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ECOTOXICIDADE DE PARABENOS EM ORGANISMOS AQUÁTICOS: A INTERAÇÃO ENTRE MICROPLÁSTICOS DE POLIETILENO E METILPARABENO NOS ESTÁGIOS INICIAIS DE DESENVOLVIMENTO DO ZEBRAFISH (*Danio rerio*)

MACEIÓ - ALAGOAS
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Dissertação apresentada ao Programa de Pós-Graduação em Diversidade Biológica e Conservação nos Trópicos, Instituto de Ciências Biológicas e da Saúde. Universidade Federal de Alagoas, como requisito para obtenção do título de Mestre em CIÊNCIAS BIOLÓGICAS, área de concentração em Conservação da Biodiversidade Tropical.

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RESUMO

Os contaminantes emergentes são substâncias de origem antrópica detectadas no ambiente em diversas concentrações, mas sem perfil ecotoxicológico bem elucidado e fora do escopo da legislação ambiental. A crescente industrialização introduz cada vez mais substâncias e materiais nas matrizes ambientais, gerando preocupação pelos possíveis efeitos deletérios à biota e à saúde humana. Destacam-se os microplásticos, partículas com tamanho menor que 5 mm e de presença ubíqua em ambientes aquáticos. Sua estrutura predominantemente hidrofóbica é capaz de adsorver substâncias orgânicas e metais pesados, concentrando-os e potencialmente aumentando sua toxicidade para organismos aquáticos. Os parabenos, ésteres do ácido p-hidroxibenzoico, são conservantes encontrados em baixas concentrações em meio aquático e que podem ter sua toxicidade ampliada ao serem adsorvidos por microplásticos. A toxicidade de microplásticos e parabenos e suas possíveis interações foram abordadas ao longo desta dissertação. Primeiramente, foi realizada uma revisão da literatura referente ao estado da arte do conhecimento acerca da bioacumulação e ecotoxicidade de parabenos em organismos aquáticos de diferentes filos. Nela, evidenciou-se uma pequena quantidade de estudos de campo que avaliavam bioacumulação e biomagnificação de parabenos em organismos aquáticos, com subrepresentação de determinados grupos e resultados que variavam de acordo com a localidade, com o tipo de organismo e tecido analisado. Além disso, verificou-se grande heterogeneidade de protocolos experimentais e concentrações utilizadas, predominância de poucas espécies-modelo e baixa utilização de controle analítico. Também foi verificado um aumento no número de estudos ao longo do tempo, com destaque para os anos de 2020 a 2023, bem como na variabilidade de biomarcadores e vias metabólicas analisadas. A seguir, testou-se a partir do teste de toxicidade embriolarval em zebrafish (ZELT) a hipótese de que a exposição de embriões e larvas de zebrafish (*Danio rerio)* a uma combinação de três diferentes concentrações ambientalmente relevantes de metilparabeno (MeP — 0,01, 0,1 e 1 μM) com microplásticos de polietileno (MPPE — ~35 μM, 3,4 mg/L) levaria a efeitos embriotóxicos mais pronunciados do que com a exposição isolada a cada um dos dois contaminantes, bem como a hipótese de que a toxicidade do MeP é concentração-dependente. Os resultados do ZELT sugerem que concentrações ambientalmente relevantes de MeP são capazes de elicitar cardiotoxicidade em embriões de zebrafish, embora a hipótese de que a mistura com MPPE amplifica sua toxicidade não tenha sido confirmada. Além disso, destaca-se a relevância do tamanho dos MPPE em seus efeitos ecotoxicológicos e a possibilidade de uma redução na toxicidade do MeP em embriões de zebrafish com o uso de partículas de tamanhos maiores. Chama-se atenção para a necessidade de mais estudos que empreguem concentrações e vias de exposição realistas, buscando um panorama mais preciso acerca das consequências da presença de tais contaminantes em meio aquático.

Palavras-chave: poluição aquática; contaminantes emergentes; biomarcadores; embriotoxicidade; peixes.

ABSTRACT

Emerging contaminants are substances of anthropogenic origin detected in the environment in various concentrations, but without a clear ecotoxicological profile and outside the scope of environmental legislation. Growing industrialization introduces more and more substances and materials into environmental matrices, raising concern about the possible harmful effects on biota and human health. Of particular note are microplastics, particles smaller than 5 mm and ubiquitous in aquatic environments. Their predominantly hydrophobic structure is capable of adsorbing organic substances and heavy metals, concentrating them and potentially increasing their toxicity to aquatic organisms. Parabens, esters of p-hydroxybenzoic acid, are preservatives found in low concentrations in aquatic environments, and their toxicity could be increased when adsorbed by microplastics. The toxicity of microplastics and parabens, along with their possible interactions, were addressed throughout this dissertation. Firstly, a literature review was carried out on the state-of-the-art knowledge on the bioaccumulation and ecotoxicity of parabens in aquatic organisms of different phyla. This revealed a small number of field studies evaluating bioaccumulation and biomagnification of parabens in aquatic organisms, with underrepresentation of certain groups and results that varied according to the location, type of organism and tissue analyzed. In addition, there was great heterogeneity in the experimental protocols and concentrations used, a predominance of a few model species and low use of analytical controls. There was also an increase in the number of studies over time, especially between 2020 and 2023, as well as in the variability of biomarkers and metabolic pathways analyzed. Subsequently, the zebrafish embryotoxicity test (ZELT) tested the hypothesis that exposure of zebrafish (*Danio rerio*) embryos and larvae to a combination of three different environmentally relevant concentrations of methylparaben (MeP — 0. 01, 0.1 and 1 μM) with polyethylene microplastics (MPPE — ~35 μM, 3 mg/L) would lead to more pronounced embryotoxic effects, 01, 0.1 and 1 μM) with polyethylene microplastics (MPPE, ~35 μM, 3.4 mg/L) would lead to more pronounced embryotoxic effects than with isolated exposure to each of the two contaminants, as well as the hypothesis that MeP toxicity is concentration-dependent. The ZELT results suggest that environmentally relevant concentrations of MeP are capable of eliciting cardiotoxicity in zebrafish embryos, although the hypothesis that mixing with MPPE amplifies its toxicity has not been confirmed. In addition, the relevance of the size of MPPE in its ecotoxicological effects and the possibility of a reduction in the toxicity of MeP in zebrafish embryos with the use of larger particle sizes are highlighted. Attention is drawn to the need for further studies using realistic concentrations and exposure routes, in order to obtain a more accurate picture of the consequences of the presence of such contaminants in aquatic environments.

Keywords: aquatic pollution; contaminants of emerging concern; biomarkers, embryotoxicity; fish.

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APRESENTAÇÃO

Esta dissertação consiste de dois capítulos e trata da ecotoxicidade de microplásticos e parabenos de forma isolada ou em conjunto através de estudos *in vivo* realizados em organismos aquáticos.

No primeiro capítulo em formato de artigo, foi realizada uma extensa revisão da literatura acerca da bioacumulação e ecotoxicidade de parabenos em organismos aquáticos. Para isso, foi feita uma análise crítica de estudos conduzidos tanto em campo, a partir da análise de tecidos animais e vegetais, quanto estudos experimentais feitos com diversos modelos de microrganismos, invertebrados e vertebrados. Este artigo foi publicado na revista Environmental Pollution em novembro de 2024.

No segundo capítulo, também em formato de artigo, foi realizado a avaliação da toxicidade embriolarval em zebrafish (*Danio rerio*) de microplásticos de polietileno de forma isolada ou em mistura com três diferentes concentrações ambientalmente relevantes de metilparabeno. Tal estudo será futuramente acrescido de outras análises realizadas com o mesmo modelo animal, destacando-se estudos comportamentais e análises morfométricas, para posterior publicação em um periódico internacional da área de ecotoxicologia e/ou conservação.

REVISÃO DA LITERATURA

Poluição ambiental e seus efeitos na biota

O conceito de “limites planetários” foi introduzido por Rockström et al (2009) como um *framework* que estabelece uma “zona segura” para o desenvolvimento das atividades humanas, dado o estado desejável dos sistemas que regem a vida no planeta Terra durante a época do Holoceno, que abrange os últimos 11 500 anos do período Quaternário. Tal conceito se baseia na ideia de que a estabilidade do ambiente global é essencial para a manutenção da vida no planeta, e identifica nove limites principais: mudanças climáticas, perda de biodiversidade genética e funcional, interferência nos ciclos biogeoquímicos (nitrogênio e fósforo), acidificação dos oceanos, uso da água doce, mudanças no uso do solo, novas entidades — referente à contaminação por substâncias e materiais que não se encontravam nas matrizes ambientais no período pré-industrial —, esgotamento da camada de ozônio e carga de aerossóis atmosféricos. No ano de 2023, apenas três desses limites não haviam sido ultrapassados: aerossóis atmosféricos, depleção do ozônio estratosférico e acidificação dos oceanos (Richardson et al., 2023).

Estima-se que existam cerca de 350 mil substâncias químicas no mercado global, com 70 mil registradas na última década. Cerca de 30 mil delas foram registradas em economias emergentes, onde a capacidade de descarte de resíduos, bem como a gestão de risco associado a eles, tendem a ser limitadas (Persson et al., 2022). A produção de tais substâncias também gera subprodutos e impurezas que geralmente não são considerados nas regulamentações e nas ações de fiscalização ambiental. Nesse contexto, surge o princípio da cautela: conceito frequentemente aplicado em políticas ambientais e de saúde, trata-se da ideia de que na ausência de consenso científico sobre os riscos relacionados a determinado produto, medida, política ou fenômeno, ações devem ser tomadas para prevenir potenciais danos ao meio ambiente e à saúde pública (Aven, 2019). Como justificativa para a adoção de tal princípio, é possível citar exemplos relevantes em que substâncias de toxicidade e impacto ambiental e social desconhecidos, outrora comercializados em larga escala

visando a solução de problemas industriais, agropecuários e relacionados à saúde humana, acabaram por tornar-se graves problemas (Bierbaum et al., 2020): os clorofluorcarbonos (CFCs), amplamente utilizados em aerossóis e sistemas de refrigeração na segunda metade do século XX, foram banidos em 1987 pelo Protocolo de Montreal após a descoberta de sua capacidade de decompor o ozônio estratosférico (Rowland, 1990). O diclorodifeniltricloroetano (DDT), herbicida e inseticida organoclorado utilizado em larga escala no combate a pragas e vetores de doenças entre os anos de 1940 e 1970, foi banido em diversos países devido à sua neurotoxicidade, carcinogenicidade e alto potencial de desregulação endócrina, bem como à sua grande persistência ambiental e efeitos deletérios no desenvolvimento e sobrevivência de espécies de aves e peixes (Carolin C et al., 2023). Similarmente ao DDT, as bifenilas policloradas (PCBs), compostos organoclorados cuja produção em larga escala se iniciou no início do século XX devido às suas diversas aplicações industriais, também foram largamente controladas e banidas em diversos países devido à sua alta toxicidade para diversos sistemas orgânicos. Possui também alta persistência ambiental, representando um risco para a saúde humana e para a perpetuação de diversas espécies de vertebrados e invertebrados marinhos e terrestres (Ngoubeyou et al., 2022). Dessa forma, entende-se atualmente que o parâmetro básico que deve ser adotado ao se referir ao limite planetário de “novas entidades” é a detecção em matrizes ambientais de substâncias e materiais cujo impacto sobre os sistemas naturais e sobre a saúde humana ainda é total ou parcialmente desconhecido (Liu et al., 2024).

Contaminantes de legado, definidos como substâncias amplamente estudadas e regulamentadas devido aos seus conhecidos impactos ambientais e à saúde humana, bem como à sua persistência ambiental e capacidade de sofrer bioacumulação e biomagnificação (Azcune et al., 2022). Dentre eles estão os metais pesados, metaloides tóxicos, pesticidas organoclorados e hidrocarbonetos aromáticos policíclicos (HPAs), possuindo por vezes tratados internacionais que restringem sua produção, uso e descarte, tal como a Convenção de Estocolmo (UN Environment Programme, 2023). Os contaminantes de preocupação emergente (CECs), por sua vez, são um conjunto de substâncias orgânicas e inorgânicas e de micropartículas de

origem antropogênica que, com o desenvolvimento industrial e crescimento populacional, têm sua presença recentemente documentada em matrizes ambientais. Dentre eles estão os produtos de cuidado pessoal (PCPs) — encontrados em cosméticos, protetores solares e produtos de higiene pessoal e limpeza doméstica —, fármacos, meios de contraste de uso médico, drogas ilícitas, hormônios naturais e sintéticos, alguns edulcorantes, diversos pesticidas, e os nanomateriais, com uma extensa variedade química e introdução contínua de novas formulações no mercado (Das et al., 2024). O relativo desconhecimento sobre seu comportamento no meio ambiente e perfil ecotoxicológico — como bioacumulação, partição no sedimento e coluna d'água e produtos de biodegradação — os tornam alvo de preocupação, dado o fato de que não são usualmente incluídos pelas tecnologias de tratamento de águas residuais nem possuem regulamentação, fiscalização e monitoramento abrangente acerca de sua produção, uso, descarte e concentrações aceitáveis nas matrizes ambientais (Li et al., 2024).

Em ecologia, o *fitness* ou aptidão de determinada população relaciona-se diretamente com sua perpetuação, diferenciando-se entre elas de acordo com as taxas de crescimento, sobrevivência e reprodução dos indivíduos (Laughlin e Messier, 2015). Nesse contexto, a presença de determinadas substâncias com potencial de desregulação endócrina (*endocrine disrupting chemicals* ou EDCs) pode prejudicar o *fitness* reprodutivo de organismos aquáticos mesmo em baixas concentrações (Xiao et al., 2023). A desregulação endócrina em decorrência da poluição ambiental não afeta apenas os organismos e sua prole, mas a população da espécie como um todo e, consequentemente, toda a comunidade biótica de um determinado local. Em um estudo multigeracional, Kidd et al. (2007) registraram o colapso de uma população do peixe norteamericano *Pimephales promelas* após a exposição ao já bem estabelecido desregulador endócrino 17-α-etinilestradiol, estrógeno sintético utilizado em contraceptivos hormonais. Juntamente aos desreguladores endócrinos, a presença em meio aquático de contaminantes com potencial de elicitar embriotoxicidade representa um risco significativo para o desenvolvimento de organismos aquáticos, como peixes (Escobar-Huerfano et al., 2020), anfíbios (Salla et al., 2024) e invertebrados (Ayari et al., 2024). Tais contaminantes podem interferir diretamente nos processos iniciais de

desenvolvimento embrionário e larval, causando malformações, atrasos no crescimento, alterações no desenvolvimento sexual e no comportamento e aumento na mortalidade dos embriões e larvas (Escobar-Huerfano et al., 2020; Dourdin et al., 2023). A exposição a essas substâncias durante as fases mais sensíveis do ciclo de vida dos organismos pode resultar em prole menos numerosa e resiliente, comprometendo a biodiversidade e a estabilidade dos ecossistemas aquáticos. A partir disso, é possível compreender a importância de se investigar substâncias com potencial de causar desregulação endócrina, alterações reprodutivas e embriotoxicidade em organismos aquáticos, considerando sua capacidade de se dispersar na coluna d'água e sua presença ubíqua em tais ambientes.

Microplásticos: caracterização e distribuição em meio aquático

A poluição por materiais plásticos é um problema ambiental de crescente preocupação na atualidade, visto que eles são os principais detritos de origem antrópica encontrados em ambientes aquáticos e estão presentes até mesmo em áreas remotas e sem presença humana (Chassignet et al., 2021). Os polímeros plásticos são leves, duráveis, maleáveis, resistentes e com baixo custo de produção, e sua utilização perpassa os mais variados produtos, desde vestimentas, embalagens, cosméticos e produtos de limpeza até componentes de veículos e equipamentos industriais (Boucher e Friot, 2017). Apesar de suas vantagens práticas para as atividades humanas, seu lado negativo torna-se cada vez mais evidente: estima-se que entre 8,8 e 11 milhões de toneladas de plástico são lançadas aos oceanos todos os anos (Fava, 2022).

A partir dos anos 1960, começou a ser detectada a presença de macroplásticos no estômago de aves marinhas e de outros animais aquáticos, bem como o aumento na quantidade desses detritos em regiões costeiras. Os problemas causados por esses materiais possuem relação com interferência física, podendo levar à morte de animais por asfixia, ferimentos, emaranhamento, constrição ou ingestão — o que pode obstruir seu trato digestório, bem como dar-lhes uma falsa sensação de saciedade e fazê-los entrar em inanição (Schmid et al., 2021).

O termo "microplásticos" (MPs) foi mencionado pela primeira vez em 2004, em um estudo de Thompson e colaboradores. Desde então, a presença de tais

partículas em águas superficiais, sedimentos e em organismos, assim como seus possíveis efeitos prejudiciais nos ecossistemas aquáticos, tornou-se uma área de pesquisa relevante (Rezania et al., 2018). Microplásticos são definidos como partículas poliméricas sintéticas com tamanho entre 1 µM e 5 mm (Fig. 1A), podendo ter origem primária ou secundária. Os MPs primários incluem *pellets* plásticos usados como matéria-prima pela indústria, agentes esfoliantes em produtos de cuidado pessoal (PCPs), além de partículas liberadas pelo desgaste de pneus ou pela lavagem de tecidos sintéticos. Já os MPs secundários são formados pela degradação de macroplásticos, através de processos químicos, exposição à luz ou ação biológica (Rezania et al., 2018; Schmid et al., 2020). Além de diferentes origens, os microplásticos também são materiais com características altamente diversificadas, podendo variar quanto a: 1. Tipo de polímero, como polietileno (PE), polipropileno (PP), poliestireno (PS), poliamida (PA), poliuretano (PU), acetato de polivinila (PVA), cloreto de polivinila (PVC), polietileno tereftalato (PET), polimetil metacrilato (PMMA), dentre outras (Fig. 1B); 2. Cor; 3. Formato, apresentando-se como esferas, fibras isoladas ou em emaranhados, fragmentos, *pellets*, espumas, filmes, dentre outros (Fig. 1C); 4. Presença de aditivos associados, como estabilizantes, plastificantes, corantes e retardantes de chama (Rochman et al., 2019).

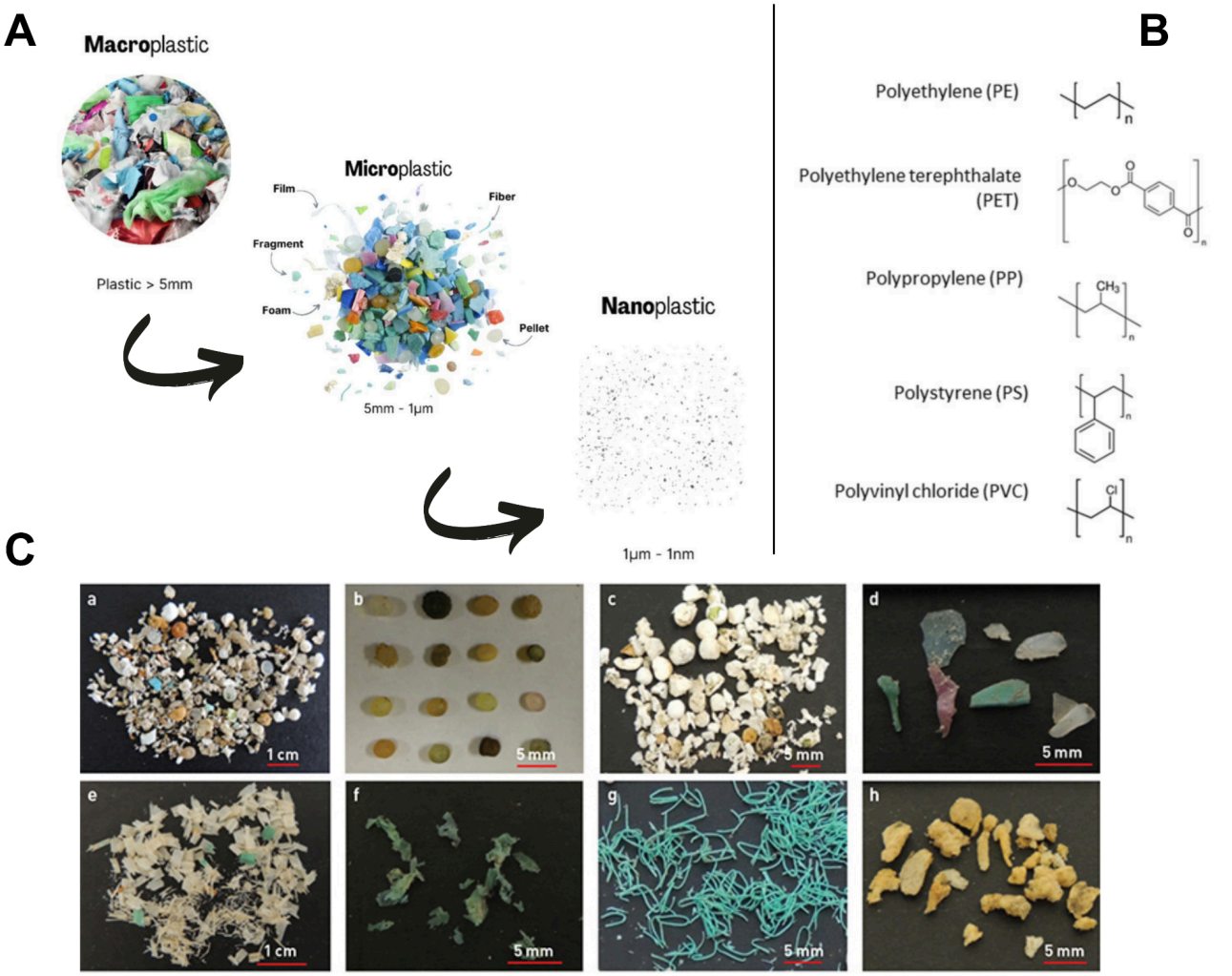

Figura 1. A) Processo de degradação de macroplásticos (> 5 mm) a microplásticos (MPs — 1 µM a 5 mm) e nanoplásticos (NPs — 1 nM a 1 µM). Fonte: Adaptado de Belmaker et al. (2024). B) Estrutura química dos monômeros de alguns dos principais tipos de polímeros plásticos. Fonte: adaptado de Urbanek et al. (2018). C) Amostras de microplásticos (MPs) coletadas do sedimento costeiro na província de Shandong, China, incluindo: (a) MPs mistos, (b) *pellets*, (c) espumas, (d) fragmentos, (e) flocos, (f) filmes, (g) fibras, e (h) esponjas. Fonte: Zhou et al. (2018).

Segundo a Associação de Produtores de Plástico da Europa (PlasticsEurope, 2020), o PE de baixa e de alta densidade (LDPE e HDPE) são, respectivamente, os polímeros termoplásticos com segunda e terceira maior demanda de mercado no continente, estando atrás apenas do PP. Tais polímeros, devido a seu baixo custo e grande versatilidade, são frequentemente usados em produtos plásticos descartáveis e de uso único, de forma que sua produção e descarte ocorrem de forma

acelerada e em larga escala (Erni-Cassola et al., 2019). Como exemplo, análises de duas amostras de águas residuais da cidade de Karlsruhe, na Alemanha, Majewski et al. (2016) relataram o polietileno como sendo o polímero mais frequentemente detectado, compondo 34% (81 mg/m^3) e 17% (257 mg/m^3) de cada uma delas. Para além da quantidade absoluta produzida e descartada, a abundância relativa de diferentes tipos de polímeros plásticos no ambiente aquático depende do local analisado: polímeros de baixa densidade (< 1 g/cm^3), como PE e PP, conseguem flutuar na coluna d'água e são frequentemente encontrados em amostras coletadas em zonas superficiais de mar aberto, porém estão presentes em menor quantidade em zonas intertidais e subtidais, bem como em regiões mais profundas da coluna d'água. Polímeros mais densos, como o poliéster, PA e o acrílico, por sua vez, são mais frequentemente encontrados em zonas subsuperficiais e em sedimentos marinhos (Boucher e Friot, 2017; Erni-Cassola et al., 2019). Os rios são uma importante fonte carreadora de detritos plásticos para ambientes estuarinos e marinhos (Fig. 2), coletando rejeitos industriais, águas de escoamento urbano e efluentes domésticos com quantidades mensuráveis de polímeros plásticos como PE, PP, PS, PA, PVA e PU em tamanhos de <100 a 3500 µM (Stanton et al., 2020). Sugere-se que partículas plásticas com tamanho entre 0,333 e 4,75 mm sejam a vasta maioria, compreendendo mais de 90% dos plásticos encontrados em águas superficiais marinhas (Trevisan et al., 2020).

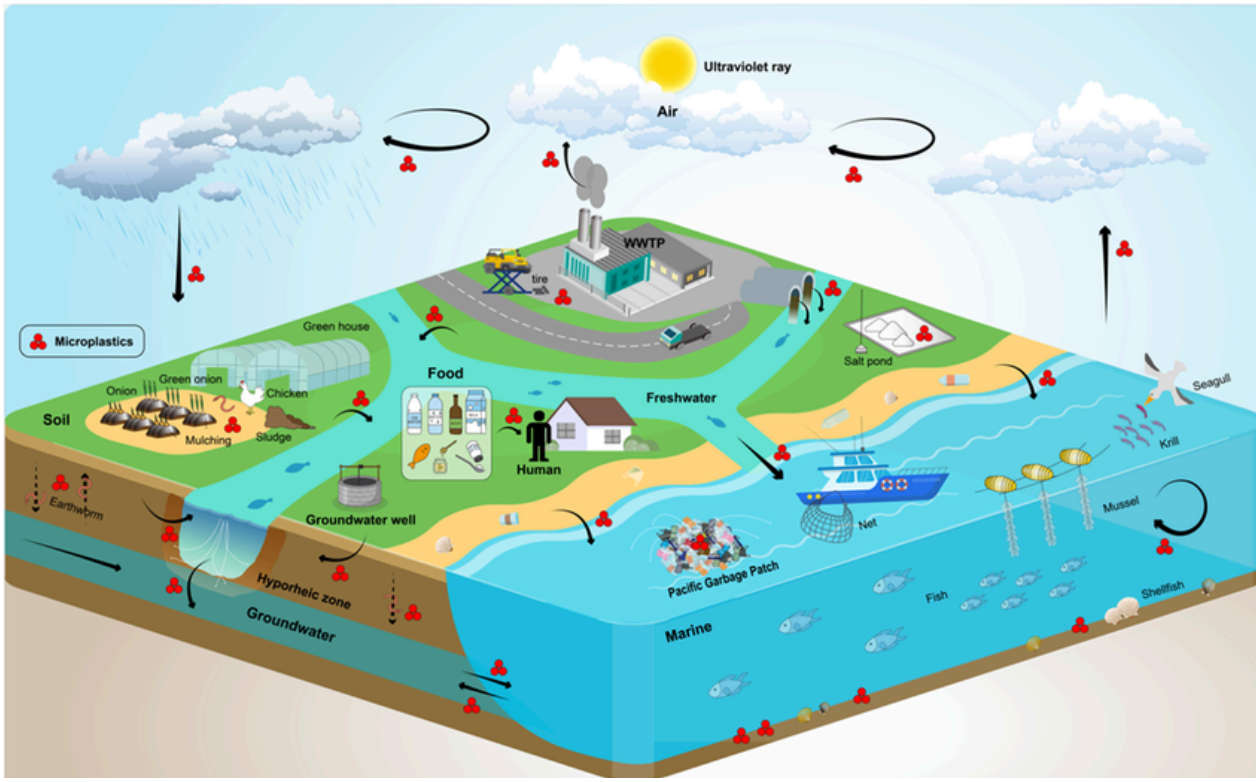

Figura 2. Esquematização da origem, dispersão e circulação de microplásticos primários e secundários entre as matrizes ambientais. Fonte: Lee et al. (2022)

Foi relatada a presença de MPs de diferentes tipos e tamanhos em ambientes marinhos de diversos países entre os anos de 2013 e 2018. Em uma revisão, Rezania et al. (2018) listaram estudos que verificaram a presença de MPs na coluna d'água e sedimentos marinhos em diferentes concentrações e em numerosas partes do mundo. Em praias da Coreia do Sul, encontrou-se uma média de 27 606/m^2 partículas de MPs, com predominância de PS. Na baía de Jinhae, também na Coreia do Sul, foram encontradas entre 33-83 partículas/L de MPs, sendo, à época (2015) a maior abundância de micropartículas plásticas flutuantes já relatada em águas superficiais. No Canadá, relatou-se a presença de 20 a 80 micropartículas plásticas a cada de 10 g de sedimento analisado no estudo. Estudos conduzidos em diversas regiões costeiras da China entre os anos de 2018 e 2020 encontraram concentrações variáveis de microplásticos, com as menores médias sendo referentes a regiões de mar aberto (0,31 itens/m^3 no Mar do Leste da China, 0,40 a 5.2 itens/m^3 no Mar Bohai) e, as maiores, a regiões estuarinas (67,5 ± 94,4 itens/m^3 no Estuário Yangtze). Foram

encontradas altas concentrações de microplásticos nos sedimentos da Baía Xiangshan (1740 ± 2150 itens/kg, peso seco) e do Mar Amarelo (2580 ± 1140 itens/kg, peso seco), contrastando com baixas concentrações em suas respectivas colunas d'água — 8.91 ± 4.7 itens/m³ e 0.33 ± 0.28 itens/m³, respectivamente (Wang et al., 2020). Em 2019, Zheng et al. (2019) encontraram microplásticos em uma concentração média de 46 ± 28 itens/m³ na coluna d´água da Baía Jiaozhou, na China, bem como uma média de 15 ± 6 itens/kg, peso seco, nos sedimentos locais. Dentre os tipos de polímeros encontrados, predominavam o PET, o PP e o PE, formando respectivamente 56,25%, 34,38% e 3,13% das partículas detectadas nas amostras de água e 51,35%, 21,62% e 8,11% daquelas detectadas nas amostras de sedimentos. Em geral, a heterogeneidade nas concentrações de microplásticos detectadas em diversos estudos reflete variações geográficas nas regiões estudadas, como conformação espacial da zona costeira, correntes marítimas, condições climáticas, variações sazonais, nível de troca de água, atividade fluvial e nível de atividade humana (Erni-Cassola et al., 2019; Wang et al., 2020).

