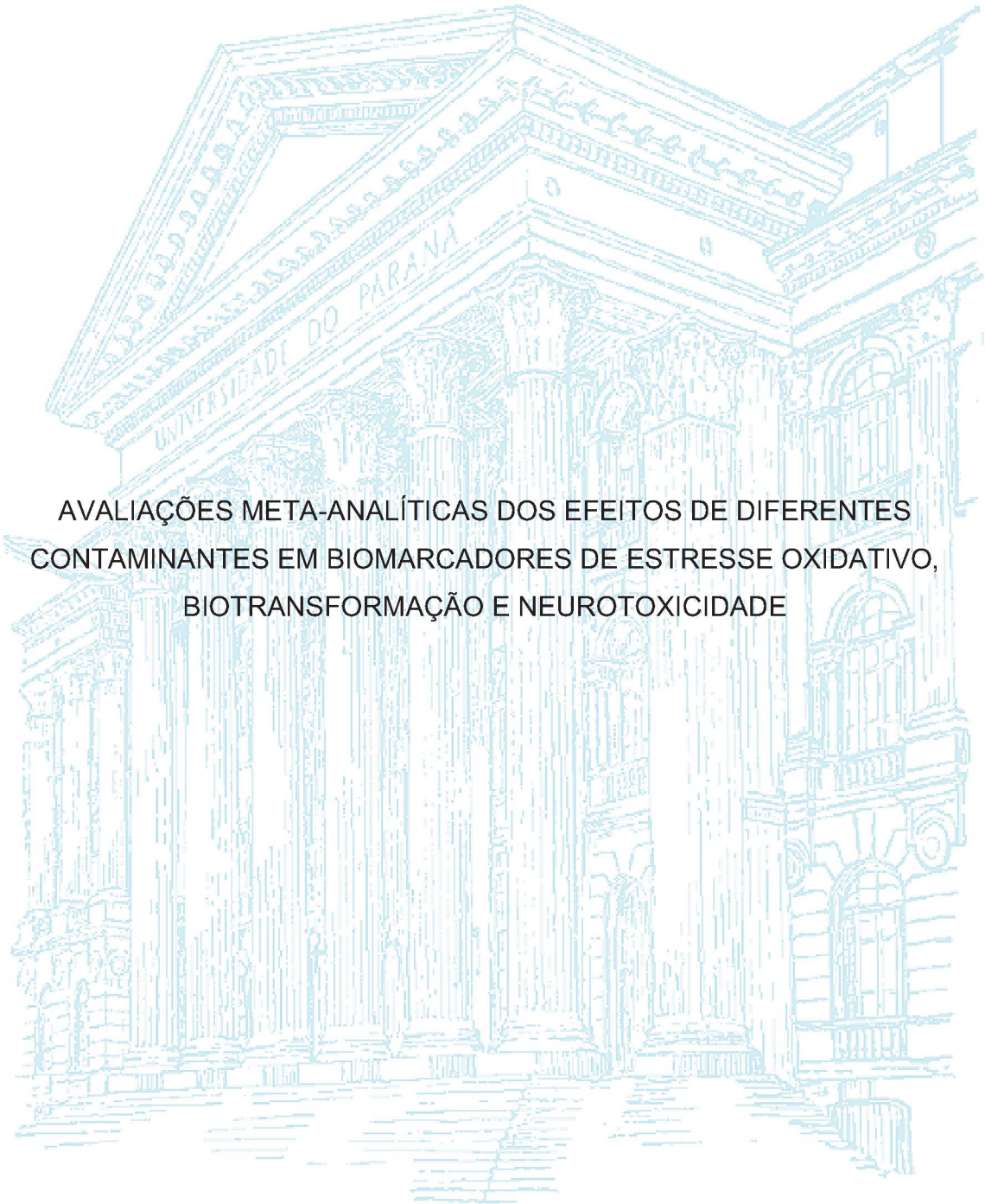
UNIVERSIDADE FEDERAL DO PARANÁ

MANUELA SANTOS SANTANA



AVALIAÇÕES META-ANALÍTICAS DOS EFEITOS DE DIFERENTES CONTAMINANTES EM BIOMARCADORES DE ESTRESSE OXIDATIVO, BIOTRANSFORMAÇÃO E NEUROTOXICIDADE

CURITIBA

2021

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Tese apresentada ao Programa de Pós-Graduação em Biologia Celular e Molecular, Departamento de Biologia Celular, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Biologia Celular e Molecular.

Orientadora: Prof.ª Dra. Maritana Mela Prodocimo
Co-orientador: Prof. Dr. Maikon Di Domenico

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“A ciência substitui preconceito privado por evidência pública, verificável”

“*Science replaces private prejudice with public, verifiable evidence*.”

Richard Dawkins

RESUMO

Existe uma grande pressão ambiental causada pela liberação de diversos compostos químicos em ecossistemas aquáticos, como poluentes orgânicos persistentes (POPs), provenientes de distintas fontes. Dioxinas e hidrocarbonetos policíclicos aromáticos (HPAs) são acidentalmente lançados ao ambiente como poluentes, enquanto compostos organoclorados ou bifenilas policloradas são intencionalmente introduzidos como agrotóxicos ou fluidos refrigerantes de equipamentos elétricos. HPAs e agrotóxicos estão entre os POPs mais comumente estudados, tendo um grande impacto em peixes e outros organismos aquáticos. A toxicidade desses compostos em peixes pode estar associada ao processo de metabolização, à indução de estresse oxidativo ou à inibição da função neuromuscular. Biomarcadores representativos dos sistemas de defesa antioxidante, de biotransformação, e de vias colinérgicas são comumente utilizados em ecotoxicologia para detectar a presença e efeito de HPAs e agrotóxicos. A fim de compreender e sintetizar os efeitos desses POPs em biomarcadores de peixes, foram conduzidas revisões sistemáticas e meta-análises, apresentadas aqui em três capítulos. O primeiro capítulo avaliou os efeitos de HPAs em biomarcadores de biotransformação e de estresse oxidativo. Além de estimar o efeito global de HPAs, buscou-se compreender a contribuição de diferentes moderadores de efeito (i.e., fontes de variação), como abordagem experimental, habitat, rota de exposição dos compostos e interação entre concentração e tempo. O segundo e terceiro capítulos tratam dos efeitos de agrotóxicos nas médias e na variabilidade biomarcadores de neurotoxicidade, e de estresse oxidativo e biotransformação, respectivamente. Assim como o primeiro capítulo, avaliou-se o efeito global e também a influência de moderadores de efeito, como a idade e tamanho do peixe na resposta de colinesterases (ChEs), e o sexo do peixe em enzimas de biotransformação e antioxidantes. O efeito global dos HPAs e agrotóxicos foi significativo para grande parte dos biomarcadores. Entre eles, a atividade da etoxiresorufina-O-desetilase (EROD) e das ChEs revelaram-se indicadores robustos dos efeitos de HPAs e agrotóxicos em peixes, respectivamente. Já a catalase (CAT), talvez devido a variabilidade inerente de sua atividade, demonstrou ser um indicador pouco consistente. Os resultados obtidos nos três capítulos demonstraram que as revisões sistemáticas e meta-análises são ferramentas poderosas para sintetizar dados ecotoxicológicos. Desta maneira, foi possível apresentar um quadro abrangente de como importantes biomarcadores respondem a esses contaminantes. Além disso, a meta-análise de variância trouxe uma nova perspectiva sobre os efeitos de contaminantes e apontou para uma nova direção de pesquisa. Por fim, as revisões identificaram lacunas na área de toxicologia, como o baixo número de estudos avaliando efeitos de agrotóxicos ambientalmente relevantes (e.g., neonicotinóides), ou mensurando níveis de glutationa (GSH) e enzimas associadas após exposição a HPAs. Novos estudos podem utilizar os resultados aqui obtidos para direcionar e definir protocolos experimentais, bem como atividades de monitoramento ambiental.

Palavras-chave: revisão sistemática, peixes, HPAs, agrotóxicos, biomarcadores, EROD, ChEs, variabilidade.

ABSTRACT

The release of several chemical compounds in aquatic ecosystems, such as persistent organic pollutants (POPs) causes an enormous environmental pressure. Dioxins and polycyclic aromatic hydrocarbon (PAHs) are accidentally released as pollutants, while organochlorine compounds and polychlorinated biphenyls (PCBs) are deliberately introduced as pesticides or coolant for electrical equipment. PAHs and pesticides are one of the most commonly studied POPs, having a great impact in fish and other aquatic organisms. Their toxicity in fish might be associated to the metabolization process, oxidative stress induction, or the inhibition of neuromuscular function. Biomarkers representing the antioxidant defense and biotransformation systems, and cholinergic pathways are frequently used in ecotoxicology to detect the presence and effects of PAHs and pesticides. To comprehend and summarize these effects in fish biomarkers, systematic reviews and meta-analyses were conducted and presented here in three chapters. The first chapter assessed PAHs effects on biotransformation and oxidative stress biomarkers. Besides estimating PAHs global effect, this chapter aimed to evaluate the influence of different moderators of effect (i.e., sources of variation), such as experimental approach, habitat, exposure route and interaction of time and concentration. The second and third chapters investigate the effects of pesticides on means and variability of biomarkers of neurotoxicity, oxidative stress and biotransformation. As with the first chapter, it was estimated the global effect and the influence of moderators, such as fish age and size on cholinesterases (ChEs) responses, fish sex on biotransformation and antioxidant enzymes. The global effect of PAHs and pesticides was significant for most biomarkers. Among them, ethoxyresorufin-O-deethylase (EROD) activity and ChEs were revealed as robust indicators of HPAs and pesticides effects on fish, respectively. Whereas catalase (CAT), perhaps due to an inherent activity variability, has demonstrated to be little consistent. The results obtained in the three chapters show systematic reviews and meta-analyses are powerful tools able to summarize ecotoxicological data. Therefore, it was possible to present a broad picture of how important biomarkers respond to these contaminants. Additionally, the meta-analysis of variance has a brought a new perspective on contaminant effects and pointed to a new direction in terms of research. Finally, the reviews identified research gaps in toxicology, including the low number of studies on the effects of environmentally relevant pesticides (e.g., neonicotinoids), or measuring levels of glutathione (GSH) and related enzymes after PAHs exposure. New studies may use the results found here to direct and define experimental protocols, as well as environmental monitoring activities.

Keywords: systematic review, fish, PAHs, pesticides, biomarkers, EROD, ChEs, variability.

ESTRUTURA DO TRABALHO

O objetivo do presente trabalho é avaliar, através de revisões sistemáticas e meta-análises, o efeito de hidrocarbonetos policíclicos aromáticos (HPAs) e agrotóxicos em biomarcadores de peixes. Para tal, o trabalho foi organizado da seguinte maneira:

- Aspectos introdutórios: Introdução geral, detalhando os contaminantes, os biomarcadores avaliados e a importância das revisões sistemáticas e meta-análises, e objetivos. Uma vez que o presente trabalho consiste em revisões sistemáticas amplas, entende-se que os resultados apresentam uma revisão da literatura, portanto, este item não estará incluso na introdução geral.

- Resultados: Apresentados como artigos científicos publicados e manuscrito a ser submetido

✓ Capítulo I – "Biomarker responses in fish exposed to polycyclic aromatic hydrocarbons (PAHs): systematic review and meta-analysis". Aritgo publicado na revista *Environmental Pollution* em julho de 2018 (Qualis-Capes A1 – Quadriênio 2013-2016. Fator de impacto: 6.792).

✓ Capítulo II – "Pesticide effects on fish cholinesterase variability and mean activity: A meta-analytic review". Publicado na revista *Science of the Total Environment* em novembro de 2020 (Qualis-Capes A2 – Quadriênio 2013-2016. Fator de impacto: 6.551).

✓ Capítulo III – "A meta-analytic review of fish antioxidant defense and biotransformation systems following pesticide exposure". Manuscrito a ser submetido à revista *Environmental Pollution* (Qualis-Capes A1 – Quadriênio 2013-2016. Fator de impacto: 6.792).

- Conclusões gerais.

SUMÁRIO

INTRODUÇÃO GERAL **12**

1. INTRODUÇÃO GERAL **13**
1.1 Hidrocarbonetos Policíclicos Aromáticos (HPAs) **14**
1.2 Agrotóxicos **19**
1.3 Biomarcadores **23**
1.3.1 Biomarcadores de Estresse Oxidativo *24*
1.3.2 Biomarcadores de Biotransformação *26*
1.3.3 Biomarcadores de Neurotoxicidade: Colinesterases (ChEs) *28*
1.4 Revisão Sistemática e Meta-Análise **30**

2. OBJETIVOS **33**
2.1 Objetivo Geral **33**
2.2 Objetivos Específicos **33**

CAPÍTULO I – *Biomarker responses in fish exposed to polycyclic aromatic hydrocarbons (PAHs): systematic review and meta-analysis* **35**
Material Suplementar – Capítulo I 49

CAPÍTULO II – *Pesticide effects on fish cholinesterase variability and mean activity: A meta-analytic review* **67**
Material Suplementar – Capítulo II 79

CAPÍTULO III – *A meta-analytic review of fish antioxidant defense and biotransformation systems following pesticide exposure* **93**
Material Suplementar – Capítulo III 135

CONCLUSÕES GERAIS **158**

REFERÊNCIAS **161**

Introdução Geral

1. INTRODUÇÃO GERAL

O aumento populacional impõe considerável pressão ambiental, uma vez que a produção agrícola e atividades industriais intensificam-se em resposta às crescentes demandas de consumo (KÖHLER; TRIEBSKORN, 2013). O impacto ambiental deve-se principalmente a liberação de uma grande diversidade de compostos químicos em ecossistemas terrestres e aquáticos. Dentre esses, estão os poluentes inorgânicos que interferem em ciclos geoquímicos, tais como elementos mineirais essenciais (e.g., carbono, nitrogênio, fósforo) e metais-traço (e.g. zinco, cobre, chumbo, cromo) (GAŁUSZKA; MIGASZEWSKI, 2018). A produção e aplicação de muitos desses poluentes inorgânicos teve início na década de 1950 e têm se intensificado desde então, como por exemplo o uso de fertilizantes nitrogenados e produção de alumínio (ELIAS, 2018). A explosão das atividades humanas, a partir da metade do século XX, deixou sinais químicos evidentes no ambiente, influenciando a estrutura e funcionamento do planeta de modo a marcar o início de uma nova época geológica, o Antropoceno (LEWIS; MASLIN, 2015; LUDWIG; STEFFEN, 2018).

Além dos poluentes inorgânicos, outros potenciais marcadores do Antropoceno são os poluentes orgânicos persistentes (POPs). Esses compostos são definidos por quatro características principais: persistência, bioacumalação, capacidade de transporte de longo alcance e toxicidade (ELIAS, 2018; FITZGERALD; WIKOFF, 2014). São altamente persistentes, por resistirem à degradação por processos naturais, como fotólise ou degradação biológica (GAUR; NARASIMHULU; PYDISETTY, 2018). Sua natureza lipofílica facilita a bioacumulação em tecidos adiposos e biomagnificação na teia alimentar

(JAMIESON et al., 2017). Por serem compostos semi-voláteis, o transporte de longo alcance é facilitado, seja pelo ar ou pela água quando adsorvidos em material particulado (FITZGERALD; WIKOFF, 2014). Há uma ampla variedade de POPs, provenientes de diversas fontes. Dioxinas e hidrocarbonetos policíclicos aromáticos (HPAs) podem ser acidentalmente lançados ao ambiente como poluentes, enquanto compostos organoclorados ou bifenilas policloradas são intencionalmente introduzidos como pesticidas ou fluidos refrigerantes de equipamentos elétricos (ELIAS, 2018).

Após lançados, POPs entram nos ambientes aquáticos diretamente, através de derrames acidentais e escoamento agrícola, ou indiretamente por transporte atmosférico e correntes oceânicas (JOHNSON et al., 2013). O destino, distribuição e a biodisponibidade para organismos aquáticos depende da estrutura e propriedades dos compostos (ERICKSON et al., 2008). HPAs e pesticidas, mais precisamente agrotóxicos, estão entre os POPs mais comumente estudados, tendo um grande impacto em peixes e outros organismos aquáticos (LUSHCHAK, 2011). Além disso, porque atividades e demandas humanas são significativamente dependentes desses compostos, é importante compreender seus mecanismos de toxicidade e quais fatores podem afetar e regular esses mecanismos.

1.1 Hidrocarbonetos Policíclicos Aromáticos (HPAs)

Hidrocarbonetos policíclicos aromáticos (HPAs) são compostos contendo pelo menos dois anéis de benzeno em suas moléculas, sendo naftaleno o representante de estrutura mais simples (i.e., dois anéis de benzeno) e menor

peso molecular (DAUNER et al., 2015; WHYTE et al., 2000). HPAs são oriundos de fontes naturais, como queimadas florestais ou erupções vulcânicas, e principalmente de fontes antrópicas, liberados por uma vasta diversidade de atividades (COLLIER et al., 2013; HESTER; WILLIS; BAKER, 2018). A origem, estrutura e as propriedades químicas são utilizadas para distinguir e classificar os HPAs em dois grupos: *(a)* HPAs petrogênicos, i.e., de menor massa molecular contendo dois ou três anéis aromáticos (DAUNER et al., 2015), associados a exploração de petróleo ou vazamentos de óleo (COLLIER et al., 2013); *(b)* HPAs pirogênicos, i.e., aqueles com maior massa e mais de três anéis aromáticos, comumente associados a combustão de incompleta de material orgânico e liberados na atmosfera ou superfície das águas (BEYER et al., 2010).

Existem mais de 1500 compostos de HPAs provenientes de uma infinidade de fontes (GALLUCCI; LUCKETT-CHASTAIN; YUCESOY, 2020). Quantidades consideráveis desses compostos são encontradas em ambientes aquáticos, muitas vezes associados à biota e aos sedimentos devido à considerável afinidade com matéria orgânica (BEYER et al., 2010; GALLUCCI; LUCKETT-CHASTAIN; YUCESOY, 2020). Organismos aquáticos podem ser expostos a HPAs através do contato cutâneo, da respiração e da ingestão de presas ou sedimento contaminados (COLLIER et al., 2013; GAD; GAD, 2014).

Muitos invertebrados são capazes de metabolizar HPAs, porém comparativamente, os peixes possuem um sistema enzimático de biotransformação bem desenvolvido, apto a converter esses compostos em metabólitos hidrossolúveis para que possam ser facilmente excretados pela urina ou pela bile (BEYER et al., 2010; SCHLENK et al., 2008). A eficiência metabólica e de eliminação observada em peixes impede que HPAs acumulem

no organismo, restando apenas concentrações insignificantes no músculo e em outros tecidos (COLLIER et al., 2013). Entretanto, essa mesma eficiência pode gerar metábolitos reativos e mais tóxicos que o composto parental, processo denominado bioativação (BOELSTERLI, 2007), que pode aumentar a susceptibilidade dos peixes aos efeitos deletérios dos HPAs (BEYER et al., 2010).

O processo de detoxificação ou bioativação de HPAs é mediado principalmente pela superfamília de heme proteínas, citocromo P450 (CYPs). São encontradas predominantemente no fígado, associadas a membrana do retículo endoplasmático liso, ou a membrana plasmática ou membrana interna das mitocôndrias (BOELSTERLI, 2007; SCHLENK et al., 2008). As isoformas das CYPs atuam como monooxigenases em reações de Fase I da metabolização, i.e., inserem um átomo de oxigênio ao substrato (CHEN, 2020; COLLIER et al., 2013). CYPs podem transformar HPAs em epóxidos reativos, que são prontamente convertidos em dihidrodióis por epóxido hidrolases, resultando na detoxificação. Entretanto, para alguns HPAs essas enzimas atuam na formação de metabólitos epóxidos de diol reativos e muitas vezes carcinógenos (SCHLENK et al., 2008).

Esses metabólitos reativos podem afetar a replicação normal de células, inativando o gene protetor p53 ao formar adutos de DNA (FORTIN; CAPDEVIELLE, 2020). Além do potencial mutagênico e carcinogênico, HPAs podem interferir o desenvolvimento embrionário e regulação endócrina (HONDA; SUZUKI, 2020; RENGARAJAN et al., 2015), bem como agir como imunossupressores ou imunoestimulantes – dependendo do tipo de tecido ou célula (GALLUCCI; LUCKETT-CHASTAIN; YUCESOY, 2020). Adicionalemnte,

durante o processo de bioativação, espécies moleculares oxidantes, i.e., espécies reativas de oxigênio (EROs), podem ser produzidas em excesso, provocando estresse oxidativo (LUSHCHAK, 2011; SCHLENK et al., 2008).

Fatores como capacidade metabólica, estratégias alimentares, idade e sexo dos organismos, podem determinar o grau de toxicidade dos HPAs (COLLIER et al., 2013; LIU; POPE, 2020)(Collier et al., 2013; Liu and Pope, 2020). Por exemplo, peixes juvenis são mais suscetíveis aos efeitos deletérios desses compostos comparados a indivíduos adultos, devido diferenças de absorção e biotransformação e de recuperação após exposição. Essas diferenças ocorrem porque órgãos importantes, como fígado e rins, estão inteiramente desenvolvidos em peixes adultos, permitindo a rápida assimilação e detoxificação de HPAs (FAJARDO; OCAMPO, 2018).

Ademais, as etapas de biotransformação e, consequentemente, os efeitos deletérios dependem das propriedades químicas e estruturas dos HPAs (DA SILVA JUNIOR et al., 2021). Por exemplo, o HPAs como o benzo[a]pireno (BaP), fenantreno e criseno são reconhecidamente carcinogênicos. A bioativação desses compostos ocorre por ação de enzimas do citocromo P450, juntamente com epóxido hidrolases, convertendo-os em epóxidos de diol altamente reativos (BISWAS; GHOSH, 2014; GAD, 2014; GRAY; HALL, 2014). A Figura 1 representa a metabolização do benzo[a]pireno (BaP), catalisada por CYPs e outras enzimas de metabolização. Vários metabólitos oxidativos podem surgir das reações de oxidação e conjugação, inclusive aqueles mais tóxicos capazes de reagir com DNA.

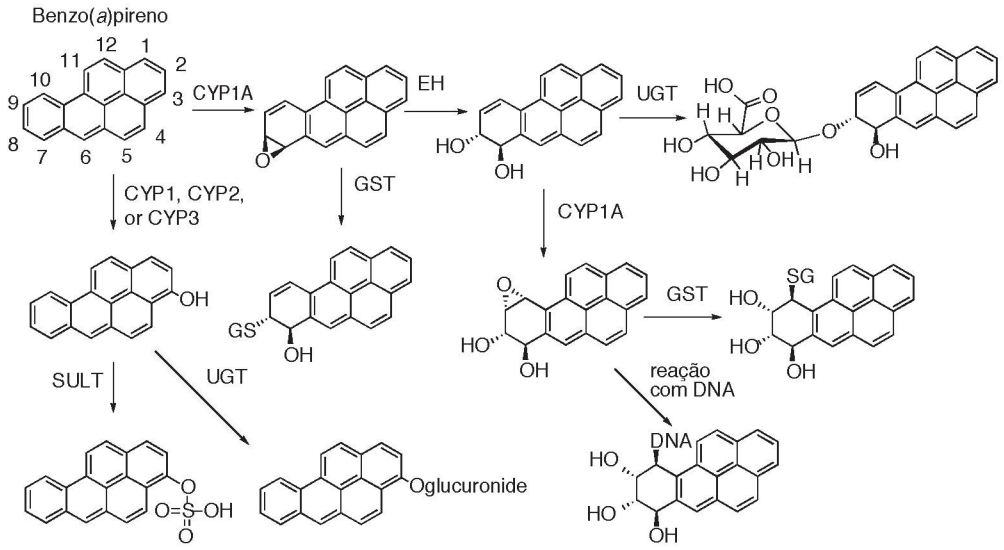


Figura 1 Biotransformação do hidrocarboneto policíclico aromático benzo[a]pireno (BaP). O composto pode ser metabolizado por diferentes famílias de citocromo P450s (CYPs), como a CYP1, CYP2 ou CYP3 e seguir diferentes vias subsequentemente. Os metabólitos podem ser biotransformados ou bioativados pela glutationa S-transferase (GST), sulfotransferases (SULTs) e uridina 5'-difosfo-glucuronosiltransferase (UGTs). (Figura adaptada de SCHLENK et al., 2008)

A presença de "embaiamento" (*bay-like region*) ou de uma região que remete um "fiorde" (*fjord-like region*) em sua estrutura (Figura 2), impede a detoxificação enzimática de alguns metabólitos, permitindo que metabólitos intermediários possam interagir com o DNA, levando a mutagênese e carcinogênese (JERINA et al., 1980; LEHR et al., 1985; SCHLENK et al., 2008; ZHANG et al., 2012). Alguns HPAs com cadeias planares, como o antraceno, por não possuírem essa região tipo baía, apresentam um menor potencial de toxicidade (DA SILVA JUNIOR et al., 2021; RUMMEL et al., 1999).

Portanto, fica claro que os mecanismos de biotransformação e detoxificação possuem um papel chave na toxicidade dos HPAs, seja por converter HPAs em metabólitos reativos, seja por promover estresse oxidativo. Considerando a contínua dependência do petróleo como fonte energética, esses compostos potencialmente perigosos estarão presentes pelas próximas

décadas. Desta maneira, é preciso compreender os mecanismos de toxicidade destes compostos para antecipar e prevenir impactos em populações e comunidades aquáticas.

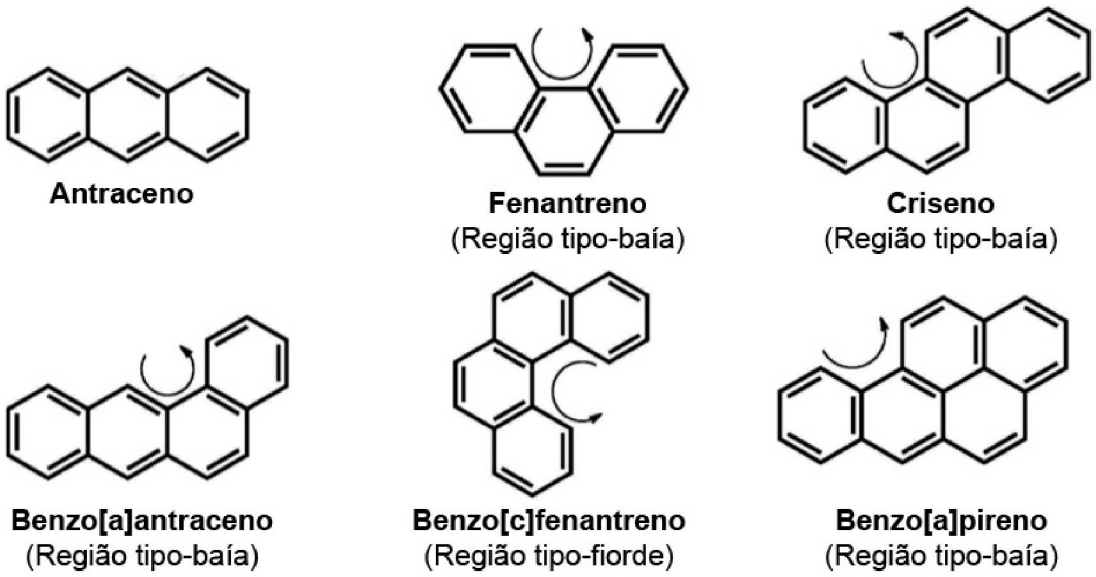


Figura 2 Estrutura química dos hidrocarbonetos policíclicos aromáticos (HPAs). A flecha curvada aponta a presença de uma região tipo baía ou tipo fiorde. (Figura adaptada de Zhang et al., 2012)

1.2. Agrotóxicos

Agrotóxicos são substâncias ou misturas de substâncias utilizadas no controle, prevenção e/ou destruição de pestes, sejam elas insetos, ervas daninhas, fungos, roedores ou outras formas de plantas aquáticas e terrestres (POPE, 2014). Podem ser classificadas sobretudo segundo a sua composição química (e.g. organoclorados, organofosforados, carbamatos, piretróides sintéticos, neonicotinóides) ou quanto a espécie-alvo (e.g. inseticida, herbicida, fungicida, rodenticida) (AZMI & NAQVI, 2011). Para aumentar a produção e eficiência de práticas agrícolas, agrotóxicos foram utilizados ampla e

indiscrimidamente. Segundo a FAO (*Food and Agriculture Organization of the United Nations*), mais 4.1 milhões de toneladas de substâncias foram aplicadas em 2018 (FAOSTAT, 2021), e esse número tende a aumentar para corresponder às crescentes demandas da população (MAGGI et al., 2019). Esse uso indiscriminado compromete seriamente o meio ambiente e a saúde humana (CUEVAS; MARTINS; COSTA, 2018), visto que são compostos químicos projetados para interferir em sistemas biológicos sem seletividade (CLASEN et al., 2018; PRIETO GARCIA et al., 2012). Estima-se que apenas 0,1% dos agrotóxicos aplicados atingem a espécie-alvo, deixando os 99,9% restantes como fonte de impacto para o meio ambiente (DAS; SARMA, 2018; PIMENTEL, 2005).

Independentemente de como é aplicado, os agrotóxicos e seus resíduos são escoados para ecossistemas aquáticos (ADEDEJI; OKOCHA, 2012; SHUMAN-GOODIER; PROPPER, 2016). Em peixes, os impactos ocorrem em diversos níveis, direta e indiretamente. Por exemplo, inseticidas podem reduzir a qualidade e quantidade presas, como insetos e outros invertebrados, enquanto herbicidas podem eliminar algas e dessa forma, reduzir os níveis de oxigênio dissolvido ao aumentar as taxas de decomposição (PIMENTEL, 2005; SCHÄFER; BRINK; LIESS, 2011). Diretamente, agrotóxicos estão associados à inibição de importantes enzimas (MELA et al., 2013; ULLAH et al., 2019), diminuição do crescimento (SANDOVAL-HERRERA et al., 2019), comprometimento imunológico (SUNANDA et al., 2016; WANG et al., 2016) e possivelmente a redução de estoques de peixes (YAMAMURO et al., 2019). Adicionalmente, assim como HPAs, alguns agrotóxicos (e.g., organoclorados) são POPs capazes de bioacumular e biomagnificar. Portanto, peixes expostos

até mesmo a pequenas frações de agrotóxicos, apresentam concentrações maiores que aquelas detectadas na água (CUEVAS; MARTINS; COSTA, 2018; GOODSELL; UNDERWOOD; CHAPMAN, 2009). Quando esses organismos contaminados são consumidos por humanos, os agrotóxicos bioacumulados podem aumentar incidência de câncer e afetar os sistemas nervoso, imunológico, endócrino e reprodutivo (KOUREAS et al., 2012; WANG et al., 2016).

Os mecanismos pelos quais agrotóxicos exercem toxicidade são similares aos descritos para HPAs. Após a absorção, agrotóxicos são distribuídos pelo organismo até atingir o local de metabolização e excreção (MAXWELL, 2020). No fígado, principal local de metabolização de xenobióticos, agrotóxicos passam por reações de oxidação, redução ou hidrólise, realizadas por enzimas de Fase I e/ou reações de conjugação catalisadas por enzimas de Fase II (BANERJEE; RAMAIAH, 2020; CHEN, 2020). A toxicidade dos agrotóxicos pode estar associada a um incremento substancial de EROs, i.e., associada a indução de estresse oxidativo. Excesso de EROs podem resultar do processo de biotransformação ou de ciclos redox (BOELSTERLI, 2007; LUSHCHAK et al., 2018). Adicionalmente, alterações no funcionamento da cadeia transportadora de elétrons mitocondrial e do retículo endoplasmático ou a inibição da atividade de antioxidantes e enzimas relacionadas podem também aumentar a produção de EROs, promovendo estresse oxidativo (LUSHCHAK et al., 2018; SLANINOVA et al., 2009).

Algumas classes de agrotóxicos inibem hidrolases importantes para a função neuromuscular, i.e., enzimas que hidrolisam o neurotransmissor acetilcolina, colinesterases (ChEs). Por exemplo, inseticidas organofosforados e carbamatos são reconhecidamente compostos anticolinesterásicos e seus

efeitos em peixes já foram descritos para diversas espécies (AGRAHARI; GOPAL; PANDLEY, 2006; GUILOSKI et al., 2013; PEREIRA et al., 2019; ULLAH et al., 2018). Outras classes ambientalmente relevantes possuem alvos moleculares distintos como receptores GABA para organoclorados, canais de Na+ dependente de voltagem para piretróides sintéticos, receptores nicotínicos de acetilcolina para neonicotinóides (CASIDA; DURKIN, 2013). Entretanto, esses inseticidas apresentaram ação anticolinesterásica (BONANSEA; WUNDERLIN; AMÉ, 2016; DA CUÑA et al., 2011; TIAN et al., 2018; ULLAH et al., 2019), bem como a indução de estresse oxidativo (HUSAK et al., 2017; MELA et al., 2013; MIŞE YONAR et al., 2014; STARA; MACHOVA; VELISEK, 2012). Inclusive, apesar de mais seletivo e menos prejudicial aos mamíferos e às aves, inseticidas piretróides são altamente tóxicos para peixes e outros espécies aquáticas (VIEIRA; DOS REIS MARTINEZ, 2018; WERNER; YOUNG, 2017).

Além da classe a qual os agrotóxicos pertencem, outros fatores podem modificar a toxicidade dos agrotóxicos. Como mencionado anteriormente, sexo, idade e estratégias alimentares podem determinar o impacto dos agrotóxicos em peixes (LIU; POPE, 2020). Porém, outro fator relevante e muitas vezes desconsiderado está associado aos compostos presentes nos agrotóxicos de uso comercial. Co-formulantes, surfactantes adjuvantes e outros ingredientes são adicionados às formulações comerciais para garantir a eficácia do ingrediente ativo (SZÉKÁCS, 2017). Porém, estudos demonstraram que surfactantes e alguns ingredientes adicionados a formulações comerciais de glifosato podem causar genotoxicidade, estresse oxidativo e citotoxicidade em peixes e outros organismos aquáticos (FOLMAR; SANDERS; JULIN, 1979; GUILHERME et al., 2012; SZÉKÁCS, 2017; TSUI; CHU, 2003).

Apesar dos novos avanços no desenvolvimento de agrotóxicos comparativamente de menor toxicidade, o herbicida glifosato é o pesticida mais utilizado globalmente (BENBROOK, 2016; MAGGI et al., 2020), enquanto os organofosforados e carbamatos permancem no topo dos inseticidas mais comercializados no mundo (CASIDA; BRYANT, 2017). Além disso, o uso de piretróides tem aumentado na tentativa de conter a produção e aplicação de composto altamente tóxicos. Logo, considerando a diversidade e quantidade de agrotóxicos constante lançados no ambiente e os impactos observados em peixes, é crucial compreender a extensão dos efeitos de agrotóxicos em diferentes marcadores de toxicidade.

1.3 Biomarcadores

HPAs e agrotóxicos são POPs que tendem a bioacumular em tecidos adiposos devido a lipofilicidade dos compostos (FITZGERALD; WIKOFF, 2014). Organismos aquáticos expostos a esses poluentes apresentam concentrações maiores que aquelas detectadas na água (CUEVAS; MARTINS; COSTA, 2018). Portanto, análises físico-químicas, que estimam o nível de contaminantes na água, podem não refletir o real impacto dos HPAs e agrotóxicos para biota. É preciso, então, buscar indicadores precoces dos efeitos de contaminação, i.e., biomarcadores. Biomarcadores são definidos por respostas quantitativas em nível celular, bioquímico, molecular ou fisiológico, que podem ser medidos em células, fluidos corporais, tecidos ou órgãos de um indivíduo, indicando uma possível exposição a xenobióticos e/ou seus efeitos (SANTANA et al., 2018; VAN DER OOST; BEYER; VERMEULEN, 2003). Dessa forma, é possível remediar

situações de risco, antes que estas se manifestem em níveis de organização biológica superiores, como populações e ecossistemas.

A toxicidade de HPAs e agrotóxicos é mediada pela biotransformação desses compostos, (i.e., detoxificação ou bioativação), processo que pode induzir estresse oxidativo através da produção de EROs em excesso (LUSHCHAK, 2011; LUSHCHAK et al., 2018; SCHLENK et al., 2008). Por esta razão, os níveis de proteínas e atividade de enzimas envolvidas na metabolização de xenobióticos, bem como na defesa antioxidante das células, são comumente utilizados como biomarcadores de exposição e efeito de contaminantes.

1.3.1 Biomarcadores de Estresse Oxidativo

Estresse oxidativo é definido como um desequilíbrio entre a produção de pro-oxidantes, como EROs ou espécies reativas de nitrogênio (ERNs), e a presença de antioxidantes (BOELSTERLI, 2007). Pequenas quantidades de EROs são geradas em condições fisiológicas normais, como por exemplo a transferência de elétrons nas mitocôndrias ou oxidação de moléculas endógenas mediadas pelas CYPs (LUSHCHAK et al., 2018). As EROs geradas a partir do oxigênio molecular são radical ânion superóxido ($O_2^{\bullet -}$), peróxido de hidrogênio (H_2O_2) e o radical hidroxila ($HO^{\bullet}$) (DAVIES, 2000). As células possuem um eficiente sistema de defesa antioxidante não-enzimático e enzimático, programado para impedir que EROs causem danos a ácidos nucleicos (i.e., oxidação do DNA), proteínas (i.e., carbonilação de proteínas), lipídios (i.e., peroxidação lipídica) (SLANINOVA et al., 2009; VAN DER OOST; BEYER; VERMEULEN, 2003).

O sistema de defesa antioxidante não-enzimático é composto por glutationa (GSH), ácido ascórbico (i.e., vitamina C), β-caroteno e vitamina E. Entre esses, o tripeptídeo GSH é a primeira e mais importante linha de defesa contra EROs. É encontrada em grandes concentrações particularmente nas mitocôndrias, onde há maior produção de EROs, mas também e no citosol das células (BOELSTERLI, 2007; LIU, 2020). É mantida em sua forma reduzida através da atividade da glutationa reductase (GR), com menos de 5% da GSH total ocorrendo na sua forma oxidada (GSSG) (LIU, 2020). A GSH atua sequestrando radicais, como co-substrato da glutationa peroxidade (GPx) degradando H_2O_2 e como co-substrato para glutationa S-transferase (GST) em reações de metabolização (DAVIES, 2000).

Além da GPx e GR descritas acima, as enzimas superóxido dismutase (SOD) e catalase (CAT) também compõem o sistema de defesa antioxidante. A SOD é uma metaloenzima que possui Cu e Zn, Mn ou Fe como metal ativo. Ocorre no citosol (Cu-Zn SOD1), na mitocôndria (Mn SOD2) e no meio extracelular (Fe SOD3) (ZHU et al., 2013), e atua na dismutação do ânion superóxido, gerando H_2O_2 e O_2 (LIU, 2020). Enquanto a catalase (CAT), encontrada essencialmente nos peroxissomos e muito abundante no fígado, atua na decomposição de H_2O_2, formando H_2O e O_2 (ZHU *et al.*, 2013). A Figura 3 resume os mecanismos de proteção à presença de EROs.

A exposição a xenobióticos, como HPAs e agrotóxicos, pode incrementar drasticamente a produção de EROs. Esses compostos podem atuar como aceptores de elétrons, recebendo o elétron do NADPH através de reações catalisadas por reductase. Uma vez reduzidos, podem entrar em um ciclo redox: caso o potencial redox permita, xenobióticos podem reduzir oxigênio molecular,

gerando ânion superóxido. Assim, estará disponível para ser novamente reduzido. O ciclo continuará até que ocorra a depleção de redutores (BOELSTERLI, 2007; LUSHCHAK et al., 2018). Portanto, uma única molécula de um composto tóxico pode gerar várias moléculas de superóxido, amplificando a geração de EROs e ativando as defesas antioxidantes.

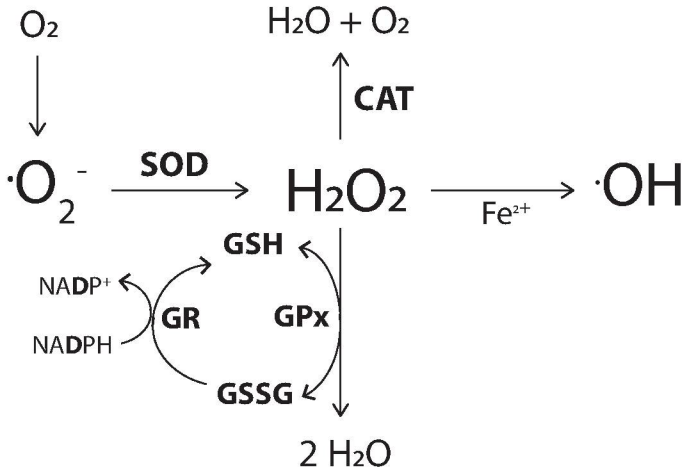


Figura 3 Espécies reativas de oxigênio (EROs) e mecanismos de defesa antioxidante. Ânion superóxido ($^{\bullet}O_2^-$), peróxido de hidrogênio (H_2O_2) e o radical hidroxila ($^{\bullet}OH$), oxigênio (O_2), glutationa reduzida (GSH), glutationa dissulfeto (glutationa oxidada, GSSG), superóxido dismutase (SOD), catalase (CAT), glutationa reductase (GR), glutationa peroxidase (GPx). (Figura adaptada de LIU, 2020)

1.3.2 Biomarcadores de Biotransformação

Como mencionado anteriormente, o citocromo P450 (CYP) é uma superfamília de heme proteínas de papel chave no processo de metabolização de xenobióticos. Existem inúmeras famílias e sub-famílias de CYPs, cada qual atuando em substratos endógenos e exógenos específicos (CHEN, 2020; SCHLENK et al., 2008). Por exemplo, as famílias CYP1, CYP2, CYP3 e CYP4 são cruciais para o metabolismo de xenobióticos e hormônios esteróides (CHEN, 2020). Mais precisamente, a subfamília CYP1A1 é responsável pela bioativação

de benzo[a]pireno na maior parte das espécies, inclusive mamíferos (SCHLENK et al., 2008).Os efeitos dos HPAs e agrotóxicos na CYP1A1 pode ser detectado através da atividade catalítica da etoxiresorufina-O-desetilase (EROD), i.e., a EROD serve como um biomarcador de biotransformação (FABRIN et al., 2018). A EROD é um indicador sensível e, geralmente, sua indução após exposição a contaminantes é rápida e quantificável (WHYTE et al., 2000).

Durante as reações de Fase I de biotransformação, as CYPs incorporam um átomo de oxigênio enquanto reduz outro. Esses processos de oxidação podem não ser suficiente para aumentar a hidrofilicidade do composto e assim facilitar a excreção (BOELSTERLI, 2007). Sendo assim, reações de conjugação, realizadas por glutationa S-transferases (GSTs), sulfotransferases (SULTs) e uridina 5'-difosfo-glucuronosiltransferase (UGTs), ocorrem na tentativa de eliminar o composto (CHEN, 2020). As SULTs transferem o sulfato do cofator 3-fosfoadenosina-5-fosfossulfato (PAPs) para o xenobiótico, enquanto UGTs realizam a glicorunidação, i.e., adicionam ácido glicurônico a partir do cofator UDP-ácido glicurônico (UDPAG) ao substrato (CHEN, 2020). As GSTs catalisam a conjugação de GSH em xenobióticos, ou seus metabólitos, em reações de Fase II (VAN DER OOST; BEYER; VERMEULEN, 2003). Além disso, são responsáveis por transporte intracelular e biossíntese de eicosanoides (HAYES; FLANAGAN; JOWSEY, 2005). Assim como as CYPs, GSTs formam uma superfamília de enzimas com diversas isoformas, sendo GSTT1 e GSTM1 envolvidas na biotransformação de HPAs (SCHLENK et al., 2008). Apesar de serem descritas como imprevisíveis (ALTENBURGER; SEGNER; VAN DER OOST, 2003; HELLOU; ROSS; MOON, 2012), GSTs possuem um importante

papel na elimanação de xenobióticos e, por isso, são amplamente utilizadas como biomarcadores.

1.3.3. Biomarcadores de Neurotoxicidade: Colinesterases (ChEs)

Entre os biomarcadores comumente utilizados, a inibição das colinesterases (ChE), especificamente acetilcolinesterase (AChE) e butirilcolinesterase (BChE) são os mais relevantes para avaliar exposição de agrotóxicos em peixes (NUNES, 2011; ROJAS-GARCÍA et al., 2011). AChE e BChE são serino hidrolases amplamente encontradas em várias espécies animais. Diferem-se pela estrutura do bolso acila na cavidade enzimática, que determina seus papéis na neutransmissão colinérgica (MANGAS et al., 2017; MASSOULIÉ; TOUTANT, 1988). A AChE possui um papel primordial na regulação da neurotransmissão em vias colinérgicas de vertebrados (POPE; BRIMIJOIN, 2018). Ocorre no cérebro, músculo esquelético e na membrana de eritrócitos, onde mantém os níveis de acetilcolina estáveis através da hidrólise desse neurotransmissor (DIAS ASSIS; SOUZA; CARVALHO JR, 2011; MANGAS et al., 2017). É extremamente eficiente, hidrolisando seu substrato em uma taxa que se aproxima do limite da difusão (MASSOULIÉ et al., 1993). Sua inibição está associada ao aumento de contrações musculares, diminuição de batimentos cardíacos, aumento de secreções exócrinas e perturbações no sistema gastrointestinal (POPE & BRIMIJOIN, 2018).

Sua enzima "irmã", BChE, divide muitas similaridades estruturais, entretanto sua função biológica ainda é elusiva (POPE & BRIMIJOIN, 2018). BChE é uma enzima com uma menor especificadade de substrato, hidrolisando butirilcolina (substrato sintético) e acetilcolina, em uma taxa comparativamente

menor (MASSOULIÉ; TOUTANT, 1988). Acredita-se que a BChE atua no metabolismo de lipoproteínas, na adesão celular, na metabolização de algumas drogas (MANGAS et al., 2017) e, potencialmente, protege a AChE de agentes anticolinesterásicos (ASSIS et al., 2011). Assim como AChE, BChE forma uma ligação reversível com inseticidas organofosforados (CHEN, 2020). Porém, a interação desses compostos com BChE é entendida como um processo de detoxificação, uma vez que minimiza a interação com a AChE e impede que efeitos subletais e até letais ocorram (ALBENDÍN et al., 2017; RÖSLER et al., 2018).

ChEs também estão presentes em tecidos não-colinérgicos, participando de eventos biológicos fundamentais, como desenvolvimento embrionário, proliferação e morte celular (FALUGI; ALUIGI, 2012). A exemplo, a AChE pode atuar como um regulador da morte celular programada, considerando que sua expressão diminui durante a proliferação celular e aumenta durante a apoptose (ZHANG et al., 2002). Acredita-se que a BChE possui papel regulador de ganho de peso e metabolismo de gorduras, já que hidrolisa o hormônio intestinal grelina, denominado "hormônio da fome" por estimular a ingestão de alimentos (BRIMIJOIN et al., 2016; POPE; BRIMIJOIN, 2018). Considerando a importância das ChEs em diversos processos biológicos e a constante aplicação de compostos neurotóxicos, é importante avaliar como diferentes classes de agrotóxicos afetam as ChEs e quais fatores podem determinar a magnitude dos efeitos.

1.4 Revisão Sistemática e Meta-Análise

A literatura na área de ecotoxicologia está repleta de estudos que discorrem sobre o mesmo problema (e.g., os efeitos de um mesmo contaminante em um conjunto de biomarcadores de peixes) e por isso, revisões atualizadas fazem-se necessárias. Revisões qualitativas baseadas na opinião de especialistas da área, i.e., revisões narrativas, foram e ainda são muito procuradas para compreender um determinado tópico (JENNIONS; LORTIE; KORICHEVA, 2013). Apresentam perspectivas amplas e desenvolvimento histórico de conceitos, porém estão sujeitas a subjetividade dos autores e possuem baixa reprodutibilidade. Além disso, não possuem ferramentas analíticas robustas para sintetizar quantitativamente os resultados do conjunto de dados selecionado. Revisões sistemáticas são práticas de síntese que incorporam métodos transparentes, reproduzíveis e estruturados para identificar e sumarizar a informação de estudos relevantes (KORICHEVA; GUREVITCH, 2013). Quando associadas a meta-análises, é possível quantificar robustamente a magnitude dos resultados e analisar causas da heterogeneidade entre os estudos primários incluídos.

A liberação constante de POPs em ambientes aquáticos, especialmente HPAs e agrotóxicos, levou a realização e publicação de uma infinidade de estudos sobre os mecanismos de toxicidade desses compostos e a extensão dos seus efeitos. Os biomarcadores descritos acima são ferramentas bem estabelecidas para avaliar o impacto por HPAs e agrotóxicos em peixes (LUSHCHAK, 2011; LUSHCHAK et al., 2018; SCHLENK, 2005; WHYTE et al., 2000). Entretanto, estudos ecotoxicológicos apresentam resultados muito

variados, com atividades enzimáticas ou níveis de proteínas permanecendo inalterado, aumentando ou reduzindo em peixes expostos a esses compostos. Meta-análises podem contribuir significativamente para a área de ecotoxicologia ao esclarecer essas inconsistências. Além de estimar o efeito global dos contaminantes em biomarcadores, é possível avaliar a influência de diferentes fontes de variação ou moderadores na magnitude do efeito (GUREVITCH et al., 2018; NOBLE et al., 2017). Dessa forma, a partir de um grande número de estudos, sobre o mesmo tópico, pode-se fazer generalizações mais amplas. Os resultados e conclusões obtidos através de meta-análises fornecem um quadro mais abrangente do que um estudo primário poderia fornecer.

Para obtenção de resultados confiáveis e robustos, é preciso seguir uma metodologia rigorosa para busca da literatura, triagem de estudos (baseada em critérios claros de inclusão), extração de dados, codificação e meta-análise (GUREVITCH et al., 2018). Diretrizes para realização adequada das etapas da revisão e apresentação dos resultados são bem estabelecidas. O PRISMA (*Preferred Reporting Items for Systematic Reviews and Meta-Analysis*), por exemplo, é um guia largamente utilizado, que auxilia pesquisadores a reportar etapas de busca e seleção de estudos e resultados, contribuindo assim para padronização e qualidade das revisões (MOHER et al., 2009).

Os dados extraídos de cada um dos estudos – avaliados e selecionados nas etapas da revisão sistemática – são quantitativamente sintetizados através de meta-análises. De cada estudo primário individual são extraídos a média, desvio padrão (ou outra medida de dispersão) e o tamanho amostral do grupo controle e do grupo tratamento. Esses dados são transformados em um único valor adimensional, que reflete a magnitude e direção do efeito de um

determinado tratamento, i.e., tamanho de efeito (BORENSTEIN et al., 2009; NAKAGAWA; CUTHILL, 2007). Os tamanhos de efeito padronizam os resultados de todos os estudos selecionados, uma vez que os colocam na mesma escala utilizando métricas variadas. Entre as métricas comumente utilizadas estão a razão de probabilidade (*odds ratio*, OR), razão de risco (risk ratio, RR), diferença padronizada das médias (*standardized mean difference*, SMD, ou hedges' *g*) ou log natural da razão de resposta (*lnRR*) (BORENSTEIN et al., 2009; ROSENBERG; ROTHSTEIN; GUREVITCH, 2013).

Essas métricas permitem que dados de uma grande variedade de estudos sejam convertidos e sintetizadas em um único efeito, i.e., efeito global (GUREVITCH et al., 2018). Além disso, utilizando ferramentas meta-analíticas mais complexas é possível incluir variáveis moderadoras para determinar o quanto elas podem explicar a heterogeneidade dos dados (NOBLE et al., 2017; VIECHTBAUER, 2007). Por seguir protocolos de buscar e obtenção rigorosos e transparentes, além de análises dos dados robustas, revisões sistemáticas associadas a meta-análises são amplamente difundidas em diferentes áreas do conhecimento, como medicina, ciências sociais e ecologia (BORENSTEIN et al., 2009; GUREVITCH et al., 2018; KORICHEVA; GUREVITCH, 2013). Porém, sua aplicação em estudos ecotoxicológicos ainda é incipiente. Assim, a adoção dessa abordagem pode contribuir para o desenvolvimento da área ao esclarecer padrões de resposta e direcionar esforços futuros.

2. OBJETIVOS

2.1 Objetivo Geral

Sintetizar quantitativamente, por meio de revisões sistemáticas e meta-análises, os efeitos de hidrocarbonetos policíclicos aromáticos (HPAs) e agrotóxicos em biomarcadores de biotransformação, estresse oxidativo, e neurotoxicidade de peixes. Adicionalmente, investigar quais fatores controlam a significância e magnitude desses efeitos.

2.2 Objetivos Específicos

- Avaliar diferenças entre peixes expostos (i.e., tratamento) e não expostos (i.e., controle) nas respostas de biomarcadores de biotransformação e de estresse oxidativo após exposição a HPAs. Especificamente, avaliar diferença na atividade média da EROD (etoxiresorufina-O-desetilase), glutationa S-transferase (GST), glutationa redutase (GR), glutationa peroxidase (GPx), catalase (CAT), superóxido dismutase (SOD), e dos níveis de glutationa (GSH), e peroxidação lipídica (LPO). Adicionalmente, investigar fatores que modificam a magnitude e direção dos efeitos, particularmente: abordagem experimental (laboratório, campo, biomonitoramento ativo), ambiente (marinho, água doce), habitat (demersal, bentopelágico, pelágico-nerítico e associado a recifes), rota de exposição (hídrica, injeção, dieta e sedimento) e a combinação de tempo e concentração.

- Avaliar os efeitos de agrotóxicos na média e variabilidade de biomarcadores de neurotoxicidade em peixes. Especificamente, quantificar a atividade de colinesterases, i.e., acetilcolinesterase (AChE) e butirilcolinesterase (BChE). Adicionalmente, investigar os principais fatores regulando a significância e extensão dos efeitos, particularmente: tipo de colinesterases, tecido utilizado para avalição (cérebro, músculo), comprimento dos peixes e idade (adulto, juvenil), tipo de pesticida (inseticida, herbicida, fungicida), classe de pesticida (e.g, organofosforado, carbamatos, piretróides, triazóis), formulação dos agrotóxicos (analítico, comercial) e combinação de concentração e tempo.
- Avaliar o efeito de agrotóxicos no sistema de biotransformação e defesa antioxidante de peixes. Especificamente, avaliar as atividades da EROD (etoxiresorufina-O-desetilase), glutationa S-transferase (GST), catalase (CAT), superóxido dismutase (SOD), e os níveis de glutationa (GSH). Adicionalmente, investigar fatores que modificam a magnitude e direção dos efeitos, particularmente: diferenças entre biomarcadores de biotransformação e estresse oxidativo, sexo (macho, fêmea, ambos), idade (adulto, juvenil) e classe de agrotóxicos (organofosforados, fosfanoglicina, piretróides, triazine, etc).

