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Sex-Specific Vulnerability of Marine, Freshwater and Terrestrial Animals to Pollutants: A Meta-Analysis Study

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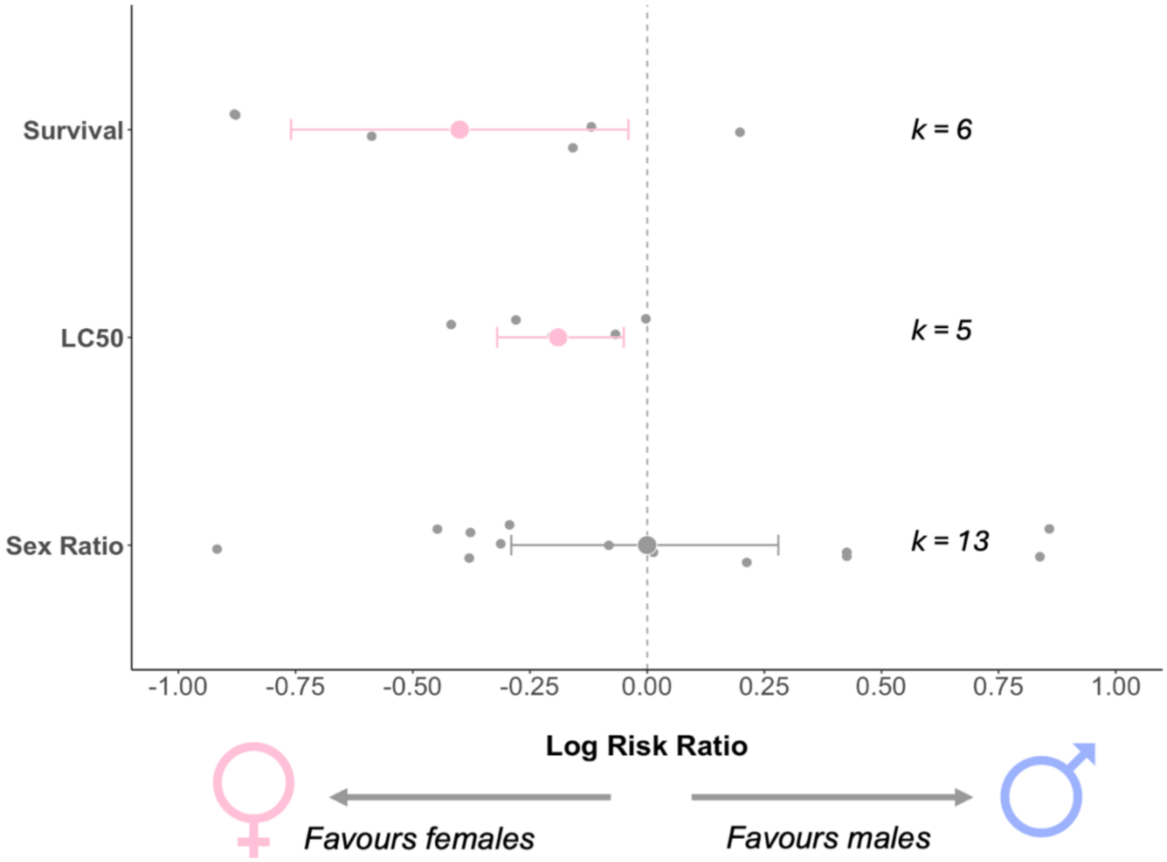

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Declaration of Artificial Intelligence Usage

In this thesis, artificial intelligence (AI) has been used. All data and personal information have been processed in accordance with the Norwegian University of Life Sciences (NMBU) regulations, and I, as the author of the document, take full responsibility for its content, claims, and references. An overview of AI usage is provided below:

- Screening: I used Rayyan AI (https://www.rayyan.com/) to remove duplicates and aid during title and abstract screening. However, I performed quality assurance by calibrating this usage, checked its validity and cross-checked with manual methods. Furthermore, I also checked that Rayyan AI had been used by a previous publication (See Pottier et al., 2021 supplementary) before applying it to this thesis.
- Debugging of R Studio code: AI was used, namely ChatGPT (https://chatgpt.com/), to help fix errors and optimise R studio code for data analysis. However, I always referred to the citations of the R packages used, and cross-checked with other sources during data analysis as well as manually checked my raw data throughout (See Appendices for further sources which aided in data analysis)

List of Abbreviations

CI – Confidence Interval

GSI – Gonado-Somatic Indices

HSI – Hepato-Somatic Indices

K-factor – condition factor (length-weight relationship/factor)

LC$_{25}$ – Lethal Lethal Concentration 25 (the concentration that kills 25% of the animal)

LC$_{50}$ – Lethal Lethal Concentration 50 (the concentration that kills 50% of the animal)

LogRR – Log Risk Ratio

MDPI – Multidisciplinary Digital Publishing Institute

MXR – multixenobiotic resistance activity

N – Sample size

NOEC – No Observed Effect Concentration

PRISMA – Preferred Reporting Items for Systematic reviews and Meta-Analyses)

SD – Standard deviation

WoS – Web of Science

Abstract

Chemical pollution is ubiquitous and impacts animals across all realms, including marine, freshwater and terrestrial ecosystems. However, chemical pollution can influence males and females differently, which could have consequences for populations, communities and ecosystems. To the best of my knowledge, no systematic reviews or meta-analyses have compared the differences between females and males on the effect of chemical pollution.

My aim was to explore sex-specific stress responses of exposed females and males. Using the Web of Science Core Collection I conducted a systematic search for relevant publications on this topic from 2010 to 2022. The search yielded 142 studies, and included sex-specific effects on species in different phyla and realms to chemical pollutants, such as pharmaceuticals, pesticides, flame retardants, and hydrocarbons. These studies were included in the systematic review. I quantified sex-specific differences in survival, LC_{50} (the concentration that kills 50% of the animal) and sex ratios following chemical exposure. I further investigated different underlying mechanisms, highlighting the different sensitivities between the sexes. Most studies were from the global North and China, with a majority of species from freshwater and marine realms, and the most common animals being a few species of fish, crustaceans, and molluscs. Using random-effects models, the results indicated females had higher survival, and tolerated higher concentrations of pollutants (LC_{50}) than males. Overall, there was no change in sex ratio after exposure to pollutants. However, most studies had sex ratios significantly biased towards more females or males in the experiment compared to the control. Among the underlying mechanisms showcasing different sensitivities between females and males, different responses in gene expressions between the sexes had the highest frequency of sex-specific effects.

Overall, the results suggest that pooling the sexes in ecotoxicological studies may confound the results, also because stress responses that alter sex ratios are likely to have long-term consequences for population fitness. Therefore, sex-specific vulnerability to pollutants should be included in ecotoxicological studies whenever possible to improve ecological risk assessments.

Keywords: Meta-analysis, systematic review, chemical pollution, contaminants, sex analysis, females, males, ecotoxicology, freshwater, marine, terrestrial

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1. Introduction

1.1. Sex in Biological Sciences and Chemical Pollution

Sex encompasses biological attributes and in the animal kingdom these attributes can differentiate living organisms as females, males, intersex and hermaphrodites (Gissi, Schiebinger, Santoleri, et al., 2023; Miguel-Aliaga, 2022; Tannenbaum et al., 2019). Differences between sexes in the animal kingdom may include morphological, physiological, behavioural, endocrine and metabolic systems (Tannenbaum et al., 2019), wherein these differences are referred to as sexual dimorphism (Ellegren & Parsch, 2007). Moreover, sexes may differ in additional characteristics such as habitat usage and segregation across time and space (Péron et al., 2016; Yusa, 2007).

