet® on Corticosterone
492 Levels, Plasma Metabolites, and Antioxidant System in the Liver and Muscle of

American Bullfrog Tadpoles. **Water, Air, and Soil Pollution**, v. 228, n. 7, 1 jul. 2017. https://doi.org/10.1007/s11270-017-3404-7

DONG, Y. et al. Aging asymmetry: systematic survey of changes in age-related biomarkers in the annual fish *Nothobranchius guentheri*. **Fish Physiology and Biochemistry**, v. 43, n. 2, p. 309–319, 2017. https://doi.org/10.1007/s10695-016-0288-1

FAUSCH, K. D. et al. Fish communities as indicators of environmental degradation. **American Fisheries Society Symposium 8**, n. July, p. 123–144, 1990.

GIESY, J. P.; DOBSON, S.; SOLOMON, K. R. Ecotoxicological Risk Assessment for Roundup® Herbicide. p. 35–120, 2000. https://doi.org/10.1007/978-1-4612-1156-3_2

GILL, J. P. K. et al. Glyphosate toxicity for animals. **Environmental Chemistry Letters** Springer Verlag, 1 Jun. 2018. https://doi.org/10.1007/s10311-017-0689-0

GLUSCZAK, L. et al. Acute exposure to glyphosate herbicide affects oxidative parameters in piava (*Leporinus obtusidens*). **Archives of Environmental Contamination and Toxicology**, v. 61, n. 4, p. 624–630, 2011. https://doi.org/10.1007/s00244-011-9652-4

GONÇALVES CDS, SOUZA UP, VOLCAN MV. The opportunistic feeding and reproduction strategies of the annual fish *Cynopoecilus melanotaenia* (Cyprinodontiformes: Rivulidae) inhabiting ephemeral habitats on southern Brazil. **Neotrop Ichthyol,** v.9, p. 191–200, 2011. https://doi.org/10.1590/S1679-62252011000100019

GODOY, R. S. et al. Age-associated liver alterations in wild populations of *Austrolebias minuano*, a short-lived Neotropical annual killifish Biogerontology, v.20, n.5, p.687-698. 2019. https://doi.org/10.1007/s10522-019-09822-5(0123456789().,-(97().,-volV)

GODOY, R.S. et al. Oxidative stress resistance in a short-lived Neotropical annual killifish. **Biogerontology**, v.21, p. 217-229. 2020. DOI 10.1007/s10522-019-09855-w

HUED, A. C.; OBERHOFER, S.; DE LOS ÁNGELES BISTONI, M. Exposure to a commercial glyphosate formulation (Roundup®) alters normal gill and liver histology and affects male sexual activity of *Jenynsia multidentata* (Anablepidae,

cyprinodontiformes). **Archives of Environmental Contamination and Toxicology**, v. 62, n. 1, p. 107–117, Jan. 2012. https://doi.org/10.1007/s00244-011-9686-7

MESSAAD, I.A. & AL ZAILAIE, K.A. Evaluation of Glyphosate Toxicity on Arabian killifish, *Aphanius dispar* Collected from Southwestern Saudi Arabia. **Global Journal of Science Frontier Research: C Biological Science.** v.7, n.3, 2017.

KAFULA, Y. A. et al. Pesticide sensitivity of *Nothobranchius neumanni*, a temporary pond predator with a non-generic life-history. **Chemosphere**, v. 291, 1 Mar. 2022. https://doi.org/ 10.1016/j.chemosphere.2021.132823

LE DU-CARRÉE, J. et al. Developmental effect of parental or direct chronic exposure to environmental concentration of glyphosate on the larvae of rainbow trout, Oncorhynchus mykiss. **Aquatic Toxicology**, v. 237, 1 ago. 2021. https://doi.org/10.1016/j.aquatox.2021.105894

LIMA, É. S.; ABDALLA, D. S. P. Peroxidação lipídica: mecanismos e avaliação em amostras biológicas. **Revista Brasileira de Ciências Farmacêuticas**, v. 37, n. 3, p. 293–303, 2001.

LOUREIRO, M. et al. Review of the family Rivulidae (Cyprinodontiformes, Aplocheiloidei) and a molecular and morphological phylogeny of the annual fish genus Austrolebias Costa 1998. **Neotropical Ichthyology**, v. 16, n. 3, p. 1–20, 2018. https://doi.org/10.1590/1982-0224-20180007

ORTIZ-ORDOÑEZ, E. et al. Effect of Yerbimat herbicide on lipid peroxidation, catalase activity, and histological damage in gills and liver of the freshwater fish *Goodea atripinnis*. **Archives of Environmental Contamination and Toxicology**, v. 61, n. 3, p. 443–452, out. 2011. https://doi.org/10.1007/s00244-011-9648-0

PANETTO, O. S. et al. The effects of Roundup® in embryo development and energy metabolism of the *zebrafish* (*Danio rerio*). **Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology**, v. 222, p. 74–81, 1 ago. 2019. https://doi.org/10.1016/j.cbpc.2019.04.007

PAPADOPOULOS, A. I. et al. Glutathione S-transferase in the insect *Apis mellifera* macedonica: Kinetic characteristics and effect of stress on the expression of GST isoenzymes in the adult worker bee. **Comparative Biochemistry and Physiology - C**

Toxicology and Pharmacology, v. 139, n. 1–3, p. 93–97, 2004. https://doi.org/10.1016/j.cca.2004.09.010

PÉREZ, G.L., VERA, M.S., MIRANDA, L. Effects of herbicide glyphosate and glyphosate-based formulations on aquatic ecosystems. Kortekamp, A. (Ed.). Herbicides andEnvironment, pp. 343–368, 2011. DOI: https://doi.org/10.5772/12877

PERSCH, T. S. P. et al. Changes in intermediate metabolism and oxidative balance parameters in sexually matured three-barbeled catfishes exposed to herbicides from rice crops (Roundup®, Primoleo® and Facet®). **Environmental Toxicology and Pharmacology**, v. 58, n. August 2017, p. 170–179, 2018. https://doi.org/10.1016/j.etap.2018.01.004

POLAČIK, M.; BLAŽEK, R.; REICHARD, M. Laboratory breeding of the short-lived annual killifish *Nothobranchius furzeri*. **Nature protocols**, v. 11, n. 8, p. 1396–413, 2016. https://doi.org/10.1038/nprot.2016.080

QUEIROZ, G. M. P. et al. Transporte de glifosato pelo escoamento superficial e por lixiviação em um solo agrícola. **Quimica Nova**, v. 34, n. 2, p. 190–195, 2011. https://doi.org/10.1590/S0100-40422011000200004

SÁNCHEZ et al. Glyphosate-based herbicides affect behaviour al patterns of the livebearer *Jenynsia multidentata*. **Environmental Science and Pollution Research**, 28, 1–13, 2021. https://doi.org/10.1007/s11356-020-11958-8/Published

THIEL, N. A. et al. Exposure to the herbicide 2,4-dichlorophenoxyacetic acid impairs mitochondrial function, oxidative status, and behavior in adult *zebrafish*. **Environmental Science and Pollution Research**, 2020. https://doi.org/10.1007/s11356-020-10497-6

VÁGÁSI, C. I. et al. Longevity and life history coevolve with oxidative stress in birds. **Funct Ecol**, v. 33, p. 152–161, 2019. https://doi.org/10.1111/1365-2435.13228

VAN HANDEL, E. Estimation of Glycogen in Small Amounts of Tissue. **ANALYTICAL BIOCHEMISTRY**. p.256-265, 1965.

VOLCAN, M. V. et al. Annual fishes (Rivulidae) from southern Brazil: a broad-scale assessment of their diversity and conservation. Em: **Annual Fishes: Life History**

Strategy, Diversity, and Evolution. Boca Raton: CRC Press, 2015. p. 185–204. https://doi.org/10.1201/b19016-14

WILKENS, A.L.L., VALGAS, A.A.N., OLIVEIRA, G.T. Effects of ecologically relevant concentrations of Boral® 500 SC, Glifosato®Biocarb, and a blend of both herbicides on markers of metabolism, stress, and nutritional condition factors in bullfrog tadpoles. **Environ Sci Poll Res.** v.26, p. 23242- 23256, 2019. https://doi.org/10.1007/s11356-019-05533-z

ZEBRAL, Y. D. et al. A glyphosate-based herbicide reduces fertility, embryonic upper thermal tolerance and alters embryonic diapause of the threatened annual fish *Austrolebias nigrofasciatus*. **Chemosphere**, v. 196, p. 260–269, 2018. https://doi.org/10.1016/j.chemosphere.2017.12.196

ZOCCHE, J. J. et al. Heavy-metal content and oxidative damage in *Hypsiboas faber*: The impact of coal-mining pollutants on amphibians. **Archives of Environmental Contamination and Toxicology**, v. 66, n. 1, p. 69–77, 2014. https://doi.org/10.1007/s00244-013-9949-6

Statements and Declarations

Funding: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão da bolsa BDC.

Competing Interests: *The authors have no relevant financial or non-financial interests to disclose.*

Author contributions: BDC: Conceptualization, Data curation, Formal analysis, Investigation, Writing- draft. **LEKL:** Collection, Contextualization, Review of the Manuscript. **RSG:** Collection, Review of manuscript. **GKC and ADB:** Data curation, Investigation. **LM:** Collection, Contextualization, Review of the Manuscript. **GTO:** Conceptualization, Formal analysis, Methodology, Supervision, Writing- review & edition.

Tables e figures (Chapter 2)

Table 1 Abiotic parameters of the collection site water. Values are expressed as mean ± standard error.

Sampling	Temperature (°C)	pH	Conductivity (Ms/cm)	ORP* (mV)	Salinity	TDS** (ppm)
June	18.12 ± 0.09	4.82 ± 0.06	0.00 ± 0.00	108.63 ± 6.72	0.18 ± 0.00	192.33 ± 1.66
November	25.06 ± 0.25	5.04 ± 0.01	0.00 ± 0.00	8.10 ± 1.94	0.26 ± 0.00	278.00 ± 1.52

* Redox-oxidation potential (ORP)
** Total dissolved solids (TDS)

Table 2 Oxidative balance analyses. Mean ± standard error data of physiological markers in young adult and senile females of *Cynopoecilus* sp. exposed to Roundup Original©. Values represent the following: Total protein of the supernatant (TP), thiobarbituric acid reactive substances (TBARS), glutathione S-transferase (GST), catalase (CAT), superoxide dismutase (SOD) and SOD/CAT ratio. (*) means the difference between age groups using Two-Way ANOVA ($p<0.05$). Values for young adults and seniles.

Developmental stages	Physiological Markers	Control	Roundup Original©		
			65 µg/L	130 µg/L	260 µg/L
Young adult	TPS	7.52 ± 0.56	6.76 ± 1.05	7.55 ± 1.16	6.83 ± 1.21
Senile		10.30 ± 1.27	10.01 ± 2.90	8.40 ± 0.80	9.37 ± 2.27
Young adult	TBARS*	40.92 ± 4.39	33.59 ± 5.99	30.63 ± 5.56	40.02 ± 6.47
Senile		5.84 ± 1.26	10.88 ± 3.00	7.32 ± 1.21	10.12 ± 2.38
Young adult	SOD*	19.99 ± 1.10	21.63 ± 2.97	21.31 ± 2.82	23.33 ± 4.40
Senile		18.87 ± 4.06	19.78 ± 5.80	17.68 ± 1.18	18.11 ± 5.30
Young adult	CAT*	30.31 ± 2.30	48.76 ± 6.94	22.33 ± 2.33	29.32 ± 7.65
Senile		5.46 ± 0.38	13.48 ± 0.58	13.45 ± 1.88	9.82 ± 2.06
Young adult	SOD/CAT*	0.67 ± 0.09	0.44 ± 0.01	0.95 ± 0.06	0.86 ± 0.13
Senile		3.39 ± 0.50	1.44 ± 0.39	1,35 ± 0.15	1.84 ± 0.30
Young adult	GST*	0.68 ± 0.04	0.63 ± 0.18	0.54 ± 0.10	0.57 ± 0.15
Senile		0.32 ± 0.02	0.32 ± 0.04	0.56 ± 0.03	0.58 ± 0.11

Table 3 Intermediate metabolism analyses. Mean ± standard error data of physiological markers in young adult and senile females of *Cynopoecilus* sp. Exposed to Roundup Original© (65 µg/L, 130 µg/L and 260 µg/L). Values represent the following: glycogen (GG), total protein (TP) and uric acid (UA) of animals. (*) means the difference between age groups using Two-Way ANOVA ($p<0.05$). Values for young adults and seniles.

Developmental stages	Physiological Markers	Control	Roundup Original©		
			65 µg/L	130 µg/L	260 µg/L
Young adult	GG* (mg/g)	0.04 ± 0.01	0.05 ± 0.00	0.03 ± 0.01	0.04 ± 0.01
Senile		0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
	TP (mg/g)	0.09 ± 0.02	0.40 ± 0.06	0.35 ± 0.03	0.28 ± 0.09
		0.23 ± 0.04	0.18 ± 0.04	0.11 ± 0.03	0.63 ± 0.06
	UA (mg/g)	0.02 ± 0.00	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
		0.06 ± 0.01	0.04 ± 0.00	0.07 ± 0.01	0.04 ± 0.00

Table 4 The initial and final total lengths (mm) of young adult and senile females of *Cynopoecilus* sp. Exposed to Roundup Original© (represented by mean ± standard error). The measurements in each experimental group (Control, 65 µg/L, 130 µg/L and 260 µg/L) were compared with the Independent Sample Test. (*) means the difference between the initial and final total length ($p<0.05$).

Developmental Stages	Groups	Initial Total Length	Final Total Length	df	F	*p*
Young adult	Control	18.92 ± 0.36	19.85 ± 0.46	22	1,476	0.127
	65 µg/L	19.89 ± 0.20	20.35 ± 0.58	16	6.369	0.468
	130 µg/L	20.11 ± 0.26	19.96 ± 0.46	16	3.012	0.785
	260 µg/L	20.33 ± 0.71	19.29 ± 0.53	16	9.593	0.091
Senile	Control*	18.63 ± 0.42	22.81 ± 0.58 (22.4%)	30	0.869	0.000
	65 µg/L*	19.91 ± 0.45	23.33 ± 0.27 (17.2%)	22	2.202	0.000
	130 µg/L*	19.17 ± 0.50	22.50 ± 0.75 (17.4%)	22	0.766	0.001
	260 µg/L*	20.28 ± 0.45	23.14 ± 0.52 (14.1%)	22	0.864	0.000

Table 5 The initial and final body mass (g) of young adult and senile females of *Cynopoecilus* sp. Exposed to Roundup Original© (represented by mean ± standard error). The measurements in each experimental group (Control, 65 µg/L, 130 µg/L and 260 µg/L) were compared with the Independent Sample Test. (*) means the difference between the initial and final body mass ($p<0.05$).

Developmental Stage	Group	Initial Body Mass	Final Body Mass	df	F	*p*
Young adult	Control*	0.07 ± 0.01	0.10 ± 0.01 (42%)	22	0.181	0.05
	65 µg/L*	0.09 ± 0.00	0.11 ± 0.00 (22%)	16	0.960	0.01
	130 µg/L*	0.07 ± 0.00	0.11 ± 0.00 (57%)	16	0.210	0.00
	260 µg/L	0.08 ± 0.00	0.09 ± 0.00 (12%)	16	0.514	0.10
Senile	Control*	0.10 ± 0.01	0.13 ± 0.01 (30%)	30	0.133	0.00
	65 µg/L*	0.10 ± 0.00	0.14 ± 0.00 (40%)	22	0.654	0.00
	130 µg/L	0.10 ± 0.01	0.12 ± 0.01 (20%)	22	0.064	0.10
	260 µg/L*	0.10 ± 0.00	0.13 ± 0.01 (30%)	22	2.337	0.00

Fig. 1. The experimental design of the toxicological test stages. Each group (control: C; exposure groups: R1, R2 and R3) contains three to four aquariums.

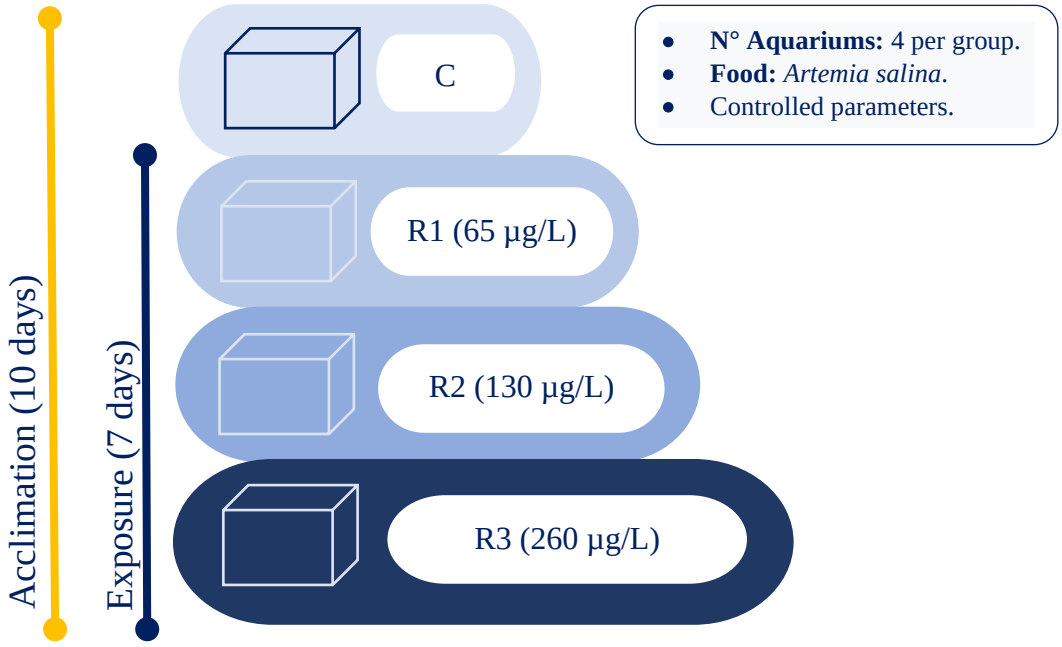


Fig. 2 Oxidative balance results of young adult females exposed to Roundup Original©. (A) Total protein of the supernatant (TPS), (B) thiobarbituric acid reactive substances (TBARS), (C) superoxide dismutase (SOD), (D) catalase (CAT), (E) SOD/CAT ratio and (F) glutathione S-transferase (GST). Bars represent means ± standard error. Different letters represent a significant difference for $p<0.05$.

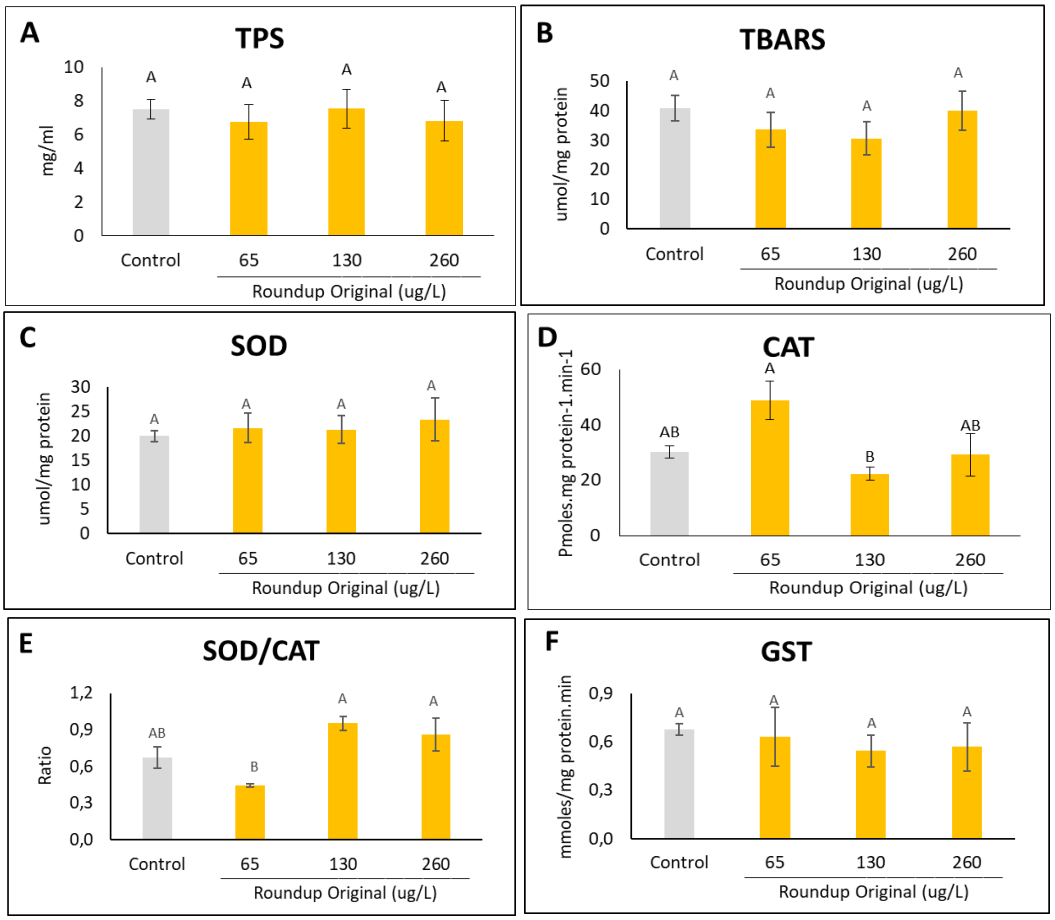


Fig. 3 Oxidative balance results of senile females exposed to Roundup Original©. (A) Total protein of the supernatant (TPS), (B) thiobarbituric acid reactive substances (TBARS), (C) superoxide dismutase (SOD), (D) catalase (CAT), (E) SOD/CAT ratio and (F) glutathione S-transferase (GST). Bars represent means ± standard error. Different letters represent a significant difference for $p<0.05$.