Capítulo I

Biomarker responses in fish exposed to polycyclic aromatic hydrocarbons (PAHs): systematic review and meta-analysis.

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Biomarker responses in fish exposed to polycyclic aromatic hydrocarbons (PAHs): Systematic review and meta-analysis☆

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ABSTRACT

Biomarkers of antioxidant and biotransformation systems are commonly used to assess polycyclic aromatic hydrocarbons (PAHs) pollution in fish. Despite their extensive application of biomarkers, contradictory results are vastly reported in the literature, even for the same species in similar contamination scenarios. This study aims to verify response patterns of biomarkers in fish exposed to PAHs. Through systematic reviews and meta-analyses, we were able to evaluate: *(i)* overall magnitude of PAHs effects on biotransformation and oxidative stress biomarkers; *(ii)* patterns of response among experimental approaches (laboratory, field and active biomonitoring), environment (marine and freshwater) and fish habitat (pelagic, demersal, etc.); *(iii)* effects of exposure route, time and concentration of PAHs; and *(iv)* which biomarkers respond best to PAHs exposure. Overall, biomarker responses were significantly affected by PAHs exposure. The activities of ethoxyresorufin-O-deethylase (EROD), glutathione S-transferase (GST), superoxide dismutase (SOD), glutathione peroxidase (GPx) and levels of oxidized glutathione (GSSG) and lipid peroxide (LPO) significantly increased in fish exposed to PAHs, whereas catalase (CAT) and glutathione reductase (GR) activities and levels of reduced glutathione (GSH) were not affected. Amongst responsive biomarkers, EROD and GST activities significantly differed among approaches and between marine and freshwater environments, but were not affected by fish habitat. GSSG levels were higher in fish from laboratory bioassays compared to the field, but did not differ between environments nor habitats. Exposure route played a major role only for GST and GPx responses. Finally, increasing PAHs concentration and exposure time had a significant effect on all assessed biomarkers, except for CAT. We conclude that EROD and GST are robust biomarkers to assess PAHs effects in fish. Contrarily, CAT is an inadequate biomarker of PAHs exposure since no significant response was observed. Our study also highlighted some research gaps in PAHs contamination studies, such as a clear lack of active biomonitoring experiments.

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1. Introduction

1.1. Polycyclic aromatic hydrocarbons in the aquatic environment

Polycyclic aromatic hydrocarbons (PAHs) represent a class of hydrophobic organic compounds ubiquitously found in freshwater and marine environments (Neff et al., 2005). Even though natural processes such as forest fires or oil seeps (Billiard et al., 2008) contribute to environmental input of PAHs, their major source is anthropogenic, mainly from the incomplete combustion of fossil fuels, wood, oil spills, and discharge from ships and sewage sludge (Suess, 1976; Douben, 2003; Abdel-Shafy and Mansour, 2016; Lawal, 2017). Because a variety of human activities are associated with the widespread contamination of PAHs, their potential toxic effects are continually and increasingly under investigation (Billiard et al., 2008; Rengarajan et al., 2015). Since 1991, PAHs have been ranked on the U.S. Agency for Toxic Substances and Disease Registry priority list (ATSDR, 1991, 2017), which suggests the risk PAHs pose to human health and environmental integrity.

PAHs may be taken up from sediments or directly from the water column into tissues of invertebrates and vertebrates,

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subsequently exerting toxicity through various mechanisms (Livingstone, 2001). Aquatic vertebrates, such as fish, have well-developed biotransformation systems capable of converting PAHs into water-soluble derivatives in order to facilitate excretion (Boelsterli, 2007). During this process, however, electrophilic reactive toxic metabolites may be generated, leading to mutagenesis, carcinogenesis, immunosuppression, as well as adverse developmental and reproductive effects (Tuvikene, 1995; Payne et al., 2003; Rengarajan et al., 2015). In addition, biotransformation of PAHs may lead to an imbalance between the production of pro-oxidant compounds and cellular antioxidants, favouring the former, and potentially leading to oxidative stress (Altenburger et al., 2003; Boelsterli, 2007).

Reactive oxygen species (ROS), such as superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$) are the most important pro-oxidants responsible for oxidative stress (Boelsterli, 2007), but potential damage to cells is normally limited by the presence of protective antioxidant defence mechanisms (Davies, 2000). Hence, several studies and monitoring programs have measured fish biotransformation and oxidative stress parameters in order to assess the quality of aquatic environments (Codi et al., 2004; Abrahamson et al., 2008; Lu et al., 2010; Barhoumi et al., 2014). These biochemical responses provide an early-warning signal of adverse effects of contamination and are commonly referred to as biomarkers (van der Oost et al., 2003).

1.2. Biotransformation and oxidative stress biomarkers

Biomarkers are early-warning signals of contamination measured in cells, body fluids, tissues and organs, and may indicate environmental impact before it can be observed at higher biological levels, such as populations and ecosystems (Vidal-Liñan et al., 2010). For the purpose of this review, we will focus on the response patterns of biochemical biomarkers commonly measured in fish livers. The liver contains a variety of PAHs biotransformation enzymes, robust antioxidant defences and is involved in the excretion of PAHs through bile (Oliveira-Ribeiro and Narciso, 2014; Boelsterli, 2007), and therefore, the majority of data on biochemical biomarkers is from fish liver.

Contaminants such as PAHs can be converted to more hydrophilic (water-soluble) compounds to facilitate excretion (Altenburger et al., 2003; Boelsterli, 2007). This biotransformation process is catalysed by phase I enzymes which oxidise, reduce or hydrolyse compounds; and phase II enzymes which catalyse the conjugation of chemical groups (cofactors) such as glutathione (GSH), sulphate and glucuronic acid. Cytochrome P450 isoforms (CYPs) are a large family of membrane-bound heme proteins involved in phase I reactions. The activity and amount of CYPs are important tools to assess environmental disturbances triggered by chemical contamination. For example, the activity of ethoxyresorufin-O-deethylase (EROD) of CYP1A subfamily members is a common and widely used biomarker of planar halogenated and polycyclic aromatic hydrocarbon contamination (Goksøyr and Förlin, 1992; van der Oost et al., 2003), although other CYPs such as CYP1B, CYP1C, and CYP3A can be involved with PAHs biotransformation (Shimada, 2006; Schlenk et al., 2008; Uno et al., 2012).

Glutathione *S*-transferase (GST) belongs to a superfamily of dimeric water-soluble enzymes responsible for a variety of functions, such as intracellular transport, biosynthesis of eicosanoids and more critically, the conjugation of glutathione (GSH) to xenobiotics in phase II reactions (George and Buchanan, 1990; Hayes et al., 2005). Even though GST activity is described as unpredictable (Altenburger et al., 2003; Hellou et al., 2012), it has been used as a biomarker in many ecotoxicological studies because of the significant role GST plays in detoxification of PAHs, DNA-reactive metabolites and oxidative stress products derived from lipid oxidation, nucleic acids and proteins (Schlenk et al., 2008; van der Oost et al., 2003). Like for CYPs, there are several isoforms of GST, with GSTT1 and GSTM1 homologs being involved in PAHs biotransformation (Shimada, 2006; Schlenk et al., 2008). However, ecotoxicological studies usually report the general activity of GSTs, assessed through enzymatic assays using a common substrate for all GST isoforms, the CDNB (2,4-dinitrochlorobenzene), rather than the activity of PAH-specific GSTs. Although there are other families of phase I and phase II enzymes involved in xenobiotic biotransformation, such as peroxidase, epoxide hydrolase, sulfotransferase (SULT) and UDP-glucuronosyltransferase (UGT), NAD(P)H quinone oxidoreductase 1, and aldo-keto reductase (Shimada, 2006; Boelsterli, 2007), the majority of studies focus on the EROD and GST activity to understand PAH contamination.

Formation of DNA and protein adducts by electrophilic PAH metabolites and induction of oxidative stress are recognised mechanisms of PAHs toxicity (Payne et al., 2003; Baird et al., 2005). In particular, CYP activity can generate large amounts of ROS that are usually neutralised or degraded by enzymatic and non-enzymatic antioxidant defence mechanisms (Altenburger et al., 2003; Boelsterli, 2007).

GSH is the most important non-enzymatic defence against ROS (Boelsterli, 2007). As a scavenger, GSH reacts with some ROS and radicals, producing GSSG (glutathione disulphide). As a cofactor, GSH is used by GST in detoxification and by glutathione peroxidase (GPx) in H_2O_2 degradation (Winston and Di Giulio, 1991; Davies, 2000). High levels of GSH are maintained by de novo synthesis and by glutathione disulphide reductase (GR)-dependent recycling of GSSG back to GSH (Zhu et al., 2013). Given this paramount role, GSH levels and related enzymes are vastly used as biochemical responses to contaminants (Hellou et al., 2012).

Superoxide dismutase (SOD) and catalase (CAT) are the first enzymatic line of antioxidant defence. SOD is responsible for converting superoxide anions ($O_2^{\bullet-}$) to H_2O_2, which in turn is converted to water by CAT (Abele et al., 2017). By decreasing the levels of $O_2^{\bullet-}$ and H_2O_2, SOD and CAT have an important role in avoiding the production of hydroxyl radicals ($HO^{\bullet}$) through Fenton/Harber-Weiss reactions (Boelsterli, 2007). Hydroxyl radicals are very unstable and reactive radicals are those that can oxidise many biomolecules, such as RNA, DNA, proteins and lipids, leading to toxicity (Farber, 1994; Di Giulio and Meyer, 2008). Thus, the activities of SOD and CAT have been measured in several studies as biomarkers for a variety of contaminants, though there is no consistent pattern of response to establish valid links between SOD-CAT activities and specific contaminants (van der Oost et al., 2003).

Although livers have robust antioxidant defences, excessive production of ROS and impairment of these defences (e.g., by inhibition of enzymatic activities) can cause oxidative damage to biomolecules (Di Guilio and Meyer, 2008). Lipid peroxidation (LPO) is a set of chain reactions triggered by radicals such as hydroxyl radical that can lead to loss of membrane integrity and secondary damage to DNA and proteins through adduct formation with toxic aldehydes produced from lipid peroxides (Davies, 2000). For these reasons, levels of LPO are also recognized as important oxidative stress biomarkers that can be evaluated by assays such as FOX (ferrous oxidation-xylenol orange; Jiang et al., 1992) for lipid hydroperoxides and TBARS (thiobarbituric acid reactive substances) for malondialdehyde (Davies, 2000; Valavanidis et al., 2006).

Even though these biomarkers are well established in the literature, the responses reported by many studies differ due to several sources of variation. Among them, experimental approach, fish species, habitat and environment (i.e. freshwater or marine) have been described as important moderators of contamination effects on fish (Whyte et al., 2000; Altenburger et al., 2003; Payne

et al., 2003; van der Oost et al., 2003; Hook et al., 2014). Therefore, it is paramount to understand how and to what degree each of these sources affect fish responses to PAHs exposure. Therefore, the aim of this review is to explore the scientific literature, in order to understand patterns of response of widely employed biotransformation and oxidative stress biomarkers from fish exposed to PAHs.

1.3. Motivation for meta-analysis

Since the mid-1980s, several studies have developed, described and recommended a broad number of biomarkers for environmental monitoring. A substantial amount of information has accumulated on fish biomarkers, including differences among experimental approaches, environments and species (Beyer et al., 1996; Kirby et al., 1999; Aas et al., 2000; Almroth et al., 2005; Jee et al., 2005; Kopecka-Pilarczyk and Correia, 2009; Lu et al., 2011; Huang et al., 2016). However, results are inconsistent with reports from similar contamination scenarios showing increased (Nogueira et al., 2011; Dussauze et al., 2015; Sadauskas Henrique et al., 2016), decreased (Deer et al., 2010; Theron et al., 2014) or even no alteration (Milinkovitch et al., 2011; Bettim et al., 2016) in biomarker responses.

There is only one quantitative review that has specifically explored EROD activity responses to PAHs (Oris and Roberts, 2007) and many narrative reviews (van der Oost et al., 2003; Whyte et al., 2000; Altenburger et al., 2003; Billiard et al., 2008) examining most of the biomarkers addressed herein. The lack of comprehensive reviews coupled with powerful and integrative analyses may be preventing the examination of general response patterns.

Thus, a thorough, systematic literature review and meta-analysis might be able to shed light on basic and recurring problems in ecotoxicological studies, quantify biomarkers response patterns and identify important sources of variation, such as experimental approaches, fish environment and habitats, route and time of exposure and PAH concentrations used in experimental assays. Additionally, these results may provide support for future experimental design protocols, requiring less sample processing and more power to detect the relationship between xenobiotic and oxidative stress.

1.4. Main questions and hypotheses

Several predictions were made about the strength and direction of PAHs effects on biomarker responses. First, we expect that the magnitude of PAHs effects will be stronger on fish exposed in laboratory compared to field-based studies due to naturally occurring and uncontrollable variables in the field, which may affect uptake and metabolism and are often not accounted for in laboratory settings, leading to an overestimation of response (Goodsell et al., 2009). For example, the bioavailable concentration of PAHs may be maintained throughout the experiment in the laboratory whereas rain, sunlight, microorganisms and sediment may decrease PAHs concentration through dilution, degradation and adsorption in the field (Burgess et al., 2003; Gong et al., 2014). It is also expected that marine and freshwater fish will respond differently to PAHs exposure due to significant physiological differences related to ionic and osmotic regulation, hepatic lipid storage and renal excretion mechanisms (Evans, 1987; Evans et al., 2005; Hinton et al., 2001; Kleinow et al., 2008). Additionally, river discharge, tidal currents and salinity are known to affect the retention, accumulation and transportation of PAHs (Liu et al., 2014), leading to differences in concentration and bioavailability of PAHs between marine and freshwater environments.

Moreover, we presume that fish habitats will influence biomarker responses, specifically, demersal species will have a stronger response to PAHs than pelagic species. Considering that sediment is regarded as an ultimate sink where several classes of contaminants deposit and persist for long periods of time (Cardoso et al., 2016), bottom-associated fish may be more vulnerable due to ingestion of contaminated invertebrates or sediment (Hugla et al., 1995; Gonçalves et al., 2014; Duarte et al., 2017). Additionally, we expect that in laboratory experiments, PAHs delivered through injection techniques (e.g., intraperitoneal injection) will elicit stronger biomarker responses compared to other routes of exposure (i.e. water, diet or sediment) as described by Whyte et al. (2000). Finally, the magnitude of PAHs effects will be stronger in fish exposed for longer periods of time, both in the field and laboratory, due to higher doses/concentrations of PAHs, as described in many primary studies included in our systematic review.

Based on these predictions, we focused on the following questions: *(i)* what is the global net magnitude of the effect of PAHs on fish biotransformation and oxidative stress biomarker responses? *(ii)* does the strength and direction of PAHs effects vary among experimental approaches, environments, habitats and exposure routes? *(iii)* does time and dose dictate the direction of PAHs effects? *(iv)* which are the most responsive biomarkers to PAHs exposure?

2. Methods

2.1. Search strategy and database

Our literature search strategy involved two approaches. First, we extensively searched the literature reporting effects of PAHs on fish biotransformation and oxidative stress biomarkers using the extended database (timespan: "all years") from *PubMed, Scopus, ScienceDirect* and *Web of Science* until September 2016. The following search strategies were applied in all databases and then cross-referenced against one another providing a final set of studies (3895 publications):

Search 1: (fish* OR teleost*) AND (contamina* OR pollut*)

Search 2: oxidative stress OR antioxidant* OR oxidant* OR oxidative damage OR biomarker* OR biotransformation OR superoxide dismutase OR catalase OR glutathione OR glutathione peroxidase OR lipid peroxidation OR glutathione S-transferase OR ethoxyresorufin-O-deethylase OR EROD OR SOD OR CAT OR GPx OR GST OR GSH OR LPO OR OR MDA OR TBARS.

Search 3: PAHs OR hydrocarbon* OR oil* OR petroleum OR polycyclic organic matter OR polynuclear aromatic hydrocarbon* OR polynuclear aromatics OR polynuclear hydrocarbon*

Second, during our scoping stage of literature search, a seminal paper widely referenced in the field of ecotoxicology was identified: van der Oost et al., 2003. We examined its reference list in order to identify studies that were not published in journals indexed in the databases we had searched. A total of 51 papers were located using this approach. Searches from databases and reference list strongly overlapped, therefore, all duplicates were removed before screening. Next, based on title and abstract, irrelevant papers were excluded and potentially suitable studies were obtained for further examination. All steps of our literature search are summarized in Fig. 1 (PRISMA flowchart – the Preferred Reporting Items in Systematic Reviews and Meta-Analysis, Moher et al., 2009).

We have not requested unpublished data from colleagues or added papers to our dataset after September 2016 since that might lead to sampling bias. Additionally, we have not searched the grey literature, seeing that unpublished studies may have quality and data reliability issues, and ultimately compromise our analysis (Kelly and Jennions, 2011).

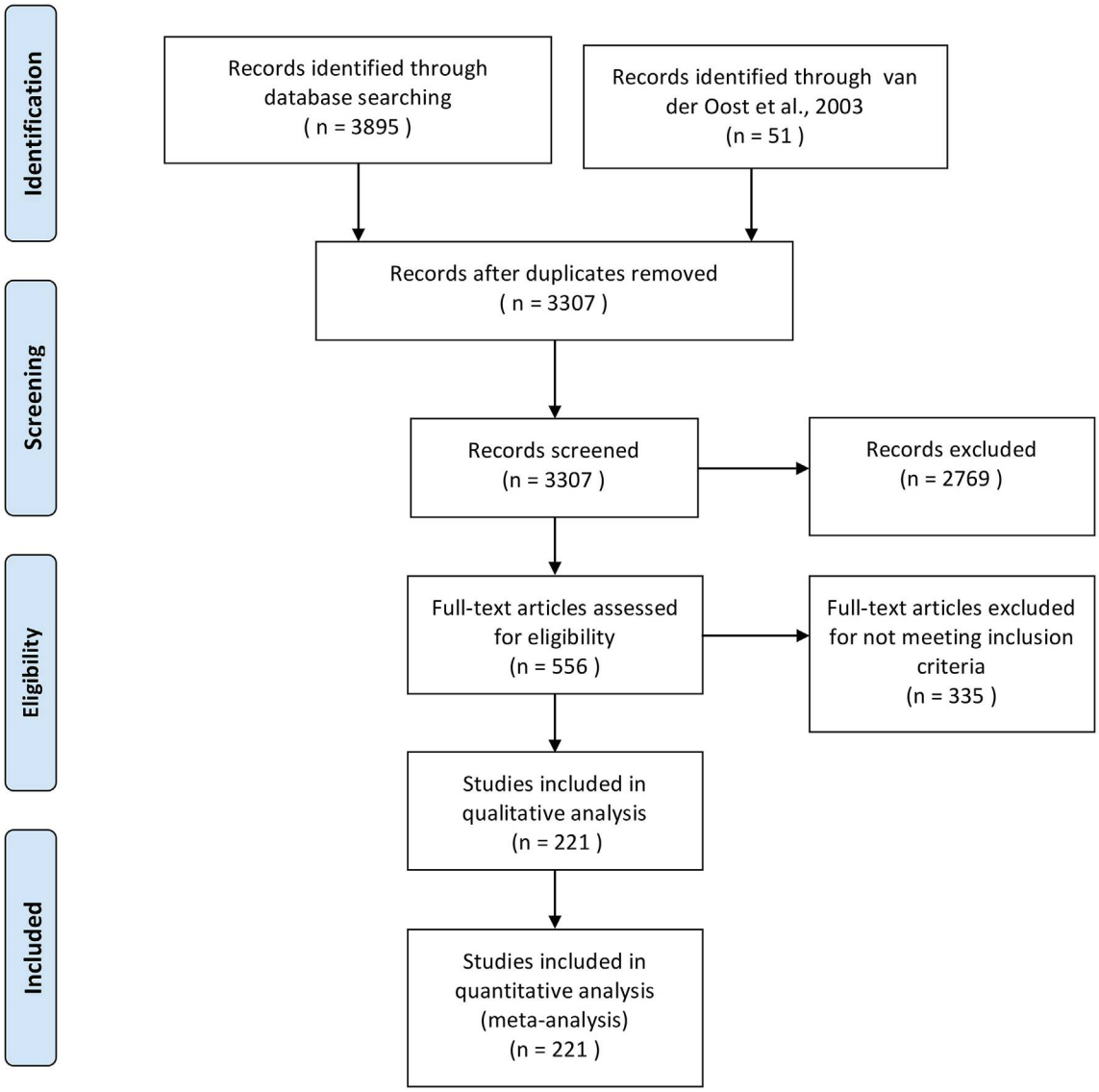


Fig. 1. The screening process used to exclude or retain articles for our meta-analysis; summarized and formatted as a PRISMA flow diagram (Moher et al., 2009).

2.2. Inclusion criteria and data extraction

All peer-reviewed articles had to meet the following inclusion criteria: *(i)* studies had to address our main research question (i.e. PAHs effects on fish biotransformation and oxidative stress biomarkers) *(ii)* studies had to contain extractable data (e.g., mean, standard deviation, sample size); as well as unambiguous details on study design; and *(iii)* both field and laboratory studies had to provide a clear comparison of biomarker responses after exposure to PAHs (impact/treatment setting), and under conditions where PAHs were absent (reference/control setting); *(iv)* only *in vivo* studies that used fish liver to assess PAHs effects were considered.

Field experiments involving the transplant and caging of fish obtained from clean sites to contaminated conditions, both in marine and freshwater environments, were referred as active biomonitoring (ABM) experiments. This practice differs from field approach because fish, usually with known histories of breeding and maintenance, are exposed for a specific amount of time to natural settings.

Since both field and laboratory experiments were included, there were specific criteria for each. For laboratory experiments, only exposure times higher than 12 h were considered, and when studies had two controls; *(i)* fish exposed to the vehicle used to solubilise PAHs, and *(ii)* fish that did not undergo any treatment, only the latter was considered. As for field studies, whenever there were two reference sites, data from both were pooled together as described by Borenstein et al. (2009).

Moreover, fish were categorized as marine or freshwater depending on how authors described the study area or the exposure medium. For example, if fish were acclimated and exposed using seawater, then the species was considered marine, even if they can be found in freshwater or estuaries. When the description was not clear, we searched for information on fish distribution in order to make a decision.

We extracted means, standard deviation and sample size from each study from text, figures and tables. If authors reported another measure of variability (e.g., confidence intervals, standard error or coefficient of variation), we converted them to standard deviations when possible. We used GraphClick (version 3.0.3, Arizona Software, 2012) to obtain data from graphs when needed. Also, we

contacted authors and requested for data whenever it was not reported for biomarkers responses.

Most studies reported response outcomes for more than one period of time, dose, impacted sites, fish species or type of PAH. Such data was extracted and treated as independent studies, which guarantees all information from included studies are accounted for, therefore increasing statistical power (Côté et al., 2013). However, this approach leads to nonindependence among estimates, a common problem in meta-analyses that can affect study conclusions by inflating significance levels for statistical tests and underestimating the standard error of the mean effect (Noble et al., 2017). We dealt with nonindependence by *(i)* treating experiments from the same paper that were conducted in different years or seasons, or in any other setting with independent controls, as independent comparisons; and by *(ii)* modelling sources of nonindependence among estimates (study ID, species and country) and fitting a variance-covariance matrix (described in Meta-analyses section).

2.3. Meta-analysis

The magnitude of PAHs effects on fish biomarker responses was evaluated by converting the data from primary studies to standardized mean difference (SMD) or Hedge's *g*. We selected this effect size because it is designed to compare two means and it includes a correction for small sample sizes (Nakagawa and Santos, 2012; Rosenberg et al., 2013). Standardized differences are >0 when PAHs exposure triggers biomarker responses in fish and their levels are higher than in fish from reference/control settings, and <0 when the opposite occurs.

We performed meta-analyses separately for each of the nine biomarkers, i.e. there is a unique data set for each biomarker. We fitted linear mixed effects models (LMM) with the effect size SMD as the response variable along with its relative sampling error V_{SMD}. We also fitted a variance-covariance matrix in all models to account for nonindependence among effect sizes, which originates from the comparison of a single control with multiple treatments.

For each data set, we ran two different models, an intercept-only model (standard meta-analysis) including only study ID as a random effect to estimate the global effect for every biomarker and a meta-regression adding moderators (i.e. fixed-effects) to the intercept-only model. We chose moderators for the meta-regressions based mostly on what fixed effects are relevant to the questions previously stated in the introduction; experimental approach, environment, habitat, route of exposure, and time and concentration.

Meta-analyses were conducted as follows:

1) Intercept-only model to assess global effect for each biomarker (only ID as random-effect);
2) Type of approach laboratory (Lab), field or active biomonitoring (ABM) was added to intercept-only model to evaluate if effects differ among them. Differences among study ID were added as random-effect. Active biomonitoring refers to the practice of transplanting and fish obtained from clean sit to contaminated field conditions.
3) Within each approach, we ran a meta-analysis assessing the environment marine or freshwater as a source of variation. We included ID, species and country as random effects. Country was added as random effects to our models because we believe that some observations were originated from related research teams from the same country, which may be a source of nonindependence among estimates.

In addition, two-sample t-tests were used to verify if there were differences between marine and freshwater fish baseline levels (i.e. the means form control groups) for each biomarker;

4) Then, we ran a meta-analysis within each environment using habitat (demersal, benthopelagic, reef-associated, demersal, pelagic) as fixed effect and ID, species and country as random effects;
5) Within ABM approach, time of exposure (days) to contaminated sites was set as a fixed effect, and again ID, species and country as random effects;
6) Finally, within Lab approach, exposure routes (diet, injection, water or sediment) and concentration × time interaction were separately assessed as sources of variation, and the type of PAHs (chemical) along with ID, species and country was added as random effects.

For the concentration X time model, we created a specific data set. Since there are different exposure routes and several PAHs, it would be unfeasible to assess the effect of each PAH-exposure route combination separately. Thus, the aforementioned data set consists of all studies that added PAHs (isolated compounds or mixtures of them) to the water tanks of experimental setups, using the same or convertible concentration units. All concentrations were standardized to mg/l.

The consistency among effect sizes was assessed by calculating the overall heterogeneity (I^2_{total}), and I^2 for each random effect as described by Nakagawa and Santos (2012). Values of 25%, 50% and 75% are defined as low, moderate and high levels of heterogeneity, respectively (Higgins et al., 2003). Additionally, total heterogeneity (Q_T) was obtained through Wald-type tests and comparisons between moderators were conducted using Cochran's Q-test (Q_m; Hedges and Olkin, 1985).

Effect size calculations and meta-analyses were performed using the metafor package (version 1.9–9; Viechtbauer, 2010) within the R open-source software environment (version 3.3.1; R Core Team, 2016).

2.4. Publication bias

We assessed potential publication bias in the global effect of each biomarker using two methods. First, we calculated Rosenberg's fail-safe number, which is a statistically improved version of Rosenthal's fail-safe number (Jennions et al., 2013). This method estimates how many additional studies with a mean centred on the null value (i.e. non-significant studies), and with the same average sample size as the included studies, are needed to change the results from significant to non-significant ($P > 0.05$).

Additionally, we visually tested all data sets using funnel plots. Effect sizes were plotted against their corresponding precision values (the inverse of the square root of the sampling variance), and the resulting plots were checked for funnel asymmetry. Publication bias analyses were also performed using the metafor package within R.

3. Results

3.1. Database and overall effects

The search protocol identified 3307 papers that had their title and abstract scanned to identify studies suitable for our meta-analyses. After this initial step, 556 papers were fully screened, i.e. their methods and results sections were read and assessed, and 221 papers were deemed eligible for inclusion. Within those, a total of 1290 estimates of effect of PAHs were computed from 471 independent studies covering several fish species, habitats and from

different experimental approaches (see Appendix A). PAHs effects were assessed mostly through laboratory experiments (69% of estimates) conducted in Europe (44%).

Overall, PAHs had a significant effect on most biomarker responses, i.e. biomarker activity and levels from fish exposed to PAHs (treatment) were higher compared to fish from control/reference settings (Figs. 2–4). EROD, GST, SOD, GPx, GSSG and LPO increased in fish exposed to PAHs, while CAT, GR and GSH levels were not altered after exposure. Given the high amount of heterogeneity (Table S1 – Appendix C), further analyses were conducted exploring differences among approaches, environments and habitats.

3.2. PAHs effects through different approaches and at different environments (marine vs freshwater)

For the biotransformation biomarkers, the effects of PAHs exposure significantly differed among approaches (Fig. 2, Table S2). EROD activity was higher in fish from laboratory bioassays than in active biomonitoring species, but there were no differences between field and other approaches ($Q_m = 12.115$, df = 2, $P = 0.0023$). GST activity from laboratory bioassays was lower and differed only from active biomonitoring experiments ($Q_m = 6.125$, df = 2, $P = 0.047$). As for oxidative stress biomarkers, no significant difference was observed between laboratory and field approaches. An exception to this was for GR activity, which was higher in fish collected in contaminated areas in the field ($Q_m = 5.153$, df = 1, $P = 0.0232$), and GSSG levels that were higher in fish from laboratory bioassays ($Q_m = 4.2622$, df = 1, $P = 0.039$).

Due to high heterogeneity, we performed further analyses within each approach to identify potential moderators of variability. First, we verified if there were differences between marine and freshwater fish baseline levels with a two-sample *t*-test. Except for GPx and GR, no differences between freshwater and marine control means were found for most biomarkers (Table S3). In laboratory experiments, significant differences between marine and freshwater fish were observed only in EROD and GST activities (Fig. 2, Table S2). EROD activity measured in fish transferred from a clean site to contaminated environments (ABM approach) did not differ between marine and freshwater species, whereas despite the absence of effect, GST activity was different between these environments (Table S5). Finally, biomarkers from fish sampled from contaminated sites (Field approach) were similar at both marine and freshwater environments, except for EROD (Fig. 2a) and GR (Fig. 3d) activities, which were higher in freshwater fish (Table S2, S4-S5). Total heterogeneity remained high, and for most biomarkers, a large proportion of it is due to variability among effect sizes (I^2_{ID}).

3.3. PAHs effects at different habitats

Within each environment, biotransformation and most of oxidative stress biomarkers showed no significant difference among habitats, except for GSH levels that were higher on benthopelagic fish compared to demersal fish sampled in contaminated freshwater environments (Figs. S1–S3 – Appendix D). We have also quantified I^2 values for the random effects added to the model and these along with other details are given in Tables S3–S5.

3.4. Exploring further sources of variation within ABM approach

Time had no significant effect on EROD ($Q_m = 0.3589$, $P = 0.5491$) and GST ($Q_m = 0.2641$, $P = 0.607$) activities from fish transferred to contaminated sites (Fig. 5). We have also estimated ID, species and country as random effects. For EROD, the residuals accounted for all variance ($I^2_{ID} = I^2_{total} = 78.35\%$), indicating high variability at the level of effect sizes. As for GST, the largest proportion of total variance was due to differences among species

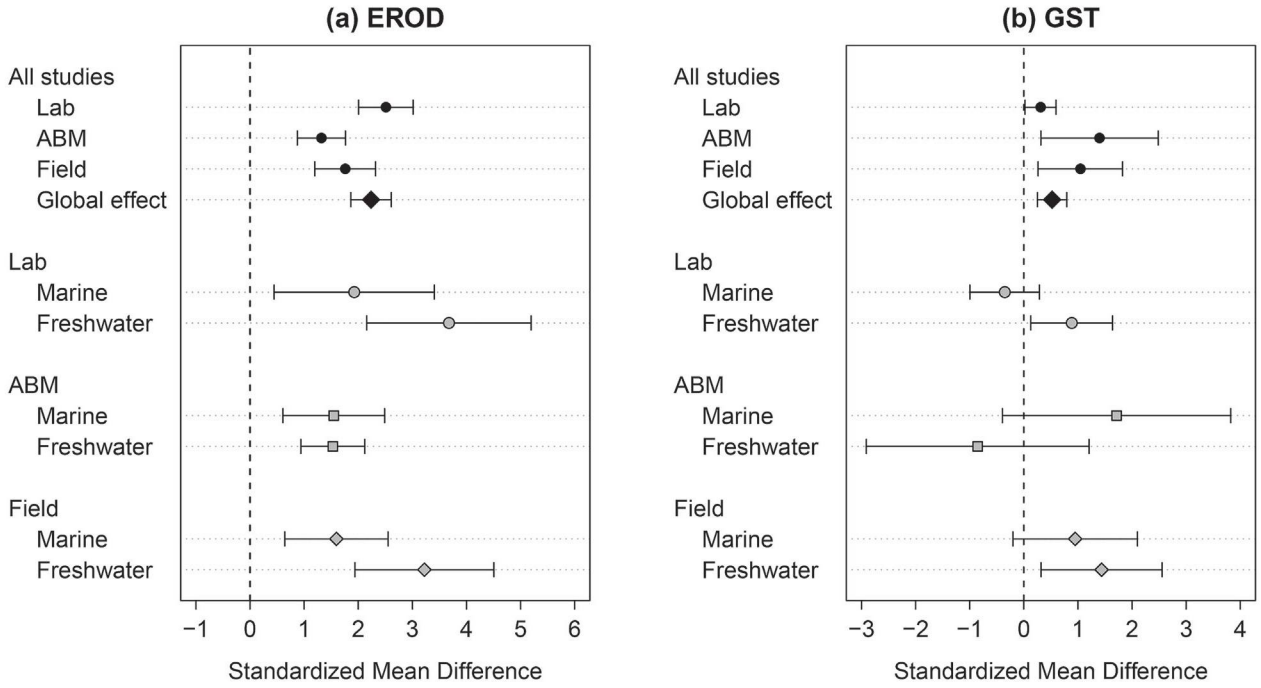


Fig. 2. Effects of polycyclic aromatic hydrocarbons (PAHs) exposure on fish biotransformation biomarkers. (a) ethoxyresorufin-O-deethylase (EROD) and (b) glutathione S-transferase (GST) from laboratory (Lab), active biomonitoring (ABM) and field experiments. Within each experimental approach, the effects on marine and freshwater fish are shown. Dots represent effect size estimates (standardized mean difference – SMD) and error bars represent 95% confidence intervals. Effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line).

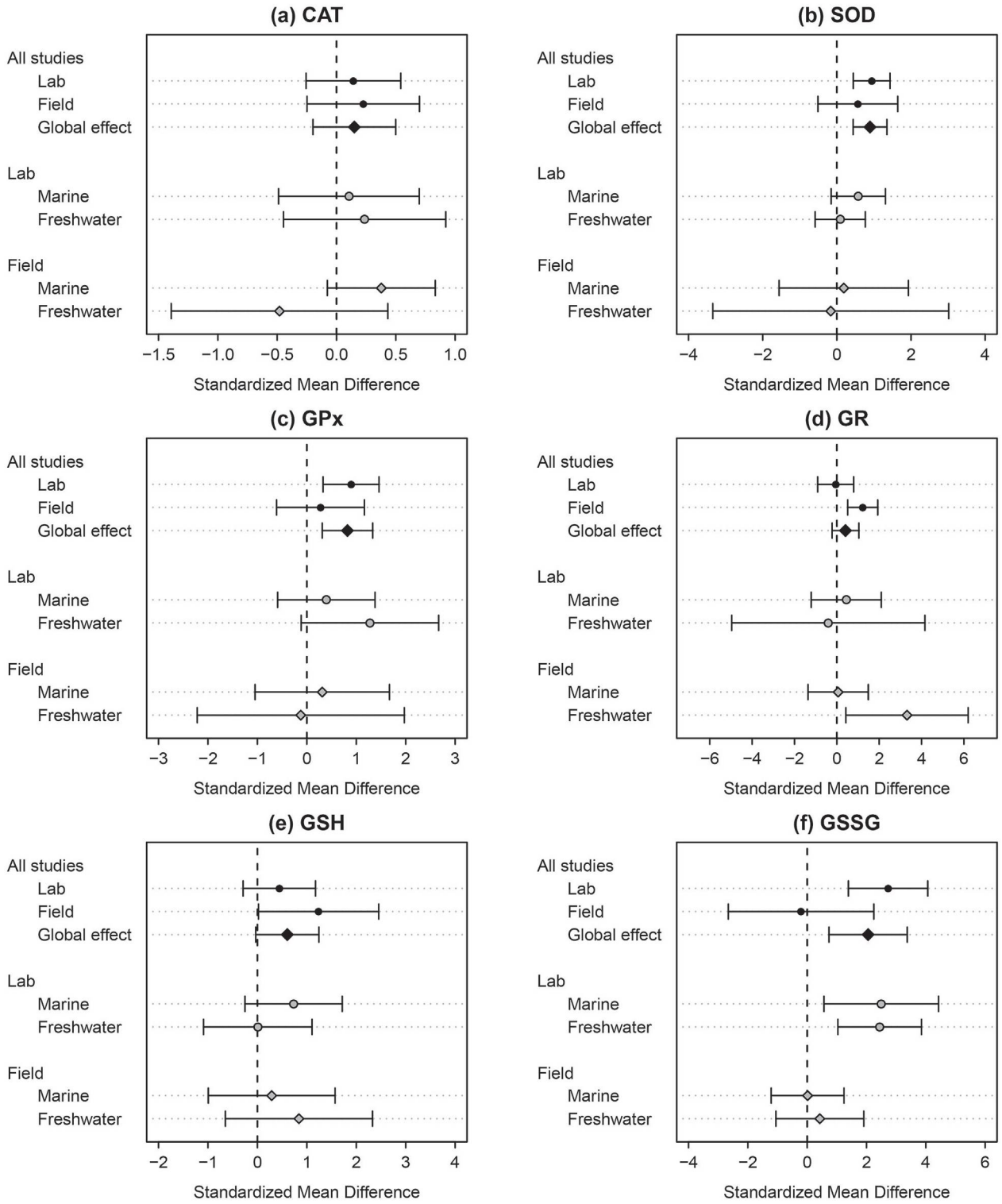


Fig. 3. Effects of polycyclic aromatic hydrocarbons (PAHs) exposure on fish oxidative stress biomarkers. (a) catalase (CAT), (b) superoxide dismutase (SOD), (c) glutathione peroxidase (GPx), (d) glutathione disulphide reductase (GR), (e) glutathione (GSH) and (f) glutathione disulphide (GSSG) from laboratory (Lab) and field experiments. Within each experimental approach, the effect on marine and freshwater fish are shown. Dots represent effect size estimates (standardized mean difference – SMD) and error bars represent 95% confidence intervals. Effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line).

($I^2_{species} = 41.39\%$, $I^2_{ID} = 22.94\%$, $I^2_{country} = 27.3\% \rightarrow I^2_{total} = 91.63\%$).

3.5. *Exploring further sources of variation within lab approach*

First, we assessed the effect of exposure route on biomarker responses, adding the types of PAHs (chemicals) as random effects along with ID, species and country. For most of them, there was no effect, except for GST and GPx activities (Table S6). GST activity was higher in fish exposed to PAHs added to sediment than fish exposed via diet, injection or water (Sediment > Diet = Injection = Water).

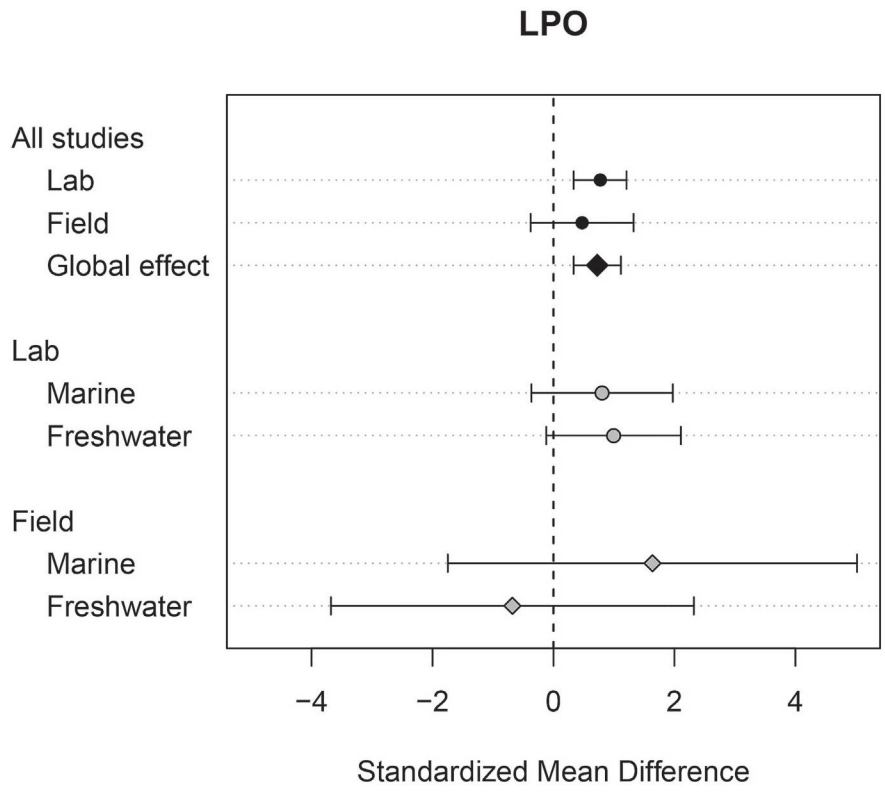


Fig. 4. Effects of polycyclic aromatic hydrocarbons (PAHs) exposure on fish lipid peroxidation (LPO) from laboratory (Lab) and field experiments. Within each experimental approach, the effect on marine and freshwater fish are shown. Dots represent effect size estimates (standardized mean difference – SMD) and error bars represent 95% confidence intervals. Effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line).

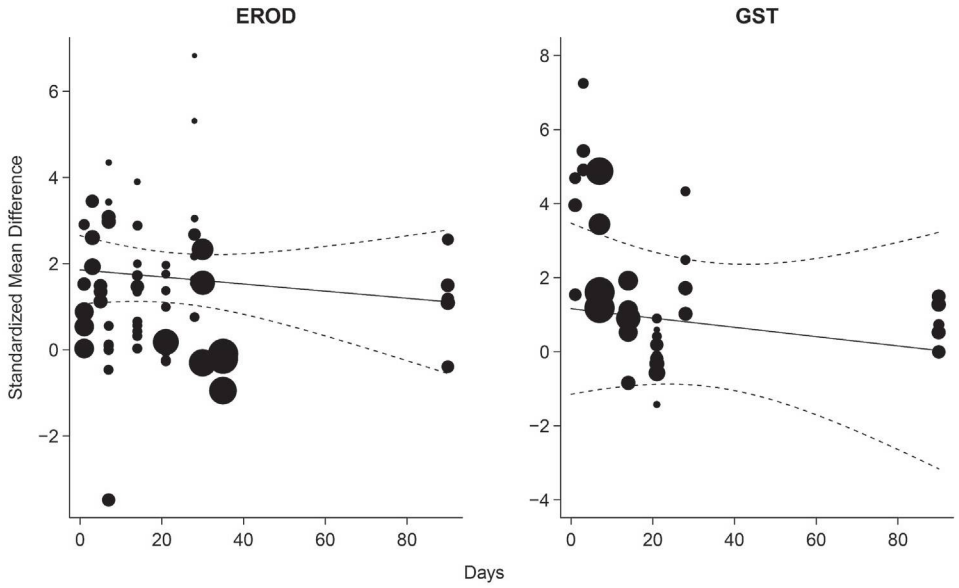


Fig. 5. Effects of polycyclic aromatic hydrocarbons (PAHs) through time in fish transferred from a clean site to a contaminated site (active biomonitoring approach). Time ranged from 12 h to 90 days. Circles represent effect size estimates (standardized mean difference – SMD) and their relative weight (circle size). Dashed lines indicate 95% confidence intervals.

Fish exposed to PAHs added to sediments also showed higher and significantly different GPx activity than fish exposed via water or injection (Sediment > Injection = Water).

The largest proportion of variance was mostly associated with the residuals for several biomarkers, as the chemicals did not explain much of the variance in most cases except for GSH and GSSG levels. For GR activity and LPO levels, the variability was mostly due to differences among countries (Table S6).

We have also assessed the effect of different concentrations of PAHs in water through time (interaction Concentration X Time) on biomarker responses, adding to the model ID, species, country and chemicals as random effects. Except for CAT, GSH and GSSG,

Concentration X Time had a significant effect on most biomarker responses (Table S7). Neither concentration, time nor interaction had a significant effect on CAT activity, and most of the variance was associated with the residuals ($Q_m = 5.982$, $P = 0.1125$). GSH levels were only affected by time, meaning that for each increase in unit of time, the difference between control and treatment means also increases ($Q_m = 110.827$, $P < 0.0001$). As for GSSG, only concentration had a significant effect on its levels, which also means that a unit increase in concentration leads to an increase in mean effect ($Q_m = 20.795$, $P = 0.0001$).

The meta-regressions also found other notable effects. First, there is a significantly negative effect of the interaction between concentration and time on SOD ($Qm = 19.558$, $P = 0.0002$) and LPO ($Q_m = 643.995$, $P < 0.0001$), i.e. the effect of PAHs concentration on these biomarkers decreases by 0.0328 and 0.288, respectively, for every unit increase in time. Additionally, the type of PAHs accounted for very little of the heterogeneity observed in most biomarkers data sets, aside from GSH, which had 36.77% of total variance associated with differences among PAHs ($I^2_{total} = 89.66\%$). Conversely, country accounted for half or more of the variance found for GST, GR, GSSG and LPO. Details on model results and the amount of heterogeneity explained by each random effect are shown in Table S7.

3.6. Publication bias

Funnel plots of all biomarkers were visually inspected (Fig. S4 – Appendix D) and a slight asymmetry was detected in EROD and GPx plots, however, only very few effect sizes (ca. 10) were responsible for this asymmetry. Rosenberg's fail-safe number for each biomarker was as follows: EROD – 930118; GST – 16976; CAT – 0; SOD – 8679; GPx – 9012; GR – 558; GSH – 0; GSSG – 1193; and LPO – 7401. For both CAT and GSH, the number of studies needed to be added was 0 because the main effect size was not statistically significant.

Although unrelated, both methods found little evidence for publication bias. Therefore, our results might be unbiased and robust.

4. Discussion

The overall effect of PAHs on biomarker responses (Table 1) was meta-analytically quantified to test their reliability as research tools since these biomarkers are commonly and widely employed to detect and assess contamination by oil-related compounds. Our meta-analyses have revealed that the overall effect of PAHs on fish is deleterious since the activity and levels of most biomarkers were altered after exposure, with the exception of CAT, GR and GSH for which PAHs showed no significant effect. These findings are compelling by themselves, but the source of variation investigated herein gives us a broader sense of how PAHs exposure is affecting fish worldwide. Below we discuss the importance of our findings for every source of variation accounted in the analyses.

4.1. The role of experimental approach, environment and habitat

Due to the established link between PAH exposure and CYP1A induction, described in several scenarios, EROD activity is expected to increase in fish captured at contaminated sites and exposed in laboratory settings. An extensive examination on EROD activity, covering mechanisms of induction by several classes of contaminants and the main factors influencing the intensity and direction of responses, has been conducted (Whyte et al., 2000; Billiard et al., 2008). Based on these narrative reviews, PAHs are regarded as a weak/moderate inducer of EROD activity both in marine and freshwater species captured in the field or experimentally exposed in laboratory (Whyte et al., 2000; Billiard et al., 2008). Similarly, we expected alterations in all biomarkers even though they are not specific to PAHs contamination. Our meta-analyses were able to corroborate several findings from primary studies and narrative reviews, such as the induction of EROD activity and overall changes in the response patterns of oxidative stress biomarkers after PAHs exposure.

Differences found between experimental approaches were expected, specifically the large magnitude of effects observed in fish from laboratory exposure. Laboratory assays and caging experiments (i.e. active biomonitoring) are designed to understand particular mechanisms of action and determine causality (Goodsell et al., 2009). Because presumed effects of contamination are isolated and hardly simulate the complexity of the natural

Table 1
Summary of biomarker responses to PAHs exposure.

Biomarker	Overall	Approach	Environment	Habitat	Exposure Route	Concentration X Time (waterborne PAHs)	Summary
EROD	✓	✓	✓	×	×	✓	↑ Lab ↑ freshwater fish ↑ concentration X time
GST	✓	✓	✓	×	✓	✓	↑ Field and ABM, ↑ freshwater fish ↑ sediment exposures ↑ concentration X time
CAT	×	×	×	×	×	×	Non-responsive
SOD	✓	×	×	×	×	✓	↓ concentration X time
GPx	✓	×	×	×	✓	✓	↑ sediment exposures ↑ concentration X time
GR	×	✓	✓	×	×	✓	↑ Field ↑ freshwater fish ↑ concentration X time
GSH	×	×	×	✓	×	✓	↑ Freshwater benthopelagic fish ↑ time
GSSG	✓	✓	×	×	×	✓	↑ Lab ↑ concentration
LPO	✓	×	×	×	×	✓	↓ concentration X time

'✓' indicates that the source of variation influences the biomarkers response. '×' indicates that the biomarkers response is not influenced by the predicted source of variation. "↑" indicates increased activity/level and "↓" indicates decreased activity/level.

environment, these approaches often overestimate the responses by fish to any contamination scenario (Forbes et al., 2006), as seen in the increased EROD activity and GSSG levels from fish exposed in the laboratory. Biotic and abiotic variables in the environment, such as rain, sunlight, microorganisms and sediment texture may determine the fate PAHs and consequently the response of organisms (Burgess et al., 2003; Gong et al., 2014). In addition, it should be mentioned that biomarkers can be extremely variable in field conditions and this have a strong impact in the required sample size to detect significant differences between sites (Gagnon and Hodson, 2012). Nonetheless, GST and GR activities were higher in the field than in laboratory exposure. Since they are both less PAH-specific than EROD (Hellou et al., 2012; van der Oost et al., 2003), their induction might be a response to other pollutants present at contaminated sites.

Freshwater fish showed increased EROD activity both in field and laboratory settings compared to marine fish. These different responses were also expected due to inherent physiological differences between marine and freshwater fish, such as osmotic regulation and renal excretion mechanisms (Evans et al., 2005; Kleinow et al., 2008). However, previous studies have recommended caution in the interpretation of these findings since EROD activity baseline levels may significantly vary among species, rendering any degree of induction to be pronounced (Flammarion and Garric, 1997; Balk et al., 1996; Whyte et al., 2000). Nevertheless, by directly comparing reference means from freshwater and marine fish using a two-sample *t*-test, no differences were observed. Therefore, our findings suggest that rather than a variation in baseline levels of EROD, the increased activity observed in freshwater fish might reflect greater susceptibility to PAHs exposure from these species compared to their marine counterparts. The same logic might be applied to GST since there is no difference between control means from freshwater and marine species. GR activity was higher in freshwater fish in the field, but the baseline levels from marine species were significantly higher than freshwater species, suggesting that the difference detected here are more related to intrinsic differences among species. It is important to note that it is difficult to generalize GR differences between environments since there was only one freshwater species studied (*Leuciscus cephalus*).

Additionally, we presumed fish habitat would play an important role in determining the magnitude of PAHs effects since benthic species are more exposed to PAHs and other persistent organic pollutants that are known to associate with aquatic sediments and accumulate in invertebrates (Logan, 2007; Gonçalves et al., 2014; Duarte et al., 2017). However, this was only true for GSH levels in freshwater fish captured at contaminated sites (Benthopelagic > Demersal, Fig. S3). Specifically, for this particular biomarker there were only two freshwater species to compare, the chub (*Leuciscus cephalus*) and the European eel (*Anguilla anguilla*). Clearly there is a lack of field experiments conducted in PAHs contaminated freshwater environments to make any assumption on species sensitivity or habitat vulnerability.

4.2. The role of PAHs exposure routes

We presumed the route of exposure would influence the strength and direction of PAHs effects on biomarkers. More specifically, fish exposed through injection techniques (i.e. intraperitoneal or intramuscular injection) were expected to show increased biomarker responses compared to fish from dietary, waterborne or sediment exposure. Although it does not represent a natural uptake mechanism, previous studies have described a significant increase of EROD activity in fish injected with several PAHs compounds (Pacheco and Santos, 1998; Reynaud et al., 2002; Banni et al., 2009). However, there were no differences among exposure routes for EROD, potentially indicating that PAHs are strong inducers of CYP1A regardless of the delivery method employed in laboratory-based experiments.

Moreover, the response of most biomarkers to PAHs administered via sediment, injection, diet or water are similar, suggesting that even though there was an overall response to PAHs exposure, the exposure route is not an important source of variation. Nevertheless, GST and GPx activities significantly varied among exposure routes. PAHs in the sediment led to a stronger induction of these enzymes compared to water, injection and dietary exposures. Differences in the composition and concentrations of PAHs present in the sediments in comparison to the other routes may explain this finding, since not all PAHs induce GST or are able to promote a redox unbalance that can induce GPx (Altenburger et al., 2003).

4.3. The effect of time and concentration

Contrary to what was expected, the period of exposure in controlled field settings (i.e. ABM experiments) did not affect EROD and GST activities. The relationship between time and these enzyme activities are not always linear and predictive, especially in the natural environment where both inducers, repressors and inhibitors may be present as a complex mixture of contaminants that influences biomarker responses (Sarkar et al., 2006). Induction of EROD activity has been described as high immediately after exposure followed by a steady decline (Whyte et al., 2000), whereas GST activity has been described as quite variable (van der Oost et al., 2003; Hellou et al., 2012). However, a visual analysis of the scatterplots (Fig. 5) indicates no clear pattern between time and biomarker activity. The lack of ABM experiments, for periods of time ranging between 40 and 90 days, is a potential research gap that needs to be fulfilled in order to properly investigate if there is a response-time relationship.

Even though there was no relationship between time of exposure and biomarker response from caging experiments in the field, in laboratory-based experiments, the combination of concentration and time significantly affected the strength of waterborne PAHs effects, as we predicted. Biomarker behaviour towards concentration and time are quite unpredictable, with some biomarkers responding only to specific compounds immediately after exposure while others responding to lower doses and longer time periods (Forbes et al., 2006). However, our analyses revealed that most biomarkers positively related to increasing exposure time and concentrations, except for SOD activity and LPO levels, which decreased with time at a given concentration.

