UNIVERSIDADE FEDERAL DE SANTA MARIA – UFSM
CENTRO DE CIÊNCIAS NATURAIS E EXATAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIODIVERSIDADE ANIMAL

Cristina Cerezer

CONSERVAÇÃO DE CRUSTÁCEOS LÍMNICOS: EFEITO DA TEMPERATURA E PESTICIDAS NO METABOLISMO OXIDATIVO DE *AEGLA LONGIROSTRI*, *IN SITU* E *EX-SITU*.

Santa Maria, RS
2021

Cristina Cerezer

CONSERVAÇÃO DE CRUSTÁCEOS LÍMNICOS: EFEITO DA TEMPERATURA E PESTICIDAS NO METABOLISMO OXIDATIVO DE *AEGLA LONGIROSTRI*, *IN SITU* E *EX-SITU*.

Tese apresentada ao Curso de Pós-Graduação em Biodiversidade Animal, Área de Concentração em Bioecologia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Doutora em Biodiversidade Animal**.

Orientador: Prof. Dr. Sandro Santos
Coorientadora: Prof. Dr. Vania Lucia Loro

Santa Maria, RS
2021

Cerezer, Cristina
CONSERVAÇÃO DE CRUSTÁCEOS LÍMNICOS: EFEITO DA TEMPERATURA E PESTICIDAS NO METABOLISMO OXIDATIVO DE AEGLA LONGIROSTRI, IN SITU E EX-SITU. / Cristina Cerezer.- 2021.
101 p.; 30 cm

Orientador: Sandro Santos
Coorientadora: Vania Lucia Loro
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Naturais e Exatas, Programa de Pós-Graduação em Biodiversidade Animal, RS, 2021

1. Biomarcador 2. Ecotoxicologia 3. Eglídeos 4. Crustáceos 5. Poluição aquática I. Santos, Sandro II. Loro, Vania Lucia III. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

Cristina Cerezer

CONSERVAÇÃO DE CRUSTÁCEOS LÍMNICOS: EFEITO DA TEMPERATURA E PESTICIDAS NO METABOLISMO OXIDATIVO DE *AEGLA LONGIROSTRI*, *IN SITU* E *EX-SITU*.

Tese apresentada ao Curso de Pós-Graduação em Biodiversidade Animal, Área de Concentração em Bioecologia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Doutora em Biodiversidade Animal**.

Aprovado em 31 de agosto de 2021:

____

Sandro Santos, Dr. (UFSM) - Videoconferência
(Presidente/Orientador)

Vania Lucia Loro, Drª. (UFSM) - Parecer
(Coorientadora)

____

André Passaglia Schuch, Dr. (UFSM) - Parecer

____

Bárbara Estevão Clasen, Drª. (UERGS) – Parecer

____

Charlene Cavalheiro de Menezes, Drª. (UFSM) - Parecer

____

Luciane Ayres Peres, Drª. (IFFar) - Parecer

Santa Maria, RS
2021

DEDICATÓRIA

Houve momentos em que me perdi de mim, briguei com a vida por ela não ser justa, cai e encontrei o fundo. Pelos que amo encontrei força, escalei aos poucos, aprendi que embora dolorosa, a vida nos traz pessoas que, apesar de não mais estarem aqui fisicamente, levaremos em nossa memória para sempre. Obrigada pelo seu último sorriso ter sido para mim, obrigada por ter sido minha segunda mãe, te amo para sempre...

Veninha, dedico essa tese a ti.

À Venilde Marchezan Zarantonelo

AGRADECIMENTOS

A pós-graduação tem de fato um percurso tortuoso, às vezes bem difícil, mas isso se torna mais leve e gratificante quando temos ao nosso lado pessoas que nos apoiam.

Gostaria de agradecer primeiramente ao meu pai Antonio Cerezer, que é meu maior incentivador, e aos demais membros da minha família: a minha mãe Leonir, meu irmão Cristiano, meu sogro Adelmo Zarantonelo e as minhas cunhadas Bruna e Michele, dos quais tive muito suporte emocional para conseguir sobreviver aos meus piores momentos.

Ao meu companheiro Charles Zarantonelo, a quem serei eternamente grata por estar comigo em todos os campos desde o mestrado, campos esses que as vezes fazíamos chorando, mas que eram também nossa válvula de escape. Te amo imensamente, meu melhor IC, meu melhor amigo, só não desisti porque tive teu apoio, tu sabes disso, te escolheria mil vezes.

À minha coorientadora Vania Loro, por ter me acolhido com muito carinho desde 2015, ter me oportunizado trabalhar em uma nova área, ter me dado acesso a toda a estrutura física do seu laboratório.

Ao meu primeiro e único Orientador Sandro Santos, que me aguenta desde 2011, que me proporcionou meu crescimento como pesquisadora e me deu o suporte necessário para a realização dessa tese, muito obrigada por não ter desistido de mim.

A minha amigona e colega Aline Marins, pela amizade, pelos puxões de orelha, pelo apoio técnico, emocional, por não desistir de mim, pelas excelentes revisões, por todos os congressos e cursos. Poderia passar horas aqui te agradecendo, és uma grande professora e Pesquisadora, me espelho muito em ti, só tenho a te agradecer, tu sempre foste meu braço direito;

Ao meu amigo e colega Eduardo Severo, obrigada pelas conversas, parceria cientifica e momentos de diversão, mesmo quando estávamos sob pressão, és um grande professor e Pesquisador, nunca te deixe pensar ao contrário disso;

Ao meu primo Felipe, não só pelo suporte técnico nas análises estatísticas, mas principalmente pelo apoio na vida, te amo Lipinho, és meu orgulho;

As minhas amigas que tornam minha vida mais leve: Bruna Palese, Bruna Ceretta Ferreira, Aline Portela, Maiara Costa, Talise Müller, Fabiana Coradini, Karine Ariotti e as minha Ramers do coração e tantos outros que são importantes na minha vida;

A Tiger Fight Clan minha eterna gratidão, levo vocês na pele e no meu coração, não fomos só uma equipe, fomos parceiros pra vida, treinar com vocês me salvou por anos; A Eliana e a Winnie, agradeço por compartilharmos tantos momentos juntas, pelo apoio e por acreditarem mais em mim do que eu mesma; e ao meu mestre e amigo Fabio, obrigada por todos os conselhos e ensinamentos, pelas palavras sempre bem colocadas após os treinos, sempre buscando o nosso equilíbrio mental e físico, nunca largaste minha mão e sempre lutou para me afastar da depressão, te amo.

Aos colegas do Labtaq, em especial a minha amiga Jossiele Leitemperger pelo apoio, revisões, conversas e por ter sido a primeira a me ensinar as técnicas bioquímicas e a Aline Blank do Amaral pelas revisões;

Aos alunos da disciplina de Toxicologia Ambiental que me ensinaram muito e fizeram despertar em mim a vontade de fazer a licenciatura e me mostraram toda a beleza da docência;

A minha psicóloga Luizara Giacomini, meu psiquiatra Álvaro Nascimento e a todos os profissionais da saúde que me atenderam e cuidaram de mim com tanto carinho;

Aos meus coautores, essenciais em todos os trabalhos;

Aos cidadãos de Faxinal do Soturno, que me permitiram o acesso aos riachos;

À minha banca por aceitarem fazer parte deste momento tão importante para mim;

À UFSM, ao PPG em Biodiversidade Animal, ao LARP, LABTAQ e à CAPES;

Aos meus doguinhos Fred e Snow e minhas gatas Safira e Dandara que me deram muito carinho e amor, sempre ao meu lado durante as infinitas horas de escrita, com suas digitais no teclado e escritas indecifráveis nos textos, me proporcionaram momentos fofos;

Agradeço a todos aqueles que direta ou indiretamente contribuíram para a concretização deste trabalho. Quero compartilhar com cada um a felicidade deste momento, que para mim simboliza uma grande superação. Para mim defender a tese não simboliza só o fim de uma etapa, mas o recomeço de uma nova caminhada, Muito obrigada!

“Aqueles que contemplam a beleza da terra, encontram reservas de força que irão perdurar enquanto a vida durar. Há algo infinitamente curativo nos refrões repetidos da natureza: a garantia de que o amanhecer vem depois da noite e a primavera depois do inverno.”

(Rachel Carson)

RESUMO

CONSERVAÇÃO DE CRUSTÁCEOS LÍMNICOS: EFEITO DA TEMPERATURA E PESTICIDAS NO METABOLISMO OXIDATIVO DE *AEGLA LONGIROSTRI*, *IN SITU* E *EX-SITU*.

AUTORA: Cristina Cerezer
ORIENTADOR: Sandro Santos
COORIENTADORA: Vania Lucia Loro

Os ecossistemas de água doce, especialmente riachos de baixa ordem, são constantemente ameaçados pelo avanço das atividades agrícolas, que acabam contaminando estes locais com pesticidas e degradando a vegetação em seu entorno, podendo causar efeitos deletérios nos organismos aquáticos. Nesses ecossistemas encontra-se crustáceos eglídeos, que são fragmentadores importantes e são sensíveis a perturbações ambientais. Desta maneira, o objetivo desta tese foi identificar e avaliar como pesticidas e variáveis abióticas afetam a sobrevivência, o comportamento e o estresse oxidativo no crustáceo límnico *Aegla longirostri*. No estudo apresentado no primeiro capítulo os animais foram expostos *in situ* em quatro riachos (local de referência, locais 1, 2 e 3). O local de referência é um riacho preservado sem interferência antropogênica, com ocorrência de eglídeos, enquanto os outros locais não exibem mais populações desses animais e são influenciados por atividades agrícolas. As exposições foram realizadas bimestralmente de novembro de 2017 a setembro de 2018 e teve duração de 96 horas. Foram medidos parâmetros abióticos e amostras de água foram coletadas durante todos os dias de exposição. Os parâmetros bioquímicos analisados foram a atividade da acetilcolinesterase (AChE) no músculo; atividade de glutationa S-transferase (GST), níveis de peroxidação lipídica (TBARS), conteúdo de proteína carbonilada (CP), níveis de tióis não proteicos (NPSH), capacidade antioxidante contra peróxidos (ACAP) e espécies reativas de oxigênio (ROS) em músculos, brânquias e hepatopâncreas. Encontramos 24 princípios ativos de pesticidas, sendo os mais frequentes Clomazone, Atrazina e Propoxur. A Bentazona foi o pesticida encontrado em maiores quantidades. Os parâmetros avaliados neste estudo, incluindo biomarcadores bioquímicos e fatores abióticos medidos na água, proporcionaram uma separação dos meses em função das condições ambientais. Houve diferença de atividade e níveis de biomarcadores ao longo do ano dentro do mesmo ponto e em alguns meses entre os pontos. A maior concentração ou variedade de pesticidas associada a dados abióticos extremos (temperaturas muito altas) foi capaz de gerar aumento do estresse oxidativo, com altos níveis de CP, TBARS e ROS em todos os tecidos, mesmo com níveis elevados de ACAP e NPSH. No capítulo 2 os animais foram expostos em condições de laboratório a 18 °C, 21 °C, 24 °C e 26 °C, durante 48 horas, buscando compreender se a temperatura sozinha afeta a taxa de sobrevivência, os biomarcadores bioquímicos e respostas comportamentais. Houve mudanças significativas nos parâmetros bioquímicos em diferentes tecidos e nos testes comportamentais em *A. longirostri*. O hepatopâncreas foi especialmente afetado pela elevação da temperatura, como demonstrado pelos altos níveis de CP. A atividade da AChE aumentou de forma dependente da temperatura no músculo. A atividade da GST diminuiu com a elevação da temperatura em todos os tecidos amostrados. Com esses dados, pretende-se alertar sobre os riscos da exposição a essas condições ambientais, tentando contribuir para a preservação da fauna límnica e principalmente dos crustáceos eglídeos, uma vez que a maioria das espécies está sob algum grau de ameaça. Os resultados obtidos neste estudo indicam que, ao avaliar a saúde de ecossistemas límnicos poluídos por meio do uso de organismos bioindicadores, deve-se considerar o efeito intrínseco de fatores abióticos, como a temperatura, sobre os biomarcadores.

Palavras-chave: Biomarcador. Ecotoxicologia. Eglídeos. Crustáceos. Poluição aquática.

ABSTRACT

CONSERVATION OF LIMNIC CRUSTACEANS: EFFECT OF TEMPERATURE AND PESTICIDES ON THE OXIDATIVE METABOLISM OF *AEGLA LONGIROSTRI*, *IN SITU* AND *EX-SITU*.

AUTHOR: Cristina Cerezer
ADVISOR: Sandro Santos
CO-ADVISOR: Vania Lucia Loro

Freshwater ecosystems, especially low-order streams, are constantly threatened by the advance of agricultural activities that end up contaminating these places with pesticides and degrading the surrounding vegetation, which can cause deleterious effects on aquatic organisms. In these ecosystems, we find eglid crustaceans that are important shredders and are sensitive to environmental disturbances. Thus, the aim of this thesis was to identify and evaluate how pesticides and abiotic variables affect survival, behavior and oxidative stress in the limnic crustacean *Aegla longirostri*. In the first chapter, the animals were exposed *in situ* in four streams (reference site, sites 1, 2, and 3). The reference site is a stream preserved without anthropogenic interference with occurrence of eglids, while the other sites no longer exhibit populations of these animals and are influenced by agricultural activities. The exposures were held bi-monthly from November 2017 to September 2018 and lasted 96 hours. Abiotic parameters were measured and water samples were collected during all exposure days. The biochemical parameters analyzed were muscle acetylcholinesterase (AChE) activity; glutathione S-transferase (GST) activity, lipid peroxidation (TBARS) levels, carbolinated protein (CP) content, non-protein thiols (NPSH) levels, antioxidant capacity against peroxides (ACAP), and reactive oxygen species (ROS) in muscles, gills, and hepatopancreas. We found 24 active pesticide ingredients, the most frequent being Clomazone, Atrazine, and Propoxur. Bentazone was the pesticide found in the greatest amounts. The parameters evaluated in this study, including biochemical biomarkers and abiotic factors measured in water, provided a separation of months depending on environmental conditions. There were differences in activity and levels of biomarkers throughout the year within the same point and in a few months between points. The highest concentration or variety of pesticides associated with extreme abiotic data (very high temperatures) was able to generate increased oxidative stress with high levels of TBARS, and ROS in all tissues, even at high levels of ACAP and NPSH. In chapter 2 the animals were exposed under laboratory conditions at 18°C, 21°C, 24°C, and 26°C for 48 hours, aiming to understand whether temperature alone affects survival rate, biochemical biomarkers and behavioral responses. There were significant changes in biochemical parameters in different tissues and in behavioral tests in *A. longirostri*. The hepatopancreas was especially affected by the rise in temperature, as demonstrated by the high levels of CP. The AChE activity increased in a temperature-dependent manner in the muscle. The GST activity decreased with increasing temperature in all tissues sampled. With these data, it is intended to alert about the risks of exposure to these environmental conditions, trying to contribute to the preservation of the limnic fauna and especially the eglid crabs, since most species are under some degree of threat. The results obtained in this study indicate that, when evaluating the health of polluted limnic ecosystems through the use of bioindicator organisms, the intrinsic effect of abiotic factors, such as temperature, on biomarkers should be considered.

Keywords: Biomarker. Ecotoxicology. Eglids. Crustaceans. Water pollution.

LISTA DE ILUSTRAÇÕES

INTRODUÇÃO

CAPÍTULO 1

CAPÍTULO 2

LISTA DE TABELAS

CAPÍTULO 1

CAPÍTULO 2

SUMÁRIO

1 INTRODUÇÃO

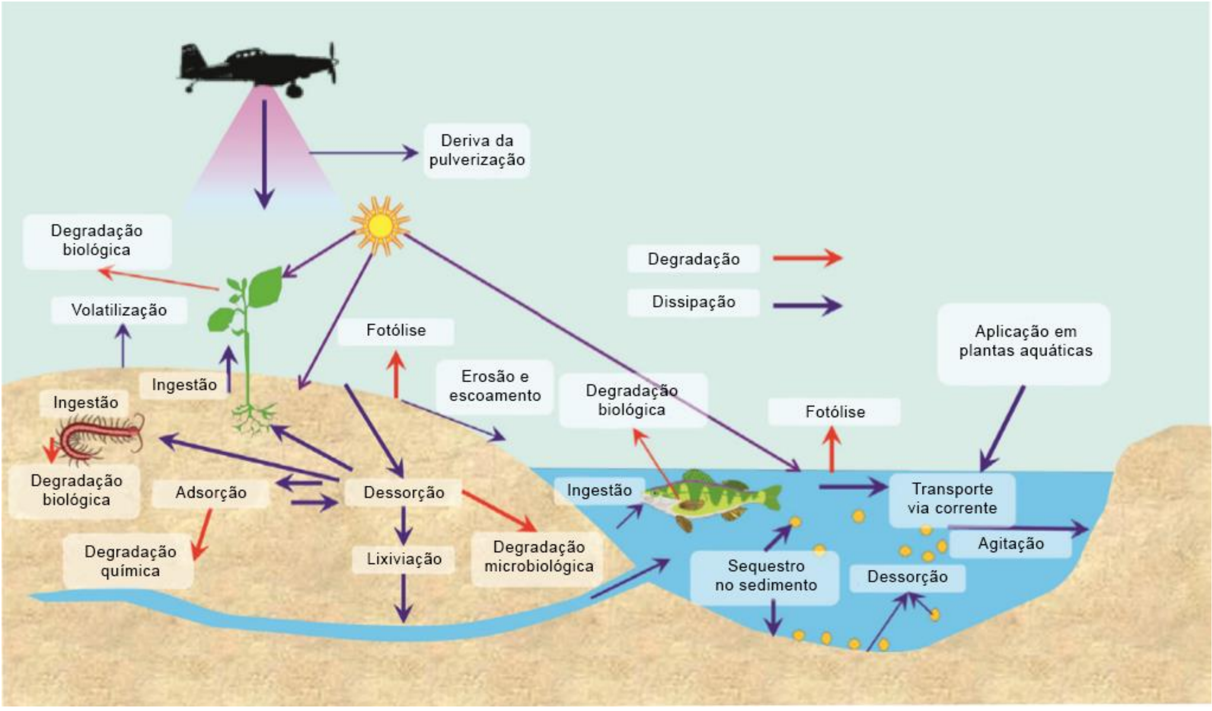
Neste tópico está abordado brevemente a temática da tese e exponho a problemática a ser desenvolvida ao longo dos capítulos.

1.1 PESTICIDAS EM AMBIENTES LÍMNICOS

O consumo indiscriminado de agroquímicos, que são utilizados durante todo o processo de produção agrícola vem sendo um problema muito preocupante. O mercado brasileiro de insumos agrícolas cresceu 190% nos últimos anos sendo hoje o maior mercado de agrotóxicos do mundo (ALBUQUERQUE et al., 2016; GHISI et al., 2017). Dentre os pesticidas, os herbicidas e os inseticidas são os mais utilizados no país (PELAEZ et al., 2009), sendo muitas vezes, aplicados indiscriminadamente sem uma conscientização adequada de suas consequências ambientais, podendo levar a extinção de espécies não alvo (ANDREA et al., 2000). Esse tipo de poluente não é monitorado com frequência apesar de apresentar potencial causador de efeitos adversos a saúde humana e a ambientes aquáticos, o que os torna poluentes emergentes (GEISSEN et al., 2015).

Os ambientes límnicos estão entre os ecossistemas mais ameaçados globalmente, sendo o uso abusivo de pesticidas uma das principais ameaças à qualidade e integridade dos recursos hídricos brasileiros (ANA, 2012; DUDGEON et al., 2006). Além disso, diversos trabalhos de monitoramento de pesticidas em recursos hídricos demonstram a presença de grande diversidade de princípios ativos (LORO et al., 2015; MARINS et al 2020; SEVERO et al., 2020). A presença de pesticidas em amostras ambientais revela que a maior parte dos pesticidas, independentemente do modo de aplicação, acaba entrando em ecossistemas aquáticos, principalmente devido aos ventos, a deposição atmosférica e a água das chuvas, que promovem o escoamento superficial, a lavagem de folhas tratadas, a lixiviação e a erosão (Figura 1) (TOMITA, BEYRUTH, 2002; SILVA, FAY, 2004).

Figura 1 – Rotas e destino de resíduos de pesticidas no ambiente



Fonte: Marins, 2020, p.23, adaptado de Solomon et al., 2013, p. 373.

O ingresso desses poluentes nos recursos hídricos implica na necessidade de uma estimativa de seus efeitos nos organismos presentes nesses locais, a curto e longo prazo (SISINNO e OLIVEIRA-FILHO, 2013). Ao adentrar nos ambientes aquáticos, os pesticidas, podem interagir com os organismos que ali habitam através das vias dérmicas, respiratórias e/ou oral, podendo causar alterações bioquímicas e fisiológicas, induzindo diferentes mecanismos de toxicidade (CORREIA et al., 2003; LUSHCHAK et al., 2018; STEINBERG et al., 2008). Dentro do organismo, essas substâncias poderão ser biotransformadas e excretadas sem causar alterações, porém, a maioria destes poluentes são conhecidos por causarem algum tipo de efeito negativo (STOLIAR e LUSHCHAK, 2012).

1.2 FATORES ABIÓTICOS E EXPOSIÇÃO

Além dos pesticidas vindos das atividades agrícolas, temos outros fatores relacionados à sazonalidade, os quais também podem influenciar a resposta de biomarcadores em estudos de ecotoxicologia de campo (DALZOCHIO e GEHLEN, 2016; SARDI et al., 2016). Fatores ambientais como pH, oxigênio dissolvido, nitrito, amônia e, principalmente, a temperatura da água são capazes de afetar o crescimento e a sobrevivência dos animais aquáticos (WANG et al., 2006).

Entre esses fatores, a temperatura é o mais evidente, especialmente se o animal-alvo for um ectotérmico, uma vez que a temperatura governa todos os seus processos metabólicos (BAGNYUKOVA et al., 2007; GANDAR et al., 2017; HEMMER-BREPSON et al., 2014; KAMYAB et al., 2017; VINAGRE et al., 2014a). Altas temperaturas podem aumentar a ingestão e toxicidade de moléculas orgânicas, aumentando o potencial tóxico de poluentes químicos, acelerando o metabolismo e esgotando as reservas de energia (LAETZ et al., 2014; NADAL et al., 2015). Além disso, um aumento na temperatura da água aumenta a demanda por oxigênio e aumenta a atividade respiratória (ISSARTEL et al., 2005). Em ambientes poluídos, uma taxa respiratória mais alta pode causar uma maior absorção de poluentes (DELORENZO, 2015; HEUGENS et al., 2001).

A exposição de animais a contaminantes, diretamente no meio ambiente (*in situ*), traz vantagens na pesquisa científica, fornecendo respostas realísticas sobre o que acontece no meio ambiente, pois integra os efeitos das variáveis ambientais com os decorrentes da exposição a contaminantes, o que não seria viável em estudos de laboratório (OIKARI, 2006). Porém, as exposições em laboratório também são importantes, pois fornecem endpoints basais da exposição a diferentes condições ambientais de maneira controlada (CAILLEAUD et al., 2007; LOUIZ et al., 2016).

1.3 BIOMARCADORES

Uma forma de se verificar futuras alterações a nível populacional é verificando alterações a nível celular, para isso tem sido utilizadas as análises bioquímicas em tecidos de organismos expostos. Biomarcadores bioquímicos relacionados ao estresse oxidativo são amplamente usados quando os organismos são expostos a estressores ambientais (AMARAL et al., 2018; MARINS, 2020; SEVERO et al., 2020). O estresse térmico ou outras variáveis abióticas alteradas associadas à exposição a xenobióticos podem aumentar a produção de espécies reativas de oxigênio (ROS) e induzir estresse oxidativo (AHMED, 2005; HALLIWELL, 1994).

As ROS são normalmente produzidas pelo corpo e neutralizadas por antioxidantes enzimáticos e não enzimáticos. O estresse oxidativo ocorre quando a concentração de ROS aumenta cronicamente, interrompendo o metabolismo e a regulação celular, causando danos aos constituintes celulares, como proteínas, lipídios e ácidos nucleicos (LUSHCHAK, 2011). As células contêm enzimas antioxidantes que limitam as reações causadas pelas ROS, sendo um importante mecanismo de proteção para minimizar o dano oxidativo celular (LEMAIRE, LIVINGSTONE, 1993; LIVINGSTONE, 2001; WINSTONE, DI GIULIO, 1991). Para verificar o estresse oxidativo em um organismo uma série de biomarcadores podem ser usados (LÓPEZ-DOVAL et al., 2015).

Entre os biomarcadores de efeitos biológicos (HOOK et al., 2014) usados para avaliar os impactos de diferentes poluentes nos ecossistemas naturais está a enzima glutationa-S-transferase, responsável pela detoxificação de xenobióticos e a enzima acetilcolinesterase, um marcador de neurotoxicidade (GONÇALVES et al., 2018; MARINS, 2020). São exemplos também as medidas de atividade de enzimas antioxidantes, como a catalase, níveis de antioxidantes não enzimáticos, como tióis não proteicos, conteúdo de proteína carbonilada, entre outros (LORO et al., 2015).