Além da presença na coluna d'água e em sedimentos de regiões costeiras e marinhas de diferentes regiões do mundo, também já foi detectada a presença de micropartículas plásticas em tecidos de diversas espécies de peixes e invertebrados marinhos. De acordo com uma revisão publicada por Sequeira et al. (2020), de amostras coletadas de 198 espécies de peixes em 24 países, sendo que apenas 14% destes eram provenientes de aquicultura, 60% continham microplásticos em seus órgãos e tecidos. Dentre os principais polímeros encontrados estavam PE (16%), PP (14%), PS (24%), PA, (11%) e PET (5%). O nível trófico e os hábitos alimentares dos animais influenciaram significativamente o número de microplásticos por indivíduo, com valores mais altos para peixes carnívoros e predadores, dando suporte à hipótese de transferência trófica para tais contaminantes. Dentre os órgãos mais frequentemente avaliados pelos estudos estavam os órgãos do sistema digestivo, as brânquias, o músculo esquelético e a pele. Em um estudo conduzido por Wang et al. (2020) na Baía Hangzhou, China, foram encontrados 70 microplásticos em 92 amostras extraídas de quatro espécies de peixes e três espécies de crustáceos. Todas as espécies de peixes analisadas possuíam maior abundância média de MPs em comparação às espécies de

crustáceos, provavelmente refletindo seus hábitos predatórios e demersais — além da transferência trófica, a maior proximidade com o sedimento, um dos principais sumidouros de MPs, poderia facilitar a ingestão de tais partículas.

A superfície dos microplásticos possui capacidade de realizar diversas interações intermoleculares com substâncias presentes nas matrizes ambientais, destacando-se as interações hidrofóbicas, as forças de Van Der Waals, as interações pi-pi, as interações eletrostáticas, as ligações covalentes, a complexação iônica e as ligações de hidrogênio. Isso permite a adsorção de diversos tipos de contaminantes em sua superfície, tais como moléculas orgânicas apolares, polares e ionizadas, bem como substâncias inorgânicas como metais pesados (Fig. 3) (Rafa et al. 2024). A capacidade de adsorção e dessorção de tais moléculas da superfície dos microplásticos depende de diversos fatores, como o tipo de polímero, o tamanho, formato e nível de degradação das partículas, a carga e polaridade do contaminante e a temperatura, salinidade e pH do ambiente onde se encontram. Sendo de tamanho diminuto e, portanto, de fácil ingestão por parte de organismos aquáticos, os microplásticos podem agir como um “Cavalo de Troia” ao funcionar como potenciais carreadores de poluentes de alta toxicidade e de microorganismos patogênicos adsorvidos em sua superfície (Hartmann et al., 2017; Rafa et al., 2024). Dentre os exemplos de contaminantes ambientais com capacidade de sofrer sorção pela superfície de microplásticos, estão HPAs (Tan et al., 2019), antibióticos (Li et al., 2018), pesticidas (Li et al., 2021), nanopartículas (Singh et al., 2021), fungicidas (Fang et al., 2019), per- e polifluoroalquilados (PFAS) (Dai et al, 2022), hormônios (Hu et al, 2020), PCPs (Zhou et al. 2020) e metais pesados como cádmio, níquel e chumbo (Tenea et al., 2024).

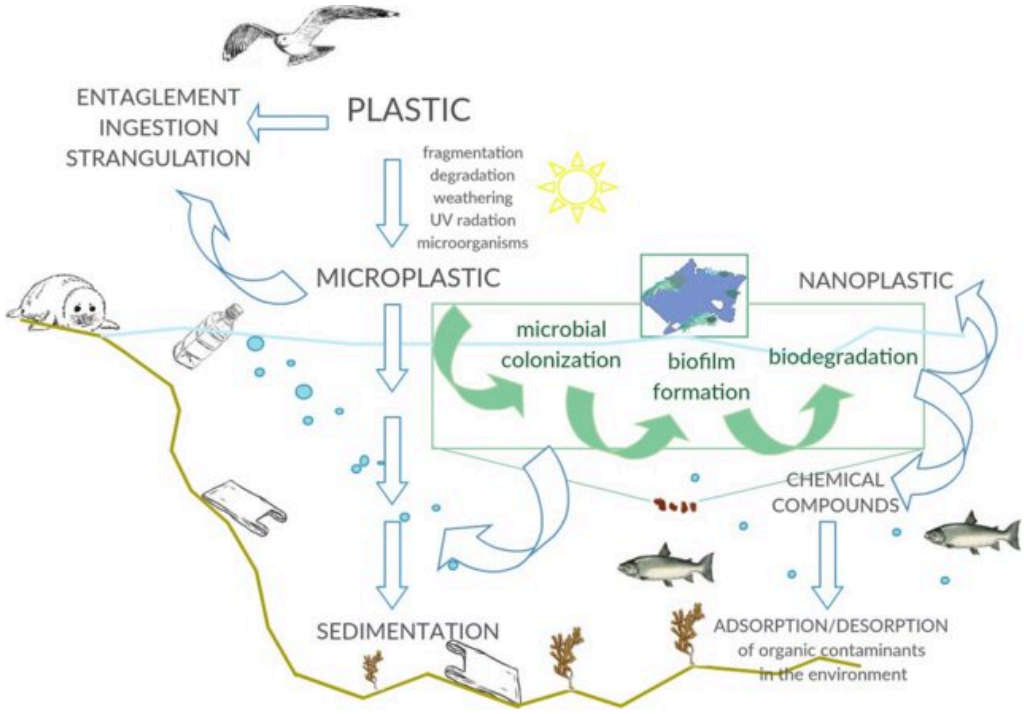

Figura 3. Resumo das rotas de degradação física e biótica de materiais plásticos em ambientes aquáticos e de suas possíveis consequências deletérias para a biota.Fonte: Urbanek et al. (2018).

Microplásticos e sua ecotoxicidade em organismos aquáticos

Diversos estudos experimentais relataram a ocorrência de consequências negativas causadas por MPs e nanoplásticos (NPs) em embriões, larvas, juvenis e indivíduos adultos de espécies de peixes como o zebrafish, *Oryzias* spp., *Tigriopus japonicas*, *Cyprinus carpio*, *Carassius* spp., *Pimephales promelas*, *Pomatoschistus microps*, *Sparus aurata*, *Acanthochromis polyacanthus,* dentre outros. Dentre elas, estavam processos inflamatórios, danos ao sistema digestório, prejuízos ao crescimento e desenvolvimento, estresse oxidativo, neurotoxicidade, alterações comportamentais e menor viabilidade e sobrevivência de embriões e larvas (Yong et al., 2020; Hasan et al., 2024).

Barboza et al. (2018) observaram uma inibição na atividade da acetilcolinesterase (AChE) cerebral e na isocitrato desidrogenase (IDH) muscular de

indivíduos juvenis do peixe *Dicentrarchus labrax* expostos a uma concentração de 0,69 mg/L de microplásticos ao longo de 96 horas, bem como aumento na peroxidação lipídica no cérebro e músculo dos animais testados. Malafaia et al. (2020) registraram efeitos negativos da exposição a micropartículas de polietileno (MPPE — 6,2 a 100 mg/L) sobre a sobrevivência e desenvolvimento de embriões e larvas de zebrafish expostos por 144h sob um regime semi-estático. Dentre estes efeitos, estavam a eclosão precoce de embriões, a maior mortalidade e alterações morfométricas de larvas, como maior altura da cabeça, maior área da vesícula óptica e do saco vitelínico e, nas maiores concentrações (50 a 100 mg/L), maior distância interocular e maior distância entre os mioseptos. Tais alterações poderiam ocasionar em perda da acuidade visual e problemas na contratilidade do músculo esquelético nos animais, prejudicando sua capacidade de nado e sua adaptabilidade comportamental. Em indivíduos juvenis do peixe marinho *Sebastes schlegelii* expostos a microplásticos de PS (MPPS) na concentração de 10^6 microesferas/L durante 14 dias, verificou-se alterações comportamentais como redução no tempo de forrageamento e de alimentação, além de inibição na atividade locomotora quanto à distância percorrida e velocidade de nado. Foram observadas também alterações histopatológicas, como congestão e hiperemia hepáticas e escurecimento biliar, além de uma menor taxa de crescimento e menor concentração de conteúdo proteico e lipídico total (Yin et al., 2018).

Em adultos de zebrafish, a exposição a micropartículas de PE e PS (100 e 1000 µg/L) durante 20 dias foi capaz de induzir alterações pró-inflamatórias, estresse oxidativo, danos na mucosa intestinal e no epitélio branquial, alterações morfológicas, redução na expressão de genes relacionados ao metabolismo energético e à função imune e mudanças no ritmo circadiano, com significativo aumento de atividade locomotora no período noturno (Limonta et al., 2019). A exposição a micropartículas de polietileno e polipropileno naturalmente desgastadas durante 21 dias (0,1 e 1 mg/L) levou adultos de zebrafish a apresentarem comportamentos sugestivos de ansiedade, além de alterações na função mitocondrial cerebral e aumento na atividade de enzimas antioxidantes hepáticas (Félix et al., 2023).

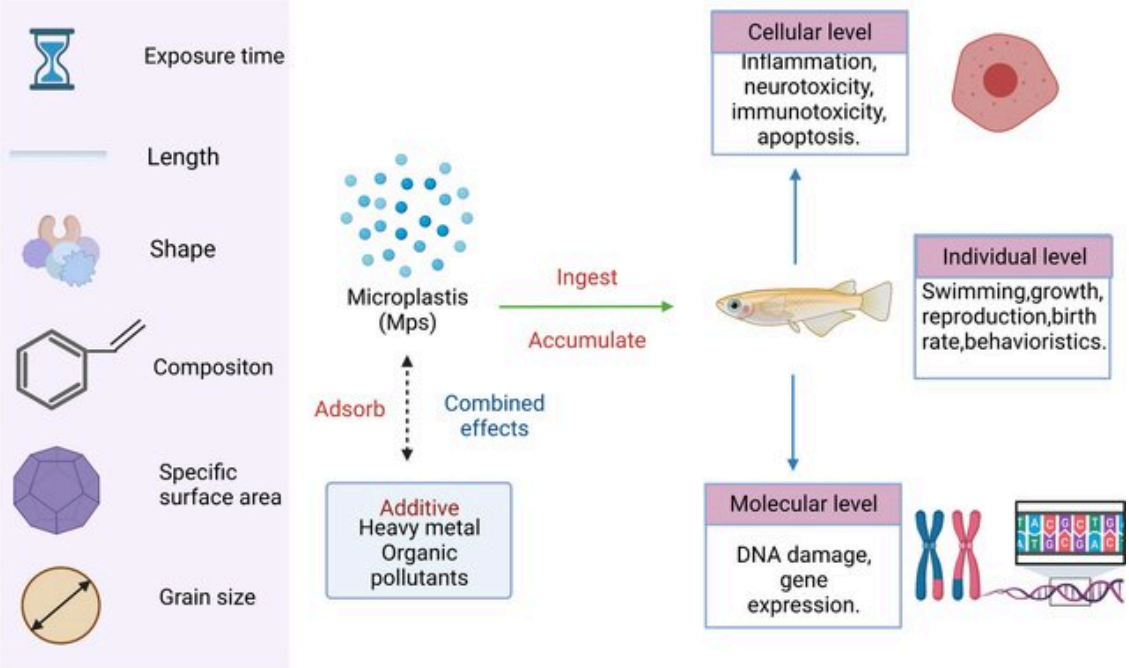

Figura 4. Efeitos biológicos de microplásticos em peixes teleósteos e fatores que influenciam em sua toxicidade. Fonte: Liu et al. (2024).

Em 2019, Wang et al. verificaram diversos marcadores de toxicidade na exposição do teleósteo medaka marinho (*Oryzias melastigma*) a MPPS (2, 20 e 200 µg/L) durante 60 dias, tais como alterações histopatológicas nas brânquias e gônadas, aumento nos marcadores de estresse oxidativo nas brânquias, intestinos, fígado e testículos de machos, desregulação nos hormônios sexuais das fêmeas e redução de sua fecundidade. Além disso, os efeitos deletérios foram transgeracionais, afetando negativamente o desenvolvimento da prole dos animais expostos aos tratamentos com MPPS. Qiang e Cheng (2021), em um estudo também com MPPS (10, 100 e 1000 µg/L), encontraram alterações histopatológicas e aumento no estresse oxidativo e nos marcadores apoptóticos nas gônadas de zebrafish expostos aos microplásticos por 21 dias.

A partir de uma análise integrada de dados de biomarcadores relacionados a estresse oxidativo e neurotoxicidade em juvenis de *D. labrax* expostos a microplásticos e a mercúrio de forma isolada ou em mistura, Barboza et al. (2018) encontraram evidência de interação significativa entre os dois contaminantes, levando a efeitos biológicos diferentes dos exibidos na exposição isolada. Em um estudo conduzido por

Bihanic et al. (2020), embriões e larvas de *Oryzias melastigma* foram expostos a MPPE (4 to 6 µM, 10 mg/L) durante 12 dias, isoladamente ou sorvidos com três contaminantes: benzo(a)pireno (BaP — 0,01 e 16,64 µg/g MP), ácido perfluoro-octanosulfônico (PFOS — 0,12 e 55,65 µg/g MP) e benzofenona-3 (BP3 — 0,14 e 24 ng/g MP). Na concentração mais alta de PFOS sorvidos em MPPE, houve redução na taxa de eclosão e no comprimento total final, ao passo que tais alterações não foram observadas na exposição ao PFOS de forma isolada. Enquanto a exposição isolada ao BaP na maior concentração não foi capaz de induzir toxicidade, a exposição conjunta com MPPE levou a uma maior incidência de anormalidades morfológicas e uma redução na atividade locomotora quanto à distância percorrida. Na exposição ao BP3 em maior concentração sorvido ao MPPE, observou-se redução no comprimento final total e no comprimento da cabeça, o que não foi observado na exposição isolada ao BP3. Não houve evidência de ingestão dos MPPE pelos animais, porém foi visualizado um acúmulo de partículas ao longo da superfície do córion, sugerindo um contato direto dos MPPE com os embriões através deste.

Em 2019, Roje e colaboradores realizaram um estudo *in vitro* realizado com linhagens celulares de adenocarcinoma mamário sensível a estrógenos expostas a uma associação de nanopartículas de poliestireno (NPPS — 1, 10 e 100 ppm) com uma mistura de parabenos (PBmix — 0,01 a 1 µg/mL). Observou-se um efeito sinérgico sobre a atividade dessas células, com a proporção de células MCF-7 em proliferação ativa mais do que dobrando ao serem expostas à concentração mais alta de NPPS em combinação com PBmix em uma concentração constante de 1 µg/mL. Foi sugerido que tais achados poderiam ser resultantes de uma interação dos parabenos com a estrutura dos NPPS, de forma a concentrar tais moléculas em sua superfície e carreá-las para o interior das células, intensificando sua atividade pró-estrogênica.

Parabenos: caracterização, distribuição ambiental e ecotoxicidade

Os parabenos, considerados como CECs, são um conjunto de substâncias orgânicas de baixo peso molecular, resultantes da reação de esterificação do ácido 4-hidroxibenzóico (4-HB). São utilizadas desde 1920 como conservantes em preparações farmacêuticas, e na atualidade são encontradas também em cosméticos,

alimentos e outros produtos industrializados (Nowak et al., 2018; Bolujoko et al., 2022). Dentre eles, os mais comumente empregados são o metilparabeno (MeP) e o propilparabeno (PrP), sozinhos ou em associação. São estáveis em soluções aquosas de baixo pH e suas propriedades variam de acordo com o tamanho de seu grupamento alquila: as maiores cadeias possuem maior atividade antimicrobiana e maior resistência à hidrólise, mas menor solubilidade em água — o MeP e o PrP possuem cadeias de um e três carbonos, respectivamente (Wei et al., 2021; Pereira et al., 2023). Devido ao seu influxo contínuo em baixas concentrações nas águas superficiais através dos efluentes antropogênicos, os parabenos podem ser considerados como contaminantes pseudopersistentes, bem como os outros PCPs em geral (Garric, 2013).

Como resultado de pesquisas conduzidas em diversos países, foi constatada a presença de parabenos em diferentes ambientes aquáticos, na água potável, em efluentes urbanos e em solos agrícolas (Feng et al., 2019). A presença de parabenos no esgoto urbano é bem documentada e, mesmo com sua baixa estabilidade, alta biodegradabilidade em condições aeróbias e uma eficácia de remoção de acima de 90% durante o processo de tratamento de água (Haman et al., 2015), eles ainda são encontrados nos efluentes tratados e na água e sedimentos de corpos d'água, assim como o seu metabólito 4-HB (Feng et al., 2019). Os parabenos também são capazes de gerar derivados clorados mais estáveis e persistentes do que as substâncias originais, principalmente devido ao processo de cloração utilizado no tratamento de água (Bolujoko et al., 2021).

No que diz respeito às concentrações de parabenos encontradas em ambientes naturais e antropizados, há grande variabilidade de acordo com o local estudado. As maiores concentrações são encontradas em efluentes urbanos, chegando a 76 900 ng/L em uma estação de tratamento de água do sul da Califórnia (Błędzka et al., 2014). No Brasil, o MeP foi detectado em córregos da cidade de Rio Grande/RS em concentrações entre 7,6 e 29,8 µg/L, e na cidade de Morro Redondo/RS, entre <1 e 134 µg/L (Penha et al., 2021). Derisso et al. (2020), ao analisar sete pontos do Rio Monjolinho na cidade de São Carlos/SP, detectaram concentrações de MeP que variavam entre 0,11 e 0,98 µg/L. Na avaliação de três rios da região de Curitiba/PR, Santos et al. (2016) encontraram concentrações de MeP de até 2875 ng/L.

Além de sua presença constatada em águas superficiais, também há evidências de bioacumulação de parabenos em tecidos animais e humanos. Xue e Kannan (2016) relataram a presença de MeP e seu metabólito 4-HB no rim, fígado e tecido muscular de águias carecas (*Haliaeetus leucocephalus*) e albatrozes (*Phoebastria* spp.) em concentrações que variavam de 580 ng/g (MeP) a 35 - 300 ng/g (4-HB), dependendo do tecido e da espécie. No mesmo estudo, relatou-se a presença de MeP, PrP e 4-HB nos tecidos hepático e cerebral de peixes da costa da Flórida, em concentrações que variaram de 11,2 ng/g para o MeP a 1130 ng/g para o 4-HB. As concentrações teciduais maiores do que as plasmáticas são sugestivas de bioacumulação, principalmente no fígado (Xue e Kannan, 2016). A bioacumulação de 4-HB e parabenos também já foi relatada em mamíferos marinhos como lontras e golfinhos, e a relação entre sua concentração e a de MeP nestes animais sugere que a fonte de tais substâncias tenha sido, de fato, antropogênica (Xue e Kannan, 2016). Chiesa et al. (2018) encontraram diversos parabenos e 4-HB em peixes pelágicos como *Salmo trutta*, *Salmo solar* e *Thunnus albacares*, bem como em bivalves, que são os invertebrados com a maior capacidade de bioacumulação dessas substâncias. A análise de invertebrados marinhos por Xue et al. (2017) demonstrou a presença de MeP em 82% das amostras, em concentrações que variavam de 9,43 a 322 ng/g, a depender da espécie. Neste estudo, chegou-se ao valor de 1,83 para o fator de magnificação trófica (TMF) do MeP a partir da análise de uma teia alimentar subtropical composta por 13 espécies, sugerindo um potencial significativo de biomagnificação para este composto.

A partir do final do século XX, com a publicação de estudos sugerindo a atividade estrogênica e antiandrogênica dos parabenos, cresceu a preocupação com seus possíveis efeitos negativos sobre o equilíbrio ecológico (Błędzka et al., 2014). A sua potencial atividade estrogênica é associada com o tamanho e o peso molecular de sua cadeia alquila ou arila, de forma que o butilparabeno (BuP), o heptilparabeno (HeP) e o benzilparabeno (BzP) possuem um maior potencial de desregulação endócrina (Routledge et al., 1998). O 4-HB é considerado o metabólito final da degradação biótica dos parabenos e também possui atividade estrogênica relatada *in vitro* e *in vivo* (Błędzka et al., 2014; Raja et al., 2019). Geração de estresse oxidativo,

embriotoxicidade, genotoxicidade, neurotoxicidade, desregulação endócrina, mudanças funcionais na microbiota intestinal e alterações metabólicas, histopatológicas e comportamentais também são possíveis efeitos deletérios destes compostos, de acordo com diversos estudos experimentais conduzidos em organismos como peixes teleósteos e crustáceos aquáticos (Merola et al., 2020; Penha et al., 2021; Lin et al., 2022; Eghan et al., 2023; Hu et al., 2023b).

Em 2017, Ateş et al. demonstraram a ocorrência de embriotoxicidade e estresse oxidativo em embriões de zebrafish (*Danio rerio*) expostos a uma concentração de 50 mg/L de MeP por 68 horas. Verificou-se aumento da mortalidade, da incidência de efeitos teratogênicos subletais e do dano oxidativo nos embriões expostos a 50 mg/L de MeP. Tais alterações também foram encontradas no estudo conduzido em zebrafish por Merola et al. (2020), sendo observadas em concentrações a partir de 30 mg/L de MeP. Dambal et al. (2017) verificaram anomalias no desenvolvimento de embriões e larvas de zebrafish expostos a concentrações de MeP a partir de 200 µM, tais como redução da frequência cardíaca, edema pericárdico, curvatura anormal da coluna e acúmulo de células sanguíneas. Observou-se também indução na expressão de vitelogenina (*vtg*), um biomarcador de desregulação endócrina, em larvas expostas a concentrações de 100 µM de MeP. Em 2019, Raja et al. observaram redução da frequência cardíaca e atraso no desenvolvimento e eclosão de embriões de zebrafish expostos a concentrações de MeP de 10 e 100 partes por bilhão (ppb). Em concentrações de MeP de 0,1 e 1 ppb, observou-se aumento dos níveis de cortisol e da exibição de comportamentos de ansiedade nos animais, bem como redução na atividade da enzima acetilcolinesterase. Bereketoglu e Pradhan (2019) registraram efeitos deletérios da exposição de embriões de zebrafish a MeP (100 e 200 µM) e PrP (10 e 25 µM) por 120 horas, como malformações embrionárias e menor expressão de genes relacionados à proteção contra o estresse oxidativo, como a superóxido dismutase 1 (*sod1*) e glutationa-S-transferase (*gst*). Também verificaram expressão alterada de genes relacionados à função endócrina, com aumento da expressão do receptor de estrógeno 2-α (*esr2a*) e redução do receptor de andrógeno (*ar*). Adicionalmente, Penha et al. (2021) verificaram em zebrafish adultos, expostos a 50 mg/L de metilparabeno por 96 horas, o aumento da peroxidação lipídica, um

biomarcador de dano oxidativo, e de micronúcleos , um biomarcador de genotoxicidade.

O zebrafish como organismo-modelo

O *Danio rerio* (Hamilton 1822), popularmente conhecido como zebrafish, é um peixe teleósteo de água doce da família dos ciprinídeos, nativo do sudeste asiático e do subcontinente indiano (Ribas e Piferrer, 2013). Há mais de 100 anos tem sido empregado como modelo bem estabelecido em pesquisas experimentais, tendo uma crescente importância em estudos de diversas áreas no Brasil (Trigueiro et al., 2020). É utilizado como um como um organismo-modelo para estudos de embriotoxicidade, teratogenicidade, neurotoxicidade, genotoxicidade, estresse oxidativo e alterações morfológicas e comportamentais devido a diversos fatores (Ribas e Piferrer, 2013; Roper e Tanguay, 2018). Os estudos realizados nesta espécie de peixe são de crescente importância para a avaliação de possíveis consequências da exposição de vertebrados a contaminantes encontrados em águas superficiais (Busch et al., 2011; Garcia et al., 2016). Embriões e larvas de zebrafish, devido à sua transparência, também têm sido cada vez mais utilizados em estudos de localização com MPs e NPs marcados por fluorocromos (Bhagat et al., 2020).

Em primeiro lugar, o genoma do zebrafish foi completamente sequenciado em 2013, demonstrando ortologia de 71% em relação ao genoma humano, e os genes relacionados a doenças chegam a 84% de homologia de sequência (Roper e Tanguay, 2018). A morfologia e fisiologia da espécie possuem similaridades significativas em relação às de outros vertebrados, bem como o seu desenvolvimento embrionário — que é altamente conservado neste subfilo e, parcialmente, em alguns outros cordados (Roper e Tanguay, 2018).

Além disso, o desenvolvimento dos embriões e larvas é de fácil observação devido à transparência dos ovos e do epitélio dos indivíduos, que permanece até sete dias após a fertilização. Além disso, seu processo de desenvolvimento é bem conhecido e documentado, o que torna a observação e análise de possíveis alterações mais simples e eficiente: as fases de gastrulação, neurulação e organogênese estão completas em cerca de 48 a 72 horas após a fertilização dos ovos, quando tem início a

eclosão. Em 72 a 96 horas após a fertilização, inicia-se o período larval (Kimmel et al., 1995).

Os peixes adultos são de pequeno tamanho (3 a 4 cm de comprimento), sendo possível manter uma grande quantidade de animais em um pequeno espaço e sem a necessidade de estrutura laboratorial de alta complexidade. Oferecem também ampla disponibilidade de embriões, com fêmeas capazes de colocar centenas de ovos a cada evento reprodutivo, que podem ocorrer mais de uma vez por semana. Além disso, os animais possuem um curto tempo geracional, alcançando a maturidade reprodutiva após cerca de três a quatro meses de vida (Roper e Tanguay, 2018). Por todas essas características, o zebrafish se apresenta como um modelo animal vantajoso em relação à questão de praticidade, facilidade de manejo e viabilidade econômica dos estudos, além de ter alto valor translacional em relação a outras espécies de vertebrados (Kalueff et al., 2013; Ribas e Piferrer, 2013).