Superoxide dismutase is essential for the dismutation of superoxide in hydrogen peroxide, which is further degraded by GPx, CAT and peroxiredoxins. There are different pools of SOD; a mitochondrial matrix pool (Mn-SOD), and other pools (Cu,Zn-SOD) that are strictly regulated to maintain appropriate levels of hydrogen peroxide and superoxide inside the cells (Fridovich, 1995; Scandalios, 2002). For these reasons, induction of SOD expression may occur immediately after exposure to neutralize the excess of superoxide anion, but once the initial insult is neutralized, SOD activity may reduce steadily, resulting in an overall decrease over time. Varying reports of SOD activity were extracted from the studies reviewed herein, with induction or inhibition occurring after 24 h or 48 h (Lemaire et al., 1996; Dussauze et al., 2015; Nogueira et al., 2013), or no significant changes occurring over time and concentrations. In many cases, a combined effect of time and concentration on biomarker response does exist, however, studies often assess concentration and time independently through one-way ANOVA or multiple t-tests, even when the experimental design requires more complex analyses (Ahmad et al., 2003; Jifa

et al., 2006; Gunawickrama et al., 2008; Nogueira et al., 2013; Frantzen et al., 2015), which may prevent the detection of time X concentration effects as identified here. Additionally, lipid peroxidation levels usually increase with time and concentration, but in our analyses, we were able to verify a decrease in LPO with time at a given concentration. Given all biomarker responses, which mostly increased, it is possible that cellular defences are constantly working to protect cells from eventual burdens.

Another noteworthy finding is that the combined effect of exposure time and concentration of waterborne PAHs did not significantly affect GSH and GSSG levels. Instead, GSH content increased over time regardless PAHs concentration and GSSG increased across concentrations regardless of the exposure time. GSH provides protection against ROS in various disease models and the ratio of GSH/GSSG is kept high in order to maintain normal cellular function and prevent oxidative stress injury (Zhu et al., 2013). Increased GSH levels and GR activity reflect the robustness of the antioxidant system, which dynamically functions to avoid damage to important biomolecules as demonstrated by the reduced levels of LPO.

The combined results of all biomarkers analysed in this dataset provide new insights on how biomarkers behave to PAHs exposure in water, and how cellular redox homeostasis may be maintained.

5. Conclusions

(1) We initially proposed to answer the question, what are the most responsive biotransformation and oxidative stress biomarkers to assess PAHs effects on fish? By covering environment, habitat, exposure route, concentration and time as sources of variation within experimental approaches, we were able to set forth a relevant suite of biomarkers to comprehend the effects induced by PAHs exposure, which may help future ecotoxicological studies.

(2) Among the biomarkers analysed herein, EROD is the most specific to PAHs exposure. Increased and strong responses occur in exposed fish regardless of habitat or route of exposure, indicating that EROD activity is a robust biomarker suitable for a variety of fish species and experimental scenarios. Conversely, CAT is an inadequate biomarker to assess and understand PAHs exposure since no significant response was observed, neither overall, nor relative, to experimental approaches. Therefore, it would not be relevant to measure this enzyme activity in order to establish a causal relationship to PAHs contamination.

(3) GST is an important, albeit, non-specific biomarker. Increased induction at contaminated sites suggests that GST strongly responds to environmental contamination, but the response is not specific to a class of contaminants. In addition, GST along with GPx were the only biomarkers that significantly differed among exposure routes, with high activities observed in experiments using contaminated sediments, suggesting that the exposure route is important to elicit a response from these enzymes.

(4) Even though GR had no overall response, freshwater fish captured at contaminated sites showed significantly increased activities. This difference between freshwater and marine fish might be due to the lack of experiments measuring this biomarker in freshwater environments contaminated by PAHs. Therefore, more research is needed to determine whether GR is indeed a reliable biomarker for PAHs contamination. Likewise, there were only a few experiments conducted in the field for GSH, hindering conclusions.

(5) Lipid peroxidation had an overall increase to PAHs exposure. Nevertheless, no difference was found among moderators, indicating that LPO is an important biomarker for PAHs effects despite approach, environment or habitat, so that LPO should be considered for environmental contamination assessments even if it is not specific to any class of contaminants.

(6) We were able to identify some research gaps to be fulfilled by future studies. First, there is a lack of active biomonitoring studies using oxidative stress biomarkers to assess PAHs contamination and exposing fish for periods of time between 40 and 80 days. This approach is important to establish links between responses found in the laboratory and field, and therefore further investigation would help us understand how fish are affected by PAHs. Additionally, biomarkers such as GSH, GR and GSSG are crucial to understand both pollutant biotransformation and oxidative stress. However, few studies measured these biomarkers on freshwater and marine species, hindering a clear conclusion.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.07.004.

References

Aas, E., Baussant, T., Balk, L., Liewenborg, B., Andersen, O.K., 2000. PAH metabolites in bile, cytochrome P4501A and DNA adducts as environmental risk parameters for chronic oil exposure: a laboratory experiment with Atlantic cod. Aquat. Toxicol. 51, 241–258.

Abdel-Shafy, H.I., Mansour, M.S.M., 2016. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egyptian J. Petroleum 25, 107–123.

Abele, D., Vázquez-Medina, J.P., Zenteno-Savín, T., 2017. Introduction to oxidative stress in aquatic ecosystems. In: Abele, D., Vázquez-Medina, J.P., Zenteno-Savín, T. (Eds.), Oxidative Stress in Aquatic Ecosystems. John Wiley & Sons, Ltd, Chichester, UK, pp. 1–5.

Abrahamson, A., Brandt, I., Brunstrom, B., Sundt, R.C., Jorgensen, E.H., 2008. Monitoring contaminants from oil production at sea by measuring gill EROD activity in Atlantic cod (Gadus morhua). Environ. Pollut 153, 169–175.

Ahmad, I., Pacheco, M., Santos, M.A., 2003. Naphthalene-induced differential tissue damage association with circulating fish phagocyte induction. Ecotoxicol. Environ. Saf. 54, 7–15.

Almroth, B.C., Sturve, J., Berglund, A., Forlin, L., 2005. Oxidative damage in eelpout (*Zoarces viviparus*), measured as protein carbonyls and TBARS, as biomarkers. Aquat. Toxicol. 73, 171–180.

Altenburger, R., Segner, H., Oost, R., 2003. Biomarkers and PAHs – prospects for the assessment of exposure and effects in aquatic ecosystems. In: Douben, P.E.T. (Ed.), PAHs: an Ecotoxicological Perspective. John Wiley & Sons, Ltd, Chichester, UK, pp. 297–328.

ATSDR, 1991. Support document to the 1991 substance priority list. In: Substance and Non-substance Addiction, vol. 1010. Springer Singapore, Singapore, pp. 1–12.

ATSDR, 2017. Support document to the 2017 substance priority list. In: Substance and Non-substance Addiction, vol. 1010. Springer Singapore, Singapore, pp. 1–12.

Baird, W.M., Hooven, L.A., Mahadevan, B., 2005. Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. Environ. Mol. Mutagen 45, 106–114.

Balk, L., Larsson, Å., Förlin, L.R., 1996. Baseline studies of biomarkers in the feral female perch (*Perca fluviatilis*) as tools in biological monitoring of anthropogenic substances. Mar. Environ. Res. 42, 203–208.

Banni, M., Bouraoui, Z., Ghedira, J., Clerandeau, C., Guerbej, H., Narbonne, J.F., Boussetta, H., 2009. Acute effects of benzo[a]pyrene on liver phase I and II enzymes, and DNA damage on sea bream Sparus aurata. Fish Physiol. Biochem. 35, 293–299.

Barhoumi, B., Clerandeau, C., Gourves, P.Y., Le Menach, K., El Megdiche, Y., Peluhet, L., Budzinski, H., Baudrimont, M., Driss, M.R., Cachot, J., 2014. Pollution

biomonitoring in the Bizerte lagoon (Tunisia), using combined chemical and biomarker analyses in grass goby, *Zosterisessor ophiocephalus* (Teleostei, Gobiidae). Mar. Environ. Res. 101, 184–195.
Bettim, F.L., Galvan, G.L., Cestari, M.M., Yamamoto, C.I., De Assis, H.C., 2016. Biochemical responses in freshwater fish after exposure to water-soluble fraction of gasoline. Chemosphere 144, 1467–1474.
Beyer, J., Sandvik, M., Hylland, K., Fjeld, E., Egaas, E., Aas, E., Skare, J.U., Goksoyr, A., 1996. Contaminant accumulation and biomarker responses in flounder (Platichthys flesus) and Atlantic cod (*Gadus morhua*) exposed by caging to polluted sediments in Sorfjorden, Norway. Aquat. Toxicol 36, 75–98.
Billiard, S.M., Meyer, J.N., Wassenberg, D.M., Hodson, P.V., Di Giulio, R.T., 2008. Nonadditive effects of PAHs on early vertebrate development: mechanisms and implications for risk assessment. Toxicol. Sci. 105, 5–23.
Boelsterli, U.A., 2007. Mechanistic Toxicology: the Molecular Basis of How Chemicals Disrupt Biological Targets, 2 ed. Taylor and Francis, London.
Borenstein, M., Hedges, L.V., Higgins, J.P.T., Rothstein, H.R., 2009. Introduction to Meta-analysis. John Wiley & Sons, Ltd.
Burgess, R.M., Ahrens, M.J., Hickey, C.W., 2003. Geochemistry of PAHs in aquatic environments: source, persistence and distribution. In: PAHs: an Ecotoxicological Perspective. John Wiley & Sons, Chichester, UK, pp. 35–45.
Cardoso, F.D., Dauner, A.L., Martins, C.C., 2016. A critical and comparative appraisal of polycyclic aromatic hydrocarbons in sediments and suspended particulate material from a large South American subtropical estuary. Environ. Pollut. 214, 219–229.
Codi, S., Humphrey, C., Klumpp, D., Delean, S., 2004. Barramundi as an indicator species for environmental monitoring in North Queensland, Australia: laboratory versus field studies. Environ. Toxicol. Chem. 23, 2737–2744.
Côté, I.M., Jennions, M.D., 2013. The procedure of meta-analysis in a nutshell. In: Koricheva, J., Gurevitch, J., Mengersen, K. (Eds.), Handbook of Meta-analysis in Ecology and Evolution. Princenton University Press, UK, pp. 14–24.
Davies, K.J.A., 2000. Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life 50, 279–289.
Deer, A.K., Henczova, M., Banka, L., Varanka, Z., Nemcsok, J., 2010. Effects of crude oil and oil fractions on the liver P450-dependent monooxygenase activities and antioxidant defence system of different freshwater fish species. Acta Biol. Hung 61, 262–273.
Di Giulio, R.T., Meyer, J.N., 2008. Reactive oxygen species and oxidative stress. In: Di Giulio, R.T., Hinton, D.E. (Eds.), The Toxicology of Fishes. CRC Press, Boca Raton, Florida, pp. 273–324.
Douben, P.E.T., 2003. Introduction. In: Douben, P.E.T. (Ed.), PAHs: an Ecotoxicological Perspective. John Wiley & Sons, Ltd, Chichester, UK, pp. 1–6.
Duarte, R.M., Sadauskas-Henrique, H., De Almeida-Val, V.M.F., Val, A.L., Nice, H.E., Gagnon, M.M., 2017. Biomarker responses and PAH ratios in fish inhabiting an estuarine urban waterway. Environ. Toxicol 32, 2305–2315.
Dussauze, M., Danion, M., Le Floch, S., Lemaire, P., Pichavant-Rafini, K., Theron, M., 2015. Innate immunity and antioxidant systems in different tissues of sea bass (*Dicentrarchus labrax*) exposed to crude oil dispersed mechanically or chemically with Corexit 9500. Ecotoxicol. Environ. Saf 120, 270–278.
Evans, D.H., 1987. The fish gill: site of action and model for toxic effects of environmental pollutants. Environ. Health Perspect. 71, 47–58.
Evans, D.H., Piermarini, P.M., Choe, K.P., 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol. Rev. 85, 97–177.
Farber, J.L., 1994. Mechanisms of cell injury by activated oxygen species. Environ. Health Perspect. 102, 17–24.
Flammarion, P., Garric, J., 1997. Cyprinids EROD activities in low contaminated rivers: a relevant statistical approach to estimate reference levels for EROD biomarker? Chemosphere 35, 2375–2388.
Forbes, V.E., Palmqvist, A., Bach, L., 2006. The use and misuse of biomarkers in ecotoxicology. Environ. Toxicol. Chem. 25, 272–280.
Frantzen, M., Hansen, B.H., Geraudie, P., Palerud, J., Falk-Petersen, I.B., Olsen, G.H., Camus, L., 2015. Acute and long-term biological effects of mechanically and chemically dispersed oil on lumpsucker (*Cyclopterus lumpus*). Mar. Environ. Res. 105, 8–19.
Fridovich, I., 1995. Superoxide radical and superoxide dismutases. Annu. Rev. Biochem. 64, 97–112.
Gagnon, M.M., Hodson, P.V., 2012. Field studies using fish biomarkers – how many fish are enough? Mar. Pollut. Bull. 64, 2871–2876.
George, S.G., Buchanan, G., 1990. Isolation, properties and induction of plaice liver cytosolic glutathione-S-transferases. Fish Physiol. Biochem 8, 437–449.
Goksøyr, A., Forlin, L., 1992. The cytochrome P450 system in fish, aquatic toxicology, and environmental monitoring. Aquatic Toxicol. 22, 287–312.
Gonçalves, C., Martins, M., Diniz, M.S., Costa, M.H., Caeiro, S., Costa, P.M., 2014. May sediment contamination be xenoestrogenic to benthic fish? A case study with Solea senegalensis. Marine Environ. Res. 99, 170–178.
Gong, Y., Zhao, X., Cai, Z., O'reilly, S.E., Hao, X., Zhao, D., 2014. A review of oil, dispersed oil and sediment interactions in the aquatic environment: influence on the fate, transport and remediation of oil spills. Mar. Pollut. Bull. 79, 16–33.
Goodsell, P.J., Underwood, A.J., Chapman, M.G., 2009. Evidence necessary for taxa to be reliable indicators of environmental conditions or impacts. Mar. Pollut. Bull. 58, 323–331. Elsevier Ltd.
Gunawickrama, S.H., Aarsaether, N., Orbea, A., Cajaraville, M.P., Goksoyr, A., 2008. PCB77 (3,3',4,4'-tetrachlorobiphenyl) co-exposure prolongs CYP1A induction, and sustains oxidative stress in B(a)P-exposed turbot, *Scophthalmus maximus*, in a long-term study. Aquat. Toxicol 89, 65–74.
Hayes, J.D., Flanagan, J.U., Jowsey, I.R., 2005. Glutathione transferases. Annu. Rev. Pharmacol. Toxicol. 45, 51–88.
Hedges, L.V., Olkin, I., 1985. Statistical Methods for Meta-analysis. Academic Press, San Diego.
Hellou, J., Ross, N.W., Moon, T.W., 2012. Glutathione, glutathione S-transferase, and glutathione conjugates, complementary markers of oxidative stress in aquatic biota. Environ. Sci. Pollut. Control Ser. 19, 2007–2023.
Higgins, J.P.T., Thompson, S.G., Deeks, J.J., Altman, D.G., 2003. Measuring inconsistency in meta-analyses. Br. Med. J. 327, 557–560.
Hinton, D.E., Segner, H., Au, D.W.T., Kullman, S.W., Hardman, R.C., 2001. Toxic responses of the liver. In: Schlenk, D., Benson, W.H. (Eds.), Target Organ Toxicity in Marine and Freshwater Teleosts. Taylor & Francis, London, pp. 248–298.
Hook, S.E., Gallagher, E.P., Batley, G.E., 2014. The role of biomarkers in the assessment of aquatic ecosystem health. Integrated Environ. Assess. Manag 10, 327–341.
Huang, G.Y., Liu, Y.S., Liang, Y.Q., Shi, W.J., Hu, L.X., Tian, F., Chen, J., Ying, G.G., 2016. Multi-biomarker responses as indication of contaminant effects in *Gambusia affinis* from impacted rivers by municipal effluents. Sci. Total Environ. 563–564, 273–281.
Hugla, J.L., Philippart, J.C., Kermess, P., Goffinet, G., Thome, J.P., 1995. PCB contamination of the common barbel, *Barbus barbus* (Pisces, Cyprinidae), in the river Meuse in relation to hepatic monooxygenase activity and ultrastructural liver change. Neth. J. Aquatic Ecol. 29, 135–145.
Jee, J.H., Kang, J.C., 2005. Biochemical changes of enzymatic defense system after phenanthrene exposure in olive flounder, *Paralichthys olivaceus*. Physiol. Res. 54, 585–591.
Jennions, M.D., Lortie, C.J., Rosenberg, M.S., Rothstein, H.R., 2013. Publication and related biases. In: Koricheva, J., Gurevitch, J., Mengersen, K. (Eds.), Handbook of Meta-analysis in Ecology and Evolution. Princenton University Press, United Kingdom, pp. 207–236.
Jiang, Z.Y., Hunt, J.V., Wolff, S.P., 1992. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxidein low density lipoprotein. Anal. Biochem. 202, 384–389.
Jifa, W., Yu, Z., Xiuxian, S., You, W., 2006. Response of integrated biomarkers of fish (*Lateolabrax japonicus*) exposed to benzo[a]pyrene and sodium dodecylbenzene sulfonate. Ecotoxicol. Environ. Safety 65, 230–236.
Kelly, C.D., Jennions, M.D., 2011. Sexual selection and sperm quantity: meta-analyses of strategic ejaculation. Biol. Rev. 86, 863–884.
Kirby, M.F., Matthiessen, P., Neall, P., Tylor, T., Allchin, C.R., Kelly, C.A., Maxwell, D.L., Thain, J.E., 1999. Hepatic EROD activity in flounder (*Platichthys flesus*) as an indicator of contaminant exposure in English estuaries. Mar. Pollut. Bull. 38, 676–686.
Kleinow, K.M., Nichols, J.W., Hayton, W.L., Mckim, J.M., Barron, M.G., 2008. Toxicokinetics in fish. In: Di Giulio, R.T., Hinton, D.E. (Eds.), The Toxicology of Fishes. CRC Press, Boca Raton, Florida, pp. 55–152.
Kopecka-Pilarczyk, J., Correia, A.D., 2009. Biochemical response in gilthead seabream (Sparus aurata) to in vivo exposure to a mix of selected PAHs. Ecotoxicol. Environ. Saf 72, 1296–1302.
Lawal, A.T., 2017. Polycyclic aromatic hydrocarbons. A review. Cogent Environ. Sci. 71, 1–89.
Lemaire, P., Förlin, L., Livingstone, D.R., 1996. Responses of hepatic biotransformation and antioxidant enzymes to CYP1A-inducers (3-methylcholanthrene, β-naphthoflavone) in sea bass (*Dicentrarchus labrax*), dab (*Limanda limanda*) and rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 36, 141–160.
Liu, F., Yang, Q., Hu, Y., Du, H., Yuan, F., 2014. Distribution and transportation of polycyclic aromatic hydrocarbons (PAHs) at the Humen river mouth in the Pearl River delta and their influencing factors. Mar. Pollut. Bull. 84, 401–410.
Livingstone, D.R., 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar. Pollut. Bull. 42, 656–666.
Logan, D.T., 2007. Perspective on ecotoxicology of PAHs to fish. Hum. Ecol. Risk Assess. 13, 302–316.
Lu, G.H., JI, Y., Zhang, H.Z., Wu, H., Qin, J., Wang, C., 2010. Active biomonitoring of complex pollution in Taihu Lake with Carassius auratus. Chemosphere 79, 588–594.
Lu, G.H., Qi, P.D., Qin, J., Wang, C., 2011. Biomarker responses in fish exposed to sediments from northern Taihu Lake. Bull. Environ. Contam. Toxicol 87, 499–505.
Milinkovitch, T., Ndiaye, A., Sanchez, W., Le Floch, S., Thomas-Guyon, H., 2011. Liver antioxidant and plasma immune responses in juvenile golden grey mullet (*Liza aurata*) exposed to dispersed crude oil. Aquat. Toxicol 101, 155–164.
Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., Prisma Group, 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 6, e1000097.
Nakagawa, S., Santos, E.A., 2012. Methodological issues and advances in biological meta-analysis. Evol. Ecol. 26, 1253–1274.
Neff, J.M., Stout, S.A., Gunstert, D.G., 2005. Ecological risk assessment of polycyclic aromatic hydrocarbons in sediments: identifying sources and ecological hazard. Integrated Environ. Assess. Manag. 1, 22–33.
Noble, D.W.A., Lagisz, M., O'dea, R.E., Nakagawa, S., 2017. Nonindependence and sensitivity analyses in ecological and evolutionary meta-analyses. Mol. Ecol 26, 2410–2425.
Nogueira, L., Da Silva, D.G., Oliveira, T.Y., Da Rosa, J.M., Felicio, A.A., De Almeida, E.A., 2013. Biochemical responses in armored catfish (*Pterygoplichthys anisitsi*) after short-term exposure to diesel oil, pure biodiesel and biodiesel blends. Chemosphere 93, 311–319.

Nogueira, L., Rodrigues, A.C., Tridico, C.P., Fossa, C.E., De Almeida, E.A., 2011. Oxidative stress in Nile tilapia (*Oreochromis niloticus*) and armored catfish (*Pterygoplichthys anisitsi*) exposed to diesel oil. Environ. Monit. Assess. 180, 243–255.

Oliveira-Ribeiro, C.A., Narciso, M.F., 2014. Histopathological markers in fish health assessment. In: Almeida, E.A., Oliveira Ribeiro, C.A. (Eds.), Pollution and Fish Health in Tropical Ecosystems. CRC Press Boca Raton, pp. 206–242.

Oris, J.T., Roberts, A.P., 2007. Statistical analysis of cytochrome P4501A biomarker measurements in fish. Environ. Toxicol. Chem. 26, 1742–1750.

Pacheco, M., Santos, M.A., 1998. Induction of liver EROD and erythrocytic nuclear abnormalities by cyclophosphamide and PAHs in *Anguilla anguilla L.* Ecotoxicol. Environ. Saf 40, 71–76.

Payne, J.F., Mathieu, A., Collier, T.K., 2003. Ecotoxicological studies focusing on marine and freshwater fish. In: PAHs: an Ecotoxicological Perspective. John Wiley & Sons, Ltd, Chichester, UK, pp. 191–224.

R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rengarajan, T., Rajendran, P., Nandakumar, N., Lokeshkumar, B., Rajendran, P., Nishigaki, I., 2015. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asian Pacific J. Tropical Biomedicine 5, 182–189.

Reynaud, S., Marionnet, D., Taysse, L., Duchiron, C., Deschaux, P., 2002. The effects of 3-methylcholanthrene on macrophage respiratory burst and biotransformation activities in the common carp (*Cyprinus carpio L.*). Fish Shellfish Immunol 12, 17–34.

Rosenberg, M.S., Rothstein, H., Gurevitch, J., 2013. Effect sizes: conventional choices and calculations. In: Koricheva, J., Gurevitch, J., Mengersen, K. (Eds.), Handbook of Meta-analysis in Ecology and Evolution. Princeton University Press, Princeton, pp. 61–71.

Sadauskas-Henrique, H., Braz-Mota, S., Duarte, R.M., De Almeida-Val, V.M.F., 2016. Influence of the natural Rio Negro water on the toxicological effects of a crude oil and its chemical dispersion to the Amazonian fish Colossoma macropomum. Environ. Sci. Pollution Res. 23, 19764–19775.

Sarkar, A., Ray, D., Shrivastava, A.N., Sarker, S., 2006. Molecular biomarkers: their significance and application in marine pollution monitoring. Ecotoxicology 15, 333–340.

Scandalios, J.G., 2002. Oxygen stress and superoxide dismutases. Plant Physiol. 101, 1–6.

Schlenk, D., Celander, M., Gallagher, E.P., George, S., James, M., Kullman, S.W., van den Hurk, P., Willett, K., 2008. Biotransformation in fishes. In: Di Giulio, R.T., Hinton, D.E. (Eds.), The Toxicology of Fishes. CRC Press, Boca Raton, Florida, pp. 153–234.

Shimada, T., 2006. Xenobiotic-metabolizing enzymes involved in activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons. Drug Metabol. Pharmacokinet. 21, 257–276.

Suess, M.J., 1976. The environmental load and cycle of polycyclic aromatic hydrocarbons. Sci. Total Environ. 6, 239–250.

Theron, M., Bado-Nilles, A., Beuvard, C., Danion, M., Dussauze, M., Ollivier, H., Pichavant-Rafini, K., Quentel, C., Le Floch, S., 2014. Chemical dispersion of crude oil: assessment of physiological, immune and antioxidant systems in juvenile turbot (*Scophthalmus maximus*). Water Air Soil Pollut. 225, 1–17.

Tuvikene, A., 1995. Responses of fish to polycyclic aromatic hydrocarbons (PAHs). Ann. Zool. Fenn. 32, 295–309.

Uno, T., Ishizuka, M., Itakura, T., 2012. Cytochrome P450 (CYP) in fish. Environ. Toxicol. Pharmacol 34, 1–13.

Valavanidis, A., Vlahogianni, T., Dassenakis, M., Scoullos, M., 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol. Environ. Safety 64, 178–189.

van der Oost, R., Beyer, J., Vermeulen, N.P., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol 13, 57–149.

Vidal-Liñan, L., Bellas, J., Campillo, J.A., Beiras, R., 2010. Integrated use of antioxidant enzymes in mussels, Mytillus galloprovincialis, for monitoring pollution in highly productive coastal areas of Galicia (Spain. Chemosphere 78, 265–272.

Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor package. J. Stat. Software 36, 1–48.

Whyte, J.J., Jung, R.E., Schmitt, C.J., Tillitt, D.E., 2000. Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. Crit. Rev. Toxicol 30, 347–570.

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

Zhu, H., Wang, J., Santo, A., Li, Y., 2013. Downregulation of antioxidants and phase 2 proteins. In: Villamena, F.A. (Ed.), Molecular Basis of Oxidative Stress: Chemistry, Mechanisms, and Disease Pathogenesis. John Wiley & Sons, New Jersey, pp. 113–121.

Material Suplementar – Capítulo I

Biomarker responses in fish exposed to polycyclic aromatic hydrocarbons (PAHs): systematic review and meta-analysis.

Supporting Figures

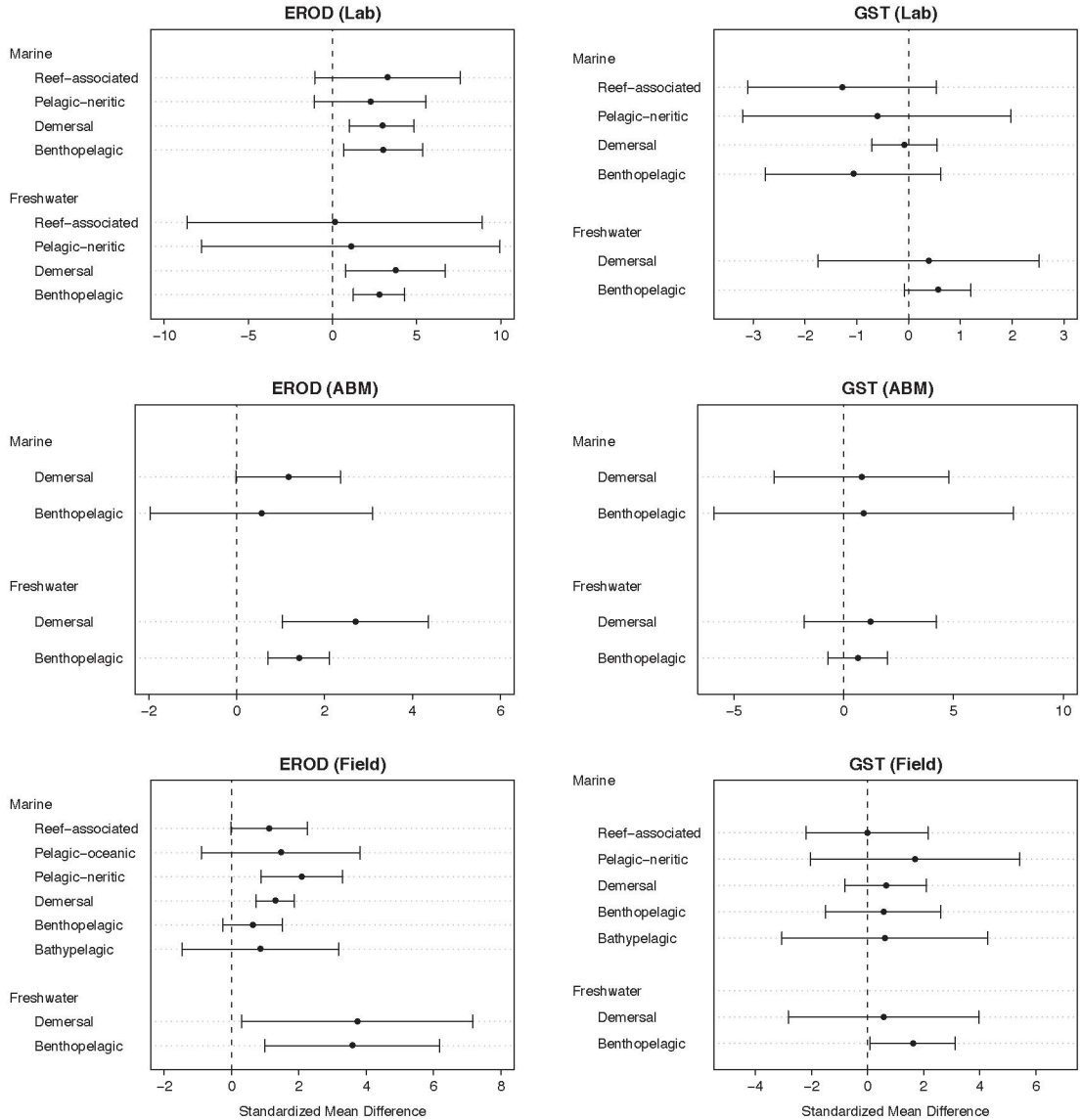


Fig. S1. Effects of polycyclic aromatic hydrocarbons (PAHs) exposure on biotransformation biomarkers of fish from different habitats within marine and freshwater environments. Ethoxyresorufin-O-deethylase (EROD) and glutathione *S*-transferase (GST) from laboratory (Lab), active biomonitoring (ABM) and field experiments. Error bars represent 95% confidence intervals. Numbers next to error bars indicate the number of effect sizes in each category. Effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line).

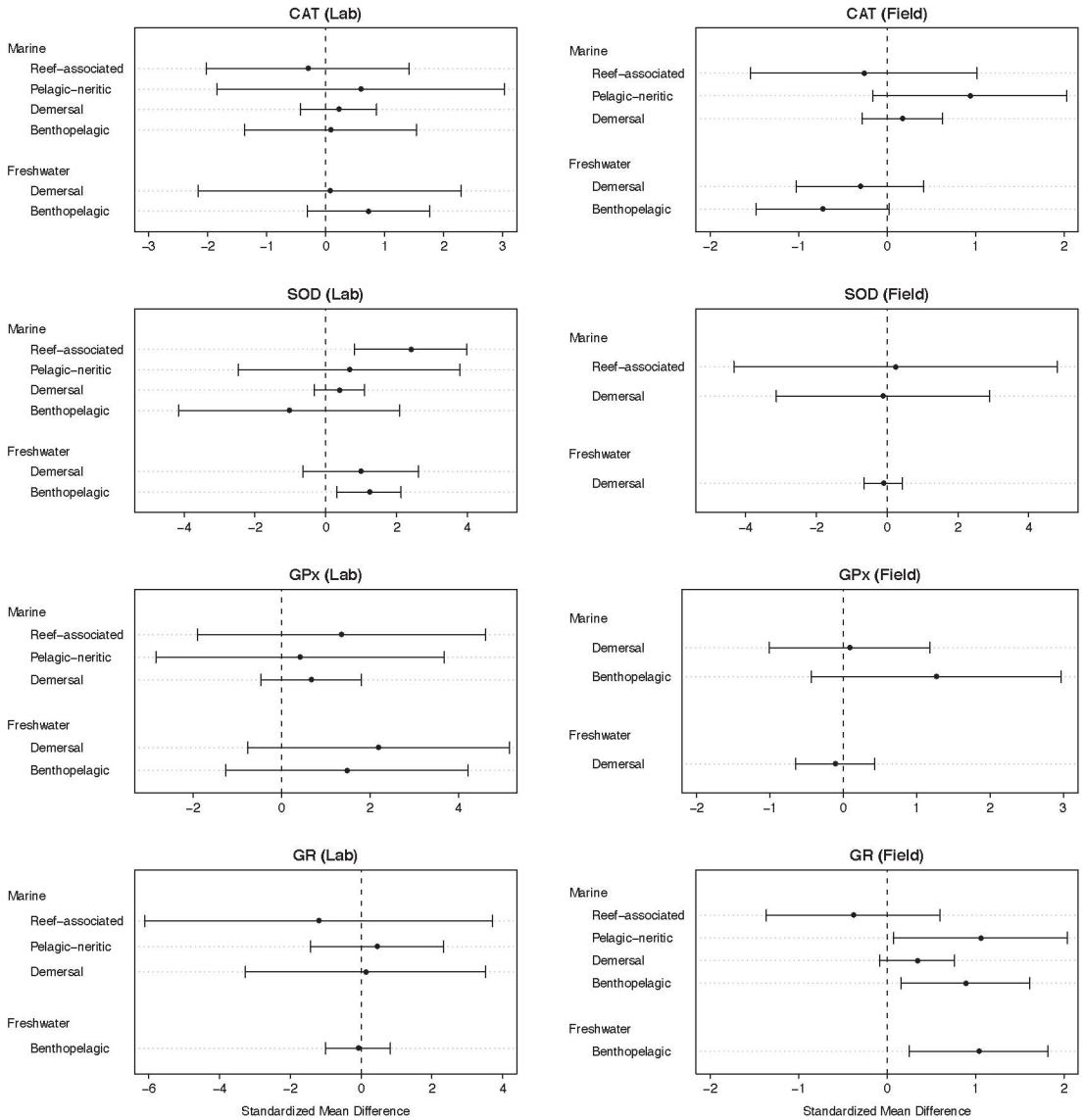


Fig. S2. Effects of PAHs exposure on oxidative stress biomarkers of fish from different habitats within marine and freshwater environments: catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione disulphide reductase (GR) from laboratory (Lab) and field experiments. Error bars represent 95% confidence intervals. Numbers next to error bars indicate the number of effect sizes in each category. Effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line).

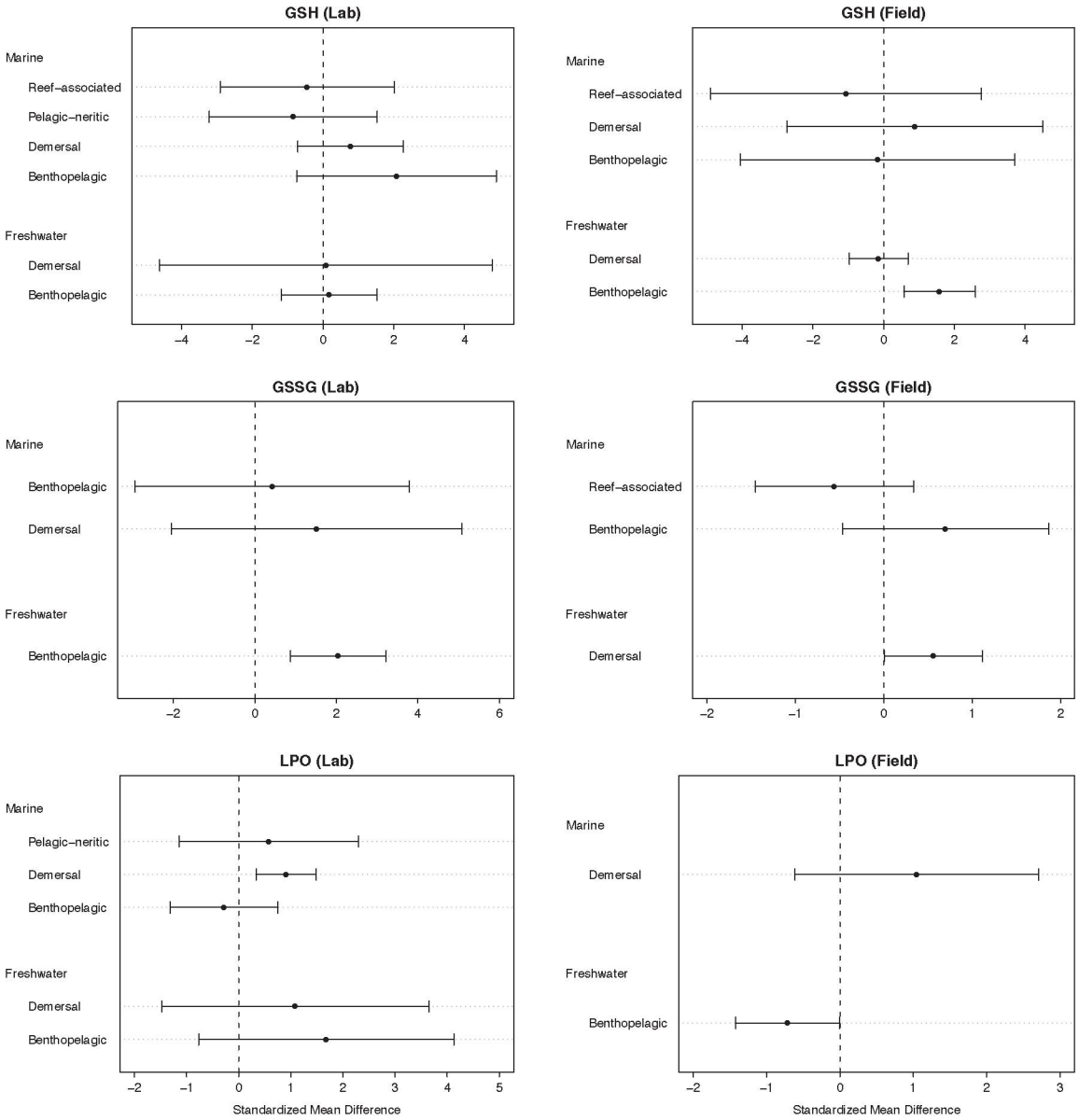


Fig. S3. Effects of PAHs exposure on oxidative stress biomarkers of fish from different habitats within marine and freshwater environments: glutathione (GSH), glutathione disulphide (GSSG) and lipid peroxidation (LPO) from laboratory (Lab) and field experiments. Error bars represent 95% confidence intervals. Numbers next to error bars indicate the number of effect sizes in each category. Effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line).

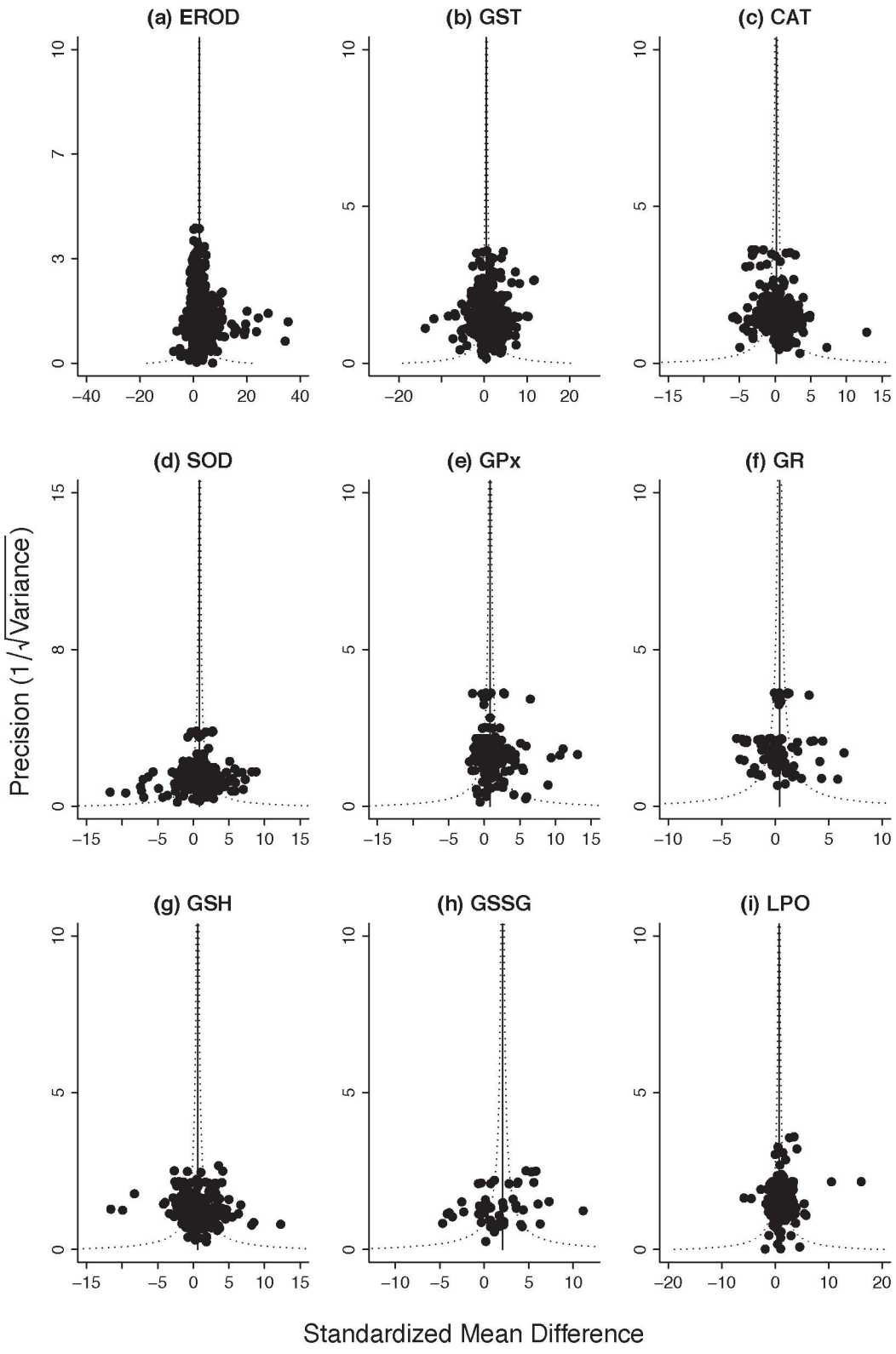


Fig. S4. Funnel plot of the overall effect size (SMD) against their precision for (a) ethoxyresorufin-O-deethylase (EROD), (b) glutathione *S*-transferase (GST), (c) catalase (CAT), (d) superoxide dismutase (SOD), (e) glutathione peroxidase (GPx), (f) glutathione disulphide reductase (GR), (g) glutathione (GSH), (h) glutathione disulphide (GSSG), and (i) lipid peroxidation (LPO). Line indicates zero.

Supporting Tables

Table S1. Summary of the nine data sets included in our meta-analyses. Total heterogeneity and observed variance among effect size identification (I^2) for the intercept-only model, i.e. global effect meta-analysis for ethoxyresorufin-O-deethylase (EROD), glutathione *S*-transferase (GST), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione disulphide reductase (GR), glutathione (GSH), glutathione disulphide (GSSG) and lipid peroxidation (LPO).

Data	*s*	*k*	*m*	Heterogeneity Q_T (*P*, df)	I^2_{total}
EROD	403	1082	92	25386.59 (<0.0001, 1081)	97.5%
GST	207	601	57	9891.303 (<0.0001, 600)	90.8%
CAT	89	265	33	3250.511 (<0.0001, 264)	87.4%
SOD	66	213	22	3435.79 (<0.0001, 212)	89%
GPx	59	191	20	5619.028 (<0.0001, 190)	92.3%
GR	27	98	14	1006.716 (<0.0001, 97)	91.8%
GSH	44	163	27	2880.797 (<0.0001, 162)	89.5%
GSSG	13	53	7	1276.74(<0.0001, 52)	92.7%
LPO	69	238	25	1050.748(<0.0001, 237)	87.8%

s=number of studies; *k*=number of effect sizes; *m*=number of species

Table S2. Summary of moderator analysis for laboratory (Lab) approach comparing fish habitat within freshwater and marine environment. *s*= number of studies; *k*= number of effect sizes; m= number of species. Q_m: heterogeneity test between moderators; I^2: amount of variance due to differences among random effects (effect size identification (ID), species and country).

	Lab Approach							
	s	*k*	*m*	Q_m (*P*, df)	$I^2_{[ID]}$	$I^2_{[species]}$	$I^2_{[country]}$	$I^2_{[total]}$
EROD	272	724	53					
Environment				8.053 (***P*=0.0045**, df=1)	50.29%	1.62%	46.19%	98.10%
Marine *(Benthopelagic/Demersal/Pelagic-neritic/Reef-associated)*	141	396	33	0.605 (*P*=0.895, df=3)	15.92%	4.78%	77.64%	98.34%
Freshwater *(Benthopelagic/Demersal/Pelagic-neritic/Reef-associated)*	131	328	21	0.797 (*P*=0.85, df=3)	71.63%	10.11%	15.89%	97.63%
GST	156	450	37					
Environment				7.450 (***P*=0.0063**, df=1)	64.16%	15.35%	10.54%	90.05%
Marine *(Benthopelagic/Demersal/Pelagic-neritic/Reef-associated)*	85	220	23	2.441 (*P*=0.486, df=3)	80.43%	0.15%	11.54%	92.12%
Freshwater *(Benthopelagic/Demersal)*	71	230	15	0.024 (*P*=0.876, df=1)	50.49%	30.10%	0%	80.59%
CAT	77	234	27					
Environment				0.085 (*P*=0.77, df=1)	74.49%	13.44%	0%	87.93%
Marine *(Benthopelagic/Demersal/Pelagic-neritic/Reef-associated)*	44	112	16	0.442 (*P*=0.931, df=3)	84.10%	7,00%	0%	91.10%
Freshwater *(Benthopelagic/Demersal)*	33	122	12	0.278 (*P*=0.598, df=1)	62.40%	24.57%	0%	86.97%

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Table S2 (*continued*)

	s	*k*	*m*	Q_m (*P*, df)	$I^2_{[ID]}$	$I^2_{[species]}$	$I^2_{[country]}$	$I^2_{[total]}$
SOD	59	195	20					
Environment				0.98 (*P*=0.322, df=1)	85.70%	0%	2.13%	87.83%
Marine *(Benthopelagic/Demersal/Pelagic-neritic/Reef-associated)*	28	56	10	6.345 (*P*=0.096, df=3)	92.73%	0%	0%	92.73%
Freshwater *(Benthopelagic/Demersal)*	31	139	11	0.074 (*P*=0.786, df=1)	70.62%	0%	9.95%	80.57%
GPx	52	172	18					
Environment				1.309 (*P*=0.253, df=1)	48.94%	6.02%	36.56%	91.52%
Marine *(Demersal/Pelagic-neritic/Reef-associated)*	32	91	11	0.19 (*P*=0.91, df=2)	38.99%	46.34%	8.65%	93.98%
Freshwater *(Benthopelagic/Demersal)*	20	81	8	0.859 (*P*=0.354, df=1)	23.30%	0%	70.25%	93.55%
GR	17	71	10					
Environment				5.153 (***P*=0.0232**, df=1)	25.69%	0%	70.46%	96.15%
Marine *(Benthopelagic/Demersal/Pelagic-neritic)*	16	57	9	0.385 (*P*=0.825, df=2)	18.02%	78.92%	0%	96.93%
Freshwater *(Benthopelagic)*	1	14	1	45.309 (*P*<0.0001, df=13)				
GSH	36	146	20					
Environment				1.204 (*P*=0.273, df=1)	75.73%	0%	11.61%	87.34%
Marine *(Benthopelagic/Demersal/Pelagic-neritic/Reef-associated)*	18	62	12	4.373 (*P*=0.224, df=3)	36.06%	0%	55.76%	91.82%
Freshwater *(Benthopelagic/Demersal)*	18	84	9	0.001 (*P*=0.971, df=1)	77.77%	0%	11.38%	89.14%

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Table S2 (*continued*)

	s	*k*	*m*	Q_m (*P*, df)	$I^2_{[ID]}$	$I^2_{[species]}$	$I^2_{[country]}$	$I^2_{[total]}$
GSSG	10	47	4					
Environment				4.262 (*P*=0.039, df=1)	60.64%	9.39%	9.39%	79.42%
Marine *(Benthopelagic/Demersal)*	3	17	2	0.189 (*P*=0.664, df=1)	0%	44.69%	44.87%	89.56%
Freshwater *(Benthopelagic)*	7	30	2	494.377 (*P*<0.0001, df=29)	68.44%	0%	0%	68.44%
LPO	58	207	22					
Environment				0.152 (*P*=0.697, df=1)	23.3%	0.29%	68.57%	92.16%
Marine *(Benthopelagic/Demersal/Pelagic-neritic)*	24	97	10	4.022 (*P*=0.134, df=2)	79.38%	0%	0%	79.38%
Freshwater *(Benthopelagic/Demersal)*	34	110	13	1.308 (*P*=0.253, df=1)	5.62%	0%	91.51%	97.13%

Table S3. Summary of t-tests comparing freshwater and marine fish baseline levels for ethoxyresorufin-O-deethylase (EROD), glutathione *S*-transferase (GST), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione disulphide reductase (GR), glutathione (GSH), glutathione disulphide (GSSG) and lipid peroxidation (LPO).

Biomarker	***t***	***P***
EROD	-0.966	0.335
GST	-1.027	0.306
CAT	-1.5897	0.118
SOD	0.26152	0.795
GPx	-4.1718	<0.001
GR	-3.116	0.025
GSH	1.0987	0.278
GSSG	1.6272	0.144
LPO	-0.085954	0.932

Table S4. Summary of moderator analysis for field approach comparing fish habitat within freshwater and marine environment. *s*= number of studies; *k*= number of effect sizes; m= number of species. Q_m: heterogeneity test between moderators; I^2: amount of variance due to differences among random effects (effect size identification (ID), species and country).

	Field Approach							
	s	*k*	*m*	Q_m (*P*, df)	$I^2_{[ID]}$	$I^2_{[species]}$	$I^2_{[country]}$	$I^2_{[total]}$
EROD	109	295	52					
Environment				9.392(*P*=**0.002**, df=1)	78.44%	0%	18.67%	97.11%
Marine *(Bathypelagic/Benthopelagic/Demersal/Pelagic-neritic/Pelagic-oceanic/Reef-associated)*	81	197	39	4.902 (*P*=0.428, df=5)	32.60%	18.41%	35.16%	86.17%
Freshwater *(Benthopelagic/Demersal*	28	98	14	0.005 (*P*=0.942, df=1)	96.55%	0%	3.02%	99.57%
GST	37	112	24					
Environment				0.359 (*P*=0.549, df=1)	95.36%	0%	0%	95.36%
Marine *(Bathypelagic/Benthopelagic/Demersal/Pelagic-neritic/Reef-associated)*	19	36	15	2.114 (*P*=0.833, df=4)	8.19%	0%	86.60%	94.79%
Freshwater *(Benthopelagic/Demersal)*	18	76	10	0.305 (*P*=0.581, df=1)	96.16%	0%	0%	96.16%
CAT	12	31	9					
Environment				2.853 (*P*=0.09, df=1)	57.55%	0%	0%	57.55%
Marine *(Demersal/Pelagic-neritic/Reef-associated)*	10	24	7	2.229 (*P*=0.328, df=2)	44.26%	0%	0%	44.26%
Freshwater *(Benthopelagic/Demersal)*	2	7	2	0.757 (*P*=0.384, df=1)	9.78%	9.78%	9.78%	29.35%

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Table S4 (*continued*)

	s	*k*	*m*	Q_m (*P*, df)	$I^2_{[ID]}$	$I^2_{[species]}$	$I^2_{[country]}$	$I^2_{[total]}$
SOD	7	18	4					
Environment				0.036 (*P*=0.849, df=1)	38.83%	26.01%	26.01%	90.85%
Marine *(Demersal/ Reef-associated)*	6	14	3	0.161 (*P*=0.899, df=1)	22.78%	35.70%	35.7%	94.18%
Freshwater *(Benthopelagic/Demersal)*	1	4	1	9.736 (*P*=0.021, df=3)				
GPx	7	19	4					
Environment				0.116 (*P*=0.733, df=1)	15.60%	4.73%	62.98%	83.31%
Marine *(Benthopelagic/Demersal)*	6	15	3	1.658 (*P*=0.198, df=1)	11.44%	30.39%	30.39%	72.22%
Freshwater *(Demersal)*	1	4	1	1.077 (*P*=0.783, df=3)				
GR	10	27	7					
Environment				3.922 (*P*=**0.048**, df=1)	12.51%	48.65%	28.14%	89.30%
Marine *(Benthopelagic/Demersal/Pelagic-neritic/Reef-associated)*	9	21	6	5.864 (*P*=0.118, df=3)	0%	16.31%	0%	16.31%
Freshwater *(Benthopelagic)*	1	6	1	45.71 (*P*<0.0001, df=5)				
GSH	8	17	8					
Environment				0.448 (*P*=0.503, df=1)	11,00%	11,00%	59.73%	81.73%
Marine *(Benthopelagic/Demersal/ Reef-associated)*	6	10	6	0.529 (*P*=0.768, df=3)	4.23%	4.23%	78.07%	86.53%
Freshwater *(Benthopelagic/Demersal)*	2	7	2	6.679 (*P*=**0.0098**, df=1)	12.20%	12.20%	12.20%	36.6%

(continued on next page)

Table S4 (*continued*)

	s	*k*	*m*	Q_m (*P*, df)	$I^2_{[ID]}$	$I^2_{[species]}$	$I^2_{[country]}$	$I^2_{[total]}$
GSSG	3	6	2					
Environment				0.323 (*P*=0.851, df=1)	24.63%	24.63%	24.63%	73.89%
Marine *(Benthopelagic/Reef-associated)*	2	2	2	2.818 (*P*=0.093, df=1)				
Freshwater *(Demersal)*	1	4	1	9.926 (*P*=0.019, df=3)				
LPO	11	31	5					
Environment				0.899 (*P*=0.343, df=1)	25.26%	0%	66.43%	91.68%
Marine *(Demersal)*	10	25	4	320.407 (*P*<0.0001, df=24)	35.55%	0%	57.94%	93.48%
Freshwater *(Benthopelagic)*	1	6	1	9.375 (*P*=0.095, df=5)				

Table S5. Summary of moderator analysis for active biomonitoring (ABM) approach comparing fish habitat within freshwater and marine environment. *s*= number of studies; *k*= number of effect sizes; m= number of species. Q_m: heterogeneity test between moderators; I^2: amount of variance due to differences among random effects (effect size identification (ID), species and country).