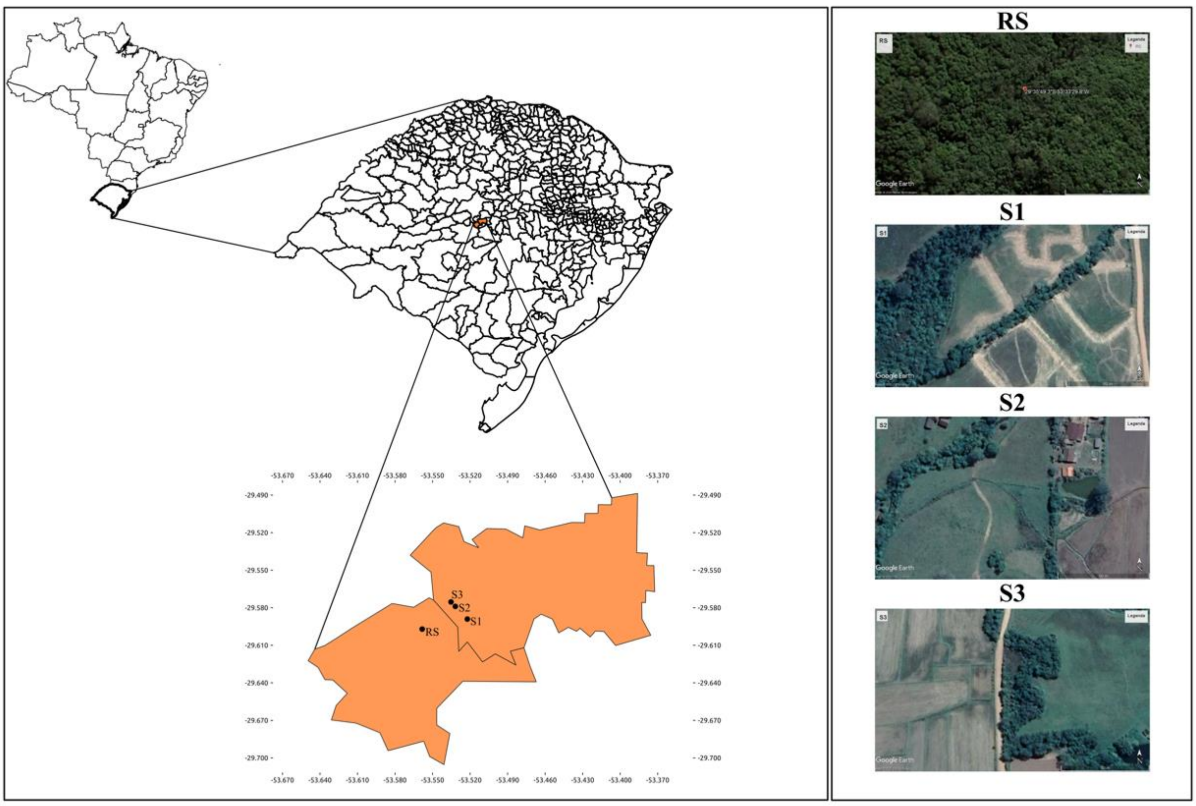
Outro biomarcador importante é o comportamental, estudos comportamentais são usados como uma ferramenta adicional para testes ecotoxicológicos e monitoramento da qualidade da água. Em geral, eles permitem a associação entre os efeitos tóxicos obtidos em níveis bioquímicos e celulares sobre os impactos observados na dinâmica de populações e comunidades (FELTEN, GUEROLD, 2001; MALTBY et al., 2002; WALLACE, ESTEPHAN, 2004). Por exemplo, as mudanças na temperatura corporal, além de afetar taxas fisiológicas e reações bioquímicas, podem afetar o comportamento de animais aquáticos ectotérmicos (BRIFFA et al., 2013).

1.4 LOCAL DE ESTUDO

Os locais utilizados nessa tese foram escolhidos por apresentarem eglídeos em sua composição faunística, segundo relatos locais e conhecimento da própria autora. Todos os pontos localizam-se a menos de 4 km do sítio referência (RS, *reference site*) e possuem fisiografia semelhante, ou seja, atendiam, a princípio, às condições básicas para a presença de eglídeos (Figura 2). O RS onde os animais foram coletados para ambos os artigos se trata de um ambiente lótico na cidade de Silveira Martins, Rio Grande do Sul (RS), Brasil (29º35'49.301 "S 53º33'29.844" W). O local apresenta uma floresta estacional semidecidual com vegetação ripária diversa e com uma aparente ausência de impactos antrópicos.

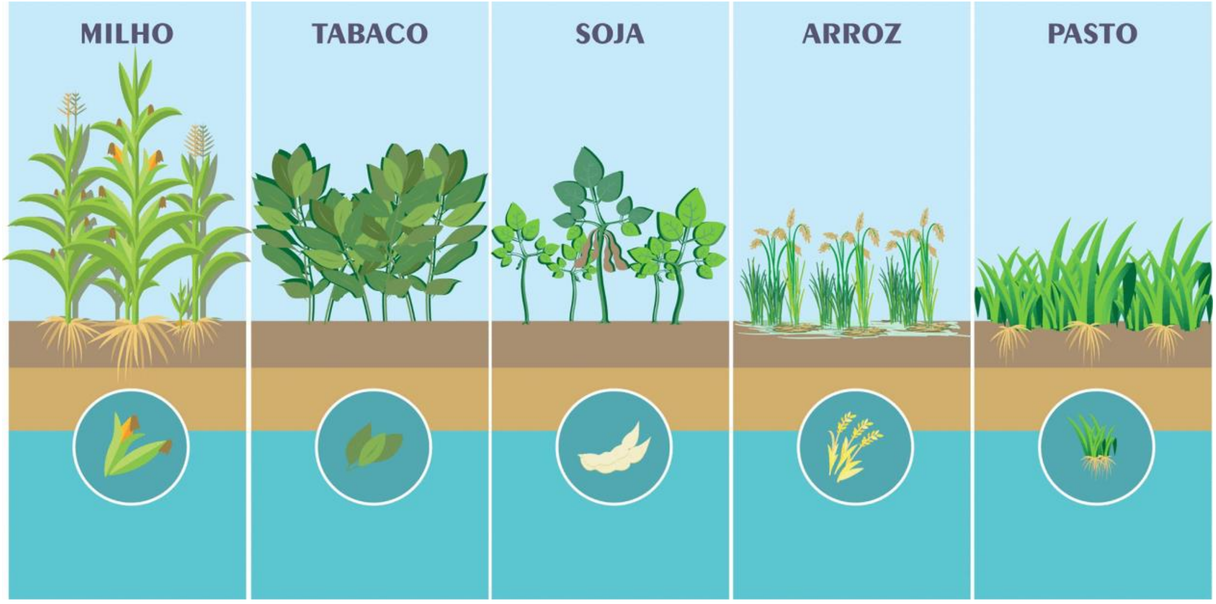
Os locais de exposição são riachos de pequeno porte, com floresta estacional semidecidual com pouca vegetação ribeirinha e impactos antrópicos semelhantes relacionados à agricultura e pecuária. As principais culturas presentes no entorno desses riachos são arroz, soja, milho, fumo, cana-de-açúcar e pastagens (Figura 3). Os riachos estão localizados no município de Faxinal do Soturno - Rio Grande do Sul, nas seguintes coordenadas geográficas: local 1 (S1) em (29º35'20.476 "S 53º31'19.826" W), local 2 (S2) em (29º34'44.026 "S 53º31'54.365" W), local 3 (S3) em (29 ° 34'31.249 "S 53 ° 32'6.814" W).

Figura 2 – Mapa do local de coleta e locais de exposição



Fonte: Autora.

Figura 3 – Ilustração das principais culturas no entorno dos riachos



Fonte: Autora

Além de regular a quantidade de água, a cobertura florestal de bacias hidrográficas desempenha um papel importante na manutenção da qualidade da água, regulando a quantidade de luz, nutrientes e sedimentos que atingem os riachos. As reduções na qualidade da água devido à perda de florestas em bacias hidrográficas podem ser exacerbadas pelas atividades que acompanham os usos de terras agrícolas adjacentes, incluindo a instalação de barragens e represas agrícolas, degradação de áreas ribeirinhas, desvio ou retirada de água e adição de fertilizantes químicos e pesticidas (PRINGLE, 2001). Na Figura 4 é possível verificar que, após a ocorrência de chuva, o sítio referência possui uma água mais límpida que os demais, isso ocorre devido à ausência de lavouras no seu entorno e da preservação da mata ciliar.

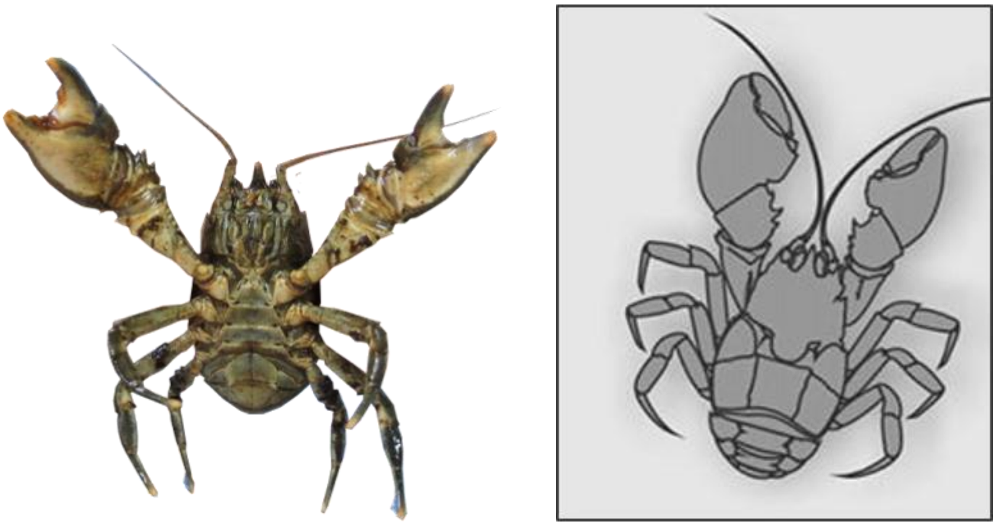
Figura 4 – Riachos depois de uma chuva

Fonte: Autora

1.5 ORGANISMOS DE ESTUDO

A maioria dos crustáceos de água doce apresenta alto risco de extinção, tornando as investigações sobre esses animais de fundamental importância (CUMBERLIDGE et al., 2009). Os eglídeos (Figura 5), por exemplo, único grupo de anomuros com ciclo de vida completo em cursos de água continentais, apresentam quase 70% das espécies sob algum grau de ameaça (SANTOS et al., 2017). Nos riachos do sul da América do Sul, os eglídeos são importantes trituradores e participam ativamente do processo de decomposição de detritos foliares, sendo espécies-chave na ciclagem de nutrientes. Eles desempenham um papel importante na cadeia trófica dos ecossistemas aquáticos, onde atuam como predadores de insetos imaturos e servem como alimento para peixes, pássaros, anfíbios e mamíferos (ARENAS, 1976; CEREZER, 2016; COGO, SANTOS 2013; MAGNI, PY-DANIEL, 1989; PARDINI, 1998). São encontrados em baixas temperaturas e riachos bem oxigenados, são sensíveis a perturbações e / ou variações ambientais abruptas, as quais podem levar à redução ou desaparecimento de suas populações (BOND-BUCKUP, SANTOS, 2007; TREVISAN et al., 2009). Portanto, por terem um papel importante na transferência de energia na cadeia alimentar e serem sensíveis às mudanças ambientais, esses animais podem atuar como bons bioindicadores da qualidade ambiental (BOND-BUCKUP, BUCKUP, 1994; FERREIRA et al., 2005; OLIVEIRA et al., 2007; TREVISAN et al., 2009). Além disso, devido à sua estrutura corporal, eles são facilmente distinguíveis de outros organismos aquáticos, exibem dimorfismo sexual (eliminando assim o fator sexual dos experimentos) e fornecem biomassa suficiente para a análise de biomarcadores bioquímicos (BOND-BUCKUP, BUCKUP, 1994; BORGES et al., 2018).

Figura 5 – Exemplar macho de *Aegla longirostri*, vista ventral, e um desenho do exemplar em vista dorsal



Fonte: Autora

2 OBJETIVOS

2.1 OBJETIVO GERAL

Identificar e avaliar como pesticidas e variáveis abióticas podem afetar a sobrevivência, comportamento e o estresse oxidativo no crustáceo límnico *Aegla longirostri*.

2.2 OBJETIVOS ESPECÍFICOS

- Verificar quais são os pesticidas encontrados em maior quantidade e frequência em riachos de baixa ordem;
- Avaliar se a combinação desses pesticidas com as demais variáveis abióticas em riachos com interferência antrópica gera alterações nos biomarcadores bioquímicos em *Aegla longirostri*, identificando possíveis vulnerabilidades para eglídeos;
- Avaliar os efeitos de diferentes temperaturas isoladamente em biomarcadores bioquímicos e no comportamento do crustáceo de água doce *Aegla longirostri.*

3 CAPÍTULO 1

Influence of pesticides and abiotic conditions on biochemical biomarkers in *Aegla aff. longirostri* (Crustacea, Anomura): implications for conservation

Cristina Cerezer[a*], Aline Teixeira Marins[a], Felipe Osmari Cerezer[a], Eduardo Stringini Severo[a], Jossiele Wesz Leitemperger[b], Nelson Miguel Grubel Bandeira[c], Renato Zanella[c], Vania Lucia Loro[a.b], Sandro Santos[a]

[a] Programa de Pós-Graduação em Biodiversidade Animal, Departamento de Ecologia e Evolução, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Avenida Roraima, 1000, Bairro Camobi, CEP 97105-900, Santa Maria, Rio Grande do Sul, Brasil.

[b] Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Avenida Roraima, 1000, Bairro Camobi, CEP 97105-900, Santa Maria, Rio Grande do Sul, Brasil.

[c] Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Avenida Roraima, 1000, Bairro Camobi, CEP 97105-900, Santa Maria, Rio Grande do Sul, Brasil.

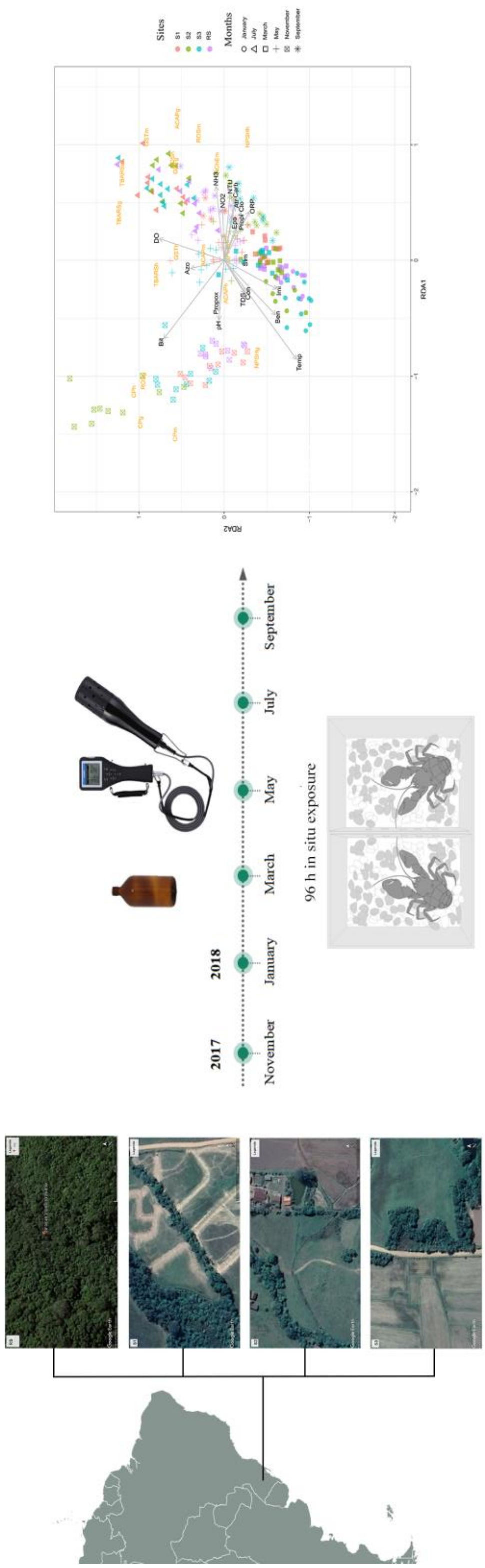
[*]Corresponding author
Cristina Cerezer
cristhinacerezer@gmail.com
55 55 30262449

O artigo foi publicado no periódico *Ecotoxicology and Environmental Safety.*
Doi: https://doi.org/10.1016/j.ecoenv.2020.110982

HIGHLIGHTS

- We found 24 active principles of pesticides in water samples.
- Bentazone was the pesticide found in the largest concentration.
- There was a difference in levels of biomarkers throughout the year.
- In situ exposure at agricultural region induces alterations in Aegla longirostri.
- Poor water quality in streams near crops threatens aquatic organisms.

GRAPHICAL ABSTRACT



ABSTRACT

Freshwater ecosystems are constantly threatened by the advance of agricultural activities. Abiotic variables (such as temperature, ammonia, and nitrite) and contaminants (e.g. pesticides) can potentially interact, increasing metabolism and the absorption of toxic substances, which can alter the ability of organisms to establish adequate stress responses. This study aimed to verify which pesticides were most frequently found and in the greatest quantities in low-order streams, and whether the combination of these pesticides with the abiotic variables altered the biological metabolism of aeglids. These freshwater crustaceans are important shredders that inhabit low-order streams and are sensitive to disturbances and/or abrupt environmental variations. The animals were exposed in situ in four streams (reference site and sites 1, 2, and 3). The reference site is a preserved stream with no apparent anthropogenic interference where aeglids still occur, while the other sites no longer exhibit populations of these animals and are influenced by agricultural activities. The exposure was performed bimonthly from November 2017 to September 2018 and lasted 96 h. Measured abiotic data and water samples were collected through all days of exposure. The analyzed biochemical parameters were acetylcholinesterase activity in muscle; and glutathione S-transferase, lipid peroxidation, protein carbonylation, non-protein thiols, antioxidant capacity against peroxides, and reactive oxygen species (ROS) in muscle, gills, and hepatopancreas. We found 24 active principles of pesticides, the most frequently being clomazone, atrazine, and propoxur. Bentazone was present at the highest amounts. The parameters evaluated in this study, including biochemical biomarkers and abiotic factors measured from the water, provided a separation of the months as a function of environmental conditions. There was a difference in activity and biomarker levels throughout the year within the same site and in some months between sites. The greater concentration or variety of pesticides associated with extreme abiotic (very high temperatures) data generated increased oxidative stress, with high levels of protein damage and considerable lipid damage in all tissues, as well as elevation in ROS, even with high levels of antioxidant capacity and non-protein thiols. With these data, we intend to warn about the risks of exposure to these environmental conditions by trying to contribute to the preservation of limnic fauna, especially aeglid crabs, because most species are under some degree of threat.

KEYWORDS: Decapods. Ecotoxicology. Oxidative stress. Pesticides. Toxicity.

1 INTRODUCTION

Limnic environments are among the most threatened ecosystems throughout the world (Dudgeon et al., 2006; Pinheiro et al., 2015). Contamination caused by agricultural activities has a marked impact on life in these places, mainly through the abusive use of pesticides to increase agricultural production (ANA, 2012; Özkara et al., 2016; Swackhamer et al., 2004). The movement of pesticides into water bodies can occur through subsurface drainage, leaching, run-off, and spray drift (Cosgrove et al., 2019). These compounds can cause biochemical and physiological changes in both non-target organisms and humans, inducing different toxicity mechanisms (Correia et al., 2003; Lushchak et al., 2018; Steinberg et al., 2008; Zhou et al., 2019).

Other environmental factors can affect the growth and survival of aquatic animals, such as pH, dissolved oxygen, nitrite, ammonia, and, in particular, water temperature (Wang et al., 2006). High temperatures can increase the intake and toxicity of organic molecules, amplifying the toxic potential of chemical pollutants, accelerating metabolism, and depleting energy reserves (Laetz et al., 2014; Nadal et al., 2015). This phenomenon can indirectly affect the ability of organisms to establish efficient defense responses against chemicals to limit cellular damage (Gandar et al., 2017, 2015; Kennedy and Ross, 2011). Aeglid crustaceans, for example, exhibit significant biochemical and behavioral changes when exposed to high temperatures or low oxygen concentrations (Cerezer, 2017; Dalosto and Santos, 2011). Thus, thermal stress or other altered abiotic variables associated with exposure to xenobiotics may increase the body's ability to produce reactive oxygen species (ROS) and cause oxidative stress (Ahmed, 2005; Halliwell, 1994).

Oxidative stress occurs when the ROS concentration increases chronically, disrupting cellular metabolism and regulation, with consequent damage to cellular constituents such as proteins, lipids, and nucleic acids (Lushchak, 2011). Cells contain antioxidant enzymes that limit the reactions caused by ROS. It is an important protection mechanism to minimize oxidative damage in cells (Lemaire and Livingstone, 1993; Livingstone, 2001; Winstone and Di Giulio, 1991). A number of biomarkers can be used to assess oxidative stress in an organism (López-Doval et al., 2015).

The exposure of animals to contaminants directly in the environment (*in situ*) has several advantages in scientific research. Indeed, this method provides answers to what really happens in the environment, besides considering previous knowledge of the exposure sites; the precise duration of exposure; selection of species and individuals by age, size, and sex; repeatability; and standardization. *In situ* studies also allow for the integration of the effects of environmental variables with those resulting from exposure to contaminants. This eventuality would not be feasible in laboratory studies (Oikari, 2006). Thus, *in situ* exposure of aquatic organisms is a good way to investigate the effect of pesticide leaching from agricultural areas to freshwater bodies (Vieira et al., 2016).

Crustaceans of the Aeglidae family are found in southern streams of Brazil. These animals are important shredders that participate in the process of decomposition of foliar debris, thus being key species in nutrient cycling (Cogo and Santos, 2013). These aeglids inhabit low-order streams and are sensitive to disturbances and/or abrupt environmental variations, as these can lead to the reduction or disappearance of their populations (Bond-Buckup and Santos, 2007; Trevisan et al., 2009). Therefore, because they have an important role in the transfer of energy in the food chain and are sensitive to environmental changes, these animals can function as good bioindicators of environmental quality (Bond-Buckup and Buckup, 1994; Ferreira et al., 2005; Oliveira et al., 2007; Trevisan et al., 2009). Furthermore, due to their body structure, they are easily distinguishable from other aquatic organisms, exhibit sexual dimorphism (thus eliminating the sex factor of experiments), and provide sufficient biomass for the analysis of biochemical biomarkers (Bond-Buckup and Buckup, 1994; Borges et al., 2018).

Thus, this study aimed to verify which pesticides are found and in the greatest quantities in low-order streams. This endeavor allowed us (1) to evaluate whether the combination of these pesticides with the other abiotic variables in streams with anthropogenic interference alters biochemical biomarkers in aeglids; (2) to analyze whether there are differences in biomarker responses to these pesticides; and (3) determine if there are any times at which time these organisms would be most vulnerable. Studies that investigate the levels of oxidative stress in aeglids in the face of different environmental conditions can be of great relevance for understanding environmental impacts in the subtropical region.

2 MATERIALS AND METHODS

2.1 DESCRIPTION OF THE STREAM AND COLLECTION OF SPECIMENS

Aegla longirostri were collected in a lotic environment in the city of Silveira Martins, Rio Grande do Sul (RS), Brazil (29º35'49.301"S 53º33'29.844"W). The site presents a semideciduous seasonal forest with diverse riparian vegetation, with an apparent absence of anthropogenic impacts. All animals were collected from the same stream. The individuals were collected with funnel traps, made with polyethylene terephthalate bottles (PET) with their conical mouth inverted and containing swine liver baits, as well as through manual collection. The traps were placed at dusk and removed the next day during the morning, since the animals are nocturnal. We only used males in intermolt for the experiments because there is a difference in the compounds in the hepatopancreas between males and females. Furthermore, during the molting stage aeglids pass through a fasting period (Ferreira et al., 2005; Oliveira et al., 2003). After collection, the length of the cephalothorax was measured using a digital caliper (accuracy: 0.01 mm) to prove that they were adults. In addition, we standardized the size of the males to an average of 20 mm of cephalothorax length, because the size indicative of its age.

2.2 DESCRIPTION OF THE EXPOSURE SITES

The collected animals were exposed *in situ* to one of three streams or the collection stream, which is the reference site (RS). The exposure sites are small-order streams, with semi-deciduous seasonal forest with little riparian vegetation and similar anthropogenic impacts related to agriculture and livestock. The main crops present in the area surrounding these streams are rice, soybean, corn, tobacco, sugar cane, and pastures. The streams are located in the municipality of Faxinal do Soturno, Rio Grande do Sul state, at the following geographical coordinates: site 1 (S1), 29º35'20.476"S 53º31'19.826"W; site 2 (S2), 29º34'44.026"S 53º31'54.365"W; and site 3 (S3), 29°34'31.249"S 53°32'6.814"W. All points are located less than 4 km from RS and have similar physiography; that is, they meet, in principle, basic conditions for the presence of aeglids. A map of the sites is provided in supplementary material (Fig. S1).