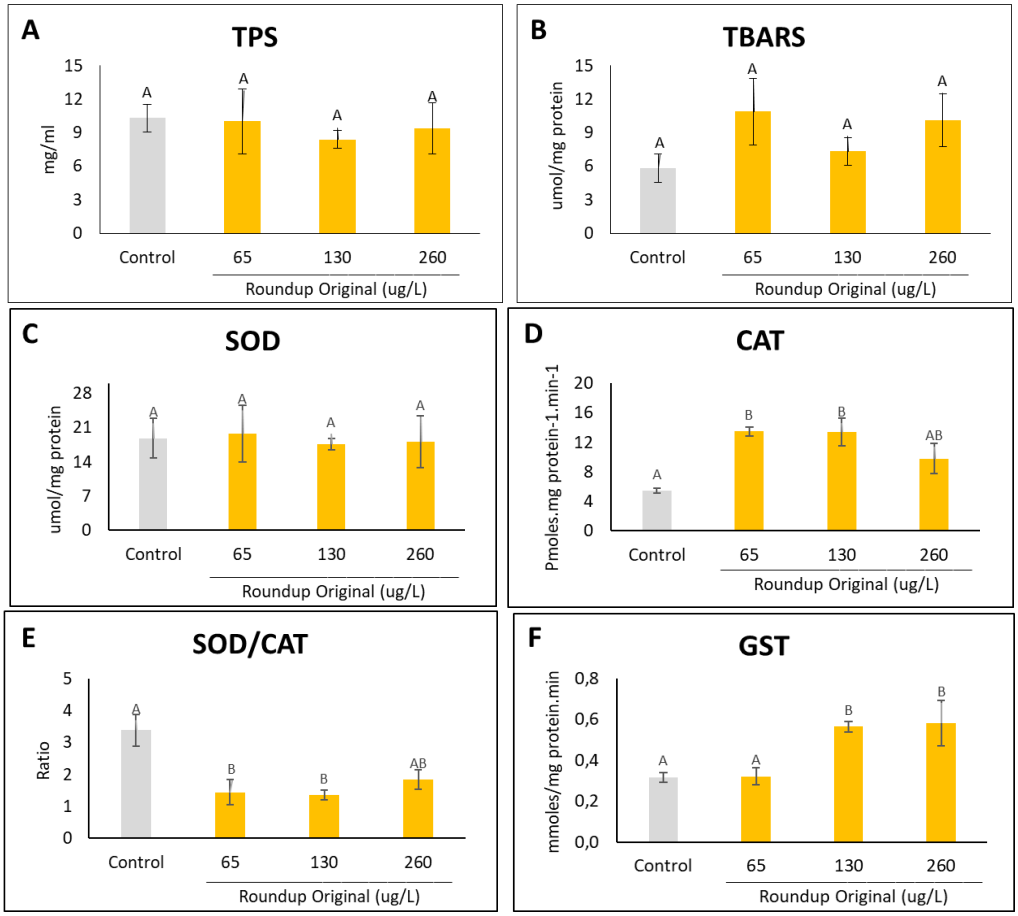
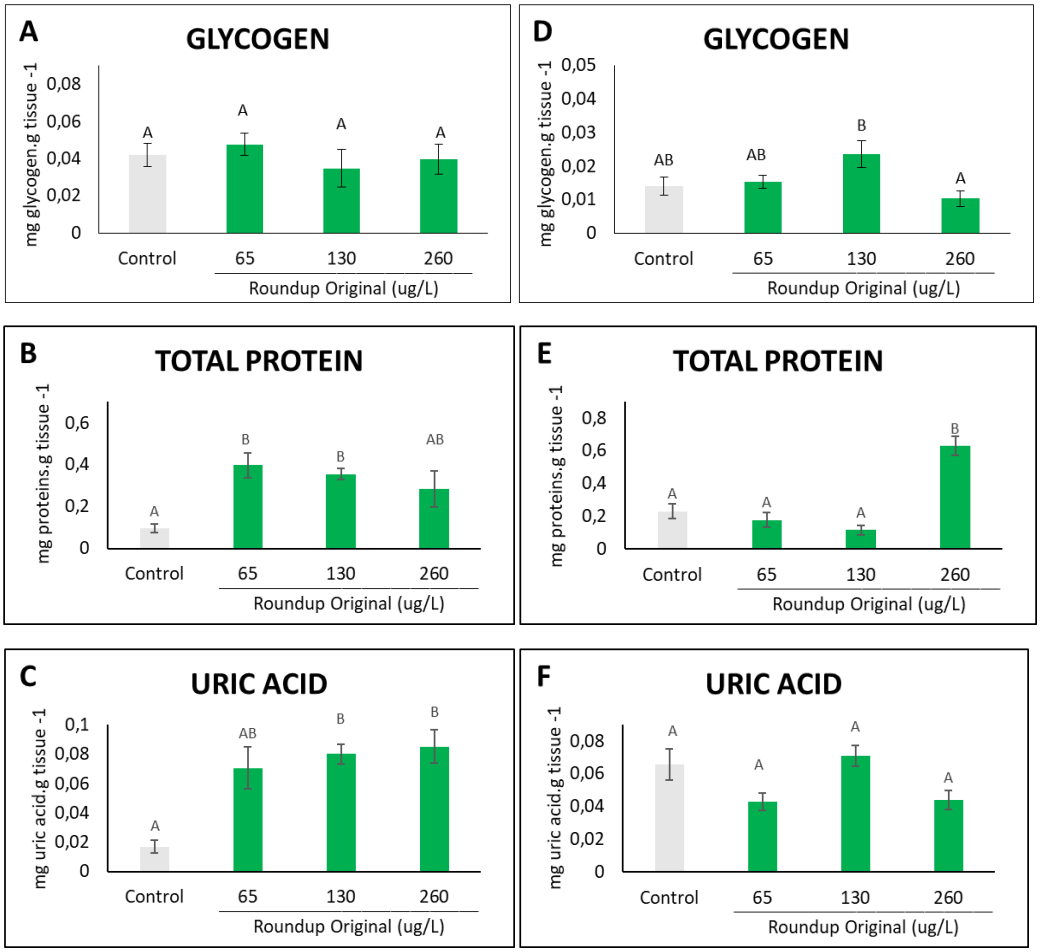


Fig. 4 Intermediate metabolism results of young adult and senile females exposed to Roundup Original©. (A) Glycogen (GG), (B) total protein (TP) and (C) uric acid (UA) of animals. Bars represent means ± standard error. Different letters represent a significant difference for $p<0.05$.



CAPÍTULO 3

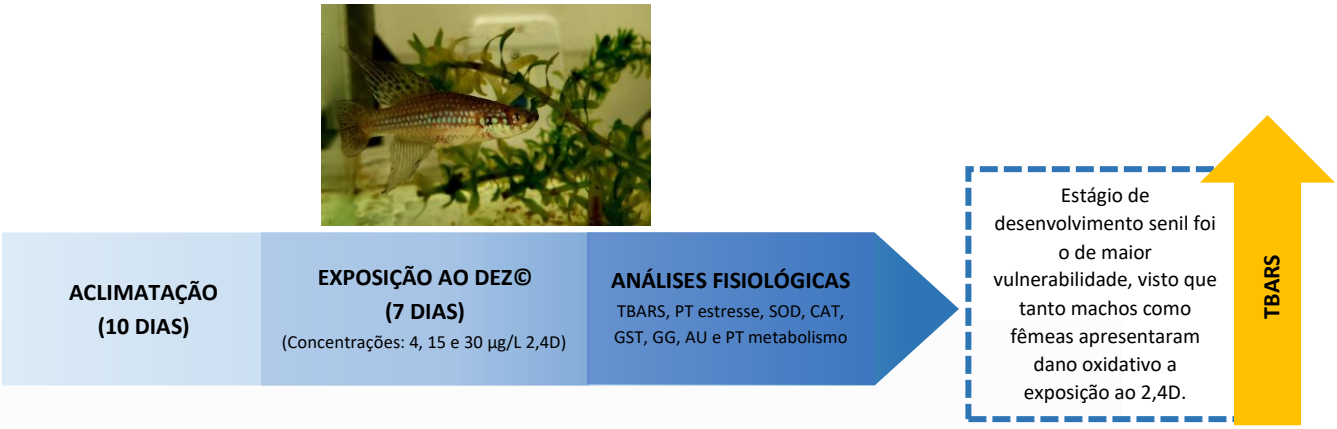
ANÁLISE DO EFEITO DO COMPOSTO QUÍMICO DEZ© (2,4 – DICLOROFENOXIACÉTICO) SOBRE O CICLO DE VIDA DE UMA ESPÉCIE DE PEIXE ANUAL NEOTROPICAL

ANÁLISE DO EFEITO DO COMPOSTO QUÍMICO DEZ© (2,4 – DICLOROFENOXIACÉTICO) SOBRE O CICLO DE VIDA DO PEIXE ANUAL NEOTROPICAL

BRUNA DUTRA DE CASTRO[1], LUIS ESTEBAN KRAUSE LANÉS[1], ROBSON SOUZA GODOY[3], GUSTAVO KASPER CUBAS[1], AMANDA DABDAB BRANDELLI[1], LEONARDO MALTCHIK[4], GUENDALINA TURCATO OLIVEIRA[1]*

[1] PUCRS, Escola de Ciências da Saúde e da Vida, Laboratório de Fisiologia da Conservação, Porto Alegre, RS, Brasil
[2] Instituto Pro-Pampa, Pelotas, RS, Brasil
[3] UNISINOS Universidade, Centro de Ciências, Programa de Biologia, Laboratório de Ecologia e Conservação de Ecossistemas Aquáticos, São Leopoldo, RS, Brasil
[4]FURG, Universidade Federal de Rio Grande, Reitoria, Pró-Reitoria de Pesquisa e Pós-Graduação, Fundação Universidade do Rio Grande, Rio Grande, RS, Brasil

** Autor correspondente:* Guendalina Turcato Oliveira
Laboratório de Fisiologia da Conservação, Departamento de Ciências Morfofisiológicas, Faculdade de Ciências da Saúde e da Vida, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681 Pd. 12, Bloco C, classe 270, 90619-900, Porto Alegre, RS, Brasil. Endereço de e-mail: guendato@pucrs.br (G.T. Oliveira).



Destaques

- Exposição ao 2,4D provocou mudanças em parâmetros oxidativos e metabólicos.
- Estágio senil foi o de maior vulnerabilidade em ambos os sexos.
- Concentrações mais elevadas foram as mais prejudiciais, indicando dano oxidativo.
- Fêmeas mostraram ser mais suscetíveis ao 2,4D do que os machos.

Resumo

Peixes anuais apresentam um ciclo de vida pós-eclosão curto, cerca de sete meses, se mantem reprodutivos até a morte produzindo ovos de resistência à seca. O objetivo deste estudo foi avaliar o efeito da formulação comercial do herbicida à base de 2,4D (Dez©) sobre diferentes fases do ciclo de vida de *Cynopoecilus* sp. Foram realizadas análises em parâmetros de estresse oxidativo e de metabolismo intermediário, em fêmeas e machos, após exposição de sete dias a diferentes concentrações de 2,4D (4, 15 e 30 µg/L). Observamos um impacto deste herbicida, onde nas fêmeas adultas-jovens temos uma diminuição da atividade da catalase e da glutationa S-transferase, e um aumento do glicogênio, das proteínas totais e do ácido úrico. Nos adultos-jovens machos somente a catalase apresentou variação significativa. Nossos resultados evidenciaram que o estágio de desenvolvimento de maior vulnerabilidade tanto para machos como para fêmeas foi a fase senil, onde observamos um incremento da atividade da catalase nas

maiores concentrações do herbicida, aliado a um aumento do dano oxidativo em lipídios (TBARS). Além disso, verificamos nas fêmeas senis um incremento dos níveis de glicogênio e de proteínas totais nos animais expostos a maior concentração do herbicida. Nos machos senis temos uma diminuição das proteínas totais seguida de um incremento do ácido úrico, sugerindo que esta molécula esteja sendo usada como um antioxidante por esta espécie. Quando comparamos as respostas entre os sexos e as fases do desenvolvimento observamos diferenças quanto ao impacto deste herbicida para todos os parâmetros, exceto para a superóxido dismutase e o ácido úrico. Já quando comparamos a interação entre grupos, sexo e fase do desenvolvimento observamos diferenças significativas para todos os parâmetros analisados. Assim, este herbicida parece impactar negativamente o fitness destes animais, levando a estresse oxidativo e possivelmente, uma redução do ciclo de vida e da capacidade reprodutiva de *Cynopoecilus* sp.

Palavras-chave

Herbicida; Estágio de desenvolvimento; Estresse oxidativo; Metabolismo intermediário; *Cynopoecilus* sp.

1. Introdução

O uso indiscriminado de agrotóxicos na agricultura é considerado um dos fatores que tem provocado o aumento da poluição ambiental (LIMA et al. 2018). A cultura agrícola tem o papel como fonte de entrada e distribuição de compostos químicos (fertilizantes, pesticidas entre outros) através da contaminação dos solos para diversos ambientes, principalmente o ambiente aquático (LIMA et al. 2018). Além disso, a dinâmica dos compostos químicos no solo influencia na entrada dos pesticidas no meio aquático podendo estes terem diferentes destinos e rotas, por exemplo: precipitação, lixiviação e escoamento superficial (COSGROVE; JEFFERSON; JARVIS, 2019; LIMA et al. 2018). Com a contaminação dos solos, resíduos desses produtos podem ser arrastados e transportados com as chuvas, como também escoados superficialmente até alcançarem corpos d'água (QUEIROZ et al. 2011). Já a lixiviação ocorre através do transporte de moléculas do poluente para camadas mais profundas provocando a contaminação do lençol freático. Cabe ressaltar que tais rotas podem resultar na detecção destes poluentes em ambientes mais distantes do que no local da aplicação (QUEIROZ et al. 2011).

Frente a isso, a entrada de agrotóxicos em ambientes aquáticos pode representar um potencial perigo para os organismos presentes nesses locais. Estudos têm mostrado a influência de poluentes químicos em diferentes espécimes aquáticas conduzindo a alterações em reservas energéticas, comportamentos, redução de massa corporal e outros efeitos deletérios, sendo um risco para os animais (DA SILVA; BORGES-MARTINS; OLIVEIRA, 2021; DE LIMA COLTRO et al. 2017; PERSCH et al. 2017, 2018). Muitas dessas respostas a exposição aos agrotóxicos podem estar relacionadas com a capacidade do organismo de buscar se adaptar a esse meio contaminado, sendo assim o animal utiliza suas reservas energéticas para essa finalidade e como consequência apresenta mudanças em processos do seu ciclo de vida. São exemplos de mudanças: inibição do crescimento, aceleração da metamorfose, comprometimento do desenvolvimento e da reprodução (DE LIMA COLTRO et al. 2017; PERSCH et al. 2018). Dessa forma, enfatiza-se a importância na utilização de análises bioquímico-funcionais para compreender as respostas desses organismos em relação a exposição a esses poluentes e, as alterações ambientais associadas (COOKE et al. 2013).

Dentre os poluentes, o 2,4-diclorofenoxiacético (2,4D) ainda é um dos mais utilizados na agricultura. Apesar de ser o primeiro herbicida comercial a ser introduzido no mercado na década de 1940, pertencendo ao químico ácido ariloxialcanóico (THIEL

et al. 2020), continua sendo um herbicida de escolha por agricultores por ser um produto de baixo custo, seletivo e de ação sistêmica, apresentando eficácia no controle de ervas daninhas, sendo amplamente utilizado nas culturas de arroz, cana-de-açúcar, milho, soja e pastagens (THIEL et al. 2020). Uma das suas formulações comerciais é o Dez©, registrado no Ministério da Agricultura, Pecuária e Abastecimento sob o nº 05009, é composto por: sal de dimetil amina do ácido diclorofenoxiacético (2,4-D- 80,6% m/v), equivalente ácido em uma proporção de 67,0% (m/v) mais outros ingredientes em uma proporção de 42,1% (m/v).

O 2,4D é absorvido pelas raízes e pela parte aérea das plantas, e então é transportado para outros locais mais afastados do local de aplicação (ISLAM et al. 2018). Tal característica é vista como um risco para o meio ambiente, pois pode contaminar superfícies, águas subterrâneas podendo alcançar os corpos d'água através das diferentes rotas atingindo e prejudicando espécies não-alvo, como os peixes (ISLAM et al. 2018; THIEL et al. 2020).

A presença de compostos químicos, como o 2,4D, na água pode modificar comportamentos e provocar alterações fisiológicas em peixes, podendo prejudicar sua resposta a esses estressores. Apesar dos peixes serem um dos grupos mais estudados, o conhecimento de aspectos toxicocinéticos e toxicodinâmicos ainda é limitado. Algumas pesquisas vêm sendo desenvolvidas com peixes como a realizada por THIEL et al. (2020) na qual foi avaliado o efeito do 2,4D em concentrações encontradas no ambiente em *Danio rerio*, e os resultados mostraram alterações no metabolismo mitocondrial, estresse oxidativo e efeitos prejudiciais na capacidade desses organismos até mesmo no seu comportamento. Estudos realizados com a mesma espécie mostraram que a exposição ao 2,4D prejudicou comportamentos essenciais de larvas guiadas visualmente, como por exemplo, a captura de presas e interferência na capacidade de percepção de risco do animal (DEHNERT; KARASOV; WOLMAN, 2019; POMPERMAIER et al. 2020). Outro estudo com a exposição ao 2,4D da espécie de peixe *Rhandia quelen* realizado por MENEZES et al. (2015) mostram uma diminuição do crescimento, glicogenólise no músculo e no rim como forma de tamponar o gasto de energia e de manter a capacidade de modulação do sistema antioxidante no fígado para evitar o dano oxidativo. Cabe destacar a importância de pesquisas ecotoxicológicas com a utilização de organismos autóctones adaptados ao ambiente local para fornecer resultados mais compatíveis com a realidade (PERSCH et al. 2017, 2018; ZEBRAL et al. 2018).

Cynopoecilus sp. é uma espécie de peixe anual nativa da região sul do Brasil. São animais adaptados a ecossistemas temporários de água doce, sendo isso intimamente relacionado ao ciclo de vida curto desses vertebrados, que investem no crescimento acelerado e manutenção de um intenso período reprodutivo, período este que se estende até o final do ciclo de vida (CELLERINO; VALENZANO; REICHARD, 2016; VRTÍLEK; REICHARD, 2015). Esses ambientes aquáticos temporários, onde peixes anuais são encontrados, situam-se muitas vezes próximos a áreas de ocorrência de atividade agrícola, como o cultivo de arroz irrigado (AMARANTE JR et al. 2002). Com isso, o objetivo deste estudo foi verificar o efeito do Dez© (2,4D) sobre diferentes fases do ciclo de vida do peixe anual neotropical, *Cynopoecilus* sp. Foram realizadas análises fisiológicas a partir de marcadores de balanço oxidativo e de metabolismo intermediário. Destacamos que tais marcadores oxidativos já foram padronizados em outros estudos com peixes anuais neotropicais pelo nosso grupo de pesquisa, mas em uma análise sobre o ciclo de vida em ambiente natural (CASTRO et al. 2021; GODOY et al. 2020).

2. Materiais e Métodos

2.1 Coleta de Peixes Anuais

Os procedimentos realizados no estudo foram aprovados pelo Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio n°68290-8) e pelo Comitê de Ética no Uso de Animais (CEUA: 9400 e 8271) da Pontifícia Universidade Católica do Rio Grande do Sul.

Para essa pesquisa, foram coletados e utilizados 160 animais no total, machos e fêmeas, de *Cynopoecilus* sp. Os peixes foram amostrados com o auxílio de rede de mão em forma de D, em piscinas naturais de Dunas Altas, Município de Palmares do Sul, Rio Grande do Sul (S 30°23’32.5” W 050°19’32.4”). A escolha da área de amostragem seguiu o critério de não apresentar um histórico de utilização de agrotóxicos no local e nas proximidades, uma vez que esta é utilizada para pecuária extensiva em pastagens naturais e silvicultura (*Pinus* spp.). Para obter diferentes fases do desenvolvimento dos animais (fêmeas e machos), estes foram amostrados nos meses de junho (adulto-jovem) e novembro (senil) de 2021. Em cada amostragem foram registrados os parâmetros físico-químicos da água do local de coleta, utilizando um dispositivo multiparâmetro

portátil: temperatura, potencial de oxidação-redox [ORP], sólidos totais dissolvidos (TDS), salinidade, pH e condutividade (Tabela 1).

2.2 Desenho Experimental

Os animais coletados em campo foram diretamente transportados em baldes com água do local de coleta para o Centro de Modelos Biológicos Experimentais (CeMBE), da PUCRS, onde foram registradas as medidas de comprimento total (CT) com paquímetro digital (precisão de 0,01 cm) e massa corporal com balança analítica (precisão de 0,001 g). O CT refere-se à distância entre a extremidade anterior da maxila à extremidade final da nadadeira caudal. Após as medições, os animais iniciaram as etapas do ensaio toxicológico: Período de Aclimatação (**Etapa 1-** 10 dias) e Período de Exposição (**Etapa 2-** 7 dias). Cabe ressaltar que em ambas as etapas os animais estavam acondicionados em aquários com areação constante, temperatura da sala controlada em 20±1°C e fotoperíodo de 12h:12h luz/escuro. Além disso, os parâmetros da água (pH, amônia e Nitrito) foram acompanhados e registrados, os animais foram alimentados *ad libitum* com *Artemia salina*.

Na **Etapa 1** (Período de Aclimatação) todos os espécimes foram sexados e acondicionados separadamente (machos e fêmeas) em aquários com água desclorada, para evitar qualquer interação entre os sexos, durante 10 dias. Após os 10 dias de aclimatação, foi iniciada a **Etapa 2** (Período de Exposição) na qual os aquários foram identificados por grupos (para cada sexo), sendo que cada grupo continha de três a quatro aquários: Controle (C), 4 µg/L (D1), 15 µg/L (D2) e 30 µg/L (D3). Nessa etapa os animais dos grupos exposição (D1, D2 e D3) de ambos os sexos foram expostos, separadamente, por sete dias ao Herbicida Dez© (ácido diclorofenoxiacético – 2,4-D) nas concentrações de 4 µg/L, 15 µg/L e 30 µg/L, e os grupos controles (de ambos os sexos) mantiveram-se apenas com água sem adição do contaminante (Figura 1).

Para o preparo da solução estoque do herbicida, utilizamos a concentração do princípio ativo descrita na embalagem da formulação comercial (DEZ®) para calcular o volume necessário. Em seguida, obtivemos uma solução com concentração (1000 mg/L de ingrediente ativo) e calculamos as concentrações nominais para cada grupo considerando o volume de água do aquário. Um laboratório comercial (Hidrolab) devidamente autorizado e certificado determinou a concentração de 2,4-D na solução estoque (Método: EPA 3510 C:1996/EPA 8270 D:2014) após cerca de 25 dias de seu preparo, tendo encontrado 883,489 mg/L de 2,4D nesta solução estoque.

No Brasil, o monitoramento de pesticidas em águas superficiais e subterrâneas ainda não é prática corrente, devido à ausência de infraestrutura laboratorial necessária e aos custos elevados envolvidos na realização das análises. Estudos pontuais vêm apresentando resultados da presença de moléculas de agrotóxicos, entre estas o 2,4D, em águas superficiais e subterrâneas (PRIMEL et al. 2005; PINHEIRO, DA SILVA e KRAISCH, 2010). A resolução CONAMA 357/2005 estabelece em 4,0 μg L^{-1} a concentração máxima de 2,4-D, em corpos d'água doce classe I, a qual compreende águas destinadas ao consumo humano (CONAMA, 2005).

O 2,4-D pode ser liberado no ar durante a sua fabricação e em aplicações por *spray*. Estes autores encontraram uma concentração de 0,5 até 74,5 μg L^{-1} em águas superficiais. A alta mobilidade no solo indica potencial para a contaminação da água subterrânea; podendo atingir águas superficiais após o uso direto próximo a ambiente aquático, por escoamento de aplicações terrestres, devido a erosão do solo, e quando é usado em plantas aquáticas. Nesses ambientes o 2,4-D não permanece por muito tempo, em cursos d'água a meia-vida do 2,4 D é de 4,5 a 7 dias (NETO et al. 2012).

Após a realização das duas etapas do ensaio experimental, as medidas dos animais foram novamente registradas (comprimento total e massa corporal) para as observações e comparações. Além disso, os espécimes foram eutanasiados em nitrogênio líquido, identificados em frascos individualizados e armazenados no freezer -80ºC para posterior análises bioquímicas.

2.3 Análise Bioquímica do Estresse Oxidativo

Para a quantificação dos parâmetros do estresse oxidativo foi utilizado os espécimes inteiros posteriormente homogeneizados em solução de tampão fosfato (20 mM, pH 7,4, acrescida de PSMF 1 mM e cloreto de potássio a 140 mM), em volume de sete vezes a massa corporal obtida (1g: 7ml) para a realização de pools (com dois animais), com o auxílio de um Ultra-Turrax® (IKA-WERK), em banho de gelo (cerca de 4°C). Após, este foi centrifugado (SORVALL RC-5B Refrigerated Superseed Centrifuge), sob refrigeração (4ºC) a 3600rpm por 10 minutos. Para a análise bioquímica, ensaio enzimático e o marcador de dano oxidativo (TBARS) foi utilizado apenas o sobrenadante. As amostras foram determinadas em triplicata para cada biomarcador.

As análises de Proteínas Totais do sobrenadante (PTS) foram quantificadas utilizando o kit comercial de Proteínas Totais da BioTécnica. A técnica é baseada na

reação das proteínas na amostra com íons de cobre, em meio alcalino. Nesse ensaio, o produto dessa reação resulta numa cor violácea que é detectada em absorbância de 550nm.