	ABM Approach							
	s	*k*	*m*	Q_m (*P*, df)	$I^2_{[ID]}$	$I^2_{[species]}$	$I^2_{[country]}$	$I^2_{[total]}$
EROD	22	63	9					
Environment				0.001 (P=0.975, df=1)	78.19%	0%	0%	78.19%
Marine	6	15	4					
(Benthopelagic/Demersal)				0.1866 (P=0.666, df=1)	81.55%	3.27%	3.27%	88.09%
Freshwater	16	48	5					
(Benthopelagic/Demersal)				2.103 (P=0.147, df=1)	74.32%	0%	2.36%	76.68%
GST	14	39	8					
Environment				9.753 (P=**0.002**, df=1)	19.20%	0%	73.44	92.64%
Marine	6	15	4					
(Benthopelagic/Demersal)				0.001 (P=0.976, df=1)	24.96%	36.44%	36.44%	97.85%
Freshwater	8	24	4					
(Benthopelagic/Demersal)				0.125 (P=0.724, df=1)	45.48%	14.89%	14.89%	75.26%

Table S6. Effect estimates (SMD) of PAHs on biomarker responses accounting for exposure route as a source of variation (moderator). We present 95% confidence intervals from linear mixed-effects models. Heterogeneities (I^2) for random effects (effect size identification (ID), species, country and type of chemical) are also shown. Significance codes: *P < 0.05; **P < 0.01; ***P < 0.001.

	Effect size (SMD)	95% CI for SMD	Q_m (P, df)	$I^2_{[ID]}$	$I^2_{[species]}$	$I^2_{[country]}$	$I^2_{[chemical]}$	$I^2_{[total]}$
EROD								
Exposure Route			2.855 (P=0.414, df=3)	44.68%	2.68%	39.83%	11.9%	98.14%
Diet	1.392	-0.554 to 3.338						
Injection	2.213	0.524 to 3.903*						
Sediment	4.464	0.806 to 8.122*						
Water	1.853	0.165 to 3.542*						
GST								
Exposure Route			22.099 (P<0.0001, df=3)	33.62%	27.13%	12.81%	20.15%	93.71%
Diet	-1.0660	-3.166 to 1.034						
Injection	-0.827	-1.822 to 0.169						
Sediment	1.352	0.021 to 2.683*						
Water	0.314	-0.685 to 1.312						
CAT								
Exposure Route			5.383 (P=0.146, df=3)	65.7%	6.08%	<0.0001%	16.57%	87.72%
Diet	-1.263	-3.136 to 0.611						
Injection	0.323	-1.513 to 0.867						
Sediment	1.697	-0.126 to 3.52						
Water	0.016	-0.642 to 0.673						
SOD								
Exposure Route			0.009 (P=0.995, df=2)	84.71%	<0.0001%	<0.0001%	3.56%	88.27%
Injection	0.477	-1.100 to 2.053						
Sediment	0.375	-0.974 to 1.723						
Water	0.42	-0.194 to 1.033						
GPx								
Exposure Route			11.116 (P=0.004, df=2)	60.07%	12.17%	16.63%	<0.0001%	88.88%
Injection	-0.22	-1.281 to 0.841						
Sediment	3.908	1.611 to 6.206***						
Water	0.852	0.036 to 1.668*						

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Table S6 (*continued*)

	Effect size (SMD)	95% CI for SMD	Q_m (P, df)	$I^2_{[ID]}$	$I^2_{[species]}$	$I^2_{[country]}$	$I^2_{[chemical]}$	$I^2_{[total]}$
GR								
Exposure Route			4.674 (P=0.097, df=2)	21.75%	<0.0001%	73.82%	<0.0001%	95.57%
Injection	-0.106	-4.392 to 4.180						
Sediment	-1.775	-4.302 to 0.752						
Water	0.707	-0.863 to 2.277						
GSH								
Exposure Route			1.678 (P=0.432, df=2)	27.41%	<0.0001%	7.3%	57.04%	91.76%
Injection	-0.623	-2.410 to 1.164						
Sediment	0.575	-1.596 to 2.746						
Water	0.349	-1.070 to 1.769						
GSSG								
Exposure Route			0.042 (P=0.838, df=1)	30.21%	<0.0001%	<0.0001%	61.26%	91.47%
Sediment	1.701	-0.708 to 4.109						
Water	2.109	-0.979 to 5.197						
LPO								
Exposure Route			4.747 (P=0.093, df=2)	8.22%	4.86%	80.07%	<0.0001%	93.15%
Injection	0.939	-0.678 to 2.555						
Sediment	0.055	-1.349 to 1.458						
Water	1.119	-0.055 to 2.294						

Table S7. Effect of waterborne PAHs concentration through time on biomarker responses. We present 95% confidence intervals from linear mixed-effects models. Heterogeneities (I^2) for random effects (effect size identification (ID), species, type of chemical and country) are also shown. Significance codes: *P < 0.05; **P < 0.01; ***P < 0.001.

	Effect size (SMD)	95% CI for SMD	Q_m (P, df)	$I^2_{[ID]}$	$I^2_{[species]}$	$I^2_{[country]}$	$I^2_{[chemical]}$	$I^2_{[total]}$
EROD (*k=245*)								
Concentration x Time			32.312 (P<0.001, df=3)	78.79%	9.88%	<0.0001%	0.99%	89.65%
Intercept	1.737	1.143 to 2.331***						
Concentration	-0.005	-0.007 to 0.003***						
Time	-0.008	-0.017 to 0.001						
Concentration x Time	0.001	0.000 to 0.002**						
GST (*k=238*)								
Concentration x Time			100.970 (P<0.001, df=3)	16.11%	15.88%	62.63%	3.09%	97.71%
Intercept	-0.009	-2.350 to 2.332						
Concentration	-0.221	-0.283 to -0.160***						
Time	0.001	-0.008 to 0.010						
Concentration x Time	0.044	0.036 to 0.053***						
CAT (*k=188*)								
Concentration x Time			5.982 (P=0.112, df=3)	75.45%	<0.0001%	<0.0001%	13.87%	89.32%
Intercept	0.241	-0.476 to 0.959						
Concentration	-0.008	-0.041 to 0.026						
Time	0.006	-0.001 to 0.014						
Concentration x Time	0.005	-0.002 to 0.012						
SOD (*k=151*)								
Concentration x Time			19.558 (P<0.001, df=3)	63.5%	<0.0001%	22.81%	5.92%	92.23%
Intercept	0.914	-0.411 to 2.239						
Concentration	0.080	0.019 to 0.142*						
Time	-0.003	-0.015 to 0.008						
Concentration x Time	-0.038	-0.057 to -0.020***						
GPx (*k=133*)								
Concentration x Time			5.229 (P=0.156, df=3)	50.54%	4.57%	32.04%	<0.0001%	87.15%
Intercept	0.957	-0.090 to 2.004						
Concentration	-0.090	-0.180 to -0.0003*						
Time	-0.009	-0.020 to 0.002						
Concentration x Time	0.041	0.005 to 0.076*						

(continued on next page)

Table S7 (*continued*)

	Effect size (SMD)	95% CI for SMD	Q_m (P, df)	$I^2_{[ID]}$	$I^2_{[species]}$	$I^2_{[country]}$	$I^2_{[chemical]}$	$I^2_{[total]}$
GR (*k=54*)								
Concentration x Time			15.502 (P=0.001, df=3)	<0.0001%	29.5%	57.27%	<0.0001%	86.77%
Intercept	0.045	-1.040 to 1.129						
Concentration	-0.510	-0.972 to -0.048*						
Time	-0.025	-0.046 to -0.003*						
Concentration x Time	0.151	0.037 to 0.265**						
GSH (*k=97*)								
Concentration x Time			110.827 (P<0.001, df=3)	7.66%	45.23%	<0.0001%	36.77%	89.66%
Intercept	-0.277	-1.892 to 1.338						
Concentration	-0.007	-0.023 to 0.008						
Time	0.097	0.078 to 0.116***						
Concentration x Time	0.001	-0.000 to 0.002						
GSSG (*k=27*)								
Concentration x Time			20.795 (P<0.001, df=3)	1.84%	47.51%	47.51%	2.11%	98.97%
Intercept	-5.810	-18.262 to 6.643						
Concentration	26.999	7.486 to 46.512**						
Time	-0.049	-0.138 to 0.039						
Concentration x Time	0.153	-0.914 to 1.219						
LPO (*k=146*)								
Concentration x Time			643.999 (P<0.001, df=3)	0.29%	1.01%	98.41%	0.01%	99.72%
Intercept	-3.047	-10.970 to 4.876						
Concentration	1.049	0.928 to 1.170***						
Time	0.120	0.084 to 0.156***						
Concentration x Time	-0.288	-0.311 to -0.265***						

Capítulo II

Pesticide effects on fish cholinesterase variability and mean activity: A meta-analytic review.

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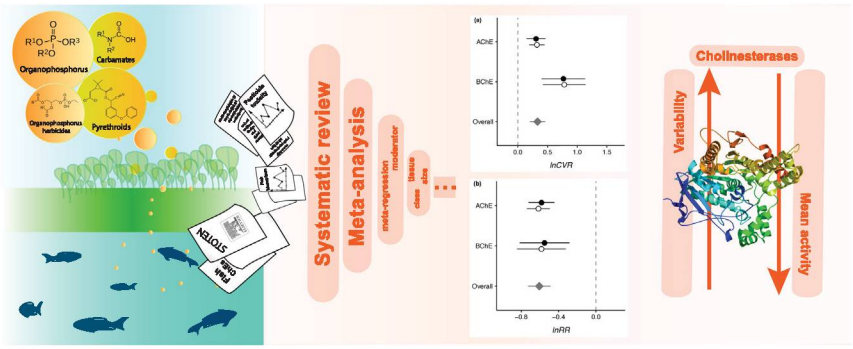
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HIGHLIGHTS

- We conducted a meta-analysis of pesticide effects on fish cholinesterases (ChEs).
- Pesticides had an overall significant effect on fish ChEs means and variability.
- BChE variability increases after exposure, implying biomarker sensitivity.
- Length of juvenile fish significantly affects ChEs variability and activity.
- OPs herbicides and oxazolidinones inhibit ChEs as strongly as pyrethroids.

GRAPHICAL ABSTRACT



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ABSTRACT

Fish cholinesterases (ChEs) – like acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) – are common biomarkers of environmental contamination due to their sensitivity to a variety of toxicants. To understand pesticide effects on fish ChEs mean activity and variability, we conducted a systematic review and meta-analyses. Our goal was to verify (*i*) if brain and muscle ChEs responded differently to pesticide exposure; (*ii*) how fish size and life stage (i.e., juvenile and adult) influence ChEs variability and mean activity; (*iii*) what type of pesticides (i.e., herbicide, insecticide, and fungicide) has the strongest effect, and if the analytical-grade compounds differ from commercial formulations; (*iv*) if increasing concentrations combined with prolonged exposure leads to stronger ChEs inhibition; and (*v*) how each class of pesticide affects these enzymes. We validated ChEs reliability as biomarkers and identified factors influencing their response. Regardless of tissue, BChE response was more variable than AChE, and no difference between their average activity was detected. The size of juvenile fish is an important factor affecting ChEs mean activity and variability, whereas pesticide had no significant effect on adult fish ChEs. Insecticides were stronger inhibitors compared to herbicides and fungicides. Analytical-grade compounds decreased ChEs mean activity to a higher degree than commercial formulations. The combined effect of concentration and time was only significant for fungicides and insecticides. Among classes, organophosphorus insecticides had the strongest effect on ChEs, followed by carbamates, organochlorines, and pyrethroids. Organophosphorus herbicides and oxazolidinones were the only herbicides to decrease ChEs mean activity significantly, and their effects were similar from those of pyrethroids and organochlorines. Additionally, our results identified research gaps, such as the small number of studies on fungicides, neonicotinoids and other relevant pesticides. These findings suggest future directions, which might help researchers identify robust cause-effect relationships between fish ChEs and pesticides.

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1. Introduction

Increasing demands on agricultural productivity, associated with an overall trend towards monoculture, has contributed to the widespread use of pesticides (particularly herbicides, insecticides, and fungicides) (Schäfer et al., 2011; WHO-FAO Report, 2019). Until 2017, more than 4 million tonnes of substances were applied globally (FAOSTAT, 2019), and given the projections of population growth for the next decades, applications are unlikely to decline (Maggi et al., 2019; Köhler and Triebskorn, 2013). It is estimated that from applied pesticide mass, less than 0.3% reaches target species, leaving the remaining 99.7% as a source of environmental impact and public health hazard (Felix et al., 2019; Pimentel, 1995), as most of these compounds interfere with biological systems of both target and non-target species in a non-selective manner (Clasen et al., 2018; Köhler and Triebskorn, 2013; Prieto Garcia et al., 2012).

Regardless of how it occurs (e.g., spray drift or precipitation and irrigation wash-off), pesticide residues reach aquatic ecosystems and affect fish health. It can affect either indirectly by modifying habitats and shaping ecosystem functioning (Gibbons et al., 2015; Maggi et al., 2019; Stanley and Preetha, 2016), or directly by inducing biochemical alterations or compromising whole systems. For instance, there is ample evidence that pesticides lead to biodiversity loss by decreasing richness and diversity of primary producers and associated fauna, which in turn leads to a loss in quantity and quality of prey for fish (Beketov et al., 2013; Relyea, 2005; Schäfer et al., 2011). Directly, pesticides may inhibit important enzymes (Mela et al., 2013; Ullah et al., 2019), reduce growth (Narra et al., 2017; Salbego et al., 2010), change behavior (Sandoval-Herrera et al., 2019; Scott and Sloman, 2004), increase susceptibility to diseases (Dupuy et al., 2019), and ultimately reduce fish stocks (Gormley et al., 2005; Yamamuro et al., 2019).

Additionally, these persistent chemicals accumulate in fish organs and other aquatic animals, mainly due to their lipophilicity (Schäfer et al., 2011). By ingesting these animals, humans are exposed to concentrations even higher than those detected in water (Cuevas et al., 2018; Schäfer et al., 2011). In humans, there is growing evidence linking pesticides to increased risk of cancer, including but not limited to neuroblastoma, stomach cancer, leukemia, and lymphoma (Mostafalou and Abdollahi, 2017). There are also reports of pesticides causing impairment of the endocrine system, pulmonary distress, diabetes (Kim et al., 2017; Mostafalou and Abdollahi, 2017) and even increased risk of Parkinson's disease (Ahmed et al., 2017; Rösler et al., 2018).

Pesticide overuse has prompted an abundance of studies investigating how and to what extent these compounds exert toxicity, especially on neural function. Several classes of pesticides are known to disrupt neuromuscular function in fish, i.e., act as anticholinesterase agents. For instance, organophosphorus (OPs) and carbamate insecticides are anticholinesterase compounds widely recognized, and their effects on fish have been described for several species (Agrahari et al., 2006; Guiloski et al., 2013; Pereira et al., 2019; Ullah et al., 2019). For this reason, cholinesterases (ChEs) – like acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) – have been commonly used as biomarkers in fish to determine the exposure to pesticides. These ChEs are sister serine hydrolases, sharing *ca* 50% amino acid identity, and similar tertiary and quaternary structures (Pope and Brimijoin, 2018). Globular and asymmetrical molecular forms of both enzymes are distributed across tissues, in membrane-bound and soluble states, throughout the animal kingdom (Massoulié et al., 1993; Pezzementi et al., 2011).

AChE and BChE are distinguished based on their substrate selectivity, determined by differences in their acyl pocket structures, which in turn determines their role in cholinergic neurotransmission (Mangas et al., 2017; Massoulié and Toutant, 1988). AChE has a fundamental role in regulating cholinergic neurotransmission in vertebrates, as it maintains the levels of acetylcholine stable through its hydrolysis in synaptic clefts. It is an extremely efficient enzyme, hydrolyzing its substrate at a rate that approaches diffusion limit (Massoulié et al., 1993; Pope and Brimijoin, 2018). BChE is a less selective enzyme, i.e., has a broader substrate specificity, hydrolyzing synthetic substrate butyrylcholine as well as acetylcholine at a comparatively minor rate (Massoulié et al., 1993; Pope and Brimijoin, 2018). It is believed that BChE acts on cellular adhesion and as a xenobiotic scavenger, potentially protecting AChE from anticholinesterase agents (Albendín et al., 2017; Rösler et al., 2018).

Furthermore, ChEs are also present in non-cholinergic tissues, taking part in critical biological events, such as embryonic development, cell proliferation, and cell death (Falugi and Aluigi, 2012). Specifically, AChE expression decreases during cell proliferation and increases during apoptosis, suggesting its role as a regulator of programmed cell death (Zhang et al., 2002). Moreover, BChE hydrolyzes the gut hormone ghrelin, known as the "hunger hormone" for its function as a stimulant of hedonic feeding, regulating weight gain and fat metabolism (Brimijoin et al., 2016; Pope and Brimijoin, 2018).

Fish ChEs activity are strongly affected not only by OPs and carbamates but also by a variety of pesticide classes. Even though organochlorines, pyrethroids, and neonicotinoids insecticides have different molecular targets (i.e., GABA receptors, voltage-gated Na+ channels, and Ach nicotinic receptors, respectively) (Casida and Durkin, 2013a), ChE inhibition has also been described for these compounds in fish (Bonansea et al., 2016; Da Cuña et al., 2011; Tian et al., 2018; Vieira et al., 2018). In fact, pyrethroids are highly toxic to fish and other aquatic organisms, despite being more selective and, therefore, seemingly harmless to mammals and birds (Vieira and dos Reis Martinez, 2018; Werner and Young, 2017). Another example is the herbicide glyphosate, which acts on a key plant enzyme involved in the synthesis of aromatic amino acids in the shikimate pathway (Mesnage and Antoniou, 2017). Theoretically, it should not act as an anticholinesterase compound; however, several studies report AChE inhibition in fish exposed to glyphosate (Cattaneo et al., 2012; de Moura et al., 2017; Salbego et al., 2010; Sanchez et al., 2017; Teixeira et al., 2018).

Despite constant innovation in the field, as presumably less toxic and more specific pesticides are developed (i.e., neonicotinoids and pesticides derived from plants or microorganisms), glyphosate is still the most applied pesticide worldwide (Benbrook, 2016; Maggi et al., 2019), while OPs and carbamates remain as the most used insecticides (Casida and Bryant, 2017; Maggi et al., 2019). Moreover, pyrethroids application is increasing in an attempt to reduce the production and use of highly toxic compounds (Vieira and dos Reis Martinez, 2018). Hence, considering the constant release of these neurotoxic compounds and the importance of ChEs to biological processes, it is crucial to assess how different classes of pesticides affect fish ChEs activity and what variables control the magnitude of pesticide effects. Furthermore, exposure to these compounds may lead not only to differences in the mean but also in the variability of ChEs activity. Acknowledging increases in variability will improve our overall understanding of pesticide impact and, consequently, validate ChEs reliability as biomarkers of exposure.

Our study presents a meta-analysis on anticholinesterase effects of pesticides in fish, including the impact on ChEs variability. We aim to answer questions related to the magnitude of pesticides effects on fish ChEs, based on the following predictions:

(i) Because ChEs have different isoforms, therefore catalytic abilities and sensibilities (Pereira et al., 2019; Pezzementi et al., 2011; Sandoval-Herrera et al., 2019), we expect that AChE and BChE will respond differently to pesticides both in brain and muscle. Specifically, since BChE is a less specific cholinesterase (Dias Assis et al., 2011; Sturve et al., 2016), pesticide exposure will increase BChE variability and differences in mean activity. Additionally, tissue distribution of AChE and BChE is variable (Pezzementi et al., 2011). AChE activity is generally described as predominant in fish brain and muscle, whereas BChE occurs mostly in muscle, liver, and serum (Nunes, 2011; Sturm et al., 1999a). Therefore, we expect more significant differences in

means of AChE activity in fish brain and of BChE activity in fish muscle.

(ii) There are reports of cholinesterase activity having an inverse relationship with fish length (Chandrasekara and Pathiratne, 2007; Chuiko et al., 1997; Fajardo and Ocampo, 2018; Flammarion et al., 2002; Lundin, 1962; Rodríguez-Fuentes et al., 2016; Tortelli et al., 2006). Additionally, the tolerance to toxicants appears to increase with fish age (i.e., fish becomes less sensitive as it matures) (Humphrey and Klumpp, 2003; McKim, 1977; Mhadhbi and Beiras, 2012). Therefore, we expect a more pronounced and less variable ChEs inhibition in younger and smaller fish than in larger, adult fish.

(iii) Because OPs and carbamates act as anticholinesterases, and pyrethroids have been described as toxic to fish (Ullah et al., 2019; Vieira and dos Reis Martinez, 2018), we expect that insecticides will have a greater impact on ChEs mean activity. Furthermore, it is known compounds used in commercial formulations of pesticides can be more toxic than the active ingredient. Therefore, we expect that the commercial formulation of pesticides will lead to greater differences in means than analytical-grade active ingredients (Mesnage et al., 2019; Sanchez et al., 2017).

(iv) Effects of concentration and time of exposure are continuously explored in primary studies (de Moura et al., 2017; Ensibi et al., 2014; Gholami-Seyedkolaei et al., 2013; Guimaraes et al., 2007; Ullah et al., 2019). Thus, we wish to verify if the combination of increased concentration and exposure duration leads to stronger ChEs inhibition.

(v) As previously stated, different classes of pesticides can affect ChEs activity despite their molecular targets. However, we expect that OPs and carbamates, known cholinesterase inhibitors, will affect ChEs mean activity to a higher degree compared to other pesticide classes.

2. Methods

2.1. Literature search and inclusion criteria

We performed a systematic search using the extended online database (timespan: "all years") of *PubMed*, *Web of Science*, and *Scopus* up to and including January 2019. All steps in this systematic review followed the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses; Moher et al., 2009). The following search strategy was applied to search on topics (i.e., title, keywords, and abstracts) in all databases: ((contaminat* OR pollut*)) AND (fish OR teleost*) AND (pesticide OR herbicide OR insecticide OR fungicide) AND (acetylcholinesterase OR AChE OR neurotoxic* OR anticholinesterase OR butyrylcholinesterase OR pseudocholinesterase OR BChE).

After removing duplicate results, 1416 peer-reviewed studies remained for screening. Retrieved records needed to provide means, sample sizes, and a measure of dispersion (interquartile ranges, standard deviations, or standard errors) and also needed to meet our inclusion criteria below:

1) Studies had to address our main research question, i.e., the effects of pesticides on fish cholinesterases.
2) Studies must report a clear and unambiguous study design description. Also, means, sample sizes, and a measure of dispersion should be provided.
3) Studies must provide ChEs activity from fish exposed to pesticides (treatment/impact group) and from fish reared or captured in conditions where pesticides were absent (control/reference groups).
4) Only synthetic chemicals used as agricultural pesticides were included. We excluded public health pesticides, biocides used as antifouling compounds, or antibiotics.
5) Only studies that measured ChEs on brain and muscle of fish were included. Studies using fish embryos, larvae, or in vitro experiments were all outside the scope of this meta-analysis.

Both field and laboratory experimental approaches were considered. For field studies, whenever two or more reference sites were used for comparisons, only the most distanced site from the contaminated areas was considered and its information extracted. Also, when laboratory studies used two control settings for comparisons, (*i*) a solvent control, in which fish was exposed to the vehicle used as solvent, and (*ii*) a regular control, in which fish remained in clean water during the experiment; only the latter was considered. We chose to compare estimates to a regular control because the majority of studies in our data set had a single, regular control.

After the screening of titles and abstracts, approximately 70% of the 1416 papers were excluded. The remaining 453 papers were fully screened, and 176 were included for data extraction. The screening process is shown in Fig. S1 in the supplementary materials. The list of included studies, data and codes are available for download in at https://osf.io/7stne/.

2.2. Data extraction

ChEs activity data were extracted from text, tables, or figures as control–treatment pairwise comparisons. To extract data from figures, we used the *metaDigitise* package (Pick et al., 2018) in *R* (v. 3.4.3; R Core Team (2018)). Additionally, we contacted authors and requested for data whenever information was incomplete or missing.

The majority of studies compared several treatments to a single control. Although a common practice in ecotoxicology studies, these experimental designs cause nonindependence among estimates. To deal with this issue, whenever several treatments had a shared control within a given paper, these estimates were assigned a unique paper ID. For each pairwise comparison, a unique estimate ID (unit of analysis) was assigned.

2.3. Moderator variables

For each pairwise comparison, we extracted information of moderator (predictor/explanatory) variables to test their influence on ChEs activity after pesticide exposure. The variables included in our analysis as moderators were: type of ChEs (AChE or BChE), tissue they were measured (brain or muscle), fish length and age (life stage: adult or juvenile), type and class of pesticide, grade of the compound used in experiments (analytical or commercial), concentration and time of exposure. Type of pesticides is a broad classification based on target species, i.e., insecticide, herbicide, or fungicide. Class of pesticides is based on the chemical nature of the compound, and 20 classes of pesticides were included in our study, e.g., organophosphorus, carbamates, triazine, and pyrethroids. For a detailed list of moderator variables, see Table S1.

2.4. Data analysis

2.4.1. Effect sizes

We calculated two effect sizes and their sampling variance to assess ChEs activity differences between a treatment and a control group of fish: the log response ratio (*lnRR*; Hedges et al., 1999) and the log coefficient of variation (*lnCVR*; Nakagawa et al., 2015). The *lnRR* was selected to test for mean differences in ChEs activity between treatment and control groups. The *lnCVR* ratio, which is the difference between coefficients of variation for treatment and control groups, was used to measure differences in ChEs activity variance. For both *lnRR* and *lnCVR*, the treatment group was specified as the numerator and the control as the denominator. Therefore, positive values represent an increase in ChEs activity mean and variance after pesticide exposure, whereas negative

values indicate a decrease in ChEs activity mean and variance after exposure.

2.4.2. Meta-analysis

Meta-analyses and meta-regressions were carried out using multilevel linear mixed-effects models. Model estimates were considered statistically significant if their 95% confidence intervals did not cross zero. The nonindependence among estimates – originated from comparing a single control to multiple treatments – were accounted for with random-effects and sampling variance-covariance matrices.

First, we ran models to decide the best random-effects structure using the maximum likelihood method. We compared models combining three random effects: effect size ID, paper ID, and species name. The variance component for effect size ID represents residual variance, for paper ID represents the between-studies variance and for species name represents the variance among species. Based on AICc values, which are shown in Table S2, we chose a model with all three random effects.

Next, we ran models without any moderator variables (i.e., meta-analytic model) to assess the overall effect of pesticides on the mean and variance of ChE activity. To control for the nonindependence among estimates, we ran models that included a variance-covariance matrix with 0.5 correlation between effect size sample variances with the same paper ID. We compared these results with models assuming no correlation, i.e., independent sample variances. Both types of models generated qualitatively similar results, therefore here we present results from the models assuming no correlation. Missing data were automatically removed from meta-regression models.

The amount of heterogeneity (I^2_{total}) for the multilevel meta-analytic models was estimated using the method described by Viechtbauer (2019). It is a measure of total heterogeneity between effect sizes that is not attributed to sampling error (Higgins et al., 2003). Since we found high levels of heterogeneity (83% and 99% for *lnCVR* and lnRR, respectively), we added moderator variables to models (i.e., meta-regression models) in an attempt to explain some of this heterogeneity. Moreover, moderator variables were selected to answer the questions and predictions earlier described. We have also quantified marginal R^2 (sensu Nakagawa and Schielzeth, 2013) for all meta-regression models, which is a measure of how much variance in ChEs activity, in response to pesticide exposure, was explained by moderators added to models.

Effect sizes calculation, as well as meta-analytic and meta-regression models, were conducted using the *metafor* package (v.2.0–0; Viechtbauer, 2010) in R (R Core Team, 2019). Codes and model fitting were adapted from O'Dea et al. (2019).

2.4.3. Publication bias

Since all of our data comes from published studies, we took three steps to determine if there is bias towards significant differences (i.e., publication bias) in our data set. First, we looked for funnel asymmetry of the 'meta-analytic' residuals (sensu Nakagawa and Santos, 2012), calculated from the model which – based on the R^2 values – best capture heterogeneity. This approach fulfills the independence assumption and helps detect bias after controlling for heterogeneity. Then, we ran Egger's regression on the same meta-analytic residuals and a trim-and-fill test to predict unpublished studies from the literature based on funnel asymmetry.

3. Results

3.1. Summary of data set

Our data set consists of 176 papers reporting pesticide effects on ChEs of 81 different species of fish. A total of 1602 effects sizes were calculated from 557 independent studies within those papers. The majority of studies focused on AChE activity both in fish brain and muscle (66% and 30% of effect sizes, respectively), while only 4% of estimates were on BChE. A large proportion of studies investigated effects on freshwater species (91%) under laboratory conditions (88%). There were no field experiments using marine species, and only a small amount of caging experiments used estuarine fish (5%). Overall, insecticides were the most evaluated type of pesticides (75%). Specifically, from a total of 20 classes, organophosphorus insecticides comprised 53% of effect sizes. Fish length ranged from 1.25 to 65 cm, and 91% of effect sizes were from fish under 20 cm. Finally, the bulk of insecticide concentrations ranged from 0 to 50 mg/l (~93%), herbicides from 0 to 100 mg/l (99.6%) and fungicides from 0 to 1 mg/l (81%). Most of these concentrations (ca. 63%) were either selected in order to determine median lethal (LC_{50}) or effective concentration (EC), or based on previously described LC_{50}. A proportion of studies (~25%) based their choice on environmentally realistic concentrations, i.e., concentration usually applied on crops, or detected in water samples, or permissible under government regulation.

3.2. Does tissue affect ChEs variability and mean activity?

Overall, pesticide exposure had a significant effect both on ChEs variability and mean activity, irrespective of fish tissue (Fig. 1, Table S3). Exposure significantly increased ChEs variability by 39.3% (*lnCVR*: 0.331, 95% confidence interval, CI: 0.203 to 0.459) and reduced mean activity values by 45.4%, i.e., inhibited ChEs activity (*lnRR*: −0.606, CI: −0.726 to −0.0486).

In particular, although BChE response was 58.5% more variable than AChE ($lnCVR_{\text{BChE-AChE slope}}$: 0.460, CI: 0.114 to 0.807, Fig. 1a, Table S4), there was no significant difference in their mean activity both in brain and muscle ($lnRR_{\text{BChE-AChE slope}}$: 0.032, CI: −0.203 to 0.268, Fig. 1b, Table S4).

3.3. Does fish length and age have an impact on ChEs activity?

There was no significant effect of fish length and age on ChEs variability ($lnCVR_{\text{adult length slope}}$: 0.001, CI: −0.163 to 0.454; $lnCVR_{\text{juvenile length slope}}$: 0.004, CI: −0.023 to 0.031, Table S5) and mean activity ($lnRR_{\text{adult length slope}}$: 0.001 CI: −0.011 to 0.014; $lnRR_{\text{juvenile length slope}}$: 0.005, CI: −0.013 to 0.022, Table S5). In other words, when considering adult and juvenile fish of all sizes, ChEs mean activity and variability did not change significantly with increasing length.

As stated earlier, the bulk of estimates originated from fish under 20 cm. For this reason, we ran post hoc meta-regressions using specific ranges of length: ≤10 cm and 10 < length ≤ 20 cm (Fig. 2a–d). For both ranges, ChEs variability and mean activity of adult fish did not show a significant relationship with length ($lnCVR_{\text{adult length≤10cm slope}}$: −0.070, CI: −0.226 to 0.086; $lnCVR_{\text{adult 10<length ≤20cm slope}}$: 0.141, CI: −0.056 to 0.226; $\text{lnRR}_{\text{adult length≤10 slope}}$: 0.042, CI: −0.055 to 0.139; $lnRR_{\text{adult 10<length ≤20cm slope}}$: −0.054, CI: −0.152 to 0.044; Table S6, Fig. 2a–d).

For fish 10 cm and smaller, there was a 23.5% significant difference between juvenile and adult variability with increasing length ($lnCVR_{\text{juvenile-adult difference length≤10 slope}}$: 0.211, CI: 0.030 to 0.391, Fig. 2a). Specifically, the variability of ChEs response in juveniles significantly increased with fish length, whereas adult fish showed a non-significant decrease of variability as fish size increased. For juveniles ranging from 10 to 20 cm, there was a significant and inverse correlation of variability and length ($lnCVR_{\text{juvenile 10<length≤20 slope}}$: -0.057, CI: −0.106 to −0.007, Table S6, Fig. 2b), i.e., considering this length range, the variability of ChEs response decreases as the length of juvenile fish increases.

Additionally, juvenile fish of both ranges showed a significant decrease in ChEs mean activity as length increased ($lnRR_{\text{juvenile length≤10 slope}}$: −0.064, CI: −0.117 to −0.011; $lnRR_{\text{juvenile 10<length≤20cm slope}}$: −0.053, CI: −0.092 to −0.014, Table S6, Fig. 2c-d), i.e., pesticide exposure leads to greater ChE inhibition as juvenile size increases.

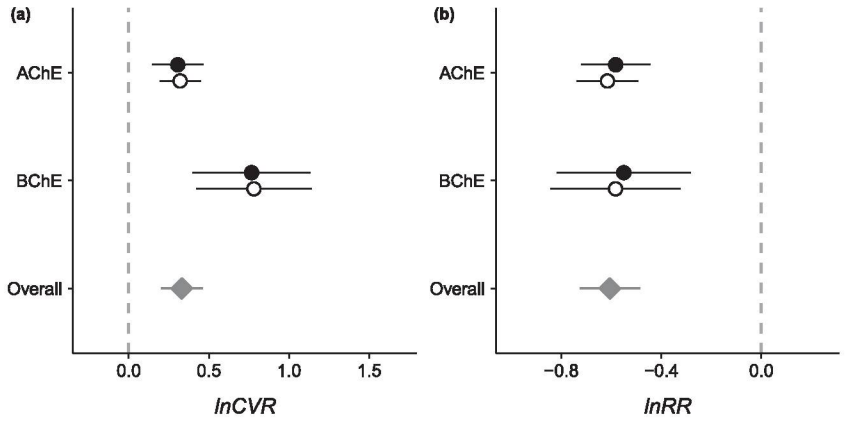


Fig. 1. Effects of pesticides on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity variability (a) and differences in means (b) assayed on brain tissue (unfilled circles) and muscle tissue (filled circles). Dots represent effect size estimates (log coefficient variation *lnCVR* and log response ration *lnRR*), and error bars represent 95% confidence intervals. Effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line).

3.4. Differences among types of pesticides and the effects of commercial formulations

There was no significant difference between analytical-grade active ingredient and commercial formulations on ChEs variability ($lnCVR_{\text{analytical-commercial slope}}$: 0.069, CI: −0.099 to 0.236, Table S6). Insecticides and herbicides significantly increased ChEs variability (Fig. 3a); however, these types of pesticides are not significantly different from each other ($lnCVR_{\text{herbicide-insecticide slope}}$: 0.005, CI: −0.181 to 0.191, Table S6). Although different from both insecticides and herbicides, fungicides did not show a significant effect on ChEs variability. Note that the estimates of fungicides are based on very little data and might not be as informative as the other types of pesticides (Table S6).

Insecticides and herbicides had strong significant effects on ChEs differences in mean activity (Fig. 3b). There is a significant difference between insecticides and herbicides. Particularly, insecticides are 27.8% stronger inhibitors than herbicides ($lnRR_{\text{herbicide-insecticide slope}}$: 0.326, CI: 0.202 to 0.450, Table S6, Fig. 3b). Also, commercial formulations and analytical-grade were significantly different from each other.

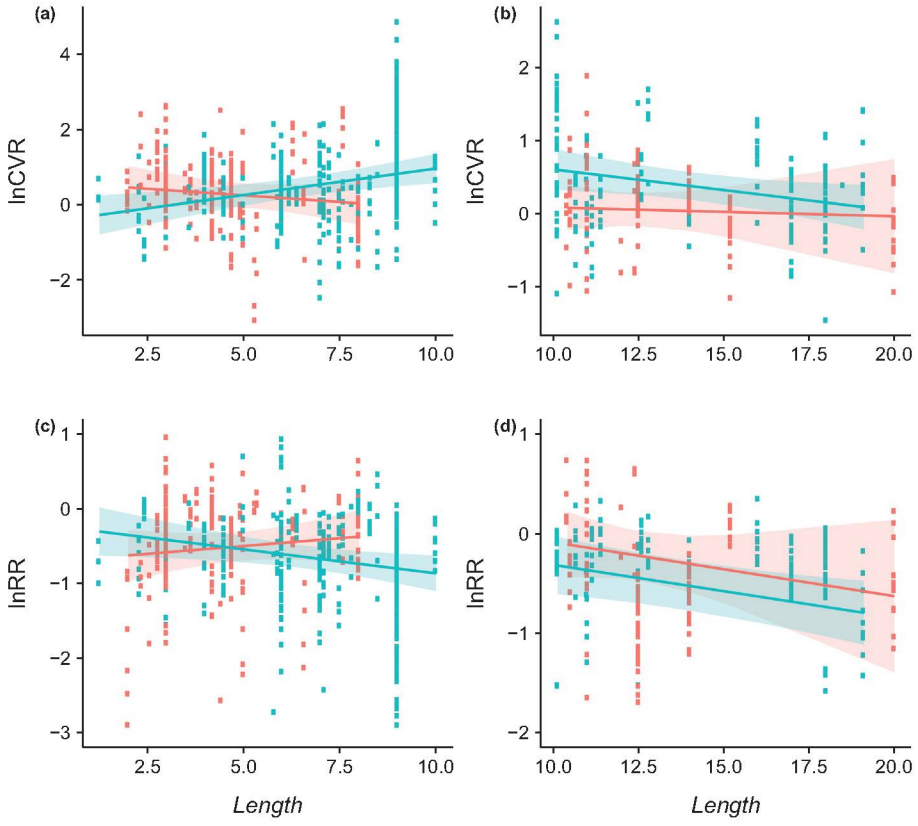


Fig. 2. Effects of pesticides on cholinesterases (ChEs) variability (a, b) and average activity (c, d) of juvenile and adult fish under 10 cm and ranging from 10 to 20 cm. Circles represent effect size estimates (log coefficient variation *lnCVR* and log response ratio *lnRR*). Juvenile fish estimates are shown in green, and adult fish estimates are shown in red. Shaded areas indicate 95% confidence intervals. To better represent estimates and confidence intervals, 6 and 3 estimates were omitted from plots (c) and (d), respectively.

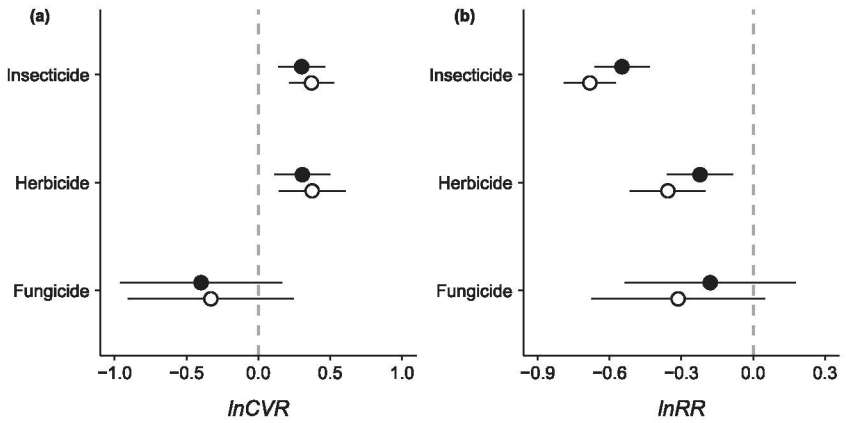


Fig. 3. Effects of herbicides, insecticides, and fungicides on ChEs activity variability (a) and differences in means (b), considering analytical-grade active ingredient (unfilled circles) and commercial formulation (filled circles). Dots represent effect size estimates (log coefficient variation *lnCVR* and log response ration *lnRR*), and error bars represent 95% confidence intervals. Effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line).

Analytical grade inhibits ChEs mean activity 14.4% more than commercial pesticides ($lnRR_{\text{analytical-commercial slope}}$: 0.134, CI: 0.026 to 0.242, Table S6, Fig. 3b).

3.5. Does increasing concentrations during longer exposure durations cause a sharp decrease in ChEs activity?

Although very small, the combined effect of concentration and exposure time of insecticides were statistically significant on both ChEs variability and mean activity (Table S7). Specifically, there was a 0.4% increase in variability, as well as a 0.4% decrease in mean activity with increasing concentrations for every unit increase in time. Also, fungicides concentrations had a strong negative effect on ChEs mean activity, i.e., increasing concentrations of fungicides decreases 28.7% ChEs mean activity for every unit increase in time (Table S7). As stated earlier, note that the amount of data on fungicides is very little. Furthermore, the combination of concentration of herbicides and exposure time had no significant effect on either ChEs variability or mean activity.

3.6. How different classes of pesticides affect ChEs activity?

Compared to all moderators selected and tested in our study, the class of pesticides was the most important, accounting for more than 13% of heterogeneity in size and magnitude of effects (*lnRR*: $Q_m = 116.446$, $p < 0.0001$, $R^2 = 13.4\%$, Table S9). From all 20 classes, only carbamate, organophosphorus insecticides, organophosphorus herbicides, and triazine significantly increased ChEs variability (Fig. 4a, Table S8). Organophosphorus herbicide and insecticides showed a more substantial effect, increasing variability by 60% and 58%, respectively.

In addition to carbamate and organophosphorus pesticides, oxazolidinones, organochlorine, and pyrethroid were the only classes of pesticides to show significant effects on mean activity differences

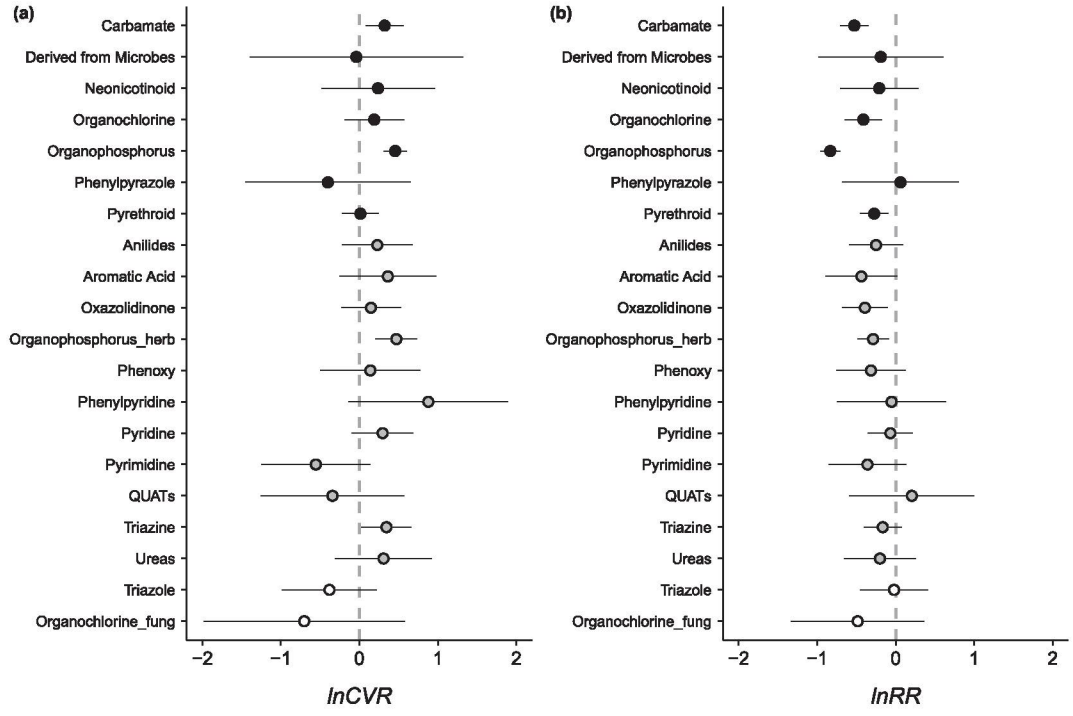


Fig. 4. Effects of specific classes of insecticides (black-filled circles), herbicides (gray-filled circles), and fungicides (unfilled circles) on ChEs activity variability (a) and difference in means (b). Dots represent effect size estimates (log coefficient variation *lnCVR* and log response ration *lnRR*), and error bars represent 95% confidence intervals. Effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line).

(Fig. 4b, Table S8). From those, organophosphorus insecticides were the strongest inhibitor, causing a 56.5% decrease in ChEs mean activity (Table S8). Even though triazine increased ChEs variability, no effect on mean activity differences was detected.

3.7. Publication bias

We were able to detect an asymmetrical distribution around the meta-analytic mean and variation by funnel plot visual inspection (Fig. S2). This asymmetry and, therefore, publication bias was confirmed by the results of Egger's regression and trim-and-fill analyses (Table S10).

4. Discussion

As expected, exposure to pesticides had a significant overall effect on ChEs average activity and variability. It has been reported that a decrease greater than 20% in ChE activity is an indication of exposure to OPs or anticholinesterase substances (Flammarion et al., 2002; Sturm et al., 1999b). Even small fractions of pesticides may cause a sharp decrease in ChEs activity, which in turn may alter behavior and ultimately compromise fish survival. For instance, studies have described erratic swimming patterns and speed (Huang et al., 2016; Laetz et al., 2013; Marigoudar et al., 2009; Qiu et al., 2017), and inability to avoid or escape predators (Sandoval-Herrera et al., 2019) as a consequence of pesticide exposure. Considering the biological importance of ChEs and the quantified magnitude of effects (i.e., > 40% inhibition), we may assume that pesticide is particularly deleterious to fish health. Next, we explore how and to what extent the selected moderator variables affect ChEs variability and average activity.

4.1. ChEs X tissue

BChE and AChE mean activities consistently decreased in exposed fish, regardless of tissue. However, as predicted, BChE showed a highly variable response pattern, represented by large coefficients of variation (*lnCVR*) and wide confidence intervals around the mean activity estimate (*lnRR*). BChE is a sensitive, non-specific detoxifying enzyme, able to scavenge several toxicants, and by doing so, it protects AChE from inhibitors (Lockridge et al., 2016; Martínez-Morcillo et al., 2019). Some argue that partial or complete inhibition of BChE happens before pesticides could reach AChE, preventing sublethal or even lethal toxic effects (Albendín et al., 2017; Rösler et al., 2018). Therefore, the varying degrees of BChE inhibition reported here might reflect not only its sensitive nature but also a protective strategy of fish to pesticide exposure. For this reason, to understand the extent of environmental contamination by pesticides or nerve agents, both ChEs should be used as biomarkers together.

We assumed brain and muscle ChEs would respond differently to pesticide exposure based on earlier reports on tissue distribution. Studies have described AChE as predominant in most tissues of freshwater and marine fish species. For instance, there are reports of AChE being the only enzyme in the brain of *Oreochromis niloticus* (Rodríguez-Fuentes and Gold-Bouchot, 2004), *Limanda limanda*, and *Platichthys flesus* (Sturm et al., 1999a); of AChE predominance in most or all tissues of *Astyanax altiparanae* (Pereira et al., 2019), *Trachurus trachurus* and *Merluccius merluccius* (Martínez-Morcillo et al., 2019); and the occurrence of both AChE and BChE in muscle tissue of *Dicentrarchus labrax* (Varò et al., 2003), *Prochilodus lineatus* (Lopes et al., 2014), *Limanda limanda* and *Platichthys flesus* (Sturm et al., 1999a). Despite evidence of differing tissue distribution, our results indicate brain and muscle ChEs responded similarly to pesticides.

While AChE is predominant in most fish species, pesticides might affect brain and muscle ChEs to a similar degree, leading to comparable decreases in mean activity after exposure, i.e., a similar magnitude of inhibition. Consequently, we might assume brain and muscle ChEs are equally reliable for detecting pesticides and assessing their effects. However, regardless of tissue, BChE detoxifying and protective role should be taken into account when interpreting results, since its inhibition might precede AChE's.

4.2. Bigger the fish, greater the inhibition?

Contrary to our expectations, when all lengths were included, ChEs average activity or variability did not correlate to juvenile and adult fish size. We believe that a relationship could not be detected because the bulk of estimates comes from small to medium size fish. Laboratory experiments usually handle smaller fish for a simple and understandable reason: logistics. It is easier to design an experiment with appropriate replicates when test organisms require less space, water volume, and toxicant mass (Mohammed, 2013). Since most estimates come from laboratory studies, we set out to investigate the influence of specific ranges of size on ChEs of both adult and juvenile fish.

While having no significant effect on adult fish, pesticide exposure affected ChEs variability and mean activity of smaller (≤ 10 cm) and larger (10 < length ≤ 20 cm) juveniles.

Increased variability in smaller juveniles, followed by a decrease in mean activity, indicates a sensitivity towards pesticides. Whereas the decrease in variability and precise inhibition (i.e., narrower confidence interval) shown by larger juveniles reveal not only that this life stage is sensitive to pesticides, but also that the exposure elicited a more homogenous, less variable response.

Although not applicable to all species, it is generally accepted that earlier life stages of fish are more sensitive to toxicants than adults (Anderson and Wever, 1975; Peters and Livingstone, 1995; Wendelaar Bonga, 1997; Whyte et al., 2000). In smaller and younger fish, detoxification and elimination of toxicants might not occur at the same rate observed in bigger and older fish, since important organs such as liver and kidneys may not be fully developed (Calfee et al., 2014; Mohammed, 2013). Additionally, due to less body fat to store lipophilic substances, and a higher ratio of body surface area to volume, which would lead to greater uptake from the environment, younger fish would be more susceptible to the effects of contaminants compared to adult fish (Mohammed, 2013). Adult fish may be more tolerant to toxicants because they can assimilate and detoxify substances at a faster rate (Fajardo and Ocampo, 2018). Our findings support this general assumption and show the importance of body size and developmental stage to pesticide toxicity in fish.

4.3. Concentration and time

Although a fairly broad classification, which encompasses several active ingredients, we hoped type of pesticides would indicate how their concentration affect fish ChEs across time in a general way. The most substantial effect was observed for fungicides, which showed a strong inhibition as concentrations and the number of exposure days increased. It is possible that such pattern emerged from a rather homogeneous small subset of data. Specifically, triazole class represents almost the totality of 16 estimates of fungicides (ca. 88%), which in turn are mainly comprised by studies on tebuconazole effects. Furthermore, almost all concentrations were based on environmentally realistic concentrations, which contributed to a narrower range of concentrations and consequently, smaller variability of responses.

Contrarily, insecticides and herbicides subsets are comprised by several active ingredients, showing a relatively wide range of concentrations based on those found in the environment or on species-specific LC_{50}, which are common endpoints of acute toxicity tests (Bidwell, 2020). Toxicity is related not only to the species, but also to the physical-chemical properties of a given compound (van den Berg et al., 2021). Hence, we argue that broad categories, such as herbicides and insecticides, are not suitable to determine the effects of concentration and time. To verify them as sources of variation, future research

synthesis should conduct meta-regressions on specific subsets of data, isolating active ingredients or at least class of pesticides. These particular analyses go beyond the scope of this present review, since our goal was to explore the relationship between concentration and time and verify general trends.

4.4. Pesticides type, class and formulation

Our results showed that, although insecticides and herbicides increased ChE variability to a similar degree, insecticides were more toxic to fish (i.e., larger inhibition of ChEs mean activity) than herbicides. This was clear not only when comparing pesticide types, but also considering the effects of increasing concentrations and exposure days. Given that the most used insecticides worldwide belong to OPs and carbamate classes (e.g., chlorpyrifos, carbaryl) (Maggi et al., 2019), which are known to inhibit fish ChEs (Casida and Bryant, 2017; Mineau, 2018), the difference between types detected here was expected. Furthermore, herbicides (e.g., glyphosate, atrazine, 2,4-d) are by far the most abundantly used type of pesticides, surpassing even the most applied insecticide (Casida and Bryant, 2017; Maggi et al., 2020). Despite inducing cytotoxicity and oxidative stress in fish (Glusczak et al., 2006; Golombieski et al., 2016; Mela et al., 2013; Miron et al., 2008), our results indicate that herbicides may not inhibit ChEs as strongly as insecticides.

Aside from identifying which type affects ChEs the most, it is crucial to assess the magnitude of effect of each class of pesticide. As expected, OPs were the strongest inhibitor, followed by carbamates, organochlorines and pyrethroids. Both OPs and carbamates act as ChEs inhibitors; however, ChE inactivation by OPs is an irreversible reaction (Casida and Durkin, 2013b; Jayaraj et al., 2016). Seeing that they share the same molecular target, it is understandable that these classes would induce a similar inhibition pattern in exposed fish.

Organochlorines and pyrethroids had no significant effect on ChEs variability and decreased ChEs mean activity similarly. In fact, we observed a precise (i.e., narrower confidence interval) effect of pyrethroids on ChEs mean activity, which supports previous findings on how toxic this insecticide may be to fish (Kaviraj and Gupta, 2014; Ullah et al., 2019; Vieira and dos Reis Martinez, 2018). Possibly, because specific organochlorines and pyrethroids compounds have the same mode of action (i.e., GABA receptor inhibition and sodium channels activation) (Casida and Durkin, 2013b; Costa, 2015), we have detected a comparable effect on ChEs variability and activity. However, organochlorines are far more hazardous than pyrethroids due to their stability and high lipid solubility, which guarantee its persistence in the environment and long-lasting toxic effects to non-target animals (Costa, 2015; Jayaraj et al., 2016).

Organophosphorus herbicides (i.e., glyphosate) and oxazolidinones (i.e., clomazone) were the only herbicides to affect ChEs mean activity. Their mode of action is directed to specific plant enzymes involved in metabolic pathways, such as the biosynthesis of aromatic amino acids and carotenoids, respectively (Mesnage and Antoniou, 2017; Pereira et al., 2013). Nonetheless, there are reports of ChE inhibition in fish exposed to glyphosate (Braz-Mota et al., 2015; de Moura et al., 2017; Modesto and Martinez, 2010) and clomazone (Miron et al., 2005; Murussi et al., 2015; Pereira et al., 2013), and our findings support these previous results. Moreover, our results indicated that the magnitude of their effects are similar to those of pyrethroids and organochlorine exposure, providing evidence of the toxic impact these classes might have on fish.