2.3 EXPOSURE *IN SITU*

The exposure of the animals in the streams was performed bimonthly over a year, beginning in November 2017 and ending in September 2018; the exposure months were November, January, March, May, July, and September. These months were chosen by considering the periods of greatest pesticide application in different seasons. A total of 360 animals were used; 60 animals were collected per month, which were distributed to one of four sites (n = 15). The animals were exposed in the streams for 96 h (Kumar et al., 2010; Soares, 2017) in boxes made with iron rods and a plastic net, with a volume of at least 2 L. Three boxes with 5 individuals were utilized in each stream. The boxes were submerged—kept close to the sediment and properly attached to the shore with the aid of ropes—and the exposure time commenced. At the same time, under the same conditions, 15 animals were kept in the reference stream for 96 h (RS). After the exposure period, aeglids were euthanized by hypothermia and their tissues (muscle, gills, and hepatopancreas) were removed and frozen at -20°C for further analysis.

2.4 ENVIRONMENTAL ANALYSES

Water sampling was performed bimonthly at the same time the animals were being exposed. We collected 120 mL per day of exposure at each location as well as a final sample at the end of the experiment. The samples were forwarded under refrigeration to the Laboratório de Análises de Resíduos de Pesticidas (LARP) at the Universidade Federal de Santa Maria (UFSM). In the laboratory, a total of 70 active principles (pesticides) were evaluated qualitatively and quantitatively by gas and liquid chromatography coupled to tandem mass spectrometry (GC–MS/MS and LC–MS/MS, respectively), following methodologies described by Donato et al. (2015). These pesticides were selected based on LARP analytical standards and regional uses. In water samples, the concentration of ammonia (expressed in μmol ammonia mL^{-1}) and nitrite (expressed in mg mL^{-1}) were quantified (Verdouw et al., 1978). At the moment of water collection, the following abiotic data were measured: temperature, pH, dissolved oxygen, turbidity, electric conductivity, oxidation/reduction potential, and total dissolved solids. These parameters were recorded with the aid of an HORIBA multiparameter device.

2.5 BIOCHEMICAL ANALYSES

Tissues were homogenized with Tris-HCl buffer (50 mM, pH 7.5), centrifuged at 1,400 *g* for 10 min, and the supernatant was kept frozen (-20°C) until use to maintain its biochemical properties. The protein concentration was determined according to Bradford (1976). The absorbance of the biomarkers was measured using a microplate reader. Ten samples were used for each biochemical analysis.

Glutathione S-transferase activity (GST) was determined according to Habig et al. (1974), expressed in μmol GS-DNB min^{-1} mg^{-1} protein. The determination of non-protein thiols (NPSH) was performed according to Ellman (1959). NPSH levels are expressed in μmol SH g^{-1} tissue. Lipid peroxidation was determined by the thiobarbituric acid reactive substances (TBARS) method, according to the technique described by Draper and Hadley (1990), and expressed as nmol MDA mg^{-1} protein. Carbonylation of proteins (CP) was determined by the method described by Yan et al. (1995) and expressed in nmol carbonyl protein mg^{-1} protein. Reactive oxygen species were determined according to the methodology described by Viarengo et al. (1999) and expressed as area of ROS mg^{-1} protein. Antioxidant capacity against peroxides (ACAP) was performed according to the methodology described by Amado et al. (2009) and expressed as relative area. Note that for ACAP, higher values on the chart mean lower antioxidant capacity. The GST, NPSH, TBARS, CP, ROS, and ACAP, assays were performed with hepatopancreas, muscle, and gills samples. Acetylcholinesterase (AChE) activity was determined in muscle, according to Ellman et al. (1961), and expressed as μmol SCh hydrolyzed min^{-1} mg^{-1} protein.

2.6 LETHALITY

During the 96-h exposure, animal deaths were counted in each of the treatments. This parameter allowed us to verify the amplitude of tolerance to the stressor of the exposed organisms. We used the percentage of lethality in each treatment.

2.7 STATISTICAL ANALYSES

The data were tested for normality and homogeneity through the Shapiro–Wilk and Levene tests, respectively. The statistical comparisons between the sample sites and between months were performed through a two-way analysis of variance (ANOVA), with Tukey's post hoc test. A P value < 0.05 was considered statistically significant.

A redundancy analysis (RDA) extracts and summarizes the disparity in a set of response variables; this measure may be explained by a set of predictor variables associated with these differences (Legendre and Anderson, 1999). The RDA was conducted to explore the main predictors (i.e., abiotic factors, pesticides) associated with certain biochemical parameters. We used 19 biochemical parameters as response variables and 27 predictive variables: 10 abiotic and 17 pesticides (6 pesticides were not considered relevant by the analysis). In November, due to the lack of reagents for analysis, clomazone and propoxur could not be analyzed; therefore, they required estimates. These missing entries in the data matrix were predicted by the iterative principal component analysis (PCA) algorithm (Josse and Husson, 2012), in which the missing data are imputed, assuming they have a Gaussian distribution with a mean and standard deviation calculated from the observed data. Pesticides that were not analyzed in most months were excluded from the analyses; these pesticides were not analyzed because the equipment presented defects.

All RDA analyses were performed in the R environment (R Core Team, 2017), using the packages missMDA (Josse and Husson, 2016), FactoMineR (Lê et al., 2008), Factoextra (Kassambara and Mundt, 2016), and vegan (Oksanen et al., 2010).

3 RESULTS

3.1 ENVIRONMENTAL ANALYSES

A total of 24 water samples were analyzed; all of them presented pesticides. There were 22 active principles: 13 fungicides, 5 herbicides, 3 insecticides, and 1 acaricide. The most to least frequently found active principles were: clomazone, atrazine, propoxur, bentazone, metalaxyl, carbofuran, imidacloprid, azoxystrobin, quinclorac, tebuconazole, and trifloxystrobin. Bentazone presented the highest amounts. The RS showed the lowest pesticide residue frequency (17), followed by S1 (24), S3 (26), and S2 (28). September showed the highest pesticide residue detection (33), while July had the lowest (6; Table 1).

The highest temperatures were found in January (22ºC in S1 and S2) and the lowest temperatures in July (14ºC). The turbidity was highest in September and lowest in November; it was higher at S1 for almost every month. The electric conductivity at S1 was high and at levels above those established as normal, so the conductivity value set as the limit for the water body to be considered altered in this work was 0.1 mS/cm. In general, the highest values of oxidation potential were found in September at RS and the lowest in November at S1. January had the lowest concentrations of dissolved oxygen. Total dissolved solids were higher at S1 and lower at RS for every month. RS had the lowest nitrite and ammonia values (Table 2). Regarding pH, January had the lowest values: between 5.6 and 5.8 at S1, S2, and S3. For the other months at S1, S2, and S3 and for all months at RS, the pH was between 6 and 9.

3.2 LETHALITY

In January, 100 % of the animals exposed to S1 died. For the other months, only 1 individual died in July and 1 died in September after exposure to S1. For the other sites, there was 100% survival.

3.3 BIOCHEMICAL ANALYSES

The gills GST activity was higher in animals exposed to S2 compared with RS in May and November, and higher in animals exposed to S1, S2, and S3 compared with RS in July. There was no difference in gills GST activity among the months in animals exposed to RS. For the polluted sites, exposed animals showed the lowest gills GST activity in November and the highest in July ($F_{15, 216} = 12.60$; $P < 0.0001$; Fig. 1). NPSH levels were lower in animals exposed to S1 compared with RS in November. There was no difference in gills NPSH levels among the months in animals exposed to S1. In general, animals exposed to RS, S2, and S3 showed higher gills NPSH levels in January and lower levels in May and July ($F_{15, 184} = 114$; $P < 0.0001$; Fig. 1). Compared to animals exposed to RS, the gills TBARS levels were higher for animals exposed to S1 in July and to S2 in November. In general, for animals exposed to S1, S2, or S3, the gills TBARS levels were highest in July and lowest in January ($F_{15, 214} = 12.11$; $P < 0.0001$;

Fig. 1). The gills CP content was higher in animals exposed to S1 and S2 compared with RS in November. For all the sites, November showed the highest gills CP content compared with the other months ($F_{15, 194} = 49.71$; $P < 0.0001$; Fig. 1). Compared with animals exposed to RS, in November the gills ROS levels were lower in animals exposed to S1 and S3 and higher for those exposed to S2. The highest gills ROS levels occurred in November for all exposure sites compared with other months ($F_{15, 214} = 5.89$; $P < 0.0001$, Fig. 1). Animals exposed to S1 compared with RS showed higher gills ACAP levels in July. In general, gills ACAP levels were highest in July and lowest in March and November ($F_{15, 216} = 5.48$; $P < 0.0001$, Fig. 1).

The hepatopancreas GST activity was lower in animals exposed to S2 and S3 in September and the highest for animals exposed to S3 in November compared with animals exposed to RS. There were no differences in hepatopancreas GST activities between animals exposed to RS or S1 among the months. In general, animals exposed to S2 or S3 showed the highest hepatopancreas GST activity in November and the lowest in January and September ($F_{15, 216} = 5.55$; $P < 0.0001$, Fig. 2). Animals exposed to the polluted sites showed no differences in hepatopancreas NPSH levels compared with animals exposed to RS. The lowest hepatopancreas NPSH levels were in November regardless of the exposure site. In general, exposed animals exhibited the highest hepatopancreas NPSH levels in March, July, and September ($F_{15, 208} = 61.84$; $P < 0.0001$, Fig. 2). The hepatopancreas TBARS levels were higher in animals exposed to S1 in May and the highest in animals exposed to S2 and S3 in November compared with animals exposed to RS. After exposure to RS or S1, the animals showed the highest TBARS levels in May. In general, after exposure to S2 or S3, the animals showed the lowest TBARS levels in January and the highest in November ($F_{15, 210} = 6.87$; $P < 0.0001$, Fig. 2). In comparison with exposure to RS, animals exposed to S3 showed higher hepatopancreas CP content in May and November. The CP content was higher in November in animals exposed to S2 compared with RS. In general, animals showed lower hepatopancreas CP contents in March and September and the highest in November ($F_{15, 216} = 40.10$; $P < 0.0001$, Fig. 2). After exposure to the polluted sites, the animals showed the lowest hepatopancreas ROS levels in September compared with animals exposed to RS. In general, exposed animals showed the highest ROS levels in May and June and the lowest levels in January, regardless of the exposure site ($F_{15, 216} = 4.71$; $P < 0.0001$, Fig. 2). The animals exposed to the polluted sites showed no differences in hepatopancreas ACAP compared with animals exposed to RS. Regardless of the month, there were no differences in hepatopancreas ACAP levels for animals exposed to RS. After exposure to S1, animals showed the highest ACAP levels in July and November and the lowest in May. In general, hepatopancreas ACAP levels were highest in March and lowest in May, July, and September for animals exposed to S2 or S3 ($F_{15, 216} = 4.15$; $P < 0.0001$, Fig. 2).

There were no differences in muscle GST activity in animals exposed to the polluted sites compared with RS. All exposed animals showed the highest muscle GST activity in July, regardless of the exposure site, with the other months showing lower values ($F_{15, 215} = 5.12$; $P < 0.0001$, Fig. 3). Animals exposed to S1 or S2 showed increased muscle NPSH levels in May compared with animals exposed to RS. For all exposure sites, animals showed the highest muscle NPSH levels in January and September and the lowest in March ($F_{15, 201} = 82.13$; $P < 0.0001$, Fig. 3). In July, muscle TBARS levels were lower in animals exposed to S1 or S2 compared with RS. Muscle TBARS levels were lower in May and September for animals exposed to S3 compared with RS. In general, muscle TBARS levels were highest in July and lowest in January, regardless of the exposure site ($F_{15, 216} = 6.87$; $P < 0.0001$, Fig. 3). Muscle CP content was higher in January in animals exposed to S2 compared with RS. Muscle CP content was highest in November in animals exposed to the polluted sites compared with RS. In general, the highest muscle CP content occurred in November, regardless of the exposure site ($F_{15, 215} = 8.08$; $P < 0.0001$, Fig. 3). In September, muscle ROS levels decreased in animals exposed to S1 or S2 compared with RS. In general, the highest muscle ROS levels occurred in September, with lower levels in January, March, May, and November, regardless of the exposure site ($F_{15, 214} = 6.88$; $P < 0.0001$, Fig. 3). Muscle ACAP levels were higher in January in animals exposed to S2 or S3 compared with RS. In May, ACAP levels increased in animals exposed to S3 compared with RS. In general, muscle ACAP levels were highest in January and July and lowest in March and September, regardless of the exposure site ($F_{15, 216} = 5.48$; $P < 0.0001$, Fig. 3). In May, there was a decrease in muscle AChE activity in animals exposed to S3 compared with RS. In general, muscle AChE activity was highest in March and July and lowest in January and November, regardless of the exposure site ($F_{15, 204} = 6.72$; $P < 0.0001$, Fig. 3).

The first two RDA axes explained 23 and 14%, respectively, of the total variation of the data, showing a clear separation of the samples in relation to the months of exposure (Fig. 4). The first RDA axis was mainly responsible for discriminating July and September samples from January and November samples. The environmental variables that most positively contributed to the separation were ammonia, turbidity, carbofuran, nitrite, atrazine, and oxidation/reduction potential, which appear to have a greater influence on ACAP levels in gills, ROS levels in muscle, GST activity in muscle, and NPSH levels in hepatopancreas. The environmental variables that most negatively contributed to the separation were temperature, bitertanol, pH, bentazone, and propoxur, which may have more influence on CP content in muscle, gills, and hepatopancreas, and ROS levels in gills.

The second RDA axis best explained the separation between July from January and March. The variables with higher positive contributions to this separation were dissolved oxygen, bitertanol, and azoxystrobin, which exerted greater influences on the levels of TBARS in gills and muscle, CP content in gills and hepatopancreas, and ROS levels in gills. The environmental variables that contributed most negatively to the separation were temperature, bentazone, and imidacloprid, which can have more influence on the levels of NPSH in gills and hepatopancreas.

4 DISCUSSION

The present study is the first to perform a year-long pesticide monitoring in low-order streams using *A. longirostri* as a bioindicator with *in situ* exposure. Due to anthropogenic activities, freshwater ecosystems have been exposed to several contaminants, which end up interacting with the organisms that live there. The proximity to different crops makes these streams receptors for diverse pesticides throughout the year. The highest pesticide detection coincided with the periods of greatest pesticide application: September to January. Furthermore, there was a greater diversity of pesticide residues in September, January, and November. The most active principles identified are related to the production of soybean and rice; these crops would perhaps most endanger the fauna of these aquatic ecosystems. However, soybeans deserve special mention because this crop has the longest pesticide application period during the year. Furthermore, it is related to at least 14 of the identified active principles (ANVISA, 2019). The lower number of active principles found in RS is mainly due to the fact that it is more distant from anthropogenic activities and the riparian forest has been preserved, a factor that also reduces the variation in abiotic data. In addition, animals exposed to RS had less oxidative damage and greater antioxidant capacity in relation to the other exposure sites. These data highlight the importance of the preservation of the riparian forest to ensure species survival. RS also showed the lowest frequency of pesticide residues in relation to the other sites. Indeed, riparian vegetation strips help to reduce the movement of pesticides and nutrients from agricultural fields (Cole et al., 2020; Prosser et al., 2020).

Clomazone was the most frequently found active principle in all samples. This finding was expected because this herbicide is used throughout the year for several crops. Studies have shown that this compound exerts toxic effects in fish via ROS production (Menezes et al., 2011). The second most commonly found pesticide was the herbicide atrazine. This compound is prohibited in Europe, but throughout the rest of the world, it is still one of the most utilized herbicides. Indeed, it was among the six best-selling herbicides in Brazil in 2017 and 2018 (IBAMA, 2020). This pesticide induces oxidative stress in crustaceans (European Union, 2004; Griboff et al., 2014; Yoon et al., 2019). Another interesting compound is the insecticide propoxur, which we detected at very significant concentrations. Hence, it is possibly being misused in agricultural areas as pest control because it is not approved for application in agricultural areas and is restricted to veterinary and domestic use. The bentazone concentration was highest among all the identified pesticides. This herbicide is widely used in rice cultivation and has high persistence in water (BASF, 2012). Another pesticide that causes concern is carbofuran; it exhibits a broad spectrum of activity against many agricultural pests and exerts very toxic effects for invertebrates and fish. It has been prohibited in Brazil (Otieno et al., 2010).

Abiotic factors, such as the water temperature, can cause alter the toxic potential of pollutants present in aquatic environments (Manciocco et al., 2014) and increase ROS production (Lushchak and Bagnyukova, 2006). These phenomena can lead to the death of animals. Besides, the National Council of the Environment (CONAMA, 2005) Resolution 357 establishes that for the protection of aquatic life, the pH must be between 6 and 9. However, in January we had a pH below the level considered normal (5.6 and 5.8) at S1, S2, and S3. Considering this fact, the death of all individuals exposed to S1 in January may have occurred due to the variety of pesticides found, especially the high bentazone concentration, associated with poor environmental conditions such as low pH, high temperatures, and lower rates of dissolved oxygen in the water. As previously mentioned, these adverse abiotic conditions are also a consequence of the lack of preservation of riparian forest around these sites.

Pesticides and other abiotic variables found in the water of these streams caused changes in many biochemical biomarkers in *A. longirostri*., in a tissue-specific pattern. The gills are the first organ to come into contact with the aquatic medium, so their changes as in antioxidant defense and oxidative stress may occur before other tissues. July and November presented greater changes in this tissue, such as the increase in levels of lipid peroxidation and CP. Lipid peroxidation would lead to increased permeability of the nuclear membrane, rendering the nucleus more susceptible to the xenobiotic-induced alterations (Lushchak, 2011).In July, the activation of GST may have detoxified xenobiotics, which generated lower levels of protein oxidation. In the biomarkers analysis, we have higher levels of oxidative stress in November, which may be related to lower biotransformation capacity due to lower GST activity. Increased CP and TBARS may be a result of increased ROS, since ROS may react with biomolecules causing damage to proteins and lipids (do Amaral et al., 2018). Several environmental and anthropogenic stressors end up contributing to the excessive production of ROS as occurred in November in gills and muscle. In this case, toxicity would be directly associated with the abiotic factors associated with pesticides in the balance between ROS production and antioxidants. Azoxystrobin is a fungicide capable of inhibiting mitochondrial respiration, thus causing an increase in the production of ROS that can affect aquatic plants and animals, which may have occurred in S2 gills in November and in RS and S3 in September (Han et al., 2016; Rodrigues et al., 2013). ACAP and TBARS levels are related, ACAP integrates the response of most enzymatic and non-enzymatic antioxidants, while LPO is indicative of the unbalance between the production of ROS and antioxidant capacity (Machado et al., 2013). Non-protein thiols show the antioxidant capacity acting against the formation of free radicals in the maintenance of the cellular redox balance and in the defense against electrophilic agents (Reischl et al., 2007). Despite the higher levels of ACAP and NPSH in November appears not to be enough to prevent damage to lipids and proteins.

In hepatopancreas, despite a low production of ROS in November, there was damage in proteins and lipids in S2 and S3. The increase in GST activity at these points was probably an attempt to detoxify this organ of endogenous lipid peroxidation products (Lushchak et al., 2005; Storey, 1996), since there was no increase in NPSH levels and ACAP was low in this month. The highest levels of TBARS were found in this tissue compared to gills and muscle. Changes in ROS content are associated with changes in the activity of various enzymes and in the concentration of antioxidant molecules to prevent subsequent deleterious oxidative damage in tissues (Lushchak, 2011). In May we had higher levels of ROS which may have caused an increase in lipid peroxidation and CP (in S3), although a readjustment in its antioxidant capacity to avoid the damages caused by the ROS generated has not been efficient.

The GST is an enzyme that conjugates xenobiotics or their metabolites to glutathione, making them less toxic and more easily excreted (Guengerich, 1990). Probably the activity of GST and the high ACAP in muscle in July was sufficient to avoid oxidative damages generated by the high production of ROS in the proteins, but it was not enough to avoid damages at the lipid level. In animals exposed to pesticides, the decrease of GST activity is possible by the direct action of the xenobiotic, and its metabolites in the structure of the enzymes (Xing et al., 2012). In November, the increase in CP content could indicate damage to enzymes, such as GST and AChE, resulting in a decrease in their activities. The reduction in the activity of GST compromises the metabolization of pesticides, increasing the permanence and the risk of these xenobiotics in causing damage to the biomolecules of the tissue. The high antioxidant capacity in September was sufficient to avoid protein damage caused by high levels of ROS but was not effective against lipid peroxidation. Elevated levels of NPSH and GST appear to have helped to prevent protein carbonylation in that month. In general, in the analysis of biomarkers, it can be observed that in muscle we have higher levels of oxidative stress in November, which may be related to lower biotransformation capacity due to lower GST activity as well as lower levels of NPSH.

AChE is an enzyme that is strongly inhibited by some pesticides even at low concentrations, which was observed in the months of November in S2 and in January in S3. Unlike in our study in January, imidacloprid caused increased AChE activity in invertebrates, acting as a neurotoxic agonist (Boily et al., 2013). Changes in lipid peroxidation in the muscle may have influenced the increase in AChE activity, showing a compensatory response to oxidative damage probably caused by pesticides found in water, as observed in *Loricariichthys anus* collected from a subtropical reservoir near agricultural areas (do Amaral et al., 2018). Murussi et al. (2015) also found an increase in AChE in muscle in animals exposed to clomazone. Increased AChE activity could lead to a decrease in acetylcholine levels at the neuromuscular junction, which would result in preventing muscle contraction, can change some behaviors of these crustaceans, such as food-seeking and defense against predation (Cerezer, 2017).

Based on the RDA, the samples were clearly separated with regard to environmental variables, namely temperature, whereby there was a distinction between colder and warmer months. Additionally, the influence of other abiotic factors, including pesticides, should not be disregarded. Thus, the biochemical biomarkers and abiotic factors evaluated in this study provide important evidence that biomarkers are seasonally affected by environmental conditions.

A. longirostri is usually found at low temperatures in well-oxygenated brooks (Bond-Buckup and Santos, 2007). Aeglids are ectothermic organisms and regulate their body temperature through the environment. Hence, temperature may have direct and indirect effects on metabolic processes closely associated with water temperature (Sokolova and Lannig, 2008).

Beyond temperature, the RDA analysis indicated the influence of other abiotic factors on biochemical variables. Hepatopancreas CP and TBARS levels, as well as increased gills ROS production, appear to have been more affected in November due to high temperatures in association with bitertanol, bentazone, azoxystrobin, and other pesticides that were not considered for the RDA construction (e.g., difenoconazole, metconazole, and pyrimethanil). The environmental conditions shown above may eventually affect all analyzed tissues by favoring oxidative damage. This eventuality suggests that November would be the month in which the animals are most vulnerable. In January, exposure to imidacloprid, propoxur, and especially bentazone, associated with high temperatures, conductivity, and total dissolved solids, was strongly correlated with high gills NPSH and ACAP levels, which helped avoid major damages in that month. However, the poor water quality combined with the pesticides in S1 possibly led to irreversible physiological stress, culminating in 100% lethality.

We cannot clearly describe correlations in May and March: While clomazone, atrazine, and propoxur were present in both months, their relation with these pesticides does not seem clear according to the RDA. March seems to have a relationship with simazine and bentazone. May appears to be related to hepatopancreas TBARS and GST levels. The ambiguous separation of May and March may be related to the fact that both months are intermediates in terms of temperature and other environmental variables.

In September, we observed the influence of abiotic factors (e.g., ammonia and turbidity) and pesticides (e.g., carbofuran) on the elevated biochemical responses such as NPSH, ACAP, ROS, AChE activity, TBARS, and GST, mainly in the muscle. These results show the notorious influence of the environmental factors on this tissue. However, there was a more pronounced relationship with the increased NPSH in the hepatopancreas. Turbidity, ammonia, and nitrite seem to have a clear relationship with elevated AChE activity. The higher levels of these variables indicate that they may be affected by fertilizers and/or agricultural effluents (e.g., nitrite and ammonia) that occur over the years as a consequence of aquatic pollution (i.e., indirect effects of pesticides). Following this line of evidence, Amaral et al. (2019) found similar results in *Loricariichthys anus*, whereby AChE activity was strongly related with variables such as pesticides, ammonia, and nitrite. These environmental variables, as well as the high dissolved oxygen values in July, may have influenced the results in this month because the only pesticide found at a high concentration was clomazone. In July, the elevation of GST and TBARS levels in all tissues was strongly associated with the highest NPSH and ROS levels in the hepatopancreas and muscle. Moreover, July had the lowest recorded temperatures, a factor that appears to have a prominent relationship with the increase in biochemical biomarkers. An attractive explanation is that the decline in temperature is the best predictor for a relationship with biomarkers observed in these two months. Specifically, at colder temperatures the body needs to adjust to keep energy production at an optimal level.