Objetivos e hipóteses

Tendo em vista a revisão da literatura feita ao longo desta seção, tem-se como objetivo geral do trabalho investigar como a presença de microplásticos de polietileno (MPPE) afeta a toxicidade de metilparabeno (MeP) sobre os estágios iniciais de desenvolvimento do zebrafish.

Os objetivos específicos, por sua vez, são:

1. Realizar uma ampla revisão da literatura acerca da bioacumulação e toxicidade de parabenos e seus metabólitos sobre organismos aquáticos de múltiplos táxons;

2. Avaliar a toxicidade de três concentrações ambientalmente relevantes de MeP (0,01 μM, 0,1 μM e 1 μM) de forma isolada ou em mistura com MPPE (3,4 mg/L) sobre embriões e larvas de zebrafish a partir do Teste de Toxicidade Embriolarval (ZELT).

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CAPÍTULO 1

BIOACCUMULATION AND ECOTOXICITY OF PARABENS IN AQUATIC ORGANISMS: CURRENT STATUS AND TRENDS

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Abstract

Parabens are preservatives commonly found in various personal care products, pharmaceuticals, and foodstuffs and mostly are unregulated chemical compounds. Given their extensive use, they are ubiquitously detected in different compartments, including aquatic environments, and classified as emerging pollutants. When parabens reach aquatic environments, they may compromise animal health. Thus, the current study aimed to review the data available concerning the bioaccumulation and ecotoxic effects of parabens on aquatic species. After the review search, which included articles published online around the world until December 2023, a total of 71 articles were systematically analyzed and summarized. The first study on paraben ecotoxicity was published in 2000. Studies were conducted mainly in laboratory conditions (80.28%) using fish, crustaceans, bivalves, algae and bacteria, among others taxa. Field studies were conducted at 82 sampling sites across five countries. Paraben bioaccumulation was detected primarily in fish muscle, liver, brain, gills, and testis. Also, aquatic animals (i.e., fish and invertebrates) were more susceptible to the effects of parabens than microorganisms such as periphyton. Parabens can cause lethal and sublethal effects on aquatic organisms, such as oxidative stress, endocrine disruption, neurotoxicity, behavioral changes, reproductive impairment, and developmental abnormalities. In addition, the toxicity of parabens depends on the species, taxon, developmental stage, exposure period, and concentrations tested. As long as its use remains massive and its detection ubiquitously, our literature overview denotes that further ecotoxicological research about the parents' parabens and their metabolites in different taxa of aquatic organisms is deeply needed.

Keywords: aquatic pollution; ecotoxicology; endocrine disruption; Esters of p-Hydroxybenzoic Acid; sentinel species; zebrafish

1.1 Introduction

Parabens are a family of organic compounds broadly used as preservatives in pharmaceuticals, personal care products (PCPs), and foodstuffs due to their antimicrobial (Terasaki et al., 2013) and fungicidal (Murata et al., 2019) activities. Also, these substances have favorable properties as colorless, odorless, active at various pH levels, and mix well with other ingredients. Parabens were produced with the intention of replacing the use of salicylic acid and benzoic acid, which had the disadvantage of being effective only at a highly acidic pH (Lück & Jager, 1997). Therefore, since the 1920s, parabens have become common additives in cosmetics and pharmaceutical products (Wei et al., 2021; Nowak et al., 2018). Additionally, the worldwide cosmetics market, worth about 500 billion EUR in 2018, is expected to increase the use of parabens and other preservatives in response to consumer demand for longer shelf life (Nowak et al., 2021).

Parabens are produced in large volumes in Europe, the USA, and Asia, with production rates as high as 5000 tons reported in the 1990s (Nowak et al., 2018). In the United States, the estimated average daily total exposure to parabens is as follows: 50 mg from personal care products (PCPs), 25 mg from pharmaceutical products, and 1 mg from food products, resulting in a cumulative exposure of 76 mg/day. For an individual weighing 70 kg, this corresponds to a daily intake of 1.26 mg kg^{-1} body weight (bw) (Vale et al., 2022; Błędzka et al., 2014). In China, monthly production of parabens is reported to be 500 tons for methylparaben (MeP) and ethylparaben (EtP) and over 10,000 tons for propylparaben (PrP), which can be attributed to the approval in March 2002 of these parabens as food additives, including a maximum allowed concentration of 0.5 g kg^{-1} in food (Ministry of Health of the People's Republic of China, 2015).

These compounds are chemically characterized as alkyl esters of the p-hydroxybenzoic acid (pHBA) or 4-hydroxybenzoic acid (4-HB) (Fig. 1), such as methylparaben (MeP), ethylparaben (EtP), n-propylparaben (PrP), isopropylparaben (iPrP), n-butylparaben (BuP), isobutylparaben (iBuP), phenylparaben (PhP), benzylparaben (BzP) and heptylparaben (HeP). The MeP and PrP being the most widely used in generic formulations, isolated or in combination (Haman et al., 2015). Parabens' physical and chemical properties are determined by their alkyl chain length, which is also directly related to their biological activity. For example, a longer carbon

chain will generally enhance their antimicrobial activity; however, a longer carbon chain can reduce their solubility in water and can increase their persistence in aquatic environments (Piao et al., 2014) and bioaccumulation in fatty tissues (Wang & Kannan, 2015). In addition, parabens are usually biodegraded within a few days (2.1 days in case of MeP and EtP to 4.5 days in case of BuP), especially those with shorter alkyl chains and under aerobic conditions, giving way to their main metabolite, 4-HB (González-Mariño et a., 2011; Santos et al., 2016).

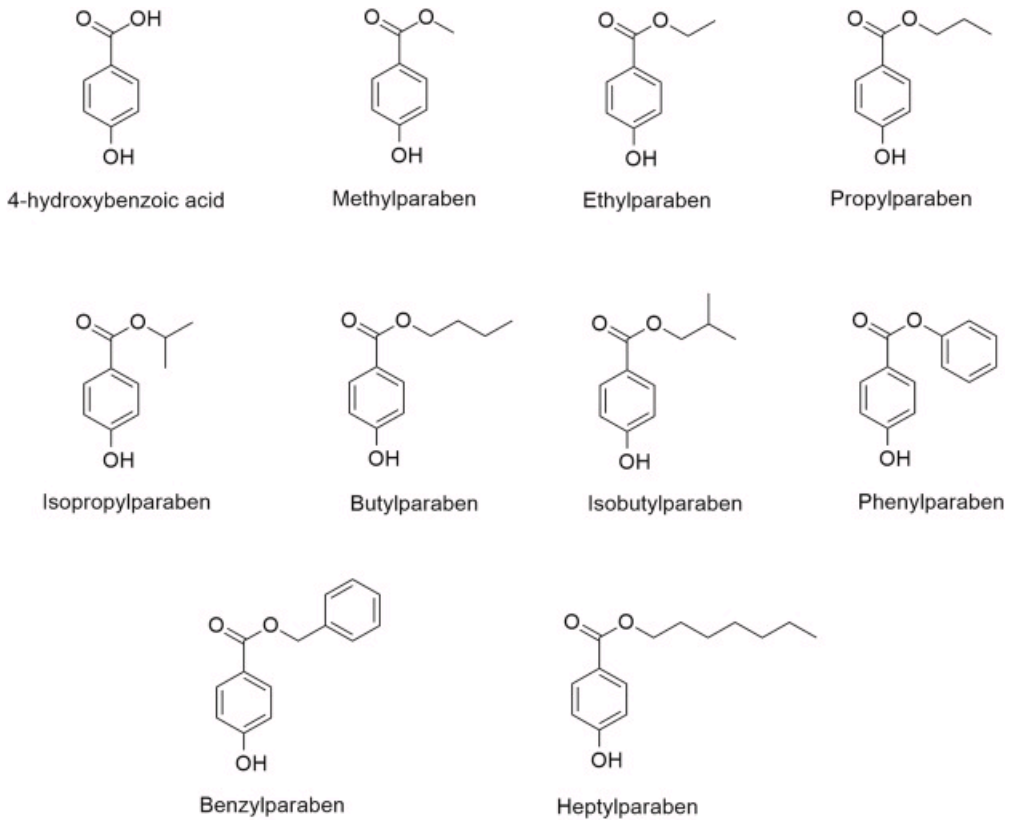

Figure 1. Chemical structure of parabens commonly employed in commercial and industrial formulations and also for *p*-hydroxybenzoic acid.

The sewage treatment may efficiently remove several parabens from urban sludge (Błędzka et al., 2014; Li et al., 2015); however, the regular input from multiple sources often outpaces their removal capacity. These remaining parabens are sometimes called a “pseudo-persistent” contaminant (Albero et al., 2012). Parabens can

react with chlorine, commonly used as a disinfectant in water treatment plants. These chlorinated derivatives are more persistent than the original molecules; however, little is known about their toxicity (Haman et al., 2015).

The growing use of PCPs and pharmaceuticals with parabens derived from their composition has been accompanied by increased environmental concentrations, specifically in aquatic habitats (Bolujoko et al., 2021; Brausch & Rand, 2011). The wash-off from human bodies during personal hygiene and the unregulated discharge of PCPs in sewage systems and regular refuse collection containers are the primary sources of parabens in urban wastewater and overall residues (Haman et al., 2015; Bolujoko et al., 2021). However, widespread regulation regarding their discharge, input, and presence in shallow waters is generally lacking. Parabens are classified as emerging pollutants (EPs) due to being detected in low concentrations (ng L^{-1} or μg L^{-1}) in aquatic environments and not being commonly included in routine monitoring of water quality (Vale et al., 2022). However, evidence regarding their potential toxic effects on aquatic organisms and human health raises worldwide concerns (Maia et al., 2023; Medkova et al., 2023).

Nonetheless, it is worth noting that the widespread use of such substances is related to their overall presumed safety to humans concerning their acute toxicity based on concentrations typically used in commercial formulations (Crovetto et al., 2022). As the market formulations, particularly from the cosmetic industry, and the world population are continually growing, the concentration of parabens in shallow waters will likely increase in the following years (Bolujoko et al., 2021). Parabens released into the aquatic ecosystem can interact with aquatic organisms and induce ecotoxic effects at different levels of biological organization, such as phytoplankton (Di Poi et al., 2017), aquatic invertebrates (Lee et al., 2017; Shore et al., 2022), and fish (Lin et al., 2022).

The toxicity of parabens is related to the disruption of the cell membrane and intracellular proteins, and the inhibition of mitochondrial function (Bolujoko et al., 2021; Crovetto et al., 2022). In addition, recent *in silico*, *in vitro,* and *in vivo* studies using zebrafish (*Danio rerio*) adults and also during the embryo-larval stage have revealed that parabens exposure results in disruption of the hypothalamic-pituitary-gonad axis (HPG), of the hypothalamic-pituitary-thyroid axis (HPT) and also for

hypothalamic-pituitary-adrenal axis (HPA) (Bereketoglu and Pradhan, 2019; Dambal et al., 2017; Hu et al., 2023; Liang et al., 2022; 2023a, b). Furthermore, another *in vivo* study evaluated parabens' effects on a copepod (*Tigriopus japonicus*) and showed that chronic exposure to MeP, EtP, and PrP induced estrogenic effects (Kang et al., 2019). All these data raise concerns about the effects of parabens on aquatic organisms and ecosystems. However, understanding the mechanism of action (MoA) and ecotoxicity of parabens in different aquatic organisms remains unclear. In this context, this review has four main objectives:

i) Gather data on the concentrations of parabens and their metabolites found in samples from several aquatic species in various locations worldwide;

ii) Build a historical and methodological perspective on the experimental ecotoxicology studies with parabens, focusing on the evolution of techniques and biomarkers since the first laboratory studies;

iii) Describe the state-of-the-art knowledge of the potential toxicity of different types of parabens and their metabolites on aquatic organisms and;

iv) Integrate background and current experimental approach in comparison to environmental findings;

Furthermore, research gaps and recommendations for future research were also identified.

1.2 Methodological approach

This review was conducted from January to December 2023. After the delimitation of the scope, keywords were expanded to capture the maximum number of articles per search. The keywords “parabens”, in combination with “ecotoxicology”, “biomarkers”, “fish”, “conservation”, “aquatic organisms”, “ecology”, “aquatic invertebrates”, “crustaceans”, “algae”, or “mollusks,” both singular and plural forms were used. The groups of crustaceans and mollusks were individually searched due to the lack of studies on other invertebrate phyla/subphyla found using the keywords “aquatic invertebrates”. The databases used were “PubMed”, “ScienceDirect”, “Web of Science”, and “SCOPUS”. Initially, a total of 5276 articles were found in the databases.

After the exclusion criteria (non-English papers, gray literature, letters/short communications, review articles, book or book chapters, duplicated documents, articles with chemical/*in vitro*/*in silico* analysis only, papers focused on human health or not aligned with the goal of this article in general), a total of 76 works were maintained for further analysis (Fig. 2).

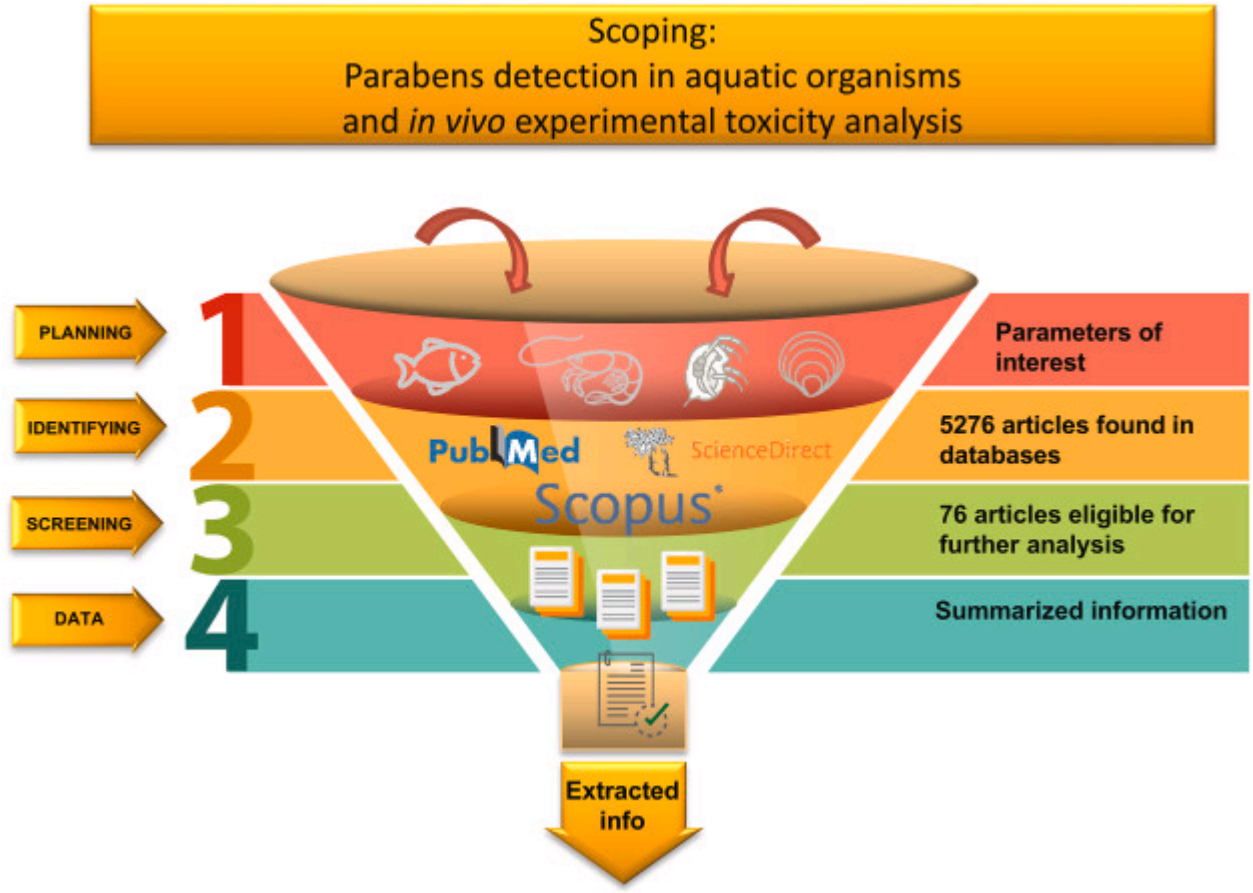

Figure 2. Summarized study methodology step by step, from the scoping to the final presentation.

As one of the goals of our study was to picture a historical retrospective of this field of study, the year of publication was not used as an exclusion criterion. Instead, each paper was analyzed according to the following parameters: a) year of publication; b) field or experimental research; c) species, sex, and developmental stage of the organisms. Furthermore, regarding the field studies, the following parameters were included: a) geographical correspondent authors' location; b) number

of species collected; c) tissues/organs investigated; d) type and concentration of parabens and their metabolites found in samples. The bioaccumulation of parabens in birds and aquatic mammals was not added. Finally, for the experimental studies, the following aspects were reviewed: a) experimental design (number of samples, treatments, and replicates); b) type of exposure (acute, chronic or subchronic exposure; static, semi-static, or flow-through); c) route of exposure (by food, gavage, or water exposure); d) type and concentration/dose of the parabens; e) analytical control (if present); f) biomarkers/parameters investigated; g) omics analysis (if present, which type); h) bioaccumulation analyses (if present); and i) biological effects found.

Graphics reporting the articles published in each country were made through the MapChat tool (https://www.mapchart.net/). The remaining data were compiled using Microsoft Excel and the graphics were organized according to the year of study publication, experimental or field study, organisms' clade, life stage, potential biomarkers, study location, and type of paraben evaluated, among others, by using the software GraphPad PRISM®.

1.3 Historical, geographical analysis and background

From 2000 to 2023, 76 studies were published (Fig. 3A). The first published study concerning the toxicity of parabens addressed their potential estrogenic effect and discussed its possible outcomes for human health. Pedersen et al. (2000) tested EtP, PrP, BuP, and their primary metabolite (4-HB) through an in vivo assay using sexually immature rainbow trout (Oncorhynchus mykiss), this being the first experimental evidence that exposure to parabens can cause endocrine disruption in fish.

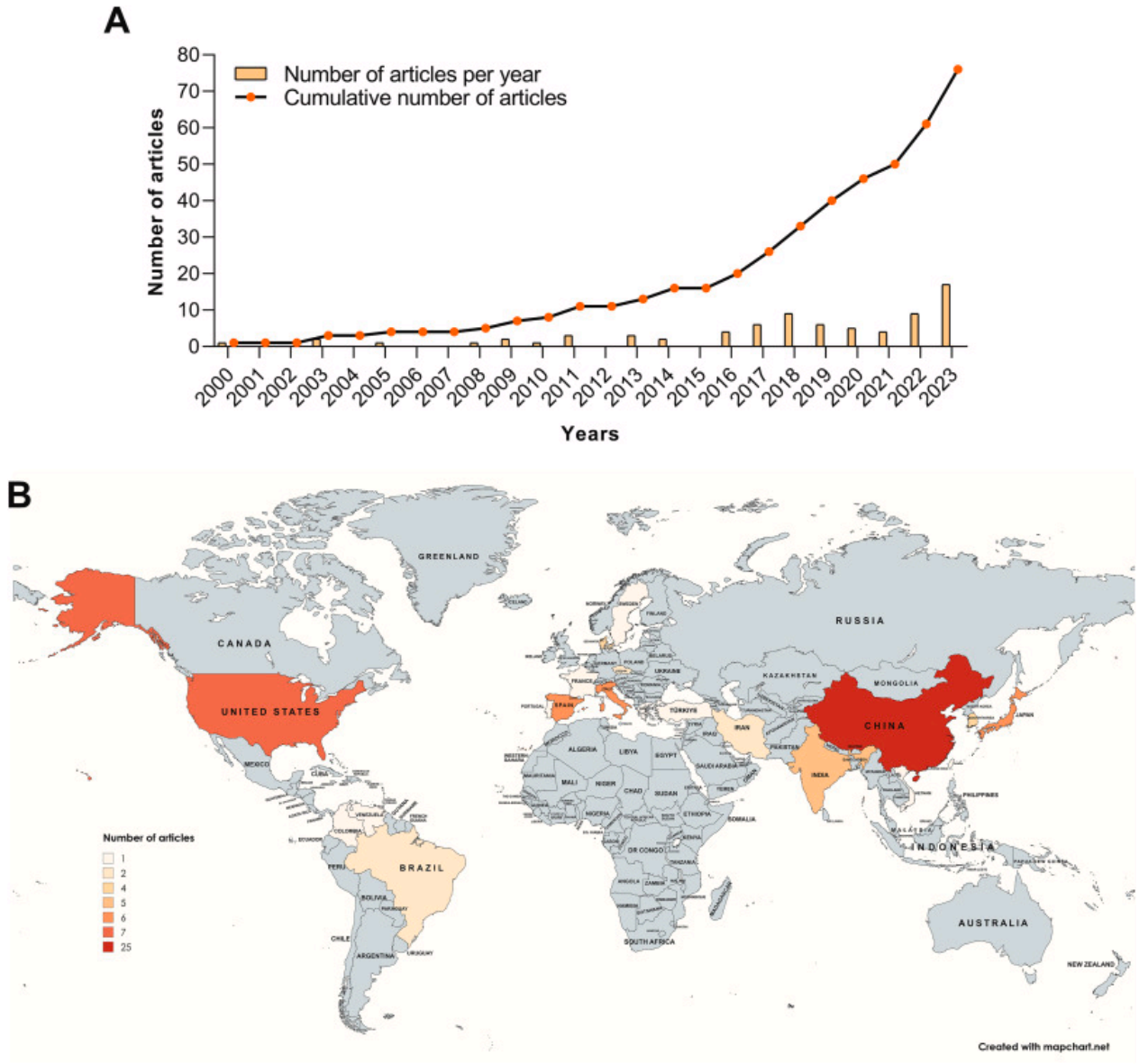

Figure 3. Number of articles published about ecotoxicological studies with parabens on aquatic organisms and their distribution across the world. A) Absolute (columns) and cumulative (line) number of articles published up to December 2023. B) Global distribution and number of papers published by corresponding author's country.

Significant growth in research interest in paraben toxicity was observed from 2016 onwards (Fig. 3A). This trend may be attributed to increased awareness of emerging pollutants and their potential impact on ecosystems. Notably, the COVID-19 pandemic accelerated this interest, as the heightened use and disposal of personal care and hygiene products may contain parabens in their composition. For instance,

Fig. 3A illustrates a marked rise in publications on the ecotoxicity of parabens to aquatic organisms in 2022 and 2023, coinciding with the scientific community's heightened focus on the environmental consequences of pandemic-related chemical use (Qualhato et al., 2023). This suggests that the pandemic may have acted as a catalyst for more urgent research into the environmental risks of parabens.

Interestingly, between 2022 and 2023, a series of studies in China investigated the ecotoxicity of MeP in zebrafish. These studies showed that MeP exposure affects gene expression in various organs, induces oxidative stress, disrupts embryonic development, and impairs endocrine functions (Hu et al., 2022a, 2022b; 2023a,b; Liang et al., 2022, 2023a; 2023b). Currently, most research has focused on areas such as water and air pollution, conservation, and the sustainable use of environmental resources, all of which have shaped investigations into the toxic potential of parabens to human and environmental health (Azeredo et al., 2023; Mejías et al., 2023; Vale et al., 2022).

Fig. 3B depicts the geographical distribution of studies investigating the ecotoxicity of parabens in aquatic environments across 19 countries. China had the highest number of authors who have researched this subject (n = 26; 34.21% of the total), followed by the USA (n = 7; 9.21%), Italy (n = 6; 7.89%), India, and Japan (n = 6; 7.89% each) (Fig. 3B). Therefore, it is essential to note that further research is needed in regions such as Africa, Oceania, Canada, Russia, South America, and others to ensure a broader understanding of the concentrations of parabens present in environmental samples.

Ecotoxicological studies on parabens and aquatic organisms were predominantly conducted under laboratory conditions (n = 57; 75%) compared to field studies (n = 19; 25%) (Fig. 4A). Laboratory studies have primarily focused on assessing multiple biomarker responses and elucidating parabens' MoA and effects in different levels of organization (i.e., organs, tissues, and cells) of various aquatic species. In contrast, field studies have primarily accessed paraben bioaccumulation or metabolites in different tissues or the entire organism. Field studies were conducted at 128 sampling sites across five countries: China (50%), USA (15.79%), Spain (10.59%), Italy, Colombia, and Vietnam (5.26% each). These data suggest the need for more

field studies in countries or regions with high biodiversity, such as Brazil, Colombia, Mexico, and Vietnam, to evaluate the bioaccumulation in various taxonomic groups, mainly in the global south countries.

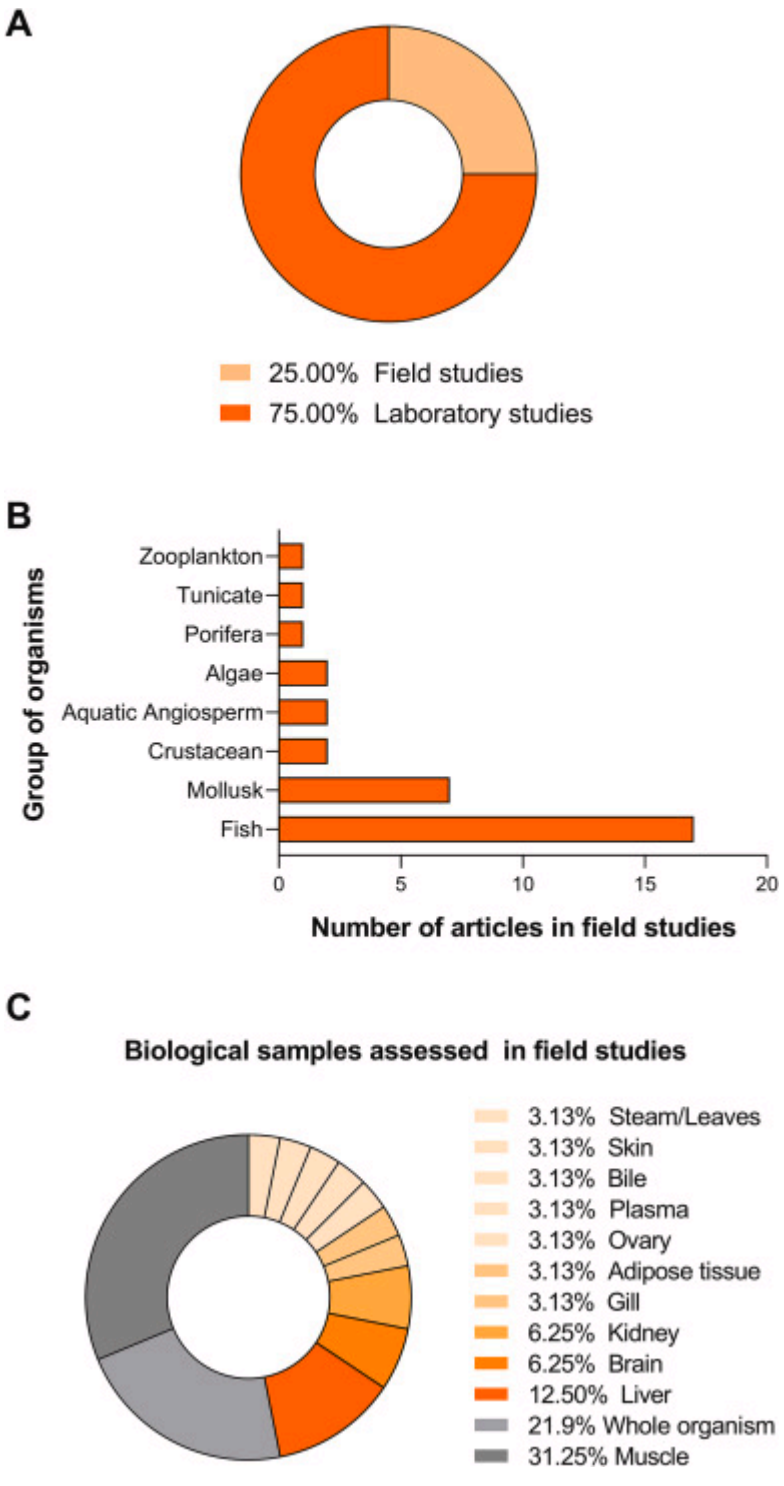

Figure 4. Details of ecotoxicological studies focused on parabens in aquatic organisms. A) Percentage of field and laboratory studies. B) Number of articles published by groups of organisms in field studies. C) Frequency of the different biological samples from aquatic organisms (invertebrates, vertebrates, and plants) whose paraben s bioaccumulation was evaluated in field studies. The 21.9% include the soft tissue of invertebrate species and also the whole body of some fish species.