Some argue that compounds found in end-use pesticides considered to be inert and innocuous are, in fact, more toxic than the active ingredient (Mesnage et al., 2019). For instance, surfactants and glyphosate-based formulations have been described as to cause genotoxicity, oxidative stress, and cytotoxicity in fish and other aquatic animals (Folmar et al., 1979; Tsui and Chu, 2003; Guilherme et al., 2012). Contrary to these findings and our initial prediction, we found that analytical-grade active ingredients were more damaging to fish ChEs means than commercial formulations.

Possibly this opposing outcome is a consequence of how experiments are conceived and conducted. There is significant heterogeneity among concentrations of both analytical-grade ingredients and commercial formulations, which might hinder comparisons. For instance, a particular study evaluated the effects of diazinon by exposing fish to 0.15 mg/l analytical-grade (Gaworecki et al., 2009), while another study also used 0.15 mg/l of a commercial formulation of diazinon as its high concentration (Sharbidre et al., 2011). An elevated amount of a pure active ingredient might have exceeded the effect of surfactants or any other ingredient present in commercial formulations. Therefore, it seems crucial to consider the kind of compound when designing experiments and selecting exposure concentrations.

4.5. Limitations and possible directions

Results from Egger's regression and trim-and-fill analysis indicate publication bias and selective reporting from studies included in our data set. Large mean differences between control and exposed fish seem to be over-represented in our data set, which might indicate that studies reporting ChEs inhibition are more likely to be published than null results (Jennions et al., 2013). Additionally, we excluded studies from our analysis because relevant information (e.g. sample size of control and/or treatment groups, as well as standard error; or details on how the experiment was conducted) was missing or was poorly reported. The exclusion of these studies might have contributed to the bias detected in our results. Thus, a change in publication practices is crucial, not only on how journals select results but also on how data is reported in studies.

Furthermore, results from our meta-regressions were limited by the amount of data we were able to extract for each moderator variable. For instance, only 939 from 1602 total had information on fish length, an essential information which studies neglected to report. The missing data could have been useful in establishing a stronger relationship between size and ChEs activity. Furthermore, most estimates came from studies on insecticides and herbicides, while only a small fraction from fungicides (i.e., 16 estimates), indicating a research gap in the field of fish toxicology. Also, most classes of pesticides had very little coverage (e.g., QUATs ($k = 2$), triazole ($k = 11$), phenylpyrazole ($k = 4$), and insecticides derived from microbes ($k = 2$)), which might point to where future studies should concentrate efforts. Considering its increasing agricultural use and debate over its environmental safety (Gibbons et al., 2015; Simon-Delso et al., 2015), future studies should focus on the effects of neonicotinoids, which were represented only by 17 estimates from 4 studies in our data set. The gaps reported here, if filled, could advance even further our understanding of pesticide effects on fish.

5. Conclusions

Cholinesterases are well-established biomarkers of environmental contamination. Due to their simple, inexpensive, and reproducible methodology, and consistent response to a variety of contaminants, ChEs are reliable tools to assess fish health. Our results not only validate their reliability but also elucidate which factors influence their response and to what extent. As stated earlier, AChE and BChE activities are complementary, and environmental monitoring practices will benefit from measuring both regardless of tissue. Moreover, the size of juvenile fish is an important source of variation affecting ChEs average activity and variability. For this reason, if not possible to use fish of similar size, the relationship between ChE and length should be factored in to avoid confounding results. Finally, our analysis identified differences among pesticide type and classes, and, as a result, we were able to highlight research gaps and directions for future studies. For instance, studies on currently relevant pesticides, such as neonicotinoids, are scarce and this lack of data makes it challenging to evaluate their effects on ChEs.

Hence, to identify robust cause-effect relationships between pesticides and fish ChEs, future studies should turn their focus on filling the gaps found here.

CRediT authorship contribution statement

Manuela S. Santana: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft. **Leonardo Sandrini-Neto:** Conceptualization, Formal analysis, Validation, Visualization. **Maikon Di Domenico:** Resources, Supervision, Writing - review & editing. **Maritana Mela Prodocimo:** Resources, Supervision, Funding acquisition, Writing - review & editing.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.143829.

References*

*Agrahari, S., Gopal, K., Pandley, K.C., 2006. Biomarkers of monocrotophos in a freshwater fish Channa punctatus (Bloch). J. Environ. Biol. 27, 453–457.

Ahmed, H., Abushouk, A.I., Gabr, M., Negida, A., Abdel-Daim, M.M., 2017. Parkinson's disease and pesticides: a meta-analysis of disease connection and genetic alterations. Biomed. Pharmacother. 90, 638–649. https://doi.org/10.1016/j.biopha.2017.03.100.

Albendín, G., Arellano, J.M., Manuel-Vez, M.P., Sarasquete, C., Arufe, M.I., 2017. Characterization and in vitro sensitivity of cholinesterases of gilthead seabream (Sparus aurata) to organophosphate pesticides. Fish Physiol. Biochem. 43, 455–464. https://doi.org/10.1007/s10695-016-0299-y.

Anderson, P.D., Wever, L.J., 1975. Toxic response as a quantitative function of body size. Toxicol. Appl. Pharmacol. 33, 471–483. https://doi.org/10.1016/0041-008X(75)90073-3.

Beketov, M.A., Kefford, B.J., Schäfer, R.B., Liess, M., 2013. Pesticides reduce regional biodiversity of stream invertebrates. Proc. Natl. Acad. Sci. U. S. A. 110, 11039–11043. https://doi.org/10.1073/pnas.1305618110.

Benbrook, C.M., 2016. Trends in glyphosate herbicide use in the United States and globally. Environ. Sci. Eur. 28, 1–15. https://doi.org/10.1186/s12302-016-0070-0.

Bidwell, J.R., 2020. In vivo ecotoxicology models. In: Pope, C.N., Liu, J.B.T.-A.I. to I.T. (Eds.), Introduction to Interdisciplinary Toxicology. Academic Press, pp. 507–523 https://doi.org/10.1016/B978-0-12-813602-7.00036-3.

Bonansea, R.I., Wunderlin, D.A., Amé, M.V., 2016. Behavioral swimming effects and acetylcholinesterase activity changes in Jenynsia multidentata exposed to chlorpyrifos and cypermethrin individually and in mixtures. Ecotoxicol. Environ. Saf. 129, 311–319. https://doi.org/10.1016/j.ecoenv.2016.03.043.

Braz-Mota, S., Sadauskas-Henrique, H., Duarte, R.M., Val, A.L., Almeida-Val, V.M., 2015. Roundup(R) exposure promotes gills and liver impairments, DNA damage and inhibition of brain cholinergic activity in the Amazon teleost fish Colossoma macropomum. Chemosphere 135, 53–60. https://doi.org/10.1016/j.chemosphere.2015.03.042.

Brimijoin, S., Chen, V.P., Pang, Y.P., Geng, L., Gao, Y., 2016. Physiological roles for butyrylcholinesterase: a BChE-ghrelin axis. Chem. Biol. Interact. 259, 271–275. https://doi.org/10.1016/j.cbi.2016.02.013.

Calfee, R.D., Little, E.E., Puglis, H.J., Scott, E., Brumbaugh, W.G., Mebane, C.A., 2014. Acute sensitivity of white sturgeon (Acipenser transmontanus) and rainbow trout (Oncorhynchus mykiss) to copper, cadmium, or zinc in water-only laboratory exposures. Environ. Toxicol. Chem. 33, 2259–2272. https://doi.org/10.1002/etc.2684.

Casida, J.E., Bryant, R.J., 2017. The ABCs of pesticide toxicology: amounts, biology, and chemistry. Toxicol. Res. (Camb). 6, 755–763. https://doi.org/10.1039/c7tx00198c.

Casida, J.E., Durkin, K.A., 2013a. Anticholinesterase insecticide retrospective. Chem. Biol. Interact. 203, 221–225. https://doi.org/10.1016/j.cbi.2012.08.002.

Casida, J.E., Durkin, K.A., 2013b. Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. Annu. Rev. Entomol. 58, 99–117. https://doi.org/10.1146/annurev-ento-120811-153645.

Cattaneo, R., Moraes, B.S., Loro, V.L., Pretto, A., Menezes, C., Sartori, G.M.S., Clasen, B., de Avila, L.A., Marchesan, E., Zanella, R., 2012. Tissue biochemical alterations of Cyprinus carpio exposed to commercial herbicide containing Clomazone under rice-field conditions. Arch. Environ. Contam. Toxicol. 62, 97–106. https://doi.org/10.1007/s00244-011-9669-8.

Chandrasekara, L.W., Pathiratne, A., 2007. Body size-related differences in the inhibition of brain acetylcholinesterase activity in juvenile Nile tilapia (Oreochromis niloticus) by chlorpyrifos and carbosulfan. Ecotoxicol Env. Saf 67, 109–119. https://doi.org/10.1016/j.ecoenv.2006.04.002.

Chuiko, G.M., Zhelnin, Y., PoD'Gornaya, V.A., 1997. Seasonal fluctuations 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. https://doi.org/10.1016/S0742-8413(97)00068-6.

Clasen, B., Loro, V.L., Murussi, C.R., Tiecher, T.L., Moraes, B., Zanella, R., 2018. Bioaccumulation and oxidative stress caused by pesticides in Cyprinus carpio reared in a rice-fish system. Sci. Total Environ. 626, 737–743. https://doi.org/10.1016/j.scitotenv.2018.01.154.

Costa, L.G., 2015. The neurotoxicity of organochlorine and pyrethroid pesticides. In: Lotti, M., Bleecker, M.L. (Eds.), Handbook of Clinical Neurology. Elsevier B.V, pp. 135–148 https://doi.org/10.1016/B978-0-444-62627-1.00009-3.

Cuevas, N., Martins, M., Costa, P.M., 2018. Risk assessment of pesticides in estuaries: a review addressing the persistence of an old problem in complex environments. Ecotoxicology 27, 1008–1018. https://doi.org/10.1007/s10646-018-1910-z.

Da Cuña, R.H., Rey Vázquez, G., Piol, M.N., Guerrero, N.V., Maggese, M.C., Lo Nostro, F.L., 2011. Assessment of the acute toxicity of the organochlorine pesticide endosulfan in Cichlasoma dimerus (Teleostei, Perciformes). Ecotoxicol. Environ. Saf. 74, 1065–1073. https://doi.org/10.1016/j.ecoenv.2011.02.002.

de Moura, F.R., Brentegani, K.R., Gemelli, A., Sinhorin, A.P., Gindri Sinhorin, V.D., 2017. Oxidative stress in the hybrid fish jundiara (Leiarius marmoratus x Pseudoplatystoma reticulatum) exposed to Roundup Original (R). Chemosphere 185, 445–451. https://doi.org/10.1016/j.chemosphere.2017.07.030.

Dias Assis, C.R., Souza, R., Carvalho Jr., L.B., 2011. Fish cholinesterases as biomarkers of organophosphorus and carbamate pesticides. In: Stoytcheva, M. (Ed.), Pesticides in the Modern World - Pests Control and Pesticides Exposure and Toxicity Assessment. InTech, Rijeka, pp. 253–278 https://doi.org/10.5772/20777.

Dupuy, C., Cabon, J., Louboutin, L., Le Floch, S., Morin, T., Danion, M., 2019. Cellular, humoral and molecular responses in rainbow trout (Oncorhynchus mykiss) exposed to a herbicide and subsequently infected with infectious hematopoietic necrosis virus. Aquat. Toxicol. 215, 105282. https://doi.org/10.1016/j.aquatox.2019.105282.

Ensibi, C., Hernandez-Moreno, D., Miguez Santiyan, M.P., Yahya, M.N.D., Soler Rodriguez, F., Perez-Lopez, M., 2014. Effects of carbofuran and deltamethrin on acetylcholinesterase activity in brain and muscle of the common carp. Environ. Toxicol. 29, 386–393. https://doi.org/10.1002/tox.21765.

Fajardo, L.J., Ocampo, P.P., 2018. Inhibition of acetylcholinesterase activities in whitegoby, Glossogobius giuris from the East Bay of Laguna Lake, Philippines. Int. J. Agric. Technol. 14, 1181–1192.

Falugi, C., Aluigi, M.G., 2012. Early appearance and possible functions of non-neuromuscular cholinesterase activities. Front. Mol. Neurosci. 5, 1–19. https://doi.org/10.3389/fnmol.2012.00054.

Felix, M., Holst, N., Sharp, A., 2019. PestTox: an object oriented model for modeling fate and transport of pesticides in the environment and their effects on population dynamics of non-target organisms. Comput. Electron. Agric. 166. https://doi.org/10.1016/j.compag.2019.105022.

Flammarion, P., Noury, P., Garric, J., 2002. The measurement of cholinesterase activities as a biomarker in chub (Leuciscus cephalus): the fish length should not be ignored. Environ. Pollut. 120, 325–330. https://doi.org/10.1016/s0269-7491(02)00137-9.

Folmar, L.C., Sanders, H.O., Julin, A.M., 1979. Toxicity of herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. Arch Environ Contam Toxicol. 8, 269–278. https://doi.org/10.1007/BF01056243.

Food and Agriculture Organization of the United Nations, 2019. Database Collection of the Food and Agriculture Organization of the United Nations (FAOSTAT). http://www.fao.org/faostat/en/#data. (Accessed July 2020).

Gaworecki, K.M., Roberts, A.P., Ellis, N., Sowers, A.D., Klaine, S.J., 2009. Biochemical and behavioral effects of diazinon exposure in hybrid striped bass. Env. Toxicol Chem 28, 105–112. https://doi.org/10.1897/08-001.1.

Gholami-Seyedkolaei, S.J., Mirvaghefi, A., Farahmand, H., Kosari, A.A., Gholami-Seyedkolaei, S.J., Gholami-Seyedkolaei, S.J., 2013. Optimization of recovery patterns in common carp exposed to roundup using response surface methodology: evaluation of neurotoxicity and genotoxicity effects and biochemical parameters. Ecotoxicol Env. Saf 98, 152–161. https://doi.org/10.1016/j.ecoenv.2013.09.009.

Gibbons, D., Morrissey, C., Mineau, P., 2015. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. Environ. Sci. Pollut. Res. 22, 103–118. https://doi.org/10.1007/s11356-014-3180-5.

Glusczak, L., dos Santos Miron, D., Crestani, M., Braga da Fonseca, M., de Araujo Pedron, F., Duarte, M.F., Vieira, V.L., 2006. Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (Leporinus obtusidens). Ecotoxicol Env. Saf 65, 237–241. https://doi.org/10.1016/j.ecoenv.2005.07.017.

Golombieski, J.I., Sutili, F.J., Salbego, J., Seben, D., Gressler, L.T., da Cunha, J.A., Gressler, L.T., Zanella, R., Vaucher Rde, A., Marchesan, E., Baldisserotto, B., 2016. Imazapyr +imazapic herbicide determines acute toxicity in silver catfish Rhamdia quelen. Ecotoxicol Env. Saf 128, 91–99. https://doi.org/10.1016/j.ecoenv.2016.02.010.

* Indicates studies included in the meta-analysis.

Gormley, K.L., Teather, K.L., Guignion, D.L., 2005. Changes in salmonid communities associated with pesticide runoff events. Ecotoxicology 14, 671–678. https://doi.org/10.1007/s10646-005-0017-5.

Guilherme, S., Gaivão, I., Santos, M.A., Pacheco, M., 2012. DNA damage in fi sh (Anguilla anguilla) exposed to a glyphosate-based herbicide–elucidation of organ-specifi city and the role of oxidative stress. Mutat Res. 743, 1–9. https://doi.org/10.1016/j.mrgentox.2011.10.017.

Guiloski, I.C., Rossi, S.C., Da Silva, C.A., Silva De Assis, H.C., 2013. Insecticides biomarker responses on a freshwater fish Corydoras paleatus (Pisces: Callichthyidae). J. Environ. Sci. Heal. Part B-Pesticides Food Contam. Agric. Wastes 48, 272–277. https://doi.org/10.1080/03601234.2013.743774.

Guimaraes, A.T., Silva de Assis, H.C., Boeger, W., 2007. The effect of trichlorfon on acetylcholinesterase activity and histopathology of cultivated fish Oreochromis niloticus. Ecotoxicol Env. Saf 68, 57–62. https://doi.org/10.1016/j.ecoenv.2006.08.005.

Hedges, L.V., Gurevitch, J., Curtis, P.S., 1999. The meta-analysis of response ratios in experimental ecology. Ecology 80, 1150–1156. https://doi.org/10.1890/0012-9658(1999)080[1150:TMAORR]2.0.CO;2.

Higgins, J.P.T., Thompson, S.G., Deeks, J.J., Altman, D.G., 2003. Measuring inconsistency in meta-analyses. Br. Med. J. 327, 557–560. https://doi.org/10.1136/bmj.327.7414.557.

Huang, Y., Zhang, J.-S., Han, X.-B., Huang, T.-L., Chan, A.K.Y., 2016. Monitoring behavioral responses of zebrafish (Danio rerio) as a biomarker for identifying cadmium and deltamethrin in water. Environ. Eng. Manag. J. 15, 2171–2179.

Humphrey, C., Klumpp, D.W., 2003. Toxicity of chlorpyrifos to the early life history stages of eastern rainbowfish Melanotaenia splendida splendida (Peters 1866) in tropical Australia. Environ. Toxicol. 18, 418–427. https://doi.org/10.1002/tox.10144.

Jayaraj, R., Megha, P., Sreedev, P., 2016. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. Interdiscip. Toxicol. 9, 90–100. https://doi.org/10.1515/intox-2016-0012.

Jennions, M.D., Lortie, C.J., Rosenberg, M.S., Rothstein, H.R., 2013. Publication and related biases (Handbook of meta-analysis in ecology and evolution). In: Koricheva, J., Gurevitch, J., Mengersen, K. (Eds.), Princeton University Press, Princeton, NJ, pp. 207–236.

Kaviraj, A., Gupta, A., 2014. Biomarkers of type II synthetic pyrethroid pesticides in freshwater fish. Biomed. Res. Int. 2014, 928063. https://doi.org/10.1155/2014/928063.

Kim, K.H., Kabir, E., Jahan, S.A., 2017. Exposure to pesticides and the associated human health effects. Sci. Total Environ. 575, 525–535. https://doi.org/10.1016/j.scitotenv.2016.09.009.

Köhler, H.R., Triebskorn, R., 2013. Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond? Science (80-.) 341, 759–765. https://doi.org/10.1126/science.1237591.

Laetz, C.A., Baldwin, D.H., Hebert, V., Stark, J.D., Scholz, N.L., 2013. Interactive neurobehavioral toxicity of diazinon, malathion, and ethoprop to juvenile coho salmon. Environ. Sci. Technol. 47, 2925–2931. https://doi.org/10.1021/es305058y.

Lockridge, O., Norgren, R.B., Johnson, R.C., Blake, T.A., 2016. Naturally occurring genetic variants of human acetylcholinesterase and butyrylcholinesterase and their potential impact on the risk of toxicity from cholinesterase inhibitors. Chem. Res. Toxicol. 29, 1381–1392. https://doi.org/10.1021/acs.chemrestox.6b00228.

Lopes, R.M., Filho, M.V.S., de Salles, J.B., Bastos, V.L.F.C., Bastos, J.C., 2014. Cholinesterase activity of muscle tissue from freshwater fishes: characterization and sensitivity analysis to the organophosphate methyl-paraoxon. Environ. Toxicol. Chem. 33, 1331–1336. https://doi.org/10.1002/etc.2556.

Lundin, S.J., 1962. Comparative studies of cholinesterases in body muscles of fishes. J. Cell. Comp. Physiol. 59, 93–105. https://doi.org/10.1002/jcp.1030590202.

Maggi, F., Tang, F.H.M., la Cecilia, D., McBratney, A., 2019. PEST-CHEMGRIDS, global gridded maps of the top 20 crop-specific pesticide application rates from 2015 to 2025. Sci. data 6, 170. https://doi.org/10.1038/s41597-019-0169-4.

Maggi, F., la Cecilia, D., Tang, F.H.M., McBratney, A., 2020. The global environmental hazard of glyphosate use. Sci. Total Environ. 717, 137167. https://doi.org/10.1016/j.scitotenv.2020.137167.

Mangas, I., Estevez, J., Vilanova, E., França, T.C.C., 2017. New insights on molecular interactions of organophosphorus pesticides with esterases. Toxicology 376, 30–43. https://doi.org/10.1016/j.tox.2016.06.006.

Marigoudar, S.R., Ahmed, R.N., David, M., 2009. Cypermethrin induced: in vivo inhibition of the acetylcholinesterase activity in functionally different tissues of the freshwater teleost, Labeo rohita (Hamilton). Toxicol. Environ. Chem. 91, 1175–1182. https://doi.org/10.1080/02772240802577282.

Martínez-Morcillo, S., Pérez-López, M., Míguez, M.P., Valcárcel, Y., Soler, F., 2019. Comparative study of esterase activities in different tissues of marine fish species Trachurus trachurus, Merluccius merluccius and Trisopterus luscus. Sci. Total Environ. 679, 12–22. https://doi.org/10.1016/j.scitotenv.2019.05.047.

Massoulié, J., Toutant, J.-P., 1988. Vertebrate cholinesterases: structure and types of interaction. In: Whittaker, V.P. (Ed.), The Cholinergic Synapse. Springer-Verlag, New York, pp. 167–224 https://doi.org/10.1007/978-3-642-73220-1_8.

Massoulié, J., Pezzementi, L., Bon, S., Krejci, E., Vallette, F.M., 1993. Molecular and cellular biology of cholinesterases. Prog. Neurobiol. 41, 31–91. https://doi.org/10.1016/0301-0082(93)90040-Y.

McKim, J.M., 1977. Evaluation of tests with early life stages of fish for predicting long-term toxicity. J. Fish. Res. Board Canada 34, 1148–1154. https://doi.org/10.1139/f77-172.

Mela, M., Guiloski, I.C., Doria, H.B., Randi, M.A., de Oliveira Ribeiro, C.A., Pereira, L., Maraschi, A.C., Prodocimo, V., Freire, C.A., Silva de Assis, H.C., 2013. Effects of the herbicide atrazine in neotropical catfish (Rhamdia quelen). Ecotoxicol Env. Saf 93, 13–21. https://doi.org/10.1016/j.ecoenv.2013.03.026.

Mesnage, R., Antoniou, M.N., 2017. Facts and fallacies in the debate on glyphosate toxicity. Front. Public Heal. 5, 1–7. https://doi.org/10.3389/fpubh.2017.00316.

Mesnage, R., Benbrook, C., Antoniou, M.N., 2019. Insight into the confusion over surfactant co-formulants in glyphosate-based herbicides. Food Chem. Toxicol. 128, 137–145. https://doi.org/10.1016/j.fct.2019.03.053.

Mhadhbi, L., Beiras, R., 2012. Acute toxicity of seven selected pesticides (alachlor, atrazine, dieldrin, diuron, pirimiphos-methyl, chlorpyrifos, diazinon) to the marine fish (turbot, Psetta maxima). Water Air Soil Pollut. 223, 5917–5930. https://doi.org/10.1007/s11270-012-1328-9.

Mineau, P., 2018. Organophosphorous and carbamate insecticides: impacts on birds. Encyclopedia of the Anthropocene. Elsevier Inc https://doi.org/10.1016/B978-0-12-809665-9.09887-6.

Miron, D.D., Crestani, M., Shettinger, M.R., Morsch, V.M., Baldisserotto, B., Tierno, M.A., Moraes, G., Vieira, V.L.P., 2005. Effects of the herbicides clomazone, quinclorac, and metsulfuron methyl on acetylcholinesterase activity in the silver catfish (Rhamdia quelen) (Heptapteridae). Ecotoxicol. Environ. Saf. 61, 398–403. https://doi.org/10.1016/j.ecoenv.2004.12.019.

Miron, D. d S., Pretto, A., Crestani, M., Glusczak, L., Schetinger, M.R., Loro, V.L., Morsch, V.M., 2008. Biochemical effects of clomazone herbicide on piava (Leporinus obtusidens). Chemosphere 74, 1–5. https://doi.org/10.1016/j.chemosphere.2008.09.070.

Modesto, K.A., Martinez, C.B., 2010. Roundup causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish Prochilodus lineatus. Chemosphere 78, 294–299. https://doi.org/10.1016/j.chemosphere.2009.10.047.

Mohammed, A., 2013. Why are early life stages of aquatic organisms more sensitive to toxicants than adults? In: Gowder, S. (Ed.), New Insights into Toxicity and Drug Testing. IntechOpen, Rijeka https://doi.org/10.5772/55187

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., Grp, P., 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement (reprinted from annals of internal medicine). Ann. Intern. Med. 151, 264–269. https://doi.org/10.1371/journal.pmed.1000097.

Mostafalou, S., Abdollahi, M., 2017. Pesticides: an update of human exposure and toxicity. Arch. Toxicol. 91, 549–599. https://doi.org/10.1007/s00204-016-1849-x.

Murussi, C.R., Costa, M., Menezes, C., Leitemperger, J., Guerra, L., Lopes, T., 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. Contam. Toxicol. 68, 646–654. https://doi.org/10.1007/s00244-015-0145-8.

Nakagawa, S., Santos, E.S.A., 2012. Methodological issues and advances in biological meta-analysis. Evol. Ecol. 26, 1253–1274. https://doi.org/10.1007/s10682-012-9555-5.

Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol. Evol. 4, 133–142. https://doi.org/10.1111/j.2041-210x.2012.00261.x.

Nakagawa, S., Poulin, R., Mengersen, K., Reinhold, K., Engqvist, L., Lagisz, M., Senior, A.M., 2015. Meta-analysis of variation: ecological and evolutionary applications and beyond. Methods Ecol. Evol. 6, 143–152. https://doi.org/10.1111/2041-210X.12309.

Narra, M.R., Rajender, K., Reddy, R.R., Murty, U.S., Begum, G., 2017. Insecticides induced stress response and recuperation in fish: biomarkers in blood and tissues related to oxidative damage. Chemosphere 168, 350–357. https://doi.org/10.1016/j.chemosphere.2016.10.066.

Nunes, B., 2011. The use of cholinesterases in ecotoxicology. In: Whitacre, D.M. (Ed.), Reviews of Environmental Contamination and Toxicology. vol 212, pp. 29–59. https://doi.org/10.1007/978-1-4419-8453-1_2.

O'Dea, R.E., Lagisz, M., Hendry, A.P., Nakagawa, S., 2019. Developmental temperature affects phenotypic means and variability: a meta-analysis of fish data. Fish Fish. 20, 1005–1022. https://doi.org/10.1111/faf.12394.

Pereira, L., Fernandes, M.N., Martinez, C.B., 2013. Hematological and biochemical alterations in the fish Prochilodus lineatus caused by the herbicide clomazone. Env. Toxicol Pharmacol 36, 1–8. https://doi.org/10.1016/j.etap.2013.02.019.

Pereira, B.V.R., Silva-Zacarin, E.C.M., Costa, M.J., Dos Santos, A.C.A., do Carmo, J.B., Nunes, B., 2019. Cholinesterases characterization of three tropical fish species, and their sensitivity towards specific contaminants. Ecotoxicol. Environ. Saf. 173, 482–493. https://doi.org/10.1016/j.ecoenv.2019.01.105.

Peters, L.D., Livingstone, D.R., 1995. Studies on cytochrome P4501A in early and adult life stages of turbot (Scophthalmus maximus L.). Mar. Environ. Res. 39, 5–9. https://doi.org/10.1016/0141-1136(94)00064-V.

Pezzementi, L., Nachon, F., Chatonnet, A., 2011. Evolution of acetylcholinesterase and butyrylcholinesterase in the vertebrates: an atypical butyrylcholinesterase from the medaka oryzias latipes. PLoS One 6. https://doi.org/10.1371/journal.pone.0017396.

Pick, J.L., Nakagawa, S., Noble, D.W.A., 2018. Reproducible, flexible and high-throughput data extraction from primary literature: the metaDigitise r package. Methods Ecol. Evol. 10, 426–431. https://doi.org/10.1111/2041-210X.13118.

Pimentel, D., 1995. Amounts of pesticides reaching target pests: environmental impacts and ethics. J. Agric. Environ. Ethics 8, 17–29. https://doi.org/10.1007/BF02286399.

Pope, C.N., Brimijoin, S., 2018. Cholinesterases and the fine line between poison and remedy. Biochem. Pharmacol. 153, 205–216. https://doi.org/10.1016/j.bcp.2018.01.044.

Prieto Garcia, F., Cortés Ascencio, S.Y., Oyarzun, J.C.G., Hernandez, A.C., Alavarado, P.V., 2012. Pesticides: classification, uses and toxicity. Measures of exposure and genotoxic risks. J. Res. Environ. Sci. Toxicol. 1, 2315–5698.

Qiu, X., Nomichi, S., Chen, K., Honda, M., Kang, I.J., Shimasaki, Y., Oshima, Y., 2017. Short-term and persistent impacts on behaviors related to locomotion, anxiety, and startle responses of Japanese medaka (Oryzias latipes) induced by acute, sublethal exposure to chlorpyrifos. Aquat. Toxicol. 192, 148–154. https://doi.org/10.1016/j.aquatox.2017.09.012.

R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria https://www.R-project.org/.

Relyea, R.A., 2005. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. Ecol. Appl. 15, 618–627. https://doi.org/10.1890/03-5342.

Rodríguez-Fuentes, G., Gold-Bouchot, G., 2004. Characterization of cholinesterase activity from different tissues of Nile tilapia (Oreochromis niloticus). Mar. Environ. Res. 58, 505–509. https://doi.org/10.1016/j.marenvres.2004.03.037.

Rodríguez-Fuentes, G., Marín-López, V., Hernández-Márquez, E., 2016. Cholinesterases in Gambusia yucatana: biochemical characterization and its relationship with sex and total length. Bull. Environ. Contam. Toxicol. 97, 776–780. https://doi.org/10.1007/s00128-016-1939-8.

Rösler, T.W., Salama, M., Shalash, A.S., Khedr, E.M., El-Tantawy, A., Fawi, G., El-Motayam, A., El-Seidy, E., El-Sherif, M., El-Gamal, M., Moharram, M., El-Kattan, M., Abdel-Naby, M., Ashour, S., Müller, U., Dempfle, A., Kuhlenbäumer, G., Höglinger, G.U., 2018. K-variant BCHE and pesticide exposure: gene-environment interactions in a case–control study of Parkinson's disease in Egypt. Sci. Rep. 8, 1–8. https://doi.org/10.1038/s41598-018-35003-4.

Salbego, J., Pretto, A., Gioda, C.R., De Menezes, C.C., Lazzari, R., Radünz Neto, J., Baldisserotto, B., Loro, V.L., 2010. Herbicide formulation with glyphosate affects growth, acetylcholinesterase activity, and metabolic and hematological parameters in Piava (leporinus obtusidens). Arch. Environ. Contam. Toxicol. 58, 740–745. https://doi.org/10.1007/s00244-009-9464-y.

Sanchez, J.A., Varela, A.S.J., Corcini, C.D., da Silva, J.C., Primel, E.G., Caldas, S., Klein, R.D., Martins, C.M., 2017. Effects of roundup formulations on biochemical biomarkers and male sperm quality of the livebearing Jenynsia multidentata. Chemosphere 177, 200–210. https://doi.org/10.1016/j.chemosphere.2017.02.147.

Sandoval-Herrera, N., Mena, F., Espinoza, M., Romero, A., 2019. Neurotoxicity of organophosphate pesticides could reduce the ability of fish to escape predation under low doses of exposure. Sci. Rep. 9, 1–11. https://doi.org/10.1038/s41598-019-46804-6.

Schäfer, R.B., Van Den Brink, P.J., Liess, M., 2011. Impacts of pesticides on freshwater ecosystems. In: Sánchez-Bayo, F., van den Brink, P.J., Mann, R.M. (Eds.), Ecological Impacts of Toxic Chemicals (Open Access). BENTHAM SCIENCE PUBL LTD, pp. 111–137 https://doi.org/10.2174/978160805121211101010111.

Scott, G.R., Sloman, K.A., 2004. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquat. Toxicol. 68, 369–392. https://doi.org/10.1016/j.aquatox.2004.03.016.

Sharbidre, A.A., Metkari, V., Patode, P., 2011. Effect of methyl parathion and chlorpyrifos on certain biomarkers in various tissues of guppy fish, Poecilia reticulata. Pestic. Biochem. Physiol. 101, 132–141. https://doi.org/10.1016/j.pestbp.2011.09.002.

Simon-Delso, N., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Chagnon, M., Downs, C., Furlan, L., Gibbons, D.W., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C.H., Liess, M., Long, E., Mcfield, M., Mineau, P., Mitchell, E.A., Morrissey, C.A., Noome, D.A., Pisa, L., Settele, J., Stark, J.D., Tapparo, A., Van Dyck, H., Van Praagh, J., Van Der Sluijs, J.P., Whitehorn, P.R., Wiemers, M., 2015. Systemic insecticides (Neonicotinoids and fipronil): trends, uses, mode of action and metabolites. Environ. Sci. Pollut. Res. 22, 5–34. https://doi.org/10.1007/s11356-014-3470-y.

Stanley, J., Preetha, G., 2016. Pesticide toxicity to non-target organisms - exposure, toxicity and risk assessment methodologies. Pesticide Toxicity to Non-target Organisms. Springer Nature https://doi.org/10.1007/978-94-017-7752-0.

Sturm, A., de Assis, H.C.D., Hansen, P.D., 1999a. Cholinesterases of marine teleost fish: enzymological characterization and potential use in the monitoring of neurotoxic contamination. Mar. Environ. Res. 47, 389–398. https://doi.org/10.1016/s0141-1136(98)00127-5.

Sturm, Armin, Wogram, J., Hansen, P.D., Liess, M., 1999b. Potential use of cholinesterase in monitoring low levels of organophosphates in small streams: natural variability in three-spined stickleback (Gasterosteus aculeatus) and relation to pollution. Environ. Toxicol. Chem. 18, 194–200. https://doi.org/10.1897/1551-5028(1999)018<0194:PUOCIM>2.3.CO;2.

Sturve, J., Scarlet, P., Halling, M., Kreuger, J., Macia, A., 2016. Environmental monitoring of pesticide exposure and effects on mangrove aquatic organisms of Mozambique. Mar. Environ. Res. 121, 9–19. https://doi.org/10.1016/j.marenvres.2016.05.005.

Teixeira, J.M.S., da Silva Lima, V., de Moura, F.R., da Costa Marisco, P., Sinhorin, A.P., Sinhorin, V.D.G., 2018. Acute toxicity and effects of roundup original® on pintado da Amazônia. Environ. Sci. Pollut. Res. 25, 25383–25389. https://doi.org/10.1007/s11356-018-2630-x.

Tian, X., Yang, W., Wang, D., Zhao, Y., Yao, R., Ma, L., Ge, C., Li, X., Huang, Z., He, L., Jiao, W., Lin, A., 2018. Chronic brain toxicity response of juvenile Chinese rare minnows (Gobiocypris rarus) to the neonicotinoid insecticides imidacloprid and nitenpyram. Chemosphere 210, 1006–1012. https://doi.org/10.1016/j.chemosphere.2018.06.083.

Tortelli, V., Colares, E.P., Robaldo, R.B., Nery, L.E.M., Pinho, G.L.L., Bianchini, A., Monserrat, J.M., 2006. Importance of cholinesterase kinetic parameters in environmental monitoring using estuarine fish. Chemosphere 65, 560–566. https://doi.org/10.1016/j.chemosphere.2006.02.047.

Tsui, M.T., Chu, L.M, 2003. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. Chemosphere . 52, 1189–1197. https://doi.org/10.1016/S0045-6535(03)00306-0.

Ullah, S., Li, Z., Ul Arifeen, M.Z., Khan, S.U., Fahad, S., 2019. Multiple biomarkers based appraisal of deltamethrin induced toxicity in silver carp (Hypophthalmichthys molitrix). Chemosphere 214, 519–533. https://doi.org/10.1016/j.chemosphere.2018.09.145.

van den Berg, S.J.P., Maltby, L., Sinclair, T., Liang, R., van den Brink, P.J., 2021. Cross-species extrapolation of chemical sensitivity. Sci. Total Environ. 753, 141800. https://doi.org/10.1016/j.scitotenv.2020.141800.

Varò, I., Navarro, J.C., Amat, F., Guilhermino, L., 2003. Effect of dichlorvos on cholinesterase activity of the European sea bass (Dicentrarchus labrax). Pestic. Biochem. Physiol. 75, 61–72. https://doi.org/10.1016/S0048-3575(03)00019-1.

Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor. J. Stat. Softw. 36, 1–48.

Viechtbauer, W., 2019. I^2 for multilevel and multivariate models. Retrieved from http://www.metafor-project.org/doku.php/tips:i2_multilevel_multivariate. Accessed on July 2019.

Vieira, C.E.D., dos Reis Martinez, C.B., 2018. The pyrethroid lambda-cyhalothrin induces biochemical, genotoxic, and physiological alterations in the teleost Prochilodus lineatus. Chemosphere 210, 958–967. https://doi.org/10.1016/j.chemosphere.2018.07.115.

Vieira, C.E.D., Pérez, M.R., Acayaba, R.D., Raimundo, C.C.M., dos Reis Martinez, C.B., 2018. DNA damage and oxidative stress induced by imidacloprid exposure in different tissues of the Neotropical fish Prochilodus lineatus. Chemosphere 195, 125–134. https://doi.org/10.1016/j.chemosphere.2017.12.077.

Wendelaar Bonga, S.E., 1997. The stress response in fish. Physiol. Rev. 77, 591–625. https://doi.org/10.1152/physrev.1997.77.3.591.

Werner, I., Young, T.M., 2017. Pyrethroid insecticides-exposure and impacts in the aquatic environment. Encyclopedia of the Anthropocene. Elsevier Inc https://doi.org/10.1016/B978-0-12-809665-9.09992-4.

Whyte, J.J., Jung, R.E., Schmitt, C.J., Tillitt, D.E., 2000. Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. Crit. Rev. Toxicol. 30, 347–570. https://doi.org/10.1080/10408440091159239.

World Health Organization & Food and Agriculture Organization of the United Nations, 2019. Global Situation of Pesticide Management in Agriculture and Public Health - Report of 2018 WHO-FAO Survey. Geneva.

Yamamuro, M., Komuro, T., Kamiya, H., Kato, T., Hasegawa, H., Kameda, Y., 2019. Neonicotinoids disrupt aquatic food webs and decrease fishery yields. Science (80-.). 366, 620–623. https://doi.org/10.1126/science.aax3442.

Zhang, X.J., Yang, L., Zhao, Q., Caen, J.P., He, H.Y., Jin, Q.H., Guo, L.H., Alemany, M., Zhang, L.Y., Shi, Y.F., 2002. Induction of acetylcholinesterase expression during apoptosis in various cell types. Cell Death Differ. 9, 790–800. https://doi.org/10.1038/sj.cdd.4401034.

Material Suplementar - Capítulo II

Pesticide effects on fish cholinesterase variability and mean activity: A meta-analytic review.

FIGURES

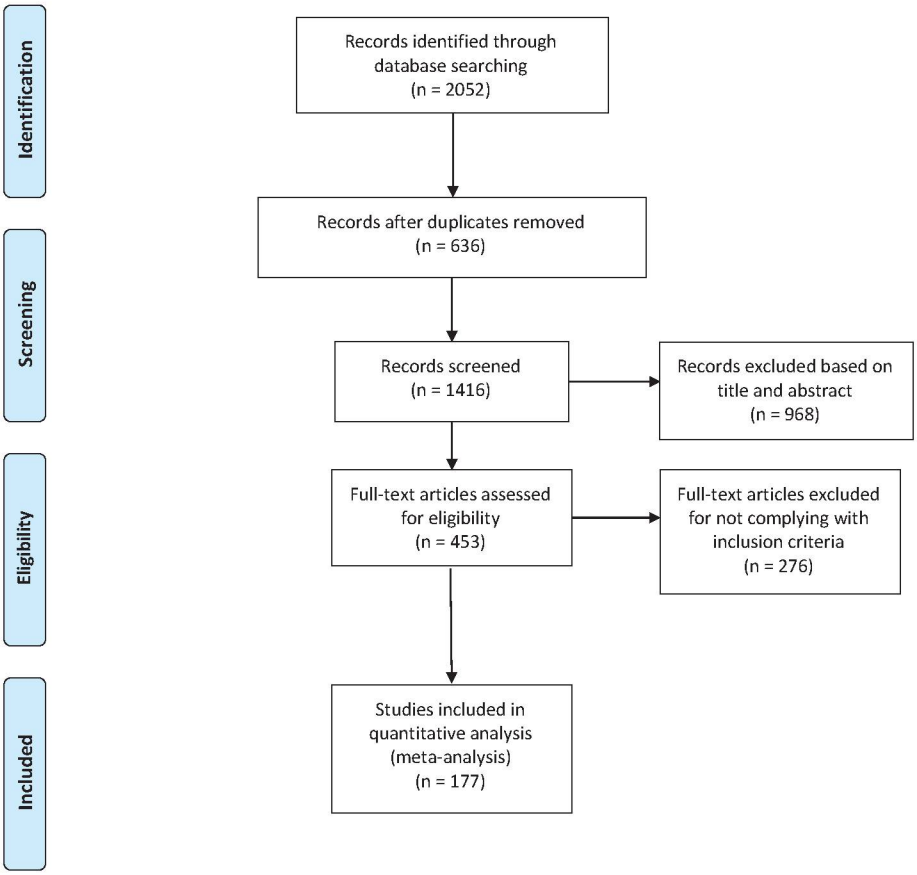


Figure S1 Screening process used to exclude or retain articles for our meta-analysis; summarized and formatted as a PRISMA flow diagram (Moher et al., 2009).

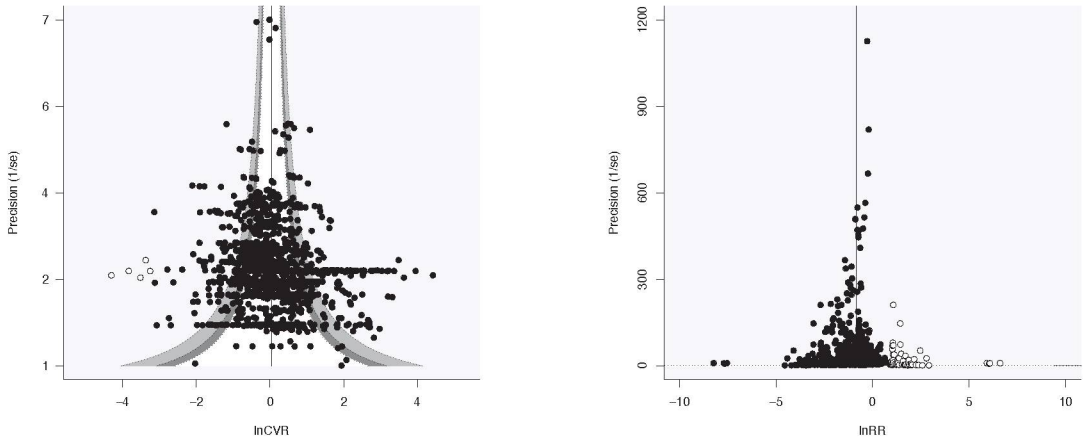


Figure S2 Funnel plots showing the distribution of effect sizes, represented by dotes, around the meta-analytic mean, for meta-analysis of (a) variance differences (*lnCVR*) and (b) mean differences (*lnRR*). The y-axis represents the precision of the estimates (inverse of the standard error) from the model which accounted for heterogeneity the most, the model with class of pesticide as moderator variable.

TABLES

Table S1

List of all extracted data and data description.

Field Name	Description
es ID	unique ID for each row of data (i.e. each effect size)
paper ID	unique ID for pairwise comparison of an independent control and treatment group within papers.
author	authors of the paper
method	method by which cholinesterase activity was measured (e.g. Ellman et al., 1961; Coppage 1971)
substrate	substrate used to measure cholinesterase activity (e.g. acetylcholine, acetylthiocholine, butyrylcholine)
year	publication year of the paper
journal	journal the paper was published
country	country where the study was conducted
species name	species name for the fish
environment	environment where the fish is commonly found (3 levels: marine, freshwater and estuarine)
length_cm	length of the fish in centimetres
weight_g	weight of the fish in grams
biomarker	type of cholinesterase measured (2 levels: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE))
age	sexual maturity of fish (2 levels: adult and juveline)
approach	experimental approach of the study (3 levels: field, field caging and laboratory)
pesticide	name of the pesticide active ingredient used in the experiment
type	classification of pesticide according of target species (3 levels: herbicide, insecticide, fungicide)
class	classification of pesticides according to chemical structure (20 levels: anilides, aromatic acid, carbamate, derived from microbes, isoxazolidinona, neonicotinoid, organochlorine, organochlorine_f (f=fungicide), organophosphorus, organophosphorus_h (h=herbicide), phenoxy, phenylpyrazole, phenylpyridine, pyrethroid, pyridine, pyrimidine, QUATs, triazine, triazole, ureas)
grade	chemical grade of the pesticide used (2 levels: analytical, commercial)
dose (mg/l)	concentration of the pesticide used in the experiment in milligrams per litre
time (days)	number of days fish was exposed to pesticides
tissue	tissue excised from exposed fish and analysed for cholinesterase activity
Nc	sample size of fish in the control group
Mc	mean of the control group

SDc	standard deviation of the control group
Nt	sample size of the treatment group
Mt	mean of the treatment group
SDt	standard deviation of the treatment group

Table S2

Model diagnostics for difference combinations of 0, 1, 2 or 3 random effects: effect size ID, paper ID and species ID.

Measure	Random Effect	N levels	Variance	AIC
lnCVR	none			8541.960
lnRR				1496993.999
lnCVR	paper ID	557	0.6126	3957.402
lnRR		557	0.3205	624496.411
lnCVR	species ID	81	0.3414	6631.626
lnRR		81	0.5386	1312088.023
lnCVR	paper ID	557	0.5560	3628.814
	es ID	1602	0.1338	
lnRR	paper ID	557	0.2996	2793.205
	es ID	1602	0.1670	
lnCVR	species	81	0.2679	4043.975
	es ID	1602	0.4613	
lnRR	species	81	0.3382	2994.567
	es ID	1602	0.3029	
lnCVR	paper ID	557	0.4035	**3572.358**
	species	81	0.1766	
	es ID	1602	0.1341	
lnRR	paper ID	557	0.1634	**2669.569**
	species	81	0.2144	
	es ID	1602	0.1661	

Table S3

Results of meta-analytic models for variances differences (*lnCVR*) and for mean differences (*lnRR*) between treatment and control groups, fit using *rma.mv*. Covariance = 0 assumes no correlation between sampling variances from the same fish group. Covariance = 0.5 assumes sampling variances for the same group have a 0.5 correlation (i.e. more conservative model). Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* - number of independent studies (paper ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in variability or mean activity.

Measure	**Model**	**Treatment**	**Coefficient**	**Covariance**	**Fixed effects**			↑↓%		
					Mean	CI.lb	CI.ub		*k*	*n*
lnCVR										
	meta-analytic	all	intercept	0	**0.331**	**0.203**	**0.460**	39.2↑	1602	557
				0.5	**0.344**	**0.217**	**0.471**	41.1↑		
lnRR										
	meta-analytic	all	intercept	0	**-0.606**	**-0.726**	**-0.486**	45.4↓	1602	557
				0.5	**-0.629**	**-0.754**	**-0.504**	46.7↓		

Measure	**Model**	**Covariance**		**Random effects**		**Heterogeneity**			
				N levels	Sigma^2	I^2_{Total}	$I^2_{paper_ID}$	$I^2_{species}$	I^2_{esID}
lnCVR	meta-analytic								
		0	paper ID	557	0.4036	83	46	21	15
			species	81	0.1812				
			es ID	1602	0.1341				
		0.5	paper ID	557	0.3822	83	43	18	23
			species	81	0.1568				
			es ID	1602	0.2025				
lnRR	meta-analytic								
		0	paper ID	557	0.1633	100	30	40	30
			species	81	0.2189				
			es ID	1602	0.1661				
		0.5	paper ID	557	0.1740	100	29	38	33
			species	81	0.2327				
			es ID	1602	0.2015				

25

26 **Table S4**

27 Results of meta-regression model assessing cholinesterases and tissue as moderators for variances
28 differences (*lnCVR*) and mean differences (*lnRR*) between treatment and control groups, fit using
29 *rma.mv*. Covariance = 0 assumes no correlation between sampling variances from the same fish
30 group. Covariance = 0.5 assumes sampling variances for the same group have a 0.5 correlation (i.e.
31 more conservative model). Effects with 95% confidence intervals (CI) not crossing zero are indicated
32 in bold. *k* - number of effect sizes included in the analysis. *n* - number of independent studies
33 (paper_ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in
34 variability or mean activity.

Measure	**Biomarker**	**Tissue**	**Coefficient**	**Covariance**	**Fixed effects**			↑↓%		
					Mean	CI.lb	CI.ub		*k*	*n*
lnCVR										
	AChE		intercept							
		Brain		0	**0.321**	**0.192**	**0.449**	37.9↑	1053	362
				0.5	**0.327**	**0.201**	**0.454**	38.7↑		
		Muscle		0	**0.306**	**0.147**	**0.465**	35.8↑	478	172
				0.5	**0.333**	**0.175**	**0.490**	39.5↑		
	BChE		intercept							
		Brain		0	**0.781**	**0.421**	**1.141**	118.4↑	35	11
				0.5	**0.830**	**0.462**	**1.197**	129.3↑		
		Muscle		0	**0.767**	**0.399**	**1.134**	115.3↑	36	12
				0.5	**0.835**	**0.461**	**1.208**	130.5↑		
	BChE-AChE difference		slope							
		Brain		0	**0.460**	**0.114**	**0.807**	58.4↑		
				0.5	**0.502**	**0.147**	**0.858**	65.2↑		
		Muscle		0	**0.460**	**0.114**	**0.807**	58.4↑		
				0.5	**0.502**	**0.147**	**0.858**	65.2↑		
lnRR										
	AChE		intercept							
		Brain		0	**-0.616**	**-0.738**	**-0.493**	46.0↓	1053	362
				0.5	**-0.638**	**-0.766**	**-0.511**	47.2↓		
		Muscle		0	**-0.583**	**-0.722**	**-0.443**	44.2↓	478	172
				0.5	**-0.607**	**-0.752**	**-0.463**	45.5↓		
	BChE		intercept							
		Brain		0	**-0.583**	**-0.844**	**-0.323**	44.2↓	35	11
				0.5	**-0.600**	**-0.877**	**-0.324**	45.1↓		
		Muscle		0	**-0.550**	**-0.817**	**-0.284**	42.3↓	36	12
				0.5	**-0.569**	**-0.852**	**-0.287**	43.4↓		
	BChE-AChE difference		slope							
		Brain		0	0.032	-0.203	0.268	3.3		
				0.5	0.038	-0.213	0.289	3.9		

	Muscle	0	0.032	-0.203	0.268	3.3
		0.5	0.038	-0.213	0.289	3.9

Table S5

Results of meta-regression model assessing fish length and fish age as moderators for variances differences (*lnCVR*) and mean differences (*lnRR*) between treatment and control groups, fit using *rma.mv*. Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* - number of independent studies (paper_ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in variability or mean activity.

Measure	**Age**	**Slope**	**Fixed effects**			↑↓%		
			Mean	CI.lb	CI.ub		*k*	*n*
lnCVR								
	adult	length_cm	0.001	-0.018	0.019	0.1↑	396	124
	juvenile	length_cm	0.004	-0.023	0.031	0.4↑	543	207
	juvenile-adult difference	length_cm	0.003	-0.029	0.036	0.3		
lnRR								
	adult	length_cm	0.001	-0.011	0.014	0.1↑	396	124
	juvenile	length_cm	0.005	-0.013	0.022	0.5↑	543	207
	juvenile-adult difference	length_cm	0.003	-0.018	0.024	0.3		

Table S6

Results of meta-regression model assessing specific ranges of fish length and fish age as moderators for variances differences (*lnCVR*) and mean differences (*lnRR*), between treatment and control groups, fit using *rma.mv*. Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* - number of independent studies (paper_ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in variability or mean activity.

Measure	Age	Slope	Fixed effects			↑↓%		
			Mean	CI.lb	CI.ub		*k*	*n*
lnCVR								
	adult	length ≤ 10cm	-0.070	-0.226	0.086	6.8↓	247	73
		10< length ≤ 20cm	-0.012	-0.112	0.088	1.2↓	125	41
	juvenile	length ≤ 10cm	**0.141**	**0.056**	**0.226**	15.1↑	358	132
		10< length ≤ 20cm	-0.057	-0.106	-0.007	5.5↓	169	67
	juvenile-adult difference	length ≤ 10cm	**0.211**	**0.030**	**0.391**	23.5↑		
	juvenile-adult difference	10< length ≤ 20cm	-0.045	-0.151	0.062	4.4↓		
lnRR								
	adult	length ≤ 10cm	0.042	-0.055	0.139	4.3↑	247	73
		10< length ≤ 20cm	-0.054	-0.152	0.044	5.3↓	125	41
	juvenile	length ≤ 10cm	**-0.064**	**-0.117**	**-0.011**	6.2↓	358	132
		10< length ≤ 20cm	**-0.053**	**-0.092**	**-0.014**	5.1↓	169	67
	juvenile-adult difference	length ≤ 10cm	-0.106	-0.218	0.006	10.0		

	juvenile-adult difference	10< length ≤ 20cm	0.0011	-0.099	0.101	0.1	

53

54

55 **Table S7**

56 Results of meta-regression model assessing type of pesticides and grade as moderators for variances
57 differences (*lnCVR*) and mean differences (*lnRR*) between treatment and control groups, fit using
58 *rma.mv*. Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* -
59 number of effect sizes included in the analysis. *n* - number of independent studies (paper_ID)
60 included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in variability or
61 mean activity.