Brazil, the world leader in the use of pesticides, lacks strict legislation to ensure their conscious use. Brazilian legislation has not established pesticide limits in freshwater for most of the active principles found in this study (CONAMA, 2005). The absence of limits for these pesticides and the effects reported in this study highlight the risks of exposure to aquatic organisms. Indeed, even short-term exposure to very low concentrations generated notable damage in these animals.

The prediction of possible consequences of the excessive use of pesticides associated with other abiotic factors is of great importance for the conservation of the aquatic organisms because most species of aeglids are threatened (Santos et al., 2017). These animals are active predators (Cerezer et al., 2016) and important shredders in streams. Changes in their niche could cause imbalances in biogeochemical cycles and aquatic food chains.

5 CONCLUSION

The frequencies of pesticides found in this study were equivalent to the periods of greater and lesser application of pesticides in crops. Bentazone was the pesticide found in the largest amounts, followed by Clomazon and Atrazine, but the frequency with which bentazon appeared was less. Some pesticides such as Imidacloprid influenced the differences in biomarkers more than the others, resulting in different responses depending on the combination of pesticides found. The pesticides combined with the most extreme abiotic factors (warmer or colder temperatures, for example) were able to generate different responses in the tested biochemical biomarkers. Furthermore, the lethality of animals exposed to poor water quality containing a wide variety of pesticides, some in high concentrations, makes evident the risks to the integrity of the species.

The conclusions of our study reinforce the need for studies with environmentally relevant concentrations of pesticides, in mixture and in association with abiotic factors (e.g.

temperature), to understand the risk of exposure to non-target organisms. The use of biomarkers at aquatic sites with interference from agricultural activity allows attitudes to be made before the harmful effects, especially of pesticides, on such species become irreversible and ecosystem integrity is affected as a whole. Moreover, the differences found between the reference site and the other sites, both in the frequency of pesticides and in various biochemical biomarkers, draws attention to the need for preservation of riparian forest and greater stringency in the current legislation with charges for the preservation of water bodies and consequently the preservation of the fauna there.

Acknowledgments

The Brazilian government financially supported this research through CAPES foundation (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) by the grant to CC and CNPq by the research grant to SS (proc. nº 311142/2014-1). We thank Dr. Barbara Clasen, Dr. Guendalina Turcato de Oliveira, Dr. Charlene Menezes, Dr. Bernardo Baldisserotto and Dr. Marcelo Marchet Dalosto by suggestions in the manuscript. We also thank Fernando Cereser, Charles Zarantonelo and Maiara Dorneles Costa for the technical support.

Declarations of 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.

REFERENCES

Ahmed, R.G., 2005. Is there a balance between oxidative stress and antioxidant defense system during development? Med. J. Islamic World A. Sci. 15, 55–63.

Amado, L.L., Garcia, M.L., Ramos, P.B., Freitas, R.F., Zafalon, B., Ferreira, J.L.R., Yunes, J.S., Monserrat, J.M., 2009. A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: application to evaluate microcystins toxicity. Sci. Total Environ. 407, 2125-2123.

Amaral, A.M.B., 2019. Biomarcadores de toxicidade em *Loricariichthys anus* (Valenciennes, 1835) e *Geophagus brasiliensis* (Quoy and Gaimard, 1824): influência de variáveis abióticas e da poluição agrícola. Tese (doutorado em Biodiversidade Animal) - Universidade Federal de Santa Maria, Santa Maria, RS, pp.88.

ANA (Agência Nacional de Águas), 2012. Panorama da qualidade das águas superficiais do Brasil/2012. Brasília, pp. 264.

ANVISA (Agência nacional de vigilância sanitária. Regularização de Produtos – Agrotóxicos), 2019. Monografias Autorizadas.

BASF Corporation, 2012. Ficha de segurança do Basagran 600R. Basf Corporation, p.9.

Boily, M., Sarrasin, B., Deblois, C., Aras, P., Chagnon, M., 2013. Acetylcholinesterase in honey bees (*Apis mellifera*) exposed to neonicotinoids, atrazine and glyphosate: laboratory and field experiments. Environ. Sci. Pollut. Rese. Int. 20, 5603– 5614.

Bond-Buckup, G., Santos, S., 2007. Crustáceos anomuros de águas continentais: diversidade e aspectos biológicos. Ciência e Ambiente. 35, 47-54.

Bond-Buckup, G., Buckup, L., 1994. A família Aeglidae (Crustacea, Decapoda, Anomura). Arquivos de Zoologia. 32, 159-346.

Borges, A.C.P, Piassão, J.F.G., Paula, M.O., Sepp, S., Bez, C.F.S., Hepp, L.U., Valduga, A.T., Pereira, A.A.M., Cansian, R.L., 2018. Characterization of oxidative stress biomarkers in a freshwater anomuran crab. Braz. J. Biol. 78, 61-67.

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

Cerezer, C., Biasi, C., Cogo, G.B., Santos, S., 2016. Avoid predation or take risks in basic activities? Predator-prey relationship in subtropical streams between decapods and caddisflies. Mar. Freshw. Res. 67, 1880-1887.

Cerezer, C., 2017. Estresse oxidativo em *Aegla longirostri* (decapoda anomura): efeito da temperatura e qualidade da água. Dissertação (mestrado em Biodiversidade Animal) - Universidade Federal de Santa Maria, Santa Maria, RS, pp. 77.

Cogo, G.B., Santos, S., 2013. The role of aeglids in shredding organic matter in Neotropical streams. J. Crustac. Biol. 33, 519–526.

Cole, L.J., Stockan, J., Helliwell, R., 2020. Managing riparian buffer strips to optimize ecosystem services: a review. Agric. Ecosyst. Environ. 296, 106891.

CONAMA – CONSELHO NACIONAL DO MEIO AMBIENTE, 2005. Resolução nº 357, de 17 de março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. Diário Oficial da União, Poder Executivo, Brasília, DF, pp. 58-63.

Correia, A.D., Costa, M.H., Luis, O.J., Livingstone, D.R., 2003. Age-related changes in antioxidant enzyme activities, fatty acid composition and lipid peroxidation in whole body *Gammarus locusta* (Crustacea: Amphipoda). J. Exp. Mar. Biol. Ecol. 289, 83–101.

Cosgrove, S., Jefferson, B., Jarvis, P., 2019. Pesticide removal from drinking water sources by adsorption: a review. Environ. Technol. Rev. 8 (1), 1-24.

Dalosto, M.M., Santos, S., 2011. Differences in oxygen consumption and diel activity as adaptations related to microhabitat in Neotropical freshwater decapods (Crustacea). Comp. Biochem. Physiol. Part A, Mol. Integ. Physiol. 160, 461-466.

do Amaral, A.M.B., Gomes, J.L.C., Weimer, G.H., Marins, A.T., Loro, V.L., Zanella, R., 2018. Seasonal implications on toxicity biomarkers of *Loricariichthys anus* (Valenciennes, 1835) from a subtropical reservoir. Chemosphere. 191, 876-885.

Donato, F.F., Martins, M.L., Munaretto, J.S., Prestes, O.D., Adaime, M.B., Zanella, R., 2015. Development of a multiresidue method for pesticide analysis in drinking water by solid phase extraction and determination by gas and liquid chromatography with triple quadrupole tandem mass spectrometry. J. Braz. Chem. Soc. 26(10), 2077-2087.

Draper, H.H., Hadley, M., 1990. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol.186, 421–431.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. Camb. Philos. Soc. 81, 163-182.

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

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

European Union, 2004. 32004D0248.2004/248/EC: Commission decision of 10 March 2004. Concerning the non-inclusion of atrazine in annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing this active substance. Pp. 731.

Ferreira, B.D.P., Hack, C., Oliveira, G.T., Bond-Buckup, G., 2005. Perfil metabólico de *Aegla platensis* Schmitt, 1942 (Crustacea, Anomura) submetida a dietas ricas em carboidratos ou proteínas. Rev. Bras. Zool. 22, 161–168.

Gandar, A., Laffaille, P., Marty-Gasset, N., Viala, D., Molette, C., Jean, S., 2017. Proteome response of fish under multiple stress exposure: effects of pesticide mixtures and temperature increase. Aquat. Toxicol. 184, 61-77.

Gandar, A., Jean, S., Canal, J., Marty-Gasset, N., Gilbert, F., Laffaille, P., 2015. Multistress effects on goldfish (*Carassius auratus*) behavior and metabolism. Environ. Sci. Pollut. Res. 23, 3184-3194.

Griboff, J., Morales, D., Bertrand, L., Bonansea, R.I., Monferrán, M.V., Asis, R., Wunderlin, D.A., Amé, M.V., 2014. Oxidative stress response induced by atrazine in *Palaemonetes argentines*: The protective effect of vitamin E. Ecotoxicol. Environ. Saf. 108, 1-8.

Guengerich, F.P., 1990. Enzymatic oxidation of xenobiotic chemicals. CRC Crit. Rev. Biochem. Mol. Biol. 25, 97–153.

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

Halliwell, B., 1994. Free radicals, antioxidants and human disease: curiosity, cause or consequence. Lancet. 344, 721–724.

Han, Y., Liu, T., Wang, J., Wang, J., Zhang, C., Zhu, L., 2016. Genotoxicity and oxidative stress induced by the fungicide azoxystrobin in zebrafish (*Danio rerio*) livers. Pestic. Biochem. Physiol. 133, 13-19.

IBAMA – INSTITUTO BRASILEIRO DO MEIO AMBIENTE E DOS RECURSOS NATURAIS, 2020. Relatórios de comercialização de agrotóxicos, boletim 2018. http://ibama.gov.br/agrotoxicos/relatorios-de-comercializacao-de-agrotoxicos#boletinsanuais, accessed May 16, 2020.

Josse, J., Husson, F., 2016. missMDA: A Package for Handling Missing Values in Multivariate Data Analysis. J. Stat. Software. 70, 1–31.

Josse, J., Husson, F., 2012. Handling missing values in exploratory multivariate data analysis methods. J. Soc. Fr. Statistique. 153, 79-99.

Kassambara, A., Mundt, F., 2016. factoextra: Extract and Visualize the Results of Multivariate Data Analyses, https://CRAN.R-project.org/package=factoextra, r package version 1.0.3.

Kennedy, C., Ross, S.P., 2011. Stress syndromes: heightened bioenergetic costs associated with contaminant exposure at warm temperatures in teleosts. Integr. Environ. Assess. Manag. 8, 202-204.

Kumar, A., Doan, H., Barnes, M., Chapman, J.C., Kookana, R.S., 2010. Response and recovery of acetylcholinesterase activity in freshwater shrimp, *Paratya australiensis* (Decapoda: Atyidae) exposed to selected anti-cholinesterase insecticides. Ecotoxicol. Environ.Saf. 73, 1503-1510.

Laetz, C.A., Baldwin, D.H., Hebert, V.R., Stark, J.D., Scholz, N.I., 2014. Elevated temperatures increase the toxicity of pesticide mixtures to juvenile coho salmon. Aquatic. Toxicol. 146, 38-44.

Lê, S., Josse, J., Husson, F., 2008. FactorMineR: An R Package for Multivariate Analysis. J. Stat. Software. 25, 1-18.

Legendre, P., Anderson, M.J., 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecol. Monogr. 69, 1- 24.

Lemaire, P., Livingstone, D.R., 1993. Pro-oxidant/antioxidant processes and organic xenobiotic interactions in marine organisms, in particular the flounder *Platichthys flesus* and the mussel *Mytillus edulis*. Trends in Comp. Biochem. Physiol. 1, 1119-1150.

Livingstone, D.R., 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar. Pollut. Bull.42, 656-666.

López-Doval, J.C., Barata, C., Díez, S., 2015. El uso de organismos como indicadores de la contaminación y evaluación del riesgo sobre el ecosistema acuático en el embalse de Flix (Cataluña, NE de España). In: POMPÊO, M. et al. Ecologia de reservatórios e interfaces. São Paulo: Instituto de Biociências da Universidade de São Paulo. 1, 1-32.

Lushchak, V.I., Bagnyukova, T.V., Lushchak, O.V., Storey, J.M., Storey, K.B., 2005. Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp (*Cyprinus carpio*) tissues. Int. J. Biochem. Cell Biol. 37, 1319–1330.

Lushchak, V. I., Bagnyukova, T.V., 2006. Temperature increase results in oxidative stress in goldfish tissues. 2. Antioxidant and associated enzymes. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol. 143, 36-41.

Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol. 101, 13-20.

Lushchak, V.I., Matviishyn, T.M., Husak, V.V., Storey, J.M., Storey, K.B., 2018. Pesticide toxicity: a mechanistic approach. EXCLI J. 17, 1101-1136.

Machado, A.A.S., Hoff, M.L.M., Klein, R.D., Cardozo, J.G., Giacomin, M.M., Pinho, G.L.L., Bianchini, A., 2013. Biomarkers of waterborne copper exposure in the guppy *Poecilia vivipara* acclimated to salt water. Aquat. Toxicol. 138-139, 60-69.

Manciocco, A., Calamandrei, G., Alleva, E., 2014. Global warming and environmental contaminants in aquatic organisms: The need of the etho-toxicology approach. Chemosphere. 100, 1– 7.

Menezes, C.C., Loro, V.L., Fonseca, M.B., Cattaneo, R., Pretto, A., Miron, D.S., Santi, A., 2011. Oxidative parameters of *Rhamdia quelen* in response to commercial herbicide containing clomazone and recovery pattern. Pestic. Biochem. Physiol. 100, 145–150.

Murussi, C.R., Costa, M., Menezes, C., Leitemperger, J., Guerra, L., Severo, E., Zanella, R., Loro, V.L., 2015. Integrated assessment of biomarker response in carp (*Cyprinus carpio*) and silver catfish (*Rhamdia quelen*) exposed to clomazone. Arch. Environ. Contamin. Toxicol. (Print). 68, 646-654.

Nadal, M., Marquès, M., Mari, M., Domingo, J.L., 2015. Climate change and environmental concentrations of POPs: a review. Environ. Res. 143, 177-185.

Oikari, A., 2006. Caging techniques for field exposures of fish to chemical contaminants. Aquat. Toxicol. 78, 370-381.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G., Solymos, P., Stevens, H., Wagner, H., 2010. Vegan: Community Ecology Package. R package version 1.15-4. http://CRAN.R-project.org/package=vegan

Oliveira, G.T., Fernandes, F.A., Bueno, A.A.P., Bond-Buckup, G., 2007. Seasonal variations in the intermediate metabolism of *Aegla platensis* (Crustacea, Aeglidae). Comp. Biochem. Physiol. Part A. 147, 600-606.

Oliveira, G.T., Fernandes, F.A., Bond-Buckup, G., Bueno, A.A., Silva, R.S.M., 2003. Circadian and seasonal variations in the metabolism of carbohydrates in *Aegla ligulata* (Crustacea:Anomura: Aeglidae). Memoirs Museum of Victoria. 60, 59-62.

Otieno, P.O., Lalah, J.O., Virani, M., Jondiko, I.O., Schramm, K.W., 2010. Carbofuran and its toxic metabolites provide forensic evidence for furadan exposure in vultures (*Gyps africanus*) in Kenya. Bull. Environ. Contamin. Toxicol. 84, 536–544.

Özkara, A., Akyil, D., Konuk, M., 2016. Pesticides, environmental pollution, and health, environmental health risk – Hazardous Factors to Living Species. DOI: 10.5772/63094.

Pinheiro, M.H.O., Carvalho, L.N., Arruda, R., Guilherme, F.A.G., 2015. Consequences of suppressing natural vegetation in drainage areas for freshwater ecosystem conservation: considerations on the new 'Brazilian forest code'. Acta Bot. Bras. 29, 262-269.

Prosser, R.S., Hoekstra, P.F., Gene, S., Truman, C., White, M., Hanson, M.L., 2020. A review of the effectiveness of vegetated buffers to mitigate pesticide and nutrient transport into surface waters from agricultural areas. J. Environ. Manage. 261, 110210.

R Code Team, 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Reischl, E., Dafre, A.L., Franco, J.L., Wilhelm-Filho, D., 2007. Distribution, adaptation and physiological meaning of thiols from vertebrate hemoglobins. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. 146, 22-53.

Rodrigues, E.T., Lopes, I., Pardal, M.A., 2013. Occurrence, fate and effects of azoxystrobin in aquatic ecosystems: a review. Environ. Int. 53, 18-28.

Santos, S., Bond-Buckup, G., Gonçalves, A.S., Bartholomei-Santos, M.L., Buckup, L., Jara, C.G., 2017. Diversity and conservation status of Aegla spp. (Anomura, Aeglidae): an update. Nauplius (in press). 25.

Soares, M.P., Jesus, F., Almeida, A.R., Zlabek, V., Grabic, R., Domingues, I., Hayd, L., 2017. Endemic shrimp *Macrobrachium pantanalense* as a test species to assess potential contamination by pesticides in Pantanal (Brazil). Chemosphere. 168, 1082-1092.

Sokolova, I.M., Lanning, G., 2008. Interactive effects of metal pollution and temperature on metabolism in aquatic ectotherms: implications of global climate change. Clim. Res. 37, 181-200.

Steinberg, C.E.W., Stürzenbaum, S.R., Menzel, R., 2008. Genes and environment - striking the fine balance between sophisticated biomonitoring and true functional environmental genomics. Sci. Total Environ. 400, 142-161.

Storey, K.B., 1996. Oxidative stress: animal adaptations in nature. Braz. J. Med. Biol. Res. 29, 1715–1733.

Swackhamer, D.L., Paerl, H.W., Eisenreich, S.J., Hurley, J., Hornbuckle, K.C., McLachlan, M., Mount, D., Muir, D., Schindler, D., 2004. Issues in Ecology: Impacts of Atmospheric Pollutants on Aquatic Ecosystems. Ecological Society of America. 12, 2-23.

Trevisan, A., Hepp, L.U., Santos, S., 2009. Abundância e distribuição de Aeglidae (Crustacea: Anomura) em função do uso da terra na bacia hidrográfica do Rio Jacutinga, Rio Grande do Sul, Brasil.

Verdouw, H., Van Echteld, C.J.A., Dekkers, E.M.J., 1978. Ammonia determinations based on indophenol formation with sodium salicylate. Water Res. 12, 399-402.

Viarengo, A., Burlando, B., Cavaletto, M., Marchi, B., Ponzano, E., Blasco, J., 1999. Role of metallothionein against oxidative stress in the mussel *Mytillus galloprovincialis*. Am. J. Physiol. 277, 1612-1619.

Vieira, C.E., Costa, P.G., Lunardelli, B., de Oliveira, F.L., Cabrera, L. da C., Risso, W.E., Primel, E.G., Meletti, P.C., Fillmann, G., Martinez, C.B., 2016. Multiple biomarker responses in *Prochilodus lineatus* subjected to short-term in situ exposure to streams from agricultural areas in Southern Brazil. Sci.Total Environ. 542, 44-56.

Wang, W.N., Wang, A.L., Liu, Y., Xiu, J., Liu, Z.B., Sun, R.Y., 2006. Effects of temperature on growth, adenosine phosphates, ATPase and cellular defense response of juvenile shrimp *Macrobrachium nipponense*. Aquaculture. 256, 624-630.

Winstone, G.W., Di Giulio, R.T., 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. Aquat. Toxicol. 19, 137-161.

Xing, H., Wang, X., Sun, G., Gao, X., Xu, S., Wang, X., 2012. Effects of atrazine and chlorpyrifos on activity and transcription of glutathione S-transferase in common carp (*Cyprinus carpio* L.). Environ. Toxicol. Pharmacol. 33, 233-244.

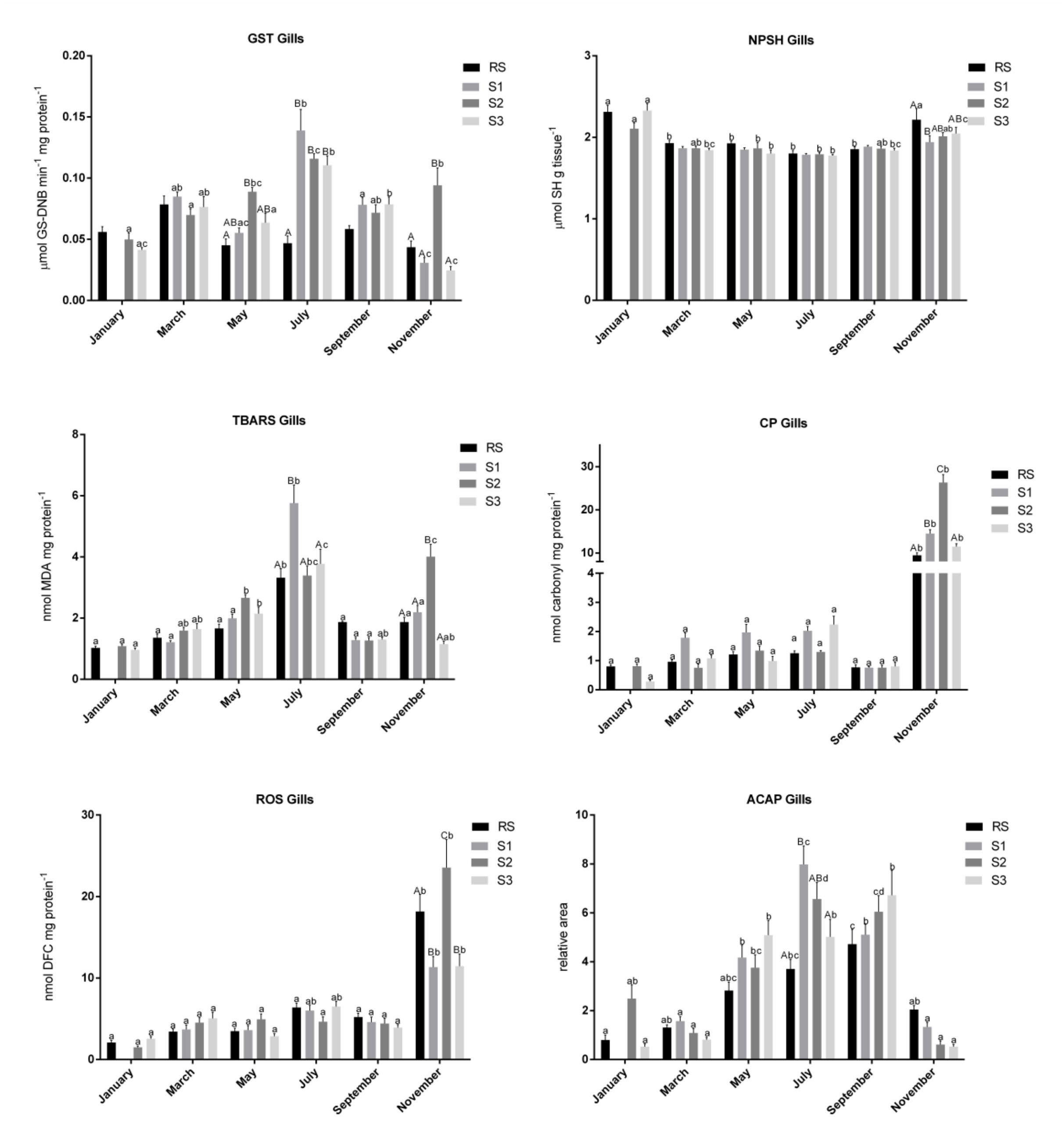
Yan, L.J., Traber, M.G., Packer, L., 1995. Spectrophotometric method for determination of carbonyls in oxidatively modified apolipoprotein B of human low-density lipoproteins. Anal. Biochem. 228, 349-351.

Yoon, D.-S., Park, J.C., Park, H.G., Lee, J.-S., Han, J., 2019. Effects of atrazine on life parameters, oxidative stress, and ecdysteroid biosynthetic pathway in the marine copepod *Tigriopus japonicus*. Aquatic. Toxicol. 213, 105213.

Zhou, J., Kang, H.-M, Lee, Y.H., Jeong, C.-B., Park, J.C., Lee, J.-S., 2019. Adverse effects of a synthetic pyrethroid insecticide cypermethrin on life parameters and antioxidant responses in the marine copepods *Paracyclopina nana* and *Tigriopus japonicus*. Chemosphere. 217, 383-392.