There is a great variation in sex determination systems across the animal kingdom. For instance, sex may be genetically or environmentally determined. Environmental sex determination for many species may be controlled by temperature or body size of adults (Adkins-Regan & Reeve 2014). For example, temperature determines the sex of green sea turtles, with warmer temperatures resulting in more females (Jensen et al., 2018). Furthermore, both day-length (photoperiod) and temperature may alter sex ratios in some fish species (Brown et al., 2014). Genetic sex determination can depend on whether the females or the males are heterogametic in the species, which refers to one in which the sex chromosomes differ (for example, males with XY and females with ZW chromosomes; Pipoly et al., 2015). Additionally, some fish species may display both female and male functional characteristics, or they can change their sex from female to male or vice versa during their lifetime (Mitcheson & Liu, 2008). Sex change may also be determined by various social and ecological factors (Ross, 1990). In the clownfish, sex change is determined by social factors. If the dominant female dies, a male can take her place by changing sex (Casas et al., 2016). The ability to change sex in some species is beneficial for a population in terms of survival and reproduction rates (Mitcheson & Liu, 2008).

Sex ratios may be influenced by sex-specific mortality (Chelyadina & Popov, 2021; Kiørboe, 2006; Yusa, 2007), and some species have naturally male- or female-biased sex ratios (Pipoly et al., 2015; Yusa, 2007). For example, adult pelagic copepods are often biased towards more females, wherein the males have higher mortalities than females, which may be attributed to risky mate-finding behaviour in males (Kiørboe, 2006). However, changes to adult sex ratios can have implications for population dynamics, which may lead to populations less resilient to stressors (Le Galliard et al., 2005; Pipoly et al., 2015; Tannenbaum et al., 2019). For instance, an experimental population study on the common lizard *Lacerta vivipara* found that a male-biased sex ratio had deleterious effects on female survival and fecundity. The authors discovered that the increased mortality of adult females may be due to increased male aggression, which in turn could lead to a long-term decline in the population (Le Galliard et al., 2005). Furthermore, in a natural lake environment, the addition of 17α-ethynyl estradiol (synthetic oestrogen) resulted in feminisation of male fish fathead minnows *Pimephales promelas*. The pollutant led to the near extinction of the fathead minnows in the experimental lake (Kidd et al., 2007). Therefore, chemical pollution is of concern as it may influence sex-specific mortality and alter sex ratios.

Chemical pollution is ubiquitous (Bernhardt et al., 2017), with over 350,000 chemicals and chemical mixtures currently registered in the global market. While chemicals provide numerous benefits for society, many also have detrimental impacts on wildlife, the environment and human health (Wang et al., 2020). Pollution ranks third among the five major anthropogenic drivers of biodiversity loss, with the impact of each major driver differing between terrestrial, marine and freshwater systems (Jaureguiberry et al., 2022; Sigmund et al., 2023). Moreover, the magnitude of chemical pollution varies geographically (Sigmund et al., 2023). For example, pharmaceuticals in the world's rivers showed highest accumulative concentrations in South America, South Asia and sub-Saharan Africa (Wilkinson et al., 2022), whereas regions of concern by pesticide risk, water scarcity and biodiversity were identified in the same areas in addition to areas in Inda, China, Australia and Mexico (Tang et al., 2021). Heavy metal pollution risks are also high in low-latitude Eurasia, southern Europe, South Asia, the Middle East and southern China (Huo et al., 2025).

Chemical pollution stems from a variety of sources, including industrial accidents and spills, mining, manufacturing, from agriculture, and consumer product usage (Thompson et al., 2024; Sigmund et al., 2023). Heavy metals are found in soil, water and atmosphere on a global scale (Briffa et al., 2020; Huo et al., 2025). In addition, flame retardants have been found in soil, water and sediments near e-waste recycling sites in China (Zapata-Corella et al., 2023). Furthermore, large-scale oil spills into the marine environment may release toxic hydrocarbons, and have been shown to negatively impact coastal communities, deep water habitats and marine species