1.4 Field studies on the bioaccumulation of parabens in aquatic organisms

Field studies are highly beneficial in identifying potential sources of contamination that may not be possible in laboratory studies (Graney et al., 2020). This approach offers the advantage of understanding the effects of realistic paraben exposure in aquatic ecosystems and organisms (Zhang et al., 2021; Haman et al., 2015). By studying the responses of organisms exposed to varying substance concentrations, researchers can assess their potential ecotoxicity and understand the long-term consequences for individual species and ecosystem health (Bom & Sá, 2021). Also, field studies using aquatic animal models allow researchers to analyze the interactions between parabens and other environmental conditions and stressors (Graney et al., 2020). This way, water bodies might be exposed to multiple contaminants and stressors, which can have greater than additive or antagonistic effects on the toxicity of parabens (Miranowicz-Dzierżawska et al., 2023). Researchers can provide a more comprehensive understanding of the environmental impact by studying paraben exposure with factors such as temperature, pH, and co-occurring pollutants in this context.

Field studies were conducted with a wide range of aquatic species, mainly fish (72.97%), followed by mollusks (14.23%), crustaceans (4.39%), aquatic angiosperms (4.39%), algae (0.34%), porifera (0.34%), tunicates (0.34%), and zooplanktons (0.3%). Among these groups, the number of articles published with fish is almost three times higher than the number of studies conducted with mollusks, as seen in Fig. 4B. Field studies have assessed 92 freshwater (50.27%) and 91 marine species (49.73%), spanning both vertebrates and invertebrates. These field studies reveal the complex interactions between parabens and aquatic ecosystems, uncovering the potential risks of their widespread presence. Therefore, aiming to give more detailed

information about paraben bioaccumulation data from field studies of different taxa of aquatic organisms, the following sections present the most relevant works in the literature covering this topic.

1.4.1 Green algae and aquatic angiosperms

Up to now, one field study has analyzed the bioaccumulation of parabens in three species of green algae and three species of aquatic angiosperms. Xue et al. (2017) investigated the presence of MeP, EtP, PrP, BuP, and BzP in the green alga Caulerpa prolifera, in marine angiosperms *Syringodium filiforme, Halodue wrightii*, and *Ruppia martima*, and the mangrove angiosperms *Avicennia germinans, Rhizophora mangle*, and *Laguncularia racemosa*, all collected from coastal waters in Florida (USA). MeP was detected in all mangrove samples at concentrations ranging from 8 to 33.3 ng g-1, and also in all seagrass samples in concentrations ranging from 4.0 to 42.2 ng g-1 (wet weight – ww), being both higher than those found in the green alga (9.6–16.5 ng g-1 ww). Other parabens, such as EtP, BuP and BzP were found in some angiosperm samples (4.5%–13.6%), at concentrations up to 7.78 ng g-1 ww for BzP in a mangrove samples (Xue et al., 2017).

Similarly, a study conducted in the Dongjiang River Basin, China, investigated the occurrence and bioaccumulation of nine types of parabens and two metabolites in samples of plants and algae (Lin et al., 2024). The study found considerable concentrations of these chemicals in all samples, in which MeP (240 ng g-1 dry wt), i-BuP (18.7 ng g-1 dry wt), and BzP (5.18 ng g-1 dry wt) were detected in all plant samples, while MeP (48.4 ng g-1 dry wt), EtP (10.8 ng g-1 dry wt), and PrP (4.03 ng g-1 dry wt) were most abundant in algae. The author also estimated the bioaccumulation factor (BAF) in plankton samples (including zooplankton), being the higher log BAF seen for EtP (5.61), followed by PrP (5.54) and MeP (4.74) (Lin et al., 2024). However, they did not calculate the log BAF for plants. Although these log BAF values denote a high potential for bioaccumulation in plankton, the authors suggested that these may have been overestimated, since they used normalized dry weight (dw) concentrations in such calculation. All these findings provide baseline information about the occurrence and fate of parabens in aquatic plant biodiversity.

Interestingly, parabens are naturally produced by certain terrestrial plants, where they act as natural preservatives to protect against microbial growth and other environmental stressors (Ali et al., 1998; Li et al., 2003; Nowak et al., 2018). However, the levels of parabens found in plants are lower than in PCPs and cosmetics, which use synthetic parabens as preservatives.

1.4.2 Aquatic invertebrates

Reviewed data showed that ten field studies analyzed the bioaccumulation of parabens in invertebrate species, mainly mollusks (n = 6) and crustaceans (n = 4). Furthermore, tunicate and poriferan species were analyzed in only one study. For these species, parabens were quantified in the whole organism, as presented in Fig. 4C. Aquatic invertebrates and benthic biodiversity research can provide valuable information about the potential exposure to parabens in aquatic environments (Zhu et al., 2024). Also, it is worth mentioning that bivalves, such as mussels and oysters, are commonly used in ecotoxicity testing as they filter organisms and can accumulate contaminants from their surrounding environment, making them valuable indicators of environmental contamination. On the other hand, bivalves can also close their shells and potentially reduce exposure to aquatic contaminants (Bom & Sá, 2021).

In a study conducted in the Chinese Bohai Sea between 2006 and 2015 by Liao & Kannan (2018), the total concentrations of parabens detected in mollusk tissue samples from *Mactra veneriformis, Mytilus edulis*, and *Cyclina sinensis* ranged from 2.66 to 299 ng g^{-1} ww, with MeP being the most commonly detected parent paraben. In addition, a gradual increase in the concentration of parabens in the samples was observed over the years (Liao & Kannan, 2018). These authors point out that the increasing paraben concentrations in mollusk samples were related to the growing production and use of parabens-containing products in China, culminating in the inevitable increase in the disposal of parabens and their metabolites in aquatic environments. Similarly, MeP was also the main paraben found in invertebrate samples from Florida coastal waters, with a detection frequency of 82% (n = 186). The concentration of MeP varied from less than 2.01 ng g^{-1} ww for shrimp *Farfantepenaeus aztecus* to 337 ng g^{-1} ww for the bivalve *Chione cancellata* (Xue et al., 2017).

In addition, Chiesa et al. (2018) evaluated two bivalves: a Mediterranean mussel (*Mytilus galloprovincialis*) and a clam species (*Chamelea gallina*) collected from fish markets in Milan, obtained from farming sites in the Mediterranean Sea. The authors identified MeP as the main paraben detected in the samples, with concentrations up to 32 ng g^{-1} (Chiesa et al., 2018). Likewise, Zhu et al. (2024) evaluated parabens' bioaccumulation in four crustacean species (n = 24) and two cephalopod species (n = 11), from the Beirpweihbu Gulf, South China Sea. The primary contaminants identified in marine organisms were MeP and 4-HB, with concentrations ranging from 0.18 to 5.03 ng g^{-1} ww for MeP in crustaceans, as well as from 1.45 to 2.67 ng g^{-1} ww for cephalopods. The log BAF of MeP in cephalopods (2.80) was similar to those found in fish (2.85), but significantly higher than that in crustaceans (2.37), denoting that fish and cephalopods have a greater capacity to enrich MeP from seawater (Zhu et al., 2024). Since most species evaluated were benthic organisms, biota-sediment accumulation factors (log BSAF) were estimated for MeP (5.51), revealing notable accumulation from sediment in the investigated environment.

Considering freshwater ecosystems, Lin et al. (2024) evaluated paraben concentrations in zooplankton samples from Dongjiang River wetland ecosystem in southern China. MeP was detected in all plankton samples, followed by EtP (95.0%) and PrP (95.0%). However, i-PrP, BuP, i-BuP, BzP, pentylparaben (PeP), and HeP were less frequently detected (<40.0%). MeP (7.37–139 ng g-1 dry wt) was the most abundant paraben detected, followed by PrP (<LOQ – 113 ng g-1 dry wt), and EtP (<LOQ – 598 ng g^{-1} dry wt). Similarly, MeP, followed by EtP and PrP, were the most abundant parabens detected in eight shellfish families (Buccinidae, Veneridae, Haliotidae, Mytilidae, Pectinidae, Ostreidae, Corbiculidae, and Pteriidae) from Shenzhen coastal waters, which represents more than 95% of the total the parabens detected (Lu et al., 2019). All these studies performed in distinct aquatic environments and countries denote the ubiquitous contamination by parabens, as needed for further field studies in elucidating the real-world exposure of aquatic organisms to parabens, which becomes important in assessing human risks in areas with high seafood consumption.

1.4.3 Fishes

A total of 17 field studies analyzed the bioaccumulation of parabens in 208 fish species. Among them, 12 studies (63.15%) were performed with freshwater species, while 7 studies 36.84%) used marine ones. The tissues or organs most evaluated in these field studies (Fig. 4C) were the muscles (28.57%), liver (14.29%), brain and kidney (7.14% each), followed by gills, fat tissue, plasma, and bile (3.57% each). Among such studies, the parabens were detected only in 16 of them, since Renz et al. (2013) did not detect paraben concentrations in the brain samples of three fish species (*Alosa pseudoharenga, Micropterus dolomieu*, and *Dorosoma cepedianum*) from the Greater Pittsburgh area, USA.

The first study devoted to the bioaccumulation of parabens in aquatic organisms was carried out by Ramaswamy et al. (2011), in which the determination of four parent parabens was investigated in muscle samples of 20 fish species (11 demersal and nine pelagic fish) from Manila Bay (Philippines). MeP, PrP, and BuP were detected in more than 90% of the fish samples analyzed, while EtP was detected in around 70% of the samples. MeP was detected in higher concentrations, ranging from <0.05 to 3600 ng g^{-1}, whereas EtP <0.011–840 ng g^{-1}, PrP <0.024–1100 ng g^{-1}, and BuP <0.003–70 ng g^{-1} pointing out the contamination of Manila Bay by parabens. Interestingly, they also observed a positive correlation between parabens concentration and fish length (Ramaswamy et al., 2011). Xue & Kannan (2016), when studying inland lakes and rivers of New York State, detected concentrations of MeP ranging from <2.01 to 690 ng g^{-1} ww in muscle and liver of the fish species *Micropterus dolomieu* and *M. salmoides*. In addition, MeP was detected at concentrations ranging from <2.01 ng g^{-1} in the muscle of *Mugil cephalus* to 735 ng g^{-1} ww in the brain of *Rhizoprionodon terraenovae* from Florida coastal waters (Xue & Kannan, 2016).

In another study conducted with 13 species of fish from four river basins in Spain, MeP and BzP were found in specimens from all basins, with concentrations ranging from 3.41 to 84.69 ng g^{-1} dw for MeP, from 0.63 to 3.46 ng g^{-1} dw for PrP, and from 0.35 to 0.54 ng g^{-1} dw for BzP, with a log BAF of 1447 for MeP and 166 for PrP. The study also reported that the predominant group of EDCs in the samples consisted

mainly of parabens (Pico et al., 2019). Consistent with these results, Wang et al. (2019) detected parabens in all fish samples from Taihu Lake, China, during an investigation conducted from 2009 to 2017. MeP, EtP, and PrP were found in all samples from the five fish species (*Culter alburnus, Carassius carassius, Hypophtalmichtys molitrix, H. nolitrix*, and *Protosalanx hyalocranius*). At the same time, BuP was detected in 47.70% of the samples, and BzP in 79.60%. The concentrations of MeP ranged from 88.1 to 1200 pg g^{-1} ww, while EtP concentrations ranged from 33.6 to 450 pg g^{-1} ww, and for PrP, from 55.3 to 543 pg g^{-1} ww. The total sum of parabens ranged from 261 to 1710 pg g^{-1} ww (Wang et al., 2019). In the Chinese Pearl River, nine species of fish were studied for the determination of phenolic EDCs and, following the abovementioned study, MeP was the main paraben found in fish samples (from 2.01 to 201 ng g^{-1} ww), and the second most frequently detected EDC, with a median concentration of 40 ng g-1 lipid weight (lw) (Peng et al., 2018). The same study found that the total concentration of parabens was higher in the liver than in abdominal fat and dorsal muscles, as well as increasing paraben bioaccumulation in parallel to increasing fish weights. The highest men log BAF was found for MeP (2.0 in dorsal muscle, 2.4 in belly fat, and 3.8 in liver), whilst the lowest was for Bup (1.3 in dorsal muscle, 1.9 in belly fat, and 2.1 in liver) (Peng et al., 2018).

Xue et al. (2017) analyzed 35 fish species from coastal Florida and showed that the MeP concentrations ranged from 2.01 ng g^{-1} (*Lagodon rhomboides*, whole homogenized fish) to 610 ng g^{-1} ww (*Lutjanus campechanus*, liver). The mean concentration of MeP in the kidney (181 ± 138 ng g^{-1} ww) was higher than in the liver (53 ± 61.4 ng g^{-1} ww) and the whole fish (30.3 ± 19.9 ng g^{-1} ww), indicating differential tissue/organ bioaccumulation. The study also detected EtP concentrations in the kidney ranging from <2.01 in *Sphyrna mokarran* to 16.8 ng g^{-1} ww in *M. cephalus*. As for the other parabens (i.e., PrP, BuP, BzP, and HeP), concentrations were generally very low: the highest concentrations were 7.29 ng g^{-1} ww of PrP in Scomberomorus maculatus and 7.32 ng g^{-1} ww of BuP in *S. mokarran*. Chiesa et al. (2018) evaluated specimens from the fish market (from farmed or wild by multiple sites) and showed that the main paraben found was also MeP, being detected in 41% of samples (n = 54), followed by EtP (3%). The other parabens were not detected in any samples except for

EtP, which was found in 3% of samples (Chiesa et al., 2018). These authors also drew attention to the high variability in the accumulations found, depending on the species, location of origin, and capture site.

MeP also was the most frequent parent paraben found in muscle samples from fish collected in Dongjiang River, China, with concentrations ranging from <LOQ–194 ng g^{-1} dw); followed by EtP (88.0%) and PrP (81.0%) (Lin et al., 2024). Other parabens (i-PrP, BuP, i-BuP, BzP, PeP, and HeP were detected with <20%). These authors also found a higher log BAF for MeP (3.77), followed by PrP (3.77), and EtP (3.43). Also, Zhu et al. (2024) detected MeP in all fish species from Beibu Gulf, South China Sea, with concentrations ranging from 0.66 to 13.77 ng g-1 ww, followed by PrP (71.70%), EtP (49.06%), BuP (25.47%), and BzP (7.55%). These authors also estimated the log BAF ranging from 2.36 to 3.37 for MeP, and from 1.42 to 2.34 for PrP, pointing to insignificant bioaccumulation. Furthermore, they found a Trophic Magnification Factor (TMF) of 2.88 and 1.17 for MeP and PrP, respectively, unveiling MeP biomagnification throughout the food web (Zhu et al., 2024).

Regarding the accumulation of polar parabens (i.e., short-chain parabens as MeP and EtP), among the 19 field studies analyzed in this review, we found no plausible explanation for the differences in the detection of MeP and EtP in different organs. In these, paraben accumulation was assessed mostly in a single organ/tissue or the entire animal (Cacua-Ortiz et al., 2020; Jakimska et al., 2013; Liao & Kanan, 2018; Lin et al., 2024; Pico et al., 2019; Renz et al., 2013; Wang et al., 2019; Xue et al., 2017; Yao et al., 2018a; Yao et al., 2019; Zhu et al., 2024). Only in three studies (Peng et al., 2018; Xue & Kannan, 2016; Xue et al., 2017) more than one organ was evaluated, not allowing us to draw up a profile for paraben bioaccumulation in different organs in aquatic organisms. Although MeP and EtP have low bioaccumulation potential (Lu et al., 2019), given the low lipophilicity for MeP (Log octanol-water partition coefficient – log KOW = 1.96) and moderate lipophilicity for EtP (log KOW = 2.47), together with PrP (log KOW = 3.04) are the most commonly detected parabens in aquatic organisms (Haman et al., 2015; Lu et al., 2019; Tran-Lam et al., 2023; Xue et al., 2017; Yao et al., 2018b). This situation can be explained by the extensive use of products containing these parabens by the world population (Bledzka et al., 2014), and

also due to MeP's higher solubility in water (Ramaswamy et al., 2011), since the solubility of parabens decreases according to the length of the ester chain (Haman et al., 2015).

1.4.4 Detection of paraben metabolites

Only eight (42.10%) field studies aimed at detecting parabens in aquatic organisms have evaluated their metabolites, even though their concentrations, especially of 4-HB, were usually much higher than those of the parent parabens. Xue et al. (2017) detected 4-HB in 99% of the biotic samples collected for the study, with concentrations up to 68,100 ng g-1 ww for the marine bivalve C. cancellata. Also, other metabolites detected in the same study were methyl-protocatechuate (OH-MeP), ethyl-protocatechuate (OH-EtP), 3,4-dihydroxybenzoic acid (3,4-DHB), and 4-HB, in all marine plant samples, at concentrations up to 13,500 ng g^{-1} ww for mangrove samples. Interestingly, the same study found higher concentrations of 4-HB in fish kidneys (4110 ± 5354 ng g^{-1} ww) in comparison with whole fish (1620 ± 1640 ng g^{-1}) and liver (738 ± 983 ng g^{-1}) (Xue et al., 2017). Consistent with these data, 4-HB was the predominant metabolite found in mollusk (gastropods and bivalves) samples in the Bohai Sea, China, between 2006 and 2015, with concentrations up to 161,000 ng g^{-1} dw in marine bivalve *Mytilus edulis* (Liao & Kannan, 2018). The metabolite 3,4-DHB was also frequently found (91.9%) at concentrations up to 4960 ng g^{-1} dw. In addition, this study detected a temporal increase in the concentrations of MeP and 4-HB in the mollusk samples collected between 2006 and 2012, suggesting a common source of parabens in these mollusks (Liao & Kannan, 2018).

Lin et al. (2024) detected 4-HB in all plankton samples (1180–24800 ng g^{-1} dw in algae, and 336–10300 ng g^{-1} dw in zooplankton), in all plant samples (1310–11800 ng g^{-1} dw), and fish muscle samples (7.34–814 ng g^{-1} dw) from Dongjiang River Basin, China. On the other hand, 3,4-DHB was more abundant in plants (<LOQ–27200 ng g^{-1} dw) in comparison with plankton (1.21–185 ng g^{-1} dw in algae and <LOQ–75.5) and fish muscle (<LOQ–14.1 ng g^{-1} dw). Also, 4-HB showed the highest log BAF (5.79) in plankton samples than in fish muscle (4.44) (Lin et al., 2024). In another study conducted in fish and bivalve samples from a fish market in Milan, Italy,

4-HB was detected in 75% of the samples, with concentrations up to 35.660 ng g^{-1} Chiesa et al. (2018). The highest amounts found in bivalve samples were attributed to the fact that they are benthic filter-feeding organisms and capable of bioaccumulating certain contaminants Chiesa et al. (2018).

Recently, Zhu et al. (2024) provided important data on the fate and transfer of 4-HB in a subtropical marine ecosystem. They investigated the parent parabens and 4-HB in a marine food web from the Beibu Gulf, South China Sea, including 4 crustacean species, 2 cephalopod species, and 19 fish species. 4-HB was one of the predominant contaminants in the marine organisms analyzed, with concentrations ranging from 13.48 to 222.24 ng g^{-1} ww, but had a low log BAF in fishes (2.73–3.24 ng g^{-1} ww), in crustaceans (2.86–3.45), and cephalopods (2.76–2.79 ng g^{-1} ww). Such a study also indicated that 4-HP has an estimated high BSAF, but has a low TMF, which suggests trophic dilution throughout a marine food web (Zhu et al., 2024). All these data highlight the importance of evaluating the bioaccumulation of parent parabens and their metabolites in aquatic organisms using species from different trophic levels and sampling periods.

1.5 Laboratory studies

Up to now, 57 laboratory studies have investigated the ecotoxicity of parabens in aquatic organisms, using 78 freshwater species (64.46 %) and 43 marine species (35.54 %). Experimental studies are considered essential techniques, especially for ecotoxicology researchers, to investigate the effects of certain substances on living organisms. These studies are carefully designed and controlled environments in which researchers can manipulate one or more variables to determine their effects on the subject under investigation (Escher et al., 2018). Experimental approaches also allow researchers to establish cause-and-effect relationships between variables. This control allows researchers to better understand the specific effects of the factor under study, and to make more accurate predictions about how it will affect the individual (Li & Xia, 2019).

In the context of parabens and their potential ecotoxicity to aquatic organisms, experimental studies can provide valuable information on the specific

mechanisms by which parabens affect these organisms, the concentration and or dose-response relationship between paraben exposure and ecotoxicity, and the potential long-term effects of chronic exposure. In this sense, the works applying different model organisms can assess their paraben-toxicity level depending on the method and strategy employed. They can also develop a comprehensive view of the relationship between paraben contamination on the environment and organisms, which foment further studies in paraben toxicity in humans.

Shore et al. (2022) evaluated MeP toxicity in an echinoderm species (Strongylocentrotus purpura) and showed adverse effects on general animal development, such as mortality and reduced body growth. This is an interesting finding since these marine organisms can indicate environmental quality and potential contamination. In addition, other studies applying in vitro assay and the marine fish species (Oryzias latipes) reported severe impacts on endocrine and reproductive systems due to exposure to MeP and PrP paraben derivatives (Kawashima et al., 2022), indicating that experimental and controlled assay methods are crucial to verify the changes related to endocrine signaling.

Aiming to organize and provide detailed information about the several bioassays that have investigated the toxicity of parabens within aquatic species, a summary is presented in Table 2. It describes their experimental details, biomarkers evaluated and the main findings, from studies performed with aquatic microorganisms, invertebrates, and vertebrate species.

1.5.1 Model species

Laboratory studies about the ecotoxicity of parabens were conducted mainly with fish (n = 40; 70.17%), followed by crustaceans and or microcrustaceans (n = 9; 15.78%), algae (n = 4; 7%), platyhelminth, algae and bacteria (n = 4; 7% each), bivalves (n = 2; 3.5%), reptile (turtles) (n = 2; 3.5%), echinoderms (n = 1; 2%), and amphibians (n = 1; 2%), as seen in Fig. 5A. Fish are the most biodiverse group within vertebrates (Volff, 2005), and have been so far the most studied group among them to investigate the ecotoxicity of parabens (Fig. 5A). Furthermore, there is a disproportionate number of studies using fish models in comparison with other groups

of vertebrates, invertebrates, and microorganisms (Fig. 5A). Six fish species were used in all the papers screened (Fig. 5B), which might be skewing the current evidence towards the sensitivity of such group to parabens, even though it may vary between species (González-Doncel et al., 2014). Among the fish model species used (Fig. 5B), the zebrafish was by far the most commonly utilized (n = 26; 65%) in such studies, followed by the medaka (O. latipes, n = 5; 12.5%), and the rainbow trout (O. mykiss, n = 3; 7.5%). Similarly, other reviews have also shown that zebrafish is the most commonly used fish species as a model system to assess the ecotoxicity of traditional and emerging pollutants (Bambino and Chu, 2017; Canedo et al., 2021; Meyers, 2018; Porto et al., 2023).

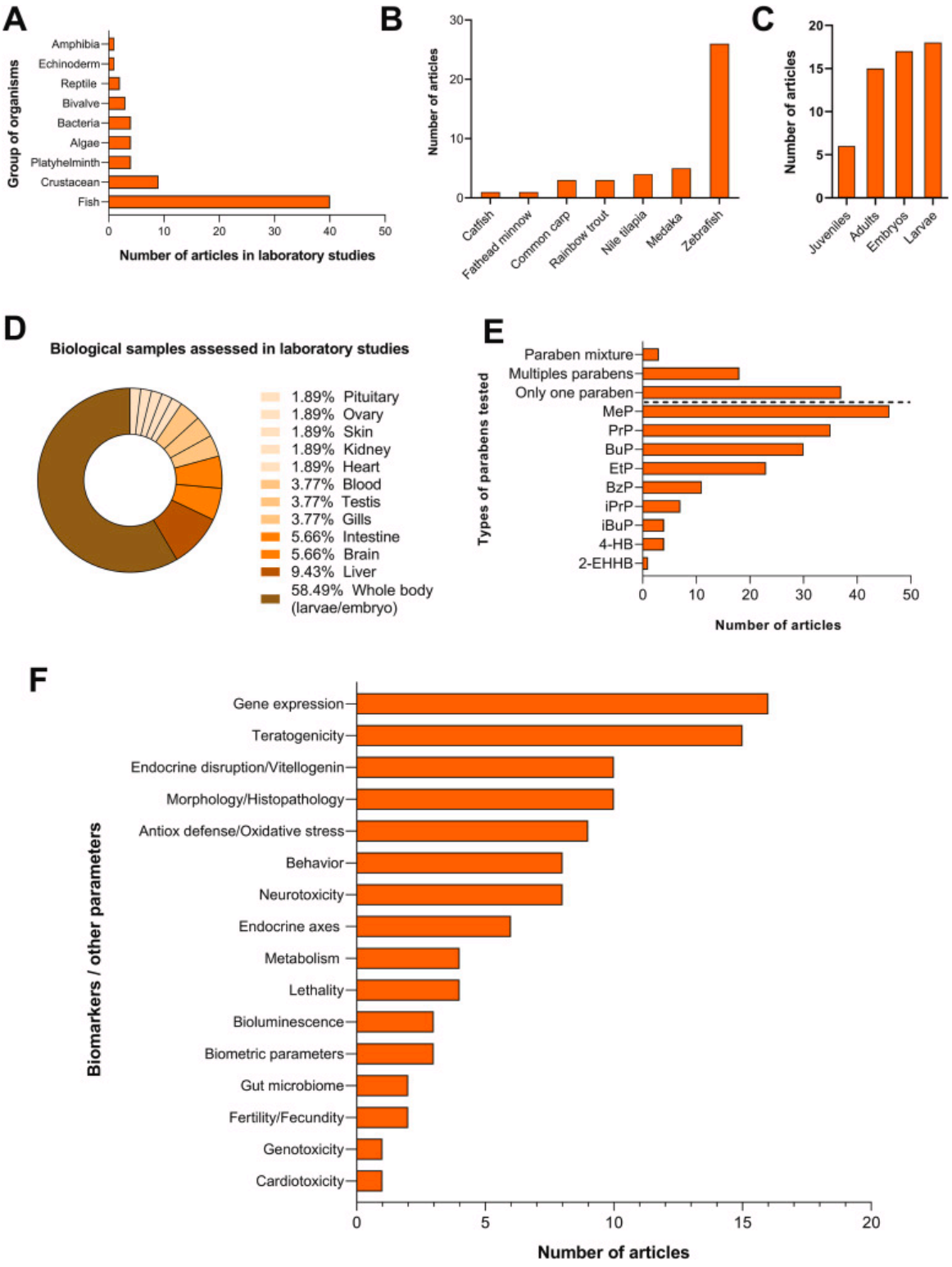

Figure 5. An overview of laboratory studies related to the ecotoxicity of parabens in aquatic organisms. A) Number of published articles per taxon. B) Number of articles published per fish species adopted in each study. C) Number of articles published by fish life cycle stage assessed in each study. D) Frequency of organs and tissues investigated in laboratory studies.

E) Number of articles by type of paraben tested in each study. F) Biomarkers and other parameters used in laboratory studies to investigate the ecotoxicological effects of parabens.