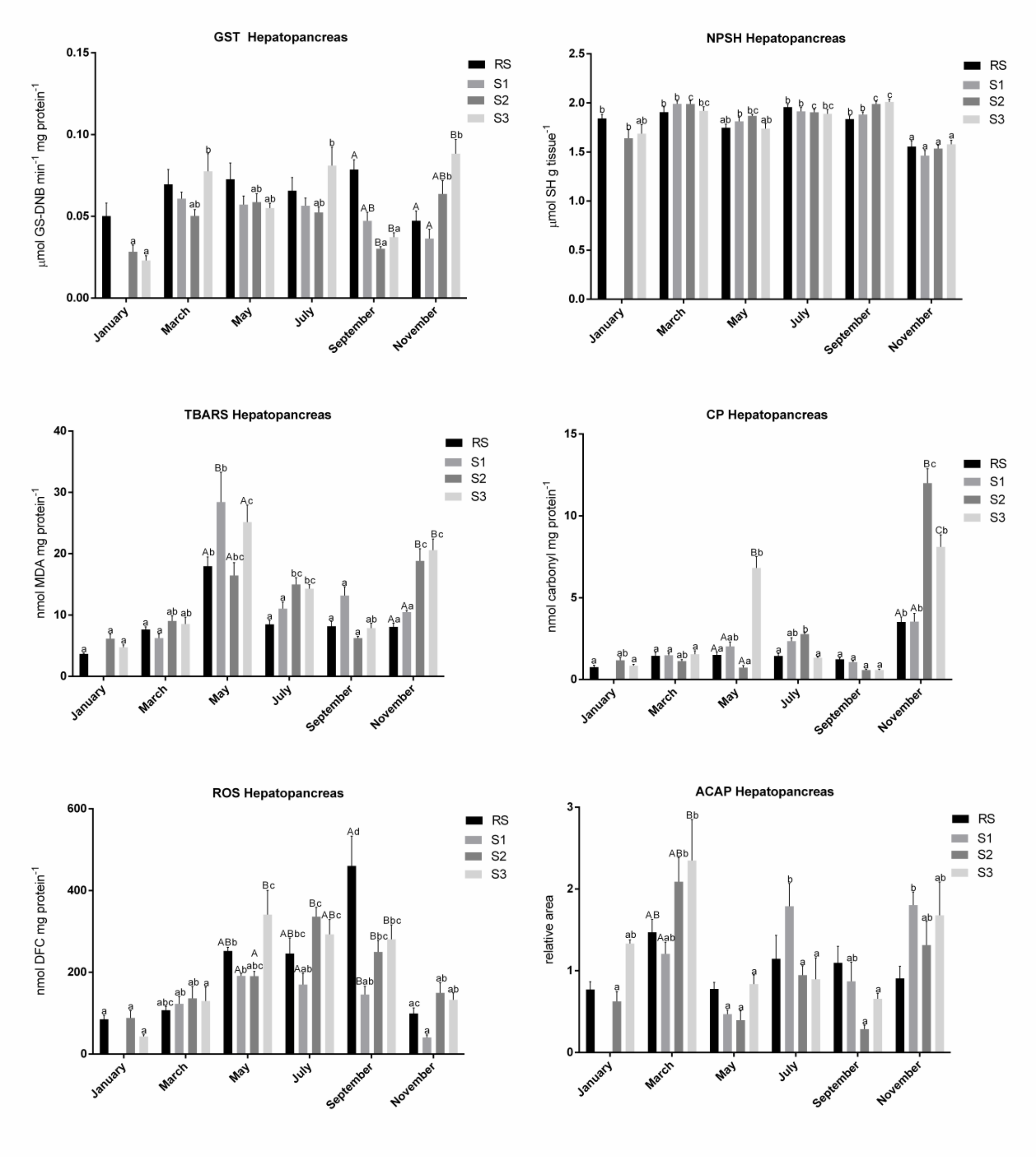
FIGURES

Figure 1. Gills of *Aegla longirostri* exposed *in situ* - GST activity, NPSH levels, TBARS levels, carbonyl protein content, ROS levels, and ACAP levels



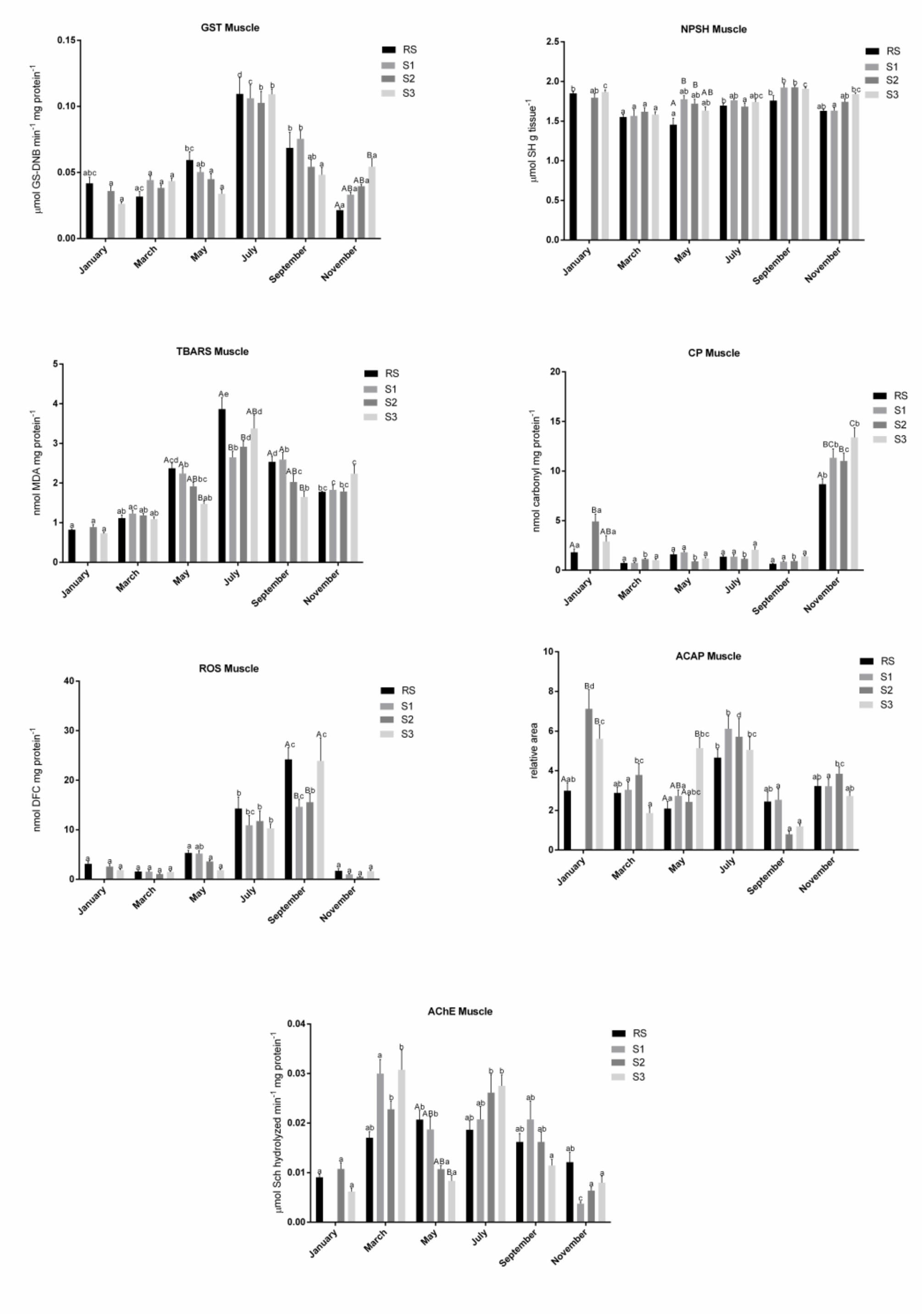
Data are reported as mean ± SEM (N = 10). Different letters indicate differences between groups, with lowercase letters representing the difference in point in different months and capital letters in the month between sites ($p < 0.05$).

Figure 2. Hepatopancreas of *Aegla longirostri* exposed *in situ* - GST activity, NPSH levels, TBARS levels, carbonyl protein content, ROS levels, and ACAP levels



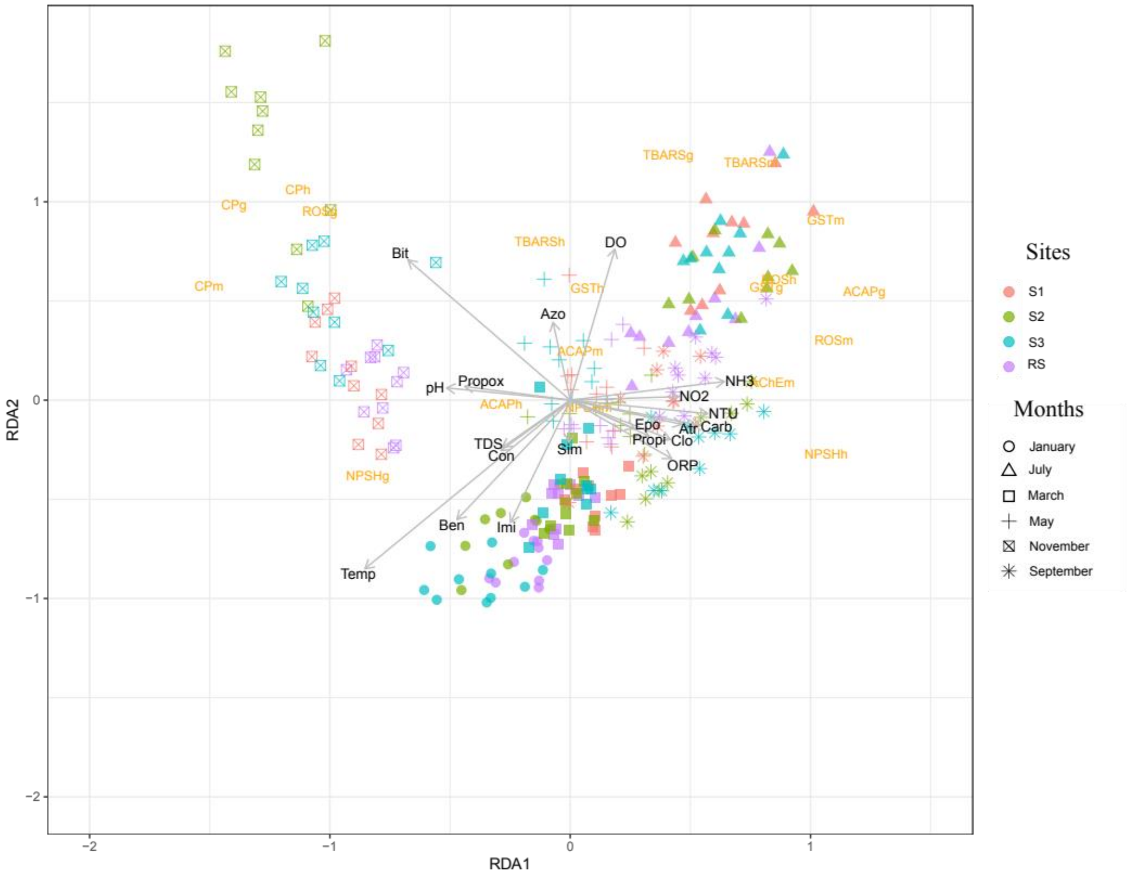
Data are reported as mean ± SEM (N = 10). Different letters indicate differences between groups, with lowercase letters representing the difference in point in different months and capital letters in the month between sites ($p < 0.05$).

Figure 3. Muscle of *Aegla longirostri* exposed *in situ* - GST activity, NPSH levels, TBARS levels, carbonyl protein content, ROS levels, ACAP levels and AChE activity



Data are reported as mean ± SEM (N = 10). Different letters indicate differences between groups, with lowercase letters representing the difference in point in different months and capital letters in the month between sites ($p < 0.05$).

Figure 4. Redundancy analysis showing sampling sites (RS, S1, S2 and S3) in each month (January, March, May, July, September and November), environmental variables and pesticides (ash arrows), toxicity biomarkers (oranges)



The letters g, h and m after the initials of the biomarkers refer to the following tissues: gills, hepatopancreas, and muscle. Abbreviations: Atr – Atrazine, Azo – azoxystrobin, Ben – Bentazone, Bit – Bitertanol, Carb – Carbofuran, Clo – Clomazone, COM – conductivity, DO – dissolved oxygen, Epo – Epoxiconazole, Imi – Imidacloprid, NH3 – ammonia, NO2 – Nitrite, NTU – turbidity, ORP – oxidation/reduction potential, Propi – propiconazole, Propox – Propoxur, Sim – simazine, Temp – temperature.

TABLES

Table 1. Pesticides measured bimonthly at the four sampling sites during a year, from November 2017 to September 2018

To be continue...

Pesticide	Class	RS						S1					
		Nov	Jan	Mar	May	Jul	Sep	Nov	Jan	Mar	May	Jul	Sep
Atrazine	H	<0.02		<0.02	0.021		0.099	<0.02	<0.02	<0.02	0.025	<0.02	0.174
Azoxystrobin	F						<0.02						
Bentazone	H							0.5	12.92	0.146			
Bitertanol	F												
Carbofuran	I						<0.02	n.a.					<0.02
Clomazone	H	n.a	0.2	0.153	0.021	0.187	0.161		0.25	0.24	0.272	0.202	0.305
Difenoconazo-le	F												
Epoxiconazole	F												
Imidacloprid	I								<0.02				
Metalaxyl	F						<0.02		<0.02				<0.02
Metconazole	F												
Piraclostrobin	F												
Pyrimethanil	F		n.a	n.a	n.a	n.a			n.a	n.a	n.a	n.a	
Propargito	A		n.a	n.a	n.a	n.a			n.a	n.a	n.a	n.a	
Propiconazole	F												
Propoxur	I	n.a		<0.02	<0.02		0.025	n.a.	<0.02	0.028	0.021		0.03
Quinclorac	H	n.a						n.a.	<0.04				
Simazine	H												
Triadimefom	F								<0.02				
Tebuconazole	F	n.a	n.a	n.a		<0.02	<0.02	n.a.	n.a	n.a			
Tetraconazole	F												
Trifloxystro-bin	F												

Class of pesticide: A – acaricide, F – fungicide, H – herbicide, I – insecticide. n.a.: not analyzed. Data in μg L^{-1}.

Table 1. Pesticides measured bimonthly at the four sampling sites during a year, from November 2017 to September 2018

conclusion.

Pesticide	Class	S2						S3					
		Nov	**Jan**	**Mar**	**May**	**Jul**	**Sep**	**Nov**	**Jan**	**Mar**	**May**	**Jul**	**Sep**
Atrazine	H	<0.02			<0.02		0.164	<0.02		<0.02	<0.02		0.178
Azoxystro-bin	F	<0.02											<0.02
Bentazone	H		0.3	0.102					0.84	0.156			
Bitertanol	F	<0.04											
Carbofuran	I						<0.02						<0.02
Clomazone	H	n.a	0.18	0.167	0.177	0.158	0.266	n.a	0.21	0.128	0.228	0.169	0.186
Difenocona-zole	F	0.03											
Epoxicona-zole	F												0.02
Imidacloprid	I		<0.02						<0.02				
Metalaxyl	F						<0.02						<0.02
Metconazole	F	<0.02											
Piraclostro-bin	F	<0.02											
Pyrimethanil	F		n.a	n.a	n.a	n.a		0.04	n.a	n.a	n.a	n.a	
Propargito	A	<0.02	n.a	n.a	n.a	n.a			n.a	n.a	n.a	n.a	
Propiconazo le	F						<0.02						0.062
Propoxur	I	n.a	<0.02	<0.02	<0.02		<0.02	n.a	<0.02		<0.02		0.062
Quinclorac	H	n.a					<0.02	n.a					0.089
Simazine	H									<0.02			
Triadime-fom	F												
Tebuconazo-le	F	n.a	n.a	n.a				n.a	n.a	n.a			0.045
Tetraconazo-le	F						0.02						0.064
Trifloxystro bin	F	<0.02					<0.02 0						0.036

Class of pesticide: A – acaricide, F – fungicide, H – herbicide, I – insecticide. n.a.: not analyzed. Data in µg L^{-1}.

Table 2. Physico-chemical and environmental parameters of the studied streams, measured bimonthly for a year, from November 2017 to September 2018

To be continued…

Variables	**RS**						**S1**					
	Nov	**Jan**	**Mar**	**May**	**Jul**	**Sep**	**Nov**	**Jan**	**Mar**	**May**	**Jul**	**Sep**
Dissolved oxygen ($mg.L^{-1}$)	9.94	8	9.6	9	9.5	9	8.6	7	7.4	9	8.5	8.9
Electric conductivity ($ms.cm^{-1}$)	0.042	0.043	0.04	0.042	0.038	0.036	0.13	0.122	0.13	0.104	0.081	0.061
Nitrite ($mg.mL^{-1}$)	0	0.001	0.001	0	0.002	0.004	0.013	0.032	0.03	0.035	0.037	0.043
Oxidation-reduction potential ($ORP.mv^{-1}$)	317	353	356	374	398	437	185	273	216	224	283	336
pH	7.7	6.3	8.3	7.9	6.1	6.8	7.4	5.6	7.4	7.4	6.2	6.4
Temperature (ºC)	17	19	17	17	14	15	20	22	20	18	14	18
Total ammonia ($\mu mol.mL^{-1}$)	0.003	0.0003	0.0003	0	0.002	0.004	0.005	0.022	0.025	0.035	0.037	0.043
Total dissolved solids ($g.L^{-1}$)	0.028	0.028	0.026	0.027	0.025	0.023	0.083	0.08	0.085	0.068	0.053	0.039
Turbidity (NTU)	0	0	0	8.8	23.6	9.4	1.4	17	4.8	16.3	33.5	239

Data reported as mean values.

Table 2. Physico-chemical and environmental parameters of the studied streams, measured bimonthly for a year, from November 2017 to September 2018

To be continued…

Variables	S2						S3					
	Nov	**Jan**	**Mar**	**May**	**Jul**	**Sep**	**Nov**	**Jan**	**Mar**	**May**	**Jul**	**Sep**
Dissolved oxygen ($mg.L^{-1}$)	9.3	7	7.6	10	9.4	9	9	6.8	7.3	8.5	9.3	9.8
Electric conductivity ($ms.cm^{-1}$)	0.052	0.077	0.076	0.065	0.058	0.045	0.062	0.088	0.094	0.062	0.055	0.051
Nitrite ($mg.mL^{-1}$)	0.017	0.025	0.02	0.01	0.002	0.053	0.012	0.041	0.035	0.026	0.079	0.05
Oxidation-reduction potential ($ORP.mv^{-1}$)	281	384	334	280	333	331	323	407	345	332	351	349
pH	7.7	5.8	7.4	7.4	6.4	7	7.6	5.8	7.6	7.4	6.4	7.4
Temperature (ºC)	19	22	20	18	14	18	19	21	20	17	14	17
Total ammonia ($\mu mol.mL^{-1}$)	0.005	0.023	0.02	0.01	0.002	0.053	0.007	0.016	0.019	0.025	0.078	0.05
Total dissolved solids ($g.L^{-1}$)	0.033	0.05	0.049	0.042	0.033	0.029	0.04	0.05	0.061	0.041	0.036	0.033
Turbidity (NTU)	0	15	0	13.2	34.8	148	0	12	0	17.5	31.8	202

Data reported as mean values.

4 CAPÍTULO 2

Raising the water temperature: consequences in behavior and biochemical biomarkers of the freshwater crab *Aegla longirostri* (Crustacea, Anomura)

Cristina Cerezer[1], Jossiele Wesz Leitemperger[2], Aline Monique Blank do Amaral[1], Bruna Ceretta Ferreira[3], Aline Teixeira Marins[1], Vania Lucia Loro[1,2], Marlise Ladvocat Bartholomei-Santos[1] and Sandro Santos[1]

[1] Programa de Pós-Graduação em Biodiversidade Animal, Departamento de Ecologia e Evolução, Universidade Federal de Santa Maria, Santa Maria, RS, CEP: 97105–900, Brazil

[2] Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, Universidade Federal de Santa Maria, Santa Maria, RS, CEP: 97105–900, Brazil

[3] Curso em Ciências Biológicas, Universidade Federal de Santa Maria, Santa Maria, RS, CEP: 97105–900, Brazil

Corresponding author:
Cristina Cerezer,
Departamento de Ecologia e Evolução
Universidade Federal de Santa Maria
97105-900 - Santa Maria, RS, Brazil.
Phone: +55 55 3220-9456
E-mail: cristhinacerezer@gmail.com

Manuscrito publicado no periódico *Environmental Science and Pollution Research.*
Doi: https://doi.org/10.1007/s11356-020-10423-w

ABSTRACT

Understanding how temperature alone affects biomarkers commonly used in ecotoxicology studies and biomonitoring programs is important to obtain a more real response in field studies, especially in freshwater. Thus, we analyzed the behavioral responses, the lethality and the biochemical biomarkers in the freshwater crustacean *Aegla longirostri* at different water temperatures. Animals were exposed under laboratory conditions, to 18 °C, 21 °C, 24 °C and 26 °C for 48 hours. There were significant changes in biochemical parameters in different tissues (hepatopancreas, gills, and muscle) and in the behavioral tests in *A. longirostri.* Hepatopancreas was especially affected by the elevation of temperature, as showed by the high levels of carbonyl proteins. The activity of acetylcholinesterase increased in a temperature-dependent manner in muscle. Glutathione S-transferase activity decreased with the elevation of temperature in all tissues sampled. The results obtained in this study indicate that when assessing the health of polluted limnic ecosystems through the use of organisms in situ, the intrinsic effect of abiotic factors, such as temperature, on biomarkers must be considered.

KEYWORDS: Oxidative stress. Acetylcholinesterase. Crustaceans. Ectothermic. Oxidative damage. Thermal stress.

1 INTRODUCTION

Studies assessing the health of freshwater ecosystems using biomarkers in animals collected in situ are important because they show the interaction of abiotic and biotic factors and, pollutants (Da Rocha et al. 2009; Dalzochio et al. 2016). However, it is difficult to separate the normal changes in biomarkers due to environmental variables from those produced because of, for example, exposure to contaminants (Cailleaud et al. 2007; Louiz et al. 2016). Elevated levels of a particular biomarker may be just a normal part of the organism's physiological cycle (Sheehan and Power 1999).

It is known that factors related to seasonality can influence biomarkers response in field ecotoxicology studies (Dalzochio and Gehlen 2016; Sardi et al. 2016). Among these confounding factors, temperature is the most evident, especially if the target animal is an ectotherm, since temperature governs all its metabolic processes (Bagnyukova et al. 2007; Hemmer-Brepson et al. 2014; Vinagre et al. 2014a; Gandar et al. 2017; Kamyab et al. 2017). For instance, an increase in water temperature increases the demand for oxygen and increases respiratory activity (Issartel et al. 2005). In polluted environments, a higher respiratory rate can cause a greater uptake of pollutants (Heugens et al. 2001; Delorenzo 2015). In addition, increased temperature is also associated with increased production of reactive oxygen species (ROS) due to increased oxygen consumption and, also because mitochondria exposed to high temperatures are less efficient (Abele et al. 2002; Hemmer-Brepson et al. 2014).

ROS are normally produced by the body and neutralized by enzymatic and non-enzymatic antioxidants. However, stressors that cause increased ROS production or deactivation of antioxidant defenses, altering the oxidative state of the organism, i.e., causing oxidative stress. Biochemical biomarkers related to oxidative stress are widely used when organisms are exposed to toxic compounds (Amaral et al. 2018; Marins et al. 2020; Severo et al. 2020). Among the biomarkers of biological effects (Hook et al. 2014) used to evaluate the impacts of different pollutants in natural ecosystems is the enzyme glutathione-S-transferase, responsible for the detoxification of xenobiotics and the enzyme acetylcholinesterase, a marker of neurotoxicity (Amaral et al. 2018; Marins et al. 2020). Since temperature can directly affect enzyme activity by changing its catalytic efficiency or binding capacity (Hochachka and Somero 1984; Cailleaud et al. 2007), it is important to understand how these biomarkers respond to temperature alone.

Changes in body temperature besides affecting physiological rates and biochemical reactions, can affect the behavior of ectotherm aquatic animals (Briffa et al. 2013). Behavioral studies are used as an additional tool for ecotoxicological testing and water quality monitoring. In general, they allow the association between the toxic effects obtained at biochemical and cellular levels on the impacts observed in the dynamics of populations and communities (Felten and Guerold 2001; Maltby et al. 2002; Wallace and Estephan 2004).

Most freshwater crabs have a high risk of extinction, making investigations on these animals of fundamental importance (Cumberlidge et al. 2009). Aeglids, for example, the only group of anomurans with a complete life cycle in continental watercourses, have almost 70% of the species under some degree of threat (Santos et al. 2017). In southern South American streams, aeglids are important shredders and actively participate in the process of decomposition of foliar debris, being key species in the cycling of nutrients. They play an important role in the trophic chain of aquatic ecosystems, where they act as predators of immature insects and serve as food for fish, birds, amphibians and mammals (Arenas 1976; Cerezer et al. 2016; Cogo and Santos 2013; Magni and Py-Daniel 1989; Pardini 1998). They are found in low temperatures and well-oxygenated streams and, are usually used as bioindicators of water quality (Bond-Buckup and Santos 2007). Low stream orders where these crustaceans can be found are subject to variations in daily and seasonal temperature and, also are a sink to pollution, especially from agricultural sources in the southern Brazil.