Estes valores de PTS foram utilizados para normatização da atividade da superóxido dismutase (SOD), catalase (CAT) e glutationa S-transferase (GST), e dos níveis de lipoperoxidação.

2.3.1 Ensaio Enzimático

A Superóxido dismutase (SOD) foi determinada em meio de reação que consiste de glicina-NaOH (50mM, pH 11) e epinefrina (1mM) (BOVERIS; CADENAS, 1982), onde ocorre a inibição da reação entre o radical superóxido e a epinefrina. Com a oxidação da epinefrina obtém-se a formação de um produto colorido (adrenocromo). Neste método as amostras foram centrifugadas em 3000rpm por 3 minutos (4°C) e foram realizadas três curvas em 40µL (960µL de tampão SOD + 40µL de amostra), 30µL (970µL de tampão SOD + 30µL de amostra) e 20µL (980µL de tampão SOD + 20µL de amostra), sempre para um volume total de 1000µL e adicionado mais 17µL de epinefrina. A atividade da SOD foi detectada a 480nm e o resultado é expresso em unidades de SOD. Min^{-1}.mg de PT^{-1}, sendo uma unidade de SOD correspondente a quantidade de enzima que inibe em 50% a velocidade de oxidação do detector utilizado (epinefrina).

A Catalase (CAT) foi determinada conforme o método descrito por BOVERIS; CHANCE (1973) sendo avaliada pelo decaimento do peróxido de hidrogênio (H_2O_2). Para a realização desse método, as amostras foram inicialmente centrifugadas a 10000rpm durante 5 minutos (4°C) e, após, foi verificada a atividade da CAT por espectrofotometria por cinética enzimática a 240nm. Nesse procedimento o meio de reação compreende de 955µL de tampão CAT, 10µL de amostra e 35µL de H2O2 (volume total de 1000µL). O resultado é expresso nmoles H2O2 min^{-1}.mg de proteínas^{-1}.

A Glutationa S-transferase (GST) foi quantificada seguindo o método descrito por BOYLAND; CHASSEAUD (1969) que consiste na conjugação do 1-cloro 2,4 dinitrobenzeno (CDNB) e glutationa reduzida (GSH). Nessa técnica as amostras foram primeiramente centrifugadas a 3000rpm por 3 minutos e o sobrenadante foi utilizado para as análises em espectrofotometria a 340nm. O resultado é expresso em nmoles de conjugado CDNB. Min^{-1}.mg de proteínas^{-1}.

2.3.2 Marcador de Dano Oxidativo

A Lipoperoxidação (LPO) foi determinada por meio da detecção de substâncias reativas ao ácido tiobarbitúrico (TBARS). Inicialmente, seguiu-se o método descrito por BUEGE; AUST (1978) onde foram adicionados em microtubos: 300µL de ácido tricloroacético (TCA), 200µL de ácido tiobarbitúrico (TBA), 100µL de água Milli-Q e 100µL do homogeneizado, sendo estes posteriormente aquecidos a 100°C durante 15 minutos e resfriados durante 10 minutos. Após, para o processo de extração do produto dessa solução foi adicionado à mistura 600µL de álcool n-butílico, agitados em Vórtex e centrifugados em 3600rpm durante 10 minutos (LIMA; ABDALLA, 2001). Para a determinação dos níveis de TBARS foi utilizado o sobrenadante e detectado a 535nm. O resultado é expresso em nmoles de TBA. Mg de proteínas^{-1}.

2.4 Análise Bioquímica do Metabolismo Intermediário

As análises metabólicas foram realizadas a partir de um homogeneizado por animal para obter as medidas de Proteínas Totais (PT), Ácido Úrico (AU) e Glicogênio (GG) conforme o método de extração de VAN HANDEL (1965). Para esse método de extração utilizou-se 2 mL de KOH 30% adicionado ao animal inteiro, a 100ºC por aproximadamente quatro horas. Em seguida, foram realizadas as análises de PT com o kit da BioTécnica (técnica baseada na reação das proteínas na amostra com íons de cobre, em meio alcalino e detectada em absorbância de 550nm) e AU com o kit comercial da BioTécnica (baseada na catalisação da oxidação do ácido úrico presente na amostra e reações produzindo um composto com coloração rosa que é determinado a 505nm. Os resultados foram expressos em mg.g^{-1}.

Para a quantificação do glicogênio (GG) foi acrescentado em cada tubo duas gotas de Sulfato de Sódio (Na2SO4) e agitado no vórtex. Após, foi adicionado 5mL de álcool etílico absoluto em cada tubo e agitado novamente em vórtex para posterior centrifugação a 3000rpm por 10 minutos. Para finalizar essa etapa, o sobrenadante foi descartado e mais dois processos de lavagem foram realizados com 2mL de Água destilada e 5mL de álcool etílico absoluto, seguindo o mesmo processo de agitação, centrifugação e descarte do sobrenadante. Na última etapa, foi realizada a ressuspensão do precipitado em 0,3 mL de água destilada e agitação em vórtex, seguida de hidrólise ácida (com 0,1 mL HCl em 0,1 mL da suspensão amostral) e aquecidos a 100ºC, por uma hora, com posterior neutralização com 0,1 mL de carbonato de sódio (Na2CO3).

Para a quantificação da glicose liberada pela hidrólise do glicogênio foi utilizado o kit comercial de Glicose da BioTécnica que é baseado no método da glicose oxidada e determinada a 505nm. O cálculo do GG feito a partir da relação de uma curva padrão obtida com concentração conhecida de glicogênio convertido em glicose e de uma curva obtida com o padrão de glicose fornecido pelo kit. Os resultados foram expressos em $mg.g^{-1}$.

2.5 Análise Estatística

Foram considerados significativos quando $p<0,05$, todos os resultados foram expressos como média ± erro padrão.Todas as análises estatísticas foram realizadas no programa SPSS Statistics para Windows, versão 26.0.

Primeiramente, foi avaliada a normalidade utilizando o teste Shapiro-Wilk, seguido do teste de homogeneidade de Levene para definir a distribuição dos dados e o teste complementar. Para dados paramétricos ($p>0,05$) foi utilizado o teste de análise de variância de uma via (ANOVA), com post-hoc de Tukey (quando o teste de Levene indicou $p>0,05$) ou Games-Howell (quando o teste de Levene indicou $p<0,05$) comparando os grupos exposição com o grupo controle em cada biomarcador. Foi utilizada uma análise de variância de duas vias, tendo como fatores fixos os grupos (controle, 4µg/L, 15µg/L e 30µg/L) e a faixa etária (adulto-jovem x senil) para cada sexo. A posteriori realizamos também, uma ANOVA de três-vias para vermos a interação entre os grupos (controle, 4µg/L, 15µg/L e 30µg/L), a idade (adulto-jovem e senil) e o sexo (fêmeas e machos). Além disso, para a comparação do Comprimento Total (CT) e Massa Corporal (MC), inicial (antes da exposição) e final (após a exposição), foi realizado o teste Independent Sample Test (Teste T) para cada grupo (controle, 4µg/L, 15µg/L e 30µg/L).

3. Resultados

3.1 Balanço Oxidativo

Os resultados obtidos em balanço oxidativo de fêmeas adultas-jovens mostraram que a exposição ao Herbicida Dez© mostrou uma diminuição significativa nos marcadores CAT ($F = 3,309$; $p = 0,017$) e GST ($F = 7,333$; $p = 0,003$) na maior concentração em relação ao grupo controle (Fig. 2 – D e F). Já os resultados para fêmeas

senis mostraram um aumento significativo na concentração de 30µg/L no marcador de dano oxidativo, o TBARS (F = 10,010; p = 0,031), e na enzima CAT (F = 4,980; p = 0,031) com a exposição ao herbicida (Fig. 3 – B e D).

Em machos adultos-jovens, os resultados apresentaram diferença significativa apenas na atividade da CAT (F = 7,426; p = 0,039) na concentração de 15µg/L, mostrando uma diminuição em relação ao grupo controle (Fig. 4 – D). E, em machos senis expostos ao Herbicida Dez© houve um aumento significativo nos níveis de TBARS (F = 3,645; p = 0,045) na concentração de 15µg/L quando comparado ao grupo controle (Fig. 5– B).

Os dados referentes a análise de variância duas vias (grupos e idade) em fêmeas mostraram uma diferença significativa na interação grupos e idade em todos os marcadores de balanço oxidativo: TBARS (F = 146,043; p = 0,000), PT (F = 16,892; p = 0,001), GST (F = 11,363; p = 0,003), SOD (F = 10,078; p = 0,005), CAT (F = 27,227; p = 0,000) e razão SOD/CAT (F = 274,995; p = 0,000) (Tabela 6). Já as análises de variância de duas vias em machos, observamos diferença significativa na interação entre os grupos e as idades apenas em CAT (F = 18,654; p = 0,000) e SOD/CAT (F = 82,564; p = 0,000) (Tabela 7). O resultado da ANOVA de três-vias mostrou uma interação significativa entre os grupos, as idades e os sexos para todos os marcadores analisados (PTS, CAT, GST- p<0,000; TBARS- p<0,004 e SOD, SOD/CAT- p<0,01).

3.2 Metabolismo Intermediário

Referente as análises de metabolismo intermediário em fêmeas adultas-jovens expostas ao Herbicida Dez©, os resultados mostraram diferenças significativas em todos os marcadores: GG, PT e AU. A exposição provocou um aumento nos níveis de GG na menor concentração (F = 7,427; p = 0,009), aumento nos níveis de PT na maior concentração (F = 7,686; p = 0,026), e em AU (F = 29,150; p = 0,020) um aumento na concentração de 15 µg/L e diminuição na concentração de 30 µg/L (Fig. 6 – A, B e C). Já em fêmeas senis, observou-se um aumento em GG na concentração de 4 µg/L (F = 13,221; p = 0,004) e um aumento nos níveis de PT na concentração de 30 µg/L (F = 43,537; p = 0,025) (Fig. 6 – D e E).

Os resultados de metabolismo da exposição de machos adultos-jovens ao Herbicida Dez©, não apresentaram diferenças significativas em nenhum marcador em relação ao grupo controle (Fig. 7 – A, B e C). Contudo, em machos senis foi possível observar alterações nos níveis de PT e AU. Em PT houve uma diminuição significativa

na concentração de 15 µg/L (F = 8,645; p = 0,006) e em AU (F = 22,212) um aumento na concentração de 4 µg/L (p = 0,013), 15 µg/L (p = 0,000) e 30 µg/L (p = 0,003) (Fig. 7 – E e F).

Além disso, as análises de variância de duas vias em fêmeas mostraram que há diferença entre as idades nos marcadores: GG (F = 12,499; p = 0,002) e PT (F = 20,915; p = 0,000) (Tabela 8). E, em machos senis houve diferença entre as idades nos marcadores de PT (F = 116,595; p = 0,000) e AU (F = 12,088; p = 0,003) (Tabela 9). O resultado da ANOVA de três-vias mostrou uma interação significativa entre os grupos as idades e os sexos para todos os marcadores analisados (PT e AU- p<0,000; GG- p<0,014).

4. Discussão

Nossos resultados demonstram pela primeira vez o impacto de concentrações ambientalmente relevantes de um herbicida à base de 2,4D sobre adultos-jovens e senis de uma espécie de peixe anual neotropical. Outros autores têm mostrado o desafio de peixes não anuais para manterem a homeostase e assim garantirem à sobrevivência frente à exposição a outros herbicidas (PERSCH et al. 2017 e 2018; URSINI; MAIORINO; FORMAN, 2016; LUSHCHAK et al. 2009; SOBJAK et al. 2017; TONI et al. 2013). Estudo realizado com *Carassius auratus* utilizou concentrações relativamente baixas do herbicida Roundup e os resultados mostraram que o herbicida provocou efeitos prejudiciais no metabolismo dos animais, especialmente em exposição prolongada (LUSHCHAK et al. 2009). De acordo com LUSHCHAK et al. (2009) mesmo as concentrações baixas de herbicidas podem provocar efeitos adversos no status antioxidante em peixes.

Além disso, fomos capazes de demonstrar que este organismo com ciclo de vida pós-eclosão curto, pode ser utilizado em estudos para o entendimento de aspectos toxicocinéticos e toxicodinâmicos em diferentes faixas etárias (adulto-jovem e senil) contribuindo para o estabelecimento de fases de maior vulnerabilidade a exposição. Estes dados reforçam resultados prévios que apontam os peixes anuais nativos como potenciais indicadores de toxicidade ambiental (CASTRO et al. 2021, 2022; GODOY et al. 2020). Estes estudos têm sugerido que estes animais mantêm um sistema antioxidante e componentes do sistema de biotransformação altamente eficiente o que pode estar relacionado aos ambientes efêmeros ocupados por estas espécies (BEROIS et al. 2012; GODOY et al. 2020; CASTRO et al. 2021, 2022).

Nesse estudo avaliamos três concentrações da formulação comercial Dez© (princípio ativo 2,4D) sobre fêmeas e machos de *Cynopoecilus* sp., em diferentes faixas etárias. Ambos os sexos mostraram alterações de marcas do sistema antioxidante, de biotransformação e das reservas energéticas tanto no estágio adulto-jovem como no senil que parecem ter permitido a sobrevivência a sete dias de exposição ao herbicida. Contudo, os resultados apontam para a presença de estresse oxidativo, com incremento dos níveis de TBARS nas concentrações mais altas do herbicida, tanto em fêmeas como em machos, na fase de senilidade o que pode determinar um encurtamento do ciclo de vida e um comprometimento da reprodução. Por habitarem ambientes efêmeros, estes pequenos peixes apresentam um ciclo de vida curto, crescimento rápido, maturidade sexual precoce, reprodução contínua e produção de produção ovos de resistência (ARENZON et al. 2001, 2002; BEROIS et al. 2012).

Sabe-se que a exposição de um animal a agrotóxicos pode desencadear a formação de espécies reativas de oxigênio (EROs) que quando em excesso provocam um desequilíbrio entre o sistema antioxidante e agentes pró-oxidantes conduzindo a uma situação de estresse oxidativo (GLUSCZAK et al. 2011). Estudos vêm utilizando como marcador de dano oxidativo em peixes a determinação dos níveis de TBARS e de proteínas carboniladas (CASTRO et al. 2022; PERSCH et al. 2018; THIEL et al. 2020). Segundo GLUSCZAK et al. (2011) os mecanismos responsáveis por combaterem as EROs constituem o sistema antioxidante. Este sistema se apresenta composto por enzimas antioxidantes, como a superóxido dismutase, catalase, glutationa peroxidase e outras, e moléculas de baixo peso molecular, como a glutationa, melatonina, ácido úrico e diferentes vitaminas. Diferentes componentes do sistema antioxidante têm sido utilizados como marcadores de contaminação, incluindo herbicidas, para o entendimento de aspectos toxicodinâmicos (FERREIRA et al. 2010; GLUSCZAK et al. 2011; SOBJAK et al. 2017).

Além disto, enzimas das vias de biotransformação têm sido utilizadas para o entendimento de aspectos toxicocinéticos de contaminantes. As glutationas transferases (GSTs) são as principais enzimas de fase II de biotransformação encontradas principalmente no citosol. Além de seu papel como catalisador a conjugação de substratos eletrofílicos à glutationa (GSH), como metabólitos da lipoperoxidação, essas enzimas também realizam uma série de outras funções (SHEEHAN et al. 2001).

Os resultados referentes as fêmeas adultas-jovens mostraram uma manutenção da atividade da SOD e decréscimo da CAT e da GST na maior concentração de 2,4D

(30µg/L). Considerando este perfil de resposta, podemos sugerir que outros componentes do sistema antioxidante estejam atuando a fim de manterem baixos os níveis de lipoperoxidação evitando assim o dano no componente lipídico; contudo, não podemos garantir a ocorrência de dano em outras moléculas, como as proteínas. Estudo de Castro et al. (2021) com *Cynopoecilus fulgens* coletados em ambiente natural encontraram baixos níveis de TBARS e altos níveis de proteínas carboniladas nesta mesma faixa etária, sugerindo uma relação inversa entre o dano lipídico e o dano proteico. Wingen (2021) trabalhando com girinos de *Squinax squalirostris* expostos a concentrações de 2,4D de 4, 15 e 30 µg/L mostraram uma manutenção dos níveis de TBARS aliado a um incremento dos níveis de proteínas carboniladas. Contudo, estes girinos apresentam um incremento da SOD, CAT e GST fato não verificado por nós para esta espécie de peixe anual o que reforça a ideia de ação de outros componentes do sistema antioxidante.

Sabe-se que a CAT é uma das enzimas mais relevantes do sistema antioxidante, sendo responsável por neutralizar a ação das EROs, especialmente do peróxido de hidrogênio, sobre sistemas biológicos (HERMES-LIMA, 2004). Também, a GST é uma enzima que apresenta uma função secundária na linha de defesa do organismo e tem um papel importante na proteção de dano por peroxidação lipídica (ROMERO et al. 2013; TU et al. 2008). Dessa forma, o decréscimo da GST na maior concentração em fêmeas pode-se sugerir uma diminuição na eliminação do herbicida 2,4D do organismo representando uma perda da capacidade funcional de biotransformação. Experimentos futuros devem ser desenvolvidos para um melhor entendimento deste conjunto de resposta.

Nas fêmeas senis os resultados também mostram alterações nos níveis de CAT; contudo, houve um aumento da atividade desta enzima na maior concentração (30 µg/L) do herbicida, diferente do obtido em fêmeas adultas-jovens. Tal resposta corrobora com estudo de THIEL et al. (2020) com *Zebrafish* que observaram um aumento da atividade da CAT na mesma concentração de 2,4D. Sendo assim, sugere-se que a exposição ao herbicida Dez© desencadeou um aumento da resposta antioxidante contra o peróxido de hidrogênio (H_2O_2); contudo, o sistema de defesa não foi capaz de evitar a produção do radical hidroxila (OH) e assim, danos aos lipídios verificados pelo aumento dos níveis de TBARS.

Em machos adultos-jovens os resultados mostraram uma diminuição da atividade da CAT, na concentração intermediária (15 µg/L), aliada a manutenção da atividade da

SOD e da GST, e ausência de lipoperoxidação; demonstrando que o sistema antioxidante do animal está atuando na defesa do organismo de uma forma mais intensa que nas fêmea. Novamente, esta ausência de dano oxidativo pode estar relacionada a uma maior eficiência do sistema antioxidante destes peixes em função do seu ciclo de vida e habitat (ARENZON et al. 2001, 2002; BEROIS et al. 2012). Frente aos resultados obtidos para ambos os sexos no estágio de desenvolvimento de adulto-jovem, cabe ressaltar que observamos que as fêmeas de *Cynopoecilus* sp. tiveram maiores alterações em níveis de marcadores enzimáticos (CAT e GST) do que os machos, o que pode representar uma maior vulnerabilidade a exposição ao Dez©, principalmente, na maior concentração do herbicida.

Machos senis também tiveram um aumento nos níveis de TBARS, mas na concentração intermediária (15 µg/L), enquanto a atividade das enzimas manteve-se constante. GODOY et al. (2020) e CASTRO et al. (2022) sugerem que peixes anuais apresentam um sistema antioxidante eficiente indicando uma capacidade de sustentar o equilíbrio oxidativo até a etapa final do ciclo de vida (senil). Entretanto, em nosso estudo percebe-se que o estágio de desenvolvimento senil foi mais impactado, tanto em fêmeas como em machos, apresentando um incremento acentuado nos níveis de TBARS o que indica uma situação de estresse oxidativo nas concentrações mais altas deste herbicida. Tal perfil pode determinar um encurtamento do ciclo de via e/ou diminuição da capacidade reprodutiva da espécie.

De acordo com FANTA et al. (2003) o estudo de parâmetros fisiológicos em peixes expostos a agrotóxicos é de extrema importância. Frente a existência de muitas substâncias químicas em diversos ambientes aquáticos que podem provocar a redução da capacidade vital do organismo acarretando a morte e/ou comprometendo a reprodução (PERSH et al. 2018). Sendo assim, para FAUSCH et al. (1990) os peixes podem indicar a qualidade do ecossistema aquático, pois são bastante sensíveis a mudanças no meio ambiente. Para isso, a análise de marcadores de metabolismo intermediário (glicogênio, proteínas totais, lipídios, triglicerídeos e outros) também são uma forma de compreender o que ocorre com a exposição do organismo ao meio contaminado. Dessa forma, as mudanças em reservas energéticas podem representar uma resposta bioquímica do animal a exposição (SANCHO; FERRANDO; ANDREU, 1997).

Em nosso estudo, os resultados referentes aos marcadores de metabolismo mostraram que fêmeas adultas-jovens e senis, e machos senis tiveram maiores alterações

nos níveis de GG, AU e PT. Para as fêmeas o GG teve aumento na menor concentração em ambos estágios de desenvolvimento, assim como a PT teve aumento na maior concentração. Tal resultado sugere que os níveis elevados de GG podem indicar o acúmulo dessa reserva visando a manutenção do balanço energético e o aumento da síntese de PT como compensatória, devido a exposição e estresse provocado pelo agrotóxico. TONI et al. (2013) afirmam que a proteína é considerada um dos principais alvos para explicação dos efeitos de agrotóxicos em peixes.

Os níveis de ácido úrico em fêmeas adultas-jovens foram elevados apenas na concentração de 15 µg/L, mostrando uma relação inversa com as proteínas, em 30 µg/L temos uma diminuição destes níveis, sugerindo que o AU pode estar sendo utilizado como um antioxidante não enzimático a fim de minorar o dano oxidativo. Nas fêmeas senis os níveis de AU não variam significativamente o que corrobora com o aumento nos níveis de TBARS verificados nesta faixa etária. Já em machos senis houve uma diminuição dos níveis de PT na concentração de 15 µg/L e um aumento do AU em todas as concentrações do herbicida. Pode-se sugerir que o catabolismo das PT seja uma estratégia do animal para garantir a síntese de AU e assim, garantir a funcionalidade do organismo apesar de não evitar o incremento da lipoperoxidação na concentração mais elevada do herbicida. Diversos estudos de nosso grupo (COLTRO et al. 2017; WILKENS et al. 2019 e DA SILVA et al. 2021) trabalhando com girinos expostos a diferentes herbicidas sugerem um papel antioxidante para o ácido úrico. O ácido úrico é o principal produto do metabolismo do nitrogênio em insetos, répteis e aves, enquanto o principal produto deste metabolismo em peixes é a ureia. Alguns trabalhos sugerem que o ácido úrico funcione como um importante antioxidante plasmático em primatas e em vários insetos (TASAKI et al. 2017). Machado et al. (2018) sugere que o ácido úrico possa ser utilizado como antioxidante em peixes, especialmente os teleósteos, e em anfíbios.

Conclusão

Esse estudo buscou elucidar o efeito de uma formulação comercial com princípio ativo 2,4D em organismos não-alvo, como uma forma de contribuir para outras pesquisas. Mostramos que utilizando concentrações ambientalmente relevantes deste herbicida provocaram mudanças em marcadores do balanço oxidativos e do metabolismo intermediário em fêmeas e machos de *Cynopoecilus* sp. em diferentes

estágios de desenvolvimento pós-embrionário. Pode-se destacar que o estágio de desenvolvimento de maior vulnerabilidade foi a fase senil, na qual os níveis de TBARS aumentados sem que o sistema antioxidante fosse capaz de conter o estresse oxidativo. Tais respostas demonstram que a exposição ao 2,4D pode provocar efeitos mais intensos quando comparado ao estudo de CASTRO et al. (2022) desenvolvido com machos destes animais expostos ao glifosato.