(Barron et al., 2020). Microplastics have a global distribution and have been detected in both aquatic and terrestrial species (Thompson et al., 2024). Moreover, pharmaceuticals are cosmopolitan in the world's rivers, influencing organisms in aquatic and terrestrial environments (Wilkinson et al., 2022). Indeed, a multitude of pharmaceuticals have been detected in aquatic invertebrates and riparian spiders (Richmond et al., 2018). Additionally, pesticides such as neonicotinoids have been linked to the decline of aquatic invertebrates in Japan (Yamamuro et al., 2019) and have been found to reduce the density of wild bees (Rundlöf et al., 2015). Furthermore, pesticide usage in Europe and Australia has been linked to negatively impacting stream invertebrate biodiversity (Beketov et al., 2013). Therefore, aquatic and terrestrial organisms are at risk due to connectivity between ecosystems and pollutants originating from various sources (Richmond et al., 2018; Yamamuro et al., 2019; Wesner et al., 2017; Wilkinson et al., 2022). In addition, several synthetic chemicals, such as pharmaceuticals, pesticides and plastics, are persistent in the environment, meaning they degrade slowly and stay in the environment for a long time (Bergmann et al., 2022; Bernhardt et al., 2017).

Different realms, such as freshwater, marine and terrestrial ecosystems may experience different environmental variabilities, and therein different selection pressures and adaptations of species (Jackson et al., 2016). Therefore, it is not unlikely that sexual dimorphism following chemical exposure can be different among freshwater, marine and terrestrial species. Indeed, a meta-analysis on wild-caught ectotherms found terrestrial females to have a higher cold tolerance plasticity than males, with this sexual dimorphism not being present in aquatic ectotherms (Pottier et al., 2021). Sex-specific effects following chemical pollution have also been reported in insects that emerge from aquatic to terrestrial environments (Wesner et al., 2017). Thus, understanding the sex-specific vulnerabilities and potential differences between realms will improve our understanding of ecological impacts following chemical exposure.

Sex-specific sensitivities to chemical pollutants have been reported in several studies (Chelyadina & Popov, 2021; Delnat et al., 2019; Kadiene et al., 2017; Lotufo & Fleeger, 1997). Some studies report females with higher survival than males due to chemical pollution (Calhôa et al., 2012; Delnat et al., 2019; Lotufo & Fleeger, 1997). For example, female copepod *Nitocra lacustris* had a significantly higher survival than males when exposed to phenanthrene (Lotufo &. Fleeger, 1997), and female crustacean *Porcellio dilatatus* had higher survival than males with exposure to cadmium (Calhôa et al., 2012). Conversely, males can have higher survival than females. For instance, males showed higher survival in the fish *Oryzias melastigma* exposed to

3,3′-Diindolylmethane than females (Chen et al., 2016). Nevertheless, for some species, the sexes depict similar mortality with exposure to chemical pollutants as found in the copepod *Schizopera knabeni* exposed to phenanthrene (Lotufo & Fleeger 1997), and in the crustacean *Austropotamobius pallipes* exposed to fluoride (Aguirre-Sierra et al., 2013). Furthermore, several studies have reported sex-specific Lethal Concentration 50 (the concentration that kills 50% of the animal; LC50) values (Kadiene et al., 2017; Lee et al., 2021; Takai et al., 2020). Exposure to cadmium in the copepod *Pseudodiaptomus annandalei* showed females tolerating higher concentrations, whereas in the copepod *Eurytemora affinis* males tolerated higher concentrations (Kadiene et al., 2017).