Thus, in this study we aimed to assess the effects of different temperatures alone in biochemical biomarkers and behavior of the freshwater crab *Aegla longirostri.*

2 MATERIAL AND METHODS

2.1 SAMPLING AND ACCLIMATION

Aegla longirostri were collected in the summer (December/2015) in a creek from the central region of the state of Rio Grande do Sul, Brazil (29°35'38"W, 53°33'17"S). The creek is located in a semideciduous seasonal forest area with a diverse riparian vegetation and presents an apparent absence of anthropic impacts. Thirty cove-type traps with liver baits, separated 10 meters from each other, were placed in the streams in the afternoon and collected in the next morning. In addition to traps, manual sampling was used. The captured individuals were previously measured for standardization using a digital caliper (accuracy: 0.01mm). Only adult males (more than 13.7 mm of length of the cephalothorax according to Colpo et al., 2005), and in intermoult period were used in the experiments because of the difference in hepatopancreas compounds between males and females, and because of aeglids pass through a fasting period in the moulting phase (Ferreira et al. 2005; Oliveira et al. 2003). After sampling, adult males were transported to the laboratory for acclimation.

During acclimation (at least 7 days), animals were accommodated individually in 2 L aquariums at 18 °C (mean water temperature of the sample site in the summer), constant aeration (dissolved oxygen by 8.9 ppm), controlled photoperiod (12/12), rock for shelter and feeding *ad libitum* (leaf litter were collected in the sample site creek).

2.2 EXPERIMENTAL DESIGN

The temperature of the treatments was defined according to the minimum and maximum water temperatures in the region where the animals were sampled (the maximum temperature 26 °C was chosen based on the rate of survival of *A. longirostri* to increasing temperature, unpublished data). In individual aquariums (2 L), sixty individuals were submitted to four different temperatures (n = 15) for 48 hours: 18 °C (mean annual temperature of the water); 21 °C, 24 °C, and 26 °C (maximum survival temperature in the laboratory pilot). The other abiotic variables were kept as in the acclimation period. After acclimation, the water temperature was increased by 1 °C per day until the desired temperature was reached. Temperatures of the water were maintained through BOD Incubator (Bio-Oxygen Demand) and controlled with thermometers.

2.3 LETHALITY

During the experiment period, mortality was recorded (in percentage per treatment). This allows verifying the amplitude of thermal tolerance of the exposed organisms.

2.4 BEHAVIOR

Behavioral observations were performed at 10 a.m. and 10 p.m. on the last day of exposure, i.e., after 32 and 44 hours of the experiment. The animals were filmed individually in the aquarium for 10 minutes (600 s; Sony® Handycam, model HDR-CX560). During the night, we used red incandescent lamps because crustaceans have low sensitivity to this wavelength (Turra and Denadai 2003). We estimated the activity of the aeglids by the average number of specific acts (inactive (score 0), low activity (score 1), moderate activity (score 2) and intense activity (score 3)), to which scores are attributed, according to Ayres-Peres et al. (2011), adapted from Dalosto and Santos (2011; Table 1). To determine the scores, 600 s were divided into 10 s intervals (totaling 60 intervals), and during each interval, the score of the predominant activity (at least 5 s, except for the tail flipping activity) was registered. The final score is the sum of the scores obtained at each interval (it can range from 0 = inactive animal to 180 = maximum activity). Also, the time (in seconds) for the animal to return to equilibrium when it was turned upside down was measured.

2.4 BIOCHEMICAL ANALYSIS

After 48 hours, the animals were cryo-euthanized. Gills, hepatopancreas and muscle were dissected and frozen at -20 °C. The tissues were homogenized (1:20 w:v) with 50 mM Tris-HCl in a Potter-Elvehjem glass/Teflon homogenizer, and centrifuged at 10,000 x *g* at 4ºC for 10 min. The supernatant was used for biochemical analysis. All analyzes were adjusted to 96-well plate (except catalase). The protein concentration of the tissues was determined according to Bradford (1976), using bovine serum albumin as standard. The activity of the enzyme glutathione S-transferase (GST) was determined in gills, hepatopancreas and muscle according to Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate and expressed in µmol GS-DNB/min/mg protein. The determination of non-protein thiols (NPSH) was performed on gills, hepatopancreas and muscle according to Ellman (1959) and expressed in µmol SH/g tissue. Catalase activity (CAT) was investigated in hepatopancreas according to the method Nelson and Kiesow (1972) and expressed in µmol/min/mg protein. Acetylcholinesterase activity (AChE) was determined in muscle according to the method described by Ellman et al. (1961) and expressed as µmol/min/mg protein. The carbonyl protein (CP) content was analyzed in gills, hepatopancreas and muscle by the method described by Yan et al. (1995) and expressed in nmol of carbonyl protein/mg protein. The level of lipid peroxidation was determined in gills, hepatopancreas and muscle by the method of thiobarbituric acid reactive substances (TBARS), according to Draper and Hadley (1990) and expressed as nmol of MDA/mg of protein.

2.5 STATISTICAL ANALYSIS

The data were previously tested for normality by the Kolmogorov-Smirnov test and homogeneity by the Levene test. One-way ANOVA with Tukey post-test was used to compare data between different temperatures in the same tissue (different tissues were not compared to each other). For non-parametric data the Kruskal-Wallis test was used. Statistical procedures were performed on GraphPad Prism 6.

3 RESULTS

3.1 LETHALITY

Lethality was higher at higher temperatures reaching approximately 30% at 26 °C. No deaths were registered at 18 °C (Table 2).

3.2 BEHAVIOR

During the day (10 a.m.) the activity was higher at the higher temperatures (24 and 26 °C) in relation to the lower temperature (18 °C, Fig. 1B). During the night (10 p.m.), animals exposed to the highest temperature (26 °C) had a lower level of activity than the intermediate temperatures (21 and 24 °C) (Fig. 1A) but not at 18 °C. It was observed that in both day and night at lower temperatures (18 and 21 °C) animals returned faster to their equilibrium (Fig. 2A and 2B).

3.3 BIOCHEMICAL ANALYSIS

In hepatopancreas, higher temperatures caused a decrease in GST activity when compared to 18 °C. In muscle and gills, GST activity decreased significantly at 24 °C compared to the other temperatures. In the muscle, there was no statistical difference between 21 and 26 °C. In the gills, there was no statistical difference between 24 and 26 °C and 21 and 26 °C (Fig. 2A).

To non-protein thiols of hepatopancreas, there was no difference between temperatures. In muscle, NPSH decreased at 21 and 24 °C compared to 18 and 26 °C. Exposure to the highest temperature 26 °C provided an increase in NPSH in gills in contrast to the other temperatures (Fig. 2B).

There was an increase in CAT activity in the hepatopancreas only at 24 °C in comparison with the other temperatures (Fig. 3A).

AChE activity in muscle showed a temperature-dependent increase, i.e. 26 °C the highest activity and 18 °C the lowest (Fig. 3B).

Carbonyl protein showed a temperature-dependent increase in the hepatopancreas. In the muscle, the carbonyl protein increased at intermediate temperatures (21 and 24 °C) compared to the others. In the gills, a similar pattern was observed, but there was no difference between 18 and 26 °C (Fig. 2D).

In hepatopancreas, there was an increase in the lipid peroxidation only at 24 °C compared to the other temperatures. In the muscle, TBARS levels showed a temperature-dependent increase, with no difference between 24 and 26 °C. Lipid peroxidation in gills increased as the temperature increased (Fig. 2C).

4 DISCUSSION

Understanding how temperature alone affects biochemical and behavior biomarkers of a bioindicator is important for conducting in situ ecotoxicological studies and for biomonitoring programs. It is already known that temperature is a confounding factor in field studies, since in aquatic ectothermic animals, water temperature governs their metabolic rates (Lushchak and Bagnyukova 2006). In their natural habitats, organisms must deal with daily and seasonal temperature fluctuations, and both their behavior and metabolism are subject to these normal changes (Sheehan and Power 1999). Separating normal responses from those induced by pollutants is extremely necessary in ecotoxicology research to avoid under- or over-estimated results (Vinagre et al. 2014a).

In this study, exposure to different temperatures was able to induce behavioral and biochemical responses in *A. longirostri*. At higher temperatures, the animals presented lower activity in relation to their "ideal" temperature (18 °C) during the night. However, during the day, activity increased at higher temperatures. The aeglids are more active in the nocturnal period (Sokolowicz et al. 2007). Thus, this result may indicate a change of behavior at an environment with higher temperatures. The reversal of the period of highest activity can be detrimental in several aspects and may interfere not only with feeding but also with reproductive habits. Besides, the return time of the animals placed with the abdomen upwards to the usual position increased with the elevation of temperature, both in the morning and at night. With greater daytime activity and slower return to equilibrium response at high temperatures, they may be more prone to predation because they will have a slower escape response, and this may affect population dynamics. Crustaceans try to avoid higher temperatures by altering their locomotor activity (Lozán 2000), generally increasing activity at the ideal temperature (Lagerspetz and Vainio 2006). This observation was corroborated by our study, in which animals decrease their activity at night at higher temperatures and have higher activity at lower temperatures. As already demonstrated in some studies, the increase in temperature affects the survival of ectotherms (Issartel et al. 2005; Maazouzi et al. 2011). The percentage of survival of *A. longirostri* was lower in higher temperatures and was 100% at the temperature considered optimal for these crustaceans. Foucreau et al. (2014) observed a lower survival in *Gammarus pulex* (Crustacea) with increasing temperature.

Association between temperature and response to thermal stress can be dependent on the magnitude of the thermal stress (Madeira et al. 2013). Our results suggest that exposure of *A. longirostri* to extremes of hyperthermia compromises the antioxidant defense system.

A central enzyme in the detoxification process, glutathione S-transferase acts on the antioxidant defense system and is also involved in the transport of hormones (Coles et al. 2001; Hamilton et al. 2003; Hayes et al. 2005; Zhou et al. 2009). In this study, GST activity varied in the different tissues, usually declining with the elevation of temperature. Enzyme activity generally increases with increasing temperature. In *Parastacus brasiliensis promatensis* collected in all seasons, the GST activity of gills was higher in summer and low in winter, whereas in hepatopancreas the activity of this enzyme was low in winter (Pinheiro and Oliveira

2016). Also, *Palaemon elegans* exposed in the laboratory to different temperatures exhibited highest whole-body GST activity at 26 °C and then the activity decreased until critical thermal maximum (33 °C) (Vinagre et al. 2014a).

Non-protein thiols are non-enzymatic antioxidants also involved in enzymatic detoxification reactions. Glutathione (GSH) is the most abundant NPSH capable of controlling ROS generation (Pisoschi and Pop, 2015). However, as NPSH are involved in enzymatic reactions besides acting as an antioxidant, their levels in the different tissues of *A. longirostri* may not reflect a direct effect of different temperatures on this parameter. Thus, the increase of NPSH at 26 °C in gills can be a protective response since temperature can affect the activity of antioxidant enzymes.

CAT is one of the most important antioxidant enzymes involved in H_2O_2 elimination, one of the products of free radical reactions. The increased activity of CAT at 24 °C in the hepatopancreas can be related to a response to ROS effects, as seen by the elevation of TBARS levels. A possible explanation for the lower CAT activity at 26 ° C relative to 24 ° C is the fact that CAT generally acts at optimum at 25 ° C. In the mud crab *Scylla serrata*, CAT activity in gills was higher in summer and lower in winter (Kong et al. 2008). In a study with coastal shrimp, CAT activity decreased at higher temperatures (Vinagre et al. 2014a).

AChE is the enzyme that hydrolyzes the neurotransmitter acetylcholine in the cholinergic synapses of vertebrates and invertebrates. AChE activity was influenced by temperature in *Lepomis macrochirus* and *Rutilus rutilus* L. (Chuiko et al. 1997; Hogan 1970) although it is considered as a biomarker of neurotoxicity to xenobiotics. AChE activity in *Mytilus* sp. was positively correlated with the water temperature (1.6 to 22.9 °C) (Pfeifer et al. 2005). Contrary to the GST activity, in this study there was an increase in AChE activity with the elevation of temperature. This could be related to the delaying of the animal to return to equilibrium and to the reduction of the locomotory activity. The increase in AChE activity may lead to a reduction of cholinergic neurotransmission efficiency due to a decrease in acetylcholine levels in the synaptic cleft (Ferreira et al., 2012).

Carbonyl protein levels are a biomarker of oxidative protein damage (Dayanand et al. 2012). The hepatopancreas is the primary organ responsible for detoxification of endogenous and exogenous substances besides being the site of enzyme synthesis and nutrient assimilation (Claybrook 1983; Gibson and Barker 1979; Johnston et al. 1998). In this study, there was an increase in hepatopancreas carbonyl proteins with the elevation of temperature. This result demonstrates that an elevation in water temperature could cause a disruption of vital function in hepatic cells. The oxidation of proteins, as well as lipids, appears to be one of the apparent toxic effects of increasing temperature.

The increase in water temperature was also able to cause damage to other biomolecules in *Aegla*. The results point out a direct relationship between temperature and lipid peroxidation with an increase in TBARS levels according to the elevation of temperature (Madeira et al. 2014; Vinagre et al. 2014a, 2014b). The increase in TBARS levels, especially in gills, indicates damage to lipids, the main constituent of cell membranes, which could lead to irreversible cell damage and function. Lipid peroxidation occurs when reactive free radicals interact with fatty acids, especially unsaturated fatty acids (Dell'anna et al. 2007; Gutteridge and Halliwell 2010). Other studies confirm that temperature influences the increase of lipid peroxidation levels (Madeira et al., 2014). In hepatopancreas, there was also an increase in the level of lipid damage at 24 °C, but at 26 °C there was a reduction. The results point out a direct relationship between temperature and lipid peroxidation with an increase in TBARS levels according to the elevation of temperature. The decrease in GST activity would result in a decrease in the functions attributed to it, such as a lower metabolism of xenobiotics, thus the organisms would be more subject to their actions in the cell, such as an increase in lipid peroxidation.

Besides being active predators (Cerezer et al. 2016), aeglids are important freshwater shredders, which participate in the process of foliar debris decomposition and nutrient cycling (Cogo and Santos 2013). Alterations in their niche could cause imbalances in biogeochemical cycles and aquatic food chains. Considering that most species of aeglids (70%) are threatened (Santos et al. 2017), it is important to understand the effects that abiotic factors like temperature have on biomarkers commonly used in assessing the health of ecosystems and organisms, especially those exposed to pollutants. Our study showed that the water temperature might influence the behavior and biochemical responses of *A. longirostri*, and these responses must be considered when these animals are used in ecotoxicological studies in the field. One must always consider the peculiarities of the species and its thermal history to obtain the real status of exposure to pollutants (Vinagre et al. 2014a).

5 CONCLUSIONS

Even in a short-term study, different temperatures were able to cause changes not only in activity but also in the equilibrium and survival rate of *A. longirostri.* This study showed that temperature alone can cause changes in biochemical and behavioral biomarkers. Therefore, caution must be taken when evaluating these biomarkers in animals collected in situ since responses to daily or seasonal temperature changes can be confused with negative effects of exposure to pollutants in the freshwater ecosystem.

Acknowledgements

The Brazilian government financially supported this research through CAPES foundation (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) by the grant to CC and CNPq by the research grant to SS (proc. nº 311142/2014-1). We thank Dr. Luis Esteban Krause Lanés, Dr. Charlene Menezes, Dr. Bernardo Baldisserotto, by suggestions in the manuscript. We also thank Fernando Cereser, Charles Zarantonelo and laboratory colleagues for the technical support.

Competing interests

The authors declare no competing or financial interests.

REFERENCES

Abele, D., Heise, K., Pörtner, H.O., Puntarulo, S., 2002. Temperature- dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*. J. Exp. Biol. 205, 18311841.

Amaral, A.M.B., Gomes, J.L.C., Weimer, G.H., Marins, A.T., Loro, V.L., Zanella, R., 2018. Seasonal implications on toxicity biomarkers of *Loricariichthys anus* (Valenciennes, 1835) from a subtropical reservoir. Chemosphere 191, 876-885.

Arenas, J., 1976. La cordillera de la costa como refugio de la fauna dulcícola preglacial. Arch. Bio. Med. Exp. 10, 1-40.

Ayres-Peres, L., Araújo, P.B., Santos, S., 2011. Description of the agonistic behavior of *Aegla longirostri* (Decapoda: Aeglidae). J. Crustacean Biol. 31, 379-388.

Bagnyukova, T.V., Lushchak, O.V., Storey, K.B., Lushchak, V.I., 2007. Oxidative stress and antioxidant defense responses by goldfish tissues to acute change of temperature from 3 to 23 °C. J. Therm. Biol. 32, 227-234.

Bond-Buckup, G., Santos, S., 2007. Crustáceos anomuros de águas continentais: diversidade e aspectos biológicos. Ciência e Ambiente. 35, 47-54.

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

Briffa, A., Bridger, D.A., Biro, P.A., 2013. How does temperature affect behavior? Multilevel analysis of plasticity, personality and predictability in hermit crabs. Anim. Behav. 86, 47-54.

Cailleaud, K., Maillet, G., Budzinski, H., Souissi, S., Forget-Leray, J., 2007. Effects of Salinity and Temperature on the Expression of Enzymatic Biomarkers in *Eurytemora affinis* (Calanoida, Copepoda). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 147, 841-849.

Cerezer, C., Biasi, C., Cogo, G.B., Santos, S., 2016. Avoid predation or take risks in basic activities? Predator-prey relationship in subtropical streams between decapods and caddisflies. Mar. Freshwater Res. 67, 1880-1887.

Chuiko, G.M., Zhehin, Y., Pod'gornaya, V.A., 1997. Seasonal fluctuation in brain acetylcholinesterase activity and soluble protein content in roach (*Rutilus rutilus* L.): a freshwater fish from northwest Russia. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 117, 251-257.

Claybrook, D.L., 1983. Nitrogen metabolism. In: Mantel, L.H. The Biology of Crustacea. Internal Anatomy and Physiological Regulation. Academic Press, New York. 5, 163-213.

Cogo, G.B., Santos, S., 2013. The role of aeglids in shredding organic matter in Neotropical streams. J. Crustacean Biol. 33, 519-526.

Coles, B., Nowell, S.A., MacLeod, S.L., Sweeney, C., Lang, N.P., Kadlubar, F.F., 2001. The role of human glutathione S-transferases (hGSTs) in the detoxification of the food-derived carcinogen metabolite N-acetoxy-PhIP, and the effect of a polymorphism in hGSTA1 on colorectal cancer risk. Mutat. Res. 482, 3-10.

Colpo, K.D., Ribeiro, L.O., Santos, S., 2005. Population biology of the freshwater anomura *Aegla longirostri* (Aeglidae) from South Brazilian streams. J. Crustacean Biol. 25, 495-499.

Cumberlidge, N., Ng, P.K.L., Yeo, D.C.J., Magalhães, C., Campos, M.R., Alvarez, F., Naruse, T., Daniels, S.R., Esser, L.J., Attipoe, F.Y.K., Clotilde-Ba, F.L., Darwall, W., McIvor, A., Baillie, J.E.M., Collen, B., Ram, M., 2009. Freshwater crabs and the biodiversity crisis: importance, threats, status, and conservation challenges. Biol. Conserv. 142, 1665-1673.

Da Rocha, A.M., De Freitas, D.P.S., Burns, M., Vieira, J.P., De La Torre, F.R., Monserrat, J.M., 2009. Seasonal and Organ Variations in Antioxidant Capacity, Detoxifying Competence and Oxidative Damage in Freshwater and Estuarine Fishes From Southern Brazil. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 150, 512-520.

Dalosto, M., Santos, S., 2011. Differences in oxygen consumption and diel activity as adaptations related to microhabitat in Neotropical freshwater decapods (Crustacea). Comp. Biochem. Physiol. Part A – Mol. Integr. Physiol. 160, 461-466.

Delorenzo, M.E., 2015. Impacts of climate change on the ecotoxicology of chemical contaminants in estuarine organisms. Curr. Zool. 61, 641-652.

Dalzochio, T., Gehlen, G., 2016. Confounding factors in biomonitoring using fish. Ecotoxicol. Environ. Contam. 11, 53-61.

Dalzochio, T., Rodrigues, G.Z.P., Petry, I.E., Gehlen, G., Silva, L.B., 2016. The use of biomarkers to assess the health of aquatic ecosystems in Brazil: a review. Int. Aquat. Res. 8, 283-298.

Dayanand, C.D., Vegi. P.K., Kutty, A.V., 2012. Protein carbonyl content as a stable oxidative stress marker in type II diabetes. Int. J. Biol. Med. Res. 3, 2362-2365.

Dell'anna, M.L., Ottaviani, M., Albanesi, V., Vidolin, A.P., Leone, G., Ferraro, C., Cossarizza, A., Rossi, L., Picardo, M., 2007. Membrane lipid alterations as a possible basis for melanocyte degeneration in vitiligo. J. Invest. Dermatol. 127, 1226-1233.

Draper, H.H., Hadley, M., 1990. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. 186, 421-431.

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

Ellman, G.L., Coutney, K.D., Andres Jr., V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88-95.

Felten, V., Guerold, F., 2001. Hyperventilation and loss of hemolymph Na and Cl in the freshwater amphipod *Gammarus fossarum* exposed to acid stress: a preliminary study. Dis. Aquat. Org. 45, 77-80.

Ferreira, B.D.P., Hack, C., de Oliveira, G.T., Bond-Buckup, G., 2005. Perfil metabólico de *Aegla platensis* Schimitt, 1942 (Crustacea, Anomura) submetida a dietas ricas em carboidratos ou proteínas. Rev. Bras. Zool. 22, 161-168.

Ferreira, G.K., Carvalho-Silva, M., Gonçalves, C.L., Vieira, J.S., Scaini, G., Ghedim, F.V., Deroza, P.F., Zugno, A.I., Pereira, T.C.B., Oliveira, G.M.T., Kist, L.W., Bogo, M.R., Schuck, P.F., Ferreira, G.C., Streck, E.L., 2012. l-Tyrosine administration increases acetylcholinesterase activity in rats. Neurochem. Int. 61, 1370-1374.

Foucreau, N., Cottin, D., Piscart, C., Hervant, F., 2014. Physiological and metabolic responses to rising temperature in *Gammarus pulex* (Crustacea) populations living under continental or Mediterranean climates. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 168, 69-75.

Gandar, A., Laffaille, P., Marty-Gasset, N., Viala, D., Molette, C., Jean, S., 2017. Proteome response of fish under multiple stress exposure: effects of pesticide mixtures and temperature increase. Aquat. Toxicol. 184, 61-77.

Gibson, O., Barker, P.L., 1979. The decapod hepatopancreas. Oceanogr. Mar. Biol. Ann. Rev. 17, 285-346.

Gutteridge, J.M., Halliwell, B., 2010. Antioxidants: molecules, medicines, and myths. Biochem. Biophysis. Res. Commun. 393, 561-564.

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

Hamilton, D.S., Zhang, X., Ding, Z., Hubatsch, I., Mannervik, B., Houk, K.N., Ganen, B., Creighton, D.J., 2003. Mechanism of the glutathione transferase-catalyzes conversion of antitumor 2-crotonyloxymethyl-2-cycloalkenones to GSH adducts. J. Am. Chem. Soc. 125, 15049-15058.

Hayes, J.D., Flanagan, J.U., Jowsey, I.R., 2005. Glutathione transferases. Annu. Rev. Pharmacol. Toxicol. 45, 51-88.

Hemmer-Brepson, C., Replumaz, L., Romestaing, C., Voituron, Y., Daufresne, M., 2014. Non-stressful temperature effect on oxidative balance and life history traits in adult fish (*Oryzias latipes*). J. Exp. Biol. 217, 274-282.

Heugens, E.H.W., Hendriks, A.J., Dekker, T., van Straalen, N.M., Admiraal, W., 2001. A Review of the Effects of Multiple Stressors on Aquatic Organisms and Analysis of Uncertainty Factors for Use in Risk Assessment. Crit. Rev. Toxicol. 31, 247-284.

Hochachka, P.W., Somero, G.M., 1984. Biochemical adaptation. Princeton University Press, New York.

Hogan, J.M., 1970. Water temperature as a source of variation in specific activity of brain acetylcholinesterase of bluegills. Bull. Environ. Contam. Toxicol. 5, 347-353.

Hook, S.E., Gallagher, E.P., Batley, G.E., 2014. The role of biomarkers in the assessment of aquatic ecosystem health. Integr. Environ. Assess. Manag. 10, 327-341.

Issartel, J., Hervant, F., Voituron, Y., Renault, D., Vernon, P., 2005. Behavioural, ventilatory and respiratory responses of epigean and hypogean crustaceans to different temperatures. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 141, 1-7.

Johnston, D.J., Alexander, C.G., Yellowhees, D., 1998. Epithelial cytology and function in the digestive gland of *Thenus orientalis* (Decapoda, Scyllaridae). J. Crustacean Biol. 18, 271-278.

Kamyab, E., Kühnhold, H., Novais, S.C., Alves, L.M.F., Indriana, L., Kunzmann, A., Slater, M., Lemos, M.F.L., 2017. Effects of thermal stress on the immune and oxidative stress responses of juvenile sea cucumber *Holothuria scabra*. J. Comp. Physiol. B 187, 51-61.

Kong, X., Wang, G., Li, S., 2008. Seasonal variations of ATPase activity and antioxidant defenses in gills of the mud crab *Scylla serrata* (Crustacea, Decapoda). Mar. Biol. 154, 269-276.