Referências

AMARANTE JR, O. P. et al. Glifosato: Propriedades, Toxicidade, Usos e Legislação. **Química Nova**, v. 25, n. 4, p. 589–593, 2002. https://doi.org/10.1590/S0100-40422002000400014

ARENZON, A; LEMOS, C. A; BOHRER, M. B. C. The influence of temperature on the embryonic development of the annual fish *Cynopoecilus melanotaenia* (Cyprinodontiformes, Rivulidae). **Brazilian journal of biology**, v. 62, n. 4B, p. 743–747, 2002. https://doi.org/10.1590/S1519-69842002000500002

ARENZON, A.; PERET, A. C.; BOHRER, M. B. C. Growth of the annual fish *Cynopoecilus melanotaenia* (Regan, 1912) based in a Temporary Water Body Population in Estado Rio Grande do Sul, Brazil (Cyprinodontiformes, Rivulidae). **Revista Brasileira de Biologia**, v. 61, p. 117–123, 2001. https://doi.org/10.1590/s0034-71082001000100015

ATLI, G.; CANLI, M. Enzymatic responses to metal exposures in a freshwater fish Oreochromis niloticus. **Comparative Biochemistry and Physiology - C Toxicology and Pharmacology**, v. 145, n. 2, p. 282–287, 2007. https://doi.org/10.1016/j.cbpc.2006.12.012

BEROIS, N. et al. Annual fish: Developmental adaptations for an extreme environment. **Wiley Interdisciplinary Reviews: Developmental Biology**, v. 1, n. 4, p. 595–602, 2012. https://doi.org/10.1002/wdev.39

BOVERIS, A.; CADENAS, E. Production of superoxide radicals and hydrogen peroxide in mitochondria. Em: OBERLEY, L. W. (Ed.). **Superoxide dismutase**. Flórida: Boca Raton: CRC Press, 1982. v. 2p. 15–30.

BOVERIS, A.; CHANCE, B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. **The Biochemical journal**, v. 134, n. 3, p. 707–716, 1973. https://doi.org/10.1042/bj1340707

BOYLAND, E.; CHASSEAUD, L. F. The Role of Glutathione and Glutathione S-transferases in Mercapturic Acid Biosynthesis. **Advances in enzymology and related areas of molecular biology**, v. 32, p. 173–219, 1969. https://doi.org/10.1002/9780470122778.ch5

BUEGE, J. A.; AUST, S. D. Microsomal Lipid Peroxidation. **Methods Enzym**, p. 302–310, 1978. https://doi.org/10.1016/s0076-6879(78)52032-6

CASTRO, B. D. et al. Biomarkers of oxidative stress in the post-embryonic characterization of the neotropical annual killifish. **Biogerontology**, v. 0, 2021. https://doi.org/10.1007/s10522-021-09931-0

CASTRO, B. D. et al. Development stage-dependent oxidative stress responses to the exposure to roundup original© in a neotropical annual killifish. **Environmental Toxicology and Pharmacology**, v. 95, p. 103976, out. 2022. https://doi.org/10.1016/j.etap.2022.103976

CELLERINO, A.; VALENZANO, D. R.; REICHARD, M. From the bush to the bench: The annual *Nothobranchius* fishes as a new model system in biology. **Biological Reviews**, v. 91, n. 2, p. 511–533, 2016. https://doi.org/10.1111/brv.12183

COOKE, S. J. et al. What is conservation physiology? Perspectives on an increasingly integrated and essential science. **Conservation Physiology**, v. 1, n. 1, p. 1–23, 2013. https://doi.org/10.1093/conphys/cot001

COSGROVE, S.; JEFFERSON, B.; JARVIS, P. Pesticide removal from drinking water sources by adsorption: a review. **Environmental Technology Reviews**, v. 8, n. 1, p. 1–24, 2019. https://doi.org/10.1080/21622515.2019.1593514

DA SILVA, P. R.; BORGES-MARTINS, M.; OLIVEIRA, G. T. *Melanophryniscus admirabilis* tadpoles' responses to sulfentrazone and glyphosate-based herbicides: an approach on metabolism and antioxidant defenses. **Environmental Science and Pollution Research**, v. 28, n. 4, p. 4156–4172, 1 jan. 2021. https://doi.org/10.1007/s11356-020-10654-x

DE LIMA COLTRO, M. et al. Influence of the Herbicide Facet® on Corticosterone Levels, Plasma Metabolites, and Antioxidant System in the Liver and Muscle of American Bullfrog Tadpoles. **Water, Air, and Soil Pollution**, v. 228, n. 7, 1 jul. 2017. https://doi.org/10.1007/s11270-017-3404-7

DEHNERT, G. K.; KARASOV, W. H.; WOLMAN, M. A. 2,4-Dichlorophenoxyacetic acid containing herbicide impairs essential visually guided behaviors of larval fish. **Aquatic Toxicology**, v. 209, p. 1–12, 1 abr. 2019. https://doi.org/10.1016/j.aquatox.2019.01.015

FANTA, E. et al. Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food. **Ecotoxicology and Environmental Safety**, v. 54, n. 2, p. 119–130, 2003. https://doi.org/10.1016/s0147-6513(02)00044-1

FAUSCH, K. D. et al. Fish communities as indicators of environmental degradation. **American Fisheries Society Symposium**, v. 8, n. July, p. 123–144, 1990.

FERREIRA, D. et al. Assessment of oxidative stress in *Rhamdia quelen* exposed to agrichemicals. **Chemosphere**, *79*(9), 914–921, 2010. https://doi.org/10.1016/j.chemosphere.2010.03.024

GLUSCZAK, L. et al. Acute exposure to glyphosate herbicide affects oxidative parameters in piava (*Leporinus obtusidens*). **Archives of Environmental Contamination and Toxicology**, v. 61, n. 4, p. 624–630, 2011. https://doi.org/10.1007/s00244-011-9652-4

GODOY, R. S. et al. Oxidative stress resistance in a short-lived Neotropical annual killifish. **Biogerontology**, v. 0123456789, 2020. https://doi.org/10.1007/s10522-019-09855-w

HERMES-LIMA, M. Role of Free Radicals. Em: STOREY, K. B. (Ed.). **Funcional Metabolism: Regulation and Adaptation**. John Wiley ed. Hoboken, 2004. p. 319–368.

ISLAM, F. et al. Potential impact of the herbicide 2,4-dichlorophenoxyacetic acid on human and ecosystems. **Environment International.** Elsevier Ltd, 1 fev. 2018. https://doi.org/10.1016/j.envint.2017.10.020

LIMA, L. B. D. DE et al. Use of biomarkers to evaluate the ecological risk of xenobiotics associated with agriculture. **Environmental Pollution**, v. 237, p. 611–624, 2018. https://doi.org/10.1016/j.envpol.2018.02.011

LIMA, É. S.; ABDALLA, D. S. P. Peroxidação lipídica: mecanismos e avaliação em amostras biológicas. **Revista Brasileira de Ciências Farmacêuticas**, v. 37, n. 3, p. 293–303, 2001.

LUSHCHAK, V. I. Environmentally induced oxidative stress in aquatic animals. In **Aquatic Toxicology** (Vol. 101, Issue 1, pp. 13–30), 2011. Elsevier B.V. https://doi.org/10.1016/j.aquatox.2010.10.006

MACHADO, A et al. A taxonomically-restricted Uric Acid transporter provides insight into antioxidant 1 equilibrium 2. **bioRxiv**, 2018. https://doi.org/10.1101/287870.

MENEZES, C. et al. Commercial formulation containing 2,4-D affects biochemical parameters and morphological indices of silver catfish exposed for 90 days. **Fish Physiology and Biochemistry,** v. 41, n. 2, p. 323–330, 2015. https://doi.org/10.1007/s10695-014-9985-9

NETO, D. M.; FROEHNER, S.; MACHADO, K. S. Avaliação do transporte do ácido 2,4-diclorofenoxiacético através de um lisímetro. **Quím. Nova** vol.35 no.9 São Paulo, 2012. https://doi.org/10.1590/S0100-40422012000900020

PERSCH, T. S. P. et al. Metabolic parameters and oxidative balance in juvenile *Rhamdia quelen* exposed to rice paddy herbicides: Roundup®, Primoleo®, and Facet®. **Chemosphere**, v. 174, p. 98–109, 2017. http://dx.doi.org/10.1016/j.chemosphere.2017.01.092

PINHEIRO, A.; SILVA, M. R. DA; KRAISCH, R. Presença de pesticidas em águas superficiais e subterrâneas na bacia do Itajaí, SC. **REGA –** Vol. 7, no. 2, p. 17-26, jul./dez. 2010. https://doi.org/10.21168/REGA.V7N2.P17-26

PERSCH, T. S. P. et al. Changes in intermediate metabolism and oxidative balance parameters in sexually matured three-barbeled catfishes exposed to herbicides from rice crops (Roundup®, Primoleo® and Facet®). **Environmental Toxicology and Pharmacology**, v. 58, p. 170–179, 1 mar. 2018. https://doi.org/10.1016/j.etap.2018.01.004

POMPERMAIER, A. et al. Waterborne agrichemicals compromise the anti-predatory behavior of *zebrafish*. **Environmental Science and Pollution Research**, v. 27, n. 31, p. 38559–38567, 1 nov. 2020. https://doi.org/10.1007/s11356-020-09862-2

QUEIROZ, G. M. P. et al. Transporte de Glifosato pelo Escoamento Superficial e por Lixiviação em um Solo Agrícola. **Quimica Nova**, v. 34, n. 2, p. 190–195, 2011. https://doi.org/10.1590/S0100-40422011000200004

ROMERO, M. C. et al. Antioxidant defenses and lipid peroxidation in two *Lithodes* species from South Atlantic during summer. **Revista de Biología Marina y Oceanografía**, v. 48, p. 227–234, 2013.

SANCHO, E.; FERRANDO, M. D.; ANDREU, E. Sublethal effects of an organophosphate insecticide on the European Eel, *Anguilla anguilla*. **Ecotoxicology and Environmental Safety**, v. 36, n. 1, p. 57–65, 1997. https://doi.org/10.1006/eesa.1996.1488

SHEEHAN, D.; MEADE, G.; FOLEY, V. M.; DOWD, C. A. Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. **Biochemical Journal**, *360*(1), 1–16, 2001. https://doi.org/10.1057/9781137378699_11

SOBJAK, T. M. et al. Assessment of the oxidative and neurotoxic effects of glyphosate pesticide on the larvae of *Rhamdia quelen* fish. **Chemosphere**, *182*, 267–275, 2017. https://doi.org/10.1016/j.chemosphere.2017.05.031

TASAKI, E. et al. Uric acid, an important antioxidant contributing to survival in termites. **PLoS ONE** 12 (6): e0179426, 2017. https://doi.org/10.1371/journal. pone.0179426

THIEL, N. A. et al. Exposure to the herbicide 2,4-dichlorophenoxyacetic acid impairs mitochondrial function, oxidative status, and behavior in adult *zebrafish*. **Environmental Science and Pollution Research**, v. 27, n. 36, p. 45874–45882, 1 dez. 2020. https://doi.org/10.1007/s11356-020-10497-6

TONI, C. et al. Oxidative stress in carp exposed to quinclorac herbicide under rice field condition. **Ecotoxicology and Environmental Safety**, v. 92, p. 27–31, 1 jun. 2013. https://doi.org/10.1016/j.ecoenv.2013.01.028

TU, H. T. et al. Oxidative stress response of black tiger shrimp (*Penaeus monodon*) to enrofloxacin and to culture system. **Aquaculture**, v. 285, n. 1–4, p. 244–248, 7 dez. 2008. https://doi.org/10.1016/j.aquaculture.2008.08.032

URSINI, F.; MAIORINO, M.; FORMAN, H. J. Redox homeostasis: The Golden Mean of healthy living. **Redox Biology**, v. 8, p. 205–215, 2016. https://doi.org/10.1016/j.redox.2016.01.010

VAN HANDEL, E. Estimation of Glycogen in Small Amounts of Tissue. **Analytical biochemistry**, v. 11, n. 2, p. 256–265, 1965. https://doi.org/10.1016/0003-2697(65)90013-8

VRTÍLEK, M.; REICHARD, M. Highly plastic resource allocation to growth and reproduction in females of an African annual fish. **Ecology of Freshwater Fish**, v. 24, n. 4, p. 616–628, 2015. https://doi.org/10.1111/eff.12175

WINGEN, N. M. A. Avaliação da toxicidade de glifosato e 2,4-D em girinos de *Pseudis minuta* e *Scinax squalirostris* coletados em ambiente natural. 2021. https://tede2.pucrs.br/tede2/handle/tede/9744

ZEBRAL, Y. D. et al. A glyphosate-based herbicide reduces fertility, embryonic upper thermal tolerance and alters embryonic diapause of the threatened annual fish *Austrolebias nigrofasciatus*. **Chemosphere**, v. 196, p. 260–269, 2018. https://doi.org/10.1016/j.chemosphere.2017.12.196

Agradecimentos

Agradecemos à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão da bolsa B.D.C, e à Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS) por todo o suporte técnico, ao Centro de Modelos Biológicos Experimentais (CeMBE) para fornecimento de alguns materiais.

Tabelas e figuras (Capítulo 3)

Tabela 1 Parâmetros abióticos da água do local de coleta utilizado neste estudo.

Campanhas de Amostragem	Temperatura (°C)	pH	Condutividade (Ms/cm)	ORP* (mV)	Salinidade	TDS** (ppm)
Junho	18.28	4.93	0.00	108.8	0.18	189
Novembro	24.74	5.06	0.00	11.08	0.27	280

* Potencial Oxidação-Redução (ORP)
** Sólidos Totais Dissolvidos (TDS)

Tabela 2 Média ± erro padrão do Comprimento Total inicial e final (mm) em fêmeas adultas jovens e senis de *Cynopoecilus* sp. exposto a Dez©. Para comparar as medidas dos animais, foi realizado o Teste de Amostra Independente em cada grupo experimental (Controle, 4, 15 e 30 µg/L). (*) significa diferença entre o Comprimento Total inicial e final dos animais ($p < 0,05$).

Estágio de Desenvolvimento	Grupo	Comprimento Total Inicial	Comprimento Total Final	df	F	*P*
Adulto-jovem	Controle	18.91 ± 0.35	19.84 ± 0.46	22	1.476	0.127
	4 µg/L	19.88 ± 0.26	19.98 ± 0.52	16	5.720	0.876
	15 µg/L	19.55 ± 0.29	20.26 ± 0.59	16	4.588	0.300
	30 µg/L	19.88 ± 0.26	20.86 ± 0.55	16	9.460	0.131
Senil	Controle*	19.80 ± 0.61	21.60 ± 0.55	22	0.068	0.041
	4 µg/L*	19.90 ± 0.62	22.51 ± 0.61	18	0.014	0.008
	15 µg/L*	20.06 ± 0.75	22.15 ± 0.63	18	0.370	0.047
	30 µg/L	21.14 ± 0.54	22.58 ± 0.50	18	0.001	0.069

Tabela 3 Média ± erro padrão do Comprimento Total inicial e final (mm) em machos adultos jovens e senis de *Cynopoecilus* sp. exposto a Dez©. Para comparar as medidas dos animais, foi realizado o Teste de Amostra Independente em cada grupo experimental (Controle, 4, 15 e 30 µg/L). (*) significa diferença entre o Comprimento Total inicial e final dos animais ($p < 0,05$).

Estágio de Desenvolvimento	Grupo	Comprimento Total Inicial	Comprimento Total Final	df	F	*P*
Adulto-jovem	Controle	24.73 ± 0,60	25.47 ± 0,48	36	1.618	0.346
	4 µg/L	25.47 ± 0.69	25.66 ± 0.65	46	0.006	0.839
	15 µg/L	25.48 ± 0.93	25.88 ± 0.87	34	0.818	0.753
	30 µg/L	24.55 ± 0.78	25.86 ± 1.04	34	2.545	0.323
Senil	Controle*	25.07 ± 0.60	29.30 ± 1.08	26	8.595	0.002
	4 µg/L*	25.66 ± 0.74	31.01 ± 1.03	34	1.112	0.000
	15 µg/L*	25.75 ± 0.85	28.54 ± 0.78	38	0.009	0.021
	30 µg/L*	24.27 ± 0.74	26.97 ± 0.93	34	0.385	0.030

Tabela 4 Média ± erro padrão da Massa Corporal inicial e final (g) em fêmeas adultas jovens e senis de *Cynopoecilus* sp. exposto a Dez©. Para comparar as medidas dos animais, o Teste de Amostra Independente foi realizado em cada grupo experimental (Controle, 4, 15 e 30 µg/L). (*) significa diferença entre o Comprimento Total inicial e final dos animais ($p < 0,05$).

Estágio de Desenvolvimento	Grupo	Massa Corporal Inicial	Massa Corporal Final	df	F	*p*
Adulto-jovem	Controle*	0.07 ± 0.00	0.10 ± 0.00	22	0.181	0.005
	4 µg/L	0.09 ± 0.00	0.10 ± 0.00	16	0.004	0.259
	15 µg/L	0.10 ± 0.00	0.11 ± 0.00	16	1.895	0.147
	30 µg/L*	0.07 ± 0.00	0.13 ± 0.00	16	3.307	0.000
Senil	Controle*	0.07 ± 0.00	0.11 ± 0.00	22	1.417	0.000
	4 µg/L*	0.08 ± 0.00	0.12 ± 0.01	18	3.927	0.005
	15 µg/L*	0.08 ± 0.00	0.12 ± 0.00	18	4.162	0.000
	30 µg/L*	0.07 ± 0.00	0.11 ± 0.01	18	1.589	0.003

Tabela 5 Média ± erro padrão da Massa Corporal inicial e final (g) em machos adultos jovens e senis de *Cynopoecilus* sp. exposto a Dez©. Para comparar as medidas dos animais, o Teste de Amostra Independente foi realizado em cada grupo experimental (Controle, 4, 15 e 30 µg/L). (*) significa diferença entre o Comprimento Total inicial e final dos animais ($p < 0,05$).

Estágio de Desenvolvimento	Grupo	Massa Corporal Inicial	Massa Corporal Final	df	F	*p*
Adulto-jovem	Controle*	0.16 ± 0.01	0.22 ± 0.01	36	1.221	0.001
	4 µg/L	0.19 ± 0.01	0.22 ± 0.01	46	2.850	0.111
	15 µg/L	0.18 ± 0.01	0.23 ± 0.01	34	1.714	0.075
	30 µg/L*	0.16 ± 0.01	0.23 ± 0.02	34	7.528	0.009
Senil	Controle*	015 ± 0.01	0.28 ± 0.02	26	12.937	0.000
	4 µg/L*	0.19 ± 0.01	0.32 ± 0.02	34	6.312	0.000
	15 µg/L*	0.19 ± 0.01	0.26 ± 0.02	38	2.782	0.008
	30 µg/L*	0.16 ± 0.01	0.23 ± 0.01	34	0.991	0.004

Tabela 6 Resultados da análise do Balanço Oxidativo. Dados de média ± erro padrão de marcadores fisiológicos em fêmeas adultas e senis de *Cynopoecilus* sp. expostas ao Dez© (4, 15 e 30 µg/L). Proteína Total (TP), Substâncias Reativas ao Ácido Tiobarbitúrico (TBARS), Glutationa S-transferase (GST), Catalase (CAT), Superóxido Dismutase (SOD) e razão SOD/CAT. (*) significa diferença entre as faixas etárias por Two-Way ANOVA ($p < 0,05$).

Estágio de Desenvolvimento	**Marcador Fisiológico**		**Dez©**		
		Controle	**4 µg/L**	**15 µg/L**	**30 µg/L**
Adulto-jovem	*PT**	6,42 ± 1,16	5,55 ± 0,71	7,84 ± 1,59	9,07 ± 0,85
Senil		11,11 ± 0,52	10,60 ± 1,13	10,39 ± 1,07	8,97 ± 1,02
	*TBARS**	42,43 ± 3,45	62,23 ± 10,50	27,10 ± 6,00	32,82 ± 2,47
		4,38 ± 0,52	3,27 ± 0,40	3,54 ± 0,36	6,48 ± 0,31
	*SOD**	21,64 ± 1,60	26,63 ± 3,84	19,97 ± 5,43	16,93 ± 2,72
		14,17 ± 1,18	4,01 ± 1,55	15,57 ± 1,93	17,01 ± 2,26
	*CAT**	33,32 ± 2,02	27,89 ± 3,62	23,68 ± 7,26	15,30 ± 3,75
		7,63 ± 0,86	15,45 ± 2,70	11,80 ± 2,33	16,46 ± 1,65
	*SOD/CAT**	0,65± 0,06	0,96 ± 0,13	0,94 ± 0,25	1,15 ± 0,14
		1,93± 0,28	0,95 ± 0,14	1,41 ± 0,32	1,05 ± 0,18
	*GST**	0,77 ± 0,03	0,66 ± 0,14	0,43 ± 0,09	0,29 ± 0,04
		0,37± 0,03	0,40 ± 0,01	0,29 ± 0,03	0,46 ± 0,06

Tabela 7 Resultados da análise do Balanço Oxidativo. Dados de média ± erro padrão de marcadores fisiológicos em machos adultos e senis de *Cynopoecilus* sp. expostos ao Dez© (4 , 15 e 30 µg/L). Proteína Total (TP), Substâncias Reativas ao Ácido Tiobarbitúrico (TBARS), Glutationa S-transferase (GST), Catalase (CAT), Superóxido Dismutase (SOD) e razão SOD/CAT. (*) significa diferença entre as faixas etárias por Two-Way ANOVA ($p < 0,05$).

Estágio de Desenvolvimento	Marcador Fisiológico	Controle	Dez©		
			4 µg/L	15 µg/L	30 µg/L
Adulto-jovem	*PT*	7,90 ± 0,99	10,92 ± 2,20	12,57 ± 0,70	7,69 ± 0,26
Senil		13,45 ± 2,18	8,72 ± 1,84	9,18 ± 1,07	7,97 ± 0,31
	TBARS	18,83 ± 6,26	15,63 ± 3,87	21,30 ± 5,03	16,57 ± 5,49
		9,87 ± 1,47	17,48 ± 4,94	27,80 ± 5,22	15,53 ± 2,76
	SOD	19,84 ± 3,65	15,58 ± 2,71	13,28 ± 1,00	21,34 ± 1,98
		11,77 ± 3,10	18,46 ± 2,70	16,26 ± 2,55	16,33 ± 1,90
	*CAT**	9,49 ± 0,71	7,28 ± 1,32	5,95 ± 0,35	10,84 ± 0,42
		9,92 ± 1,10	17,57 ± 4,13	17,78 ± 2,66	13,13 ± 1,16
	*SOD/CAT**	2,05 ± 0,24	2,15 ± 0,18	2,23 ± 0,12	1,96 ± 0,13
		1,14 ± 0,16	1,14 ± 0,16	0,93 ± 0,12	1,29 ± 0,24
	GST	0,56 ± 0,09	0,41 ± 0,06	0,38 ± 0,03	0,46 ± 0,05
		0,32 ± 0,07	0,53 ± 0,07	0,52 ± 0,06	0,42± 0,01

Tabela 8 Resultados da análise do Metabolismo Intermediário. Dados de média ± erro padrão de marcadores fisiológicos em fêmeas adultas e senis de *Cynopoecilus* sp. expostas a Dez© (4, 15 e 30 µg/L). Glicogênio (GG), Proteína Total (PT) e Ácido Úrico (UA). (*) significa diferença entre as faixas etárias por Two-Way ANOVA ($p < 0,05$).

Estágio de Desenvolvimento	Marcador Fisiológico	Controle	Dez©		
			4 µg/L	15 µg/L	30 µg/L
Adulto-jovem Senil	*GG**	1,14 ± 0,20	3,47 ± 0,55	2,44 ± 0,59	1,31 ± 0,15
	(mg/g)	1,20 ± 0,07	2,07 ± 0,19	0,96 ± 0,08	1,14 ± 0,15
	*PT**	0,27 ± 0,02	0,23 ± 0,03	0,24 ± 0,04	0,44 ± 0,03
	(mg/g)	0,13 ± 0,00	0,09 ± 0,00	0,15 ± 0.03	0,42 ± 0,02
	AU	0,03 ± 0,00	0,04 ± 0,01	0,12 ± 0,01	0,02 ± 0,00
	(mg/g)	0,07 ± 0.00	0,06 ± 0.00	0.05 ± 0.00	0.04 ± 0.00

Tabela 9 Resultados da análise do Metabolismo Intermediário. Dados de média ± erro padrão de marcadores fisiológicos em machos adultos e senis de *Cynopoecilus* sp. expostos a Dez© (4, 15 e 30 µg/L). Glicogênio (GG), Proteína Total (PT) e Ácido Úrico (UA). (*) significa diferença entre as faixas etárias por Two-Way ANOVA ($p < 0,05$).