Moreover, several studies have shown changes to sex ratios due to chemical pollution in both experimental studies (Ferreira-Junior et al., 2017; Park et al., 2015; Qin et al., 2016; Wu et al., 2017) and in natural populations (Chelyadina & Popov, 2021). Sex ratios can become more female-biased due to chemical exposure, such as in the insect *Spodoptera litura* when exposed to perchlorate (Qin et al., 2016), and the annelid *Perinereis nuntia* with exposure to benzo[a]pyrene (Wu et al., 2017). Conversely, sex ratios may skew towards more males as found in the insect *Chironomus xanthus* larvae with exposure to glyphosate (Ferreira-Junior et al., 2017), and the mollusc *Gomphina veneriformis* with exposure to tributyltin (Park et al., 2015). In a natural population, sex-specific mortality of female mollusc *Mytilus galloprovincialis* was higher than males in a polluted site near the Black Sea, which could partly explain the increase in male-biased sex ratios over the past two decades (Chelyadina & Popov, 2021).

Aside from sex-specific lethal effects, females and males may differ in behavioural, physiological, biochemical and morphological responses following chemical exposure (Dinh et al., 2019; McClellan-Green et al., 2007; Michelangeli et al., 2022; Saaristo et al., 2018; Sigmund et al., 2023; Zhang et al., 2019). For instance, reduction of grazing rate in copepod *Calanus finmarchicus* was found to be stronger in females exposed to pyrene compared to males (Dinh et al., 2019), and glufosinate-ammonium herbicide exposure to lizard *Eremias argus* revealed males to be more sensitive than females with a more pronounced effect on neurotoxic behaviour, locomotor performance and body weight (Zhang et al., 2019). Furthermore, female fish *Poecilia reticulata* showed differential behavioural response with exposure to 17β-trenbolone, whereas male fish did not (Tomkins et al., 2016). Moreover, another study exposed imagoes of the mayfly *Baetis tricaudatus* to various concentrations of zinc. Zinc concentration in tissue varied between female and male imagoes, with the male imagoes having higher zinc tissue concentrations than female imagoes (Wesner et al., 2017).

The past decade has seen an increase in sex-based studies across biological sciences, yet many researchers still do not analyse their data by sex (Woitowich et al., 2020). In some cases, analysing data by sex is not feasible as determining the sex of individuals can be difficult and sometimes lead to misidentification (Yusa, 2007). Moreover, for some species the sexes can occupy different habitats (Péron et al., 2016; Yusa, 2007), or sex ratios can be naturally skewed towards one sex (Kiørboe, 2006). Nonetheless, many researchers have pooled the sex of organisms (McClellan-Green et al., 2007), however, pooling responses of females and males in experimental studies assumes that differences between the sexes are absent or not relevant. This can mask biologically relevant information and potential sex-specific effects (Garcia-Sifuentes & Maney 2021; Tannenbaum et al., 2019). Conversely, issues may arise when studies claim sex-specific differences without statistical evidence to support their finding (Garcia-Sifuentes & Maney 2021). Investigating sex-specific differences will enhance our knowledge of the vulnerability of organisms and populations to environmental stressors that may otherwise be overlooked (Gissi, Schiebinger, Hadly, et al., 2023; Gissi, Schiebinger, Santoleri, et al., 2023; Tannenbaum et al., 2019), which can improve the accuracy of findings and potentially improve ecological risk assessments.

1.2. Knowledge Gaps and Aims of the Study

Previous reviews on sex in the animal kingdom have revealed the underrepresentation of including sex in ecological and biological sciences (Ellis et al., 2017; Pottier et al., 2021). A systematic review and meta-analysis on thermal acclimation of ectotherms reported that 77% of the studies identified from their search confounded the sexes or did not report them (Pottier et al., 2021). Similarly, a systematic review on ocean acidification found that 85% of studies did not mention or account for sex, with only 4% of studies statistically assessing the effect of sex. This is unfortunate, however, as the studies that did account for sex reported sex-specific differences (Ellis et al., 2017). Another review on sex-specific differences in marine biology reported that in ~90% of studies that have incorporated sex analysis, an effect of sex was found in morphological, physiological and behavioural mechanisms (Gissi, Schiebinger, Santoleri, et al., 2023). These reviews highlight both the lack of studies that account for sex and the need to further investigate this issue, as females and males may have different sensitivities when exposed to stressors.