Lagerspetz, K.Y., Vainio, L.A., 2006. Thermal behaviour of crustaceans. Biol. Rev. Camb. Philos. Soc. 81, 237-258.

Loiuz, I., Hassine, O.K.B., Palluel, O., Ben-Attia, M., Aït-Aïssa, S., 2016. Spatial and temporal variation of biochemical biomarkers in *Gobius niger* (Gobiidae) from a southern Mediterranean lagoon (Bizerta lagoon, Tunisia): Influence of biotic and abiotic factors. Mar. Poll. Bull. 107, 305-314.

Lozán, J.L., 2000. On the threat to the European Crayfish: a contribution with the study of the activity behaviour of four crayfish species (Decapoda: Astacidae). Limnologica. 30, 156-161.

Lushchak, V.I., Bagnyukova, T.V., 2006. Temperature increase results in oxidative stress in goldfish tissues. 1. Indices of oxidative stress. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 143, 30-35.

Maazouzi, C., Piscart, C., Legier, F., Hervant, F., 2011. Ecophysiological responses to temperature of the “killer shrimp” *Dikerogammarus villosus*: is the invade really stronger than the native *Gammarus pulex*? Comp. Biochem. Physiol. A Mol. Integr. Physiol. 159, 268-274.

Madeira, D., Narciso, L., Cabral, H.N., Vinagre, C., Diniz, M.S., 2013. Influence of temperature in thermal and oxidative stress responses in estuarine fish. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 166, 237-243.

Madeira, D., Narciso, L., Cabral, H.N., Diniz, M.S., Vinagre, C., 2014. Role of thermal niche in the cellular response to thermal stress: lipid peroxidation and HSP70 in coastal crabs. Ecol. Indic. 36. 601-606.

Magni, S.T., Py-Daniel, V., 1989. *Aegla platensis* Schmitt, 1942 (Decapoda, Anomura) um predador de imaturos de Simullidae (Diptera, Culicomorpha). Rev. Saúde Publ. 23, 258-259.

Maltby, L., Clayton, S.A., Wood, R.M., McLoughlin, N., 2002. Evaluation of the *Gammarus pulex* in situ feeding assay as a biomonitor of water quality: robustness, responsiveness and relevance. Environ. Toxicol. Chem. 21, 361-368.

Marins, A.T., Severo, E.S., Leitemperger, J.W., Cerezer, C., Muller, T.E., Costa, M.D., Weimer, G.H., Bandeira, N.M.G., Prestes, O.D., Zanella, R., Loro, V.L., 2020. Assessment of River Water Quality in an Agricultural Region of Brazil Using Biomarkers in a Native Neotropical Fish, *Astyanax* Spp. (Characidae). Bull. Environ. Contam. Toxicol. 104, 575-581.

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

Oliveira, G.T., Fernandes, F.A., Bond-Buckup, G., Bueno, A.A., Silva, R.S.M., 2003. Circadian and seasonal variations in the metabolism of carbohydrates in *Aegla ligulate* (Crustacea: Anomura: Aeglidae). Memoirs Museum of Victoria. 60, 59-62.

Pardini, R., 1998. Feeding ecology of the neotropical river otter *Lontra longicaudis* in an Atlantic Forest Stream, south-eastern Brazil. J. Zool. 245, 385-391.

Pfeifer, S., Schiedek, D., Dippner, J.W., 2005. Effect of temperature and salinity on acetylcholinesterase activity, a common pollution biomarker, in *Mytilus* sp. from the south-western Baltic Sea. J. Exp. Mar. Biol. Ecol. 320, 93-103.

Pinheiro, L.C., Oliveira, G.T., 2016. Oxidative Status Profle in Different Tissues of *Parastacus brasiliensis promatensis* (Crustacea, Decapoda, Parastacidae) over a Seasonal Cycle. J. Exp. Zool. 325, 318-328.

Pisoschi, A.M., Pop, A., 2015. The role of antioxidants in the chemistry of oxidative stress: a review. Eur. J. Med. Chem. 97, 55-74.

Santos, S., Bond-Buckup, G., Gonçalves, A.S., Batholomei-Santos, M.L., Buckup, L., Jara, C.G., 2017. Diversity and conservation status of *Aegla* spp. (Anomura, Aeglidae): an update. Nauplius. 25, 1-14.

Sardi, A.E., Renaud, P.E., Lana, P.C., Camus, L., 2016. Baseline levels of oxidative stress biomarkers in species from a subtropical estuarine system (Paranaguá Bay, southern Brazil). Mar. Poll. Bull. 113, 496-508.

Severo, E.S., Marins, A.T., Cerezer, C., Costa, D., Nunes, M., Prestes, O.D., Zanella, R., Loro, V.L., 2020. Ecological Risk of Pesticide Contamination in a Brazilian River Located Near a Rural Area: A Study of Biomarkers Using Zebrafish Embryos. Ecotoxicol. Environ. Saf. 190, 110071.

Sheehan, D., Power, A., 1999. Effects of seasonality on xenobiotic and antioxidant defence mechanisms of bivalve molluscs. Comp. Biochem. Phys. C 123, 193-199.

Sokolowicz, C.C., López-Greco, L.S., Gonçalves, R., Bond-Buckup, G., 2007. The gonads of *Aegla plantensis* Schimitt (Decapoda, Anomura, Aeglidae): a macroscopic and histological perspective. Acta Zoologica. 88, 71-79.

Turra, A., Denadai, M.R., 2003. Daily activity of four tropical intertidal hermit crabs from southeastern Brazil. Braz. J. Biol. 63, 537-544.

Vinagre, C., Madeira, D., Mendonça, V., Dias, M., Roma, J., Diniz, M.S., 2014a. Effect of temperature in multiple biomarkers of oxidative stress in coastal shrimp. J. Thermal Biol. 41, 38-42.

Vinagre, C., Madeira, D., Mendonça, V., Dias, M., Roma, J., Diniz, M.S., 2014b. Effect of increasing temperature in the differential activity of oxidative stress biomarkers in various tissues of the Rock goby, *Gobius paganellus*. Mar. Environ. Res. 97, 10-14.

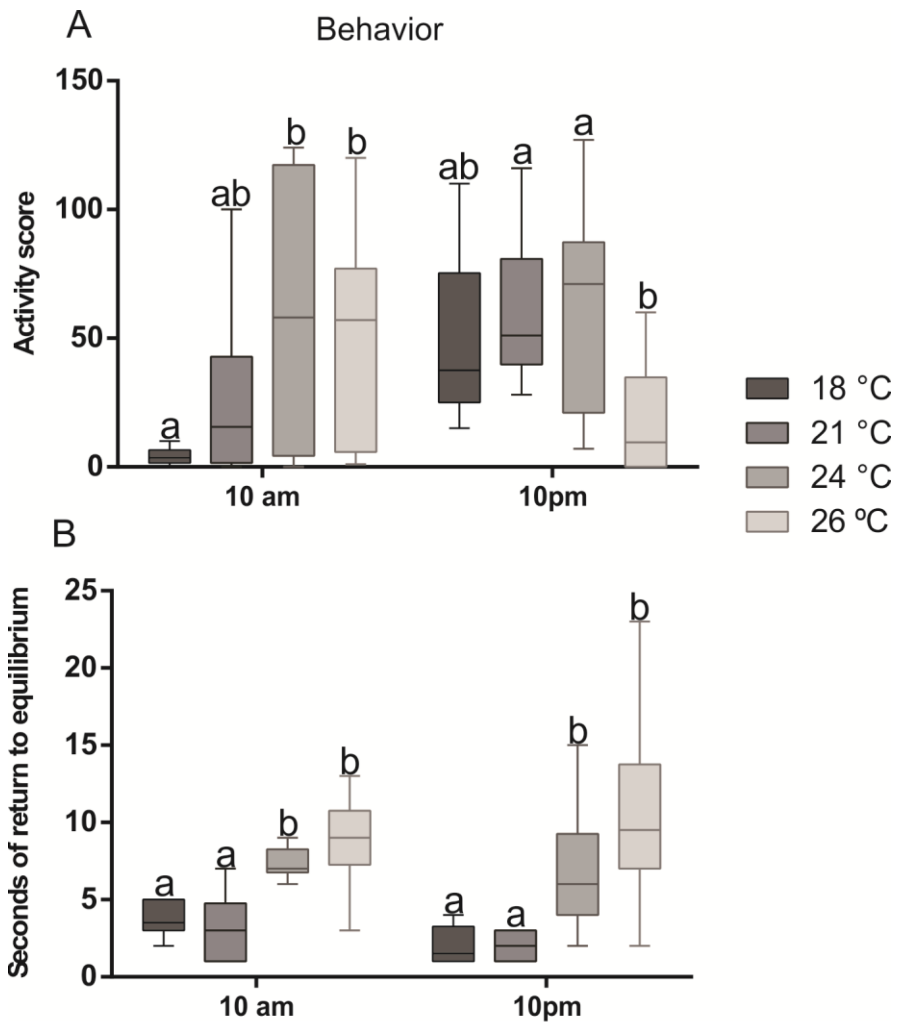
Wallace, W.G., Estephan, A., 2004. Differential susceptibility of horizontal and vertical swimming activity to cadmium exposure in a gammaridean amphipod (*Gammarus lawrencianus*). Aquat. Toxicol. 69, 289-297.

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

Zhou, J., Wang, W.N., Wang, A.L., He, W.Y., Zhou, Q.T., Liu, Y., Xu, J., 2009. Glutathione S-transferase in the white shrimp *Litopenaeus vannamei*: characterization and regulation under pH stress. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 150, 224-230.

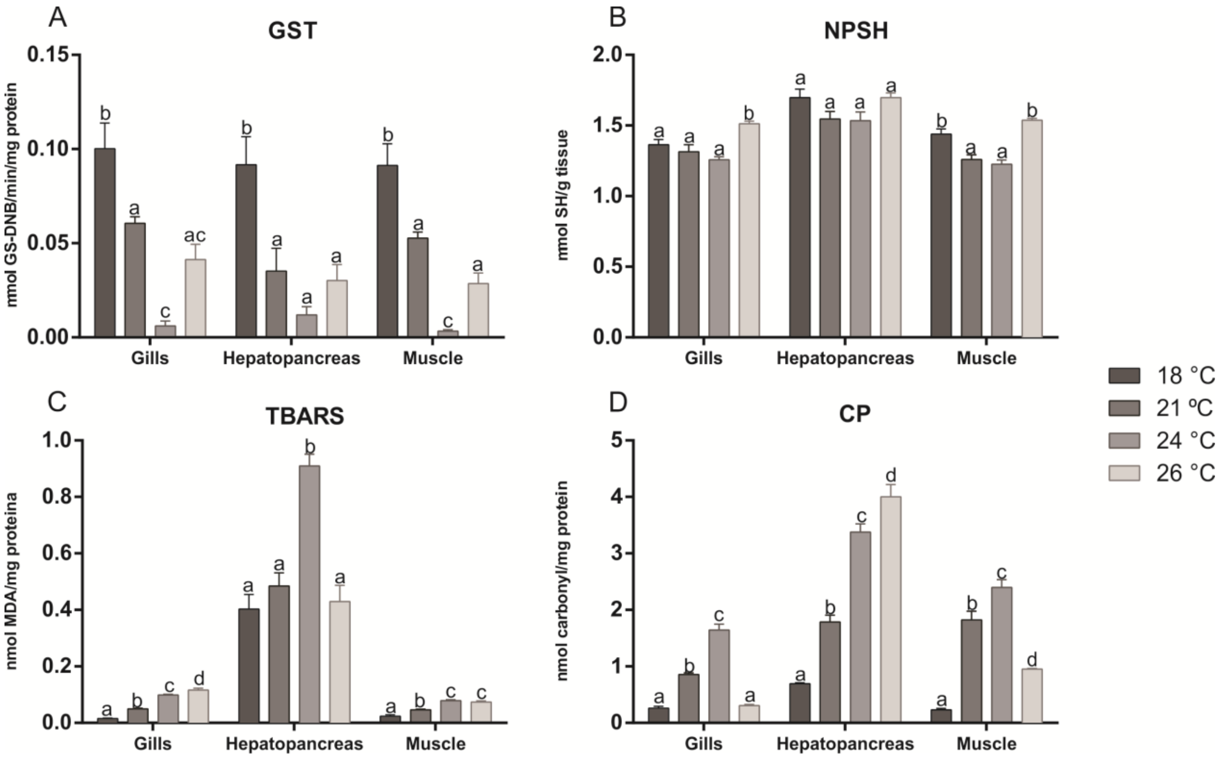
FIGURES

Fig. 1 Activity score (A), time (s) taken by the freshwater crab *Aegla longirostri* to return to equilibrium (B) at different temperatures at 10 a.m. and 10 p.m



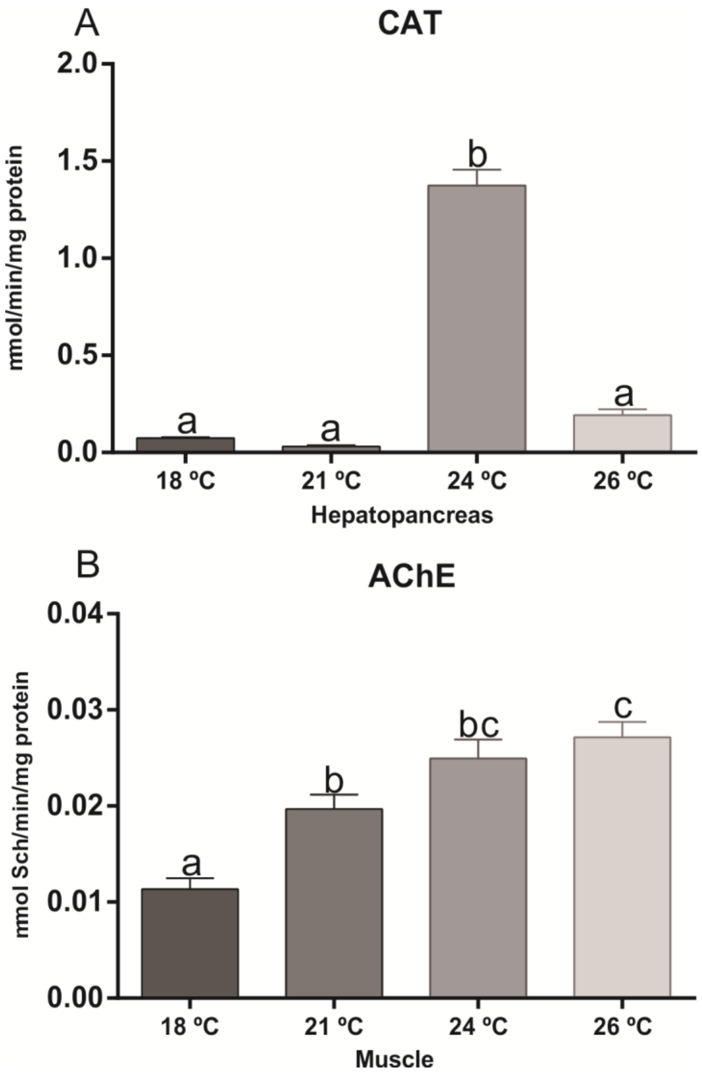
Data are reported as Box and Whiskers ± min to max (n = 10). Different letters indicate differences between groups ($p < 0.05$).

Fig. 2 GST activity (A), NPSH levels (B), CP content (C) and TBARS levels (D) in hepatopancreas, muscle and gills of the freshwater crab *Aegla longirostri* at different temperatures



Data are reported as mean ± standard error (n = 10). Different letters indicate differences between groups ($p < 0.05$).

Fig. 3 CAT activity in hepatopancreas (A) and AChE activity in muscle (B) of the freshwater crab *Aegla longirostri* at different temperatures



Data are reported as mean ± standard error (n = 10). Different letters indicate differences between groups ($p < 0.05$)

TABLES

Table 1. Description of the behaviors analyzed in the freshwater crab *Aegla longirostri* and their respective scores

Behavior	Description	Score
Inactive	Absence of apparent movement or only movements of cephalic appendages (antennas, antennules, and / or maxillipeds)	0
Low activity	Movements of chelipods, pereopods, pleopods and / or short movements of animals	1
Moderate activity	Active movement of the animal through the aquarium	2
Intense activity	Tail flipping	3

Table 2. Percentage of survival and lethality of the freshwater crab *Aegla longirostri* at each temperature tested including the maximum survival temperature

	Temperature (°C)			
	18	**21**	**24**	**26**
% Survival	100	93.4	80	73.3
Lethality	0	6.6	20	26.7

5 DISCUSSÃO

O desenvolvimento das atividades antrópicas e o crescimento populacional acabam gerando impactos ambientais negativos, que afetam os ecossistemas como um todo. Devido às exigências de consumo terem aumentado, houve um aumento na produção de alimentos, expansão das culturas e a eliminação da vegetação natural. Com essa necessidade de aumentar a produtividade agrícola foram necessários novos produtos que controlassem as “pragas” que dificultam o desenvolvimento da produção. O Brasil aprovou o registro de 493 agrotóxicos em 2020 sendo, o maior número documentado pelo Ministério da Agricultura e os registros vêm crescendo anualmente no país desde 2016 (MAPA, 2021). Esse aumento não só no registro, mas também no consumo de pesticidas gera uma preocupação sobre o destino desses produtos nos ecossistemas aquáticos. Além disso estudos sobre a presença desses defensivos agrícolas em riachos são raras, tornando assim pesquisas sobre esses contaminantes nesses locais de suma importância e urgência.

Devido às atividades antrópicas, os ecossistemas de água doce têm sido expostos a diversos contaminantes, que acabam interagindo com os organismos que ali vivem. Como consequência, a aplicação de pesticidas afeta também organismos não-alvos, ou seja, organismos que não interferem no processo de produção (SCHÄFER et al., 2011). Organismos não alvos podem assimilar e reter os contaminantes através da absorção direta, absorção indireta (através do alimento contaminado), ou a partir do ambiente, acarretando efeitos danosos para sua saúde, podendo levá-los à morte (AMÉRICO et al., 2015; LINS et al. 2010).

A proximidade com diferentes culturas agrícolas torna riachos receptores para diversos pesticidas ao longo do ano. Em encontro com essa problemática, a presente tese apresenta no artigo 1 um total de 24 amostras de água analisadas, onde todas elas apresentavam pesticidas. Foram detectados 22 princípios ativos: 13 fungicidas, 5 herbicidas, 3 inseticidas e 1 acaricida. Os princípios ativos mais frequentes estão relacionados à produção de soja e arroz; essas culturas talvez sejam as que mais ameacem a fauna desses ecossistemas aquáticos. No entanto, a soja merece destaque, pois é a cultura com maior período de aplicação de agrotóxicos no ano. Além disso, está relacionado a pelo menos 14 dos princípios ativos identificados (ANVISA, 2019).

O Clomazone foi o princípio ativo mais frequentemente encontrado em todas as amostras, o que era esperado pois ele é usado ao longo do ano em várias culturas. O segundo pesticida mais comumente encontrado foi o herbicida Atrazina, composto que é proibido na Europa, mas no resto do mundo ainda é um dos herbicidas mais utilizados, sendo um dos herbicidas mais vendidos no Brasil (IBAMA, 2020). Outro composto interessante é o inseticida Propoxur, que detectamos em concentrações muito significativas. Assim sendo, possivelmente está sendo mal utilizado em áreas agrícolas como controle de pragas, porque não é aprovado para aplicação em áreas agrícolas e é restrito ao uso veterinário e doméstico. A concentração de bentazona foi a mais alta entre todos os pesticidas identificados. Este herbicida é amplamente utilizado na cultura do arroz e possui alta persistência em água (BASF, 2012). Outro pesticida que causa preocupação é o Carbofurano, pois esse foi proibido no Brasil, e exibe um amplo espectro de atividade contra muitas pragas agrícolas e exerce efeitos muito tóxicos para invertebrados e peixes (OTIENO et al., 2010).

No artigo 1 vimos que o menor número de princípios ativos encontrado no Sítio Referência se deve principalmente ao fato de estar mais distante das atividades antrópicas e a mata ciliar ter sido preservada, fator que também reduz a variação dos dados abióticos. Esses dados destacam a importância da preservação da mata ciliar para garantir a sobrevivência das espécies. A mata ripária atua como uma barreira ao escoamento superficial e lixiviação de contaminantes das culturas adjacentes, reduzindo o movimento de pesticidas e nutrientes de campos agrícolas (COLE et al., 2020; PROSSER et al., 2020; VIEIRA et al., 2016). Além disso, os animais expostos ao Sítio Referência apresentaram menor dano oxidativo e maior capacidade antioxidante em relação aos demais locais de exposição.

Estes poluentes acabam desencadeando uma cascata de respostas biológicas causando uma situação de estresse nos organismos aquáticos. Apesar de existirem diferentes métodos de avaliação da qualidade ambiental, os mais utilizados atualmente são os de análises químicas e testes de toxicidade utilizando organismos. Assim, a ecotoxicologia tem sido empregada por autores da área para descrever os efeitos adversos causados aos organismos vivos pelas substâncias químicas liberadas no ambiente, considerando variáveis abióticas e biológicas e suas interações, apresentando respostas integrativas especialmente a misturas complexas de poluentes (COSTA, 2010; GHISI et al., 2014). Biomarcadores são análises bioquímicas, moleculares, comportamentais que avaliam desvios do estado normal do organismo em função de exposição a xenobióticos (VAN DER OOST et al., 2003) e complementam avaliações físico-químicas da água do ambiente de estudo. Nessa tese avaliamos biomarcadores bioquímicos além da análise de resíduos de pesticidas. Pesticidas e outras variáveis abióticas encontradas na água desses riachos causaram alterações em diversos biomarcadores bioquímicos em *A. longirostri*, em um padrão tecido-específico.

Com base na RDA, as amostras foram claramente separadas no que diz respeito às variáveis ambientais, nomeadamente a temperatura, havendo uma distinção entre meses mais frios e meses mais quentes. Assim, os resultados deste estudo fornecem evidências importantes de que os biomarcadores são sazonalmente afetados pelas condições ambientais. Além da temperatura, a análise RDA indicou a influência de outros fatores abióticos nas variáveis bioquímicas.

As margens de riachos, que são áreas de preservação permanente, muitas vezes não são respeitadas, o que torna a contaminação desses locais ainda maior. Além disso, a ausência de vegetação nas margens desses ecossistemas aumenta a incidência de luz e consequentemente a temperatura desses locais. Sabe-se que fatores abióticos, como a temperatura da água, podem alterar o potencial tóxico de poluentes presentes em ambientes aquáticos (MANCIOCCO et al., 2014) e aumentar a produção de ROS (LUSHCHAK e BAGNYUKOVA, 2006). Esses fenômenos podem levar à morte de animais. Um aumento na temperatura da água causa um aumento no consumo de oxigênio e provavelmente um aumento na produção de espécies reativas e na captação de compostos (LAETZ et al., 2014). Como a toxicidade de vários produtos químicos agrícolas mostrou ser dependente da temperatura (COATS et al., 1989; LYDY et al., 1999; WILLMING, et al 2013), é importante avaliar essas interações com compostos ainda não estudados, mas além disso é necessário saber qual é a resposta a diferentes temperaturas de forma isolada.

Sabe-se que para muitos organismos aquáticos a temperatura é uma importante variável ambiental que pode influenciar os mecanismos fisiológicos em níveis enzimáticos e celulares, resultando em alterações nas taxas metabólicas (CAIRNS et al., 1975; WARD, STANFORD, 1982). Tais efeitos de temperatura podem modificar a capacidade de um organismo de detoxificar xenobióticos, alterando as taxas de absorção, eliminação ou biotransformação de contaminantes (HOOPER et al., 2013).

A. longirostri é geralmente encontrada em baixas temperaturas em riachos bem oxigenados (BOND-BUCKUP, SANTOS, 2007). Eglídeos são organismos ectotérmicos e regulam sua temperatura corporal por meio do meio ambiente. Portanto, a temperatura pode ter efeitos diretos e indiretos nos processos metabólicos intimamente associados à temperatura da água (SOKOLOVA, LANNIG, 2008). Em seus habitats naturais, os organismos precisam lidar com as flutuações de temperatura diárias e sazonais, e tanto seu comportamento quanto seu metabolismo estão sujeitos a essas mudanças naturais (SHEEHAN, POWER, 1999). Separar as respostas naturais daquelas induzidas por poluentes é extremamente necessário na pesquisa de ecotoxicologia para evitar resultados sub ou superestimados (VINAGRE et al., 2014a). Tendo isso em vista, no artigo 2 diferentes temperaturas foram capazes de causar mudanças não apenas na atividade, mas também no equilíbrio e na taxa de sobrevivência de *A. longirostri.* Sendo assim, esse estudo mostra que a temperatura por si só pode causar alterações nos biomarcadores bioquímicos e comportamentais.