Considering the experimental studies carried out with fish, the most commonly used life stages cycle were the early developmental stages (embryo-larval stage) (Fig. 5C). During this stage, fish are more sensitive to toxicants, allowing for the observation of morphological abnormalities and facilitating the development of large-scale studies (Porto et al., 2023). Furthermore, laboratory analyses have examined various organs and tissues from adult fish species to elucidate the effects and MoA of parabens (Fig. 5D). Among the samples evaluated, the liver (9.43%), brain (5.66%), and intestine (5.66%) were the most extensively investigated organs, followed by gills, testis, and blood (3.77% each) (Fig. 5D). Bioaccumulation was also investigated in a few laboratory studies, as detailed in section 5.3.

In addition to fish, other vertebrates such as turtles and amphibians have recently been used to evaluate paraben ecotoxicity. As seen for the Chinese striped-neck turtle *Mauremys sinensis* (n = 2; 3.5%) (Ding et al., 2023) and for the African clawed frog *Xenopus laevis* (n = 1; 2%) (Medkova et al., 2023). Chelonians are excellent bioindicator species and models for assessing the impacts of anthropogenic pollution, due to their sedentary lifestyle, long life cycle, multiple feeding habits, and capacity for bioaccumulation and biomagnification of contaminants (Adams et al., 2016; Ding et al., 2023). Similarly, amphibians, given their dual aquatic-terrestrial life stages, sensitivity to both water and soil contaminants, and vulnerability to environmental stressors (Do Amaral et al., 2019; He et al., 2014).

Microorganisms and invertebrates were also utilized to assess the ecotoxicological impact of parabens. Specifically, the green algae *Pseudokirchneriella subcapitata* (n = 2; 3.8%), the bacterium *V. fischeri* (n = 2; 3.8%), and the freshwater microcrustacean *D. magna* (n = 6; 11%). Other aquatic arthropods, such as *Artemia franciscana, C. dubia*, and *Tigriopus japonicu*s, appear in various studies. Beyond crustaceans, invertebrate model organisms used included the sea urchins *Strongylocentrotus purpuratus* and *Paracentrotus lividus*, the planarian *Dugesia japonica*, and the oyster *Crassostrea gigas* (n = 1; 1.8% each).

In this context, a few studies (n = 8; 15%) used microorganisms to assess paraben ecotoxicology. Similarly, studies involving invertebrates represented approximately 20% of all the research analyzed in this review. Including microorganisms in future studies is particularly interesting due to their rapid reproduction rates and simple cellular structures, which allow for high-throughput screening and detailed studies. Additionally, microorganisms often serve as primary producers or decomposers in aquatic ecosystems, making them crucial indicators of ecological health (Sivapragasam et al., 2020). Notably, the sensitivity of microorganisms and invertebrates to parabens may surpass that of some vertebrates under similar conditions (Yamamoto et al., 2011). Therefore, it is pertinent to consider including these species in future ecotoxicological assessments of parabens, as they can provide early warning signs of environmental contamination and contribute to a more comprehensive understanding of the ecological impacts of these chemicals.

1.5.2 Types of parabens tested

Most published studies only investigated a single paraben (n = 37; 86.04 %) (Fig. 5E). Still, some studies have tested more than one paraben individually (n = 18; 41.86 %), while few studies (n = 2; 4.65 %) have used mixtures of parabens as the focus of research (Fig. 5E). The paraben type most commonly employed in experimental studies was MeP (57.74% of 71 studies), followed by PrP (42.25%) (Fig. 5E). Also, BuP was the third most employed paraben in the revised experimental studies (Fig. 5E), exhibiting higher ecotoxicity than parabens of shorter alkyl chains (Merola et al., 2020b). MeP and PrP are the most frequently investigated and detected parabens in surface water and sediments, as well as in wastewater, effluent wastewater treatment plants, and sewage sludge (Haman et al., 2015), due to the continuous consumption of products containing parabens and their final destination in aquatic environments. Given this, researchers have focused their work mainly on these two parabens.

Regarding their metabolites, Pedersen et al. (2000) failed to find any toxicity related to 4-HB in *O. mykiss* exposed via intraperitoneal injection. Since this was the earliest paraben-related toxicity study made with an aquatic organism, it may explain

why only a single study employed paraben metabolites after that, even though there are some other than 4-HB that might need to be investigated. Some of those were detected at high concentrations in fish samples, such as OH-MeP, OH-EtP, and 4-DHB (Xue et al., 2017). Additionally, studies conducted over the last decade have detected 4-HB levels ranging from 4.58 to 2380 ng L^{-1} in surface waters from China (Li et al., 2015; Ma et al., 2018; Zhao et al., 2019), and from 1380 to 31,400 ng L^{-1} in wastewater from India (Karthikraj et al., 2017). These findings underscore the need for further research on the ecotoxicity of paraben metabolites across different groups of organisms. Thus, understanding these contaminants' ecological and health impacts is essential, given their widespread presence in aquatic environments.

1.5.3 Bioaccumulation in model species

Among the laboratory studies analyzed in this review, only four evaluated the paraben bioaccumulation in aquatic organisms. In *O. mykiss* exposed to water containing 225 µg L^{-1} for 12 days, PrP accumulation was investigated and detected in the liver (6700 µg kg^{-1}) and muscle (870 µg kg^{-1}) (Bjerregaard et al., 2003), denoting that that bioaccumulation in the liver was almost eight times greater than in the muscles. However, in this same study, when specimens were orally exposed to 7.2–1830 mg kg^{-1} PrP by food for 10 days, the PrP bioaccumulation was detected only in the liver (37 mg g^{-1} ww) from specimens exposed to the highest dose. In addition, although muscle samples were also collected after the oral exposure experiment, in such a study the authors could not detect PrP in this tissue (Bjerregaard et al., 2003), confirming that the bioaccumulation of parabens in fish also depends on the route of exposure.

Alslev et al. (2005) also conducted a study with *O. mykiss*, in which juvenile specimens were orally exposed to 4–74 mg kg^{-1} of BuP by food for 10 days. BuP accumulation was not detected in the muscle tissue sampled, whilst on the liver range of 0.6–5 µg g^{-1} ww, presenting a discrete dose-dependent increase in PrP concentrations in this organ (Alslev et al., 2005). In a second experimental condition, BuP concentrations of 9 and 183 µg g^{-1} were detected in the plasma samples of fish exposed to water containing the nominal concentrations of 50 and 250 µg L^{-1},

respectively, over 12 days (Alslev et al., 2005). Although liver and muscle samples from *O. mykiss* were also collected in the second experiment by water exposure, the authors could not assess BuP concentrations in them due to technical problems. In both studies conducted with *O. mykiss* and paraben exposure by food, PrP and BuP accumulation represented less than 1% of administered doses, suggesting that when administered orally to fish, these two parabens are rapidly metabolized or poorly absorbed. In mammals, parabens are rapidly absorbed by the gastrointestinal tract and metabolized by esterases, producing p-hydroxybenzoic and other metabolites. This metabolization may occur in the skin, subcutaneous adipose tissue, and in the digestive tract (Bledzka et al., 2014; Soni et al., 2005). However, a study with five fish species detected esterase activity in the intestine, liver, and bile. Nevertheless, *O. mykiss* presented the lowest activity among the species investigated (Li & Fan, 1997).

In the study conducted by Barse et al. (2010), adult males of *Cyprinus carpio* were exposed to water containing 0.84, 1.68 and 4.20 mg/L of MeP - which is equivalent to 1/143, 1/71st and 1/29 of LC50 of MeP calculated for this species, respectively, for 28 days. MeP bioaccumulation was investigated and detected in gill (~0.48 and ~0.84 mg kg^{-1}), brain (~0.9 and ~2.3 mg kg^{-1}), liver (~1.0 and ~1.7 mg kg^{-1}), muscle (~0,48 and ~0,84 mg kg^{-1}) and testes (~0.7 and ~1.4 mg kg^{-1}), presenting a concentration-dependent bioaccumulation in the organs of fish exposed to the two lowest concentrations (0.84 and 1.68 mg L^{-1}), respectively. Such data corroborated the findings in *O. mykiss* on the bioaccumulation of parabens in fish liver and muscle after oral exposure, as well as being the first published study to indicate that parabens can bioaccumulate in multiple organs of aquatic organisms and, consequently, cause some disturbance in their activity.

In addition to the three studies mentioned, only another study evaluated the bioaccumulation of parabens in a different group of aquatic organisms. In it, adult females of the turtle species *M. sinens* were exposed to different concentrations of BuP (5, 50, and 500 µg L^{-1}) for 20 weeks, and the bioaccumulation of BuP only in the intestine was assessed (Ding et al., 2023). A concentration-dependent bioaccumulation was observed for this organ (~4.1, ~60, and ~1000 ng g^{-1} of BuP), respectively (Ding et al., 2023). In such study, turtles were exposed to BuP through

water ingestion and, although the authors stated that BuP bioaccumulation in the intestine was low, this was sufficient to induce dysbiosis as well as changes in the intestinal physiology and structure of *M. sinens*. Among the articles analyzed in this review, we did not find any laboratory studies that evaluated the bioaccumulation of paraben metabolites, reinforcing that the bioaccumulation of parent parabens and their metabolites should be investigated in further studies.

1.5.4 Ecotoxicological effects on aquatic organisms

Unlike articles based on field studies, in which only the presence and amounts of parabens in biota samples were investigated, in the laboratory articles reviewed here, several biological effects caused by paraben contamination were found, as shown in Fig. 5. These effects were evaluated using several biomarkers or parameters presented in Fig. 5F and also detailed in Table 2, which were chosen according to the type of aquatic organism used, the stage of the life cycle evaluated, and the objectives of each investigation.

In ecotoxicology, biomarkers are carefully chosen based on the expected responses resulting from exposure, thereby providing valuable data for conducting biomarker-based ecological monitoring projects (Porto et al., 2023). Specifically, studies utilizing aquatic animals as models have identified a diverse range of biomarkers that indicate exposure to parabens or their effects (Fig. 5F and Table 2), which encompass molecular to physiological endpoints (Pedersen et al., 2000; Thakkar et al., 2022; Ates et al., 2018; Torres et al., 2016). As demonstrated in Fig. 5C, endocrine disruption, oxidative stress, developmental toxicity, and reproductive impairment are the major effects promoted by parabens in aquatic organisms in the articles analyzed, which will be described in more detail below.

1.5.4.1 Effects on development and growth

The earliest study conducted on fish during the embryonic phase was performed by Dobbins et al. (2009) using the freshwater fish *Pimephales promelas* to explore the growth rate and seven paraben compounds (MeP, EtP, PrP, isoPrP, Bup, iso-BuP, and BzP). Using 24 h post-hatch *P. promelas* and a static exposure for 48 h,

the LC50 values found ranged from 3.0 to 160 mg L^{-1}, which correspond to the LC50 values calculated for MeP and BzP, respectively. Also, exposure to parabens containing longer alkyl chains was associated with reduced growth in fish (Dobbins et al., 2009). Similarly, the developmental toxicity induced by parabens was reported in the Japanese medaka (*O. latipes*) (González-Doncel et al., 2014), and zebrafish (Bereketoglu & Pradhan, 2019; Torres et al., 2016). Furthermore, O. latipes embryos and larvae exposed to PrP (40, 400, 1000, and 4000 mg L^{-1}) showed decreased survival rate, developmental delay, increased morphological changes, and histological abnormalities (González-Doncel et al., 2014). Besides, zebrafish embryos and larvae exposed to MeP (100–1000 µM) for 96 h showed several malformations, and a decline in heart rate (cardiotoxicity) and hatching rate (Dambal et al., 2017). Bereketoglu & Pradhan (2019), using a semi-static exposure, also observed that MeP (≥100 µM) and PrP (≥10 µM) exposure promotes concentration-dependent toxicity in zebrafish embryos, in parallel to the induction of morphological abnormalities. They also found that PrP is more toxic to zebrafish embryos than MeP. In addition to these endpoints, the study conducted by Merola et al. (2020a) demonstrated the occurrence of blood stasis, reduction in blood circulation, and also reduced heartbeat in zebrafish larvae exposed to MeP (1–80 mg L^{-1}) during 96 h, drawing attention to the potential ecotoxicological impact of parabens during the fish early developmental stages.

Similar to fish, *Xenopus laevis* embryos exposed to MeP, PrP, and BtP showed 100% mortality for the higher concentrations tested (5000 and 100,000 µg L^{-1}), hatching delay, developmental abnormalities, and gene expression downregulation (Medkova et al., 2023). To our knowledge, there are no studies addressing the effects of parabens on the development of other aquatic vertebrates, such as turtles, different classes of amphibians, as well as in neotropical fish species.

Paraben exposure, including MeP, EtP, and PrP, has been associated with developmental alterations in aquatic invertebrates, such as developmental delays in the microcrustacean *Tigriopus japonicus*. The study involved acute exposure to concentrations raning from 5000 to 20,000 µg L^{-1} (Kang et al., 2019). In another study with the sea urchin *Paracentrotus lividus*, PrP exposure decreased larval length and induced several morphological changes. The experiment utilized acute exposure with

concentrations ranging from 10 to 10,000 μg L^{-1} (Torres et al., 2016). Therefore, all these data suggest that paraben exposure, particularly to those with longer alkyl chains and higher concentrations, may compromise the development of aquatic animals.

1.5.4.2 Effects on endocrine system

The initial biomarkers used in experimental studies involving fish were primarily focused on endocrine disruption, particularly related to sex hormones and induction of vitellogenin (*vtg*) secretion. Pedersen et al. (2000) performed a bioassay using EtP, PrP, BuP, and 4-HB in juveniles of *O. mykiss* through an intraperitoneal injection dose of 1 mg kg^{-1}, as the exposure method. Blood samples were obtained on days 0, 6 and 12 after the injection. The results indicated that all the tested parabens presented estrogenicity (increased plasma levels of *vtg*) in doses between 100 and 300 mg kg^{-1} (Pedersen et al., 2000). However, according to these authors, PrP and BuP were approximately six times more potent than EtP, having an estrogenic potential comparable to that previously reported for the plastic additive bisphenol A. It is important to mention that in this study, neither the route of exposure nor the concentrations used were meant to be environmentally realistic.

In the following studies, using *O. mykiss* as a model organism, Bjerregaard et al. (2003) and Alslev et al. (2005) tested the potential estrogenicity of PrP and BuP, respectively, as depicted in section 5.3. Both studies used the oral administration protocol of the paraben by food gavage. These studies found that the *vtg* synthesis in *O. mykiss* was a more sensitive biomarker of estrogenic activity, which may have opened the path for more experimental studies using this biomarker in other fish species. It was also found that orally administered BuP is quickly metabolized by the liver and that water exposure also leads to detectable concentrations of BuP in fish bloodstream. In addition, the authors pointed out that absorption of parabens through water exposure might be more damaging to the HPG axis due to the lack of first-passage effect involved in oral administration (Alslev et al., 2005).

Subsequently, Barse et al. (2010) also detected an increased *vtg* production in adult male common carp (*C. carpio*) exposed to three MeP concentrations (0.84, 1.68, and 4.20 mg L^{-1}). The first study where *vtg* induction was assessed by gene

expression was developed by Yamamoto et al. (2011) in *O. latipes*. As from liver microarray analyses, they detected upregulation of *vtg* and choriogenin (*chg*) genes after exposure to 10 µg L^{-1} of MeP. Additionally, using the medaka vitellogenin test, they found increased *vtg* plasma levels in fish exposed to 630 µg L^{-1}.

Since then, endocrine disruption has been reported in fish in many studies. It has been observed in zebrafish larvae that MeP, EtP, PrP, and BuP (100, 200, 400, 800 and 1000 µM) exposure during 96 h (Dambal et al., 2017) or to different concentrations (20 and 100 µM of MeP, 20 and 50 µM of EtP, 2 µM of PrP and 1 and 2 µM of BuP) during to 120 h (Liang et al., 2023a), promotes *vtg* upregulation, demonstrating its estrogenic effect. Nonetheless, an opposite effect was observed in adult zebrafish, in which they detected a reduction in *vtg* levels in males and females exposed to MeP (1, 3, and 10 µg L-1) for 28 days (Hu et al., 2023a,b). Additionally, such exposures also alter the gene expression of key factors related to the hypothalamic-pituitary-gonadal axis in zebrafish larvae and adults, disrupting the secretion of steroid hormones (Hu et al., 2023b; Liang et al., 2023a), which can have negative consequences for the sexual differentiation, gonad differentiation and also in the reproductive success of adult animals.

Furthermore, recent studies evidenced that paraben exposure can also disrupt other endocrine axes. Using embryo-larval stages of zebrafish exposed to MeP (20–200 µM), EtP (20–100 µM), PrP (5–20 µM), and BuP (2 µM) for 120h, a reduction in thyroid hormone levels (T3 and T4) was detected (Liang et al., 2022). The transcription levels of several target genes along the hypothalamic-pituitary-thyroid (HPT) axis (Liang et al., 2022) also was disturbed. Using molecular docking, the authors also showed that all tested parabens acted as thyroid receptor agonists (Liang et al., 2022).

Parabens can also disrupt the HPA axis. In adults of zebrafish exposed to environmentally realistic concentrations of MeP (1, 3, and 10 µg L^{-1}) for 28 days, both sexes exhibited alterations in the HPA axis activity, including reduced transcription levels of corticotropin-releasing hormone (CRH) and its binding protein, as well as decreased blood cortisol concentrations (Hu et al., 2023a). Similarly, the exposure of zebrafish embryos and larvae to MeP, EtP, PrP, and BuP during 120 h increased

adrenocorticotropic hormone (ACTH) levels and reduced cortisol levels, as well as upregulates several genes related to stress response signaling (Liang et al., 2023b). Also, the activation of zebrafish glucocorticoid receptors (Gr) by parabens was demonstrated *in silico* and *in vitro* (Liang et al., 2023b). Brown et al. (2018) also investigated the effects of BuP exposure on zebrafish development, focusing on the development of the endocrine pancreas, using insulin: GFP transgenic zebrafish embryos exposed to BuP (250, 500, 1,000, and 3000 nM), over 7 days. After 96 h of exposure, they detected an increase in pancreatic islet area at the lowest BuP concentration, with 70% of islets presenting variant morphology, as fragmented islet clusters and ectopic beta cells. Also, they found alterations in GSH content and the transcripts of GSH-related genes (Brown et al. (2018), evidencing that BuP exposure affects the development of pancreatic islets and disrupts the redox balance, resulting in several developmental abnormalities. Such findings indicate that paraben exposure may affect multiple endocrine axes at different stages of the fish life cycle and act as an EDC. Notwithstanding, it is important that future studies also evaluate the protein levels of the various hormones and receptors involved, as well as other proteins related to the several endocrine axes, which can also be linked to functional studies, to fully clarify the mechanisms of endocrine disruption triggered by parabens. Furthermore, the extent to which parabens may impact the endocrine axes in neotropical fish species or even in other groups of aquatic organisms remains unclear.

1.5.4.3 Effects on reproductive system and reproduction

Using a histopathological approach in zebrafish testes, Hassanzadeh (2017) revealed that chronic exposure (21 days) to MeP (0.001–10 mg L^{-1}) decreased the gonadosomatic index (GSI), leading to testicular atrophy, germ cell impairments, proliferation in spermatogonia, and decrease in the proportion of the spermatozoa, Leydig cell hyperplasia, interstitial fibrosis, and apoptosis of Sertoli cells. However, no hormone analysis was carried out. Such results are in agreement with those found by Barse et al. (2010) in males of *C. carpi*o, in which all MeP concentrations tested (0.84, 1.68, and 4.2 mg L^{-1}) reduced the GSI, increased the occurrence of areas with

inflammatory infiltrate and fibrosis, and reduced the interstitial compartment and the number of sperm in the lumen of the seminiferous tubules.

In another study conducted by Hu et al. (2023b), both males and females of zebrafish exposed to MeP for 28 days exhibited higher GSI only at the highest tested concentration (10 μg L^{-1}), while all treatments (1, 3, and 10 μg L^{-1}) resulted in a blockade of gametogenesis, accompanied by an imbalance in sex hormone (low levels for T, 11-keto testosterone and estradiol). By evaluating the expression of several genes related to the HPG axis, a disturbance in steroidogenesis and feedback regulation mechanisms was demonstrated, evidencing an antiestrogenic activity for MeP (Hu et al., 2023b). In males, all concentrations inhibited the spermatogenesis process, increasing the percentage of spermatogonia and spermatocytes, but reducing spermatozoa. In females, the low estradiol levels downregulated the hepatic expression of VTG, resulting in a deficiency in production and, consequently, affecting the vitellogenesis process in the ovary (Hu et al., 2023b). Interestingly, this did not reflect on egg production, egg weight and protein, but induced a high mortality rate in larvae and also precocious hatching of offspring larvae derived from females exposed to 3 and 10 μg L^{-1} groups (Hu et al., 2023b). These data provided important insights about the effects of MeP on fish reproductive systems. However, there are still no studies on the effects of parabens on the quality of gametes in fish and other aquatic organisms. Also, Hu et al. (2022c) suggested the occurrence of maternal transfer of parabens to the offspring, which may interfere with the survival and development of the offspring. To our knowledge, the bioaccumulation of parabens has already been evidenced only in fish ovaries (Peng et al., 2018) and testes (Barse et al., 2010).

1.5.4.4 Effects on nervous system and behavior

Zebrafish exposed to MeP (0.1, 1, 10, and 100 ppb) during the embryo-larval stages (over 144 h) exhibited inhibited acetylcholinesterase (AChE) activity in all treatments, as well elevated cortisol levels and induced anxiety-like behavior in the two MeP lowest concentrations (Luzeena-Raja et al., 2019). In addition to these results, Merola et al. (2020a) also reported that embryos exposed to 10 and 30 mg L^{-1} presented a low number of spontaneous contractions, which is used as a

biomarker of neurotoxicity in zebrafish embryos. In adult zebrafish exposed to MeP (1, 11 and 110 ppb of the LC50, over 30 days), Thakkar et al. (2022) found a concentration-dependent reduction in AChE activity in the brain for both sexes following chronic sub-lethal methylparaben exposure and altered the expression of two genes involved in neuronal differentiation (ntrk2a and pax6b). Nonetheless, sex-specific responses in the brain have also been observed in adult zebrafish. Females showed an increase in serotonin levels, while males showed a decrease (Thakkar et al., 2022). Similarly, Hu et al., 2023a reported enhanced glutamatergic neural signaling in the male zebrafish brain exposed to MeP (1, 3, and 10 mg L^{-1}, during 28 days), while blockage of synaptic neurotransmission was observed in females.

O. niloticus (with no information regarding the sex or life cycle stage of the specimens used) exposed to BuP (5, 50, 500, and 5000 ng -1, over 56 days) demonstrated reduced dopamine and γ-aminobutyric acid content in the brain, along with the induction of pathways related to skin pigmentation (Liu et al., 2023a). To date, no studies have evaluated whether exposure to parabens can affect the mechanisms of cell proliferation and death (apoptosis) in the nervous system of aquatic organisms, nor have they investigated specific brain regions.

1.5.4.5 Effects on the digestive system

The effects of MeP have already been investigated in adult zebrafish, focusing on morphological and physiological biomarkers in the gut and its microbiome. De Carvalho Penha et al. (2021) conducted an innovative study introducing an intestinal microbiota analysis through the evaluation of the use of carbon sources by the microbial community. After the exposure of adult male zebrafish to 30 and 50 mg L^{-1} of MeP for 96 h, no significant changes were observed in the diversity or abundance of gut microbiota, despite concerns about MeP's antimicrobial properties. However, the animals had an increase in carbon source utilization, suggesting a potential metabolic adaptation to MeP exposure (De Carvalho Penha et al., 2021).

Furthermore, Hu et al. (2022b) exposed zebrafish to environmentally relevant concentrations of MeP (1, 3, and 10 μg L^{-1}) for 28 days to assess its impact on

gut microbiota and overall health. High-throughput amplicon sequencing revealed that subchronic MeP exposure significantly disrupted the composition and diversity of the gut microbial community. Interestingly, MeP caused sex-specific intestinal effects: males exhibited increased goblet cell density, elevated tight junction protein (Tjp2) expression, and higher serotonin levels, while females showed reduced goblet cell density, reduced Tjp2 expression, and decreased serotonin levels, alongside up-regulated pro-inflammatory cytokines transcription. Additionally, intestinal catalase (CAT) activity was elevated under MeP stress, contributing to oxidative stress mitigation (Hu et al., 2022b). These findings showed how MeP exposure may impair gut barrier function and intestinal health, emphasizing the need for risk assessments of this contaminant. Another study using the same MeP concentrations and exposure time in adult zebrafish conducted by Hu et al. (2022a) demonstrated that MeP subchronic exposure induced hepatotoxic effects, such as hepatocellular vacuolization, changes in antioxidant system and lipid metabolism and, interesting, also promotes increased cortisol levels in the liver and as well as inhibiting the synthesis and conjugation of primary bile acid. The authors also performed a metabolomic analysis, which showed that MeP mainly alters the composition of fatty acids, retinoids, and steroids (Hu et al., 2022a). Similarly, Barse et al. (2010) also detected an increase in vacuoles and areas of focal necrosis in the liver of C. carpio exposed to the higher MeP concentrations (1.68 and 4.2 mg L-1).

Ding et al. (2023) investigated the effects in the gut of the turtle *M. sinensis* exposed to several BuP concentrations (5, 50, and 500 μg L-1) over 20 weeks. The results revealed changes in gut microbiome composition, with the genus *Edwardsiella* emerging uniquely in BuP-exposed turtles, particularly absent in the control group. Structural changes in the intestines included shortened villi, a thinner muscular layer, and a marked reduction in goblet cell numbers. Immune responses were notably affected, with increased numbers of neutrophils and natural killer cells in the lamina propria of the intestinal mucosa, especially at higher BuP concentrations (500 μg L-1). This was accompanied by a marked upregulation of pro-inflammatory cytokines, particularly IL-1β, which was strongly correlated with the abundance of *Edwardsiella*. They also showed that the presence of Edwardsiella was inversely related to goblet

cell counts, further indicating compromised gut barrier function (Ding et al., 2023). These findings highlight the impacts of BuP, a long alkyl chain paraben, on intestinal homeostasis in aquatic turtles, as it induces gut dysbiosis, triggers inflammatory responses, and weakens the physical gut barrier (Ding et al., 2023), demonstrating that parabens also may promote toxicity to the digestive tract and affect the organism systemically.

1.5.4.6 Effects on metabolism

Oxidative stress, primarily detected in the liver due to its involvement in the degradation of xenobiotics, has been extensively investigated in response to exposure to various parabens. Silva et al. (2018) observed an adaptive response in *O. niloticus* exposed to 4 mg L^{-1} of MeP, EtP, PrP, and BuP over 6 and 12 days. CAT activity only was increased in the liver of animals exposed to MeP and EtP for six days. After 6 days of exposure, decreased glutathione (GSH) levels in the liver and gills were seen. However, after 12 days, the levels increased compared to controls, suggesting an adaptation of the antioxidant response in animals exposed to sublethal concentrations of parabens. The malondialdehyde (MDA) content was only increased in animals exposed to EtP and BuP for 12 days (Silva et al., 2018). In a second study in *O. niloticus*, but in juveniles exposed to BzP (5, 50, 500, and 5000 ng L-1) for eight weeks, Lin et al. (2022) detected metabolic disorders in hepatic glycerol phospholipids, glycerolipids, and sphingomyelins, as well as increased crude fat content, oxidative stress, and liver tissue inflammation. In the same way, Lite et al. (2022) focused their study on oxidative stress and found lower levels of antioxidant enzymes and GSH in zebrafish exposed to PrP and BuP (0.1, 1, and 10 ppb) over 96 h.