Estágio de Desenvolvimento	Marcador Fisiológico	Controle	Dez©		
			4µg/L	15µg/L	30µg/L
Adulto-jovem	*GG*	0,59 ± 0,07	0,54 ± 0,09	0,80 ± 0,35	1,30 ± 0,34
Senil	*(mg/g)*	0,62 ± 0,09	0,49 ± 0,07	0,52 ± 0,11	0,66 ± 0,14
	*PT**	0,81 ± 0,19	1,11 ± 0,04	1,34 ± 0,30	1,13 ± 0,09
	(mg/g)	3,97 ± 0,18	4,74 ± 0,74	2,02 ± 0,22	3,07 ± 0,23
	*AU**	0,02 ± 0,00	0,02 ± 0,00	0,02 ± 0,00	0,02 ± 0,00
	(mg/g)	0,01 ± 0,00	0,03 ± 0,00	0,04 ± 0,00	0,03 ± 0,00

Fig. 1. Representação do delineamento experimental com as etapas do teste toxicológico com machos e fêmeas de *Cynopoecilus* sp. Em ambos os sexos, cada grupo (C, D1, D2 e D3) contém de três a quatro aquários.

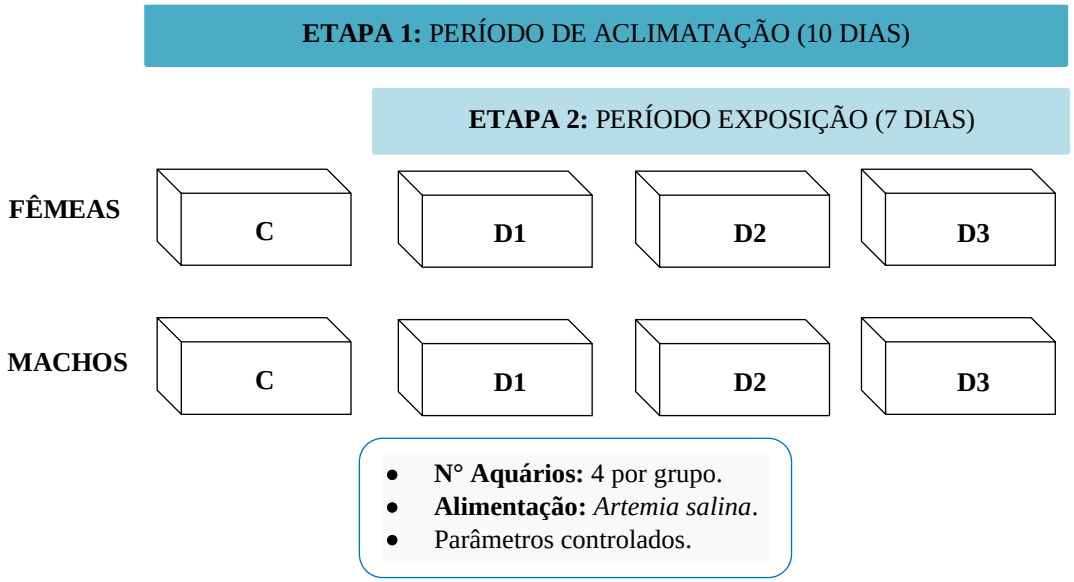


Fig. 2 Efeitos do Herbicida Dez© (4µg/L, 15µg/L e 30µg/L) sobre parâmetros de balanço oxidativo em fêmeas adultas jovens de *Cynopoecilus* sp. (A) Proteína Total do sobrenadante (TP), (B) Substâncias reativas ao ácido tiobarbitúrico (TBARS), (C) Superóxido dismutase (SOD), (D) Catalase (CAT), (E) relação SOD/CAT e (F) Glutationa S -transferase (GST). As barras representam médias ± erro padrão. (*) Significativamente diferente do grupo controle ($p < 0,05$).

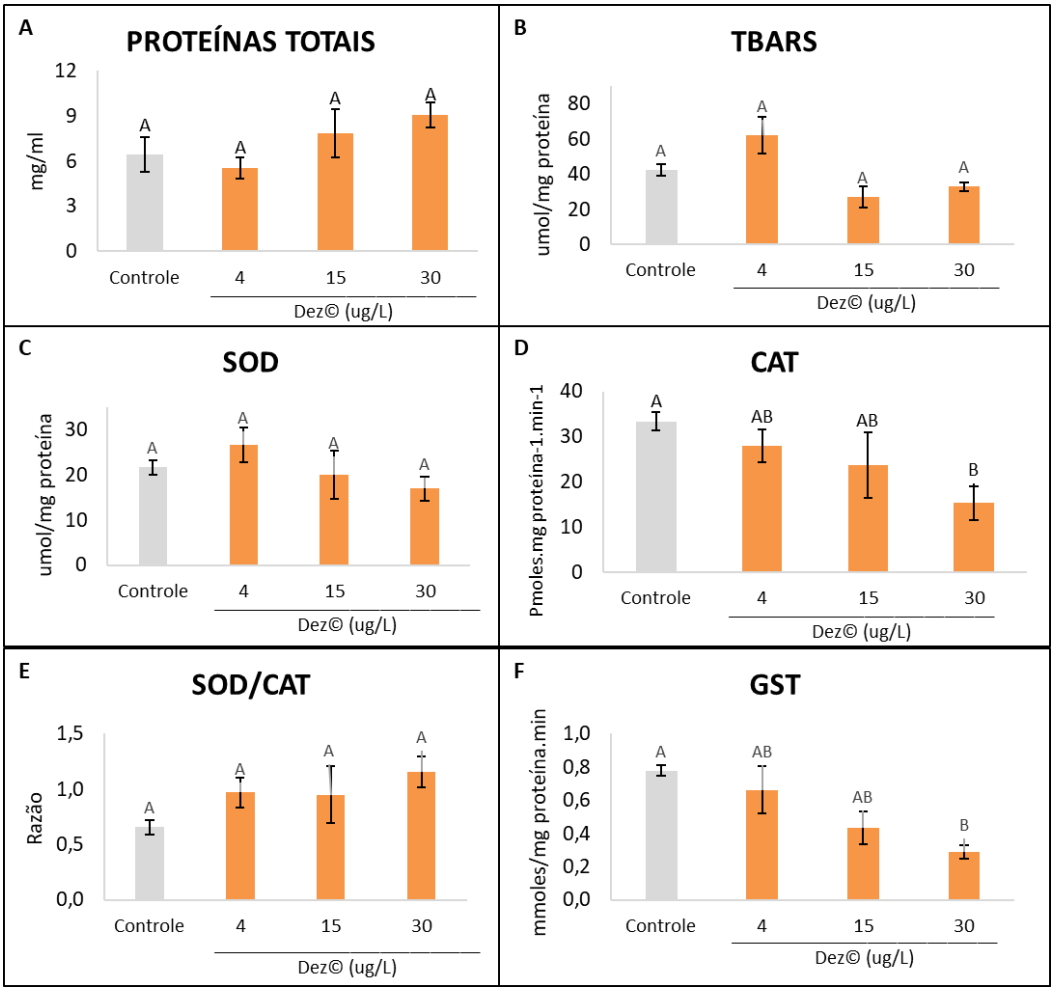


Fig. 3 Efeitos do Herbicida Dez© (4µg/L, 15µg/L e 30µg/L) sobre parâmetros de balanço oxidativo em fêmeas senis de *Cynopoecilus* sp. (A) Proteína Total do sobrenadante (TP), (B) Substâncias reativas ao ácido tiobarbitúrico (TBARS), (C) Superóxido dismutase (SOD), (D) Catalase (CAT), (E) relação SOD/CAT e (F) Glutationa S -transferase (GST). As barras representam médias ± erro padrão. (*) Significativamente diferente do grupo controle ($p < 0,05$).

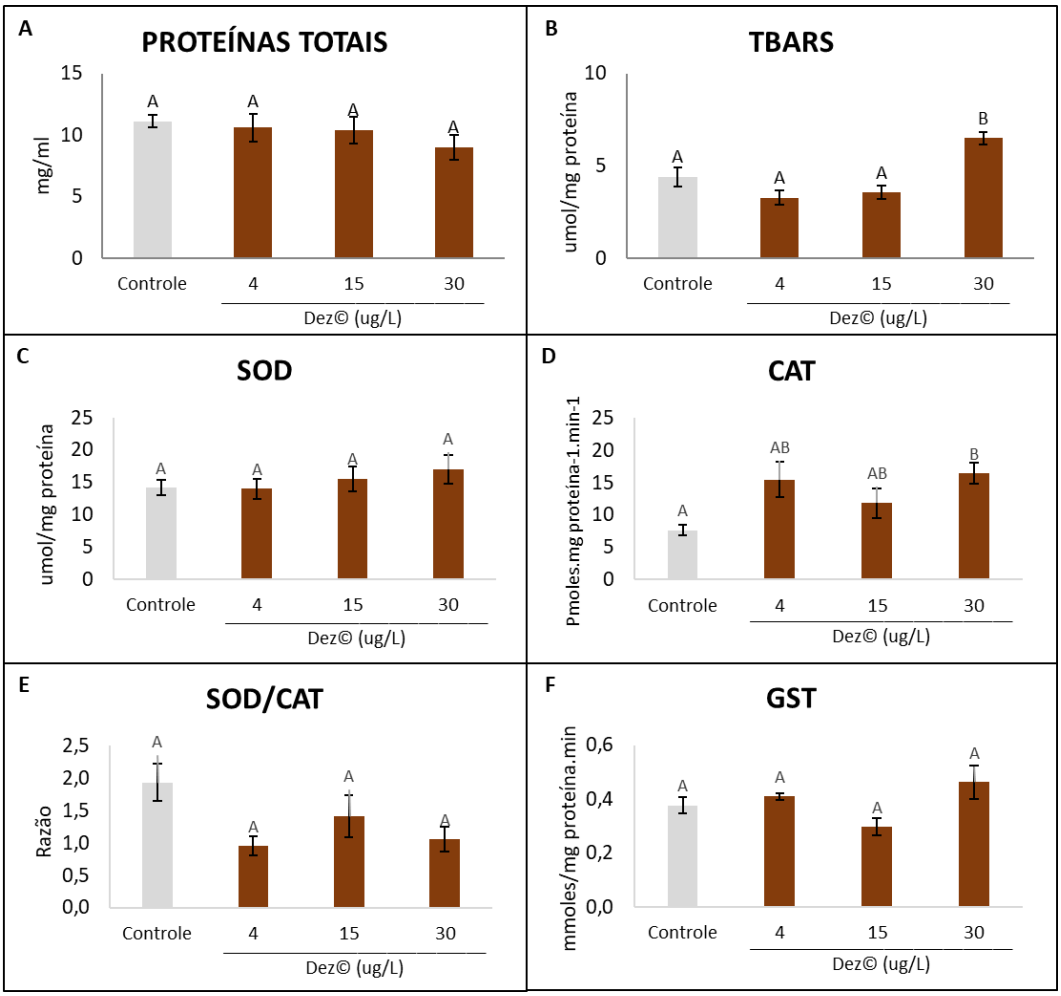


Fig. 4 Efeitos do Herbicida Dez© (4µg/L, 15µg/L e 30µg/L) sobre parâmetros de balanço oxidativo em machos adultos-jovens de *Cynopoecilus* sp. (A) Proteína Total do sobrenadante (TP), (B) Substâncias reativas ao ácido tiobarbitúrico (TBARS), (C) Superóxido dismutase (SOD), (D) Catalase (CAT), (E) relação SOD/CAT e (F) Glutationa S -transferase (GST). As barras representam médias ± erro padrão. (*) Significativamente diferente do grupo controle ($p < 0,05$).

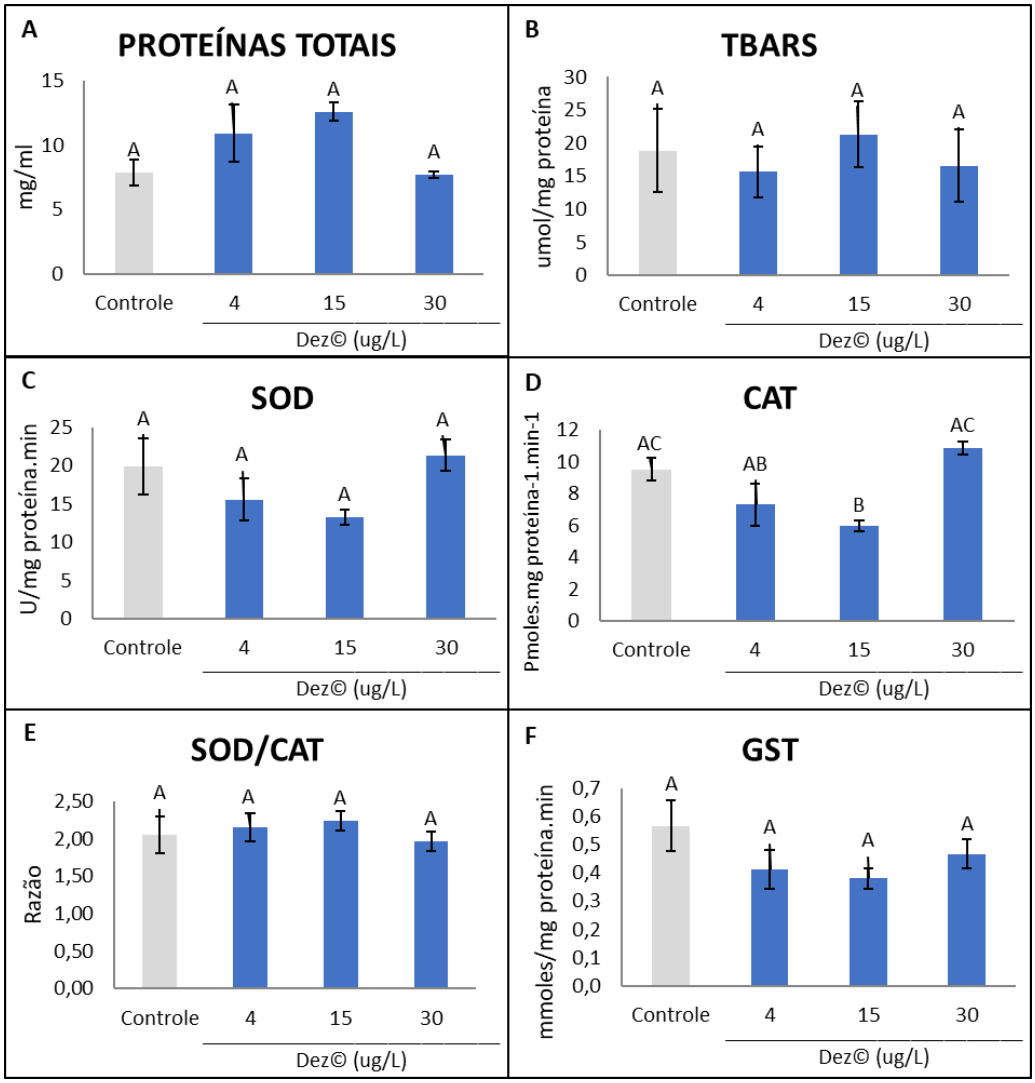


Fig. 5 Efeitos do Herbicida Dez© (4µg/L, 15µg/L e 30µg/L) sobre parâmetros de balanço oxidativo em machos senis de *Cynopoecilus* sp. (A) Proteína Total do sobrenadante (TP), (B) Substâncias reativas ao ácido tiobarbitúrico (TBARS), (C) Superóxido dismutase (SOD), (D) Catalase (CAT), (E) relação SOD/CAT e (F) Glutationa S -transferase (GST). As barras representam médias ± erro padrão. (*) Significativamente diferente do grupo controle ($p < 0,05$).

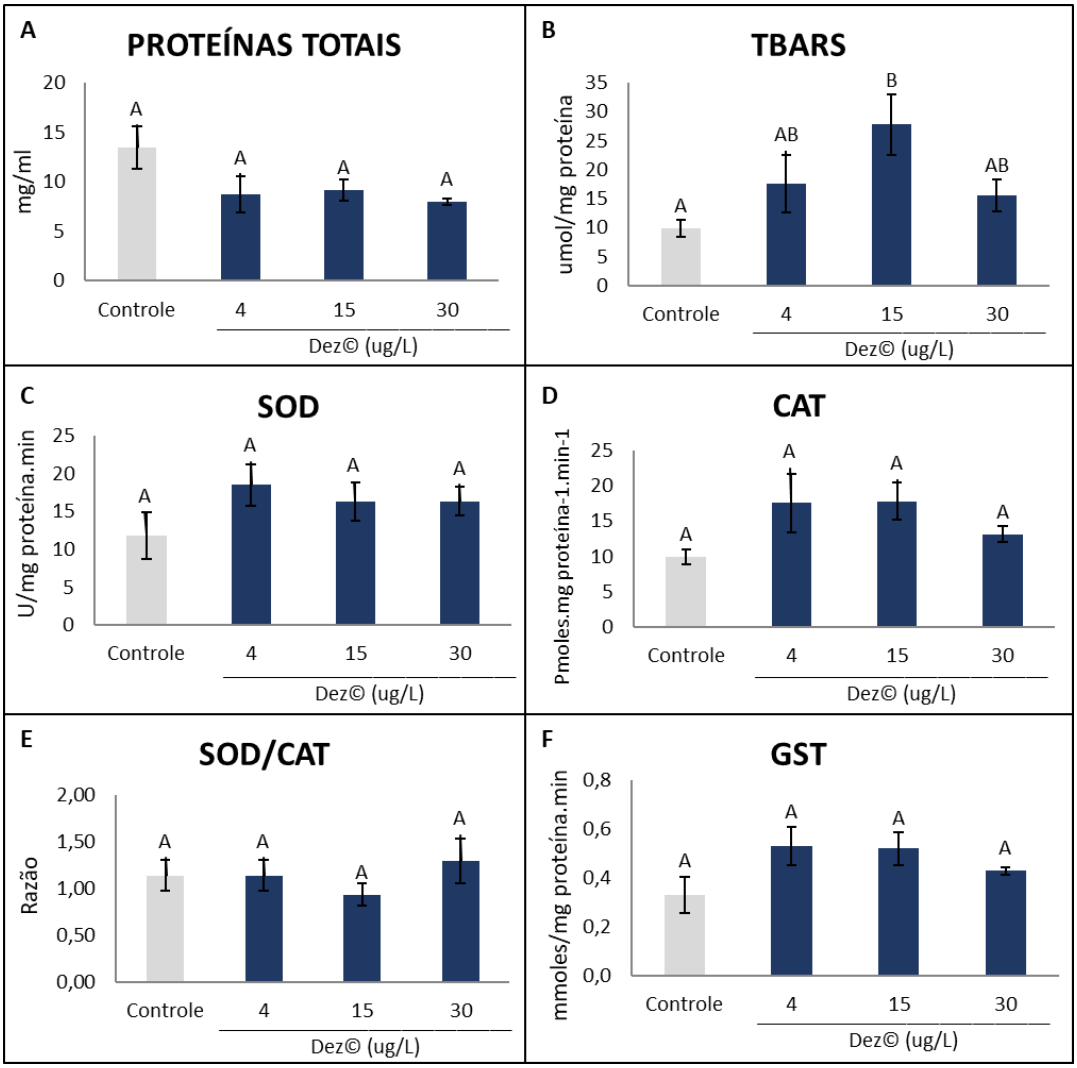


Fig. 6 Resultados da análise do Metabolismo Intermediário para as fêmeas adultas jovens e senis de *Cynopoecilus* sp. expostas ao Herbicida Dez©. (A) Glicogênio adulto-jovem (GG), (B) Proteína total adulto-jovem (TP), (C) Ácido úrico adulto-jovem (UA), (D) Glicogênio senil, (E) Proteína total senil (TP) e (F) Ácido Úrico Senil (AU). As barras representam médias ± erro padrão. (*) Significativamente diferente do grupo controle ($p < 0,05$).

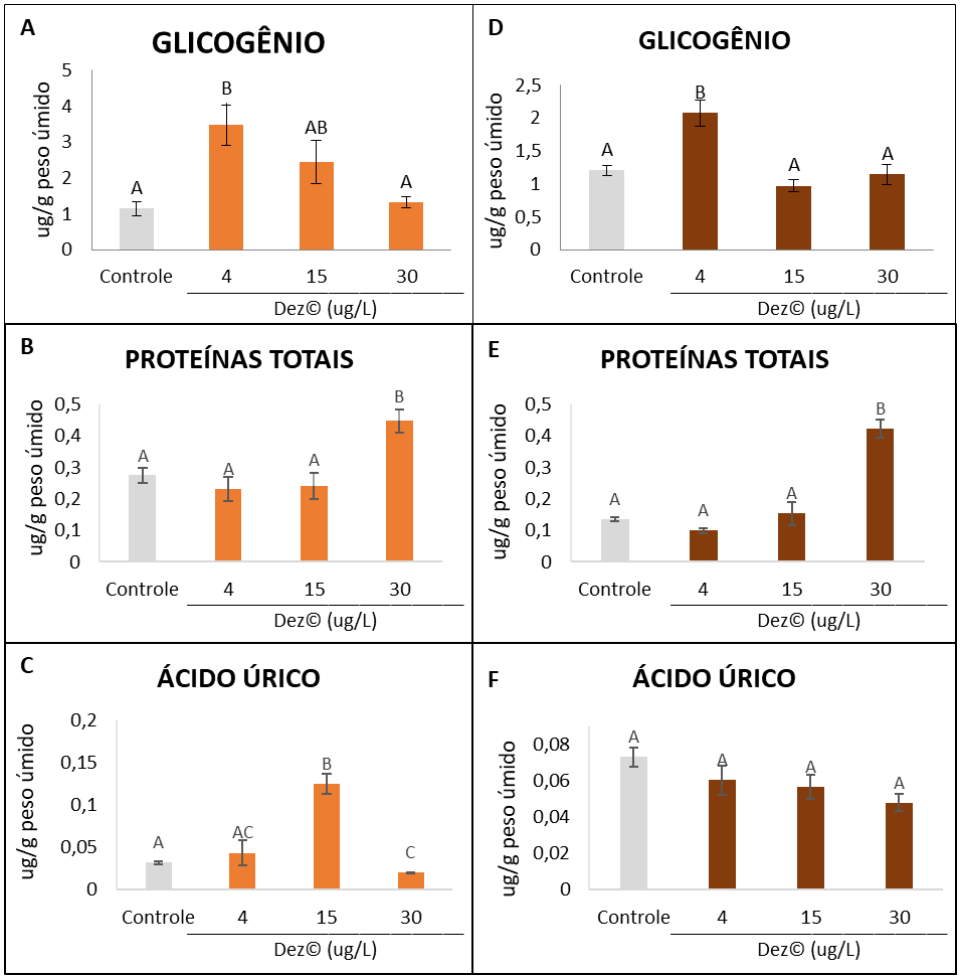
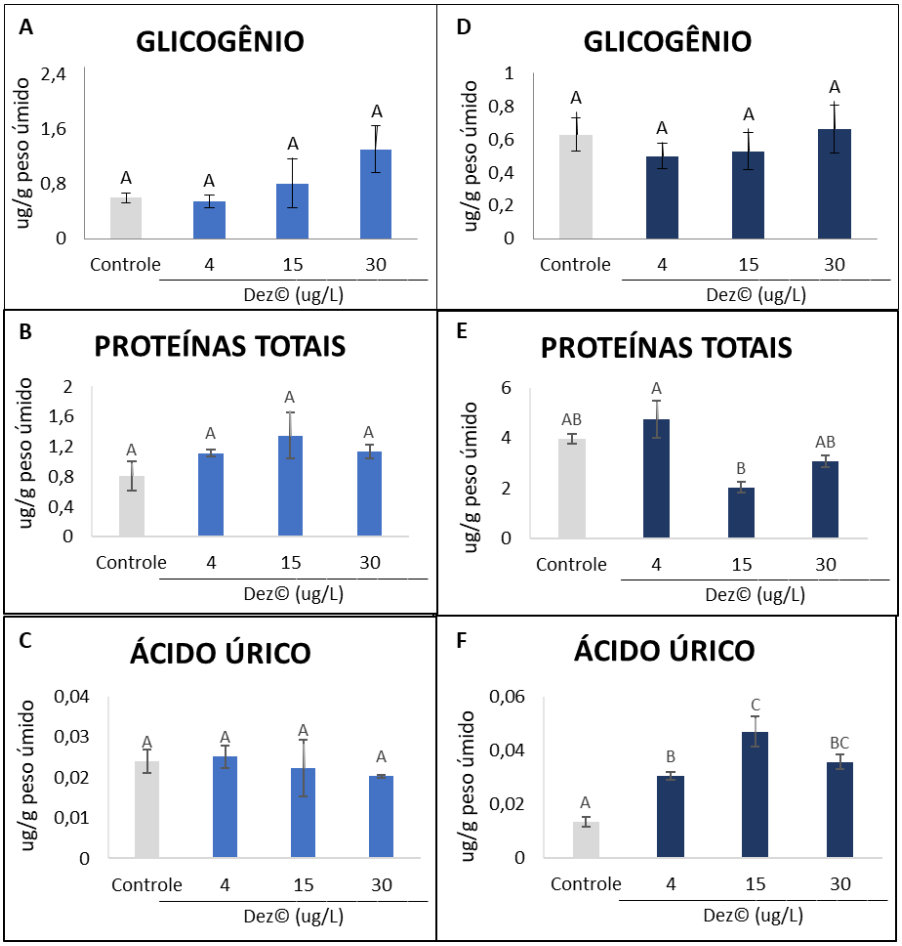


Fig. 7 Resultados da análise do Metabolismo Intermediário para os machos adultos-jovens e senis de *Cynopoecilus* sp. expostos ao Herbicida Dez©. (A) Glicogênio adulto-jovem (GG), (B) Proteína total adulto-jovem (TP), (C) Ácido úrico adulto-jovem (UA), (D) Glicogênio senil, (E) Proteína total senil (TP) e (F) Ácido Úrico Senil (AU). As barras representam médias ± erro padrão. (*) Significativamente diferente do grupo controle ($p < 0,05$).