Nossos resultados sugerem que a exposição de *A. longirostri* a extremos de hipertermia compromete o sistema de defesa antioxidante. Também vimos a alteração comportamental em relação a elevação da temperatura, onde animais diminuíram sua atividade à noite em temperaturas mais altas e apresentaram maior atividade em temperaturas mais baixas. Como já foi demonstrado em alguns estudos, o aumento da temperatura afeta a sobrevivência dos ectotérmicos (ISSARTEL et al., 2005; MAAZOUZI et al., 2011). A porcentagem de sobrevivência de *A. longirostri* foi menor em altas temperaturas e foi de 100% na temperatura considerada ótima para esses crustáceos. Como vimos no capítulo 1 a alta temperatura da água combinada com os agrotóxicos em S1 possivelmente levou a um estresse fisiológico irreversível, culminando em 100% de letalidade.

O que gera preocupação em relação aos resultados do capítulo 2 é a reversão do período de maior atividade, o que pode ser prejudicial em vários aspectos e pode interferir não só na alimentação, mas também nos hábitos reprodutivos desses animais. Além disso, o tempo de retorno dos animais colocados com o abdômen para cima à posição usual aumentava com a elevação da temperatura, tanto pela manhã quanto à noite. Com maior atividade diurna e retorno mais lento à resposta de equilíbrio em altas temperaturas, eles podem estar mais sujeitos à predação porque terão uma resposta de fuga mais lenta, e isso pode afetar a dinâmica populacional.

Embora os pesticidas sejam utilizados para beneficiar a vida humana por meio do aumento da produtividade agrícola, quando aplicados de maneira negligente, seus efeitos adversos superaram os benefícios associados ao seu uso. A discussão acima destaca algumas possíveis consequências do uso indiscriminado de pesticidas em riachos. O Brasil, líder mundial no uso de pesticidas, carece de uma legislação rígida para garantir seu uso consciente. A legislação brasileira não estabeleceu limites de agrotóxicos em água doce para a maioria dos princípios ativos encontrados neste estudo (CONAMA, 2005). A ausência de limites para esses compostos e os efeitos relatados neste estudo destacam os riscos de exposição aos organismos aquáticos. Na verdade, mesmo a exposição de curto prazo a concentrações muito baixas gerou danos notáveis nesses animais. Além disso, os poluentes podem causar efeitos nos indivíduos que não são aparentes até que aconteçam mudanças à nível de população ou ecossistema, que são mais difíceis de remediar (LINDE-ARIAS et al., 2008). A exposição a poluentes pode diminuir a capacidade dos organismos de responder às mudanças ambientais uma vez que ativar sistemas de defesa tem um alto custo energético (GANDAR et al., 2017).

Sabemos que suspender completamente o uso de pesticidas é impossível, porém é sabido que existem de fato estratégias para a redução do uso, usos corretos e usos alternativos desses pesticidas. O momento requer o uso adequado de destes produtos para proteger o meio ambiente, o que demanda estratégias alternativas de controle de pragas para reduzir o número e a quantidade de aplicações. Além disso, abordagens avançadas, como biotecnologia e nanotecnologia, podem facilitar o desenvolvimento de genótipos resistentes ou pesticidas com menos efeitos adversos (GILL, GARG., 2014). Se faz necessário também um desenvolvimento comunitário e programas de extensão (conversa entre universidade e comunidade com assistência técnica e educação ambiental, por exemplo), que poderiam educar e encorajar os agricultores a adotarem estratégias inovadoras que reduzem o impacto deletério dos pesticidas no meio ambiente.

A previsão das possíveis consequências do uso excessivo de pesticidas associados a outros fatores abióticos é de grande importância para a conservação dos organismos aquáticos, pois grande parte das espécies de eglídeos estão ameaçadas (SANTOS et al., 2017). Esses animais são predadores ativos (CEREZER et al., 2016) e importantes trituradores em riachos. Mudanças em seu nicho podem causar desequilíbrios nos ciclos biogeoquímicos e nas cadeias alimentares aquáticas.

Entender os efeitos que os fatores abióticos, como a temperatura, têm sobre os biomarcadores comumente usados na avaliação da saúde de ecossistemas e organismos, especialmente aqueles expostos a poluentes, é muito importante. Nosso estudo mostrou que a temperatura da água pode influenciar o comportamento e as respostas bioquímicas de *A. longirostri*, e essas respostas devem ser consideradas quando esses animais são usados em estudos ecotoxicológicos em campo. Deve-se sempre considerar as peculiaridades da espécie e sua história térmica para obter o real estado de exposição aos poluentes (VINAGRE et al., 2014).

Além disso, futuros estudos laboratoriais devem levar em conta as flutuações diárias de temperatura (WILLMING et al., 2013).

6 CONCLUSÃO

- As frequências de pesticidas encontradas neste estudo foram equivalentes aos períodos de maior e menor aplicação nas lavouras.
- Os pesticidas combinados com os fatores abióticos foram capazes de gerar diferentes respostas nos biomarcadores bioquímicos testados.
- A letalidade de animais expostos a água de baixa qualidade contendo grande variedade de pesticidas, alguns em altas concentrações, evidencia os riscos à integridade da espécie.
- Fica clara a necessidade de estudos com concentrações de pesticidas ambientalmente relevantes, em mistura e em associação com fatores abióticos, para compreender o risco de exposição a organismos não visados.
- O uso de biomarcadores em sítios aquáticos com interferência da atividade agrícola permite que atitudes sejam tomadas antes que os efeitos deletérios, principalmente dos pesticidas, sobre tais espécies se tornem irreversíveis e a integridade do ecossistema seja afetada como um todo.
- As diferenças encontradas entre o sítio de referência e os demais sítios, tanto na frequência dos pesticidas quanto nos diversos biomarcadores bioquímicos, chamam a atenção para a necessidade de preservação da mata ciliar e maior rigor na legislação vigente e fiscalização, a fim de assegurar a preservação dos corpos hídricos e consequentemente a preservação da fauna aquática.
- A temperatura *per se* pode causar alterações nos biomarcadores bioquímicos e comportamentais.
- Mesmo em um estudo de curto prazo, diferentes temperaturas foram capazes de causar mudanças não apenas na atividade, mas também no equilíbrio e na taxa de sobrevivência de *A. longirostri.*
- As respostas às mudanças diárias ou sazonais de temperatura podem ser confundidas com os efeitos negativos da exposição a poluentes no ecossistema de água doce.

REFERÊNCIAS

AHMED, R.G. Is there a balance between oxidative stress and antioxidant defense system during development? **Medical Journal of Islamic World Academy of Sciences,** 15: 55–63, 2005.

ALBUQUERQUE, A. F. Pesticides in Brazilian freshwaters: a critical review. **Environmental Science: Processes and Impacts**, 18: 779-787.
AMÉRICO, J. H. P. et al. 2015. O uso de agrotóxicos e os impactos nos ecossistemas aquáticos. **Revista Científica ANAP Brasil,** 8: 101-115, 2016.

AMARAL, A. M. B., et al. Seasonal implications on toxicity biomarkers of *Loricariichthys anus* (Valenciennes, 1835) from a subtropical reservoir. **Chemosphere,** 191: 876-885, 2018.

ANVISA - Agência nacional de vigilância sanitária. Regularização de Produtos – Agrotóxicos. Monografias Autorizadas, 2019.

ANA - Agência Nacional de Águas. Panorama da qualidade das águas superficiais do Brasil/2012, p. 264. Brasília, 2012;

ANDREA, M.M., PERES, T.B., LUCHINI, L.C., PETTINELLI JUNIOR, A. Impact of long-term pesticide application on some soil biological parameters. **Journal of Environmental Science and Health**, 35: 297-307, 2000.

ARENAS, J. La cordillera de la costa como refugio de la fauna dulcícola preglacial. **Archivos de biología y medicina experimentales,** 10:1–40, 1976.

BAGNYUKOVA, T.V., LUSHCHAK, O.V., STOREY, K.B., LUSHCHAK, V.I. Oxidative stress and antioxidant defense responses by *goldfish* tissues to acute change of temperature from 3 to 23 °C. **Journal of Thermal Biology,** 32: 227–234, 2007.

BOND-BUCKUP, G. & BUCKUP, L. Bond-Buckup, G., Buckup, L., 1994. A família *Aeglidae* (Crustacea, Decapoda, Anomura). **Arquivos de Zoologia** (São Paulo), 32: 159–346, 1994.

BOND-BUCKUP, G. & SANTOS, S. Crustáceos anomuros de águas continentais: diversidade e aspectos biológicos. **Ciência e Ambiente,** 35: 47–54, 2007.

BRIFFA, A., BRIDGER, D.A., BIRO, P.A. How does temperature affect behavior? Multilevel analysis of plasticity, personality and predictability in hermit crabs. **Animal Behavior,** 86:47–54, 2013.

BORGES, A. C. P., et al. Characterization of oxidative stress biomarkers in a freshwater anomuran crab. **Brazilian Journal of Biology,** 78: 61–67, 2018.

CAIRNS, J., HEATH, A.G., PARKER, B.C. The effects of temperature upon the toxicity of chemicals to aquatic organisms. **Hydrobiologia**, 47:135-171, 1975.

CAILLEAUD, K., et al. Effects of salinity and temperature on the expression of enzymatic biomarkers in *Eurytemora affinis* (Calanoida, Copepoda). Comparative Biochemistry and Physiology, Part A: **Molecular and Integrative Physiology,** 147:841–849, 2007.

CEREZER, C., BIASI, C., COGO, G.B., SANTOS, S. Avoid predation or take risks in basic activities? Predator-prey relationship in subtropical streams between decapods and caddisflies. **Marine and Freshwater Research**, 67:1880–1887, 2016.

COATS, J.R., et al. Toxicology of synthetic pyrethroids in aquatic organisms: An overview. **Environmental Toxicology and Chemistry**, 8:671-679, 1989.

COGO & SANTOS. The role of aeglids in shredding organic matter in Neotropical streams. **Journal of Crustacean Biology**, 33:519–526, 2013.

COLE, L.J., Stockan, J., Helliwell, R. Managing riparian buffer strips to optimize ecosystem services: a review. **Agriculture, Ecosystems & Environment,** 296: 106891, 2020.

CONAMA - Conselho Nacional Do Meio Ambiente, 2005. Resolução nº 357, de 17 de março de 2005. Dispõe sobre a classificação dos corpos d'água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. Diário Oficial da União, Poder Executivo, Brasília, DF, pp. 58–63, 2005.

CORPORATION, B.A.S.F. Ficha de segurança do Basagran 600R. **Basf Corporation**, p. 9, 2012.

CORREIA, A.D., COSTA, M.H., LUIS, O.J., LIVINGSTONE, D.R. Age-related changes in antioxidant enzyme activities, fatty acid composition and lipid peroxidation in whole body *Gammarus locusta* (Crustacea: Amphipoda). **Journal of Experimental Marine Biology and Ecology,** 289: 83–101, 2003.

COSTA, R. N. Ecotoxicologia como ferramenta para análise da qualidade ambiental do estuário do rio Macaé (Macaé – RJ). 80 f. 2010. Dissertação (Mestrado) – Programa de Pós-Graduação em Engenharia Ambiental, Instituto Federal de Educação, Ciência e Tecnologia Fluminense, Macaé, 2010.

CUMBERLIDGE, N., et al. Freshwater crabs and the biodiversity crisis: importance, threats, status, and conservation challenges. **Biological Conservation,** 142:1665–1673, 2009.

DALZOCHIO, T., GEHLEN, G.Confounding factors in biomonitoring using fish. **Ecotoxicology and Environmental Contamination**, 11:53–61, 2016.

DELORENZO, M.E. Impacts of climate change on the ecotoxicology of chemical contaminants in estuarine organisms. **Current Zoology**, 61:641–652, 2015.

DUDGEON, D., et al. Freshwater biodiversity: importance, threats, status and conservation challenges. **Biological reviews of the Cambridge Philosophical Society,** 81:163–182, 2006.

FELTEN, V. & GUEROLD F. Hyperventilation and loss of hemolymph Na and Cl in the freshwater amphipod *Gammarus fossarum* exposed to acid stress: a preliminary study. **Diseases of Aquatic Organisms**, 45:77–80, 2001.

FERREIRA B.D.P., HACK, C., OLIVEIRA, G.T., BOND-BUCKUP, G. Perfil metabólico de *Aegla platensis* Schmitt, 1942 (Crustacea, Anomura) submetida a dietas ricas em carboidratos ou proteínas. **Revista Brasileira de Zoologia**, 22: 161–168, 2005.

GANDAR A., et al. Proteome response of fish under multiple stress exposure: effects of pesticide mixtures and temperature increase. **Aquatic Toxicology,** 184: 61–77, 2017.

GEISSEN, V., et al.Emerging pollutants in the environment: A challenge for water resource management. **International Soil and Water Conservation Research**, 3: 57-65, 2015.

GHISI, N. C., et al. In situ assessment of a neotropical fish to evaluate pollution in a river receiving agricultural and urban wastewater. **Bulletin of Environmental Contamination and Toxicology**, 93: 699-709, 2014.

GILL, H. K., & GARG, H. Pesticides - Toxic Aspects- capter 8; Pesticides: Environmental Impacts and Management Strategies, 187-230. http://dx.doi.org/10.5772/57399, 2014.

GONCALVES, C.R. et al. Biochemical Responses in Freshwater Fish Exposed to Insecticide Propoxur. **Bulletin of Environmental Contamination and Toxicology**, 100: 524–528, 2018.

HALLIWELL, B. Free radicals, antioxidants and human disease: curiosity, cause or consequence. **Lancet**, 344: 721–724, 1994.

HEMMER-BREPSON, C., et al. Non-stressful temperature effect on oxidative balance and life history traits in adult fish (*Oryzias latipes*). J Exp Biol 217:274– 282, 2014.

HEUGENS, E. H. W., et al. A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. **Critical Reviews in Toxicology,** 31:247–284, 2001.

HOOK, S. E., GALLAGHER, E. P., BATLEY, G. E. The role of biomarkers in the assessment of aquatic ecosystem health. **Integrated Environmental Assessment and Management**, 10:327–341, 2014.

HOOPER, M.J., et al. Interactions between chemical and climate stressors: a role for mechanistic toxicology in assessing climate change risks. **Environmental Toxicology and Chemistry,** 32:32-48, 2013.

IBAMA – Instituo Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (2019) – Relatórios de comercialização de agrotóxicos. http://www.ibama.gov.br/agrotoxicos/relatorios-de-comercializacao-de-agrotoxicos#boletinsanuais. Acessado 11 outubro de 2020.

ISSARTEL, J., et al. Behavioural, ventilatory and respiratory responses of epigean and hypogean crustaceans to different temperatures. **Comparative Biochemistry and Physiology,** 141:1–7, 2005.

KAMYAB, E., et al. al. Effects of thermal stress on the immune and oxidative stress responses of juvenile sea cucumber *Holothuria scabra*. **Journal of Comparative Physiology B,** 187:51–61, 2017.

LAETZ, C.A., et al. Elevated temperatures increase the toxicity of pesticide mixtures to juvenile coho salmon. **Aquatic Toxicology,** 146: 38–44, 2014.

LEMAIRE, P., LIVINGSTONE, D.R. Pro-oxidant/antioxidant processes and organic xenobiotic interactions in marine organisms, in particular the flounder Platichthys flesus and the mussel Mytillus edulis. **Trends in Comparative Biochemistry & Physiology,** 1: 1119–1150, 1993.

LINDE-ARIAS, A. R., et al. Multibiomarker approach in fish to assess the impact of pollution in a large Brazilian river, Paraíba do Sul. **Environmental Pollution,** 159: 974-979, 2008.

LIVINGSTONE, D. R. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. **Marine Pollution Bulletin,** 42: 656–666, 2001.

LINS, J. A. P. N., et al. Uso de peixes como biomarcadores para monitoramento ambiental aquático. **Revista Acadêmica, Ciências Agrárias e Ambientais**, 8: 469-484, 2010.

LÓPEZ-DOVAL, J. C., BARATA, C., DÍEZ, S. El uso de organismos como indicadores de la contaminación y evaluación del riesgo sobre el ecosistema acuático en el embalse de Flix (Cataluña, NE de España). In: POMPÊO, M. et al. **Ecologia de reservatórios e interfaces.** São Paulo: Instituto de Biociências da Universidade de São Paulo. cap. 1, p.1-32., 2015.

LORO, V. L. et al. Spatial and temporal biomarkers responses of *Astyanax jacuhiensis* (Cope, 1894) (Characiformes: Characidae) from the middle rio Uruguai, Brazil. **Neotropical Ichthyology**, 13: 569-578, 2015.

LOUIZ, I., et al. Spatial and temporal variation of biochemical biomarkers in *Gobius niger* (Gobiidae) from a Southern Mediterranean lagoon (Bizerta lagoon, Tunisia): influence of biotic and abiotic factors. **Marine Pollution Bulletin**, 107: 305-314, 2016.

LUSHCHAK, V. I. Environmentally induced oxidative stress in aquatic animals. **Aquatic Toxicology,** 101: 13-30, 2011.

LUSHCHAK, V. I.; BAGNYUKOVA, T. V. Temperature increase results in oxidative stress in goldfish tissues. Antioxidant and associated enzymes. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,** 143: 36-41, 2006.

LUSHCHAK, V.I., et al. Pesticide toxicity: a mechanistic approach. **EXCLI Journal,** 17:1101–1136, 2018.

LYDY, M. J., BELDEN, J. B., TERNES, M. A. Effects of Temperature on the Toxicity of MParathion, Chlorpyrifos, and Pentachlorobenzene to *Chironomus tentans*. **Archives of Environmental Contamination and Toxicology**, 37:542-547, 1999.

MAAZOUZI, C., PISCART, C., LEGIER, F., HERVANT, F. Ecophysiological responses to temperature of the “killer shrimp” *Dikerogammarus villosus*: is the invade really stronger than the native *Gammarus pulex*? **Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology**, 159:268–274,2011.

MAGNI, S. T., PY-DANIEL, V. *Aegla platensis* Schmitt, 1942 (Decapoda, Anomura) um predador de imaturos de Simullidae (Diptera, Culicomorpha). **Revista de Saúde Pública**, 23:258–259, 1989.

MALTBY, L., CLAYTON, S. A., WOOD, R. M., MCLOUGHLIN, N. Evaluation of the *Gammarus pulex* in situ feeding assay as a biomonitor of water quality: robustness, responsiveness and relevance. **Environmental Toxicology and Chemistry,** 21:361–368, 2002.

MANCIOCCO, A., CALAMANDREI, G., ALLEVA, E. Global warming and environmental contaminants in aquatic organisms: the need of the etho-toxicology approach. **Chemosphere,** 100: 1–7, 2014.

MAPA - Ministério da Agricultura, Pecuária e Abastecimento. Acesso em https://www.gov.br/pt-br/orgaos/ministerio-da-agricultura-pecuaria-e-abastecimento acessado em 09/08/2021.

MARINS, A.T. **Biomonitoramento do Baixo Jacuí: avaliação de Pesticidas e seus efeitos em peixes.** Tese apresentada ao Curso de Pós-Graduação em Biodiversidade Animal, Área de Concentração em Bioecologia, como requisito parcial para obtenção do grau de Doutora em Biodiversidade Animal. Universidade Federal de Santa Maria, Santa Maria, 125 p., 2020.

NADAL, M., MARQUES, M., MARI, M., DOMINGO, J. L. Climate change and environmental concentrations of POPs: a review. **Environmental Research,** 143: 177–185, 2015.

OIKARI, A. Caging techniques for field exposures of fish to chemical contaminants. **Aquatic Toxicology**, 78:370–381, 2006.

OLIVEIRA, G. T., FERNANDES, F. A., BUENO, A. A. P., BOND-BUCKUP, G. Seasonal variations in the intermediate metabolism of *Aegla platensis* (Crustacea, Aeglidae). **Comparative Biochemistry and Physiology, A,** 147: 600–606, 2007.

OTIENO, P.O., et al. Carbofuran and its toxic metabolites provide forensic evidence for furadan exposure in vultures (*Gyps africanus*) in Kenya. **Bulletin of Environmental Contamination and Toxicology** 84, 536–544, 2010.

PARDINI, R. Feeding ecology of the neotropical river otter *Lontra longicaudis* in an Atlantic Forest stream, South-Eastern Brazil. **Journal of Zoology,** 245:385–391, 1998.

PELAEZ, V.; TERRA, F.H.B; SILVA, L.R. A regulamentação dos agrotóxicos no Brasil: entre o poder de mercado e a defesa da saúde e do meio ambiente. Artigo apresentado no XIV Encontro Nacional de Economia Política / Sociedade Brasileira de Economia Política - São Paulo/SP, de 09/06/2009 a 12/06/2009. 22 p.

PRINGLE C. M. Hydrologic connectivity and the management of biological reserves: A global perspective. **Ecological Applications**, 11: 981-998, 2001.

PROSSER, R.S., et al. A review of the effectiveness of vegetated buffers to mitigate pesticide and nutrient transport into surface waters from agricultural areas. **Journal of Environmental Management,** 261: 110210, 2020.

SANTOS, S., BOND-BUCKUP, G., GONÇALVES, A. S., BATHOLOMEI-SANTOS, M. L., BUCKUP, L., JARA, C.G. Diversity and conservation status of *Aegla spp.* (Anomura, Aeglidae): an update. **Nauplius,** 25:1–14, 2017.

SARDI, A. E., RENAUD, P. E., LANA, P. C., CAMUS, L. Baseline levels of oxidative stress biomarkers in species from a subtropical estuarine system (Paranaguá Bay, southern Brazil). **Marine Pollution Bulletin,** 113:496– 508, 2016.

SCHÄFER, R. B., BRINK P. J. V. D., & LIESS, M. Impacts of Pesticides on Freshwater Ecosystems. **Ecological Impacts of Toxic Chemicals**, 111-137, 2011.

SEVERO, E. S, ET AL. Ecological risk of pesticide contamination in a Brazilian river located near a rural area: A study of biomarkers using zebrafish embryos. **Ecotoxicology and Environmental Safety,** 190:110071, 2020.

SHEEHAN, D., POWER A. Effects of seasonality on xenobiotic and antioxidant defense mechanisms of bivalve molluscs. **Comparative Biochemistry and Physiology - Part C**, 123:193–199,1999.

SILVA, C. M. M. S.; FAY, E. F. Agrotóxico e ambiente. Brasília: Embrapa Informação Tecnológica, 400 p., 2004.

SISINNO, C.L.S.; OLIVEIRA-FILHO, E.C. **Princípios de toxicologia ambiental: conceitos e aplicações**. Rio de Janeiro: Interciência, 2013.

SOKOLOVA, I.M., LANNING, G. Interactive effects of metal pollution and temperature on metabolism in aquatic ectotherms: implications of global climate change. **Climate Research.** 37:181-200, 2008.

STEINBERG, C.E.W., STÜRZENBAUM, S.R., MENZEL, R. Genes and environment - striking the fine balance between sophisticated biomonitoring and true functional environmental genomics. **Science of the Total Environment**, 400:142–161, 2008.

STOLIAR, O. B.; LUSHCHAK, V. I. Environmental pollution and oxidative stress in fish. In: LUSHCHAK, V. I. (Ed.). Oxidative Stress Environmental Induction and Dietary Antioxidants. 1 ed. Rijeka: **InTech Europe**, cap. 7: 131-166, 2012.

TOMITA, R. Y.; BEYRUTH, Z. Toxicologia de agrotóxicos em ambiente aquático. **Biológico,** 64: 135-142, 2002.

TREVISAN, A., HEPP, L.U., SANTOS, S. Abundância e distribuição de *Aeglidae* (Crustacea: Anomura) em função do uso da terra na bacia hidrográfica do Rio Jacutinga. Rio Grande do Sul, Brasil, 2009.

VAN DER OOST, R., BEYER, J., VERMEULEN, N. P. E. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. **Environmental Toxicology and Pharmacology,** 13: 57-149, 2003.

VIEIRA, C.E., et al. Multiple biomarker responses in *Prochilodus lineatus* subjected to short-term in situ exposure to streams from agricultural areas in Southern Brazil**. Science of the Total Environment**, 542, 44–56, 2016.

VINAGRE, C., et al.Effect of temperature in multiple biomarkers of oxidative stress in coastal shrimp. **Journal of Thermal Biology**, 41:38–42, 2014.

WALLACE, W. G., ESTEPHAN, A. Differential susceptibility of horizontal and vertical swimming activity to cadmium exposure in a gammaridean amphipod (*Gammarus lawrencianus*). **Aquatic Toxicology**, 69:289–297, 2004.

WANG, W.N., et al. Effects of temperature on growth, adenosine phosphates, ATPase and cellular defense response of juvenile shrimp *Macrobrachium nipponense.* **Aquaculture**, 256: 624–630, 2006.

WARD, J. V., STANFORD, J.A. Thermal responses in the evolutionary ecology of aquatic insects. **Annual Review of Entomology**, 27:97-117, 1982.

WILLMING, M.M., QIN, G. & MAUL, J. D. Effects of environmentally realistic daily temperature variation on pesticide toxicity to aquatic invertebrates. **Environmental Toxicology and Chemistry**, 32: 2738-45, 2013.

WINSTONE, G.W., DI GIULIO, R.T. Prooxidant and antioxidant mechanisms in aquatic organisms. **Aquatic Toxicology,** 19: 137–161, 1991.