In adult zebrafish exposed to 30 and 50 mg L^{-1} of MeP over 96 h, an increase of MDA levels and for ethoxyresorufin O-deethylase activity (EROD) activity were detected in gills at the higher treatment, but no changes in the liver, as well as no lipid peroxidation, were detected in larvae exposed to 30 and 60 mg L^{-1} over 168 h (Carvalho Penha et al., 2021). However, in a second study in zebrafish adults, but in a subchronic exposure over 28 days, Hu et al. (2022a) detected elevated hepatic ROS levels and GPX activity in females exposed to 10 µg L^{-1} of MeP, in parallel to reduced

activity for CAT and for GSH content. Also, an increase in HSI was seen in females exposed to 1 and 10 μg L^{-1} of MeP, but not for the intermediate concentration of 3 μg L^{-1} (Hu et al., 2022a). Furthermore, the effects of parabens exposure have also been detected along the embryo larval development in zebrafish. After exposure to 50 mg L^{-1} of MeP over 72 h, Ates et al. (2018) detected a reduction in GST and NO levels, but a slight increase in lipid peroxidation was observed. However, De Carvalho Penha et al. (2021) did not detect changes in lipid peroxidation in zebrafish larvae exposed to higher MeP concentrations (30 and 60 mg L^{-1}) and a longer exposure time (168 h). Interestingly, at the transcriptional level, Bereketoglu & Pradhan (2019) found that, depending on paraben tested (MeP or PrP) and its concentration, they can alter several genes related to the oxidative stress *(nrf2, keap1, gst, mgst, sod1, hsp70, mt1*) and fatty acid metabolism (*apoab*, *apoeb*, apoa4, *fasn, ldlr, lpl, lipc*), as well to apoptosis (*bax, bcl2, casp3a, dap3*), cell proliferation (*p21, p38*), to DNA damage pathways (*gadd45a, rad51, apex1* and *xpc*), to endocrine (*ar, esr2a, thraa* and *thrb*) and immune function (*tnfa* and *il8*) (Bereketoglu & Pradhan (2019), drawing attention to the fact that exposure to parabens can systemically impact animal physiology, negatively affecting the zebrafish development.

The alanine aminotransferase (ALT) was also evaluated in zebrafish blood samples by Hu et al. (2022a), since this enzyme is released by the liver after injury to hepatocytes (Liu et al., 2014). An increased ALT activity was observed in males and females exposed to the intermediate concentration of MeP tested (3 μg L^{-1}). In addition, Barse et al. (2010) also evaluated the activity of hepatic enzymes in muscle tissue of *C. carpio* exposed to MeP (0.84, 1.68, and 4.2 mg L^{-1}, over 28 days), being found an increase in alkaline phosphatase (ALK) and alanine aminotransferase (ALN), but a reduction on acid phosphatase (ACP) and aspartate aminotransferase (AST) activity, which denotes a change in muscle tissue metabolism. Furthermore, Yin et al. (2023) investigated the effects of BuP (5, 50, and 500 μg L-1, over a 20-week) on the liver of the turtle *M. sinensis*. They detected a decreased antioxidant enzyme activity (SOD, CAT, GSH-Px) in the liver, as well as increased levels of MDA at the highest concentrations tested, signaling compromised oxidative defense and oxidative damages. The Nrf2-Keap1 pathway-related genes, initially upregulated, declined at

higher concentrations, indicating oxidative stress overload. Heat shock proteins (HSP70, HSP90) and inflammatory markers (BAFF, IL-6, P50, P65) were elevated, suggesting cellular stress and inflammation. BuP exposure also induced apoptosis, with increased pro-apoptotic gene expression (BAX, CytC, Caspase3 and Caspase9) and decreased anti-apoptotic Bcl2 levels (Yin et al., 2023). Collectively, such data indicate that exposure to parabens may also interfere with the metabolism of aquatic organisms, as well such biomarkers are important to understand the ecotoxicological effects caused by them.

1.5.5 Types, exposure pathways, and analytical monitoring

The exposure methods employed in laboratory studies are crucial as they can significantly impact the outcomes and interpretations of the research (Zhang et al., 2019). These methods can be broadly categorized into acute and chronic exposures, each serving different purposes and offering unique insights depending on the study's aims. In this review, most of the studies used acute exposure methods. The prevalence of acute studies can be attributed to several factors, such as faster results and cost-effectiveness, as well as the fact that many of the experimental studies employ the initial stages of zebrafish development, as validated by Guide no. 239, Fish Acute Embryo Toxicity (FET) Test by OECD (2013). Acute exposure studies, which are shorter in duration, focus on immediate toxic effects and help identify lethal concentrations and immediate responses. However, acute exposure studies have limitations. The short duration may not capture delayed effects or the cumulative impact of a substance, such as those investigated throughout male and female gametogenesis, potentially overlooking long-term consequences (Erhirhie et al., 2018). In contrast, chronic exposure involves prolonged exposure to a substance or condition, often at lower levels, to simulate real-world scenarios more closely (Silva et al., 2020). Chronic exposure studies are essential for understanding the long-term effects and potential continuous or repeated exposure risks (Zhang et al., 2019). The choice between acute and chronic exposure methods depends on each study's specific objectives and experimental models.

Considering the routes of exposure, interestingly, the initial studies employed exposure to parabens exposure through intraperitoneal injection or by food using the gavage method (Pedersen et al., 2000; Bjerregaard et al., 2003; Alslev et al., 2005). However, these substances can be metabolized by the liver when administered orally, which probably interferes with the absorption rates and generates higher sensitivity than in experiments with mice (Bjerregaard et al., 2003). From this, most studies have been carried out with vertebrates via exposure to water. Regarding this, most studies have employed the exposure using a static system (n = 41; 71.9 %), rather than semi-static (with renewal, n = 14; 24.5%) or flow-through system (n = 2; 3.50%). Since paraben's half-life ranges from minutes to days varying according to the physicochemical conditions considered (Bledzka et al., 2014; González-Mariño et al., 2011b; Wu et al., 2017), depending on the model species adopted and the duration of the test, a semi-static system should preferably be adopted, where water changes occur every 24–48 h during the exposure time, since in this type of bioassay the concentration of the chemical under study is expected to remain within ±20% of the nominal values, ensuring the experimental results' validity (Erhirhie et al., 2018). In addition, a few experimental studies (n = 10; 17.5%) measured the paraben concentration in their treatments, denoting that further experimental studies related to paraben ecotoxicity should be concerned with this type of analytical validation, as recommended by OECD (2013) and also by the leading journals in the field of ecotoxicology.

In addition to the traditional exposure methods, recent advances in analytical techniques, such as magnetic solid-phase extraction (MSPE), have enhanced the detection and quantification of personal care products, including parabens, in environmental samples. A novel porphyrin-based magnetic covalent organic framework (PCOF) has demonstrated high efficiency in extracting a wide range of analytes, with log Kow values ranging from 1.96 to 7.60. This method, which offers enhanced extraction efficiency due to the COF's functional groups, has shown great promise in identifying parabens in aquatic environments with low detection limits (0.4–0.9 ng mL^{-1}) (Ning et al., 2023).

1.5.6 Environmental relevance of experimental studies and confounding factors

Although this review does not aim to compare paraben concentrations previously detected in distinct aquatic environments across the world with those used in the several laboratory studies reviews, it is important for future research to address this gap. Such comparisons would enhance the realism of environmental risk assessments. Paraben concentrations in bioassays published until now vary widely, from a few nanograms per liter (ng L^{-1}) (Lin et al., 2022) to several milligrams per liter (mg L^{-1}) (Fan et al., 2022). This variability likely reflects differences in study objectives, as human exposure to parabens tends to be higher than that of aquatic organisms (Bledzka et al., 2014). Moreover, inconsistencies in measurement units in different studies, such as molar concentrations (Dambal et al., 2017; Bereketoglu and Pradhan, 2019) and parts per billion (ppb) (Luzeena Raja et al., 2019; Thakkar et al., 2022), complicate direct comparisons of tested concentrations. The diversity of experimental protocols, the lack of standardization in exposure durations, and the imprecision in measuring paraben concentrations during in vivo studies further complicates the interpretation of these studies.

1.6 Conclusions and future perspectives

This is the first study to review and systematize the current knowledge about the bioaccumulation and ecotoxicity of parabens in aquatic organisms. Our review indicates that parabens, particularly MeP, PrP, BuP, and their derivatives, can adversely affect aquatic organisms. Parabens can induce endocrine disruption, oxidative stress, and developmental and reproductive impairments. The ecotoxicity of parabens varies based on species, life cycle stage, and experimental design, such as exposure period, concentrations tested, and type of exposure. Some studies suggest that certain organisms, such as fish and crustaceans, exhibit greater susceptibility to paraben effects than others. Additionally, the persistence of parabens in the environment and their potential for bioaccumulation in organisms raise significant concerns regarding their long-term impact on aquatic ecosystems, mainly due to the potential growth of the world population and the use of paraben-based products. While further research is necessary to comprehensively understand paraben ecotoxicity in

aquatic organisms using environmentally relevant concentrations of parabens, the current evidence highlights the need for more stringent monitoring and regulation of these chemicals. Given their status as chemicals of emerging pollutants, enhancing regulatory frameworks to mitigate parabens' risks to aquatic ecosystems is imperative.

For a more comprehensive understanding of bioaccumulation and ecotoxicity of parabens in aquatic organisms, future research should focus on the key aspects listed below.

a) Standardizing experimental protocols related to paraben bioassays among different research groups;

b) Perform laboratory assays using environmentally relevant concentrations of parabens, their metabolites, and chlorinated derivatives;

c) Evaluate paraben ecotoxicity in representative aquatic species from different taxa;

d) Evaluate paraben bioaccumulation in samples of aquatic organisms from different aquatic environments, and also from distinct geographic regions or countries;

e) Combine multiple biomarkers;

f) In addition to gene expression analyses, also adopt the quantification of proteins and other biomolecules or their metabolites in experimental studies (Multi-omics approaches);

g) Conduct multi- and transgenerational studies;

h) Measure parents' parabens and their metabolites in tissue samples from field and laboratory studies;

i) Develop analytical methods to measure paraben concentrations in biological samples with small volumes, such as for zebrafish organs;

j) Measure the real paraben concentration in water throughout the bioassay;

k) Assessment of the interactive effects of parabens with traditional and emerging pollutants;

l) Analyzing the effects of parabens on microbiota from aquatic organisms.

Such efforts will improve the relevance and applicability of experimental findings to real-world scenarios and increase our understanding of the ecotoxicity of parabens in aquatic organisms.

CRediT authorship contribution statement

Felipe Felix Costa Lima da Silveira: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Viviane Amaral Porto:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Bianca Leite Carnib de Sousa:** Writing – original draft, Visualization, Software, Formal analysis, Data curation. **Emilly Valentim de Souza:** Writing – original draft, Software, Formal analysis, Data curation. **Fabiana Laura Lo Nostro:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Thiago Lopes Rocha:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Lázaro Wender Oliveira de Jesus:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

All data used for the research are described or cited in the article.

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Capítulo 2

INTERACTIVE EFFECTS OF METHYLPARABEN AND MICROPLASTICS IN THE DEVELOPING ZEBRAFISH (*Danio rerio*)

Abstract

Among the contaminants of emerging concern (CECs) are parabens, esters of the p-hydroxybenzoic acid which are commonly used as preservatives in industrial preparations. Microplastics, in turn, are particles up to 5 mm in diameter made up of various types of organic polymers, among which polyethylene (PE) is one of the most widely used and found in aquatic environments. These particles have the ability to adsorb substances on their surface, potentially modifying the toxicity of the adsorbed molecules. This study sought to understand the toxicity of the interaction between methylparaben (MeP) and polyethylene microplastics (MPPE) on zebrafish (*Danio rerio*) in its early stages of development. To achieve this, the embryolarval toxicity test (ZELT), morphometric analysis and behavioral analysis were carried out using the environmentally relevant concentrations of 0.01, 0.1 and 1 µM of MeP separately or in a mixture with a suspension of MPPE at a concentration of 3.4 mg/L. There were no differences between the treatments regarding mortality, hatching rates and spontaneous movements during the 144 hours of experiment, but there was a significant difference in the heart rate of groups exposed to the concentrations of parabens in comparison to negative and solvent controls and to the group exposed to MPPE alone, which is an evidence that MeP is capable of eliciting cardiotoxicity in zebrafish embryos at environmentally relevant concentrations. However, the lower concentration of MeP in mixture with MPPE didn't elicit cardiotoxicity, which might be a signal that MPPE could, in certain conditions, reduce the toxicity of contaminants adsorbed to its surface. This phenomenon indicates not only that MPPE is, in fact, capable of interacting with MeP and produce different effects than the isolate exposure, but also that the variability of microplastics as a contaminant suite must be taken into account, especially regarding size, shape and type of polymer. Further studies addressing such variables should be carried in order to form a more comprehensive panoram of the interactions between microplastics and parabens.

Keywords: contaminants of emerging concern, ecotoxicology, parabens, plastic residues,conservatives, trojan horse effect.

2.1 Introduction

Contaminants of emerging concern (CECs) are a set of substances and microparticles of anthropogenic origin that are found ubiquitously in various environmental matrices due to industrial development and population growth. They include personal care products (PCPs) and pharmaceuticals, which have a wide range of chemicals and new formulations continuously introduced to the market (Wilkinson et al., 2017). Although present in low concentrations in their environments (µg/L or ng/L), their behavior in the environment and ecotoxicological profile are not well understood. This information gap raises concerns, especially since they are not typically removed by wastewater treatment technologies (Gao et al., 2021). Furthermore, their potential impacts have not been thoroughly explained in terms of their effects on individual, population and community levels of aquatic ecosystems.

Parabens, which belong in the group of CECs, are a group of low molecular weight organic substances generally defined as the alkyl and aryl esters of the 4-hydroxybenzoic acid (4-HB). They have been widely used as preservatives in pharmaceutical preparations since the 1920s and are now also found in cosmetics, foodstuffs, and other industrial products (Nowak et al., 2018; Bolujoko et al., 2022). Among them, the most commonly used are methylparaben (MeP) and propylparaben (PrP) — often found under the trade names of Nipagin™ and Nipasol™, respectively —, either alone or in combination. They are stable in acidic aqueous solutions and their properties vary according to the size/molecular weight of their alkyl group: longer chains have greater antimicrobial activity and resistance to hydrolysis, but lower water solubility (Błędzka et al, 2014). Parabens, like other PCPs, can be considered pseudopersistent contaminants due to their continuous influx in low concentrations into surface waters through anthropogenic effluents (Garric, 2013).

Parabens have been found in various aquatic environments, drinking water, urban effluents, and agricultural soils as a result of research conducted in different countries (Feng et al., 2019; Pompei et al., 2019). The presence of parabens in urban

wastewater is well documented, and despite their low stability, high biodegradability under aerobic conditions, and removal efficiency of over 90% during water treatment processes (Haman et al, 2015), they are still found in treated effluents and in water and sediments of water bodies, as well as their metabolite 4-HB (Feng et al., 2019).

Regarding the concentrations of parabens found in natural and anthropized environments, there is great variability depending on the location studied. The highest concentrations are found in urban effluents, reaching 76 900 ng/L (equivalent to ~ 0.5 µM) in a Southern California water treatment plant (Błędzka et al, 2014). In Brazil, MeP was detected in streams in the city of Rio Grande/RS at concentrations between 7.6 and 29.8 µg/L (equivalent to ~ 0.05 to 0.2 µM), and in the city of Morro Redondo/RS, between <1 and 134 µg/L (~ 0.88 µM) (Penha et al, 2021). Derisso et al (2020), when analyzing seven points of the Monjolinho River in the city of São Carlos/SP, detected MeP concentrations ranging from 0.11 to 0.98 µg/L. In an assessment of three rivers in the Curitiba/PR region, Santos et al. (2016) found MeP concentrations of up to 2875 ng/L. In the Lobo reservoir, São Carlos/SP, MeP was detected in all water samples in a mean concentration of 170.87 µg/L (~ 1.12 µM) and a maximum concentration of 1192.39 µg/L (~ 7.85 µM) (Pompei et al., 2019). In addition to their presence in surface water, there is also evidence of bioaccumulation of parabens in animal tissues. Xue and Kannan (2016) reported the presence of MeP and its metabolite 4-HB in the kidney, liver and muscle tissue of bald eagles and albatrosses at concentrations ranging from 580 ng/g for MeP to between 35 and 300 ng/g for 4-HB, depending on the tissue and species. In the same study, the presence of MeP, PrP and 4-HB was reported in the liver and brain tissues of fish from the Florida coast, at concentrations ranging from 11.2 ng/g for MeP to 1130 ng/g for 4-HB. Tissue concentrations higher than plasma concentrations are suggestive of bioaccumulation, especially in the liver (Xue; Kannan, 2016). So far, parabens have been detected in muscle, liver, kidney, fat tissue, muscle, brain, plasma, gill, ovary and testicle samples of freshwater and marine fish collected from multiple sites (da Silveira et al., 2024).

From the end of the 20th century, with the publication of studies suggesting the estrogenic and antiandrogenic activity of parabens, concern grew about their possible negative effects on the ecological balance (Błędzka et al, 2014). Their potential

estrogenic activity is associated with the size of their alkyl chain, so that butylparaben (BuP), heptylparaben (HeP) and benzylparaben (BzP) have a greater potential for endocrine disruption, according to *in silico*, *in vitro* and *in vivo* approaches (Routledge et al, 1998; Watanabe et al., 2013; Wei et al., 2022; Liang et al., 2023). 4-HB is considered the final metabolite of the biotic degradation of parabens and also has estrogenic activity reported in vitro and in vivo (Błędzka et al, 2014; Raja et al, 2019). The generation of oxidative stress, embryotoxicity, neurotoxicity and cardiotoxicity are also possible deleterious effects of these compounds (Merola et al, 2020; Penha et al, 2021; Fan et al., 2022; Hu et al., 2023). However, studies aimed at clarifying the cellular, metabolic, physiological and ecological consequences of fish exposure to sub-lethal concentrations of parabens are still incipient.

Plastic pollution is a pressing environmental issue due to its prevalence as the primary anthropogenic debris in aquatic environments, even in remote areas without human presence (Chassignet et al, 2021). Although plastic has practical advantages, such as malleability, durability, and lightness, its negative impact is becoming increasingly apparent, since 8 to 11 million tons of plastic are dumped into the oceans annually (Fava, 2022). The term 'microplastics' (MPs) was first introduced in 2004 by Thompson et al., referring to plastic particles that are 5 mm or smaller in size. Since then, researchers have been investigating their presence in surface waters, sediments, and organisms, as well as their potential harmful effects on aquatic ecosystems (Rezania et al., 2018). MPs can be of primary origin, such as plastic pellets used as raw materials by industries or as exfoliating agents in PCPs. They can also be released by the abrasion of tires or the washing of synthetic fabrics. Alternatively, they can be of secondary origin, generated from the fragmentation of macroplastics by chemical, luminous, or biotic degradation phenomena (Rezania et al., 2018; Schmid et al., 2020). The surface of such polymers has a strong attraction to hydrophobic molecules, enabling them to adsorb pollutants with this property, such as polycyclic aromatic hydrocarbons (PAHs) (Hou et al., 2023), polychlorinated biphenyls (PCBs) (Llorca et al., 2020), antibiotic drugs (Li et al., 2018), dyes (Xia et al., 2020) and steroid hormones (Lu et al., 2020; Lara et al., 2021). Due to their small size, aquatic organisms can easily ingest them, making them a potential carrier of highly toxic pollutants and pathogenic

microorganisms, acting as a 'Trojan Horse' (Trevisan et al., 2020; Kinigopoulou et al., 2022; Rafa et al., 2024).

According to Plastics Europe (2020), low-density and high-density polyethylene are the thermoplastic polymers with the second and third largest market demand in Europe, respectively, after polypropylene. Majewski et al. (2016) analyzed two wastewater samples from the city of Karlsruhe in Germany and reported that polyethylene was the most common polymer, accounting for 34% (81 mg/m³) and 17% (257 mg/m³) of each sample. Yong et al. (2020) lists the negative consequences of MPs and NPs on embryos, larvae, and adult individuals of various fish species, including zebrafish, medaka (*Oryzias* spp.), Japanese medaka (*Tigriopus japonicas*), carp (*Cyprinus carpio*), sea bream (*Carassius auratus*), *Pimephales promelas*, and wild species. Such adverse effects include inflammatory processes, damage to the digestive system, reduced growth, oxidative stress, behavioral changes, and reduced viability and survival of embryos and larvae. In addition, MPs can also be adsorbed by primary producers in aquatic environments, such as algae and plankton, affecting their photosynthesis and respiration rate, thus inhibiting their growth (Saud et al., 2023). The bioaccumulation of multiple toxicants carried by MPs is also a growing concern, especially concerning mollusks, which are filter feeders and more propense to bioaccumulate substances (Rafa et al., 2024).

Considering such information, understanding how MPs and other organic substances present in aquatic environments interact and exert toxic effects on the local biota is an important matter that must be further researched. In this way, the present study sought to understand the toxicity of the interaction between methylparaben (MeP) and polyethylene microplastics (MPPE) on zebrafish (*Danio rerio*) in its early stages of development. The expected outcomes are a higher toxicity of mixtures in comparison to the isolated contaminants, as well as a concentration-dependent response to the exposure to MeP.

2.2 Materials and Methods

2.2.1 Reagents

Polyethylene microplastics (MPPE) purchased from Sigma Aldrich were used. They had been previously characterized by Dias (2023) through electron microscopy, presenting a diameter of 35.46 µM ± 18.17 µM, heterogeneous shape and irregular surface. The MPPE concentrations used in the treatments were prepared from a stock solution with a concentration of 34 mg/L (10x). Polyethylene was chosen as representative of MPs because it is one of the most widely used polymers in various industrial applications, as well as one of the most found in the aquatic environment and with the greatest resistance to biodegradation (Horton et al, 2017; Sequeira et al, 2020). The other reagents such as methylparaben (MeP), dimethylsulfoxide (DMSO), dichloroaniline (3,4-dichloroaniline) were also obtained from Sigma, with a high degree of purity.

2.2.2 Maintenance of adult zebrafish and collection of eggs

The adult zebrafish of AB strain were kept in the Instituto de Patologia Tropical e Saúde Pública of the Universidade Federal de Goiás (IPTSP — UFG) Fish Nursery, in 3 L injected polycarbonate tanks (SLZF 110 - Scienlabor), at a ratio of 5 animals/L, at 27 ºC in a recirculating water system, on a 14:10h light-dark cycle, as recommended by Reed and Jennings (2010). The tanks were filled with reconstituted water (deionized water, sodium bicarbonate, magnesium sulphate, calcium chloride and potassium chloride) and cleaned regularly. The fish were fed twice a day with flake feed and once a day with brine shrimp. For breeding, the animals were placed in multiple brooders (Tecniplast) with a ratio of 1:1 between males and females in each. After breeding early the next morning, the embryos were collected and the viable embryos separated. Different groups of animals were used for breeding in order to reduce the genetic influence on the parameters evaluated. All the experiments were carried out under the approval of UFAL's Ethics Committee on the Use of Animals — nº 15/2022.

2.2.3 Exposure

The newly fertilized zebrafish embryos were placed in 24-well plates (Kasvi), one embryo per well, in a total of 10 embryos for each treatment below: I) Negative control (reconstituted water only — NC); II) Solvent control (0.05% DMSO) — SC; III) Positive control (3,4-DCA at 4 mg/L — which promotes 30 to 100% embryo mortality) — PC; IV) Polyethylene plastic microparticles (MPPE - 3.4 mg/L); V) MeP (0.01 µM); VI) MeP (0.01 µM) + MPPE (3.4 mg/L); VII) MeP (0.1 µM); VIII) MeP (0.1 µM) + MPPE (3.4 mg/L); IX) MeP (1 µM); X) MeP (1 µM) + MPPE (3.4 mg/L).

The plates were incubated in an embryo chamber (Scienlabor) and kept at the same temperature and photoperiod as the adults. Exposure was semi-static for 144 hours and the solutions were changed every 24 hours. The concentrations of 0.01 and 0.1 µM of MeP have environmental relevance (Penha et al, 2021), while the concentration of 1 µM (equivalent to 152 µg/L) represents the highest average concentrations found in current literature for surface waters (Bolujoko et al., 2022). The concentration of 3.4 mg/L of MPPE also has environmental relevance (Koelmans et al., 2019) and might elicit mild toxicity throughout the embryolarval period of zebrafish (Malafaia et al., 2020).

2.2.4 Zebrafish embryo-larval toxicity test (ZELT)

The test was adapted from OECD standards (2013), assessing lethal, non-lethal and teratogenic parameters. All tests were carried out in triplicate, using batches of embryos obtained from different groups of adults. Throughout the exposure period, the embryos/larvae were analyzed every 24 hours using a (ZEISS® Stemi 508) with an associated image capture system (ZEISS® Axiocam 105 color). Embryo mortality was assessed daily and considered based on four possible results, according to OECD standards (2013): a) Embryo coagulation; b) Absence of somites; c) Non-detachment of the tail; d) Absence of a heartbeat.

The non-lethal parameters analyzed were: Hatching rate (48, 72, 96 hours post-fertilization — hpf); Spontaneous movements/min (24 hpf), related to neurotoxicity; Heartbeats/min (48 hpf), related to cardiotoxicity; and embryo pigmentation (24, 48, 72, 96, 120 and 144 hpf). The teratogenic effects verified were: Presence of scoliosis (24 to 144 hpf); Vitelinic deformation (24 to 144 hpf); Growth retardation in general (24 to 144

hpf); Eye, otolith and tail defects (24 to 144 hpf); and pericardial and vitelinic edema (24 to 144 hpf).

2.2.5 Behavioral analysis

The methodology for behavioral analysis was adapted from Pinheiro-Da-Silva et al. (2020). After 144 hours of exposure to MPPEs with or without the MeP concentrations listed in section 2.3, 15 larvae (5 larvae from each replicate) were transferred to 12-well microplates (KASVI®) with 3 mL of reconstituted water per well, resulting in 15 larvae per experimental group, except for the positive control. The larvae were acclimatized to the recording room temperature of 26 ºC for 30 minutes before the 1-minute recordings. The recordings were conducted in a mini-studio (24.5 cm x 24.5 cm x 24.5 cm) using a Logitech C922 Pro® webcam mounted on top of a Puluz Photo Light Box®. The videos were analyzed using ZebTrack software, developed by Pinheiro-da-Silva et al. (2016) and implemented in MATLAB (R2014a; MathWorks, Natick, MA). The locomotor behavioral parameters assessed were total distance traveled (DT), mean speed (MS), maximum speed (Vmax), and peripheral time (PTime).

2.2.6 Morphological analysis

After the ZELT and the recordings for behavioral analysis, the specimens were euthanized by immersion in a 0.1% benzocaine solution and fixed in 4% paraformaldehyde for 24 hours. After fixation, the specimens were washed thrice in 0.2 M PBS buffer at pH 7.2 and kept in 70% alcohol at 4 ºC until biometry was carried out. Pictures from a lateral and dorsal view of each randomly selected individual (n = 15 per treatment) were taken using a stereomicroscope (ZEISS® Stemi 508) with an associated image capture system (ZEISS® Axiocam 105 color). The images were analyzed using ImageJ software and morphometric parameters were divided into four categories: i. sensory (eye diameter; maximum and minimum distances between the eyes); ii. physiological (swimming bladder, yolk sac and pericardial sac diameters); iii. skeletal structural (height, head width and depth, and distances from mouth to anus); iv. muscle structural (angle and distance between myotomes), according to Malafaia et al

(2020) and Ribeiro et al (2020).

4.2.7 Statistical analysis

The fish parameters measured were compared between treatments through Generalized Linear Model Analysis, using the best-fitted model. For some of these data, the best-fit model was attained to Gaussian distribution. For the hatching rate, the best-fitted model was attained to quasibinomial distribution. The predictive factors used were treatments and, when appropriate, time. Pairwise comparisons were performed using a posteriori Tukey tests. To verify the mortality rates in the different treatments over time, a Survival Analysis was performed using the Kaplan-Meier curve.