Anexo (Capítulo 3)

HIDROLAB
Análises Ambientais

RELATORIO DE ENSAIO Cod.: 7790.2022_AT Rev_1

Este Relatório anula e substitui o relatório 7790.2022_AT

Canoas, 17 de outubro de 2022

DADOS DO CLIENTE

Cliente: Guendalina Turcato Oliveira
Endereço: Av. Ipiranga pd. 12C- sala 250, 6681
CPF: 60739517015
Cidade: Porto Alegre , Rio Grande do Sul
CEP: 90.619-900

DADOS DA AMOSTRA E DO LOCAL DE AMOSTRAGEM

Amostra: 7790.2022_AT_1_1
Matriz: Água Tratada
Endereço Coleta: Av. Ipiranga pd. 12C- sala 250, 6681- Partenon- Porto Alegre
Ponto de Amostragem: Solução Mãe
Data Recebimento: 23/09/2022 16:00:00
Condições Climáticas: Ensolarado
Tipo de Amostra: Água Tratada
Coletor: Cliente
Data Coleta: 23/09/2022

PARÂMETRO	RESULTADO	UNIDADE
2,4 D *	883489	µg/L

DADOS COMPLEMENTARES DO ENSAIO

PARÂMETRO	LQ	LD	U95%	MÉTODO	DATA DE REALIZAÇÃO
2,4 D	0,05	0,02	±0,01	EPA 3510 C:1996/EPA 8270 D:2014	17/10/2022

Nota 01: SMWW - Standard Methods for the Examination of Water and Wastewater, Ed. 23º, 2017.
Nota 02: LQ - Limite de Quantificação
Nota 03: LD - Limite de Detecção
Nota 04: U95% - Incerteza do ensaio com 95% de probabilidade.
Nota 05: O(s) resultados(s) desta(s) análise(s) tem significado restrito e se aplica somente a amostra analisada.
Nota 06: O Relatório de Ensaio somente pode ser reproduzido por completo e sem nenhuma alteração.
Nota 07: PG 17(Amostragem), IT 029(Manual de Amostragem) e PE 090(Ensaios Realizados na Amostragem), conforme revisão atual. A identificação do plano de amostragem tem o mesmo código do "Protocolo da Amostra".
Nota 08: (*) Serviço Provido Externamente.
Nota 09: (**) Análises realizadas nas instalações do cliente.
Nota 10: 1 CU(Unidade de Cor) = 1Hz(Unidade de Hazen) = 1Pt-Co(Unidade de Platino-Cobalto).
Nota 11: A regra de decisão padrão, para declaração de conformidade dos resultados, não considera a incerteza analítica associada ao ensaio.
Nota 12: Os Resultados da tabela Taxonômica são emitidos conforme padrão do ITIS.
Nota 13: Os dados dos parâmetros: Alumínio na CTC efetiva, Alumínio trocável, Cálcio na CTC efetiva, Cálcio trocável, CTC efetiva, CTC pH 7,0, Hidrogênio extraível, Magnésio na CTC efetiva, Magnésio trocável, Matéria orgânica, % de saturação por alumínio, % de saturação de bases, % de sódio trocável, Potássio na CTC efetiva, Potássio trocável, Relação cálcio/magnésio, Relação cálcio/potássio, Relação magnésio/potássio, Soma de bases, Bases trocáveis, Sódio na CTC efetiva e Sódio trocável, são parâmetros calculados de acordo com o método EMBRAPA, não apresentando assim Limite de Quantificação (LQ) e Incerteza (U95%).

Ordem Serviço nº: 7790/2022 FI 090 Rev.11 Página 1 de 2

Rua Ary Dias Ferreira, 260 • Pq. Ind. Jorge Lanner, Bairro Niterói • 92130-500 Canoas - RS
(51) 3475-5656 hidrobrasil@hidrobrasil.com www.hidrobrasil.com

HIDROLAB
Análises Ambientais

RELATORIO DE ENSAIO Cod.: 7790.2022_AT Rev_1

Verifique a autenticidade deste documento no endereço abaixo ou no QR-Code ao lado.:

http://hidrolab.glabnet1.com.br/valida.php

Código: 7790.2022 - Chave de autenticação: 7RP-0K6C-1Z5

Dra. Fernanda do Amarante
Signatária Autorizada

Quim. Michele Beza
Signatária Autorizada
Responsável Técnica

Rua Ary Dias Ferreira, 260 • Pq. Ind. Jorge Lanner, Bairro Niterói • 92130-500 Canoas - RS
(51) 3475-5656 hidrobrasil@hidrobrasil.com www.hidrobrasil.com

CONSIDERAÇÕES FINAIS DA TESE

Frente a constante entrada e a exposição de agrotóxicos em ecossistemas aquáticos diversos organismos como os peixes anuais podem ser considerados vulneráveis por ocorrerem em áreas que podem estar inseridas ou próximas a matrizes agrícolas que fazem uso de agrotóxicos. Além disto, os peixes anuais são organismo de distribuição geográfica restrita, ocupam habitats efêmeros e por isto apresentam ciclo de vida pós-embrionário muito curto, rápido crescimento e reprodução até o final do ciclo de vida com a produção de ovos de resistência. Neste estudo desenvolvido com *Cynopoecilus* sp. em diferentes faixas etárias e sexos mostramos pela primeira vez o impacto da exposição a dois tipos de formulações comerciais de herbicidas tendo como princípio ativo o glifosato (Roundup Original®) e o 2,4D (Dez©), sendo estes os dois herbicidas mais comercializados no Brasil. Foi evidenciado que a exposição ao agrotóxico modula o sistema antioxidante e o metabolismo destes animais a fim de evitar a mortalidade ao longo da exposição (7 dias). As respostas foram relacionadas a faixa de desenvolvimento e ao sexo dos animais. Nossa pesquisa mostrou que os marcadores fisiológicos estabelecidos, bem como a espécie, parecem serem promissores para serem utilizados em estudos futuros de senescência e toxicologia. O AU parece atuar como uma molécula antioxidante nesta espécie apesar de não ser a excreta nitrogenada principal em peixes.

Nossos resultados em relação ao herbicida glifosato (Roundup Original®) mostram que em machos de *Cynopoecilus* sp. a capacidade do sistema antioxidante de foi eficiente nas duas fases do desenvolvimento biológico analisadas neste estudo, embora os idosos apresentem níveis 2 vezes maiores de peroxidação lipídica associados à manutenção dos níveis de atividade da SOD e da CAT mesmo após a exposição ao herbicida. No entanto, indivíduos senis foram afetados negativamente pela exposição a formulações comerciais de Roundup Original© e isto foi relacionado a redução da atividade da GST. Para as fêmeas as respostas mais intensas também foram observadas nos animais senis, onde temos um aumento de CAT e da GST, uma diminuição da relação SOD/CAT, combinado com um aumento de PT e AU quando comparados ao grupo controle. A análise two-way mostrou uma diferença significativa entre as faixas etárias e os grupos para vários dos biomarcadores reforçando a maior sensibilidade das fêmeas senis ao glifosato.

Já a exposição ao herbicida 2,4D (Dez©) provocou mudanças intensas nos parâmetros oxidativos e metabólicos em *Cynopoecilus* sp., em ambos os sexos. Observamos um impacto deste herbicida nas fêmeas adultas-jovens provocando uma diminuição da CAT e da GST, e um aumento do GG, PT e AU. Já nos adultos-jovens machos, somente a CAT apresentou uma variação significativa. Novamente, evidenciamos que o estágio de desenvolvimento de maior vulnerabilidade, tanto para machos como para fêmeas, foi a fase senil, onde observamos um incremento da atividade da CAT nas maiores concentrações do herbicida, aliado a um aumento do dano oxidativo em lipídios (TBARS). Assim, podemos afirmar que o 2,4D provocou estresse oxidativo nestes animais mesmo em concentrações ambientalmente relevantes. Além disso, verificamos nas fêmeas senis um incremento dos níveis de GG e PT nos animais expostos a maior concentração do herbicida. Nos machos senis temos uma diminuição das PT seguida de um incremento do AU.

Cabe ressaltar que para ambos os herbicidas utilizados o estágio de desenvolvimento de maior sensibilidade foi a fase senil, a qual apresentou níveis de TBARS mais elevados, uma perda da capacidade funcional de enzimas e até mesmo um esgotamento de reservas energéticas. Entretanto, a exposição ao herbicida com o princípio ativo 2,4D mostrou ser mais prejudicial a espécie do que o herbicida glifosato. Sendo assim, a determinação da exposição em diferentes estágios de desenvolvimento contribuiu para ressaltar as mudanças e efeitos em cada faixa etária, demonstrando o estágio mais suscetível. Tais resultados obtidos que destacam a maior vulnerabilidade no estágio senil podem estar relacionados com a possível deterioração da integridade fisiológica na fase de senescência, possivelmente evidenciando disfunções mitocondriais que normalmente ocorrem no processo de envelhecimento.

Por fim, nossos estudos mostram que a utilização dos peixes anuais neotropicais é muito promissora, representando um excelente modelo de vertebrado em estudos toxicológicos. Como também, os parâmetros analisados mostraram ser importantes na compreensão dos efeitos provocados com a exposição. Dessa forma, enfatiza-se a necessidade de desenvolvimento de métodos e estratégias para conservação do ambiente aquático e dos organismos ali presentes. Esperamos que os resultados das pesquisas possam contribuir para futuros estudos envolvendo efeito de misturas, da influência de fatores ambientais com a exposição ou até mesmo um período prolongado de exposição dos animais com o intuito na conservação dos ecossistemas aquáticos.

NORMAS DAS REVISTAS

ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 1382-6689

DESCRIPTION

Environmental Toxicology and Pharmacology publishes the results of studies concerning **toxic** and **pharmacological effects** of (human and veterinary) **drugs** and of **environmental contaminants** in animals and man.

Areas of special interest are: molecular **mechanisms** of toxicity, biotransformation and **toxicokinetics** (including toxicokinetic modelling), molecular, biochemical and physiological mechanisms explaining **differences in sensitivity** between species and individuals, the characterisation of **pathophysiological models** and mechanisms involved in the development of effects and the identification of **biological markers** that can be used to study exposure and effects in man and animals.

In addition to full length papers, short communications, full-length reviews and mini-reviews, *Environmental Toxicology and Pharmacology* will publish in depth assessments of special problem areas. The latter publications may exceed the length of a full length paper three to fourfold. A basic requirement is that the assessments are made under the auspices of international groups of leading experts in the fields concerned. The information examined may either consist of data that were already published, or of new data that were obtained within the framework of collaborative research programmes. Provision is also made for the acceptance of minireviews on (classes of) compounds, toxicities or mechanisms, debating recent advances in rapidly developing fields that fall within the scope of the journal.

Benefits to authors
We also provide many author benefits, such as free PDFs, a liberal copyright policy, special discounts on Elsevier publications and much more. Please click here for more information on our author services .

Please see our Guide for Authors for information on article submission. If you require any further information or help, please visit our Support Center

GUIDE FOR AUTHORS

INTRODUCTION

Environmental Toxicology and Pharmacology publishes the results of studies concerning toxic and pharmacological effects of (human and veterinary) drugs and of environmental contaminants in animals and man.

Areas of special interest are: molecular mechanisms of toxicity, biotransformation and toxicokinetics (including toxicokinetic modelling), molecular, biochemical and physiological mechanisms explaining differences in sensitivity between species and individuals, the characterisation of pathophysiological models and mechanisms involved in the development of effects and the identification of biological markers that can be used to study exposure and effects in man and animals.

Types of paper

In addition to full length papers, short communications, full-length reviews and mini-reviews, *Environmental Toxicology and Pharmacology* will publish in depth assessments of special problem areas. The latter publications may exceed the length of a full length paper three to fourfold. A basic requirement is that the assessments are made under the auspices of international groups of leading experts in the fields concerned. The information examined may either consist of data that were already published, or of new data that were obtained within the framework of collaborative research programmes. Provision is also made for the acceptance of minireviews on (classes of) compounds, toxicities or mechanisms, debating recent advances in rapidly developing fields that fall within the scope of the journal.

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
- E-mail address
- Full postal address

All necessary files have been uploaded:
Manuscript:
- Include keywords
- All figures (include relevant captions)
- All tables (including titles, description, footnotes)
- Ensure all figure and table citations in the text match the files provided
- Indicate clearly if color should be used for any figures in print

Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

Further considerations
- Manuscript has been 'spell checked' and 'grammar checked'
- All references mentioned in the Reference List are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Internet)
- A competing interests statement is provided, even if the authors have no competing interests to declare
- Journal policies detailed in this guide have been reviewed
- Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

BEFORE YOU BEGIN

Ethics in publishing

Please see our information on Ethics in publishing.

Studies in humans and animals

If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The manuscript should be in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals and aim for the inclusion of representative human populations (sex, age and ethnicity) as per those recommendations. The terms sex and gender should be used correctly.

Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

All animal experiments should comply with the ARRIVE guidelines and should be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Research Council's Guide for the Care and Use of Laboratory Animals and the authors should clearly indicate in the manuscript that such guidelines have been followed. The sex of animals must be indicated, and where appropriate, the influence (or association) of sex on the results of the study.

Declaration of competing interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/ registrations, and grants or other funding. Authors should complete the declaration of competing interest statement using this template and upload to the submission system at the Attach/Upload Files step. **Note: Please do not convert the .docx template to another file type. Author signatures are not required.** If there are no interests to declare, please choose the first option in the template. More information.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify compliance, your article may be checked by Crossref Similarity Check and other originality or duplicate checking software.

Preprints

Please note that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints e.g. on a preprint server will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Use of inclusive language

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Content should make no assumptions about the beliefs or commitments of any reader; contain nothing which might imply that one individual is superior to another on the grounds of age, gender, race, ethnicity, culture, sexual orientation, disability or health condition; and use inclusive language throughout. Authors should ensure that writing is free from bias, stereotypes, slang, reference to dominant culture and/or cultural assumptions. We advise to seek gender neutrality by using plural nouns ("clinicians, patients/clients") as default/wherever possible to avoid using "he, she," or "he/she." We recommend avoiding the use of descriptors that refer to personal attributes such as age, gender, race, ethnicity, culture, sexual orientation, disability or health condition unless they are relevant and valid. When coding terminology is used, we recommend to avoid offensive or exclusionary terms such as "master", "slave", "blacklist" and "whitelist". We suggest using alternatives that are more appropriate and (self-) explanatory such as "primary", "secondary", "blocklist" and "allowlist". These guidelines are meant as a point of reference to help identify appropriate language but are by no means exhaustive or definitive.

Reporting sex- and gender-based analyses

Reporting guidance

For research involving or pertaining to humans, animals or eukaryotic cells, investigators should integrate sex and gender-based analyses (SGBA) into their research design according to funder/ sponsor requirements and best practices within a field. Authors should address the sex and/or gender dimensions of their research in their article. In cases where they cannot, they should discuss this as a limitation to their research's generalizability. Importantly, authors should explicitly state what definitions of sex and/or gender they are applying to enhance the precision, rigor and reproducibility of their research and to avoid ambiguity or conflation of terms and the constructs to which they refer (see Definitions section below). Authors can refer to the Sex and Gender Equity in Research (SAGER) guidelines and the SAGER guidelines checklist. These offer systematic approaches to the use and editorial review of sex and gender information in study design, data analysis, outcome reporting and research interpretation - however, please note there is no single, universally agreed-upon set of guidelines for defining sex and gender.

Definitions

Sex generally refers to a set of biological attributes that are associated with physical and physiological features (e.g., chromosomal genotype, hormonal levels, internal and external anatomy). A binary sex categorization (male/female) is usually designated at birth ("sex assigned at birth"), most often based solely on the visible external anatomy of a newborn. Gender generally refers to socially constructed roles, behaviors, and identities of women, men and gender-diverse people that occur in a historical and cultural context and may vary across societies and over time. Gender influences how people view themselves and each other, how they behave and interact and how power is distributed in society. Sex and gender are often incorrectly portrayed as binary (female/male or woman/man) and unchanging whereas these constructs actually exist along a spectrum and include additional sex categorizations and gender identities such as people who are intersex/have differences of sex development (DSD) or identify as non-binary. Moreover, the terms "sex" and "gender" can be ambiguous—thus it is important for authors to define the manner in which they are used. In addition to this definition guidance and the SAGER guidelines, the resources on this page offer further insight around sex and gender in research studies.

Author contributions

For transparency, we encourage authors to submit an author statement file outlining their individual contributions to the paper using the relevant CRediT roles: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing. Authorship statements should be formatted with the names of authors first and CRediT role(s) following. More details and an example.

Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Article transfer service

This journal uses the Elsevier Article Transfer Service to find the best home for your manuscript. This means that if an editor feels your manuscript is more suitable for an alternative journal, you might be asked to consider transferring the manuscript to such a journal. The recommendation might be provided by a Journal Editor, a dedicated Scientific Managing Editor, a tool assisted recommendation, or a combination. If you agree, your manuscript will be transferred, though you will have the opportunity to make changes to the manuscript before the submission is complete. Please note that your manuscript will be independently reviewed by the new journal. More information.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

For gold open access articles: Upon acceptance of an article, authors will be asked to complete a 'License Agreement' (more information). Permitted third party reuse of gold open access articles is determined by the author's choice of user license.

Author rights

As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing

Find out how you can share your research published in Elsevier journals.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement, it is recommended to state this.

Open access

Please visit our Open Access page for more information.

Elsevier Researcher Academy

Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's Author Services.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

Suggesting reviewers

Please submit the names and institutional e-mail addresses of several potential reviewers.

You should not suggest reviewers who are colleagues, or who have co-authored or collaborated with you during the last three years. Editors do not invite reviewers who have potential competing interests with the authors. Further, in order to provide a broad and balanced assessment of the work, and ensure scientific rigor, please suggest diverse candidate reviewers who are located in different countries/ regions from the author group. Also consider other diversity attributes e.g. gender, race and ethnicity, career stage, etc. Finally, you should not include existing members of the journal's editorial team, of whom the journal are already aware.

Note: the editor decides whether or not to invite your suggested reviewers.

Experimental procedures

All animal experiments should be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, the European Communities Council Directive of 24 November 1986 (86/609/EEC) or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and the authors should clearly indicate in the manuscript that such guidelines have been followed. ***All animal studies need to ensure they comply with the ARRIVE guidelines. More information can be found at http://www.nc3rs.org.uk/page.asp?id=1357.***

PREPARATION

Drugs: generic names should be used. Trade names may be mentioned in parentheses the first time the name of the drug appears in the text. The form of the drug used should be indicated.

Queries

For questions about the editorial process (including the status of manuscripts under review) or for technical support on submissions, please visit our Support Center.

Peer Review

This journal operates a single anonymized review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. Editors are not involved in decisions about papers which they have written themselves or have been written by family members or colleagues or which relate to products or services in which the editor has an interest. Any such submission is subject to all of the journal's usual procedures, with peer review handled independently of the relevant editor and their research groups. The Editor must not be involved in decisions about papers which s/he has written him/herself or have been written by family members or colleagues or which relate to products or services in which the editor has an interest. Further, any such submission must be subject to all of the journal's usual procedures, peer review must be handled independently of the relevant author/Editor and their research groups, and there must be a clear statement to this effect on any such paper that is published.More information on types of peer review.

Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.

To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods
Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Theory/calculation
A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results
Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Glossary
Please supply, as a separate list, the definitions of field-specific terms used in your article.

Appendices
If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**
• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Highlights

Highlights are mandatory for this journal as they help increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the examples here: example Highlights.

Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if

essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself. **Abstract must not exceed 150 words.**

Graphical abstract
Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. You can view Example Graphical Abstracts on our information site.

Keywords

Immediately after the abstract, type 3-6 keywords or short phrases suitable for indexing. If possible. keywords should be selected from Index Medicus or Excerpta Medica Index. First category key-words (e.g. Hyperphagia; G-Strophantidin; Fiber shortening) will all be listed and cross-indexed. Second category keywords (e.g. Rat, Cold, Metabolite) will only be listed under the index entry for the first category keywords. Use American spelling and avoid general and plural terms and multiple concepts (avoid, for example, "and", "of"). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These terms will be printed at the end of the abstract. If the editors feel that keywords should be adjusted to the standards of the journal this will be done without consultation of the author(s).

Abbreviations
Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements
Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources
List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, it is recommended to include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Nomenclature and units
Follow internationally accepted rules and conventions: use the international system of units (SI). If other quantities are mentioned, give their equivalent in SI. You are urged to consult IUPAC: Nomenclature of Organic Chemistry for further information.

Footnotes
Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors can build footnotes into the text, and this feature may be used. Otherwise, please indicate the position of footnotes in the text and list the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Artwork

Electronic artwork
General points
• Make sure you use uniform lettering and sizing of your original artwork.
• Embed the used fonts if the application provides that option.
• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Provide captions to illustrations separately.
• Size the illustrations close to the desired dimensions of the published version.
• Submit each illustration as a separate file.
• Ensure that color images are accessible to all, including those with impaired color vision.

A detailed guide on electronic artwork is available.
You are urged to visit this site; some excerpts from the detailed information are given here.
Formats
If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.
Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings, embed all used fonts.
TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.
Please do not:
• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
• Supply files that are too low in resolution;
• Submit graphics that are disproportionately large for the content.