Measure	**Type**	**Grade**	**Coefficient**	**Fixed effects**			↑↓%		
				Mean	CI.lb	CI.ub		*k*	*n*
lnCVR									
	Herbicide		Intercept						
		Analytical		**0.375**	**0.144**	**0.606**	45.5↑	42	26
		Commercial		**0.307**	**0.111**	**0.502**	35.9↑	278	126
	Insecticide		Intercept						
		Analytical		**0.370**	**0.214**	**0.527**	44.8↑	517	166
		Commercial		**0.302**	**0.181**	**0.191**	35.3↑	424	142
	Fungicide		Intercept						
		Analytical		-0.330	-0.907	0.247	28.1↓	8	2
		Commercial		-0.398	-0.963	0.166	32.8↓	8	5
	Herbicide-Fungicide difference		Slope	**0.705**	**0.145**	**1.265**	102.4		
	Herbicide - Insecticide difference		Slope	0.005	-0.181	0.191	0.5		
	Insecticide-Fungicide difference		Slope	**0.700**	**0.143**	**1.258**	101.4		
	Analytical – Commercial difference		Slope	0.069	-0.099	0.236	7.1		

Measure	**Type**	**Grade**	**Coefficient**	**Fixed Effects**			↑↓%		
				Mean	CI.ub	CI.lb		*k*	*n*
lnRR									
	Herbicide		Intercept						

	Analytical		**-0.356**	**-0.514**	**-0.198**	30.0↓	42	26
	Commercial		**-0.222**	**-0.360**	**-0.083**	19.9↓	278	126
Insecticide		Intercept						
	Analytical		**-0.682**	**-0.791**	**-0.573**	49.4↓	517	166
	Commercial		**-0.548**	**-0.663**	**-0.433**	42.2↓	424	142
Fungicide		Intercept						
	Analytical		-0.313	-0.677	0.050	26.9↓	8	2
	Commercial		-0.179	-0.535	0.177	16.4↓	8	5
Herb- Fung difference		Slope	-0.043	-0.393	0.307	4.2		
Herb -Insec difference		Slope	**0.326**	**0.202**	**0.450**	27.8		
Insec- Fung difference		Slope	**-0.369**	**-0.716**	**-0.021**	30.9		
Analytical – Commercial difference		Slope	**0.134**	**0.026**	**0.242**	12.5		

Table S8

Results of meta-regression model assessing the interaction of administered dose (mg/l) of pesticides and time of exposure (days) as moderators for variances differences (*lnCVR*) and mean differences (*lnRR*) between treatment and control groups, fit using *rma.mv*. Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* - number of independent studies (paper ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in variability or mean activity.

lnCVR	**Estimate**	**CI.lb**	**CI.ub**	**↑↓%**	**Qm(*P*)**	**k**	**n**	**R^2**
Herbicide								
Intercept	0.179	-0.015	0.379		0.934 (0.817)	323	149	0.8%
Dose (mg/l)	-0.000	-0.001	0.001					
Time (days)	-0.002	-0.008	0.004					
Dose * Time	-0.0001	-0.0003	0.0004	0.0				
Insecticide								
Intercept	0.324	0.184	0.464		**39.253 (<0.001)**	1011	337	8.9%
Dose (mg/l)	-0.004	-0.008	-0.000					
Time (days)	0.010	0.005	0.014					

Dose * Time	**0.004**	**0.002**	**0.006**	0.4↑				
Fungicide								
Intercept	-1.018	-3.112	1.075		2.317 (0.509)	16	7	10.2%
Dose (mg/l)	-1.376	-4.208	1.457					
Time (days)	0.086	-0.215	0.387					
Dose * Time	0.362	-0.343	1.068	43.6↑				

lnRR	**Estimate**	**CI.lb**	**CI.ub**	**↑↓%**	**Qm(*P*)**	**k**	**n**	**R^2**
Herbicide								
Intercept	-0.173	-0.249	-0.098		0.180 (0.981)	323	149	0.1%
Dose (mg/l)	-0.000	-0.000	0.000					
Time (days)	-0.000	-0.003	0.003					
Dose * Time	0.000	-0.000	0.000	0.0				
Insecticide								
Intercept	-0.684	-0.830	0.539		**38.779 (<0.0001)**	1011	337	4.4%
Dose (mg/l)	0.005	0.002	0.008					
Time (days)	-0.004	-0.008	0.000					
Dose * Time	**-0.004**	**-0.006**	**-0.003**	0.4↓				
Fungicide								
Intercept	0.607	0.031	1.183		**73.552 (<0.0001)**	16	7	43.2%
Dose (mg/l)	1.298	0.401	2.195					
Time (days)	-0.122	-0.200	-0.043					
Dose * Time	**-0.338**	**-0.562**	**-0.114**	28.7↓				

Table S9

Results of meta-regression model assessing class of pesticides as moderator for variances differences (*lnCVR*) and mean differences (*lnRR*) between treatment and control groups, fit using *rma.mv*. Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* - number of independent studies (paper_ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in variability or mean activity.

Measure	**Class of pesticide**	**Coefficient**	**Fixed effects**			**↑↓%**		
			Mean	CI.lb	CI.ub		*k*	*n*

lnCVR		Intercept						
	Anilides		0.229	-0.219	0.677	25.7↑	18	11
	Aromatic Acid		0.363	-0.251	0.977	43.8↑	10	6
	Carbamate		**0.332**	**0.083**	**0.562**	39.4↑	109	50
	Derived from microbes		-0.039	-1.397	1.320	3.8↓	2	2
	Oxazolidinone		0.148	-0.231	0.527	16.0↑	47	25
	Neonicotinoid		0.238	-0.484	0.959	26.9↑	17	4
	Organochlorine		0.175	-0.167	0.516	19.1↑	51	19
	Organochlorine_fung		0.702	-1.983	0.579	101.8↑	5	1
	Organophosphorus		**0.456**	**0.308**	**0.605**	57.8↑	737	265
	Organophosphorus_herb		**0.472**	**0.206**	**0.737**	60.3↑	134	61
	Phenoxy		0.141	-0.494	0.775	15.1↑	16	6
	Phenylpyrazole		-0.401	-1.453	0.650	33.0↓	4	2
	Phenylpyridine		0.878	-0.135	1.891	140.6↑	10	2
	Pyrethroid		0.013	-0.219	0.246	1.3↑	116	44
	Pyridine		0.296	-0.094	0.686	34.4↑	34	20
	Pyrimidine		-0.554	-1.248	0.140	42.5↓	14	6
	QUATs		0.342	-1.254	0.570	40.8↑	2	2
	Triazine		**0.346**	**0.028**	**0.663**	41.3↑	36	22
	Triazole		0.381	-0.984	0.223	46.4↑	11	6
	Ureas		0.308	-0.306	0.922	36.1↑	10	6

Measure	**Class of pesticide**	**Coefficient**	**Fixed Effects**			**↑↓%**		
			Mean	CI.lb	CI.ub		*k*	*n*
lnRR		Intercept						
	Anilides		-0.250	-0.592	0.091	22.1↓	18	11
	Aromatic Acid		-0.437	-0.893	0.019	35.4↓	10	6
	Carbamate		**-0.527**	**-0.710**	**-0.345**	41.0↓	109	50
	Derived from microbes		-0.190	-0.987	0.607	17.3↓	2	2
	Oxazolidinone		**-0.392**	**-0.678**	**-0.106**	32.4↓	47	25
	Neonicotinoid		-0.210	-0.707	0.287	18.9↓	17	4
	Organochlorine		**-0.294**	**-0.539**	**-0.048**	25.5↓	51	19
	Organochlorine_fung		-0.484	-1.334	0.365	38.4↓	5	1
	Organophosphorus		**-0.833**	**-0.957**	**-0.709**	56.5↓	737	265
	Organophosphorus_herb		**-0.288**	**-0.488**	**-0.087**	25.0↓	134	61
	Phenoxy		-0.315	-0.751	-0.122	27.0↓	16	6
	Phenylpyrazole		0.062	-0.679	0.802	6.4↑	4	2
	Phenylpyridine		-0.053	-0.747	0.642	5.2↓	10	2
	Pyrethroid		**-0.274**	**-0.456**	**-0.093**	24.0↓	116	44
	Pyridine		-0.067	-0.352	0.219	6.5↓	34	20
	Pyrimidine		-0.359	-0.853	0.134	30.2↓	14	6

QUATs	0.206	-0.591	1.001	22.9↑	2	2
Triazine	-0.165	-0.408	0.079	15.2↓	36	22
Triazole	-0.021	-0.453	0.412	2.1↓	11	6
Ureas	-0.200	-0.657	0.256	18.1↓	10	6

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80 **Table S10**

81 Test of moderators (coefficients) based on the omnibus Wald-type test of all linear combinations, for

82 *metafor* models.

Moderators	**Measure**	**QM statistic**	**p-value**	R^2
ChEs + Tissue	*lnCVR*	**6.786**	**0.034**	**1.3**
	lnRR	0.537	0.764	0.1
fish length (cm) * fish age	*lnCVR*	**8.454**	**0.038**	**3.6**
	lnRR	**7.708**	**0.052**	**2.5**
length ≤ 10cm * fish age	*lnCVR*	**12.356**	**0.006**	**7.9**
	lnRR	7.479	0.060	4.1
10 < length ≤ 20cm * fish age	*lnCVR*	**11.974**	**0.008**	**13.8**
	lnRR	**15.552**	**0.001**	**12.6**
type of pesticide + grade	*lnCVR*	6.892	0.075	0.9
	lnRR	**44.001**	**< 0.0001**	**8.2**
class of pesticide	*lnCVR*	**44.257**	**0.0009**	**5.6**
	lnRR	**116.446**	**<0.0001**	**13.4**
Herbicide dose(mg/l) * time (days) of exposure	*lnCVR*	0.934	0.817	0.8
	lnRR	0.180	0.981	0.1
Insecticide dose(mg/l) * time (days) of exposure	*lnCVR*	**39.253**	**<0.0001**	**8.9**
	lnRR	**38.779**	**<0.0001**	**4.4**
Fungicide dose(mg/l) * time (days) of exposure	*lnCVR*	2.317	0.509	10.2

lnRR	**73.551**	**<0.0001**	**43.2**

Table S11

Results of Egger's regression and trim-and-fill analysis conducted on meta-analytic residuals after controlling for heterogeneity, using the model with class of pesticides as moderator.

	Egger's regression			Trim-and-fill		
	z-value	df	p-value	Missing effect sizes	SE	p-value
lnCVR	3.592	1381	**0.0003**	5	3.464	**0.016**
lnRR	-6.682	1381	**< 0.0001**	54	10.488	**< 0.0001**

Capítulo III

A meta-analytic review of fish antioxidant defense and biotransformation systems following pesticide exposure.

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A meta-analytic review of fish antioxidant defense and biotransformation systems following pesticide exposure

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Abstract

Pesticides are a constant risk to aquatic life due to their intensive application and non-selective mode of action. Once released, they reach aquatic ecosystems and interact with various targets in cells of fish and other living organisms. In fish, toxicity might originate during the metabolization process, which may produce toxic metabolites or reactive oxygen species (ROS). Ethoxyresorufin-*O*-deethylase (EROD), glutathione S-transferase (GST), catalase (CAT), superoxide dismutase (SOD) activities, and levels of reduced glutathione (GSH) indicate toxicants interacted with drug-metabolizing and antioxidant systems, i.e., they are biomarkers of biotransformation and oxidative stress. We meta-analytically quantified the impact of pesticides on the mean response and variability of these biomarkers. Our goals were to verify (*i*) the overall effect of pesticides on oxidative stress and biotransformation, as well how each biomarker respond to exposure; (*ii*) how the life stage of fish (i.e., juvenile and adult) influence biomarkers variability and mean activity; (*iii*) to what extent fish sex (i.e., male, female or mixed-sex groups) modify pesticides toxicity; (*iv*) how different classes of pesticides affect each biomarker. Overall, pesticides induced oxidative stress and the biotransformation system. Regardless of life stage, EROD mean activity increased significantly. In exposed juveniles, CAT and GST variability decreased and increased, respectively. CAT mean activity was higher in females, while EROD and GST activities increased in males after pesticide exposure. Organophosphorus and organochlorine insecticides, along with imidazole and triazole fungicides, affected biomarkers the most. Notably, imidazoles and triazoles classes increased EROD by more than 100%. Additionally, we identified research gaps, such as the lack of effect estimates of relevant pesticides on EROD (e.g., pyrethroids and neonicotinoids) and the small number of studies evaluating GSH on female fish. Future researchers may use these gaps as a guide towards enhanced experimental designs and, consequently, a better understanding of pesticide toxic effects on fish.

Keywords: Oxidative stress; systematic review; biomarkers; EROD; CAT.

1 Introduction

Towards enhancing yield and harvest efficiency, an enormous and diverse load of pesticides are used globally, placing environmental pressure on terrestrial and aquatic life (Stanley and Preetha, 2016). According to FAO, over 4.1 million tonnes of substances were applied globally in 2018 (FAOSTAT, 2021), and this number is likely to increase due to the high demands from an ever-growing population (Maggi et al., 2019). Most herbicides, insecticides, and fungicides are non-selective toxicants, interfering with the molecular and cellular function of both target and non-target species (Felix et al., 2019; Schäfer et al., 2011). Once released, pesticides reach aquatic ecosystems and interact with various targets in the cells of fish and other living organisms to exert their toxic effects (Schäfer et al., 2011). In fish, toxicity might originate during the metabolization process, which may produce toxic metabolites or oxidizing molecular species (Lushchak et al., 2018; Schlenk, 2005).

Fish have well-developed biotransformation systems, converting lipophilic toxicants into more water-soluble metabolites to facilitate excretion (Beyer et al., 2010; Santana et al., 2018). Following absorption, pesticides move through the circulatory system of fish until it reaches the site of metabolization (liver) and ultimate excretion (kidney) (Maxwell, 2020). As pesticides enter hepatocytes – the major site of xenobiotics metabolism – they undergo Phase I (oxidation, reduction, and hydrolysis reactions) and/or Phase II (conjugation reactions) of the biotransformation process (Banerjee and Ramaiah, 2020).

Cytochrome P450s (CYPs) are the most critical Phase I metabolizing enzymes. They form a large family of heme-containing proteins, mostly found in the liver cells associated with the endoplasmic reticulum and mitochondria (Schlenk et al., 2008). The catalytic activity of ethoxyresorufin-*O*-deethylase (EROD) acts as a proxy to the subfamily CYP1A, i.e., effects on CYP1A can be detected by measuring EROD activity (Fabrin et al., 2018). Drug-metabolizing CYPs incorporate an oxygen atom while reducing another. This oxidation process might not be enough to increase polarity since most compounds require more than

an oxidation step to increase hydrophilicity. Therefore, Phase II conjugation of chemical groups (cofactors) ensues.

Glutathione *S*-transferase (GST) is a superfamily of enzymes, mostly present in the cytosol of cells, but also found bound to the endoplasmic reticulum membrane (Boelsterli, 2007). They catalyze the conjugation of glutathione (GSH) to xenobiotics in Phase II reactions. They have broad substrate specificity, conjugating several toxic xenobiotics such as benzo(a)pyrene, aflatoxin B_1 and different classes of pesticides (Schlenk et al., 2008). Beyond their role as Phase II enzymes, GSTs have a variety of functions inside cells. Notably, they detoxify oxidative damage products, act as organic peroxidase, and catalyze biosynthetic reactions of eicosanoids (Schlenk et al., 2008). Both steps in the biotransformation process (Phase I and II reactions) might lead to reactive intermediates or metabolites with more significant biological activity than the parent. Furthermore, CYPs oxidative reactions might act as a source of oxidizing molecular species, such as reactive oxygen species (ROS) (Di Giulio and Meyer, 2008; Lushchak et al., 2018).

Superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\bullet}$) are ROS occurring in cells under normal physiological conditions. The cellular antioxidant defense maintains ROS levels steadily low to prevent an imbalance between pro-oxidants and antioxidants, i.e., oxidative stress. ROS has natural, endogenous cellular sources, such as electron transport during mitochondrial respiration and oxidative metabolism of endogenous compounds by cytochrome P450 (Bidwell, 2020; Boelsterli, 2007). Otherwise, ROS can be generated by ultraviolet light, bacterial or viral infection, and, most importantly, environmental contaminants (Di Giulio and Meyer, 2008; Liu, 2020). Contamination by pesticides, for instance, can generate ROS and enhance oxidative stress, prompting a response from the antioxidant defense system (Lushchak et al., 2018; Slaninova et al., 2009).

Enzymatic and non-enzymatic antioxidants are important indicators of contaminant exposure. Among them, superoxide dismutase (SOD), catalase (CAT), and glutathione

(GSH) are widely recognized and applied as biomarkers of oxidative stress. SOD converts superoxide anions ($O_2^{\bullet-}$) to H_2O_2, which in turn is converted to water by CAT (Liu, 2020). By decreasing the levels of $O_2^{\bullet-}$ and H_2O_2, SOD, and CAT have an important role in avoiding the production of hydroxyl radicals ($HO^{\bullet}$) (Boelsterli, 2007; Santana et al., 2018). GSH is the first and most important non-enzymatic defense against ROS, abundantly found in the mitochondria and cytosol of cells (Boelsterli, 2007; Davies, 2000; Liu, 2020). May act as a radical scavenger, as a cofactor for the enzymatic antioxidant glutathione peroxidase (GPx), and a co-substrate for GST (Davies, 2000; Liu, 2020). Pesticides may exert their toxicity by increasing ROS levels during biotransformation processes, by inhibiting antioxidant enzymes, or the biosynthesis of GSH (Lushchak et al., 2018).

There is constant innovation in the agro-food industry, with the development of novel, promising classes of pesticides considered less threatening to humans and non-target species (Maggi et al., 2019; Santana et al., 2021). However, different classes of pesticides may have distinct effects on antioxidant and biotransformation biomarkers. For instance, organophosphorus (OP) insecticides and carbamates – widely recognized as anticholinesterase agents (Santana et al., 2021) – have been shown to elicit the activity of biotransformation enzymes (Almeida et al., 2010; Guiloski et al., 2013; Hernandez-Moreno et al., 2011; Martínez-Lara et al., 1996; Ullah et al., 2018; Xu et al., 2018) and promote oxidative stress in fish (Clasen et al., 2014; Isik and Celik, 2008; Jia et al., 2018; Narra, 2016; Oruç and Üner, 2000). There have also been reports of redox balance disruption triggered by organochlorine (Ballesteros et al., 2009; Safari et al., 2016; Zhang et al., 2008) and pyrethroid insecticides (Bhattacharjee and Das, 2017; Bhutia et al., 2015; Narra, 2016), several herbicides (e.g., glyphosate, atrazine, clomazone) (Mela et al., 2013; Murussi et al., 2015; Pereira et al., 2013) and fungicides (e.g., prochloraz, propiconazole, azoxystrobin) (Han et al., 2016; Sanchez et al., 2008b; Tabassum et al., 2016).

Despite the abundance of studies, data on the extent of pesticide effects on the biomarkers described here is too heterogenous to be consistent. Moreover, the volume and

diversity of pesticides used yearly and their associated environmental burden call for in-depth review and quantification of effects. Thus, our study presents a meta-analysis on the impact of pesticides on biomarkers representing antioxidant defense and biotransformation systems. We evaluated not only the impact on biomarkers at a mean level but also at a variance level (i.e., if and to what extent pesticides impact affect biomarkers mean response and variability). We aim to understand the magnitude of pesticides effects on fish biomarkers based on the following question and predictions:

(i) Do pesticides have an overall effect on antioxidant defense and biotransformation systems? We did not predict a non-zero overall effect, i.e., we do not assume that pesticides will either increase or decrease biotransformation or oxidative stress. Instead, we expected high heterogeneity among estimates resulting from the various contexts in which oxidative stress and biotransformation metabolism is measured and the multiple mechanisms that may drive their responses. Furthermore, we aim to quantify pesticide effects on individual biomarkers representing oxidative stress and biotransformation, i.e., CAT, SOD, GSH, EROD, and GST.

(ii) Does the life stage affect the magnitude of pesticide effects on oxidative stress and biotransformation biomarkers? It is widely recognized that the life stage of fish is an important intraspecific factor modifying or modulating biomarker response (Liu and Pope, 2020). Young individuals tend to be more susceptible to toxicants than adults (Humphrey and Klumpp, 2003; Liu and Pope, 2020; Santana et al., 2021). Possibly, differences in toxicokinetic and toxicodynamic (i.e., differences in absorption and/or biotransformation, different recovery capabilities from a toxicant exposure) might contribute to a greater sensitivity in juvenile fish (Pope, 2010). Therefore, we expect a more pronounced and less variable biomarker response in younger fish than in adult fish. Specifically, we presume that the activity of both antioxidant and biotransformation biomarkers will increase while GSH levels will decrease.

(iii) Do pesticides have differential effects on antioxidants and biotransformation biomarkers in males and females? Sex-related differences are also recognized as an important factor influencing biomarkers responses (Sopinka et al., 2016; Liu and Pope, 2020). Oxidative damage, regulation of antioxidants, and biotransformation process frequently differ between males and females (Costantini, 2018; Shao et al., 2012; Whyte et al., 2000). These differences might result from toxicodynamic and toxicokinetic factors or how specific substances impact endogenous signaling pathways (Liu and Pope, 2020). Although sex-specific differences in biomarkers are widely described (Dong et al., 2009; Ibor et al., 2019; Jakšić et al., 2008; Strehlow et al., 2003), there is no general trend of response. Therefore, we did not predict an overall difference between males and females. However, seeing it is an important factor, it should be quantified.

(iv) How do specific classes of pesticides affect fish biomarkers? There is high heterogeneity of biomarker responses to different classes of pesticides. For instance, there are reports of pyrethroids increasing (Bhattacharjee and Das, 2017) and decreasing antioxidant activity (Davico et al., 2020; Mu et al., 2014). Similarly, OP insecticides have a variable impact on biotransformation and antioxidant biomarkers, inducing (Chen and Huang, 2011) or inhibiting enzymatic activity (Abdelkhalek et al., 2017; Almeida et al., 2010). Again, due to this heterogeneity, we did not make specific assumptions on differences in biomarker response among classes. However, the class of pesticides is an important factor influencing antioxidant and biotransformation systems, and therefore, it should also be quantified.

2 Methods

2.1 Systematic review and data collection

Searches were conducted on *Web of Science*, *Scopus,* and *Pubmed* (timespan: "all years", up to and including January 2019) with queries designed to identify studies on biotransformation and oxidative stress of fish exposed to pesticides. The following search strategy was applied to search on topics (i.e., title, keywords, and abstracts) in all databases: ((contaminat* OR pollut*) AND (fish OR teleost*)) AND (pesticide OR herbicide OR insecticide OR fungicide) AND (oxidative stress OR biomarker OR biotransformation OR superoxide dismutase OR SOD OR catalase OR CAT OR glutathione OR GSH OR glutathione S-transferase OR GST OR ethoxyresorufin-O-deethylase OR EROD).

After the removal of duplicates, 3467 peer-reviewed studies remained for screening. Retrieved records needed to provide means, sample sizes, and a measure of dispersion (interquartile ranges, standard deviations, or standard errors) and also required to meet our inclusion criteria below:

1) Studies had to address our main research question, i.e., oxidative stress and biotransformation of fish exposed to pesticides.
2) Studies must report an unambiguous study design description. Also, means, sample sizes, and a measure of dispersion should be provided.
3) Studies must provide GSH levels, CAT, SOD, GST, and EROD activities from fish exposed to pesticides (treatment/impact group) and from fish reared or captured in conditions where pesticides were absent (control/reference groups).
4) Only synthetic chemicals used as agricultural pesticides were included. We excluded public health pesticides, biocides used as antifouling compounds, or antibiotics.
5) Only studies that measured the biomarkers mentioned above on the liver of fish were included. Studies using fish embryos, larvae, or *in vitro* experiments were all outside the scope of this meta-analysis.

We considered both laboratory and field experimental approaches. For field studies, whenever two or more reference sites were selected for comparisons, we only extracted data from those sites located furthest from contaminated areas. Moreover, laboratory studies often compared two control settings, *(i)* a solvent control, in which fish was exposed to the vehicle used as the solvent, and *(ii)* a regular control, in which fish remained in clean water during the experiment. Whenever this occurred, only the latter was considered.

During the screening of titles and abstracts, 2981 records were excluded. The remaining 486 papers were fully screened, and 204 were included for data extraction. The screening process is shown in Figure S1 in the supplementary materials.

2.2 Data extraction

For each biomarker of interest, data required to calculate effect sizes, such as means, standard error (SE), or standard deviation (SD), together with sample sizes (*N*), were extracted from text, tables, and/or figures as control–treatment pairwise comparisons. Data from figures were extracted using the *metaDigitise* package (Pick et al., 2018) in R (v. 3.4.3; R Development Core Team, 2018). Most studies compared several treatments to a single control, a common practice in ecotoxicology that causes nonindependence among estimates. Therefore, whenever several treatments had a shared control within a given paper, these estimates were assigned a unique control ID (ctrl ID). This ctrl ID was accounted for as a factor in the random structure of meta-analytic and meta-regression models. In addition, for each pairwise comparison, a unique effect size ID (es ID, unit of analysis) was assigned and added as a random factor.

2.3 Effect size calculation

The mean effects of pesticides on fish biomarkers were analyzed using log response ratio (*lnRR*, Hedges et al., 1999), while the variance effects were analyzed using the log coefficient of variation ratio (*lnCVR*, Nakagawa et al., 2015). These effect sizes, and their

corresponding sampling variances, were calculated for each pairwise comparison between the control and treatment groups. For both *lnRR* and *lnCVR*, the treatment group was specified as the numerator and the control as the denominator. Therefore, positive values represent an increase in biomarkers mean activity/levels and variance after pesticide exposure. In contrast, negative values indicate a decrease in variability and activity/levels of biomarkers after exposure.

2.4 Data-analysis: main-effects and hypothesis-testing model

We have divided our data set into two subsets: *(i)* oxidative stress data, which comprises the antioxidants CAT, SOD, and GSH; and *(ii)* biotransformation data, which comprises EROD and GST. We decided to work with two separate subsets to guarantee a clear and direct presentation of results and discussion.

First, we ran models to decide the best random-effects structure using the maximum likelihood method. We compared models combining three random effects: es ID, ctrl ID, species, and paper ID. The variance component for es ID represents residual variance, for ctrl ID represents variance among treatments sharing a single control, for paper ID represents the between-studies variance and for species represents the variance among species. Based on AIC values (Akaike information criterion), shown in Table S2-S3, we chose a model with es ID and ctrl ID as random factors for oxidative stress and biotransformation data.

Next, multilevel intercept-only meta-analytic models were fitted for each effect size, assessing the overall effect of pesticides on oxidative stress and biotransformation at a mean and variance level. We fitted meta-analytic models that included a variance-covariance matrix with 0.5 correlation between effect size sample variances with the same ctrl ID. Then we compared these results with models assuming no correlation, i.e., independent sample variances. Both models generated qualitatively similar results; therefore, we present results from the models assuming no correlation.

Finally, we tested our predictions using multilevel meta-regression models for both *lnRR* and *lnCVR,* including random effects as above. For each subset, we first included "*biomarker*" as the moderator variable to evaluate how each biomarker responds to pesticides individually. Then, we fitted a meta-regression including both "*biomarker*" and "*life stage*" to assess differences between adult and juvenile fish among biomarkers. Next, both "*biomarker*" and "*sex*" tested for differences among female, male and mixed-sex fish (female and male) for each biomarker. Lastly, to test for differences among pesticide classes, we created biomarker-specific subsets comprising environmentally relevant classes for which enough data were available from multiple studies (≥10 estimates). The environmental relevance was based upon which classes are applied massively in dominant crops (Maggi et al. 2019), which are considered highly hazardous to human and fish health (Jepson et al., 2020), and/or which raises concerns due to environmental persistence (i.e., organochlorines) (Martyniuk et al., 2020; Newton, 2018).

Effect size calculation, as well as meta-analytic and meta-regression models, were conducted using the *metafor* package (v.2.0-0; Viechtbauer, 2010) in R (R Core Team, 2019). We examined total, residual, and random-effect specific relative heterogeneity for main-effects models by calculating I^2 values (Nakagawa and Santos, 2012) via function '*i2_ml*' in *R* package *oRchard* (Nakagawa et al., 2020; Noble, 2020). It measures total heterogeneity between effect sizes that are not attributed to sampling error (Higgins et al., 2003). For meta-regression models, we calculated the $R^2_{marginal}$, the percentage of heterogeneity explained by the inclusion of moderators in each model (Nakagawa and Schielzeth, 2013); via function '*r2_ml*' also in R package *oRchard*. Residual heterogeneity Q_E and moderator-specific heterogeneity Q_M (i.e., an omnibus test of coefficients) were calculated using the function '*rma.mv*' via R package *metafor*.

305 *2.5 Publication bias*

306 We took three steps to determine if there is a bias towards significant differences
307 (i.e., publication bias) in our data set, comprised exclusively of published studies. First, we
308 looked for funnel asymmetry of the 'meta-analytic' residuals (*sensu* Nakagawa and Santos,
309 2012), calculated from the meta-analytic models. Then, we ran Egger's regression (Egger et
310 al., 2015) on the same meta-analytic residuals and a trim-and-fill test to predict unpublished
311 studies from the literature based on funnel asymmetry (Duval and Tweedie, 2000).

312 Furthermore, we included publication year as a continuous moderator variable in
313 meta-regression models to test for time-lag effect (Jennions and Møller, 2002), i.e., to
314 assess whether early studies tend to publish larger effect sizes than later studies.

315 Since studies included in our meta-analysis were focused mainly on effects at the
316 mean level, we did not expect publication bias for *lnCVR*. Therefore, all tests mentioned
317 above were conducted solely for *lnRR*.

318

319 **3 Results**

320

321 *3.1 Description of the data set*

322 After the full-text screening, 204 papers reporting the effects of pesticides on fish
323 biomarkers were included in our meta-analysis. These 204 papers amounted to 2623
324 estimates of effect calculated from 1007 independent experiments within papers. The
325 subsets created from the original data set consisted of 1765 estimates of effects on oxidative
326 stress and 858 estimates on biotransformation. Within oxidative stress data, 45% and 40%
327 of estimates were of CAT and SOD activities, respectively. While within biotransformation
328 data, EROD activity amounted to only 22% of estimates.

329 Our results describe biomarkers responses under controlled settings since 97% of
330 estimates correspond to data from laboratory experiments. Adult fish comprised 58% of
331 estimates, while juvenile fish 42%. Most estimates originated from experiments using fish
332 from both sexes (45%). Only 28% of data was on female fish responses alone, and 27% on

male fish. Also, most studies focused on the effects of insecticides (47% of estimates), mainly OPs insecticides, which represented 23% of estimates from a total of 35 classes of pesticides.

3.2 Do pesticides affect oxidative stress and biotransformation?

There was a significant, albeit small, global effect of pesticides on oxidative stress mean difference (*lnRR*: 0.039, 95% confidence interval, CI: 0.003 to 0.075; Figure 1a, Table S4). However, pesticides did not affect stress biomarkers individually. SOD, CAT, and GSH estimates were very small, and their confidence intervals substantially overlapped zero (Table S4).

Both EROD and GST mean activities significantly increased after pesticide exposure (Figure 1c, Table S6). The effect on EROD was expressive, corresponding to a 42.4% increase in activity (*lnRR*: 0.353, CI: 0.226 to 0.481). GST had a significantly smaller response, showing a 7.5% increase due to pesticides (*lnRR*: 0.072, CI: 0.009 to 0.135). Accordingly, the overall estimate for mean differences was significantly high, corresponding to a 13.7% increase in biotransformation means.

For *lnCVR*, estimates from individual biomarkers and overall effect were very small, with large confidence intervals overlapping zero (Figure 1b and 1d, Table S5 and S7). Therefore, there was no significant effect of pesticides on oxidative stress and biotransformation variability.

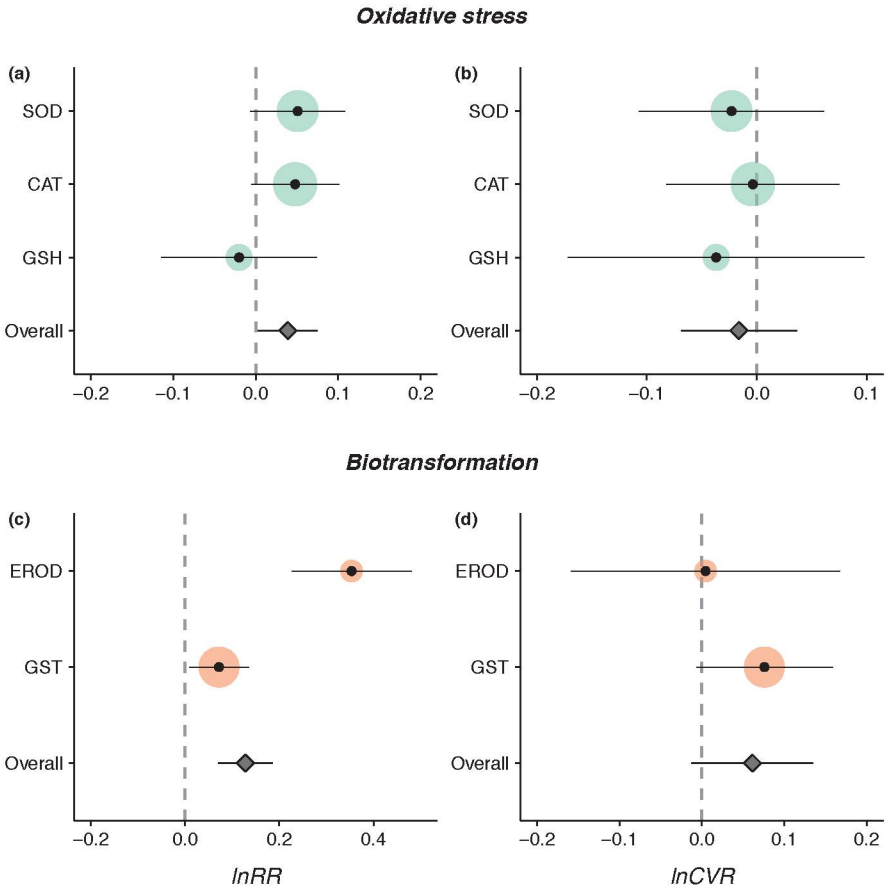


Figure 1 Effects of pesticides on oxidative stress and biotransformation biomarkers superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), ethoxyresorufin-O-deethylase (EROD), and glutathione-S-transferase. Effects on differences in means (a, c) and variability (b, d). Dots represent effect size estimates (log coefficient variation *lnRR* and log response ratio (*lnCVR*), and error bars represent 95% confidence intervals. Effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line). Areas of the green and red-shaded circles are proportional to the number of effect sizes (*k*) used.

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356 *3.3 How do pesticides affect biomarkers in adult and juvenile fish?*

357 For *lnRR*, estimates of stress biomarkers from adult and juvenile fish were small and
358 non-significant (Figure 2a, Table S8). However, most mean estimates of biotransformation
359 were large and significant (Figure 2c, Table S9). Pesticides substantially increased EROD
360 activity both in adult and juvenile, corresponding to a 63.6% and 50.3% increase,
361 respectively ($lnRR_{\text{EROD adult}}$: 0.492, CI: 0.327 to 0.657; $lnRR_{\text{EROD juvenile}}$: 0.407, CI: 0.246 to
362 0.569), while having a small and non-significant effect on GST activity in juvenile fish. Within
363 biotransformation data, life stage and biomarkers explained a large amount of heterogeneity

364 in *lnRR* ($R^2_{marginal}$= 7.90%, Table S17) while explaining very little within oxidative stress data
365 ($R^2_{marginal}$= 0.39%, Table S17).

366 As for *lnCVR*, pesticides only affected CAT and GST variability of juvenile fish
367 (Figure 2b and 2d, Table S8 and S9). Specifically, pesticides decreased variance of CAT
368 activity by 13%, while increased GST variability by 14.8% in juveniles ($lnCVR_{CAT\ juvenile}$: -
369 0.140, CI: -0.247 to 0.032; $lnCVR_{GST\ juvenile}$: 0.138, CI: 0.013 to 0.264). While there was no
370 significant effect on biomarkers of oxidative stress individually, adult and juvenile fish
371 differed by 17.3% from each other, i.e., the effects of pesticides on variance were 17.3 times
372 higher on oxidative stress response of adult fish ($lnCVR_{adult\text{-}juvenile\ difference}$: 0.160, CI: 0.041 to
373 0.278).

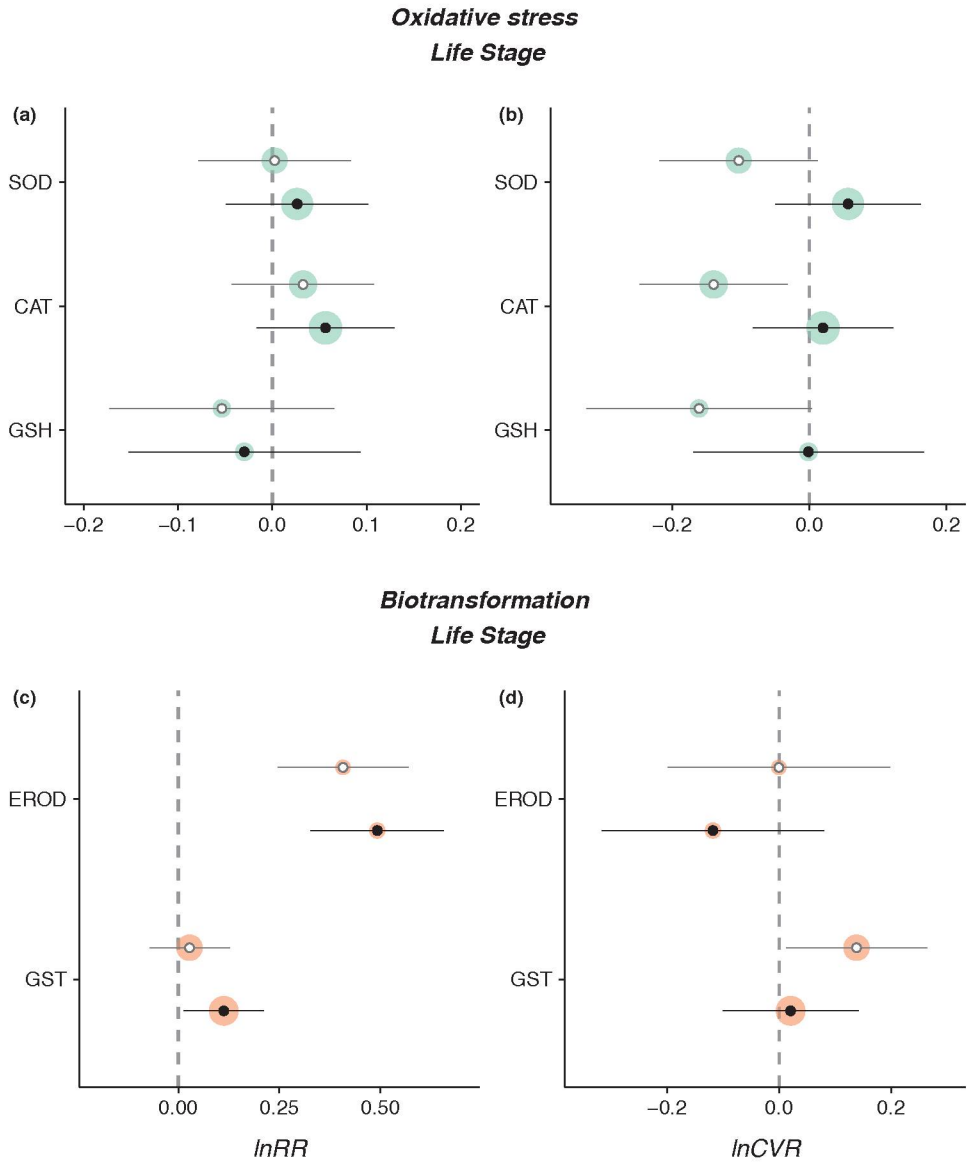


Figure 2 Effects of pesticides on oxidative stress and biotransformation biomarkers superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), ethoxyresorufin-O-deethylase (EROD), and glutathione-S-transferase, considering fish life stage as a moderator. Effects on differences in means (log response ratio *lnRR*) (a, c) and variability (log coefficient variation *lnCVR*) (b, d) on juvenile and adult fish are represented by white and black dots, respectively. Error bars represent 95% confidence intervals, and effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line). Areas of the green and red-shaded circles are proportional to the number of effect sizes (*k*) used.

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379 *3.4 Sex differences in pesticide effects*

380 CAT activity of female fish was significantly affected by pesticides, corresponding to
381 a 16% increase in means ($lnRR_{CAT\ female}$: 0.148, CI: 0.019 to 0.277, Figure 3a, Table S10).
382 Whereas a 19.6% decrease in GSH mean levels was observed in mixed-sex groups
383 (male+female) ($lnRR_{GSH\ mixed}$: -0.219, CI: -0.396 to -0.041, Figure 3a, Table S10). No
384 significant effect on SOD means was detected. In general, considering oxidative stress
385 biomarkers, pesticide effects were similarly stronger in female and male fish compared to
386 mixed-sex groups (Table S10). Also, effects on CAT mean activity were significantly different
387 and higher than GSH mean levels ($lnRR_{CAT\text{-}GSH\ difference}$: 0.196, CI: 0.008 to 0.384, Table S10).

388 Within biotransformation data, EROD means of all sex groups were strongly affected
389 by pesticides (Figure 3c, Table S11). The highest and most significant effect was observed
390 in male fish, which showed an 81.5% increase after pesticide exposure ($lnRR_{EROD\ male}$: 0.596,
391 CI: 0.372 to 0.821, Table S11). GST means were only significantly different from zero in
392 male fish ($lnRR_{GST\ male}$: 0.240, CI: 0.074 to 0.406, Figure 3c, Table S11), while female and
393 mixed-sex groups showed small estimates, with confidence intervals crossing zero.

394 For *lnCVR*, estimates of biotransformation biomarkers were all small and non-
395 significant (Figure 3d, Table S11). Among oxidative stress biomarkers, only SOD variance
396 was significantly affected by pesticides, which increased in mixed-sex groups by 17.1%
397 ($lnCVR_{SOD\ mixed}$: 0.158, CI: 0.000 to 0.316, Figure 3b, Table S10).

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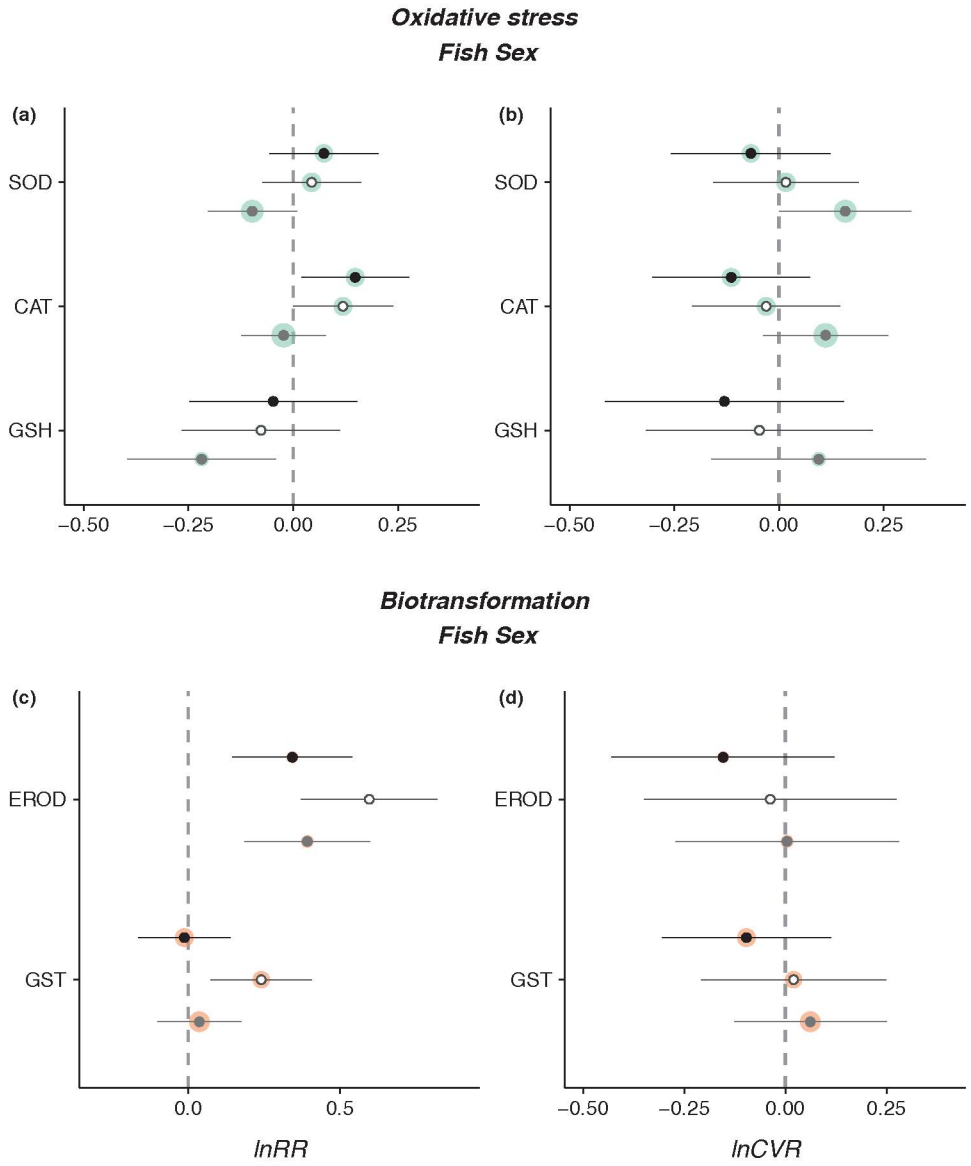


Figure 3 Effects of pesticides on oxidative stress and biotransformation biomarkers superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), ethoxyresorufin-O-deethylase (EROD), and glutathione-S-transferase, considering fish sex as a moderator. Effects on differences in means (log response ratio *lnRR*) (a, c) and variability (log coefficient variation *lnCVR*) (b, d) on female, male, and mixed-sex (male+female) fish are represented by black, white, and grey dots, respectively. Error bars represent 95% confidence intervals, and effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line). Areas of the green and red-shaded circles are proportional to the number of effect sizes (*k*) used.

406 *3.5 Oxidative stress biomarkers X Pesticide class:*

407 OPs insecticides and strobilurin fungicides had a significant effect on CAT means,
408 increasing activities by 13.2% and 18.2%, respectively ($lnRR_{CAT\ OPs}$: 0.124, CI: 0.023 to
409 0.225; $lnRR_{CAT\ strobilurin}$: 0.167, CI: 0.010 to 0.325; Figure 4a, Table S12). Triazine strongly
410 increased, while triazole decreased CAT variance ($lnCVR_{CAT\ triazine}$: 0.420, CI: 0.127 to 0.713;
411 $lnCVR_{CAT\ triazole}$: -0.357, CI: -0.709 to -0.004, Figure 4b, Table S12).

412 GSH means and variability was not significantly affected by any pesticide class
413 (Figure 4c and 4d, Table S13), whereas SOD mean activity increased after carbamate and
414 OPs exposure and decreased 30.2% with neonicotinoids ($lnRR_{SOD\ imidazole}$: 0.328, CI: 0.109 to
415 0.547; $lnRR_{SOD\ OPs}$: 0.203, CI: 0.093 to 0.313; $lnRR_{SOD\ neonicotinoids}$: -0.359, CI: -0.623 to -0.095,
416 Figure 4e, Table S14). Thus, although none of the pesticide classes affected SOD variability
417 and only a few affected means, the "*class*" moderator explained almost 10% of
418 heterogeneity among SOD *lnRR* estimates ($R^2_{marginal}$=9.56%, Table S17).

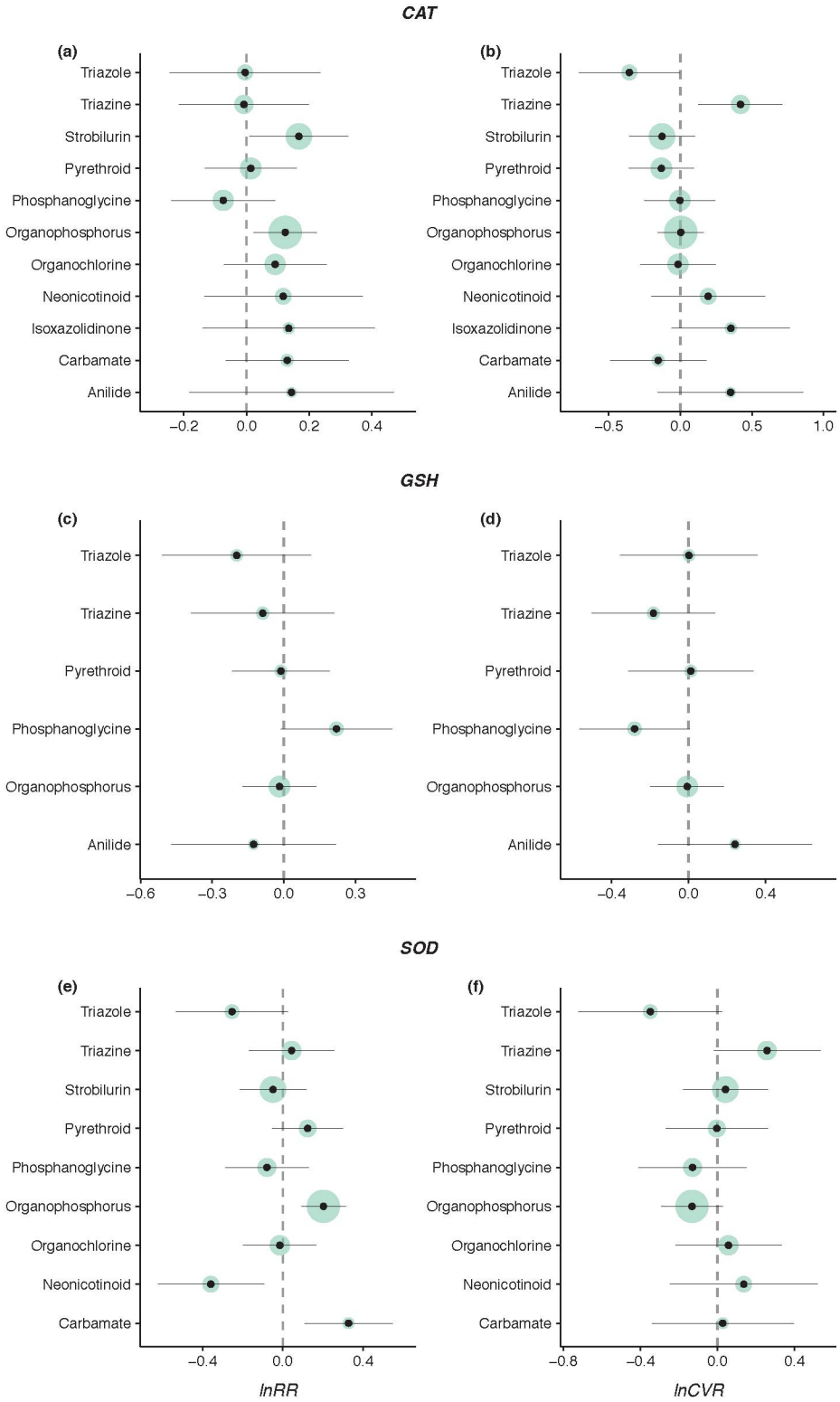


Figure 4 Estimates for log response ratio (*lnRR*) and log coefficient of variation ratio (*lnCVR*) with meta-regressions testing the effect of different classes of pesticides on catalase, CAT (a, b), glutathione, GSH (b, c), and superoxide dismutase, SOD (d, e). *lnRR* estimates are presented on the left and *lnCVR* on the right. Error bars represent 95% confidence intervals, and effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line). The areas of the green-shaded circles are proportional to the number of effect sizes (k) used.

419

420 *3.6 Biotransformation biomarkers X Pesticide class:*

421 Pesticide class as a moderator explained a substantial amount of heterogeneity
422 among EROD *lnRR* estimates ($R^2_{marginal}$=36.07%, Table S17). Imidazole, OCs, and triazole
423 considerably affected EROD mean activity (Figure 5a, Table S15). Imidazole and triazole
424 fungicides increased EROD means by 120.8% and 628.6%, respectively ($lnRR_{EROD\ imidazole}$:
425 0.792, CI: 0.246 to 1.339; $lnRR_{EROD\ triazole}$: 1.986, CI: 1.273 to 2.699). However, there was no
426 significant effect on EROD variance (Figure 5b, Table S15).
427 Imidazole and OCs, along with OPs, also affected GST means (Figure 5c, Table
428 S16). Imidazole had the highest effect, corresponding to a 33.1% increase in GST means
429 after exposure ($lnRR_{GST\ imidazole}$: 0.286, CI: 0.000 to 0.572), while OPs had a smaller effect by
430 comparison ($lnRR_{GST\ OPs}$: 0.093, CI: 0.012 to 0.173). Only QUATs affected GST variance
431 significantly, increasing *lnCVR* estimates by 155% ($lnCVR_{GST\ QUATs}$: 0.936, CI: 0.560 to
432 1.313, Figure 5d, Table S16).