A significance level of 5% was used. All analyses were performed in the R environment (R core Team, 2024) using the following packages: multcomp (Hothorn et al., 2018), survival (Therneau, 2024), ggsurvfit (Sjoberg et al., 2024), flexsurv (Jackson, 2016) and survminer (Kassambara et al., 2021).

2.3 Results and Discussion

Regarding the survival of the embryos (Fig. 1A), both the negative control group (NC) and the solvent control group (SC) had a survival rate of 96,7% in the first 48 h, while the survival of the positive control group (PC) was significantly lower ($p < 0.0001$). At the 48 hpf time stamp, all the embryos from the PC group were dead. Additionally, there were no differences between the survival rate of any treatment group and the NC and SC groups. This result was expected, since previous studies have established high LC_{50} values for MeP in zebrafish — ranging from 72.67 mg/L (Merola et al., 2020a) to 211.12 mg/L for 96h larvae (Penha et al., 2021), which are approximately 470 to 1,380 times higher than the highest concentration used in this study. Furthermore, MeP is generally regarded as having lower toxicity in comparison to other parabens due to its shorter side alkyl chain (Liang et al., 2023). In a study conducted with zebrafish larvae (up to 24 hours post hatching — hph) and adults exposed to an environmentally relevant concentration of MeP (30 µg/L, equivalent to ~ 0.2 µM) for 168 h and 96 h, respectively, survival rate also wasn't altered in comparison to controls (Penha et al., 2021). Moreover, lower survival rate wasn't observed in

zebrafish embryos exposed to MeP at the concentration of 1 mg/L (Merola et al., 2020a), which is near the highest environmental findings for MeP (1192.39 µg/L) (Pompei et al., 2019).

In accordance with our findings, the exposure of zebrafish embryos to concentrations of MPPE (38.26 ± 15.64 µM) that ranged from 6.2 to 100 mg/L did not alter the survival rates when a semi-static protocol (exposure solution changed every 24 h) was used (Malafaia et al., 2020). Contrasting with these results, a lower 24 h survival rate was observed in zebrafish embryos exposed to MPPE (52 to 74 µM) in concentrations that ranged from 10^2 to 10^6 particles/L (converted from ~ 0.04 to 437.47 mg/L) using a semi-static protocol (Chen et al., 2023). This result is explained by the author as resulting from the low density of MPPE, which due to buoyancy form a kind of hydrophobic film on the water surface and can potentially undermine gas exchange with the atmosphere. Despite the similarities in concentrations, sizes, polymer and exposure protocol, the MPPE used in both studies were different in a potentially relevant aspect: in the study conducted by Malafaia et al. (2020), the MPPE had irregular shapes and a rough surface, similarly to the ones used in this study; the ones used by Chen et al. (2023), though, were nearly spherical and had a smoother surface. Further studies should be conducted in order to investigate factors such as shape, surface texture, color and aging on the toxicity of MPPE to aquatic organisms in early development stages, given that their influence on harmful biological outcomes is not as well elucidated as other aspects such as concentration, size and presence of sorbed contaminants.

In zebrafish, hatching occurs at 48 to 72 hpf, and individuals that have spontaneously hatched generally are not more developmentally advanced than the ones that remain in their chorions (Kimmel et al., 1995). The relative plasticity in hatching time is thought to enhance fitness of zebrafish embryos by balancing the benefits and costs of emerging as a free-swimming larva, as opposed to remaining bound within the chorion (Wisenden et al., 2022). Hatching time can be influenced by various environmental variables, such as temperature, oxygen availability, chemical signals and the presence of environmental hazards, such as predators and pollutants, being mainly driven by metabolic rate (Silva et al., 2022). The mechanisms that lead to early hatching of zebrafish embryos exposed to environmental contaminants aren’t well

elucidated. However, it is speculated that MP adherence to the chorionic membrane might lead to mechanical damage and to physiological changes, such as hindering of gas exchange and consequent reduction of oxygen supply (Malafaia et al., 2020).

The hatching of eggs started at 48 hpf and ended at 96 hpf for all treatments (Fig. 1B), except for the group exposed to MPPE alone, in which the start of hatching was observed only at 72 hpf. In a study conducted by Merola et al. (2020a), the exposure of zebrafish embryos to a higher, but still environmentally relevant concentration of MeP (1 mg/L, ~ 6.5 μM) did not lead to alterations in hatching when compared to controls. In a study conducted by La Pietra et al. (2024), the exposure to MPPS (1 and 3 μM, 0.01 to 10 mg/L) also did not alter survival or the normal hatching process in zebrafish embryos in any of the concentrations tested. In a study conducted by Malafaia et al (2020), the exposure to MPPE at concentrations from 6.2 to 100 mg/L in a static protocol resulted in early hatching and in lower survival rates in the concentrations of 25, 50 and 100 mg/L, which was not observed in the semi-static exposure system for any of the concentrations tested. This suggests that the exposure protocol might have an equal or greater impact on detrimental development outcomes in comparison to the concentrations of MPPE used and is in consonance with our findings.

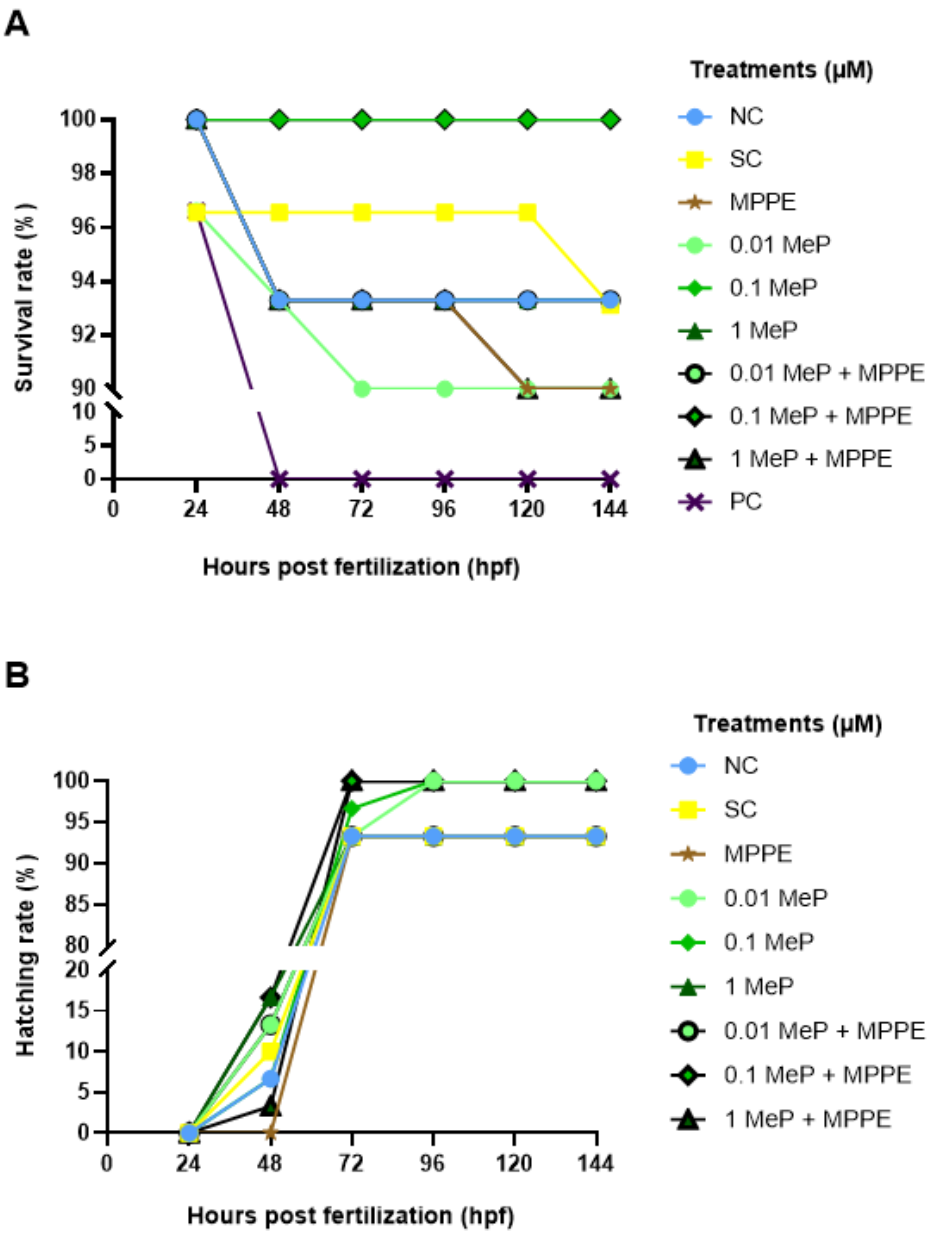

Figure 1. A) Survival rate of zebrafish embryos and larvae exposed to methylparaben (MeP) in environmentally relevant concentrations either alone or in mixture with polyethylene microplastics (MPPE) during the course of the 144h. B) Hatching rate of zebrafish embryos exposed to MeP with or without MPPE during the course of 144h.

In the first 24 hours of development of zebrafish embryos, an analysis of spontaneous movements was conducted, being related to neurological development. At that point, embryos exhibit slow and rhythmic movements driven by glutamatergic

signaling and tonic glycinergic impulses that cause contraction of muscle fibers throughout the body, along with the development of the brain into five distinct lobes (Norton, 2012; Mrinalini et al., 2023). There were no significant differences in spontaneous movements between any treatment groups except for the positive control ($p < 0.01$) (Fig. 2A), suggesting lack of neurotoxicity of both MPPE and MeP in environmentally relevant concentrations as well as their mixtures. Dambal et al (2017) and Merola et al (2020), in contrast, observed slowed or absent spontaneous movements in zebrafish embryos exposed to MeP alone, though in much higher concentrations (100 to 1000 μM and 1 to 80 mg/L — equivalent to 6.5 and 526 μM, respectively). This result also contrasts with data obtained from adult zebrafish exposed to environmentally relevant concentrations (1, 3 or 10 μg/L) of MeP for 28 days, in which brain proteome disruption, oxidative stress, reduction of the brain-somatic index (BSI) and neural signaling disturbances were observed, along with brain inflammation in the male fish (Hu et al., 2023). The longer duration of exposure to MeP along with the use of molecular biomarkers, which are generally more sensitive than those of higher biological organization levels and can provide earlier signs of harmful effects (Ryan; Hightower, 1996), may account for such differences. Regarding previous findings for MPPE alone, Malafaia et al. (2020) also did not observe alteration of the number of spontaneous movements per minute in 24 hpf zebrafish embryos exposed to concentrations of MPPE that ranged from 6.2 to 100 mg/L.

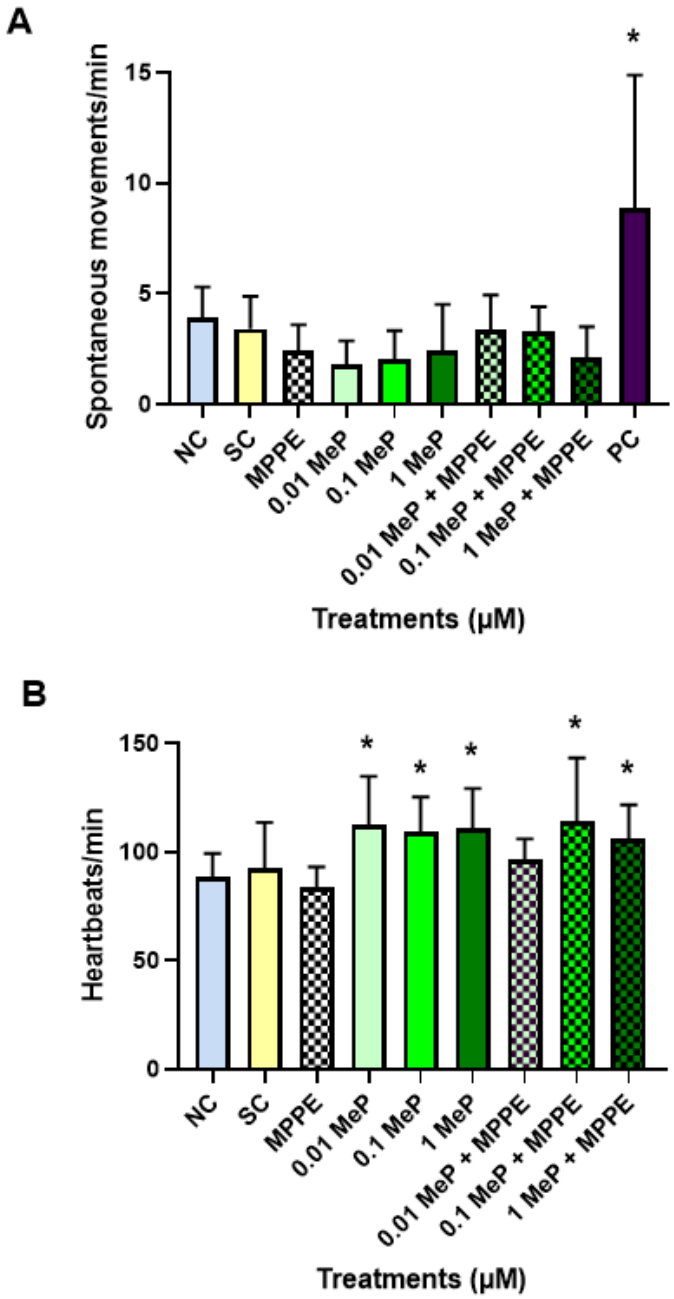

Figure 2. A) Number of spontaneous movements per minute of 24 hpf zebrafish embryos exposed to environmentally relevant concentrations of methylparaben (MeP) with or without the presence of polyethylene microplastics (MPPE) ($p < 0.01$). B) Number of heartbeats per minute of 48 hpf zebrafish embryos exposed to MeP and MPPE either alone or in mixture ($p < 0.05$).

After 48 hours of development, an analysis of embryonic heart rate was conducted. In zebrafish, the heart development starts at 48 hpf, in which the peristaltic linear heart tube is formed, and after another 48 hours a separate atrium and ventricle

are formed by looping (Teranikar et al., 2023). Malformations during that time period, which can be induced by environmental pollutants, may affect the hemodynamic performance of the embryonic heart, which comprises the heart rate and stroke volume as components of total cardiac output (Teranikar et al., 2023). All the treatments with MeP alone had a significantly higher heart rate when compared to both NC and SC groups and also to the group exposed to MPPE alone ($p < 0.05$) (Fig. 2B). The two mixture groups with higher concentrations of MeP (0.1 and 1 μM + MPPE) also had a higher heart rate in comparison to the group exposed to MPPE alone ($p < 0.001$) (Fig. 2B). This suggests that environmentally relevant concentrations of MeP are capable of eliciting toxicity effects over zebrafish heart function, even though there wasn't a difference in heartbeats per minute between MeP concentrations tested. This result differs from previous findings in the sense that, although also altering heart function in 48 hpf zebrafish embryos, the exposure to MeP was observed to induce bradycardia (Dambal et al., 2017; Merola et al., 2020a), while in our findings it appears to induce tachycardia in comparison to controls. This discrepancy may be attributed to the fact that the concentrations employed in the present study are 6.5 to 100 times lower than those utilized in the aforementioned works. Consequently, it is plausible that a distinct mechanism of cardiotoxicity may be involved depending on the MeP concentrations. In the study conducted by Malafaia et al (2019), the exposure of zebrafish embryos to MPPE in various concentrations did not elicit cardiotoxicity in neither a static or semi-static exposure protocol, which is in accordance with our findings, since the group exposed to MPPE alone did not differ from the control groups regarding heart rate.

On the other hand, the group exposed to 0.01 μM MeP alone had a significantly lower heart rate in comparison to the group exposed to 0.01 μM MeP + MPPE. This could suggest that the association of MPPE with lower concentrations of MeP might reduce cardiotoxic effects of the latter in zebrafish embryos. In fact, contrasting results regarding the effects of microplastics on the toxicity of associated contaminants have been described, with size and shape of particles being relevant variables to the biological outcomes. In juveniles of the marine fish *Dicentrarchus labrax*, it was observed a reduction in immunotoxicological effects when animals were fed with PFOS (4.83 μg/kg) adsorbed to MPPE (150 to 500 μM, 100 mg/kg) in

comparison to those fed with PFOS alone (Espinosa-Ruiz et al., 2023). In larvae of the aquatic midge *Chironomus riparius*, MPPE either reduced or increased the toxicity of the bioinsecticide *Bacillus thuringiensis israelensis* (Bti) depending on particle size (Khan & Johnson, 2024). Our findings are also in consonance with a study conducted in zebrafish embryos regarding the toxicity of mercury (Hg, 0.1 mg/L) alone or in combination with NPPS (100 nM, 10 mg/L) or MPPS (157 μM, 10 mg/L). While no difference in heart rate was observed in embryos exposed to MPPS either alone or in combination with Hg in comparison to controls, exposure to Hg alone was able to significantly decrease the heart rate of 48 hpf embryos. These results are also attributed to the size of particles, which might be contained by the chorionic barrier, thus decreasing the bioavailability of adsorbed Hg to zebrafish embryos (Wang et al., 2022).

The appearance of morphological malformations wasn't observed in any of the treatment groups (Fig. 3). Merola et al (2020a) observed multiple lethal and sublethal alterations in zebrafish embryos and larvae exposed to MeP alone, such as pericardial and yolk edema and notochord curvature. However, the concentrations in which such alterations were observed in the aforementioned study (10 to 80 mg/L) were from 65 to 526 times higher than the highest concentration used in our study and do not have environmental relevance (Bolujoko et al., 2021). Malafaia et al. (2020) observed a concentration-dependent response regarding malformations in embryos and larvae of zebrafish exposed to MPPE in a semi-static manner, with stronger teratogenic effects in concentrations ranging from 50 to 100 mg/L. The malformations found included pericardial and vitelline sac edema, spinal curvature and caudal flexure, possibly being related to chorionic pore clogging and consequent hypoxia induced by the MPPE. In the study, MPPE were observed to adhere to the external surface and gastrointestinal tract of the larvae, which might be associated with the toxicity mechanism regarding teratogenesis. Such findings, nonetheless, contrast with our study, in which malformations were not observed in the group exposed to MPPE alone. These differences might be related to the lower concentration used in the present study, which is in accordance with a concentration-dependent pattern of response.

Microplastics dispersed in the water might be ingested by aquatic organisms, being detected in their gastrointestinal tract (Malafaia et al., 2019) and causing physical

damage and inflammation to the digestive organs (Rafa et al., 2024). The lack of measurable alterations in the group exposed to MPPE alone, apart from the low concentration used in the present study, might also be related to the fact that zebrafish larvae only open their mouths widely after 72 hours of development (Kimmel et al., 1995), thus allowing ingestion of particles present in the water, but reducing the effective duration of exposure to MPPE. It is likely that smaller particles could cross the chorion more promptly during the pre-hatching stages, bypassing the limitation inflicted by the lack of active ingestion during, at least, the first 48 hours of zebrafish development (Chen et al., 2024).

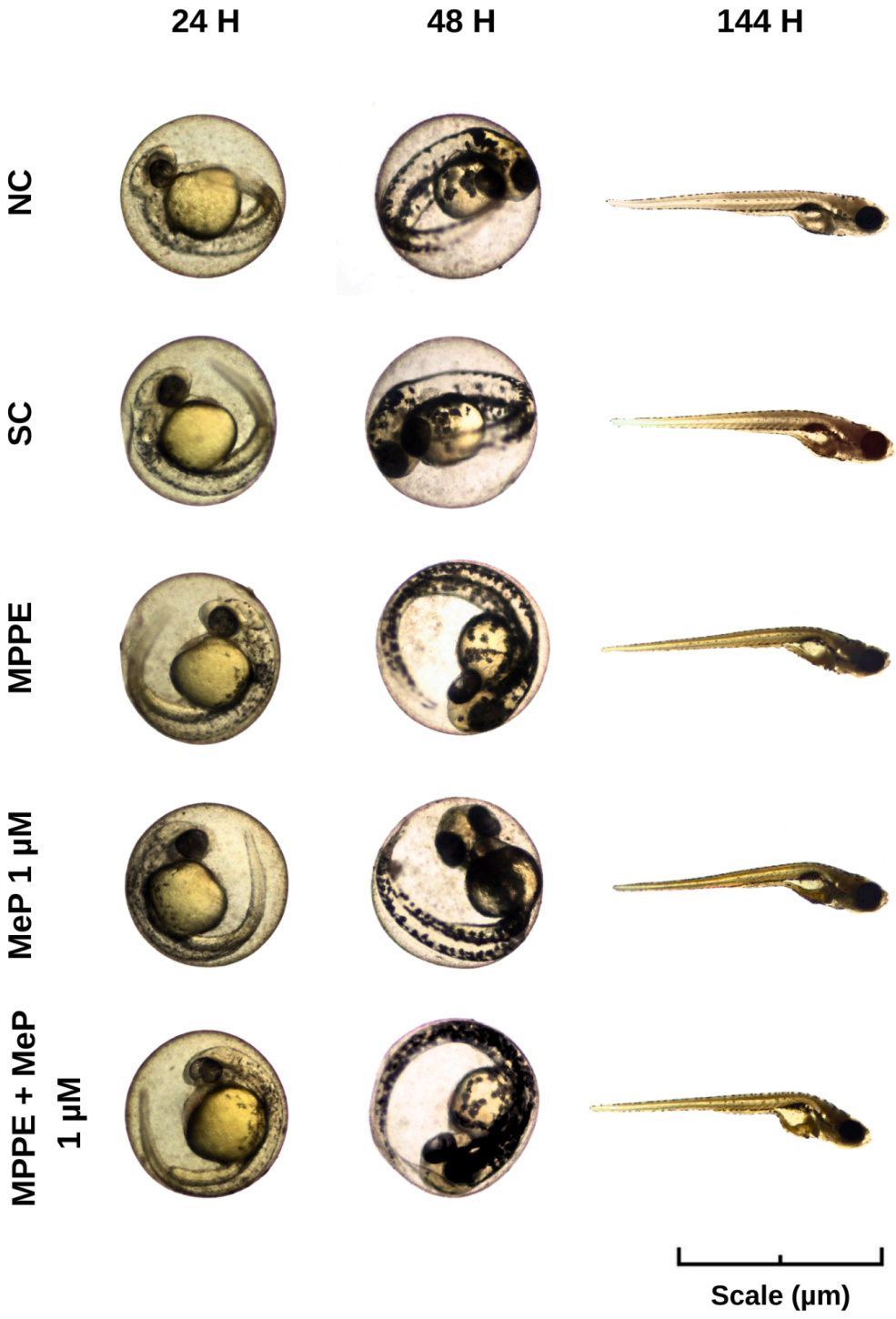

Figure 3. Photographic sheet of zebrafish embryos and larvae from five different treatment groups (NC; SC; MPPE; MeP 1 µM; and MPPE + MeP 1 µM) at 24, 48 and 144 hpf. Scale bar represents 1000 µM for 24 and 48 hpf embryos and 4000 µM for 144 hpf larvae.

As a matter of fact, the chorion of unfertilized zebrafish embryos have evenly spaced pores with approximately 0.2 μM, while the chorionic pores of fertilized embryos in the gastrula stage (5 to 10 hpf) have a diameter that varies between 0.5 to 0.7 μM (Pelka et al., 2017) — comparatively, the MPPE used in this study were roughly 70 times larges (~ 35 μM). Whether a molecule is able to cross the chorion seems to depend on its physical and chemical properties, as well as on its size and molecular weight. Besides, the stability and permeability of the chorion appears to vary with age, with blastula and gastrula-stage embryos requiring more force to puncture the chorion than pre-hatching embryos, which is due to the increase of proteolytic activity with time (Kim et al., 2024); On the other hand, the chorion of embryos in later stages of development (> 24 hpf) seem to be more permeable to small molecules such as DMSO (Kais et al., 2013). A dechorionation process of zebrafish embryos has been proposed as a tool to enhance the sensitivity of ZELT, avoiding false-negatives due to the chorion functioning as a barrier to certain materials and molecules, such as some nanomaterials and bulkier polymeric structures (Pelka et al., 2017; Pereira et al., 2023). This process could be performed after 24 hpf by a mechanical process with > 90% survival and < 5% rate of sublethal effects, having shown to significantly increase the sensitivity of embryos to the deleterial biological effects of a polymeric substance of high molecular weight (Luviquat HM 552, ~ 400 kDa) (Henn & Braunbeck, 2011). Hence, dechorionation could be performed in future studies that seek to evaluate the toxicity of MPs and NPs in zebrafish embryos, aiming to analyze whether this measure has significant impacts on test sensitivity and biological outcomes.

In contrast to our findings, a study made with smaller PS microplastics (1 and 3 μM in size) in different concentrations (0.01, 1 and 10 mg/L) was able to observe malformations, tachycardia and apoptotic processes related to oxidative stress in zebrafish embryos (La Pietra et al., 2024). A work conducted by Sun et al. (2021) showed significant cardiovascular dysfunction, oxidative stress and systemic inflammation elicited by NPPE of approximately 191 nm in diameter size; in comparison, the plastic particles used by Malafaia et al. (2020) and in this study were nearly two hundred times larger (~ 38 μM). In fact, particle size seems to have a relevant influence on the type and intensity of toxicity elicited by micro and nanoplastics. In a study

conducted by Chen et al (2024) with different sizes of NPs in embryonic and juvenile zebrafish, only the larger particles (500 nM) elicited oxidative stress, whereas only the smaller particles (80 nM) were capable of increasing the expression of neural and optical-specific mRNAs. The exposure of the clam *Scrobicularia plana* to MPPE (1 mg/L) of two different sizes (4-6 and 20-25 μM) alone or with PFOS adsorbed (55.7 ± 5.3 and 46.1 ± 2.9 μg/g) led to increased accumulation of larger MPPE in whole soft tissues in comparison to the smaller MPPE. Furthermore, higher levels of lipid peroxidation in the gills were detected for the larger MPPE, in both isolated form and with PFOS absorbed, in comparison to the smaller MPPE (Islam et al., 2021). This suggests that the size of microplastic particles has a significant influence on its toxicity mechanisms and biological effects. To further investigate this phenomenon, it might be relevant to carry a study with MPs and parabens using different particle sizes, rather than, or in addition to, different concentrations of either contaminants. Further investigation on the dynamics of MP diffusion, absorption and ingestion by embryos and larvae could also be conducted by using particles dyed with specific stains, such as Nile red, along with fluorescence microscopy (Bhagat et al., 2020; Malafaia et al., 2020; Konings et al., 2024).

2.4 Conclusion

Based on the embryotoxicity data alone, the hypothesis that the mixture of MeP and MPPE would cause greater toxicity than the exposure to either MeP or MPPE alone could not be confirmed. Alterations in neurological, cardiac and general development parameters, as well as in survival and hatching rates, were not observed in the group exposed to MPPE alone when compared to negative and solvent controls. However, an increased heart rate was observed for almost all the groups exposed to MeP, with or without the presence of MPPE, except for the group exposed to 0.01 μM MeP + MPPE. This suggests that MPPE in the size and concentration used in this study have low toxicity to zebrafish embryos and larvae until 144 hpf and might even reduce the cardiotoxicity of low MeP concentrations in such individuals. With the conclusion of further analysis, which will comprise morphometric measurements and locomotor behavioral assessment, it will be possible to form a better understanding of potential

outcomes of the exposure to these pollutants and how they might interact to elicit toxicity in the earlier stages of zebrafish development.