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF) or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) in addition to color reproduction in print. Further information on the preparation of electronic artwork.

Figure captions
Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables
Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

References
Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links
Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, Crossref and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is highly encouraged.

A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884. Please note the format of such citations should be in the same style as all other references in the paper.

Web references
As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Data references
This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

Preprint references
Where a preprint has subsequently become available as a peer-reviewed publication, the formal publication should be used as the reference. If there are preprints that are central to your work or that cover crucial developments in the topic, but are not yet formally published, these may be referenced. Preprints should be clearly marked as such, for example by including the word preprint, or the name of the preprint server, as part of the reference. The preprint DOI should also be provided.

References in a special issue
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software
Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley. Using citation plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript. More information on how to remove field codes from different reference management software.

Reference formatting
There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume and issue/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style
Text: All citations in the text should refer to:
1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;
2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication.

Citations may be made directly (or parenthetically). Groups of references can be listed either first alphabetically, then chronologically, or vice versa.
Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999)…. Or, as demonstrated (Jones, 1999; Allan, 2000)… Kramer et al. (2010) have recently shown …'
List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.
Examples:
Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59. https://doi.org/10.1016/j.Sc.2010.00372.
Reference to a journal publication with an article number:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:
Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.
Reference to a chapter in an edited book:
Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.
Reference to a website:
Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003).
Reference to a dataset:
[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.
Reference to software:
Coon, E., Berndt, M., Jan, A., Svyatsky, D., Atchley, A., Kikinzon, E., Harp, D., Manzini, G., Shelef, E., Lipnikov, K., Garimella, R., Xu, C., Moulton, D., Karra, S., Painter, S., Jafarov, E., & Molins, S., 2020. Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88). Zenodo. https://doi.org/10.5281/zenodo.3727209.

Journal abbreviations source
Journal names should be abbreviated according to the List of Title Word Abbreviations.

Video
Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Data visualization
Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

Supplementary material
Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to

supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

Research data

This journal requires and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. When sharing data in one of these ways, you are expected to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking

If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Research Elements

This journal enables you to publish research objects related to your original research – such as data, methods, protocols, software and hardware – as an additional paper in Research Elements.

Research Elements is a suite of peer-reviewed, open access journals which make your research objects findable, accessible and reusable. Articles place research objects into context by providing detailed descriptions of objects and their application, and linking to the associated original research articles. Research Elements articles can be prepared by you, or by one of your collaborators.

During submission, you will be alerted to the opportunity to prepare and submit a Research Elements article.

More information can be found on the Research Elements page.

Data statement

To foster transparency, we encourage you to state the availability of your data in your submission. This may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you will have the opportunity to indicate why during the submission process, for example by stating that the research data is confidential. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page.

Additional information

Short communications should be prepared as described under 2, except for the following:
(a) The average length of a short communication should not exceed 4 pages in print (approx. 2000-2300 words, including abstract, captions and references). A maximum of 2 illustrations (figures or tables) is allowed.
(b) An abstract of not more that 100 words should be provided and 3-6 keywords should be listed immediately below the abstract.

Reviews
The organization and subdivision of review articles can be arranged at the author's discretion. Tables, figures and references are to be arranged in the same way as research articles in the journal. Please contact the Editor before submitting an article for this section.

Mini-reviews
(a) The total length of a mini-review should be about 30 double-spaced typewritten pages, including references, tables and figures.
(b) A brief introduction to the subjects, comprising not more than 500 words, in which reference is made to recent key articles or reviews on the topic should be given.
(c) Conclusions should be summarized in 500-1000 words at the end of this article. They should be preferentially outlined in a flow diagram or a graph.
(d) A maximum of 40 references may be used.
We have now renamed the Article Type "Mini-Review" as "Review Article". If you want to submit a paper for "Mini-Review", please select "Mini-Review" in the section category field while submitting the paper using the article type "Review Article".

Assessments (Opinion Paper)
The format of an assessment will be decided upon in consultation with the editors.
If you want to submit an article for "Assessments", kindly select the article type as "Opinion Paper".

Correspondence
The total length of a letter should not exceed one page. Please name three potential reviewers making sure to include their name, affiliation and email address.

AFTER ACCEPTANCE

Online proof correction
To ensure a fast publication process of the article, we kindly ask authors to provide us with their proof corrections within two days. Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.
If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.
We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints
The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's Author Services. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

AUTHOR INQUIRIES
Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.
You can also check the status of your submitted article or find out when your accepted article will be published.

CHEMOSPHERE

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 0045-6535

DESCRIPTION

Chemosphere is an international journal designed for the publication of original communications as well as review articles on chemicals in the environment. Chemosphere, as a multidisciplinary journal, offers maximum dissemination of investigations related to all aspects of the identification, quantification, behavior, fate, toxicology, treatment, and remediation of chemicals in the bio-, hydro-, litho- and atmosphere.

Chemosphere will publish:
- Original communications (Research Papers) describing important new discoveries or further developments in important fields of investigation
- Review Articles, mainly of new developing areas
- Short communications
- Letters to the Editor
- Special, themed issues on relevant topics

All papers should demonstrate a high level of novelty, originality and uniqueness. The following sections and subject fields are included:

Environmental Chemistry

This section will publish manuscripts dealing with fundamental processes in the environment that are related to the analysis, behavior, fate, and alteration of organic and inorganic contaminants focused on the dynamics of contaminants in environmental compartments such as water, soil, sediment, particulate matter, organisms, dust and indoor/outdoor air. Only studies that are of significance to an international audience, include sites of particular global interest, or lend themselves to interpretation at the global level should be submitted.

Topics of specific interest include, but not limited to, are:
- All aspects of emerging contaminants, such as pharmaceuticals, pesticides, flame retardants, other industrial chemicals, persistent organic pollutants, endocrine disruptors, etc.
- All aspects of trace metals, organometals, metalloids (e.g., arsenic) and radionuclides
- Environmental fate studies including transport, biodegradation, bio-accumulation and/or deposition, atmospheric (photo)chemical processes, hydrolysis, adsorption/desorption
- Transformation and mineralisation of chemicals (e.g., by bio- and photo degradation, redox processes and hydrolysis)
- Novel environmental analytical methods including case studies

• Environmental modelling and quantitative structure-activity relationships to study fate and environmental dynamics
• Monitoring studies presenting new strategies, report of novel contaminants, findings or interpretations of interest for an international readership.
• Passive sampling
• Non-target and suspect screening (e.g. effect-directed analysis)
• Natural marine toxins

The following studies are not considered for publication: studies on (micro)organisms (unless chemicals are clearly involved), monitoring studies based on standard methodology, and/or only of regional importance, studies dealing only with nutrients in agricultural ecosystems, pesticide application studies, plant physiology studies, studies on improvement of crops and purely analytical methodology studies. As regards papers on air pollution, we focus on contaminants in air, particulate matter studies and also consider papers on NO_X, SO_X or ozone.

Toxicology and Risk Assessment

The section on Environmental Toxicology and Risk Assessment covers all aspects of toxicology, i.e., the science of adverse effects of chemicals on living organisms including humans, and the scientific risk assessment.

Topics of specific interest include, but not limited to, are:
• Adverse effects of chemicals in environmental, aquatic and terrestrial, organisms
• Similar studies in experimental organisms (under laboratory conditions)
• Epidemiological studies on effects of chemicals in humans
• Biochemical studies related to mechanisms of adverse effects
• Toxicokinetics and metabolic studies on chemicals related to adverse effects
• Development and validation of testing methods based on living organisms or biological materials
• Nanopolymers, nanocomposites, and microplastics in the environment
• Adaptation
• Human biomonitoring
• Elucidation of mechanisms of toxic effects
• DNA and protein adducts
• In vitro assays and omics techniques
• Phytotoxicity

Not considered are, e.g., studies that report only concentrations of chemicals in the environment, living organisms, food or other materials etc. and studies on biochemical effects of chemicals non-relevant to toxicology.

Treatment and Remediation

This section deals with papers about technologies that manage and/or reduce environmental contaminants, including reuse and recycling processes. The technology must be beyond a basic laboratory study or have obvious implications for current or potential treatment or remediation technologies and, for example, for any advanced oxidation process, the intermediates and/or the extent of mineralization of the targeted compound(s) and wastes must be quantified.

Topics of specific interest include, but not limited to, are:
• Advanced water and wastewater treatment processes and sludge management
• Produced water
• Drinking water
• Incineration
• Remediation including bio/phytoremediation employing new strategies
• Hydraulic fracturing
• Use of biochar amended soil to bind (e.g., herbicides)
• Nanotechnology
• Advanced oxidation processes
• Photolysis/photocatalysis and electrochemical and photo-assisted electrochemical methods
• Sonolysis/sonocatalysis

GUIDE FOR AUTHORS

Your Paper Your Way

We now differentiate between the requirements for new and revised submissions. You may choose to submit your manuscript as a single Word or PDF file to be used in the refereeing process. Only when your paper is at the revision stage, will you be requested to put your paper in to a 'correct format' for acceptance and provide the items required for the publication of your article.
To find out more, please visit the Preparation section below.

INTRODUCTION

Submission of Papers
All manuscripts should be submitted electronically through Editorial Manager which can be accessed at https://www.editorialmanager.com/CHEMOSPHERE/default.aspx.

During submission papers should be marked for the attention of a subject Editor or the relevant section, if possible. Failure to provide this information will significantly delay processing of the manuscript.

Types of article

Chemosphere accepts the following article types. Please note that papers with a routine nature and lacking originality, novelty and uniqueness will not be accepted for publication.

Research papers that meet the Aims and Scope of the journal reporting original and previously unpublished work. The Editors generally encourage brevity for all Research Papers should preferably not exceed 6,500 words (excluding refs.)

Review articles should preferably be 10,000 words or less (excluding refs.)

Short communications must not exceed 2,000 words and will be given priority for rapid publication. A Short Communication should be of significant scientific merit (a novel finding that warrants immediate publication).

Letter to the Editor are a written discussion of papers published in the journal. Letters are accepted on the basis of new scientific insights on the particular topic, critical additional information, relevance to the published paper and timeliness. Authors will be invited to submit a **Reply** to respond to points raised. The Editor will decide on the publication of Letters and Replies based on scientific merit, importance to the raised issues, and interest to the general audience. Letters and Replies of an unprofessional or unscientific nature, or containing personal invective, will not be considered.

Proposals for Special Issues composed predominantly of Research Papers that focus on a specific topic or theme will also be considered. Full information can be found here. **Editorials** for Special issues are submitted by invitation only through the Editorial Manager portal.

Each figure or table should be considered equal to 300 words. The number of figures and/or tables should not exceed a total amount of seven. Every page of the manuscript, including the title page, references, tables, etc. should be numbered. However, in the text no reference should be made to page numbers; if necessary, one may refer to sections.In case you have serious reasons for submitting a paper that is longer that the aforementioned word limits, make sure you include convincing arguments in your cover letter. Please avoid lengthy submissions if possible.

Submission checklist
You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
- E-mail address
- Full postal address

LINE and PAGE NUMBERING (NEW AND REVISED SUBMISSIONS):

Please ensure the text of your paper is double-spaced and has consecutive(continuous) LINE numbering. Please also ensure to add PAGE numbers to the source file- this is an essential peer review requirement.

All necessary files have been uploaded:
Manuscript:
• Include keywords
• All figures (include relevant captions)
• All tables (including titles, description, footnotes)
• Ensure all figure and table citations in the text match the files provided
• Indicate clearly if color should be used for any figures in print
Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

Further considerations
• Manuscript has been 'spell checked' and 'grammar checked'
• All references mentioned in the Reference List are cited in the text, and vice versa
• Permission has been obtained for use of copyrighted material from other sources (including the Internet)
• A competing interests statement is provided, even if the authors have no competing interests to declare
• Journal policies detailed in this guide have been reviewed
• Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

BEFORE YOU BEGIN

Ethics in publishing

Please see our information on Ethics in publishing.

Studies in humans and animals

If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The manuscript should be in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals and aim for the inclusion of representative human populations (sex, age and ethnicity) as per those recommendations. The terms sex and gender should be used correctly.

Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

All animal experiments should comply with the ARRIVE guidelines and should be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Research Council's Guide for the Care and Use of Laboratory Animals and the authors should clearly indicate in the manuscript that such guidelines have been followed. The sex of animals must be indicated, and where appropriate, the influence (or association) of sex on the results of the study.

Declaration of competing interest

Corresponding authors, on behalf of all the authors of a submission, must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. All authors, including those *without* competing interests to declare, should provide the relevant information to the corresponding author (which, where relevant, may specify they have nothing to declare). Corresponding authors should then use this tool to create a shared statement and upload to the submission system at the Attach Files step. **Please do not convert the .docx template to another file type. Author signatures are not required.**

Submission declaration

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Submission declaration

Submission of an article implies that the work described has **not been published previously**(except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint, see https://www.elsevier.com/sharingpolicy), that it is **not under consideration for publication elsewhere**, that its publication is **approved by all authors** and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere including electronically in the same form, in English or in any other language, without the written consent of the copyright-holder. The authors are asked to state this explicitly in their accompanying letter.

Preprints

Please note that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints e.g. on a preprint server will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Preprint posting on SSRN

In support of Open Science, this journal offers its authors a free preprint posting service. Preprints provide early registration and dissemination of your research, which facilitates early citations and collaboration.

During submission to Editorial Manager, you can choose to release your manuscript publicly as a preprint on the preprint server SSRN once it enters peer-review with the journal. Your choice will have no effect on the editorial process or outcome with the journal. Please note that the corresponding author is expected to seek approval from all co-authors before agreeing to release the manuscript publicly on SSRN.

You will be notified via email when your preprint is posted online and a Digital Object Identifier (DOI) is assigned. Your preprint will remain globally available free to read whether the journal accepts or rejects your manuscript.

For more information about posting to SSRN, please consult the SSRN Terms of Use and FAQs.

Use of inclusive language

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Content should make no assumptions about the beliefs or commitments of any reader; contain nothing which might imply that one individual is superior to another on the grounds of age, gender, race, ethnicity, culture, sexual orientation, disability or health condition; and use inclusive language throughout. Authors should ensure that writing is free from bias, stereotypes, slang, reference to dominant culture and/or cultural assumptions. We advise to seek gender neutrality by using plural nouns ("clinicians, patients/clients") as default/wherever possible to avoid using "he, she," or "he/she." We recommend avoiding the use of descriptors that refer to personal attributes such as age, gender, race, ethnicity, culture, sexual orientation, disability or health condition unless they are relevant and valid. When coding terminology is used, we recommend to avoid offensive or exclusionary terms such as "master", "slave", "blacklist" and "whitelist". We suggest using alternatives that are more appropriate and (self-) explanatory such as "primary", "secondary", "blocklist" and "allowlist". These guidelines are meant as a point of reference to help identify appropriate language but are by no means exhaustive or definitive.

Reporting sex- and gender-based analyses

Reporting guidance

For research involving or pertaining to humans, animals or eukaryotic cells, investigators should integrate sex and gender-based analyses (SGBA) into their research design according to funder/ sponsor requirements and best practices within a field. Authors should address the sex and/or gender

dimensions of their research in their article. In cases where they cannot, they should discuss this as a limitation to their research's generalizability. Importantly, authors should explicitly state what definitions of sex and/or gender they are applying to enhance the precision, rigor and reproducibility of their research and to avoid ambiguity or conflation of terms and the constructs to which they refer (see Definitions section below). Authors can refer to the Sex and Gender Equity in Research (SAGER) guidelines and the SAGER guidelines checklist. These offer systematic approaches to the use and editorial review of sex and gender information in study design, data analysis, outcome reporting and research interpretation - however, please note there is no single, universally agreed-upon set of guidelines for defining sex and gender.

Definitions

Sex generally refers to a set of biological attributes that are associated with physical and physiological features (e.g., chromosomal genotype, hormonal levels, internal and external anatomy). A binary sex categorization (male/female) is usually designated at birth ("sex assigned at birth"), most often based solely on the visible external anatomy of a newborn. Gender generally refers to socially constructed roles, behaviors, and identities of women, men and gender-diverse people that occur in a historical and cultural context and may vary across societies and over time. Gender influences how people view themselves and each other, how they behave and interact and how power is distributed in society. Sex and gender are often incorrectly portrayed as binary (female/male or woman/man) and unchanging whereas these constructs actually exist along a spectrum and include additional sex categorizations and gender identities such as people who are intersex/have differences of sex development (DSD) or identify as non-binary. Moreover, the terms "sex" and "gender" can be ambiguous—thus it is important for authors to define the manner in which they are used. In addition to this definition guidance and the SAGER guidelines, the resources on this page offer further insight around sex and gender in research studies.

Author contributions

For transparency, we encourage authors to submit an author statement file outlining their individual contributions to the paper using the relevant CRediT roles: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing. Authorship statements should be formatted with the names of authors first and CRediT role(s) following. More details and an example.

Changes to authorship

This policy concerns the addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts.

Before the accepted manuscript is published in an online issue: Requests to add or remove an author, or to rearrange the author names, must be sent by the corresponding author of the accepted manuscript to the Journal Manager and must include: (a) the reason the name should be added or removed, or the author names rearranged and (b) **written confirmation (e-mail, fax, letter) from all authors that they agree with the addition, removal or rearrangement.** In the case of addition or removal of authors, **this includes confirmation from the author being added or removed.** Requests that are not sent by the corresponding author will be forwarded by the Journal Manager to the corresponding author, who must follow the procedure as described above. Note that: (1) Journal Managers will inform the Journal Editors of any such requests and (2) publication of the accepted manuscript in an online issue is suspended until authorship has been agreed. After the accepted manuscript is published in an online issue requests to add, delete, or rearrange author names in an article published in an online issue will not be accepted anymore.

Dual first authorship

If requested, we can provide dual-first authorship if two authors have contributed equally to a paper. In that case a footnote will be added to the author names and an explanation will be given.

Article transfer service

This journal uses the Elsevier Article Transfer Service to find the best home for your manuscript. This means that if an editor feels your manuscript is more suitable for an alternative journal, you might be asked to consider transferring the manuscript to such a journal. The recommendation might be provided by a Journal Editor, a dedicated Scientific Managing Editor, a tool assisted recommendation, or a combination. If you agree, your manuscript will be transferred, though you will have the opportunity to make changes to the manuscript before the submission is complete. Please note that your manuscript will be independently reviewed by the new journal. More information.

Copyright

This journal offers authors a choice in publishing their research: Open Access and Subscription.

For Subscription articles:

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (for more information on this and copyright, see https://www.elsevier.com/copyright). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations (please consult https://www.elsevier.com/permissions). If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases: please consult https://www.elsevier.com/permissions.

For Open Access articles:

Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (for more information see https://www.elsevier.com/OAauthoragreement). Permitted reuse of open access articles is determined by the author's choice of user license (see https://www.elsevier.com/about/policies/open-access-licenses).

Retained author rights

As an author you (or your employer or institution) retain certain rights. For more information on author rights for: Subscription articles please see https://www.elsevier.com/journal-authors/author-rights-and-responsibilities. Open access articles please see https://www.elsevier.com/OAauthoragreement.

Elsevier supports responsible sharing
Find out how you can share your research published in Elsevier journals.

Funding Source Disclosure

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Open access

Please visit our Open Access page for more information.

This journal has an embargo period of 24 months.

Elsevier Researcher Academy
Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these).**In case of doubt, let your manuscript check by a native English or American colleague, or a professional service**. Regularly, manuscripts are rejected before review because of poor language. Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop(https://webshop.elsevier.com/language-editing-services/language-editing/) or visit our customer support site (https://service.elsevier.com) for more information.

Submission

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts source files to a single PDF file of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to PDF files at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail removing the need for a paper trail.

LINE and PAGE NUMBERING (NEW AND REVISED SUBMISSIONS)

Please ensure the text of your paper is double-spaced and has consecutive(continuous) LINE numbering. Please also ensure to add PAGE numbers to the source file- this is an essential peer review requirement.

Referees

With the submitted manuscript authors are requested to provide full contact details of **six potential reviewers** including email addresses. Please suggest potential reviewers for this submission and provide specific reasons for your suggestion in the comments box for each person. Please note that the editorial office may not use your suggestions, but your help is appreciated and may speed up the selection of appropriate reviewers. Of the six potential reviewers, please suggest two members of the **Editorial Board** (but NOT **Editors**) of *Chemosphere* as reviewer candidates. The other suggested reviewers should not be from the same institution as the author, or co-authors/collaborators on any books, articles, reports, papers, or projects of the author. Not more than one should come from the same country as the author. It should also be avoided to suggest referees that are living in a different country but have the same nationality as the author. *Please provide institutional email addresses only; if this is not possible then the potential reviewer's institution should be clearly stated.*

PREPARATION

Queries

For questions about the editorial process (including the status of manuscripts under review) or for technical support on submissions, please visit our Support Center.

NEW SUBMISSION

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process.

As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or lay-out that can be used by referees to evaluate your manuscript. It should contain high enough quality figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual figure files larger than 10 MB must be uploaded separately. If you use the Your Paper Your Way service, please make sure that you also provide your highlights and the six suggested referees in this document.

References

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/ book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

Formatting requirements

There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript, for example **Abstract, Keywords, Introduction, Materials and Methods, Results, Conclusions, Artwork and Tables with Captions**.

If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes.

Divide the article into clearly defined sections.

Figures and tables embedded in text
Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. The corresponding caption should be placed directly below the figure or table.

Peer review

This journal operates a single anonymized review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. Editors are not involved in decisions about papers which they have written themselves or have been written by family members or colleagues or which relate to products or services in which the editor has an interest. Any such submission is subject to all of the journal's usual procedures, with peer review handled independently of the relevant editor and their research groups. More information on types of peer review.

REVISED SUBMISSIONS

Use of word processing software
Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: https://www.elsevier.com/guidepublication). See also the section on Electronic artwork. To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor. **For revised submissions: always include one copy of the new text with changes clearly indicated (in red or bold or track change) and one version with all changes accepted, and a letter with your response to the comments of the reviewers. When submitting the revised manuscript, please make sure that you upload the final version of the paper. Please remove the old version(s) of the manuscript before submitting the revised version.**

Article structure

Subdivision - numbered sections
Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction
State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Include specific objectives/testable hypotheses in the last paragraph of Introduction.

Material and methods
Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

The QA/QC of the data must be clearly presented, particularly for low/trace concentrations or lower recovery efficiencies of some compounds. Authors of toxicological submissions are asked to report analytical confirmation of their experimental test concentrations.

Theory/calculation
A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results
Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Include a paragraph dealing with study limitations in the discussion section.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices
If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible. Please limit your title to 20 words or below - editors may ask for titles which are overly long to be shortened.
• ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that phone numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author. .**
• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Highlights

Highlights are mandatory for this journal as they help increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the examples here: example Highlights.

Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

Abstract

A concise and factual abstract is required, maximum length 300 words. **The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone**. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself (and then later again when used in the text, see Abbreviations).

Mandatory graphical abstract

A graphical abstract is mandatory for all Research Papers, Review Articles and Short Communications submitted to this journal. It does not need to be uploaded with the initial submission but must be supplied with any subsequent revisions. It should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the article. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. You can view Example Graphical Abstracts on our information site.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Nomenclature and units

Follow internationally accepted rules and conventions: **use the international system of units (SI).** If other quantities are mentioned, give their equivalent in SI. You are urged to consult IUPAC: Nomenclature of Organic Chemistry: http://www.iupac.org/ for further information.

Math formulae

Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many wordprocessors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Table footnotes

Indicate each footnote in a table with a superscript lowercase letter.

Artwork

Electronic artwork

General points

- Make sure you use uniform lettering and sizing of your original artwork.
- Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier.
- Number the illustrations according to their sequence in the text.
- Use a logical naming convention for your artwork files.
- Indicate per figure if it is a single, 1.5 or 2-column fitting image.
- For Word submissions only, you may still provide figures and their captions, and tables within a single file at the revision stage.
- Please note that individual figure files larger than 10 MB must be provided in separate source files.