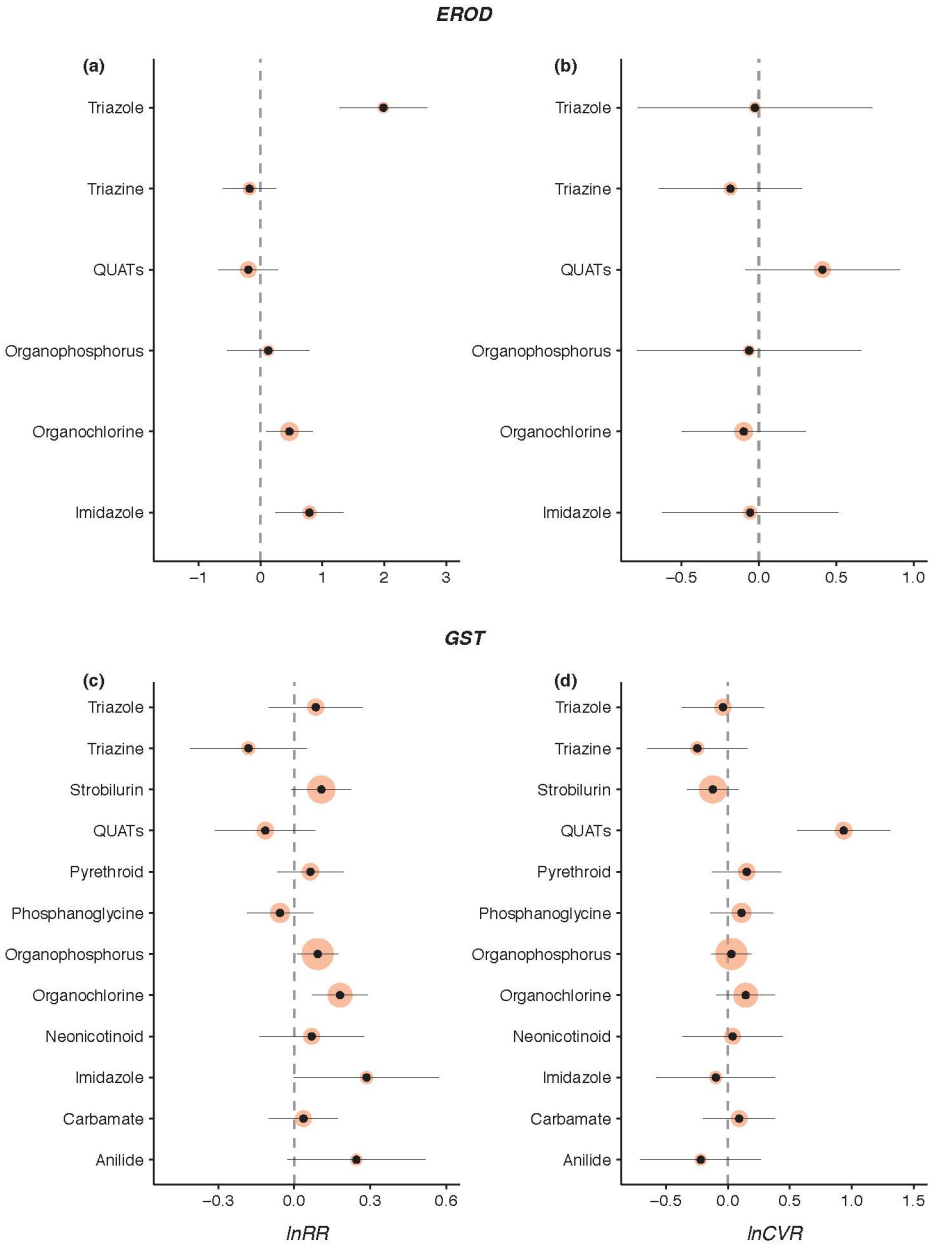


Figure 5 Estimates for log response ratio (*lnRR*) and log coefficient of variation ratio (*lnCVR*) with meta-regressions testing the effect of different classes of pesticides on ethoxyresorufin-O-deethylase, EROD (a, b), and glutathione-S-transferase, GST (b, c). *lnRR* estimates are presented on the left and *lnCVR* on the right. Error bars represent 95% confidence intervals, and effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line). The areas of the green-shaded circles are proportional to the number of effect sizes (k) used.

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438 *3.7 Publication bias*

439 We could not detect an asymmetrical distribution around the meta-analytic mean by
440 funnel plot visual inspection, either for oxidative stress or biotransformation data (Figure 6).
441 The symmetry assumed from funnel plots was confirmed by the trim-and-fill analysis.
442 However, the results of Egger's regression indicate the presence of publication bias in our
443 data sets (Table S18).

444 Furthermore, there was evidence of time-lag bias in the biotransformation data, with
445 effect sizes decreasing over time (Table S19). On the other hand, there was no such
446 evidence in the oxidative stress data, i.e., a very small estimate, with confidence intervals
447 crossing zero (Table S19).

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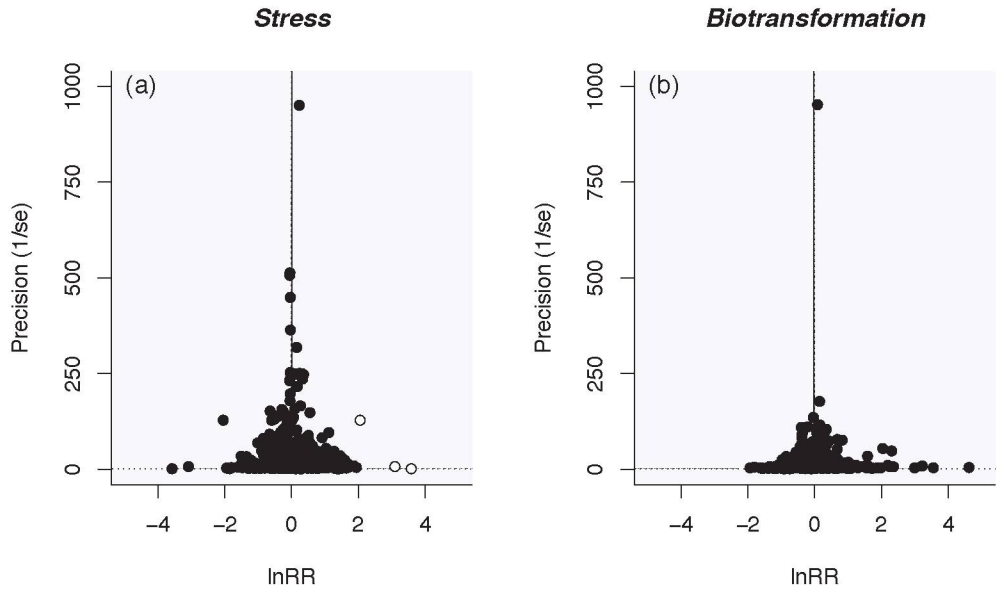


Figure 6 Funnel plots showing the distribution of effect sizes, represented by dotes, around the meta-analytic mean, for meta-analysis of mean differences (*lnRR*) for oxidative stress data (a) and biotransformation data (b). The y-axis represents the precision of the estimates (inverse of the standard error), which is the square root of the sampling variance. The black dots represent published data, and the white dots represent 'missing' data as calculated by the trim-and-fill analysis.

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4 Discussion

Despite initial assumptions, we found an overall effect on fish stress and biotransformation systems. Fish exposed to a range of pesticides are likely to show increased mean activities of the biomarkers evaluated here, especially EROD and GST. Our results indicate pesticides consistently interact with drug-metabolizing enzymes, triggering both Phase I and Phase II biotransformation systems. Since pesticide toxicity is related to oxidative stress (Lushchak et al., 2018), which in turn might result from biotransformation activities (Luschack et al., 2018, Schlenk et al., 2008; Boelsterli, 2003), the small overall increase in oxidative stress might be related to the considerable rise in biotransformation biomarkers.

The lack of overall effect on biomarkers variability (*lnCVR*), both of biotransformation and stress, might suggest consistent pesticide effects on means. However, due to the high heterogeneity in *lnRR* estimates, mainly among treatments sharing a single control (ctrl ID), it is important to consider intrinsic and extrinsic factors regulating toxicity when quantifying and interpreting biomarkers. Below we explore how selected moderator variables affect biotransformation and oxidative stress biomarkers at mean and variance levels.

4.1 Intrinsic factors modulating pesticide toxicity: fish life stage and sex

Contrary to our expectations, no difference between adult and juvenile was found in individual oxidative stress and biotransformation biomarkers. However, the substantial increase of EROD means (>50%) observed in adult and juvenile fish indicates a high sensitivity of this biomarker towards pesticides. Additionally, EROD variability did not increase after exposure, providing further support for its use as an indicator of pesticide and other chemical exposures.

Differences in variability help us better understand our results. For instance, the decrease in CAT variability indicates not only that juveniles might be sensitive to pesticides but also that the exposure may elicit a more homogenous response among young fish.

480 Considering GST responses, increased variability in juveniles might explain why there was
481 no significant effect on means. At the same time, the lack of variance in adults reflects a
482 greater biomarker sensibility in this life stage.

483 Based on previous studies, we had assumed juveniles would be more sensitive to
484 pesticides than adults (Peters and Livingstone, 1995; Pope, 2010; Santana et al., 2021;
485 Whyte et al., 2000). However, our results found a general and opposite trend, with pesticides
486 showing a slightly higher – although not always significant – effect on adult fish compared to
487 juveniles. Adult fish might exhibit increased biotransformation activity because important
488 organs such as the liver and kidneys are fully developed (Mohammed, 2013), allowing for
489 faster assimilation and detoxification of substances by drug-metabolizing enzymes (Fajardo
490 and Ocampo, 2018).

491 Since the activity of CYPs might act as a source of ROS, it is expected that
492 antioxidant defense would increase as a response. Smaller fish are more susceptible to the
493 effects of pesticides, showing a steeper inhibition of cholinesterases, for instance (Santana
494 et al., 2021), which in turn might compromise fish survival by affecting swimming, speed,
495 and predator avoidance (Marigoudar et al., 2013; Qiu et al., 2017; Sandoval-Herrera et al.,
496 2019). However, the elevated biotransformation activity, along with increased stress markers
497 in adult fish, suggests not only the presence of pesticides or other toxicants but also
498 important and beneficial protection mechanisms against contaminant-induced toxicity.

499 Sex-related differences on stress biomarkers were only detected for CAT mean
500 activity, which increased in females. Previous studies described elevated ROS, SOD, and
501 CAT activities in females, which points to their sensitivity to toxicants and ability to prevent
502 oxidative damage (Han et al., 2016; Shao et al., 2012). These sex-specific differences have
503 been attributed to hormonal regulation, specifically estrogen and mitochondrial stress
504 regulation. Estrogens might protect cells from damage by enhancing the expression and
505 activities of antioxidant enzymes (Han et al., 2016; Strehlow et al., 2003). While it has been
506 reported that mitochondria from female rats have higher levels of GSH and antioxidant
507 activities, leading to reduced oxidative damage (Borrás et al., 2003).

508 Similarly, drug-metabolizing enzymes are sex-specific, with male fish showing higher
509 expression and activities of CYPs and GST (Dong et al., 2013; Ibor et al., 2019; Solé et al.,
510 2002). Changes in sex steroid levels and exposure to estrogenic compounds might explain
511 these sex-related effects (Ibor et al., 2019). CYP protein levels and catalytic activity change
512 seasonably, having an inverse relationship with plasma 17-β estradiol (E2) levels in females,
513 (i.e., high levels of E2 act as inhibitors of EROD activity) (Navas and Segner, 2001). Sex-
514 related differences detected here in biotransformation markers are in accordance with
515 previous studies and highlight the importance of sex as a moderator of effect.
516
517 *4.2 Pesticide class as an extrinsic modifying factor*
518 Our results show that sensitivity towards pesticides may be affected by fish sex and
519 life stage. Beyond these intrinsic factors, various classes of pesticides may have different
520 effects on fish. Among the environmentally relevant classes considered in our analysis,
521 organophosphorus (OP) and organochlorine (OC) insecticides affected most biomarkers
522 analyzed here. OCs are a highly toxic class of insecticides known for their chemical stability
523 and environmental persistency (Newton, 2018). OPs, along with carbamates, exert toxicity
524 by targeting cholinesterases activity, which is why both classes are widely recognized as
525 cholinesterase-inhibitor insecticides (Lushchak, 2011; Mineau, 2018; Slaninova et al., 2009).
526 However, before interacting with these important enzymes, OP insecticides must first
527 undergo metabolic pathways mediated by several drug-metabolizing enzymes, including
528 CYPs and GST (Chambers et al., 2010; Hodgson, 2010). Similarly, OC insecticides are
529 subjected to metabolic activation during Phase I and Phase II reactions (Lushchak, 2011).
530 Biotransformation of OP and OC insecticides might generate excess ROS, prompting a
531 response from the antioxidant defense (Liu, 2020; Slaninova et al., 2009), as demonstrated
532 by our results. Although having no significant effect on EROD, OP insecticides increased
533 GST, CAT, and SOD, indicating conjugation reactions might be an important
534 biotransformation step and that antioxidant defense might be activated to prevent oxidative
535 damage.

536 EROD mean activity was substantially affected by imidazole and triazole, structurally
537 related fungicides commonly applied for foliar pathogen control. Both fungicides act on CYP-
538 dependent 14α-demethylase activity, preventing the conversion of lanosterol to ergosterol,
539 an essential component of fungal biological membranes (Husak et al., 2017). Additionally,
540 both can induce biotransformation and detoxification processes in fish, including the CYP1A
541 system and GST (Konwick et al., 2006; Sanchez et al., 2008a; Wang et al., 2018). Here, we
542 found that triazoles (e.g., propiconazole, tebuconazole, paclobutrazol) considerably affected
543 EROD, increasing mean activity by more than 600%. While imidazoles had a significant
544 effect on EROD and GST, supporting previous evidence of the interaction between these
545 fungicides with Phase I and Phase II enzymes.
546 Strobilurin fungicides and neonicotinoid insecticides had substantial effects on CAT
547 and SOD mean activities, respectively. Strobilurins comprise an important class of
548 fungicides, widely applied for plant disease control worldwide (Jia et al., 2018). Strobilurin
549 acts on fungal mitochondrial respiration by binding at the so-called Qo site of cytochrome b
550 and consequently inhibiting electron transfer and halting ATP production (Bartlett et al.,
551 2002). This mode of action might produce excess ROS and lead to oxidative stress. The
552 increase in CAT observed here and in previous studies (Han et al., 2016; Jia et al., 2018)
553 suggests that strobilurins trigger fish's antioxidant defense.
554 Neonicotinoids are a relatively new class of systemic insecticides, rising fast in the
555 global market due to their presumed low-risk to non-target organisms (Simon-Delso et al.,
556 2015). However, there is a growing concern about their effects on pollinators and their
557 widespread presence in aquatic environments (Sánchez-Bayo, 2018; Stehle and Schulz,
558 2018). Although having a specific molecular target, i.e., nicotinic acetylcholine
559 neurotransmitter (Gibbons et al., 2015), neonicotinoids might affect antioxidant enzymes
560 (Iturburu et al., 2018; Yan et al., 2015), as seen here in the significant decrease in SOD
561 mean activity. This decrease might result from oxidative damage in the active site of the
562 enzyme or negative feedback related to high concentrations of ROS produced by these
563 insecticides (Iturburu et al., 2018; Pigeolet et al., 1990).

564

565 *4.3 Research gaps and limitations*

566 Despite their importance, GSH and EROD had the least estimates (*k*) compared to
567 CAT, SOD, and GST, potentially affecting results and preventing response patterns from
568 being observed. For instance, few studies assessed GSH levels in females and males
569 (Table S10), accounting for less than a third of estimates from CAT and SOD. Considering
570 the importance of sex-related and life-stage differences found here, conducting experiments
571 with single-sex groups of fish would be important to understand pesticide toxicity. Likewise,
572 when evaluating class as a moderator variable, there were very few estimates of EROD
573 responses to important pesticide classes, such as pyrethroids, neonicotinoids, and
574 phosphanoglycine (i.e., glyphosate). Pyrethroids are highly toxic to fish and other aquatic
575 organisms (Werner and Young, 2017), while there is evidence of glyphosate – the most
576 applied herbicide worldwide – affecting fish cholinesterases as strongly as OCs (Santana et
577 al., 2021). Hence, it is essential to understand the impact of current-use pesticides on
578 biotransformation systems, seeing that most pesticides exert their toxic effects after being
579 subjected to metabolization processes (Chen, 2020; Schlenk, 2005).

580 Results from Egger's regression indicate publication bias and selective reporting
581 from studies included in our data set, although no asymmetry was detected either visually in
582 funnel plots. Trim-and-fill analyses suggested that some low-precision studies reporting
583 positive effects of pesticides on oxidative stress biomarkers are missing from the published
584 literature. Perhaps, studies that contradict expectations are less likely to be published, or
585 heterogeneity in the data was not accounted for in our analyses (Nakagawa & Santos,
586 2012). We have also found that the effect estimates from the biotransformation data have
587 been decreasing over time, and recently published effects were, on average, no longer
588 distinguishable from zero. Hence, we advise caution when interpreting our results since
589 average differences might be over-estimated.

590

591

5 Conclusions

Fish biomarkers are early warning indicators of contamination, i.e., they indicate an interaction between toxicants and biological molecules has occurred. Thus, they are useful to anticipate future harm to higher levels of organization (e.g., population and communities) (Thompson, 2018; van den Brink et al., 2008). Apart from evaluating the overall effect of pesticides on the most commonly used biomarkers, we were able to quantify the influence of intrinsic and extrinsic factors modifying their responses.

Meta-regression models demonstrate the relevance of life stage and sex as moderator variables. While having little effect on antioxidant biomarkers, pesticides affected CAT variability in juveniles, suggesting further attention should be given to variance as opposed to means when considering this particular biomarker. The detected sex-related differences, such as increased biotransformation activity in males, and increased CAT in females, indicate the importance of working with single-sex groups of fish when assessing these biomarker responses to pesticides. Furthermore, our results indicate EROD activity is a consistent biomarker, responding to pesticides in both adult and juvenile fish.

Finally, pesticide class was an important factor modifying biomarker responses. However, the effects of relevant insecticides and herbicides were not estimated on all biomarkers, especially on EROD. Pesticides toxicity is contingent on how fish distribute and metabolize these compounds, which in turn respond to life stage and sex. Thus, to understand the cause-effect relationship between pesticides and fish biomarkers, future studies should focus on filling the gaps identified here.

Acknowledgments

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620 **REFERENCES**

621

622 Abdelkhalek, N.K.M., Eissa, I.A.M., Ahmed, E., Kilany, O.E., El-Adl, M., Dawood, M.A.O.,
623 Hassan, A.M., Abdel-Daim, M.M., 2017. Protective role of dietary Spirulina platensis
624 against diazinon-induced Oxidative damage in Nile tilapia; Oreochromis niloticus.
625 Environ. Toxicol. Pharmacol. 54, 99–104. https://doi.org/10.1016/j.etap.2017.07.002
626 Almeida, J.R., Oliveira, C., Gravato, C., Guilhermino, L., 2010. Linking behavioural
627 alterations with biomarkers responses in the European seabass *Dicentrarchus labrax l.*
628 exposed to the organophosphate pesticide fenitrothion. Ecotoxicology 19, 1369–1381.
629 https://doi.org/10.1007/s10646-010-0523-y
630 Ballesteros, M.L., Wunderlin, D.A., Bistoni, M.A., 2009. Oxidative stress responses in
631 different organs of *Jenynsia multidentata* exposed to endosulfan. Ecotoxicol. Environ.
632 Saf. 72, 199–205. https://doi.org/10.1016/j.ecoenv.2008.01.008
633 Banerjee, A., Ramaiah, S.K., 2020. Hepatic Toxicology, in: Pope, C.N., Liu, J. (Eds.), An
634 Introduction to Interdisciplinary Toxicology. Academic Press, London, pp. 153–162.
635 https://doi.org/10.1201/b18343-14
636 Bartlett, D.W., Clough, J.M., Godwin, J.R., Hall, A.A., Hamer, M., Parr-dobrzanski, B., 2002.
637 The strobilurin fungicides † 662. https://doi.org/10.1002/ps.520
638 Beyer, J., Jonsson, G., Porte, C., Krahn, M.M., Ariese, F., 2010. Analytical methods for
639 determining metabolites of polycyclic aromatic hydrocarbon (PAH) pollutants in fish bile:
640 A review. Environ. Toxicol. Pharmacol. 30, 224–244.
641 https://doi.org/10.1016/j.etap.2010.08.004
642 Bhattacharjee, P., Das, S., 2017. Deltamethrin Induced Alteration of Biochemical
643 Parameters in *Channa punctata*, Bloch and its Amelioration by Quercetin. Bull. Environ.
644 Contam. Toxicol. 98, 763–769. https://doi.org/10.1007/s00128-017-2092-8
645 Bhutia, D., Rai, B.K., Pal, J., 2015. Hepatic Cytochrome P450 as Biomarkers of
646 Cypermethrin Toxicity in Freshwater Teleost, *Channa punctatus* (Bloch). Brazilian Arch.
647 Biol. Technol. 58, 131–136. https://doi.org/10.1590/s1516-8913201400049

Bidwell, J.R., 2020. In vivo ecotoxicology models, in: Pope, C.N., Liu, J.B.T.-A.I. to I.T. (Eds.), Introduction to Interdisciplinary Toxicology. Academic Press, pp. 507–523. https://doi.org/https://doi.org/10.1016/B978-0-12-813602-7.00036-3

Boelsterli, U., 2007. Mechanistic Toxicology - The molecular basis of how chemicals disrupt biological targets, Second Edition, 2nd ed. CRC Press, Boca Raton.

Borrás, C., Sastre, J., García-Sala, D., Lloret, A., Pallardó, F. V., Viña, J., 2003. Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. Free Radic. Biol. Med. 34, 546–552. https://doi.org/10.1016/S0891-5849(02)01356-4

Chambers, J.E., Meek, E.C., Chambers, H.W., 2010. The Metabolism of Organophosphorus Insecticides, in: Krieger, R.B.T.-H.H. of P.T. (Third E. (Ed.), Hayes' Handbook of Pesticide Toxicology. Academic Press, New York, pp. 1399–1407. https://doi.org/https://doi.org/10.1016/B978-0-12-374367-1.00065-3

Chen, H. bin, Huang, H. qing, 2011. Proteomic analysis of methyl parathion-responsive proteins in Sparus latus liver. Fish Shellfish Immunol. 30, 800–806. https://doi.org/10.1016/j.fsi.2011.01.003

Chen, G., 2020. Xenobiotic metabolism and disposition, in: Pope, C.N., Liu, J. (Eds.), An Introduction to Interdisciplinary Toxicology. Academic Press, London, pp. 31–42. https://doi.org/10.1016/b978-0-12-813602-7.00003-x

Clasen, B., Leitemperger, J., Murussi, C., Pretto, A., Menezes, C., Dalabona, F., Marchezan, E., Adaime, M.B., Zanella, R., Loro, V.L., 2014. Carbofuran promotes biochemical changes in carp exposed to rice field and laboratory conditions. Ecotoxicol Env. Saf 101, 77–82. https://doi.org/10.1016/j.ecoenv.2013.12.012

Costantini, D., 2018. Meta-analysis reveals that reproductive strategies are associated with sexual differences in oxidative balance across vertebrates. Curr. Zool. 64, 1–11. https://doi.org/10.1093/cz/zox002

Davico, C.E., Loteste, A., Parma, M.J., Poletta, G., Simoniello, M.F., 2020. Stress oxidative and genotoxicity in *Prochilodus lineatus* (Valenciennes, 1836) exposed to commercial

formulation of insecticide cypermethrin. Drug Chem. Toxicol. 43, 79–84. https://doi.org/10.1080/01480545.2018.1497643

Davies, K.J.A., 2000. Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life 50, 279–289. https://doi.org/10.1080/15216540051081010

Di Giulio, R.T., Meyer, J.N., 2008. Reactive oxygen species and oxidative stress. Toxicol. Fishes 273–324. https://doi.org/10.1201/9780203647295

Dong, M., Zhu, L., Shao, B., Zhu, S., Wang, Jun, Xie, H., Wang, Jinhua, Wang, F., 2013. The effects of endosulfan on cytochrome P450 enzymes and glutathione S-transferases in zebrafish (*Danio rerio*) livers. Ecotoxicol. Environ. Saf. 92, 1–9. https://doi.org/10.1016/j.ecoenv.2012.10.019

Dong, X., Zhu, L., Wang, Jinhua, Wang, Jun, Xie, H., Hou, X., Jia, W., 2009. Effects of atrazine on cytochrome P450 enzymes of zebrafish (*Danio rerio*). Chemosphere 77, 404–412. https://doi.org/10.1016/j.chemosphere.2009.06.052

Fabrin, T.M.C., Diamante, N.A., Mota, T.F.M., Ghisi, N. de C., Prioli, S.M.A.P., Prioli, A.J., 2018. Performance of biomarkers metallothionein and ethoxyresorufin-O-deethylase in aquatic environments : A meta-analytic approach 205, 339–349. https://doi.org/10.1016/j.chemosphere.2018.04.069

Fajardo, L.J., Ocampo, P.P., 2018. Inhibition of acetylcholinesterase activities in white goby, *Glossogobius giuris* from the East Bay of Laguna Lake, Philippines. Int. J. Agric. Technol. 14, 1181–1192.

Felix, M., Holst, N., Sharp, A., 2019. PestTox: An object oriented model for modeling fate and transport of pesticides in the environment and their effects on population dynamics of non-target organisms. Comput. Electron. Agric. 166. https://doi.org/10.1016/j.compag.2019.105022

Gibbons, D., Morrissey, C., Mineau, P., 2015. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. Environ. Sci. Pollut. Res. 22, 103–118. https://doi.org/10.1007/s11356-014-3180-5

704 Guiloski, I.C., Rossi, S.C., Da Silva, C.A., Silva De Assis, H.C., 2013. Insecticides biomarker
705 responses on a freshwater fish *Corydoras paleatus* (Pisces: Callichthyidae). J. Environ.
706 Sci. Heal. Part B-Pesticides Food Contam. Agric. Wastes 48, 272–277.
707 https://doi.org/10.1080/03601234.2013.743774
708 Han, Y., Liu, T., Wang, Jinhua, Wang, Jun, Zhang, C., Zhu, L., 2016. Genotoxicity and
709 oxidative stress induced by the fungicide azoxystrobin in zebrafish (Danio rerio) livers.
710 Pestic. Biochem. Physiol. 133, 13–19. https://doi.org/10.1016/j.pestbp.2016.03.011
711 Hedges, L. V., Gurevitch, J., Curtis, P.S., 1999. The meta-analysis of response ratios in
712 experimental ecology. Ecology 80, 1150–1156. https://doi.org/10.1890/0012-
713 9658(1999)080[1150:TMAORR]2.0.CO;2
714 Hernandez-Moreno, D., Perez-Lopez, M., Soler, F., Gravato, C., Guilhermino, L., 2011.
715 Effects of carbofuran on the sea bass (Dicentrarchus labrax L.): Study of biomarkers
716 and behaviour alterations. Ecotoxicol. Environ. Saf. 74, 1905–1912.
717 https://doi.org/10.1016/j.ecoenv.2011.07.016
718 Higgins, J.P.T., Thompson, S.G., Deeks, J.J., Altman, D.G., 2003. Measuring inconsistency
719 in meta-analyses. Br. Med. J. 327, 557–560. https://doi.org/10.1136/bmj.327.7414.557
720 Hodgson, E., 2010. Metabolism of Pesticides, in: Krieger, R. (Ed.), Hayes' Handbook of
721 Pesticide Toxicology. Academic Press, London, pp. 893–921.
722 Humphrey, C., Klumpp, D.W., 2003. Toxicity of chlorpyrifos to the early life history stages of
723 eastern rainbowfish *Melanotaenia splendida splendida* (Peters 1866) in tropical
724 Australia. Environ. Toxicol. 18, 418–427. https://doi.org/10.1002/tox.10144
725 Husak, V. V., Mosiichuk, N.M., Storey, J.M., Storey, K.B., Lushchak, V.I., 2017. Acute
726 exposure to the penconazole-containing fungicide Topas partially augments antioxidant
727 potential in goldfish tissues. Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 193,
728 1–8. https://doi.org/10.1016/j.cbpc.2016.12.003
729 Ibor, O.R., Adeogun, A.O., Regoli, F., Arukwe, A., 2019. Xenobiotic biotransformation,
730 oxidative stress and obesogenic molecular biomarker responses in Tilapia guineensis
731 from Eleyele Lake, Nigeria. Ecotoxicol. Environ. Saf. 169, 255–265.

https://doi.org/10.1016/j.ecoenv.2018.11.021

Isik, I., Celik, I., 2008. Acute effects of methyl parathion and diazinon as inducers for oxidative stress on certain biomarkers in various tissues of rainbow trout (*Oncorhynchus mykiss*). Pestic. Biochem. Physiol. 92, 38–42. https://doi.org/10.1016/j.pestbp.2008.06.001

Iturburu, F.G., Bertrand, L., Mendieta, J.R., Amé, M. V., Menone, M.L., 2018. An integrated biomarker response study explains more than the sum of the parts: Oxidative stress in the fish *Australoheros facetus* exposed to imidacloprid. Ecol. Indic. 93, 351–357. https://doi.org/10.1016/j.ecolind.2018.05.019

Jakšić, Ž., Hamer, B., Landeka, N., Batel, R., 2008. Western mosquitofish as a bioindicator of exposure to organochlorine compounds. Ecotoxicol. Environ. Saf. 71, 426–435. https://doi.org/10.1016/j.ecoenv.2007.11.006

Jennions, M.D., Møller, A.P., 2002. Relationships fade with time: A meta-analysis of temporal trends in publication in ecology and evolution. Proc. R. Soc. B Biol. Sci. 269, 43–48. https://doi.org/10.1098/rspb.2001.1832

Jepson, P.C., Murray, K., Bach, O., Bonilla, M.A., Neumeister, L., 2020. Selection of pesticides to reduce human and environmental health risks: a global guideline and minimum pesticides list. Lancet Planet. Heal. 4, e56–e63. https://doi.org/10.1016/S2542-5196(19)30266-9

Jia, W., Mao, L., Zhang, L., Zhang, Y., Jiang, H., 2018. Effects of two strobilurins (azoxystrobin and picoxystrobin) on embryonic development and enzyme activities in juveniles and adult fish livers of zebrafish (Danio rerio). Chemosphere 207, 573–580. https://doi.org/10.1016/j.chemosphere.2018.05.138

Konwick, B.J., Garrison, A.W., Avants, J.K., Fisk, A.T., 2006. Bioaccumulation and biotransformation of chiral triazole fungicides in rainbow trout (Oncorhynchus mykiss). Aquat. Toxicol. 80, 372–381. https://doi.org/10.1016/j.aquatox.2006.10.003

Liu, J., Pope, C.N., 2020. Intrinsic and extrinsic factors that can modify toxicity, in: Pope, C.N., Liu, J. (Eds.), An Introduction to Interdisciplinary Toxicology. Academic Press,

760 London, pp. 285–293. https://doi.org/10.1016/b978-0-12-813602-7.00021-1
761 Liu, L., 2020. Cellular responses to toxicants, in: Pope, C.N., Liu, J. (Eds.), An Introduction
762 to Interdisciplinary Toxicology. Academic Press, London, pp. 59–68.
763 https://doi.org/10.1016/b978-0-12-813602-7.00005-3
764 Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat.
765 Toxicol. 101, 13–30. https://doi.org/10.1016/j.aquatox.2010.10.006
766 Lushchak, V.I., Matviishyn, T.M., Husak, V. V., Storey, J.M., Storey, K.B., 2018. Pesticide
767 toxicity: A mechanistic approach. EXCLI J. 17, 1101–1136.
768 https://doi.org/10.17179/excli2018-1710
769 Maggi, F., Tang, F.H.M., la Cecilia, D., McBratney, A., 2019. PEST-CHEMGRIDS, global
770 gridded maps of the top 20 crop-specific pesticide application rates from 2015 to 2025.
771 Sci. data 6, 170. https://doi.org/10.1038/s41597-019-0169-4
772 Marigoudar, S.R., Ahmed, R.N., David, M., 2013. Ultrastructural responses and oxidative
773 stress induced by cypermethrin in the liver of Labeo rohita. Chem. Ecol. 29, 296–308.
774 https://doi.org/10.1080/02757540.2012.748754
775 Martínez-Lara, E., Toribio, F., López-Barea, J., Bárcena, J.A., 1996. Glutathione-S-
776 transferase isoenzyme patterns in the gilthead seabream (*Sparus aurata*) exposed to
777 environmental contaminants. Comp. Biochem. Physiol. - C Pharmacol. Toxicol.
778 Endocrinol. 113, 215–220. https://doi.org/10.1016/0742-8413(95)02090-X
779 Martyniuk, C.J., Mehinto, A.C., Denslow, N.D., 2020. Organochlorine pesticides:
780 Agrochemicals with potent endocrine-disrupting properties in fish. Mol. Cell. Endocrinol.
781 507, 110764. https://doi.org/10.1016/j.mce.2020.110764
782 Maxwell, L., 2020. Absorption, distribution, and excretion in complex organisms, in: Pope,
783 C.N., Liu, J. (Eds.), An Introduction to Interdisciplinary Toxicology. Academic Press,
784 London, pp. 17–29. https://doi.org/10.1016/b978-0-12-813602-7.00002-8
785 Mela, M., Guiloski, I.C., Doria, H.B., Randi, M.A., de Oliveira Ribeiro, C.A., Pereira, L.,
786 Maraschi, A.C., Prodocimo, V., Freire, C.A., Silva de Assis, H.C., 2013. Effects of the
787 herbicide atrazine in neotropical catfish (Rhamdia quelen). Ecotoxicol Env. Saf 93, 13–

21. https://doi.org/10.1016/j.ecoenv.2013.03.026

Mineau, P., 2018. Organophosphorous and carbamate insecticides: Impacts on birds, Encyclopedia of the Anthropocene. Elsevier Inc. https://doi.org/10.1016/B978-0-12-809665-9.09887-6

Mohammed, A., 2013. Why are Early Life Stages of Aquatic Organisms more Sensitive to Toxicants than Adults?, in: Gowder, S. (Ed.), New Insights into Toxicity and Drug Testing. IntechOpen, Rijeka. https://doi.org/10.5772/55187

Mu, X., Wang, K., Chen, X., Pang, S., Zhu, L., Yang, Y., Zhang, J., Li, X., Wang, C., 2014. Impact of environmental concentrations of beta-cypermethrin on the antioxidant system in the brain and liver of zebrafish (Danio rerio). Chem. Ecol. 30, 643–652. https://doi.org/10.1080/02757540.2014.889121

Murussi, C.R., Costa, M., Menezes, C., Leitemperger, J., Guerra, L., Lopes, T., 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. Contam. Toxicol. 68, 646–654. https://doi.org/10.1007/s00244-015-0145-8

Nakagawa, S., Lagisz, M., O'Dea, R.E., Rutkowska, J., Yang, Y., Noble, D.W.A., Senior, A.M., 2020. The orchard plot: Cultivating a forest plot for use in ecology, evolution, and beyond. Res. Synth. Methods 1–9. https://doi.org/10.1002/jrsm.1424

Nakagawa, S., Poulin, R., Mengersen, K., Reinhold, K., Engqvist, L., Lagisz, M., Senior, A.M., 2015. Meta-analysis of variation: Ecological and evolutionary applications and beyond. Methods Ecol. Evol. 6, 143–152. https://doi.org/10.1111/2041-210X.12309

Nakagawa, S., Santos, E.S.A., 2012. Methodological issues and advances in biological meta-analysis. Evol. Ecol. 26, 1253–1274. https://doi.org/10.1007/s10682-012-9555-5

Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol. Evol. 4, 133–142. https://doi.org/10.1111/j.2041-210x.2012.00261.x

Narra, M.R., 2016. Single and cartel effect of pesticides on biochemical and haematological status of *Clarias batrachus*: A long-term monitoring. Chemosphere 144, 966–974.

816 https://doi.org/10.1016/j.chemosphere.2015.09.065
817 Navas, J.M., Segner, H., 2001. Estrogen-mediated suppression of cytochrome P4501A
818 (CYP1A) expression in rainbow trout hepatocytes: Role of estrogen receptor. Chem.
819 Biol. Interact. 138, 285–298. https://doi.org/10.1016/S0009-2797(01)00280-0
820 Newton, I., 2018. Organochlorine Pesticides, Rachel Carson, and the Environmental
821 Movement, in: Dellasala, D.A., Goldstein, M.I.B.T.-E. of the A. (Eds.). Elsevier, Oxford,
822 pp. 97–104. https://doi.org/https://doi.org/10.1016/B978-0-12-809665-9.09989-4
823 Oruç, E.Ö., Üner, N., 2000. Combined effects of 2,4-D and azinphosmethyl on antioxidant
824 enzymes and lipid peroxidation in liver of Oreochromis niloticus. Comp. Biochem.
825 Physiol. - C Pharmacol. Toxicol. Endocrinol. 127, 291–296.
826 https://doi.org/10.1016/S0742-8413(00)00159-6
827 Pereira, L., Fernandes, M.N., Martinez, C.B., 2013. Hematological and biochemical
828 alterations in the fish Prochilodus lineatus caused by the herbicide clomazone. Env.
829 Toxicol Pharmacol 36, 1–8. https://doi.org/10.1016/j.etap.2013.02.019
830 Peters, L.D., Livingstone, D.R., 1995. Studies on cytochrome P4501A in early and adult life
831 stages of turbot (*Scophthalmus maximus L.*). Mar. Environ. Res. 39, 5–9.
832 https://doi.org/10.1016/0141-1136(94)00064-V
833 Pick, J.L., Nakagawa, S., Noble, D.W.A., 2018. Reproducible, flexible and high-throughput
834 data extraction from primary literature: The metaDigitise r package. Methods Ecol. Evol.
835 10, 426–431. https://doi.org/10.1111/2041-210X.13118
836 Pigeolet, E., Corbisier, P., Houbion, A., Lambert, D., Michiels, C., Raes, M., Zachary, M.-D.,
837 Remacle, J., 1990. Glutathione peroxidase, superoxide dismutase, and catalase
838 inactivation by peroxides and oxygen derived free radicals. Mech. Ageing Dev. 51,
839 283–297. https://doi.org/https://doi.org/10.1016/0047-6374(90)90078-T
840 Pope, C., 2010. The influence of age on pesticide toxicity, in: Krieger, R. (Ed.), Hayes'
841 Handbook of Pesticide Toxicology. Academic Press, London, pp. 819–835.
842 Qiu, X., Nomichi, S., Chen, K., Honda, M., Kang, I.J., Shimasaki, Y., Oshima, Y., 2017.
843 Short-term and persistent impacts on behaviors related to locomotion, anxiety, and

startle responses of Japanese medaka (*Oryzias latipes*) induced by acute, sublethal exposure to chlorpyrifos. Aquat Toxicol 192, 148–154. https://doi.org/10.1016/j.aquatox.2017.09.012

R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria https://www.R-project.org/

Safari, R., Khalili, M., Imanpour, M.R., Pourkazemi, M., 2016. The effects of endosulfan on P450 1A gene expression, antioxidant enzymes activity and histopathological alterations in liver of Persian sturgeon (Acipenser persicus Borodin, 1987). J. Appl. Ichthyol. 32, 636–642. https://doi.org/10.1111/jai.13072

Sánchez-Bayo, F., 2018. Systemic Insecticides and Their Environmental Repercussions, in: Dellasala, D.A., Goldstein, M.I.B.T.-E. of the A. (Eds.), . Elsevier, Oxford, pp. 111–117. https://doi.org/https://doi.org/10.1016/B978-0-12-809665-9.09895-5

Sanchez, W., Piccini, B., Ditche, J.-M., Porcher, J.-M., 2008a. Assessment of seasonal variability of biomarkers in three-spined stickleback (Gasterosteus aculeatus L.) from a low contaminated stream: Implication for environmental biomonitoring. Environ. Int. 34, 791–798. https://doi.org/10.1016/j.envint.2008.01.005

Sanchez, W., Piccini, B., Porcher, J.M., 2008b. Effect of prochloraz fungicide on biotransformation enzymes and oxidative stress parameters in three-spined stickleback (Gasterosteus aculeatus L.). J. Environ. Sci. Heal. - Part B Pestic. Food Contam. Agric. Wastes 43, 65–70. https://doi.org/10.1080/03601230701735151

Sandoval-Herrera, N., Mena, F., Espinoza, M., Romero, A., 2019. Neurotoxicity of organophosphate pesticides could reduce the ability of fish to escape predation under low doses of exposure. Sci. Rep. 9, 1–11. https://doi.org/10.1038/s41598-019-46804-6

Santana, M.S., Sandrini-Neto, L., Di Domenico, M., Prodocimo, M.M., 2021. Pesticide effects on fish cholinesterase variability and mean activity: A meta-analytic review. Sci. Total Environ. 757, 143829. https://doi.org/10.1016/j.scitotenv.2020.143829

Santana, M.S., Sandrini-Neto, L., Filipak Neto, F., Oliveira Ribeiro, C.A., Di Domenico, M., Prodocimo, M.M., 2018. Biomarker responses in fish exposed to polycyclic aromatic

872 hydrocarbons (PAHs): Systematic review and meta-analysis. Environ. Pollut. 242.
873 https://doi.org/10.1016/j.envpol.2018.07.004
874 Schäfer, R.B., Brink, P.J. Van Den, Liess, M., 2011. Impacts of Pesticides on Freshwater
875 Ecosystems, in: Sánchez-Bayo, F., van den Brink, P.J., Mann, R.M. (Eds.), Ecological
876 Impacts of Toxic Chemicals (Open Access). BENTHAM SCIENCE PUBL LTD, pp. 111–
877 137. https://doi.org/10.2174/978160805121211101010111
878 Schlenk, D., 2005. Pesticide biotransformation in fish. Biochem. Mol. Biol. Fishes 6, 171–
879 190. https://doi.org/10.1016/S1873-0140(05)80009-8
880 Schlenk, D., Celander, M., Gallagher, E.P., George, S., James, M., Kullman, S.W., Van Den
881 Hurk, P., Willett, K., 2008. Biotransformation in fishes, 1st ed, The Toxicology of Fishes.
882 CRC Press. https://doi.org/10.1201/9780203647295
883 Shao, B., Zhu, L., Dong, M., Wang, J., Wang, J., Xie, H., Zhang, Q., Du, Z., Zhu, S., 2012.
884 DNA damage and oxidative stress induced by endosulfan exposure in zebrafish (Danio
885 rerio). Ecotoxicology 21, 1533–1540. https://doi.org/10.1007/s10646-012-0907-2
886 Simon-Delso, N., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Chagnon, M., Downs,
887 C., Furlan, L., Gibbons, D.W., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P.,
888 Krupke, C.H., Liess, M., Long, E., McField, M., Mineau, P., Mitchell, E.A., Morrissey,
889 C.A., Noome, D.A., Pisa, L., Settele, J., Stark, J.D., Tapparo, A., Van Dyck, H., Van
890 Praagh, J., Van der Sluijs, J.P., Whitehorn, P.R., Wiemers, M., 2015. Systemic
891 insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites.
892 Env. Sci Pollut Res Int 22, 5–34. https://doi.org/10.1007/s11356-014-3470-y
893 Slaninova, A., Smutna, M., Modra, H., Svobodova, Z., 2009. A review: oxidative stress in
894 fish induced by pesticides. Neuro Endocrinol Lett 30 Suppl 1, 2–12.
895 Solé, M., Barceló, D., Porte, C., 2002. Seasonal variation of plasmatic and hepatic
896 vitellogenin and EROD activity in carp, Cyprinus carpio, in relation to sewage treatment
897 plants. Aquat. Toxicol. 60, 233–248. https://doi.org/10.1016/S0166-445X(02)00009-7
898 Stanley, J., Preetha, G., 2016. Pesticide toxicity to non-target organisms - Exposure, toxicity
899 and risk assessment methodologies, Pesticide Toxicity to Non-target Organisms.

Springer Nature. https://doi.org/10.1007/978-94-017-7752-0

Stehle, S., Schulz, R., 2018. The Impact of Pesticides on Our Freshwater Resources, in: Dellasala, D.A., Goldstein, M.I.B.T.-E. of the A. (Eds.), . Elsevier, Oxford, pp. 127–132. https://doi.org/https://doi.org/10.1016/B978-0-12-809665-9.09978-X

Strehlow, K., Rotter, S., Wassmann, S., Adam, O., Grohé, C., Laufs, K., Böhm, M., Nickenig, G., 2003. Modulation of antioxidant enzyme expression and function by estrogen. Circ. Res. 93, 170–177. https://doi.org/10.1161/01.RES.0000082334.17947.11

Tabassum, H., Khan, J., Salman, M., Raisuddin, S., Parvez, S., 2016. Propiconazole induced toxicological alterations in brain of freshwater fish *Channa punctata* Bloch. Ecol. Indic. 62, 242–248. https://doi.org/10.1016/j.ecolind.2015.11.001

Thompson, E.L., 2018. Indicators of Anthropogenic Change and Biological Risk in Coastal Aquatic Environments☆, in: Dellasala, D.A., Goldstein, M.I.B.T.-E. of the A. (Eds.), . Elsevier, Oxford, pp. 97–124. https://doi.org/https://doi.org/10.1016/B978-0-12-809665-9.09106-0

Ullah, S., Li, Z., Hasan, Z., Khan, S.U., Fahad, S., 2018. Malathion induced oxidative stress leads to histopathological and biochemical toxicity in the liver of rohu (*Labeo rohita*, Hamilton) at acute concentration. Ecotoxicol. Environ. Saf. 161, 270–280. https://doi.org/10.1016/j.ecoenv.2018.06.002

van den Brink, P.J., Sibley, P.K., Ratte, H.T., Baird, D.J., Nabholz, J.V., Sanderson, H., 2008. Extrapolation of effects measures across levels of biological organization in ecological risk Assessment, in: D. de Zwart Solomon, K.R., Brock, T.C.M., Posthuma, H.S.L., Richards, S.M. (Eds.), Extrapolation Practice for Ecotoxicological Effect Characterization of Chemicals. SETAC CRC Press, Boca Raton, Florida, pp. 105–134. https://doi.org/10.1201/b13610-4

Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor. J. Stat. Softw. 36, 1–48.

Wang, C., Harwood, J.D., Zhang, Q., 2018. Oxidative stress and DNA damage in common

927 carp (Cyprinus carpio) exposed to the herbicide mesotrione. Chemosphere 193, 1080–
928 1086. https://doi.org/10.1016/j.chemosphere.2017.11.148
929 Werner, I., Young, T.M., 2017. Pyrethroid insecticides-Exposure and impacts in the aquatic
930 environment, Encyclopedia of the Anthropocene. Elsevier Inc.
931 https://doi.org/10.1016/B978-0-12-809665-9.09992-4
932 Whyte, J.J., Jung, R.E., Schmitt, C.J., Tillitt, D.E., 2000. Ethoxyresorufin-O-deethylase
933 (EROD) activity in fish as a biomarker of chemical exposure. Crit. Rev. Toxicol. 30,
934 347–570. https://doi.org/10.1080/10408440091159239
935 Xu, X., Cui, Z., Wang, S., 2018. Joint toxicity on hepatic detoxication enzymes in goldfish
936 (Carassius auratus) exposed to binary mixtures of lead and paraquat. Environ. Toxicol.
937 Pharmacol. 62, 60–68. https://doi.org/10.1016/j.etap.2018.06.005
938 Yan, S., Wang, Jinhua, Zhu, L., Chen, A., Wang, Jun, 2015. Toxic effects of nitenpyram on
939 antioxidant enzyme system and DNA in zebrafish (Danio rerio) livers. Ecotoxicol.
940 Environ. Saf. 122, 54–60. https://doi.org/10.1016/j.ecoenv.2015.06.030
941 Zhang, M., Yin, D., Kong, F., 2008. The changes of serum testosterone level and hepatic
942 microsome enzyme activity of crucian carp (Carassius carassius) exposed to a
943 sublethal dosage of pentachlorophenol. Ecotoxicol Env. Saf 71, 384–389.
944 https://doi.org/10.1016/j.ecoenv.2007.10.014

Material Suplementar - Capítulo III

A meta-analytic review of fish antioxidant defense and biotransformation systems following pesticide exposure.

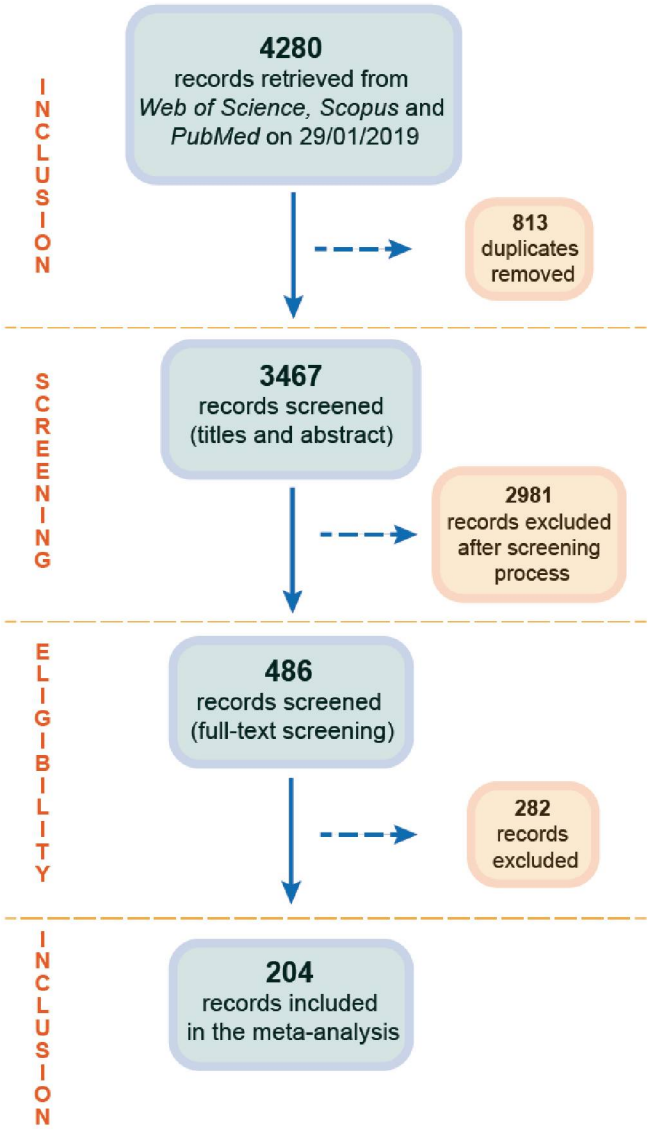


Figure S1 The stages of searching and screening to find the studies included in this meta-analysis.

Table S1

List of all extracted data and data description.

Field Name	Description
es ID	unique ID for each row of data (i.e. each effect size)
ctrl ID	unique ID for pairwise comparison of an independent control and treatment group within papers.
paper ID	unique ID for each paper
author	authors of the paper
journal	journal the paper was published
year	publication year of the paper
country	country where the study was conducted
title	title of the paper
species name	species name for the fish
fish length	length of the fish in centimetres
fish weight	weight of the fish in grams
sex	sex of fish (3 levels: female (F), male (M) and both female and male (M+F))
life stage	sexual maturity of fish (2 levels: adult and juveline)
approach	experimental approach of the study (2 levels: field and laboratory)
pesticide name	name of the pesticide active ingredient used in the experiment
type	classification of pesticide according of target species (5 levels: acaricide, biocide, herbicide, insecticide, fungicide)
class	classification of pesticides according to chemical structure (35 levels: anilides, benzoylurea, botanical-based, carbamate, dicarboximides, dinitroanilide, inorganic, isoxazolidinona, neonicotinoid, organic dication, organochlorine, organochlorine_f (f=fungicide), organofluorine, organophosphorus, organotin, phenoxy, phenoxypyrazole, phenylpyrazole, phenylpyridine, phenylurea, phosphanoglycine, pyrethroid, pyridine, QUATs, quinoline, strobilurin, sulfonylurea, thiocarbamate, triazine, triazole, triazolopyrimidines, triketone)
grade	chemical grade of the pesticide used (2 levels: analytical, commercial)
exposure route	Route by which fish were exposed to pesticides (3 levels: water, diet and intraperitoneal (IP) injection)
concentration mg/l	concentration of the pesticide used in the experiment in milligrams per litre
time days	number of days fish was exposed to pesticides
biomarker	biomarker measured (5 levels: catalase, CAT; glutathione, GSH; superoxide dismutase, SOD; glutathione-S-transferase, GST; ethoxyresorufin-O-deethylase, EROD)
biomarker category	2 categories: oxidative stress and biotransformation
Nc	sample size of fish in the control group
Mc	mean of the control group

SDc	standard deviation of the control group
Nt	sample size of the treatment group
Mt	mean of the treatment group
SDt	standard deviation of the treatment group

Table S2

Model diagnostics of oxidative stress data for different combinations of 0, 1 or 2 random effects: effect size ID (es_ID), paper ID, species ID and control ID (ctrl_ID). Lowest AIC values were shown in bold were selected as random factors.

Measure	**Random Effect**	**N levels**	**AIC**
lnCVR	none		8062.39
lnRR			369146.82
lnCVR	paper ID	164	7085.68
lnRR	paper ID	164	181170.93
lnCVR	ctrl_ID	686	4620.58
lnRR	ctrl_ID	686	101456.05
lnCVR	species	50	7866.24
lnRR	species	50	312548.76
lnCVR	species	50	4299.20
	es ID	1765	
lnRR	species	50	2481.02
	es ID	1765	
lnCVR	ctrl_ID	686	**3956.25**
	es ID	1765	
lnRR	ctrl_ID	686	**1706.90**
	es ID	1765	
lnCVR	paper ID	164	4190.06
	es ID	1765	
lnRR	paper ID	164	2000.66
	es ID	1765	

Table S3

Model diagnostics of biotransformation data for different combinations of 0, 1 or 2 random effects: effect size ID (es_ID), paper ID, species ID and control ID (ctrl_ID). Lowest AIC values were shown in bold were selected as random factors.

Measure	Random Effect	N levels	AIC
lnCVR	none		3962.92
lnRR			88456.31
lnCVR	paper ID	133	3246.42
lnRR	paper ID	133	36112.70
lnCVR	ctrl_ID	322	2297.38
lnRR	ctrl_ID	322	24596.51
lnCVR	species	56	3580.01
lnRR	species	56	62552.28
lnCVR	species	56	2006.05
	es ID	858	
lnRR	species	56	1346.36
	es ID	858	
lnCVR	ctrl_ID	322	**1867.71**
	es ID	858	
lnRR	ctrl_ID	322	**1039.32**
	es ID	858	
lnCVR	paper ID	133	1968.95
	es ID	858	
lnRR	paper ID	133	1197.87
	es ID	858	

Table S4

Results of meta-analytic and meta-regression models for mean differences (*lnRR*) between treatment and control groups, from the oxidative stress data. Covariance = 0 assumes no correlation between sampling variances from the same fish group. Covariance = 0.5 assumes sampling variances from treatments having the same control have a 0.5 correlation (i.e. more conservative model). Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* - number of independent studies (ctrl ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in mean activity.