Sex analysis is often ignored in biological sciences; however, not accounting for sex may under- or overestimate the risk on the effects of chemical pollutants on natural populations (Tannenbaum et al., 2019). To the best of my knowledge no systematic review or meta-analysis has summarised the influence of sex in animals exposed to chemical pollutants. This is important, as several studies that have investigated the influence of chemical pollution on both females and males have found an effect of sex at both lethal and sub-lethal levels (Calhôa et al., 2012; Chelyadina & Popov, 2021; Dinh et al., 2019; Kadiene et al., 2017; Zhang et al., 2019). Moreover, sex-specific responses to chemical pollution may have consequences for populations, communities and ecosystems (Chelyadina & Popov, 2021; Kidd et al., 2007).

My aim was to explore sex-specific stress responses of artificially exposed females and males to chemical pollutants. I conducted a systematic review and meta-analysis on the effect of chemical pollution between females and males in the animal kingdom across marine, freshwater and terrestrial systems with the aim to extract all relevant sex-specific information that fit my eligibility criteria. First and foremost, to highlight potential knowledge gaps, I researched the diversity of chemical pollutants, the species used, the realms the animals were from, where the experimental animals originated (laboratory or field), and from which parts of the globe this topic was explored. Furthermore, I calculated effect sizes for data on sex-specific survival, LC_{50} and sex ratios following chemical exposure. My aim was also to summarise underlying mechanisms that highlight the differences between the sexes, such as gene expressions, hormones, and behaviours. Specifically, I aimed to answer the following questions:

1. Do males and females have different sensitivities in survival and LC_{50} to chemical pollutants?
2. Do sex ratios change in response to chemical pollutants, and if so, do they cause more male- or female-skewed sex ratios?
3. What are the mechanisms underlying sex-specific vulnerabilities to chemical pollutants?
4. Are sex-specific sensitivities to chemical pollutants similar or different among marine, freshwater and terrestrial species?

2. Methods

2.1. Screening

I used the database Web of Science Core Collection (WoS; https://www.webofscience.com/wos/woscc/basic-search) to conduct a systematic search for relevant publications on this topic, from 2010 to 2022. I restricted the search to the last decade to ensure a manageable set of studies to screen within the limited time available, and potentially with the most up-to-date knowledge. I accessed WoS from May 2024 through to October 2024 and filtered out review articles in WoS before export to full-text Excel file. My search strings were “All fields”: “Male*” AND “Female*” AND “Pollut*” AND “Effect*”, and “All fields”: “Freshwater*” OR “Marine*” OR “Terrestrial*”, and “Year Published”: (2010-2022) (Appendix 1). I also conducted a pilot study from January 2024 to May 2024 to ensure an appropriate search string to cover this topic and a manageable set of articles to screen due to limited time.

I uploaded full-text records from WoS into Rayyan AI (http://rayyan.qcri.org), which I used to remove duplicates and assist during title and abstract screening (Ouzzani et al., 2016). To calibrate the usage of Rayyan, I checked if the articles from the freshwater realm search string (*n* = 84) matched with the articles in the Excel file downloaded from WoS and exported articles to Rayyan. The calibration confirmed that the articles from the freshwater realm search in both Rayyan and Excel matched. Thus, since the search matched for both Rayyan and Excel for the freshwater realm, I only screened in Rayyan during the title and abstract screening for the marine and terrestrial realms (Appendix 2). I performed full-text screening from October 2024 until February 2025 without AI assistance.

I followed PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) 2020-compliant flow diagrams with modifications for the analysis to ensure transparency (Haddaway et al., 2022; O’Dea et al., 2021).