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CONSIDERAÇÕES GERAIS

Os microplásticos são uma classe de contaminantes extremamente variável em suas características físicas e químicas, havendo evidências crescentes de sua ubiquidade em ambientes aquáticos, bem como de sua ecotoxicidade e dos efeitos biológicos de sua interação com outros tipos de contaminantes orgânicos e inorgânicos encontrados em águas superficiais. Dentre os contaminantes que poderiam interagir com microplásticos e alterar sua toxicidade, os parabenos se destacam por serem substâncias detectadas em águas residuais, águas superficiais e em tecidos animais de organismos aquáticos de diversas localizações, com efeitos tóxicos documentados a partir de estudos experimentais realizados em microrganismos, invertebrados e vertebrados de variadas espécies. Esta dissertação provê uma extensa revisão da literatura acerca de todo o conhecimento atual que diz respeito à bioacumulação e ecotoxicidade de parabenos e seus metabólitos em organismos aquáticos. Além disso, conduz um estudo preliminar que busca investigar a interação de um polímero plástico de ampla produção e descarte com o parabeno mais extensamente encontrado em águas superficiais a partir de concentrações ambientalmente relevantes de ambos os contaminantes. Dessa forma, traz conhecimento inovador a respeito do comportamento e ecotoxicidade destes contaminantes ambientais emergentes em um cenário realista, buscando contribuir para com o arcabouço de informações que sustenta regulações e políticas públicas acerca da produção, descarte e remediação de tais contaminantes em matrizes ambientais.

APÊNDICE I

Figura 1. Captura de tela da primeira página do artigo de revisão publicado na revista Environmental Pollution em novembro de 2024.

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Review

Bioaccumulation and ecotoxicity of parabens in aquatic organisms: Current status and trends☆

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ABSTRACT

Parabens are preservatives widely used in personal care products, pharmaceuticals, and foodstuffs. However, they are still unregulated chemical compounds. Given their extensive use and presence in different environmental compartments, parabens can adversely affect animal health. Thus, the current study aimed to summarize and critically analyze the bioaccumulation and ecotoxicity of parabens in aquatic species. Studies have been mostly conducted in laboratory conditions (75%), using mainly fish and crustaceans. Field studies were carried out across 128 sampling sites in six countries. Paraben bioaccumulation was predominantly detected in fish muscle, liver, brain, gills, ovary, and testes. Among the parent parabens, methylparaben (MeP), ethylparaben (EtP), and propylparaben (PrP) have been detected frequently and more abundantly in tissues of marine and freshwater specimens, as well as the metabolite 4-hydroxybenzoic acid (4-HB). Parabens can induce lethal and sublethal effects on aquatic organisms, such as oxidative stress, endocrine disruption, neurotoxicity, behavioral changes, reproductive impairment, and developmental abnormalities. The toxicity of parabens varied according to species, taxonomic group, developmental stage, exposure time, and concentrations tested. This study highlights the potential bioaccumulation and ecotoxicological impacts of parabens and their metabolites on aquatic invertebrates and vertebrates. Additionally, future research recommendations are provided to evaluate the environmental risks posed by paraben contamination more effectively.

APÊNDICE II

Table 1. Chemical and physical properties of main parabens∗.

CHARACTERISTICS/ PARABENS	4-HB/PHBA	MeP	EtP	PrP	BuP	PhP	BzP	HeP
Chemical name	4-hydroxybenzoic acid	Methylparaben	Ethylparaben	Propylparaben	Butylparaben	Phenylparaben	Benzylparaben	Heptylparaben
Chemical formula	$C_7H_6O_3$	$C_8H_8O_3$	$C_9H_{10}O_3$	$C_{10}H_{12}O_3$	$C_{11}H_{14}O_3$	$C_{13}H_{10}O_3$	$C_{14}H_{12}O_3$	$C_{14}H_{20}O_3$
Molecular weight (g M-1)	138.12	152.15	166.17	180.2	194.23	214.22	228.25	236.31
CAS n.	99-96-7	99-76-3	120-47-8	94-13-3	94-26-8	17696-62-7	94-18-8	6259-77-4
Solubility in water at 25°C (mg L-1) (MG/l)	5000	2500	885	500	~150	~10	~20	~1
Log octanol-water (Log KOW)	1.58	1.96	~2.47	3.04	3.57	~3.5	3.71	4.52
Log acid dissoc. constant (pKA)	~4.54	~8.17	8.1	~8.2	8.37	~8.4	~8.4	~8.4
Half-life (hours)	—	35.2#	27.5#	20.3#	9.6#	–	10–19h##	–
Biodegradability (in water) ∗∗	Readily	Readily	Readily to slower rate	Readily to slower rate	Readily to slower rate	Slow rate	Slow rate	Slow rate

Data obtained from Barabasz et al. (2019) Gonzalez-Marino et al. (2011)a,b, Kim et al. (2023), Nowak et al. (2018), Vale et al. (2022), and Yamamoto et al. (2007). (**) Paraben's longer alkyl chain makes it more lipophilic and less water-soluble, which can lead to a slower degradation rate in the environment. (#) Data from wastewater test. (##) Data from activated sludge batch. (¡) Data not available.

APÊNDICE III

Table 2. Summary of experimental studies with parabens in aquatic organisms regarding the types and concentrations of parabens used, biomarkers investigated and model species.

Species	Stage of development	Type of parabens	Concentrations	Biomarkers	Exposure period	Exposure Type	Analytical control	Bioaccumulation Analysis	Effects	Reference
				MICROORGANISMS						
Vibrio fischeri	—	MeP, EtP, PrP, iPrP, BuP, iBuP, BzP and their chlorinated compounds	Unspecified	Bioluminescence	5, 15 min	Static	No	No	↓bioluminescence	Terasaki et al., 2009
Pseudokirchneriella subcapitata	—	MeP, EtP, PrP, iPrP, BuP, iBuP,	Unspecified (established	Growth rate	72 h	Static	No	No	↓growth rate for longer alkyl	Yamamoto et al., 2011

		BzP	according to LOEC)						chain PBs at high concentrations	
	—	MeP	Unspecified (EC_{50} = 35.25 mg/L)	Growth inhibition	72 h	Static	No	No	↑growth inhibition	Di Poi et al., 2017
Aliivibrio fischeri	—	MeP, EtP, PrP, 4-HB	Unspecified	Bioluminescence, respiration inhibition	5, 15 min	Static	No	No	↓bioluminescence, respiration	Ortíz de García et al., 2014
Aliivibrio fischeri	—	MeP, PrP	1.75, 2.5, 5, 10 µg/L and 0.25, 0.5, 1, 3.75, 7.5, 15, 30 mg/L	Bioluminescence	5, 15 min	—	No	No	↓bioluminescence	Dailianis et al., 2023
Acinetobacter calcoaceticus	—	MeP, PrP, BuP, mixture	150 ng/L	Virulence factors, cellular culturability, density, and thickness	7-26 d	Semi-static for (2 d) for 26 d-exposure	No	No	↑cellular culturability and density	Pereira et al., 2023
Freshwater biofilm	—	MeP, BuP	Unspecified	Type of cell deatlh, cell wall damage, ROS generation	24 h	Static	No	No	BuP promoted necrosis while MeP apoptosis/both ↑ cell wall dagame and ROS production	Liu et al., 2023b
Periphyton biofilm	—	BuP	0,5, 50 and 5000 µg/L	Total biomass, chlorophyll *a*, algae diversity and biovolume, photosynthetic efficiency, carbon source utilizing capacity	32 d	Flow-through (12h)	No	No	No effect at environmentally relevant concentrations (0,5 µg/L), ↓algae growth, algal diversity and photosynthetic efficiency at the highest concentration	Song et al., 2016

Stenotrophomonas maltophilia	—	MeP, PrP, BuP, mixture	150 ng/L	Virulence factors, cellular culturability, density, and thickness	7-26 d	Semi-static for (2 d) for 26 d-exposure	No	No	↑cellular density and thickness, protease and gelatinase production	Pereira et al., 2023
				INVERTEBRATES						
Daphnia magna	Neonates	MeP, EtP, PrP, iPrP, BuP, iBuP, BzP and their mono- and dichlorinated compounds	Unspecified	EC_{50} (immobilization)	48 h	Static	No	No	↑mortality with chlorinated and longer alkyl chain PBs	Terasaki et al., 2008
	Neonates	MeP, EtP, PrP, iPrP, BuP, iBuP, BzP	Unspecified (calculated accordingly to LC_{50})	LC_{50}, mortality, reproduction	48 h (acute exposure), 10 d (subchronic exposure)	Static	Yes	No	↑mortality for higher MeP concentration (12 mg/L), ↓growth for longer alkyl chain PBs	Dobbins et al., 2009
	Neonates	MeP, EtP, PrP, iPrP, BuP, iBuP, BzP	Unspecified (calculated accordingly to LOEC)	Immobilization	48 h (acute exposure), 21 d (chronic exposure, MeP only)	Static	No	No	↑immobilization for longer alkyl chain PBs and higher concentrations of shorter alkyl chain PBs	Yamamoto et al., 2011
	Neonates	MeP	Acute exposure: 0, 0.37, 0.75, 1.50, 3.12, and 6.25 mg/L Chronic exposure: 0, 0.1, 0.3, 1.0, 3.2, and 10.0 mg/L with or without UV light	Immobilization, growth, number of living offspring, gene expression	48 h (acute exposure), 21 d (chronic exposure)	Static	Yes	No	↑transcription of genes related to oxidative stress (with UV light), ↓growth and number of offspring in a dose-dependent manner, ↓population growth rate	Lee et al., 2017
	Neonates	MeP	Unspecified (EC_{50} = 41.23 mg/L)	Immobilization	48 h	Static	No	No	↑immobilization	Di Poi et al., 2017
	Juvenile	MeP, EtP, PrP, BuP	Acute exposure: 0, 0.1, 1.0, 10 mg/L	Behavior, neurotoxicity,	48 h	Static	No	No	Disruption of cardio and	Eghan et al., 2023

				cardiotoxicity and gene expression					neurobehavioral functions	
Acartia tonsa	Nauplii	MeP, EtP, PrP, BuP, BzP	Unspecified (use of wastewater)	Larval development	5 d	Static	No	No	↑inhibition of development	Kusk et al., 2011
Artemia franciscana	Nauplii	MeP	0.0085 and 0.017 mg/L	LC_{50}, oxidative stress, AChE	24 h (LC_{50}) and 9 d (chronic exposure)	Static	No	No	↓CAT	Comeche et al., 2017
Tigriopus japonicus	Nauplii	MeP, EtP, PrP	Acute exposure: 0, 5000, 7500, 10,000, 15,000, and 20,000 µg/L (MeP and EtP); 0, 100, 200, 300, 400, and 500 µg/L (PrP) Chronic exposure: 0, 10, 100, 1000, and 10,000 µg/L (MeP); 0, 3.75, 37.5, 375, and 3750 µg/L (EtP); 0, 0.05, 0.5, 5, and 50 µg/L (PrP)	LC_{50}, development, reproduction rate	96 h (acute exposure)	Static	No	No	Developmental delay, ↓reproduction rate in higher concentrations	Kang et al., 2019
Ceriodaphnia dubia	Adults and neonates	MeP, PrP, iPrP, BzP and their chlorinated compounds	Unspecified	EC_{50} (Mortality, offspring number and first brood)	7 d	Static	Yes	No	↓offspring number for MeP, BzP and dichlorinated BzP ↑mortality with longer alkyl chains and nonchlorinated PBs	Terasaki et al., 2013
Paracentrotus lividus	Embryos and larvae	PrP	10, 64, 100, 160, 400, 1000 and 10000 µg/L	Larvae length, malformations	48 h	Static	No	No	↓larvae length ↑malformations	Torres et al., 2016
Crassostrea gigas	Embryos and larvae	MeP	Unspecified (EC50 = 18.57 mg/L — embryotoxicity; EC_{50} = 7.88 —	Embryotoxicity, metamorphosis	36 h (embryotoxicity), 24 h (metamorph	Static	No	No	↑abnormal larvae, ↓metamorphosis rate	Di Poi et al., 2017

			metamorphosis)		osis)					
Dugesia japonica	—	MeP, EtP, PrP, BuP	0, 6.25, 12.5, 25, 50 (MeP); 0, 50, 100, 200, 400 (EtP); 0, 1, 5, 10, 25 (PrP); 0, 1, 10, 25, 50 (BuP)	Mobility	10 min	Static	Yes	No	↓mobility in higher concentrations	Li, 2020
Mytilus galloprovicialis	—	MeP, PrP	0.3, 2.0 µg/L	Oxidative stress, lysosomal membrane integrity, genotoxicity	4 days	Semi-static (24 h)	Yes	No	disturbance of lysosomal membrane integrity, ↑ superoxides, nitric oxides, lipid peroxidation, superoxides, and micronuclei.	Dailianis et al., 2023
Strongylocentrotus purpuratus	Larvae	MeP	0.1, 0.5, 1 and 5 mg/L	Fertilization success, survival, development, body length	40 min (fertilization) , 3 h (larval developmen t), 96 h (larval growth and survival)	Static	No	No	↓survival, ↓body length in the highest dose	Shore et al., 2022
				VERTEBRATES						
Oncorhynchus mykiss	Juvenile	EtP, PrP, BuP, 4-HB	100 and 300 mg/kg (EtP, PrP and 4-HB), 50, 150 and 200 mg/kg (BuP)	VTG	Intraperitone al injections at days 0 and 6	Static	No	No	↑VTG and mortality	Pedersen et al., 2000
	Juvenile	PrP	7 to 1830 mg/kg/2 days (oral administration) 50 and 225 µg/L (water exposure)	VTG, toxicokinetics	10 d (oral administrati on), 12 d (water exposure)	Static	No	Liver and muscle	↑VTG	Bjerregaard et al., 2003
	Juvenile	BuP	4 to 74 mg/kg/2 days (oral administration)	LC_{50}, VTG, tissue concentration	10 d (oral administrati on), 12 d	Static	No	Liver and muscle	↑VTG	Alslev et al., 2005

Cyprinus carpio	Juvenile	MeP	35 and 201 µg/L (water exposure) 0, 0.84, 1.68 and 4.20 mg/L	LC_{50}, histopathology, VTG, behavior, organ mass, bioaccumulation, hepatic enzymes	(water exposure) 96 h (acute exposure), 28 d (subchronic exposure)	Static	No	Testes, liver, brain, gills and muscle	↓ASP, ACP, testiculosomatic index, ↑ALT, ALK, liver size, VTG, bioaccumulation in testis, liver, brain, gills and muscle	Barse et al., 2010
	Embryos	MeP, PrP, BuP	MeP 0.5; 50; 500; 5000; 100,000 µg/L; PrP 0.1; 10; 100; 1000; 100,000 µg/L; BuP 0.1; 10; 100; 1000; 100,000 µg/L	Mortality, hatching, development, oxidative damage, and gene expression.	96 h	Semi-static (24h)	No	No	↑Mortality, ↓hatching rates, ↑malformations, gene expression downregulation	Medkova et al., 2023
Pimephales promelas	Larvae	MeP, EtP, PrP, iPrP, BuP, iBuP, BzP	Unspecified (calculated accordingly to LC_{50})	LC_{50}, mortality, growth	48 h (acute exposure), 7 d (subchronic exposure)	Static	No	No	↓growth for longer alkyl chain PBs	Dobbins et al., 2009
Oryzias latipes	Adults	PrP	0.055, 0.55, 5.5 and 55 mM	VTG, gene expression	1 week	Static	No	No	↑VTG, ERS1 and CHG	Inui et al., 2003
	Larvae	MeP, EtP, PrP, iPrP, BuP, iBuP, BzP	Unspecified (established accordingly to LOEC)	VTG, gene expression	96 h (acute exposure), 14 d (chronic exposure, MeP only)	Static	No	No	↑VTG in males, upregulation of 12 genes (including *vtg2*, choriogenin and *esr1*), downregulation of 10 genes	Yamamoto et al., 2011
	Embryos, eleuthero embryos and larvae	PrP	40, 400, 1000 and 4000 µg/L	Embryotoxicity, histopathology, EROD, post-eclosion development	10 d	Static	No	No	↓development rate, ↑malformations, weakness, histological defects and mortality at the highest concentration,	González-Doncel et al., 2014

									EROD, gallbladder area	
	Adults	MeP and PrP	0.400, 2.00 and 10.0 mg/L (MeP); 0.320, 1.00 and 3.20 mg/L (PrP)	Fecundity and fertility (spawning status), VTG, secondary sex characteristics, body length and weight, ESR1, Arβ	21 d	Static	Yes	No	Agonistic activity towards ESR1, ↑VTG in males, ↓fecundity	Kawashima et al., 2021
	Adults and parental generation	2-EHHB	Unspecified (established according to OCSPP MEOGRT 890.2200)	Fecundity and fertility (spawning status), VTG, secondary sex characteristics, body length and weight	32 weeks	Flow-through (72 min)	Yes	No	Testicular hypoplasia/atrophy, reduced liver glycogen, and effects on body weight and length	Matten et al., 2023
Oreochromis niloticus	Adults	MeP, EtP, PrP, BuP, BzP, MeP + PrP	4.0 mg/L (all parabens alone), 6.0 mg/L MeP + 1.7 mg/L PrP	LC_{50}, oxidative stress in gills and liver	12 d	Semi-static	Yes	No	↓GSH (6 days), ↑SOD, GPx, GR, GSH (12 days)	Silva et al., 2018
	Juvenile	BzP	0, 5, 50, 500 and 5000 ng/L	Lipid metabolism, hepatic morphology, oxidative stress, brain AChE	8 weeks	Semi-static (24h)	No	No	↑metabolic disorders of hepatic glycerol phospholipids, glycerolipids and sphingomyelins, crude fat content, oxidative stress and liver tissue inflammation ↓brain AChE	Lin et al., 2022
	Adults	BuP	5, 50, 500 and 5000 ng/L	Histology, neurotransmitters, gene expression	56 d	Semi-static (24h)	Yes	No	Darker skin pigmentation, ↑Tyr, Arr3a ↓dopamine, Asip2,	Liu et al., 2023a

Danio rerio	Juvenile	PrP	500, 1000 or 2000 mg/kg	Length and weight, VTG, sex ratio	20 d (VTG), 45 d (sex ratio)	Semi-static (24h)	Yes	No	↑proportion of females in the lowest concentration	Mikula et al., 2009
	Embryos	PrP	10, 100, 1000, 3500, 6000, 8500 and 10000 µg/L	Embryotoxicity	80 h	Static	No	No	↑developmental delay, malformations, mortality at the highest concentration ↓heart rate, hatching rate	Torres et al., 2016
	Embryos and larvae	MeP	100, 200, 400, 800 and 1000 µM	LC_{50}, embryotoxicity, gene expression (VTG-I only)	96 h	Static	No	No	↑malformations, VTG-I ↓heart rate, hatching rate	Dambal et al., 2017
	Embryos and larvae	MeP	50 mg/L	Embryotoxicity, oxidative stress, gene expression, behavior, apoptosis	68 h	Static	No	No	↑malformations, mortality, lipid peroxidation, *myca* and *ccnd1* ↓GST, NO, distance swam	Ateş et al., 2017
	Adults	MeP	0.001, 0.01, 1 and 10 mg/L	Survival, length, weight, gonadosomatic index, histopathology of the testis	21 d	Semi-static (24h)	No	No	Testicular atrophy, ↓gonadosomatic index, multinucleated gonocytes, impaired germ cells, Leydig cell hyperplasia, interstitial fibrosis, apoptosis of Sertoli cells	Hassanzadeh, 2017
	Embryos and larvae	BuP	0, 250, 500, 1000 and 3000 nM	NOEC/LOEC, embryotoxicity, pancreatic malformations,	165 h	Static	No	No	↑malformations, beta cell area, aberrant pancreatic islet	Brown et al., 2018

			oxidative stress, gene expression,					morphologies, GSH, *gsr* ↓*pdx1*	
Embryos and larvae	MeP	0.1, 1, 10 and 100 ppb	Embryotoxicity, neurotoxicity (AChE, cortisol), behavior	142 h	Static	No	No	↑cortisol ↓AChE, latency to reach and time spent in the upper part of the tank, heart rate, hatching rate	Luzeena-Raja et al., 2018
Embryos and larvae	MeP and PrP	1, 10, 25, 50, 100 and 200 µM (embryotoxicity), 1 and 10 µM (gene expression)	Embryotoxicity, gene expression	118 h	Static	No	No	↑malformations, mortality ↓hatching rate, altered expression of 30 genes related to cell cycle, DNA damage, inflammation, fatty acid metabolism and endocrine function	Bereketoglu and Pradhan, 2019
Embryos and larvae	MeP	1, 10, 30, 60 and 80 mg/L	LC_{50}, embryotoxicity	96 h	Static	No	No	↑malformations ↓heart rate, survival at the highest concentrations	Merola et al., 2020a
Embryos and larvae	EtP, BuP	5, 10, 20 and 30 mg/L (EtP), 1, 2.5 mg/L (BuP)	LC_{50}, embryotoxicity, behavior	96 h	Static	No	No	↑malformations, behavioral abnormalities ↓heart rate, blood circulation, hatching rate	Merola et al., 2020b
Embryos and larvae	PrP	1, 2, 4, 6 and 8 mg/L	Embryotoxicity, lipid metabolism	96 h	Static	No	No	↑malformations, yolk sac size ↓hatching rate, swim bladder size, embryo length, head length, survival	Perugini et al., 2020

								at the highest concentrations, PLA_2	
Embryos and larvae	MeP, EtP, BuP	100, 1000 e 10000 µg/L (MeP), 50, 500 e 5000 µg/L (EtP), 5, 50 e 500 µg/L (BuP)	Behavior	96 h	Static	No	No	↑thigmotaxis (EtP and BuP)	Merola et al., 2021
Embryos and larvae	MeP, EtP, PrP, BuP	20 to 200 µM (MeP), 20 to 100 µM (EtP), 5 to 20 µM (PrP), 2 to 10 µM (BuP)	Embryotoxicity, endocrine dysregulation (thyroid), gene expression	120 h	Static	No	No	↓survival (EtP, PrP and BuP), T3 and T4 ↑malformations, cell proliferation	Liang et al., 2021
Larvae and adults	MeP	30 µL, 50 mg/L (adults), 50 mg/L (larvae)	LC_{50}, NOEC, oxidative stress, genotoxicity, gut microbiome	96 h (adults), 168 h (larvae)	Static	No	No	↓EROD in adults (gills) at high concentration ↑lipid peroxidation, kidney nuclei and micronuclei (erythrocytes) in adults, carbon sources utilized by gut microbiota in adults	Penha et al., 2021
Adults	MeP	1, 10 and 110 ppb	Neurotoxicity (AChE, 5-HT), gene expression, behavior	30 d (24h)	Semi-static	No	No	↓AChE ↑5-HT, anxiety-like behavior in females Dysregulation of cardiac hypoxia and neuronal differentiation-related genes	Thakkar et al., 2022
Embryos and larvae	PrP	10 and 10000 µg/L	Oxysterols	24 h	Static	No	No	↑27-OH, ↓7a-OH and 7b-OH at 8hpf, ↑24-OH at 24 hpf, non-detection of	Merola et al., 2022

Embryos and larvae	PrP, BuP	0.1, 1 and 10 ppb	Embryotoxicity, anxiety behavior, oxidative stress in the brain, apoptosis in the head, AChE, NO	96 h	Static	No	No	22-OH and 25-OH at 8 and 24 hpf ↓hatching rate (PrP), heart rate, SOD, CAT, GPx, GST, GSH, AChE ↑mortality, malformations, scototaxis, NO, ROS, lipid peroxidation, apoptosis in the head	Lite et al., 2022
Adults	MeP	0, 1, 3 and 10 µg/L	Gene expression, histopathology of liver, oxidative stress, metabolomic profile of liver	28 d	Semi-static (24h)	No	No	↑hepatic cortisol in males, ↓synthesis and conjugation of primary bile acid, ↑degradation of estradiol and retinoic acid, ↑hepatocellular vacuolization, ↑redox imbalance	Hu et al., 2022a
Adults	MeP	0, 1, 3 and 10 µg/L	Growth, gut microbiome, 5-HT, TJP2, goblet cells, proinflammatory genes, oxidative stress	28 d	Semi-static (24h)	No	No	↑body length and weight in females, ↑proinflammatory cytokines in females, ↑intestinal dysbiosis, ↑goblet cells in males, ↓TJP2 and serotonin in females	Hu et al., 2022b
Embryos	EtP	0.1, 0.5, 1, 2, 3, 4, 5, 6, 10, 15, 18, 20, 25, 30, 42, 50, 80 and 100 mg/L	Heart morphology and histopathology, heart rate, gene	90 h	Static	No	No	↑abnormalities in heart morphology and function,	Fan et al., 2022

			expression					disruption of retinoic acid signaling pathway, ↓gene related to myocardial contraction	
Embryos and Adults	MeP	0, 1, 3 and 10 μg/L	Embryotoxicity, gonad histology, sex hormones, gene expression	28 d	Semi-static (24h)	No	No	↑gonadosomatic index, ↑mortality of offspring, early hatching, blockage of oogenesis, disbalance of sex hormones, upregulation of 17 genes, downregulation of 29 genes	Hu et al., 2022c
Embryos	MeP, EtP, PrP and BuP	20, 100 and 200 μM (MeP), 20, 50 and 100 μM (EtP), 2, 5 and 10 (PrP), 1, 2 and 5 μM (BuP)	Hormones, gene expression	120 h	Static	No	No	Downregulation of 14 genes, upregulation of 6 genes, ↑VTG, ↑estradiol (BuP), ↓testosterone	Liang et al., 2023a
Adults	MeP	1, 3 and 10 μg/L	Neural proteome, oxidative stress, AChE, glutamate, gene expression	28 d	Semi-static (24h)	No	No	Downregulation of 33 differential proteins in males and 88 in females, upregulation of 31 differential proteins in males and 89 in females, downregulation of two genes, ↑glutamate in the male brain, ROS ↓glutamate in the female	Hu et al., 2023

									brain, CAT, GSH	
	Embryos and larvae	MeP, EtP, PrP, BuP	20, 100 and 200 µM (MeP), 20, 50 and 100 µM (EtP), 2, 5 and 10 µM (PrP), 1, 2 and 5 µM (BuP)	Swimming behavior, AChE, cortical hormones, gene expression	118 h	Static	No	No	Downregulation of four genes (MeP), upregulation of two genes (EtP), ↑AChE in two exposure groups ↓ACTH, total movement distance, mean velocity	Liang et al. 2023b
	Embryos	MeP, PrP, BuP	MeP 0.5; 50; 500; 5000; 100,000 µg/L; PrP 0.1; 10; 100; 1000; 100,000 µg/L; BuP 0.1; 10; 100; 1000; 100,000 µg/L	Lethal and sublethal endpoints and gene expression	96 h	Static	No	No	↑Mortality, ↓hatching rates, ↑malformations (at higher concentrations), gene expression downregulation	Medkova et al., 2023
	Embryos and Larvae	MeP, EtP, PrP	5, 10, 20, 40, 80, 150 and 300 µM (embryotoxicity test) Non-specified for other tests (based on embryonic mortality)	Embryotoxicity, neurotoxicity, behavior, gene expression	120 h	Static	No	No	Abnormal embryonic development; behavioral hyperactivity	Tran et al., 2023
	Embryos	BuP	0.6 mg/L, 1.2 mg/L, and 1.8 mg/L	Cardiotoxicity, oxidative stress, and gene expression	72 h	Static	No	No	Cardiac morphological defects and functional impairment; Cardiac oxidative stress and immunosuppression	Zhu et al., 2023
Mauremys sinensis	Adults	BuP	5, 50 and 500 µg/L	Gut microbiome, gut histopathology, cytokines, gene expression	20 weeks	Semi-static (48h)	No	Intestine	Gut microbiome dysbiosis; inflammatory response,	Ding et al., 2023

	Adults	BuP	5, 50 and 500 µg/L	Liver oxidative stress and gene expression	20 weeks	Semi-static (48h)	No	No	shortened intestinal villi ↑ MDA, ↓ SOD, CAT, GSH, dysregulation of genes related to Nrf2-Keap1 pathway, inflammatory and apoptosis, several histopathological findings	Yin et al.. 2023
Xenopus laevis	Embryos and larvae	MeP, PrP, BuP	MeP 0.5; 50; 500; 5000; 100,000 µg/L; PrP 0.1; 10; 100; 1000; 100,000 µg/L; BuP 0.1; 10; 100; 1000; 100,000 µg/L	Lethal and sublethal endpoints and gene expression	96 h	Static	No	No	↑Mortality (at higher concentrations), gene expression downregulation	Medkova et al., 2023