Measure	Model	Treatment	Coefficient	Covariance	Fixed effects			↑↓%		
					Mean	CI.lb	CI.ub		*k*	*n*
lnRR										
	meta-analytic	all	intercept	0	**0.039**	**0.003**	**0.075**	4.0↑	1765	686
				0.5	0.032	-0.005	0.070	3.3↑		
	meta-regression	CAT	intercept	0	0.048	-0.006	0.101	4.9↑	790	317
				0.5	0.046	-0.009	0.100	4.7↑		
		GSH	intercept	0	-0.020	-0.114	0.074	2.0↑	272	102
				0.5	0.031	-0.127	0.066	3.0↑		
		SOD	intercept	0	0.051	-0.007	0.108	5.2↑	703	267
				0.5	0.040	-0.019	0.099	4.1↑		

Measure	Model	Covariance		Random effects		Heterogeneity		
				N levels	Sigma^2	I^2_{Total}	$I^2_{ctrl_ID}$	$I^2_{es_ID}$
lnRR	meta-analytic							
		0	ctrl_ID	686	0.198	100	78	22
			es_ID	1765	0.055			
		0.5	ctrl_ID	686	0.197	100	72	28
			es_ID	1765	0.075			

Table S5
Results of meta-analytic and meta-regression models for variances differences (*lnCVR*) between treatment and control groups, from the oxidative stress data. Covariance = 0 assumes no correlation between sampling variances from the same fish group. Covariance = 0.5 assumes sampling variances from treatments having the same control have a 0.5 correlation (i.e. more conservative model). Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* - number of independent studies (ctrl ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in variability.

Measure	Model	Treatment	Coefficient	Covariance	Fixed effects			↑↓%		
					Mean	CI.lb	CI.ub		*k*	*n*
lnCVR										
	meta-analytic	all	intercept	0	-0.016	-0.069	0.036	1.6↓	1765	686
				0.5	-0.004	-0.062	0.054	0.4↓		
	meta-regression	CAT	intercept	0	-0.004	-0.082	0.075	0.4↓	790	317
				0.5	0.002	-0.081	0.085	0.2↑		
		GSH	intercept	0	-0.037	-0.172	0.098	3.6↓	272	102
				0.5	0.001	-0.145	0.147	0.1↑		
		SOD	intercept	0	-0.023	-0.107	0.061	2.3↓	703	267
				0.5	-0.014	-0.103	0.076	1.3↓		

Measure	Model	Covariance		Random effects		Heterogeneity		
				N levels	Sigma^2	I^2_{Total}	$I^2_{ctrl_ID}$	$I^2_{es_ID}$
lnCVR	meta-analytic							
		0	ctrl_ID	686	0.310	75	50	25
			es_ID	1765	0.157			
		0.5	ctrl_ID	686	0.289	76	44	32
			es_ID	1765	0.210			

Table S6

Results of meta-analytic and meta-regression models for mean differences (*lnRR*) between treatment and control groups, from the biotransformation data. Covariance = 0 assumes no correlation between sampling variances from the same fish group. Covariance = 0.5 assumes sampling variances from treatments having the same control have a 0.5 correlation (i.e. more conservative model). Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* - number of independent studies (ctrl ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in mean activity.

Measure	Model	Treatment	Coefficient	Covariance	Fixed effects			↑↓%		
					Mean	CI.lb	CI.ub		*k*	*n*
lnRR										
	meta-analytic	all	intercept	0	**0.128**	**0.070**	**0.186**	13.7↑	858	322
				0.5	**0.120**	**0.061**	**0.178**	12.7↑		
	meta-regression	EROD	intercept	0	**0.353**	**0.226**	**0.481**	42.4↑	186	69
				0.5	**0.313**	**0.186**	**0.439**	36.7↑		
		GST	intercept	0	**0.072**	**0.009**	**0.135**	7.5↑	672	253
				0.5	**0.069**	**0.004**	**0.134**	7.2↑		

Measure	Model	Covariance		Random effects		Heterogeneity		
				N levels	Sigma^2	I^2_{Total}	$I^2_{ctrl_ID}$	$I^2_{es_ID}$
lnRR	meta-analytic							
		0	ctrl_ID	322	0.239	100	79	21
			es_ID	858	0.065			
		0.5	ctrl_ID	322	0.215	100	66	34
			es_ID	858	0.112			

Table S7
Results of meta-analytic and meta-regression models for variances differences (*lnCVR*) between treatment and control groups, from the biotransformation data. Covariance = 0 assumes no correlation between sampling variances from the same fish group. Covariance = 0.5 assumes sampling variances from treatments having the same control have a 0.5 correlation (i.e. more conservative model). Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* - number of independent studies (ctrl ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in variability.

Measure	**Model**	**Treatment**	**Coefficient**	**Covariance**	**Fixed effects**			↑↓%		
					Mean	CI.lb	CI.ub		*k*	*n*
lnCVR										
	meta-analytic	all	intercept	0	0.061	-0.013	0.135	6.3↑	858	322
				0.5	0.049	-0.032	0.130	5.0↑		
	meta-regression	EROD	intercept	0	0.005	-0.158	0.168	0.5↑	186	69
				0.5	-0.027	-0.199	0.146	2.6↓		
		GST	intercept	0	0.079	-0.007	0.159	7.9↑	672	253
				0.5	0.069	-0.022	0.159	7.1↑		

Measure	**Model**	**Covariance**		**Random effects**		**Heterogeneity**		
				N levels	Sigma^2	I^2_{Total}	$I^2_{ctrl_ID}$	$I^2_{es_ID}$
lnCVR	meta-analytic							
		0	ctrl_ID	322	0.277	76	49	27
			es_ID	858	0.155			
		0.5	ctrl_ID	322	0.274	79	44	35
			es_ID	858	0.223			

Table S8
Results of meta-regression models with biomarker and life stage as fixed effects, for mean differences (*lnRR*) and variance differences (*lnCVR*) between treatment and control groups from the oxidative stress data. Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* – number of independent studies (ctrl ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in mean activity and variability.

Measure	**Biomarker**	**Life Stage**	**Coefficient**	**Fixed effects**			↑↓%		
				Mean	CI.lb	CI.ub		*k*	*n*
lnRR									
	CAT		Intercept						
		Adult		0.057	-0.017	0.130	5.8↑	324	120
		Juvenile		0.033	-0.043	0.108	3.3↑	252	116
	GSH		Intercept						
		Adult		-0.030	-0.153	0.093	2.9↓	99	29
		Juvenile		-0.054	-0.173	0.066	5.2↓	96	40
	SOD		Intercept						
		Adult		0.026	-0.049	0.102	2.7↑	334	110
		Juvenile		0.003	-0.079	0.084	0.2↑	210	91
	CAT-SOD difference		Slope	0.030	-0.060	0.120	3.1		
	GSH-SOD difference		Slope	-0.056	-0.188	0.076	5.5		
	CAT-GSH difference		Slope	0.086	-0.043	0.215	9.0		
	Adult-juvenile difference		Slope	0.0239	-0.060	0.108	2.4		

Measure	**Type**	**Grade**	**Coefficient**	**Fixed Effects**			↑↓%		
				Mean	CI.ub	CI.lb		*k*	*n*
lnCVR									
	CAT		Intercept						
		Adult		0.020	-0.082	0.122	2.0↑	324	120
		Juvenile		**-0.140**	**-0.247**	**-0.032**	13.0↓	252	116

GSH		Intercept						
	Adult		-0.001	-0.170	0.167	0.1↓	99	29
	Juvenile		-0.161	-0.325	0.003	14.9↓	96	40
SOD		Intercept						
	Adult		0.057	-0.049	0.163	5.8↑	334	110
	Juvenile		-0.103	-0.219	0.012	9.8↓	210	91
CAT-SOD difference		Slope	-0.037	-0.164	0.091	3.6		
GSH-SOD difference		Slope	-0.058	-0.240	0.124	5.6		
CAT-GSH difference		Slope	0.0214	-0.157	0.200	2.2		
Adult-juvenile difference		Slope	**0.160**	**0.041**	**0.278**	17.3		

Table S9
Results of meta-regression models with biomarker and life stage as fixed effects, for mean differences (*lnRR*) and variance differences (*lnCVR*) between treatment and control groups from the biotransformation data. Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* – number of independent studies (ctrl ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in mean activity and variability.

Measure	**Biomarker**	**Life Stage**	**Coefficient**	**Fixed effects**			↑↓%		
				Mean	CI.lb	CI.ub		*k*	*n*
lnRR									
	EROD		Intercept						
		Adult		**0.492**	**0.327**	**0.657**	63.6↑	72	22
		Juvenile		**0.407**	**0.246**	**0.569**	50.3↑	64	29
	GST		Intercept						
		Adult		**0.112**	**0.013**	**0.212**	11.9↑	281	88
		Juvenile		0.028	-0.071	0.127	2.8↑	217	97
	EROD-GST difference		Slope	**0.380**	**0.213**	**0.546**	46.2		
	Adult-juvenile difference		Slope	0.085	-0.048	0.217	8.8		

Measure	**Type**	**Grade**	**Coefficient**	**Fixed Effects**			↑↓%		
				Mean	CI.ub	CI.lb		*k*	*n*
lnCVR									
	EROD		Intercept						
		Adult		-0.118	-0.317	0.080	11.1↓	72	22
		Juvenile		-0.000	-0.199	0.198	0.0	64	29
	GST		Intercept						
		Adult		0.021	-0.101	0.142	2.1↑	281	88
		Juvenile		**0.138**	**0.013**	**0.264**	14.8↑	217	97
	EROD-GST difference		Slope	-0.139	-0.342	0.064	13.0		
	Adult-juvenile difference		Slope	-0.118	-0.282	0.047	11.1		

Table S10
Results of meta-regression models with biomarker and fish sex as fixed effects, for mean differences (*lnRR*) and variance differences (*lnCVR*) between treatment and control groups from the oxidative stress data. Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* – number of independent studies (ctrl ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in mean activity and variability.

Measure	Biomarker	Sex	Coefficient	Fixed effects			↑↓%		
				Mean	CI.lb	CI.ub		*k*	*n*
lnRR									
	CAT		Intercept						
		Female		**0.148**	**0.019**	**0.277**	16.0↑	102	30
		Male		0.119	-0.000	0.238	12.6↑	101	37
		Mixed		-0.023	-0.124	0.078	2.3↓	181	63
	GSH		Intercept						
		Female		-0.048	-0.248	0.153	4.7↓	24	5
		Male		-0.077	-0.265	0.111	7.4↓	31	10
		Mixed		**-0.219**	**-0.396**	**-0.041**	19.6↓	53	15
	SOD		Intercept						
		Female		0.073	-0.057	0.204	7.6↑	95	28
		Male		0.044	-0.073	0.161	4.5↑	108	40
		Mixed		-0.098	-0.204	0.009	9.3↓	155	51
	CAT-SOD difference		Slope	0.075	-0.043	0.193	7.8		
	GSH-SOD difference		Slope	-0.121	-0.310	0.068	11.4		
	CAT-GSH difference		Slope	**0.196**	**0.008**	**0.384**	21.6		
	Female-Male difference		Slope	0.029	-0.123	0.182	3.0		
	Female-Mixed difference		Slope	**0.171**	**0.030**	**0.312**	18.6		
	Male-Mixed difference		Slope	**0.142**	**0.012**	**0.272**	15.2		

Measure	Type	Grade	Coefficient	Fixed Effects			↑↓%		
				Mean	CI.ub	CI.lb		*k*	*n*
lnCVR									
	CAT		Intercept						
		Female		-0.114	-0.302	0.074	10.8↓	102	30
		Male		-0.031	-0.207	0.146	3.1↓	101	37
		Mixed		0.111	-0.037	0.260	11.7↑	181	63
	GSH		Intercept						
		Female		-0.130	-0.416	0.155	12.2↓	24	5
		Male		-0.047	-0.317	0.223	4.6↓	31	10
		Mixed		0.095	-0.161	0.351	10.0↑	53	15
	SOD		Intercept						
		Female		-0.067	-0.258	0.123	6.5↓	95	28
		Male		0.016	-0.157	0.190	1.6↑	108	40
		Mixed		**0.158**	**0.000**	**0.3158**	17.1↑	155	51
	CAT-SOD difference		Slope	-0.047	-0.258	0.123	4.6		
	GSH-SOD difference		Slope	-0.063	-0.335	0.209	6.1		
	CAT-GSH difference		Slope	0.016	-0.254	0.286	1.6		
	Female-Male difference		Slope	-0.084	-0.306	0.139	8.1		
	Female-Mixed difference		Slope	**-0.225**	**-0.431**	**-0.019**	20.1		
	Male-Mixed difference			-0.142	-0.334	0.050	13.2		

Table S11
Results of meta-regression models with biomarker and fish sex as fixed effects, for mean differences (*lnRR*) and variance differences (*lnCVR*) between treatment and control groups from the biotransformation data. Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* – number of independent studies (ctrl ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in mean activity and variability.

Measure	**Biomarker**	**Life Stage**	**Coefficient**	**Fixed effects**			↑↓%		
				Mean	CI.lb	CI.ub		*k*	*n*
lnRR									
	EROD		Intercept						
		Female		**0.343**	**0.146**	**0.541**	40.9↑	32	16
		Male		**0.596**	**0.372**	**0.821**	81.5↑	16	9
		Mixed		**0.392**	**0.185**	**0.599**	48.0↑	39	10
	GST		Intercept						
		Female		-0.013	-0.166	0.140	1.3↓	99	31
		Male		**0.240**	**0.074**	**0.406**	27.1↑	83	27
		Mixed		0.036	-0.102	0.174	3.7↑	125	41
	EROD-GST difference		Slope	**0.356**	**0.159**	**0.553**	42.8		
	Female-Male difference		Slope	**-0.253**	**-0.468**	**-0.039**	22.4		
	Female-Mixed difference		Slope	-0.049	-0.244	0.145	4.8		
	Male-Mixed difference		Slope	0.204	-0.004	0.413	22.6		

Measure	**Type**	**Grade**	**Coefficient**	**Fixed Effects**			↑↓%		
				Mean	CI.ub	CI.lb		*k*	*n*
lnCVR									
	EROD		Intercept						
		Female		-0.154	-0.429	0.121	14.3↓	32	16
		Male		-0.037	-0.349	0.274	3.6↓	16	9
		Mixed		0.004	-0.272	0.281	0.4↑	39	10
	GST		Intercept						
		Female		-0.096	-0.306	0.113	9.2↓	99	31

	Male		0.020	-0.209	0.249	2.0↑	83	27
	Mixed		0.062	-0.127	0.250	6.4↑	125	41
EROD-GST difference		Slope	-0.058	-0.327	0.212	5.6		
Female-Male difference		Slope	-0.117	-0.414	0.181	11.0		
Female-Mixed difference		Slope	0.062	-0.127	0.250	6.4		
Male-Mixed difference		Slope	-0.042	-0.328	0.245	4.1		

Table S12
Results of meta-regression models from catalase (CAT) data with pesticide class as fixed effects, for mean differences (*lnRR*) and variance differences (*lnCVR*) between treatment and control groups. Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* – number of independent studies (ctrl ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in mean activity and variability.

Measure	**Class**	**Coefficient**	**Fixed effects**			↑↓%		
			Mean	CI.lb	CI.ub		*k*	*n*
lnRR								
		Intercept						
	Anilide		0.144	-0.183	0.470	15.5↑	14	7
	Carbamate		0.130	-0.066	0.326	13.9↑	22	20
	Isoxazolidinone		0.135	-0.141	0.410	14.5↑	19	15
	Neonicotinoid		0.117	-0.135	0.370	12.4↑	40	13
	Organochlorine		0.092	-0.072	0.255	9.6↑	68	27
	Organophosphorus		**0.124**	**0.023**	**0.225**	13.2↑	180	80
	Phosphanoglycine		-0.074	-0.240	0.092	7.1↓	68	32
	Pyrethroid		0.014	-0.133	0.160	1.4↑	72	37
	Strobilurin		**0.167**	**0.010**	**0.325**	18.2↑	110	32
	Triazine		-0.008	-0.215	0.199	0.8↓	54	19
	Triazole		-0.004	-0.245	0.236	0.4↓	36	16

Measure	**Class**	**Coefficient**	**Fixed Effects**			↑↓%		
			Mean	CI.ub	CI.lb		*k*	*n*
lnCVR								

	Intercept						
	Anilide	0.351	-0.156	0.856	42.0↑	14	7
	Carbamate	-0.155	-0.491	0.181	14.4↓	22	20
	Isoxazolidinone	0.353	-0.060	0.766	42.3↑	19	15
	Neonicotinoid	0.194	-0.202	0.589	21.4↑	40	13
	Organochlorine	-0.016	-0.280	0.247	1.6↓	68	27
	Organophosphorus	0.003	-0.156	0.162	0.3↑	180	80
	Phosphanoglycine	-0.003	-0.251	0.244	0.3↓	68	32
	Pyrethroid	-0.133	-0.359	0.094	12.5↓	72	37
	Strobilurin	-0.128	-0.356	0.100	12.0↓	110	32
	Triazine	**0.420**	**0.127**	**0.713**	52.2↑	54	19
	Triazole	**-0.357**	**-0.709**	**-0.004**	30.0↓	36	16

Table S13
Results of meta-regression models from glutathione (GSH) data with pesticide class as fixed effects, for mean differences (*lnRR*) and variance differences (*lnCVR*) between treatment and control groups. Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* – number of independent studies (ctrl ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in mean activity and variability.

Measure	**Class**	**Coefficient**	**Fixed effects**			↑↓%		
			Mean	CI.lb	CI.ub		*k*	*n*
lnRR								
		Intercept						
	Anilide		-0.126	-0.470	0.218	11.8↓	15	6
	Organophosphorus		-0.018	-0.171	0.136	1.8↓	71	28
	Phosphanoglycine		0.221	-0.012	0.455	24.7↑	29	14
	Pyrethroid		-0.012	-0.216	0.193	1.2↓	21	16
	Triazine		-0.088	-0.387	0.211	8.4↓	23	8
	Triazole		-0.197	-0.509	0.115	17.9↓	20	7

Measure	**Class**	**Coefficient**	**Fixed Effects**			↑↓%		
			Mean	CI.ub	CI.lb		*k*	*n*

lnCVR							
	Intercept						
	Anilide	0.241	-0.158	0.639	27.3↑	15	6
	Organophosphorus	-0.007	-0.198	0.184	0.7↓	71	28
	Phosphanoglycine	-0.280	-0.565	0.004	24.4↓	29	14
	Pyrethroid	0.011	-0.313	0.336	1.1↑	21	16
	Triazine	-0.182	-0.502	0.137	16.6↓	23	8
	Triazole	0.001	-0.354	0.357	0.1↑	20	7

Table S14
Results of meta-regression models from superoxide dismutase (SOD) data with pesticide class as fixed effects, for mean differences (*lnRR*) and variance differences (*lnCVR*) between treatment and control groups. Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* – number of independent studies (ctrl ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in mean activity and variability.

Measure	**Class**	**Coefficient**	**Fixed effects**			↑↓%		
			Mean	CI.lb	CI.ub		*k*	*n*
lnRR								
		Intercept						
	Carbamate		**0.328**	**0.109**	**0.547**	28.8↑	18	17
	Neonicotinoid		**-0.359**	**-0.623**	**-0.095**	30.2↓	40	13
	Organochlorine		-0.015	-0.197	0.168	1.5↓	62	24
	Organophosphorus		**0.203**	**0.093**	**0.313**	22.5↑	178	75
	Phosphanoglycine		-0.079	-0.286	0.128	7.6↓	53	22
	Pyrethroid		0.124	-0.052	0.301	13.2↑	46	27
	Strobilurin		-0.049	-0.216	0.118	4.8↓	111	32
	Triazine		0.044	-0.168	0.256	4.5↑	57	20
	Triazole		-0.253	-0.532	0.025	22.4↓	30	13

Measure	**Class**	**Coefficient**	**Fixed Effects**			↑↓%		
			Mean	CI.ub	CI.lb		*k*	*n*
lnCVR								
		Intercept						

	Carbamate	0.027	-0.341	0.395	2.7↑	18	17
	Neonicotinoid	0.136	-0.248	0.520	14.6↑	40	13
	Organochlorine	0.057	-0.218	0.332	5.9↑	62	24
	Organophosphorus	-0.132	-0.292	0.027	12.4↓	178	75
	Phosphanoglycine	-0.130	-0.409	0.150	12.2↓	53	22
	Pyrethroid	-0.004	-0.267	0.259	0.4↓	46	27
	Strobilurin	0.041	-0.179	0.261	4.2↑	111	32
	Triazine	0.257	-0.020	0.533	29.3↑	57	20
	Triazole	-0.348	-0.721	0.024	29.4↓	30	13

Table S15
Results of meta-regression models from ethoxyresorufin-O-deethylase (EROD) data with pesticide class as fixed effects, for mean differences (*lnRR*) and variance differences (*lnCVR*) between treatment and control groups. Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* – number of independent studies (ctrl ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in mean activity and variability.

Measure	**Class**	**Coefficient**	**Fixed effects**			↑↓%		
			Mean	CI.lb	CI.ub		*k*	*n*
lnRR								
		Intercept						
	Imidazole		**0.792**	**0.246**	**1.339**	120.8↑	21	7
	Organochlorine		**0.468**	**0.095**	**0.841**	59.7↑	41	16
	Organophosphorus		0.126	-0.535	0.786	13.4↑	13	4
	QUATs		-0.196	-0.676	0.285	17.8↓	32	9
	Triazine		-0.177	-0.604	0.250	16.2↓	19	13
	Triazole		**1.986**	**1.273**	**2.699**	628.6↑	13	4

Measure	**Class**	**Coefficient**	**Fixed Effects**			↑↓%		
			Mean	CI.ub	CI.lb		*k*	*n*
lnCVR								
		Intercept						
	Imidazole		-0.055	-0.624	0.513	5.4↓	21	7
	Organochlorine		-0.098	-0.496	0.301	9.3↓	41	16

	Organophosphorus	-0.062	-0.786	0.661	6.0↓	13	4
	QUATs	0.412	-0.086	0.909	51.0↑	32	9
	Triazine	-0.183	-0.645	0.278	16.7↓	19	13
	Triazole	-0.025	-0.781	0.732	2.5↓	13	4

Table S16
Results of meta-regression models from glutathione-S-transferase (GST) data with pesticide class as fixed effects, for mean differences (*lnRR*) and variance differences (*lnCVR*) between treatment and control groups. Effects with 95% confidence intervals (CI) not crossing zero are indicated in bold. *k* - number of effect sizes included in the analysis. *n* – number of independent studies (ctrl ID) included in the analysis. ↑ indicate the percent increase and ↓ the percent decrease in mean activity and variability.

Measure	**Class**	**Coefficient**	**Fixed effects**			↑↓%		
			Mean	CI.lb	CI.ub		*k*	*n*
lnRR								
		Intercept						
	Anilide		0.246	-0.027	0.518	27.9↑	15	6
	Carbamate		0.037	-0.099	0.172	3.8↑	32	22
	Imidazole		**0.286**	**0.000**	**0.572**	33.1↑	18	5
	Neonicotinoid		0.069	-0.137	0.275	7.1↑	32	9
	Organochlorine		**0.181**	**0.072**	**0.289**	19.8↑	76	28
	Organophosphorus		**0.093**	**0.012**	**0.173**	9.7↑	132	59
	Phosphanoglycine		-0.056	-0.187	0.075	8.9↓	47	27
	Pyrethroid		0.064	-0.067	0.195	6.6↑	35	22
	QUATs		-0.114	-0.312	0.083	10.8↓	35	9
	Strobilurin		0.107	-0.011	0.225	11.3↑	100	28
	Triazine		-0.181	-0.411	0.049	16.6↓	21	8
	Triazole		0.085	-0.099	0.270	8.9↑	33	13

Measure	**Class**	**Coefficient**	**Fixed Effects**			↑↓%		
			Mean	CI.ub	CI.lb		*k*	*n*
lnCVR								
		Intercept						
	Anilide		-0.218	-0.704	0.267	19.6↓	15	6
	Carbamate		0.092	-0.198	0.382	9.6↑	32	22

Imidazole	-0.097	-0.576	0.382	9.2↓	18	5
Neonicotinoid	0.039	-0.366	0.444	4.0↑	32	9
Organochlorine	0.145	-0.090	0.379	15.6↑	76	28
Organophosphorus	0.029	-0.133	0.191	2.9↑	132	59
Phosphanoglycine	0.111	-0.145	0.366	11.7↑	47	27
Pyrethroid	0.153	-0.128	0.433	16.5↑	35	22
QUATs	**0.936**	**0.560**	**1.313**	155.0↑	35	9
Strobilurin	-0.121	-0.327	0.086	11.4↓	100	28
Triazine	-0.246	-0.651	0.158	21.8↓	21	8
Triazole	-0.039	-0.371	0.293	3.8↓	33	13

Table S17
Test of moderators (coefficients) based on the omnibus Wald-type test of all linear combinations.

Stress				
Moderator(s)	Effect Size	Q_E (residual)	Q_M (moderator)	$R^2_{marginal}$ (%)
Biomarker	lnRR	370742.32 P<0.0001	1.79 P=0.408	0.25
	lnCVR	7511.77 P<0.0001	0.22 P=0.898	0.033
Biomarker+Life Stage	lnRR	295736.97 P<0.0001	2.15 P=0.541	0.39
	lnCVR	5817.71 P<0.0001	7.82 P=0.050	1.52
Biomarker+Sex	lnRR	235918.45 P<0.0001	12.20 P=0.016	3.76
	lnCVR	4380.60 P<0.0001	5.31 P=0.257	1.80
Class				
CAT	lnRR	169799.36 P<0.0001	7.92 P=0.636	2.35
	lnCVR	2831.79 P<0.0001	20.00 P=0.029	7.00
GSH	lnRR	6465.11 P<0.0001	5.88 P=0.318	6.95
	lnCVR	500.07 P<0.0001	5.63 P=0.344	6.21
SOD	lnRR	82655.79	30.71	9.56

		P<0.0001	P=0.0002	
	lnCVR	2446.84	10.84	4.41
		P<0.0001	P=0.211	

Biotransformation

Moderator(s)	Effect Size	Q_E (residual)	Q_M (moderator)	$R^2_{marginal}$ (%)
		79589.32	15.05	
	lnRR	P<0.0001	P=0.0001	4.44
Biomarker		3828.19	0.59	
	lnCVR	P<0.0001	P=0.444	0.20
		57083.54	21.35	
	lnRR	P<0.0001	P<0.0001	7.90
Biomarker+Life Stage		2993.12	3.72	
	lnCVR	P<0.0001	P=0.156	1.54
		46803.69	17.54	
	lnRR	P<0.0001	P=0.001	9.81
Biomarker+Sex		2344.46	1.67	
	lnCVR	P<0.0001	P=0.644	1.07
Class				
		16289.94	33.94	
	lnRR	P<0.0001	P<0.0001	36.07
EROD		516.46	3.51	
	lnCVR	P<0.0001	P=0.623	7.13
		46997.31	20.98	
	lnRR	P<0.0001	P=0.034	7.66
GST		2436.73	29.15	
	lnCVR	P<0.0001	P=0.002	13.71

Table S18
Results of Egger's regression and trim-and-fill analysis conducted on meta-analytic residuals for both oxidative stress and biotransformation data.

Stress

	Egger's regression			Trim-and-fill		
	z-value	df	p-value	Missing effect sizes	SE	p-value
lnRR	**-4.196**	**1763**	**< 0.0001**	3	2.828	0.063

Biotransformation

	Egger's regression			Trim-and-fill		
	z-value	df	p-value	Missing effect sizes	SE	p-value
lnRR	**-2.207**	**856**	**0.0273**	0	1.414	0.500

Table S19
Meta-regression results for the effects of publication year on mean differences *(lnRR)* of the stress and biotransformation data. *k* - number of effect sizes included in the analysis. *n* - number of controls (ctrl ID) included in the analysis.

Stress						
Measure	**Slope**	**Fixed effects**				
		Mean	CI.lb	CI.ub	*k*	*n*
lnRR	Publication year	-0.005	-0.013	0.003	1765	686

Biotransformation						
Measure	**Slope**	**Fixed Effects**				
		Mean	CI.lb	CI.ub	*k*	*n*
lnRR	Publication year	**-0.017**	**-0.027**	**-0.006**	858	322

Conclusões Gerais

CONCLUSÕES GERAIS

Este trabalho teve como objetivo fornecer uma síntese dos efeitos de HPAs e agrotóxicos nos principais biomarcadores bioquímicos de peixes. Por meio de revisões sistemáticas e meta-análises, foi possível apresentar um quadro abrangente de como indicadores de biotransformação, estresse oxidativo e neurotoxicidade respondem a esses contaminantes. O efeito global dos HPAs e agrotóxicos foi significativo para grande parte dos biomarcadores avaliados, entretando associado a uma grande heterogeneidade. As análises de moderadores além de responderem a predições estabelecidas *a priori*, foram importantes para avaliar, e talvez explicar, parte dessa heterogeneidade.

Entre os biomarcadores avaliados, EROD demonstrou ser uma ferramenta importante para avaliar efeitos de HPAs e agrotóxicos em peixes. As respostas desse marcador de biotransformação foram consistentes e robustas, inclusive considerando diferentes fontes de variação. Além da EROD, os agrotóxicos afetaram significantemente a atividade média das ChEs. A resposta desses biomarcadores neurotóxicos também foi consistente, havendo evidência de que AChE e BChE são enzimas complementares e a avaliação de ambas seria uma poderosa ferramenta para monitoramento ambiental.

A CAT, uma das enzimas que representam a defesa antioxidante das células e indica estresse oxidativo, foi o biomarcador menos robusto. Diferenças na sua atividade média, entre controle e tratamento, mostraram-se inadequadas para avaliação de impactos tanto por HPAs como por agrotóxicos. Possivelmente, a heterogeniedade das respostas da CAT impede que padrões sejam identificados. Através das meta-análises de variância, identificou-se um

aumento da variabilidade de CAT entre peixes juvenis expostos a agrotóxicos. Talvez fosse mais indicado avaliar alterações na variabilidade desse biomarcador, ao invés de utilizar diferenças entre médias para estimar efeitos.

Além de demonstrar a consistência ou robustez de biomarcadores após exposição a HPAs e agrotóxicos, outra contribuição importante do presente trabalho foram as meta-análises de variância. Quando se observa uma diminuição de variabilidade, presume-se que a exposição leva uma resposta mais homogênea dos biomarcadores. Já um aumento da variabilidade indica uma incerteza na resposta do biomarcador, especialmente se esse aumento for acompanhado de um efeito não-significativo nas médias. Nota-se que ao considerar os efeitos de contaminantes na variação dos biomarcadores pode ser um caminho interessante, ainda não trilhado na ecotoxicologia.

Outra contribuição relevante deste trabalho foi a identificação de lacunas de pesquisa na área de ecotoxicologia ambiental. Essas lacunas podem auxiliar pesquisadores a definir e planejar novos estudos. Por exemplo, nota-se um pequeno número de estudos avaliando os efeitos de HPAs em biomarcadores importantes, como GSH, GR e GSSG. Já para agrotóxicos, estudos considerando classes ambientalmente relevantes, como neonicotinóides ou piretróides (associados a EROD), são escassos. Novos estudos podem utilizar os resultados aqui obtidos para direcionar e definir protocolos experimentais e atividades de monitoramento ambiental.

Revisões sistemáticas e meta-análises demonstraram ser ferramentas poderosas para sintetizar dados ecotoxicológicos. Os resultados apresentados trazem novas percepções para área, principalmente ao avaliar os efeitos de diferentes moderadores. Os capítulos trataram de forma inovadora como

mensurar os riscos associados a produção, uso e lançamento de contaminantes no ambiente. Ao revelar padrões de importantes biomarcadores, é possível compreender como HPAs e agrotóxicos afetam a saúde de peixes e, assim, antecipar impactos em níveis de organização biológica superiores.

REFERÊNCIAS

ADEDEJI, O. B.; OKOCHA, R. O. Overview of pesticide toxicity in fish. **Advances in Environmental Biology**, v. 6, n. 8, p. 2344–2351, 2012.

AGRAHARI, S.; GOPAL, K.; PANDLEY, K. C. Biomarkers of monocrotophos in a freshwater fish Channa punctatus (Bloch). **Journal of Environmental Biology**, v. 27, n. 2, p. 453–457, 2006.

ALBENDÍN, G. et al. Characterization and in vitro sensitivity of cholinesterases of gilthead seabream (Sparus aurata) to organophosphate pesticides. **Fish Physiology and Biochemistry**, v. 43, n. 2, p. 455–464, 2017.

ALTENBURGER, R.; SEGNER, H.; VAN DER OOST, R. **Biomarkers and PAHs — Prospects for the Assessment of Exposure and Effects in Aquatic SystemsPAHs: An Ecotoxicological Perspective**: Wiley Online Books., 14 mar. 2003. Disponível em: <https://doi.org/10.1002/0470867132.ch16>

BANERJEE, A.; RAMAIAH, S. K. Hepatic Toxicology. In: POPE, C. N.; LIU, J. (Eds.). **An Introduction to Interdisciplinary Toxicology**. London: Academic Press, 2020. p. 153–162.

BENBROOK, C. M. Trends in glyphosate herbicide use in the United States and globally. **Environmental Sciences Europe**, v. 28, n. 1, p. 1–15, 2016.

BEYER, J. et al. Analytical methods for determining metabolites of polycyclic aromatic hydrocarbon (PAH) pollutants in fish bile: A review. **Environmental Toxicology and Pharmacology**, v. 30, n. 3, p. 224–244, 2010.

BISWAS, S.; GHOSH, B. Chrysene. In: WEXLER, P. B. T.-E. OF T. (THIRD E. (Ed.). . Oxford: Academic Press, 2014. p. 959–962.

BOELSTERLI, U. **Mechanistic Toxicology - The molecular basis of how chemicals disrupt biological targets, Second Edition**. 2nd. ed. Boca Raton: CRC Press, 2007.

BONANSEA, R. I.; WUNDERLIN, D. A.; AMÉ, M. V. Behavioral swimming effects and acetylcholinesterase activity changes in Jenynsia multidentata exposed to chlorpyrifos and cypermethrin individually and in mixtures. **Ecotoxicology and Environmental Safety**, v. 129, p. 311–319, 2016.

BORENSTEIN, M. et al. **Introduction to meta-analysis**. [s.l.] John Wiley and Sons Inc., 2009.

BRIMIJOIN, S. et al. Physiological roles for butyrylcholinesterase: A BChE-ghrelin axis. **Chemico-Biological Interactions**, v. 259, p. 271–275, 2016.

CASIDA, J. E.; BRYANT, R. J. The ABCs of pesticide toxicology: Amounts, biology, and chemistry. **Toxicology Research**, v. 6, n. 6, p. 755–763, 2017.

CASIDA, J. E.; DURKIN, K. A. Neuroactive insecticides: Targets, selectivity, resistance, and secondary effects. **Annual Review of Entomology**, v. 58,

p. 99–117, 2013.

CHEN, G. Xenobiotic metabolism and disposition. In: POPE, C. N.; LIU, J. (Eds.). . **An Introduction to Interdisciplinary Toxicology**. London: Academic Press, 2020. p. 31–42.

CLASEN, B. et al. Bioaccumulation and oxidative stress caused by pesticides in Cyprinus carpio reared in a rice-fish system. **Science of the Total Environment**, v. 626, p. 737–743, 2018.

COLLIER, T. K. et al. Effects on Fish of Polycyclic Aromatic HydrocarbonS (PAHS) and Naphthenic Acid Exposures. In: TIERNEY, K. B.; FARRELL, A. P.; BRAUNER, C. J. B. T.-F. P. (Eds.). . **Organic Chemical Toxicology of Fishes**. [s.l.] Academic Press, 2013. v. 33p. 195–255.

CUEVAS, N.; MARTINS, M.; COSTA, P. M. Risk assessment of pesticides in estuaries: a review addressing the persistence of an old problem in complex environments. **Ecotoxicology**, v. 27, n. 7, p. 1008–1018, 2018.

DA CUÑA, R. H. et al. Assessment of the acute toxicity of the organochlorine pesticide endosulfan in Cichlasoma dimerus (Teleostei, Perciformes). **Ecotoxicology and Environmental Safety**, v. 74, n. 4, p. 1065–1073, 2011.

DA SILVA JUNIOR, F. C. et al. A look beyond the priority: A systematic review of the genotoxic, mutagenic, and carcinogenic endpoints of non-priority PAHs. **Environmental Pollution**, v. 278, 2021.

DAS, K.; SARMA, G. C. Residue analysis of organophosphorus pesticide malathion after the growth of Anabaena spiroides and Nostoc punctiformae in culture media under laboratory condition. v. 7, n. 9, p. 1275–1280, 2018.

DAUNER, A. L. L. et al. Molecular characterisation of anthropogenic sources of sedimentary organic matter from Potter Cove, King George Island, Antarctica. **Science of the Total Environment**, v. 502, p. 408–416, 2015.

DAVIES, K. J. A. Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. **IUBMB Life**, v. 50, n. 4–5, p. 279–289, 2000.

DIAS ASSIS, C. R.; SOUZA, R.; CARVALHO JR, L. B. Fish Cholinesterases as Biomarkers of Organophosphorus and Carbamate Pesticides. In: STOYTCHEVA, M. (Ed.). . **Pesticides in the Modern World - Pests Control and Pesticides Exposure and Toxicity Assessment**. 1st. ed. Rijeka: InTech, 2011. p. 253–278.

ELIAS, S. A. Finding a “Golden Spike” to Mark the Anthropocene. In: DELLASALA, D. A.; GOLDSTEIN, M. I. B. T.-E. OF THE A. (Eds.). . Oxford: Elsevier, 2018. p. 19–28.

ERICKSON, R. J. et al. Bioavailabilty of chemical contaminants in aquatic systems. In: DI GIULIO, R. T.; HINTON, D. E. (Eds.). . **The Toxicology of Fishes**. 1st. ed. [s.l.] CRC Press, 2008. p. 9–54.

FABRIN, T. M. C. et al. Performance of biomarkers metallothionein and ethoxyresoru fi n O -deethylase in aquatic environments : A meta-analytic approach. v. 205, p. 339–349, 2018.

FAJARDO, L. J.; OCAMPO, P. P. Inhibition of acetylcholinesterase activities in whitegoby, Glossogobius giuris from the East Bay of Laguna Lake, Philippines. **International Journal of Agricultural Technology**, v. 14, n. 7, p. 1181–1192, 2018.

FALUGI, C.; ALUIGI, M. G. Early appearance and possible functions of non-neuromuscular cholinesterase activities. **Frontiers in Molecular Neuroscience**, v. 5, n. APRIL, p. 1–19, 2012.

FITZGERALD, L.; WIKOFF, D. S. Persistent Organic Pollutants. In: **Encyclopedia of Toxicology: Third Edition**. Third Edit ed. [s.l.] Elsevier, 2014. v. 3p. 820–825.

FOLMAR, L. C.; SANDERS, H. O.; JULIN, A. M. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. **Archives of Environmental Contamination and Toxicology**, v. 8, n. 3, p. 269–278, 1979.

FORTIN, M.; CAPDEVIELLE, M. Chapter 24 - Toxicology in the workplace. In: POPE, C. N.; LIU, J. B. T.-A. I. TO I. T. (Eds.). . [s.l.] Academic Press, 2020. p. 327–338.

GAD, S. C.; GAD, S. E. Polycyclic Aromatic Hydrocarbons (PAHs). In: WEXLER, P. B. T.-E. OF T. (THIRD E. (Ed.). . Oxford: Academic Press, 2014. p. 1040–1042.

GAD, S. E. Phenanthrene. In: WEXLER, P. B. T.- (Ed.). . **Encyclopedia of Toxicology: Third Edition**. Oxford: Academic Press, 2014. v. 3p. 865–867.

GALLUCCI, R.; LUCKETT-CHASTAIN, L.; YUCESOY, B. Immunotoxicology. In: POPE, C. N.; LIU, J. B. T.-A. I. TO I. T. (Eds.). . [s.l.] Academic Press, 2020. p. 233–244.

GAŁUSZKA, A.; MIGASZEWSKI, Z. M. Chemical Signals of the Anthropocene. In: DELLASALA, D. A.; GOLDSTEIN, M. I. B. T.-E. OF THE A. (Eds.). . Oxford: Elsevier, 2018. p. 213–217.

GAUR, N.; NARASIMHULU, K.; PYDISETTY, Y. Recent advances in the bio-remediation of persistent organic pollutants and its effect on environment. **Journal of Cleaner Production**, v. 198, p. 1602–1631, 2018.

GOODSELL, P. J.; UNDERWOOD, A. J.; CHAPMAN, M. G. Evidence necessary for taxa to be reliable indicators of environmental conditions or impacts. **Marine Pollution Bulletin**, v. 58, n. 3, p. 323–331, 2009.

GRAY, J. P.; HALL, G. J. Benz[a]anthracene. In: WEXLER, P. B. T.-E. OF T. (THIRD E. (Ed.). . Oxford: Academic Press, 2014. p. 413–414.

GUILHERME, S. et al. Differential genotoxicity of Roundup((R)) formulation and its constituents in blood cells of fish (Anguilla anguilla): considerations on chemical interactions and DNA damaging mechanisms. **Ecotoxicology**, v. 21, n. 5, p. 1381–1390, 2012.

GUILOSKI, I. C. et al. Insecticides biomarker responses on a freshwater fish Corydoras paleatus (Pisces: Callichthyidae). **Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes**, v. 48, n. 4, p. 272–277, 2013.

GUREVITCH, J. et al. Meta-analysis and the science of research synthesis. **Nature**, v. 555, n. 7695, p. 175–182, 2018.

HAYES, J. D.; FLANAGAN, J. U.; JOWSEY, I. R. Glutathione transferases. **Annual Review of Pharmacology and Toxicology**, v. 45, p. 51–88, 2005.

HELLOU, J.; ROSS, N. W.; MOON, T. W. Glutathione, glutathione S-transferase, and glutathione conjugates, complementary markers of oxidative stress in aquatic biota. **Environmental Science and Pollution Research**, v. 19, n. 6, p. 2007–2023, 2012.

HESTER, M. W.; WILLIS, J. M.; BAKER, M. C. Oil Spills in Coastal Wetlands. In: DELLASALA, D. A.; GOLDSTEIN, M. I. B. T.-E. OF THE A. (Eds.). . Oxford: Elsevier, 2018. p. 67–76.

HONDA, M.; SUZUKI, N. Toxicities of polycyclic aromatic hydrocarbons for aquatic animals. **International Journal of Environmental Research and**

Public Health, v. 17, n. 4, 2020.

HUSAK, V. V. et al. Acute exposure to the penconazole-containing fungicide Topas partially augments antioxidant potential in goldfish tissues. **Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology**, v. 193, p. 1–8, 2017.

JAMIESON, A. J. et al. Bioaccumulation of persistent organic pollutants in the deepest ocean fauna. **Nature Ecology and Evolution**, v. 1, n. 3, p. 24–27, 2017.

JENNIONS, M. D.; LORTIE, C. J.; KORICHEVA, J. Role of Meta-analysis in Interpreting the Scientific Literature. In: KORICHEVA, J.; GUREVITCH, J.; MENGERSEN, K. (Eds.). . **Handbook of Meta-analysis in Ecology and Evolution**. [s.l.] Princeton University Press, 2013. p. 364–380.

JERINA, D. M. et al. **Carcinogenicity of Polycyclic Aromatic Hydrocarbons: The Bay-Region Theory**. (B. Pullman, P. O. P. Ts'o, H. Gelboin, Eds.)Carcinogenesis: Fundamental Mechanisms and Environmental Effects. **Anais**...Dordrecht: Springer Netherlands, 1980

JOHNSON, L. L. et al. Effects of Legacy Persistent Organic Pollutants (POPs) in Fish—Current and Future Challenges. In: TIERNEY, K. B.; FARRELL, A. P.; BRAUNER, C. J. B. T.-F. P. (Eds.). . **Organic Chemical Toxicology of Fishes**. [s.l.] Academic Press, 2013. v. 33p. 53–140.

KÖHLER, H. R.; TRIEBSKORN, R. Wildlife ecotoxicology of pesticides: Can we track effects to the population level and beyond? **Science**, v. 341, n. 6147, p. 759–765, 2013.

KORICHEVA, J.; GUREVITCH, J. Place of Meta-analysis among Other Methods of Research Synthesis. In: KORICHEVA, J.; GUREVITCH, J.; MENGERSEN, K. (Eds.). . **Handbook of Meta-analysis in Ecology and Evolution**. [s.l.] Princeton University Press, 2013. p. 3–13.

KOUREAS, M. et al. Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. **Toxicology Letters**, v. 210, n. 2, p. 155–168, 2012.

LEHR, R. E. et al. Bay Region Theory of Polycyclic Aromatic Hydrocarbon Carcinogenesis. **ACS Symposium Series**, p. 63–84, 1985.

LEWIS, S. L.; MASLIN, M. A. Defining the Anthropocene. **Nature**, v. 519, n. 7542, p. 171–180, 2015.

LIU, J.; POPE, C. N. Intrinsic and extrinsic factors that can modify toxicity. In: POPE, C. N.; LIU, J. (Eds.). . **An Introduction to Interdisciplinary Toxicology**. London: Academic Press, 2020. p. 285–293.

LIU, L. Cellular responses to toxicants. In: POPE, C. N.; LIU, J. (Eds.). . **An Introduction to Interdisciplinary Toxicology**. London: Academic Press, 2020. p. 59–68.

LUDWIG, C.; STEFFEN, W. The 1950s as the Beginning of the Anthropocene. In: DELLASALA, D. A.; GOLDSTEIN, M. I. B. T.-E. OF THE A. (Eds.). . Oxford: Elsevier, 2018. p. 45–56.

LUSHCHAK, V. I. Environmentally induced oxidative stress in aquatic animals. **Aquatic Toxicology**, v. 101, n. 1, p. 13–30, 2011.

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

MAGGI, F. et al. PEST-CHEMGRIDS, global gridded maps of the top 20 crop-specific pesticide application rates from 2015 to 2025. **Scientific data**, v. 6,

n. 1, p. 170, 2019.

MAGGI, F. et al. The global environmental hazard of glyphosate use. **Science of the Total Environment**, v. 717, p. 137167, 2020.

MANGAS, I. et al. New insights on molecular interactions of organophosphorus pesticides with esterases. **Toxicology**, v. 376, p. 30–43, 2017.

MASSOULIÉ, J. et al. Molecular and cellular biology of cholinesterases. **Progress in Neurobiology**, v. 41, n. 1, p. 31–91, 1993.

MASSOULIÉ, J.; TOUTANT, J.-P. Vertebrate Cholinesterases: Structure and Types of Interaction. In: WHITTAKER, V. P. (Ed.). . **The cholinergic synapse**. New York: Springer-Verlag, 1988. p. 167–224.

MAXWELL, L. Absorption, distribution, and excretion in complex organisms. In: POPE, C. N.; LIU, J. (Eds.). . **An Introduction to Interdisciplinary Toxicology**. London: Academic Press, 2020. p. 17–29.

MELA, M. et al. Effects of the herbicide atrazine in neotropical catfish (Rhamdia quelen). **Ecotoxicol Environ Saf**, v. 93, p. 13–21, 2013.

MIŞE YONAR, S. et al. Malathion-induced changes in the haematological profile, the immune response, and the oxidative/antioxidant status of Cyprinus carpio carpio: Protective role of propolis. **Ecotoxicology and Environmental Safety**, v. 102, n. 1, p. 202–209, 2014.

MOHER, D. et al. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement (Reprinted from Annals of Internal Medicine). **Annals of Internal Medicine**, v. 151, n. 4, p. 264–269, 2009.

NAKAGAWA, S.; CUTHILL, I. C. Effect size, confidence interval and statistical significance: A practical guide for biologists. **Biological Reviews**, v. 82, n. 4, p. 591–605, 2007.

NOBLE, D. W. A. et al. Nonindependence and sensitivity analyses in ecological and evolutionary meta-analyses. **Molecular Ecology**, p. 2410–2425, 2017.

PEREIRA, B. V. R. et al. Cholinesterases characterization of three tropical fish species, and their sensitivity towards specific contaminants. **Ecotoxicology and Environmental Safety**, v. 173, n. October 2018, p. 482–493, 2019.

PIMENTEL, D. Environmental and economic costs of the application of pesticides primarily in the United States. **Environment, Development and Sustainability**, v. 7, n. 2, p. 229–252, 2005.

POPE, C. Pesticides. In: WEXLER, P. (Ed.). . **Encyclopedia of Toxicology: Third Edition**. [s.l.] Academic Press, 2014. v. 3p. 826–827.

POPE, C. N.; BRIMIJOIN, S. Cholinesterases and the fine line between poison and remedy. **Biochemical Pharmacology**, v. 153, n. January, p. 205–216, 2018.

PRIETO GARCIA, F. et al. Pesticides: classification, uses and toxicity. Measures of exposure and genotoxic risks. **Journal of Research in Environmental Science and Toxicology**, v. 1, n. 11, p. 2315–5698, 2012.

RENGARAJAN, T. et al. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. **Asian Pacific Journal of Tropical Biomedicine**, v. 5, n. 3, p. 182–189, 2015.

ROSENBERG, M. S.; ROTHSTEIN, H. R.; GUREVITCH, J. Effect Sizes: In: GUREVITCH, J.; KORICHEVA, J.; MENGERSEN, K. (Eds.). . **Handbook of Meta-analysis in Ecology and Evolution**. [s.l.] Princeton University Press, 2013. p. 61–71.

RÖSLER, T. W. et al. K-variant BCHE and pesticide exposure: Gene-environment interactions in a case–control study of Parkinson's disease in

Egypt. **Scientific Reports**, v. 8, n. 1, p. 1–8, 2018.
RUMMEL, A. M. et al. Polycyclic aromatic hydrocarbons with bay-like regions inhibited gap junctional intercellular communication and stimulated MAPK activity. **Toxicological Sciences**, v. 49, n. 2, p. 232–240, 1999.
SANDOVAL-HERRERA, N. et al. Neurotoxicity of organophosphate pesticides could reduce the ability of fish to escape predation under low doses of exposure. **Scientific Reports**, v. 9, n. 1, p. 1–11, 2019.
SANTANA, M. S. et al. Diffuse sources of contamination in freshwater fish: Detecting effects through active biomonitoring and multi-biomarker approaches. **Ecotoxicology and Environmental Safety**, v. 149, n. November 2017, p. 173–181, 2018.
SCHÄFER, R. B.; BRINK, P. J. VAN DEN; LIESS, M. Impacts of Pesticides on Freshwater Ecosystems. In: SÁNCHEZ-BAYO, F.; VAN DEN BRINK, P. J.; MANN, R. M. (Eds.). . **Ecological Impacts of Toxic Chemicals (Open Access)**. [s.l.] BENTHAM SCIENCE PUBL LTD, 2011. p. 111–137.
SCHLENK, D. Pesticide biotransformation in fish. **Biochemistry and Molecular Biology of Fishes**, v. 6, n. C, p. 171–190, 2005.
SCHLENK, D. et al. **Biotransformation in fishes**. 1st. ed. [s.l.] CRC Press, 2008.
SHUMAN-GOODIER, M. E.; PROPPER, C. R. A meta-analysis synthesizing the effects of pesticides on swim speed and activity of aquatic vertebrates. **Science of the Total Environment**, v. 565, p. 758–766, 2016.
SLANINOVA, A. et al. A review: oxidative stress in fish induced by pesticides. **Neuro Endocrinol Lett**, v. 30 Suppl 1, p. 2–12, 2009.
STARA, A.; MACHOVA, J.; VELISEK, J. Effect of chronic exposure to simazine on oxidative stress and antioxidant response in common carp (Cyprinus carpio L.). **Environmental Toxicology and Pharmacology**, v. 33, n. 2, p. 334–343, 2012.
SUNANDA, M. et al. Effects of chlorpyrifos (An organophosphate pesticide) in fish. **International Journal of Pharmaceutical Sciences Review and Research**, v. 39, n. 1, p. 299–305, 2016.
SZÉKÁCS, A. Mechanism-related Teratogenic, Hormone Modulant and other Toxicological effects of Veterinary and agricultural surfactants. **Insights in Veterinary Science**, v. 1, n. 1, p. 024–031, 2017.
TIAN, X. et al. Chronic brain toxicity response of juvenile Chinese rare minnows (Gobiocypris rarus) to the neonicotinoid insecticides imidacloprid and nitenpyram. **Chemosphere**, v. 210, p. 1006–1012, 2018.
TSUI, M. T. K.; CHU, L. M. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. **Chemosphere**, v. 52, n. 7, p. 1189–1197, 2003.
ULLAH, S. et al. Malathion induced oxidative stress leads to histopathological and biochemical toxicity in the liver of rohu (Labeo rohita, Hamilton) at acute concentration. **Ecotoxicology and Environmental Safety**, v. 161, n. May, p. 270–280, 2018.
ULLAH, S. et al. Multiple biomarkers based appraisal of deltamethrin induced toxicity in silver carp (Hypophthalmichthys molitrix). **Chemosphere**, v. 214, p. 519–533, 2019.
VAN DER OOST, R.; BEYER, J.; VERMEULEN, N. P. E. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. **Environmental Toxicology and Pharmacology**, v. 13, n. 2, p. 57–149, 2003.
VIECHTBAUER, W. Accounting for heterogeneity via random-effects models and

moderator analyses in meta-analysis. **Journal of Psychology**, v. 215, n. 2, p. 104–121, 2007.

VIEIRA, C. E. D.; DOS REIS MARTINEZ, C. B. The pyrethroid lambda-cyhalothrin induces biochemical, genotoxic, and physiological alterations in the teleost Prochilodus lineatus. **Chemosphere**, v. 210, p. 958–967, 2018.

WANG, X. et al. Permethrin-induced oxidative stress and toxicity and metabolism. A review. **Environmental Research**, v. 149, p. 86–104, 2016.

WERNER, I.; YOUNG, T. M. **Pyrethroid insecticides-Exposure and impacts in the aquatic environment**. [s.l.] Elsevier Inc., 2017. v. 1–5

WHYTE, J. J. et al. Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. **Critical Reviews in Toxicology**, v. 30, n. 4, p. 347–570, 2000.

YAMAMURO, M. et al. Neonicotinoids disrupt aquatic food webs and decrease fishery yields. **Science**, v. 366, n. 6465, p. 620–623, 2019.

ZHANG, L. et al. The role of human aldo-keto reductases in the metabolic activation and detoxication of polycyclic aromatic hydrocarbons: Interconversion of PAH catechols and PAH o-quinones. **Frontiers in Pharmacology**, v. 3 NOV, n. November, 2012.

ZHANG, X. J. et al. Induction of acetylcholinesterase expression during apoptosis in various cell types. **Cell Death and Differentiation**, v. 9, n. 8, p. 790–800, 2002.

ZHU, H. et al. **Downregulation of Antioxidants and Phase 2 ProteinsMolecular Basis of Oxidative Stress**: Wiley Online Books., 8 jul. 2013. Disponível em: <https://doi.org/10.1002/9781118355886.ch5>