A detailed guide on electronic artwork is available.

You are urged to visit this site; some excerpts from the detailed information are given here.

Formats

Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.

TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.

TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.

TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.

Please do not:

• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low.
• Supply files that are too low in resolution.
• Submit graphics that are disproportionately large for the content.

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article**. Please indicate your preference for color: in print or online only. For further information on the preparation of electronic artwork, please see https://www.elsevier.com/artworkinstructions.

Please note: Because of technical complications which can arise by converting color figures to 'gray scale' (for the printed version should you not opt for color in print) please submit in addition usable black and white versions of all the color illustrations.

Figure captions
Ensure that each illustration has a caption. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables
Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

References
Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links
Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, Crossref and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is highly encouraged.

A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884. Please note the format of such citations should be in the same style as all other references in the paper.

Data references
This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

Preprint references
Where a preprint has subsequently become available as a peer-reviewed publication, the formal publication should be used as the reference. If there are preprints that are central to your work or that cover crucial developments in the topic, but are not yet formally published, these may be referenced. Preprints should be clearly marked as such, for example by including the word preprint, or the name of the preprint server, as part of the reference. The preprint DOI should also be provided.

References in a special issue
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software
This journal has standard templates available in key reference management packages EndNote (http://www.endnote.com/support/enstyles.asp) and Reference Manager (http://refman.com/support/rmstyles.asp). Using plug-ins to wordprocessing packages, authors only need to select the appropriate journal template when preparing their article and the list of references and citations to these will be formatted according to the journal style which is described below.

Reference formatting
There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/ book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style
Text: All citations in the text should refer to:
1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;
2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication.
Citations may be made directly (or parenthetically). Groups of references should be listed first alphabetically, then chronologically.
Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999). Kramer et al. (2010) have recently shown ....'
List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.
Examples:
Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59.
Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. http://dx.doi.org/10.17632/xwj98nb39r.1.
Reference to a book:
Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.
Reference to a chapter in an edited book:
Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Journal abbreviations source
Journal names should be abbreviated according to the List of Title Word Abbreviations.

Video
Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum

size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Data visualization

Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

Supplementary data

Elsevier accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, high resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. In order to ensure that your submitted material is directly usable, please provide the data in one of our recommended file formats. Authors should submit the material in electronic format together with the article and supply a concise and descriptive caption for each file. For more detailed instructions please visit our artwork instruction pages at https://www.elsevier.com/artworkinstructions.

Data at PANGAEA

Electronic archiving of supplementary data enables readers to replicate, verify and build upon the conclusions published in your paper. We recommend that data should be deposited in the data library PANGAEA (http://www.pangaea.de). Data are quality controlled and archived by an editor in standard machine-readable formats and are available via Open Access. After processing, the author receives an identifier (DOI) linking to the supplements for checking. As your data sets will be citable you might want to refer to them in your article. In any case, data supplements and the article will be automatically linked as in the following example: https://doi.org/10.1016/0016-7037(95)00105-9. Please use PANGAEA's web interface to submit your data (http://www.pangaea.de/submit/).

Research data

This journal requires and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. When sharing data in one of these ways, you are expected to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking

If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Research Elements

This journal enables you to publish research objects related to your original research – such as data, methods, protocols, software and hardware – as an additional paper in Research Elements.

Research Elements is a suite of peer-reviewed, open access journals which make your research objects findable, accessible and reusable. Articles place research objects into context by providing detailed descriptions of objects and their application, and linking to the associated original research articles. Research Elements articles can be prepared by you, or by one of your collaborators.

During submission, you will be alerted to the opportunity to prepare and submit a Research Elements article.

More information can be found on the Research Elements page.

Data statement

To foster transparency, we require you to state the availability of your data in your submission if your data is unavailable to access or unsuitable to post. This may also be a requirement of your funding body or institution. You will have the opportunity to provide a data statement during the submission process. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page..

Submission checklist

The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:

- E-mail address
- Full postal address

All necessary files have been uploaded, and contain:

- Keywords
- All figure captions
- All tables (including title, description, footnotes)

Further considerations

- Manuscript has been 'spell-checked' and 'grammar-checked'
- All references mentioned in the Reference list are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Web)
- Color figures are clearly marked as being intended for color reproduction on the Web (free of charge) and in print, or to be reproduced in color on the Web (free of charge) and in black-and-white in print
- If only color on the Web is required, black-and-white versions of the figures are also supplied for printing purposes .

For any further information please visit our customer support site at https://service.elsevier.com.

AFTER ACCEPTANCE

Online proof correction

Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.

If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.

We will do everything possible to get your article published quickly and accurately - please upload all of your corrections within 48 hours. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility. Note that Elsevier may proceed with the publication of your article if no response is received.

Offprints

The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

AUTHOR INQUIRIES

For inquiries relating to the submission of articles (including electronic submission) please visit this journal's homepage.

Revision submissions:

If you have any specific questions related due date extensions for revision, please visit our Support Center.

For detailed instructions on the preparation of electronic artwork, please visit https://www.elsevier.com/artworkinstructions. Contact details for questions arising after acceptance of an article, especially those relating to proofs, will be provided by the publisher. You can track accepted articles at https://www.elsevier.com/trackarticle. You can also check our Author FAQs at https://www.elsevier.com/authorFAQ and/or contact Customer Support via https://service.elsevier.com.

REFERÊNCIAS DA TESE

Amarante, O. P., Santos, T. C. R. dos, Brito, N. M., & Ribeiro, M. L. (2002). Glifosato: Propriedades, Toxicidade, Usos e Legislação. *Química Nova*, *25*(4), 589–593.

Apel, K., & Hirt, H. (2004). REACTIVE OXYGEN SPECIES: Metabolism, Oxidative Stress, and Signal Transduction. *Annual Review of Plant Biology*, *55*(1), 373–399. https://doi.org/10.1146/annurev.arplant.55.031903.141701

Barbosa, K. B. F., Costa, N. M. B., de Cássia Gonçalves Alfenas, R., de Paula, S. O., Minim, V. P. R., & Bressan, J. (2010). Estresse oxidativo: Conceito, implicações e fatores modulatórios. *Revista de Nutricao*, *23*(4), 629–643. https://doi.org/10.1590/S1415-52732010000400013

Barreiros, A. L. B. S., David, J. P., & David, J. (2006). Estresse Oxidativo: Relação entre Geração de Espécies Reativas e Defesa do Organismo. *Química Nova*, *29*(1), 113–123.

Berois, N., Arezo, M. J., Papa, N. G., & Clivio, G. a. (2012). Annual fish: Developmental adaptations for an extreme environment. *Wiley Interdisciplinary Reviews: Developmental Biology*, *1*(4), 595–602. https://doi.org/10.1002/wdev.39

BOMBARDI, L. M. (2017). GEOGRAFIA DO USO DE AGROTÓXICOS NO BRASIL E CONEXÕES COM A UNIÃO EUROPEIA. In E. Penha (Ed.), *FFLCH - USP* (1° Edição, Vol. 1). FFLCH - USP. https://doi.org/10.1097/SMJ.0b013e31818da807

Braghirolli, F. M., Oliveira, M. R., & Oliveira, G. T. (2016). Seasonal variability of metabolic markers and oxidative balance in freshwater amphipod Hyalella kaingang (Crustacea, Amphipoda). *Ecotoxicology and Environmental Safety*, *130*, 177–184. https://doi.org/10.1016/j.ecoenv.2016.04.021

Cericato, L., Neto, J. G. M., Kreutz, L. C., Quevedo, R. M., da Rosa, J. G. S., Koakoski, G., Centenaro, L., Pottker, E., Marqueze, A., & Barcellos, L. J. G. (2009). Responsiveness of the interrenal tissue of Jundiá (Rhamdia quelen) to an in vivo ACTH test following acute exposure to sublethal concentrations of agrichemicals. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, *149*(3), 363–367. https://doi.org/10.1016/j.cbpc.2008.09.002

Cnubben, N. H. P., Rietjens, I. M. C. M., Wortelboer, H., Van-Zanden, J., & van Bladeren, P. J. (2001). The interplay of glutathione related processes in antioxidant defense. *Environ. Toxicol. Pharmacol.*, *10*, 141–152.

Coltro, M., da Silva, P. R., Valgas, A. A. N., Miguel, C., de Freitas, B. S., & Oliveira, G. T. (2017). Influence of the Herbicide Facet on Corticosterone Levels, Plasma Metabolites, and Antioxidant System in the Liver and Muscle of American Bullfrog Tadpoles. *Water, Air, & Soil Pollution*, *228*(7), 241. https://doi.org/10.1007/s11270-017-3404-7

Costa, W. J. E. M. (1998). Phylogeny and Classification of Revulidae Revisited: Origin and Evolution of Annualism and Miniaturization in Rivulid Fishes (Cyprinodontiformes: Aplocheiloidei). *Journal of Comparative Biology*, *3*(1), 1–62. http://www.killiclub.org/pagina/articulos/Gran_trabajo_anuales_Costa.pdf

Costantini, D. (2014). Oxidative Stress and Hormesis in Evolutionary Ecology and Physiology. In *Springer Berlin Heidelberg*. Springer Heidelberg New York Dordrecht London. https://doi.org/10.1007/978-3-642-54663-1

Cooke, S. J., Sack, L., Franklin, C. E., Farrell, a. P., Beardall, J., Wikelski, M., & Chown, S. L. (2013). What is conservation physiology? Perspectives on an increasingly integrated and essential science. **Conservation Physiology**, *1*, 1–23. https://doi.org/10.1093/conphys/cot001

Da Silva, P. R., Borges-Martins, M., & Oliveira, G. T. (2021). Melanophryniscus admirabilis tadpoles' responses to sulfentrazone and glyphosate-based herbicides: an approach on metabolism and antioxidant defenses. *Environmental Science and Pollution Research*, *28*(4), 4156–4172. https://doi.org/10.1007/s11356-020-10654-x

Dantzer, B., Fletcher, Q. E., Boonstra, R., & Sheriff, M. J. (2014). Measures of physiological stress: A transparent or opaque window into the status, management and conservation of species? *Conservation Physiology*, *2*(1), 1–18. https://doi.org/10.1093/conphys/cou023

Dowling, D. K., & Simmons, L. W. (2009). Reactive oxygen species as universal constraints in life-history evolution. *Proceedings. Biological Sciences / The Royal Society*, *276*(1663), 1737–1745. https://doi.org/10.1098/rspb.2008.1791

Fanta, E., Rios, F. S., Romão, S., Vianna, A. C. C., & Freiberger, S. (2003). Histopathology of the fish Corydoras paleatus contaminated with sublethal levels of organophosphorus in water and food. *Ecotoxicology and Environmental Safety*, *54*(2), 119–130. https://doi.org/10.1016/S0147-6513(02)00044-1

Ferreira, D., Motta, a. C. D., Kreutz, L. C., Toni, C., Loro, V. L., & Barcellos, L. J. G. (2010). Assessment of oxidative stress in Rhamdia quelen exposed to agrichemicals. *Chemosphere*, *79*(9), 914–921. https://doi.org/10.1016/j.chemosphere.2010.03.024

Gaaied, S., Oliveira, M., Domingues, I., & Banni, M. (2020). 2,4-Dichlorophenoxyacetic acid herbicide effects on *zebrafish* larvae: development, neurotransmission and behavior as sensitive endpoints. *Environmental Science and Pollution Research*, *27*(4), 3686–3696. https://doi.org/10.1007/s11356-019-04488-5

Genade, T., Benedetti, M., Terzibasi, E., Roncaglia, P., Valenzano, D. R., Cattaneo, A., & Cellerino, A. (2005). Annual fishes of the genus Nothobarnchius as a model system for aging research. *Aging Cell*, *4*(5), 223–233. https://doi.org/10.1111/j.1474-9726.2005.00165.x

Gervais J.A., Luukinen B., Buhl K., Stone D. 2,4-D Technical Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services, 2008.

Giesy, J. P., Dobson, S., & Solomon, K. R. (2000). *Ecotoxicological Risk Assessment for Roundup® Herbicide*. 35–120. https://doi.org/10.1007/978-1-4612-1156-3_2

Hermes-Lima, M. (2004). ROLE OF FREE RADICALS. In K. B. Storey (Ed.), *Funcional Metabolism: Regulation and Adaptation* (John Wiley, pp. 319–368). Hoboken.

Hwang, H., Alegre, G. F., Borrely, S. I., & Flynn, M. N. (2009). Avaliação de Toxicidade em Sedimentos do Rio Juqueri (SP) com VIBRIO FISCHERI e HYALELLA AZTECA. *Revista Intertox de Toxicologia, Risco Ambiental e Sociedade*, *2*(2), 18–28.

Ibama. (2021). *Consolidação de dados fornecidos pelas empresas registrantes de produtos técnicos, agrotóxicos e afins, conforme art. 41 do Decreto n° 4.074/2002*.

Isaksson, C. (2010). Pollution and its impact on wild animals: A meta-analysis on oxidative stress. *EcoHealth*, *7*(3), 342–350. https://doi.org/10.1007/s10393-010-0345-7

Islam, F., Wang, J., Farooq, M. A., Khan, M. S. S., Xu, L., Zhu, J., Zhao, M., Muños, S., Li, Q. X., & Zhou, W. (2018). Potential impact of the herbicide 2,4-dichlorophenoxyacetic acid on human and ecosystems. In *Environment International* (pp. 332–351). Elsevier Ltd. https://doi.org/10.1016/j.envint.2017.10.020

Kaster Garcez, D., de Oliveira Fernandes, M., da Rosa Ozório, G., Vieira Volcan, M., & Robe, L. J. (2020). Phylogenetic structure of Neotropical annual fish of the genus Cynopoecilus (Cyprinodontiformes: Rivulidae), with an assessment of taxonomic implications. *Journal of Zoological Systematics and Evolutionary Research*, *September 2019*, 1–12. https://doi.org/10.1111/jzs.12389

Lanés, L. E. K. (2011). *Dinâmica e Conservação de Peixes Anuais (Cyprinodontiformes: Rivulidae) no Parque Nacional da Lagoa do Peixe*.

Laufer, G., Arim, M., Loureiro, M., Piñeiro-Guerra, J. M., Clavijo-Baquet, S., & Fagúndez, C. (2009). Diet of four annual killifishes: An intra and interspecific comparison. *Neotropical Ichthyology*, *7*(1), 77–86. https://doi.org/10.1590/S1679-62252009000100010

Loureiro, M., Sá, R. de, Serra, S. W., Alonso, F., Lanés, L. E. K., Volcan, M. V., Calviño, P., Nielsen, D., Duarte, A., & Garcia, G. (2018). Review of the family Rivulidae (Cyprinodontiformes, Aplocheiloidei) and a molecular and morphological phylogeny of the annual fish genus Austrolebias Costa 1998. *Neotropical Ichthyology*, *16*(3), 1–20. https://doi.org/10.1590/1982-0224-20180007

Lucas-sá, A., Almaida-pagá, P. F., Mendiola, P., & Costa, J. de. (2014). Nothobranchius as a model for aging studies. A review. *Aging and Disease*, *5*(4), 281–291. https://doi.org/10.14336/AD.2014.0500281

Machado, A. M., Filipe, L., & Castro, C. (2018). *A taxonomically-restricted Uric Acid transporter provides insight into antioxidant equilibrium*. https://doi.org/10.1101/287870

Martins-Gomes, C., Silva, T. L., Andreani, T., & Silva, A. M. (2022). Glyphosate vs. Glyphosate-Based Herbicides Exposure: A Review on Their Toxicity. *Journal of Xenobiotics*, *12*(1), 21–40. https://doi.org/10.3390/jox12010003

Menezes, C., Fonseca, M. B., Leitemperger, J., Pretto, A., Moraes, B. S., Murussi, C. R., Baldisserotto, B., & Loro, V. L. (2015). Commercial formulation containing 2,4-D affects biochemical parameters and morphological indices of silver catfish exposed for 90 days. *Fish Physiology and Biochemistry*, *41*(2), 323–330. https://doi.org/10.1007/s10695-014-9985-9

Michael C. Newman. (2015). *FUNDAMENTALS OF ECOTOXICOLOGY. The Science of Pollution* (4° Edição).

Neto, D. M.; Froehner, S.; Machado, K. S (2012). Avaliação do transporte do ácido 2,4-diclorofenoxiacético através de um lisímetro. Quím. Nova, vol.35 no.9 São Paulo. https://doi.org/10.1590/S0100-40422012000900020

Queiroz, G. M. P., Silva, M. R. da, Bianco, R. J. F., Pinheiro, A., & Kaufmann, V. (2011). Transporte de Glifosato pelo Escoamento Superficial e por Lixiviação em um Solo Agrícola. *Quimica Nova*, *34*(2), 190–195. https://doi.org/10.1590/S0100-40422011000200004

RED, 2005. Reregistration Eligibility Decision (RED) 2,4-D; EPA 738-R-05-002. U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office, Washington, DC.

Rocha, G. M., & Grisolia, C. K. (2018). Why pesticides with mutagenic, carcinogenic and reproductive risks are registered in Brazil. *Developing World Bioethics*, *19*(3), 148–154. https://doi.org/10.1111/dewb.12211

Rodrigues, B. N.; Almeida, F. S (2005). Guia de Herbicidas. 5ª ed. ed. Londrina: IAPAR, p. 648pp.

Sies, H. (1991). Oxidative stress: From basic research to clinical application. *The American Journal of Medicine*, *91*(3 SUPPL. 3), 31–38. https://doi.org/10.1016/0002-9343(91)90281-2

Sies, H. (1993). Strategies of antioxidant defense. *Pharmacological Research*, *31*, 161. https://doi.org/10.1016/1043-6618(95)86904-2

Sies, H. (2018). On the history of oxidative stress: Concept and some aspects of current development. *Current Opinion in Toxicology*, *7*, 122–126. https://doi.org/10.1016/j.cotox.2018.01.002

Sies, H. (2019). Oxidative Stress: Eustress and Distress in Redox Homeostasis. In *Stress: Physiology, Biochemistry, and Pathology*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-813146-6.00013-8

Sies, H., & Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature Reviews Molecular Cell Biology*, *21*(7), 363–383. https://doi.org/10.1038/s41580-020-0230-3

Panetto, O. S., Gomes, H. F., Fraga Gomes, D. S., Campos, E., Romeiro, N. C., Costa, E. P., do Carmo, P. R. L., Feitosa, N. M., & Moraes, J. (2019). The effects of Roundup® in embryo development and energy metabolism of the *zebrafish* (Danio rerio). *Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology*, *222*(April), 74–81. https://doi.org/10.1016/j.cbpc.2019.04.007

Persch, T. S. P., Weimer, R. N., Freitas, B. S., & Oliveira, G. T. (2017). Metabolic parameters and oxidative balance in juvenile Rhamdia quelen exposed to rice paddy herbicides: Roundup®, Primoleo®, and Facet®. *Chemosphere*, *174*, 98–109. https://doi.org/10.1016/j.chemosphere.2017.01.092

Persch, T. S. P., Silva, P. R. da, Santos, S. H. D. dos, Freitas, B. S. de, & Oliveira∗, G. T. (2018). Changes in intermediate metabolism and oxidative balance parameters in sexually matured three-barbeled catfishes exposed to herbicides from rice crops (Roundup®, Primoleo® and Facet®). *Environmental Toxicology and Pharmacology*, *58*, 170–179. https://doi.org/10.1016/j.etap.2018.01.004

Peterson, M. A., McMaster, S. A., Riechers, D. E., Skelton, J., & Stahlman, P. W. (2016). 2,4-D Past, Present, and Future: A Review. *Weed Technology*, *30*(2), 303–345. https://doi.org/10.1614/wt-d-15-00131.1

Pinheiro, L. C., & Oliveira, G. T. (2016). Oxidative Status Profile in Different Tissues of Parastacus brasiliensis promatensis (Crustacea, Decapoda, Parastacidae) over a Seasonal Cycle. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *325*(5), 318–328. https://doi.org/10.1002/jez.2019

Podrabsky, J. E., & Hand, S. C. (1999). THE BIOENERGETICS OF EMBRYONIC DIAPAUSE IN AN ANNUAL KILLIFISH, AUSTROFUNDULUS LIMNAEUS. *The Journal of Experimental Biology*, *202*, 2567–2580. http://jeb.biologists.org/content/202/19/2567.full.pdf\npapers3://publication/uuid/7895BDBF-A7FA-4718-B978-A6D27FF4FB9B

Polacik, M., & Podrabsky, J. E. (2015). Temporary Environments. *Extremophile Fishes: Ecology, Evolution, and Physiology of Teleosts in Extreme Environments*, 1–29. https://doi.org/10.1007/978-3-319-13362-1

Tasaki, E., Sakurai, H., Nitao, M., Matsuura, K., & Iuchi, Y. (2017). Uric acid, an important antioxidant contributing to survival in termites. *PLoS ONE*, *12*(6). https://doi.org/10.1371/journal.pone.0179426

Thiel, N. A., Sachett, A., Schneider, S. E., Garbinato, C., Decui, L., Eichwald, T., Conterato, G. M. M., Latini, A., Piato, A., & Siebel, A. M. (2020). Exposure to the herbicide 2,4-dichlorophenoxyacetic acid impairs mitochondrial function, oxidative status, and behavior in adult *zebrafish*. *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-020-10497-6

Toni, C., Menezes, C., Clasen, B., Leitemperger, J., Pretto, A., Adaime, M. B., Leonardo Martins, M., Zanella, R., & Lucia Loro, V. (2013). Oxidative stress in carp exposed to quinclorac herbicide under rice field condition. *Ecotoxicology and Environmental Safety*, *92*, 27–31. https://doi.org/10.1016/j.ecoenv.2013.01.028

Ursini, F., Maiorino, M., & Forman, H. J. (2016). Redox homeostasis: The Golden Mean of healthy living. *Redox Biology*, *8*, 205–215. https://doi.org/10.1016/j.redox.2016.01.010

Van Gestel, C. a. M., & van Brummelen, T. C. (1996). Incorporation of the biomarker concept in ecotoxicology calls for a redefinition of terms. *Ecotoxicology*, *5*(4), 217–225. https://doi.org/10.1007/BF00118992

Vasconcelos, S. M. L., Goulart, M. O. F., Moura, J. B. D. F., Manfredini, V., Benfato, M. D. S., & Kubota, L. T. (2007). Espécies reativas de oxigênio e de nitrogênio, antioxidantes e marcadores de dano oxidativo em sangue humano: principais métodos analíticos para sua determinação. *Quimica Nova*, *30*(5), 1323–1338. https://doi.org/10.1590/S0100-40422007000500046

Volcan, M. V., Gonçalves, A. C., & Lanés, L. E. K. (2011). Distribution, habitat and conservation status of two threatened annual fishes (Rivulidae) from southern Brazil. *Endangered Species Research*, *13*(2), 79–85. https://doi.org/10.3354/esr00316

Wourms, J. P. (1972). The Developmental Biology of Annual Fishes. *J. Exp. Zool.*, *182*, 389–414. https://doi.org/10.1016/0002-9149(87)91006-X

Zebral, Y. D., Lansini, L. R., Costa, P. G., Roza, M., Bianchini, A., & Robaldo, R. B. (2018). A glyphosate-based herbicide reduces fertility, embryonic upper thermal tolerance and alters embryonic diapause of the threatened annual fish Austrolebias nigrofasciatus. *Chemosphere*, *196*, 260–269. https://doi.org/10.1016/j.chemosphere.2017.12.196

Pontifícia Universidade Católica do Rio Grande do Sul
Pró-Reitoria de Pesquisa e Pós-Graduação
Av. Ipiranga, 6681 – Prédio 1 – Térreo
Porto Alegre – RS – Brasil
Fone: (51) 3320-3513
E-mail: propesq@pucrs.br
Site: www.pucrs.br