2.2. Eligibility Criteria

Both females and males had to be included in the experiment, with data from both sexes reported and compared to a control. Therefore, I excluded studies with intersex, hermaphrodites and imposex. Sex-specific data had to be reported statistically, and accompanied by a description in text, in figures, a table or a combination. “Study” refers to the primary research article where I extracted the data and information. Sometimes an individual article included more than one species, more than one chemical pollutant or more than one experiment. Therefore, a single article could be duplicated, or more, to include several “studies” (See Jackson et al., 2016). I only included experimental studies, such as manipulative laboratory studies, experimental field mesocosm or field experimental studies. I excluded multiple stressors and interactive stressor studies. I excluded Multidisciplinary Digital Publishing Institute (MDPI) articles due to a concern with the quality of results (Oviedo-García, 2021).

All species in the animal kingdom, from marine, freshwater and terrestrial systems were eligible for analysis. I included studies that exposed the animals to the pollutant via water, food, injection or sediment. Further, I attempted to report all pollutants in µg/L or mg/kg, thus, whenever possible, I converted the units provided by the authors to my chosen units. Exposure methods via water were all reported in µg/L whereas the remaining exposure methods (food, injection and sediment) were reported in mg/kg. This was to ensure the pollutants were as comparable as possible across studies. I only included studies that reported organismal-level response for a single generation.

If a study reported sex-specific effects across multiple concentrations for the same pollutant and/or time intervals, I only considered the sex-specific effects at the end of the exposure duration, and the highest pollutant concentration reported by the authors. This was to keep it consistent and comparable across all studies. For LC_{50}, I used the highest reported time interval up to 96 hrs to keep it as similar as possible across the studies. However, the second highest pollutant concentration was used if the highest concentration resulted in 100% mortality and/or data were not comparable between the sexes. If the species were exposed to the stressor at the beginning of the experiment, and the effect of the stressor was measured later, I still included that study. However, I excluded studies that reported sex-specific effects after what the authors reported as “recovery time”.

I had a few studies with minor deviations from my eligibility criteria, but I included them in the results and analysis as they were relevant to this study. Ideally, both sexes had to be kept in equal conditions. However, I had two exceptions where the studies exposed females and males to different concentrations. Merad et al. (2018) based the concentrations on sex-specific LC_{25} (Lethal Concentration 25; the concentration that kills 25% of the animals), and Lee et al. (2021) based the concentrations on sex-specific NOEC (No Observed Effect Concentration) and LC_{50}. These studies otherwise fit the criteria and were included in the results as the authors provided a reasoning behind the sex-specific concentrations (Merad et al., 2018; Y. Lee et al., 2021). Furthermore, there was one study I could not report the pollutant concentrations in µg/L or mg/kg due to the pollutant being first taken up by a prey before the polluted prey was fed to the fish. The study otherwise fit all the criteria (Deane et al., 2014). Nonetheless, I noted these studies down as a “concern” since the different concentrations between the sexes may influence the results, and thus not providing a precise concentration of pollutants. To minimise bias, I included studies that mentioned testing both sexes before pooling the data for both sexes due to no difference or effect between the sexes.

Through the search, I obtained a total of 321 records after duplicates were removed. After abstract and title screening, I removed 186 articles because they were not relevant and identified 135 articles that were assessed for eligibility through full text screening (Fig. 1). After full-text screening I removed 37 articles because they were not relevant due to different criteria, while 98 articles were included in the data extraction. These 98 articles reported results from a total of 142 studies, which resulted in 24 effect sizes used for the meta-analysis on survival, LC_{50} and sex ratio, while 131 studies investigated sex-specific underlying mechanisms (See Appendix 3 for exclusion reasons; See Appendix 4 for full access to the included studies).

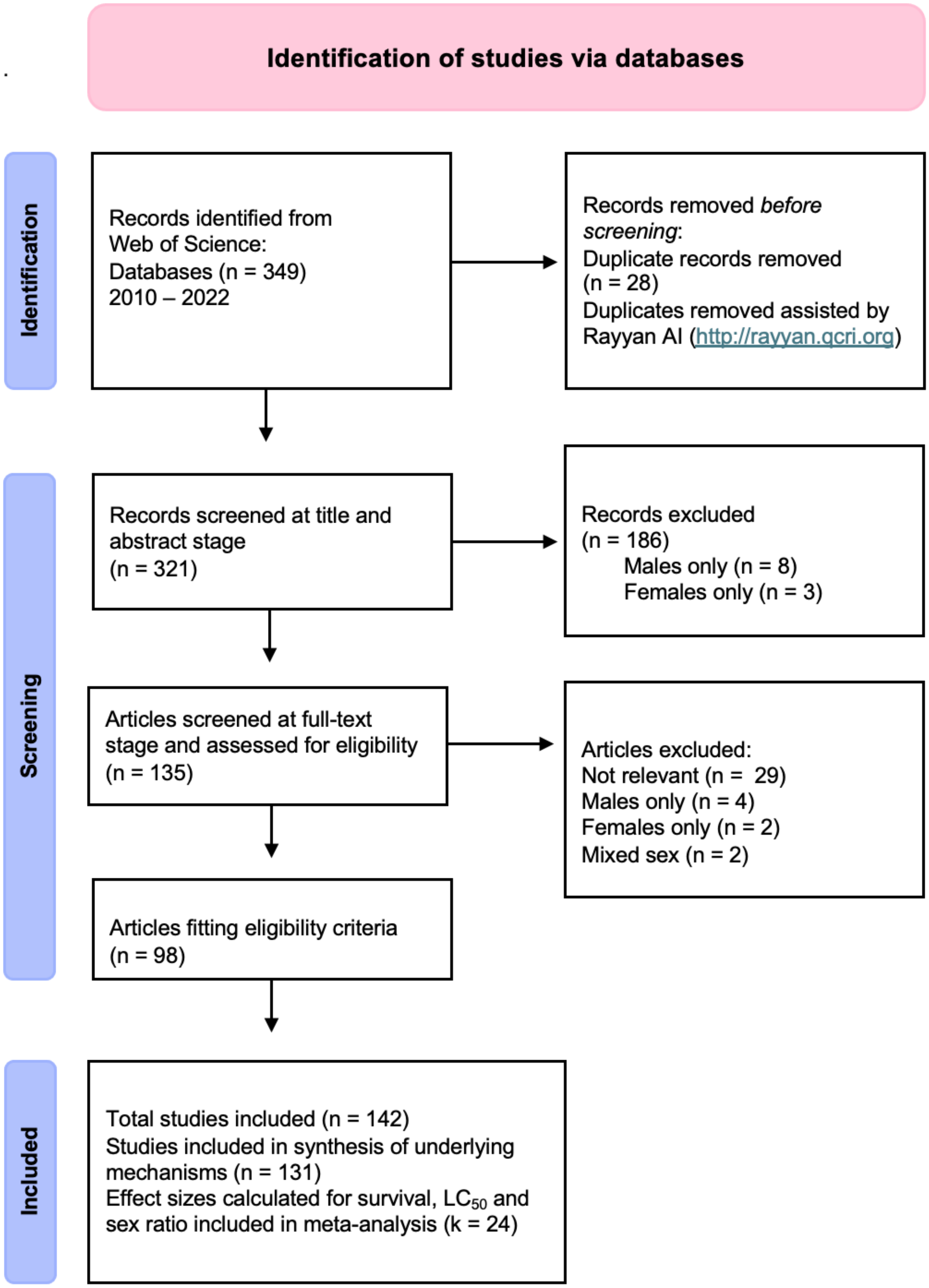

Figure 1. *PRISMA flow diagram showing the exclusion and inclusion of articles at each stage of the screening process. Also depicting sex-specific removal of records. Adapted flow diagram with modifications from Haddaway et al. (2022) CC-BY-4.0. Details on exclusion reasons are provided by Appendix 3. Access to included studies are provided by Appendix 4.*

2.3. Data Extraction

For each study, I collected general information such as year published, country, taxonomic information, origin of animals and habitat. I also extracted quantitative and qualitative information on the pollutants, pollutant classes, exposure methods and exposure duration. I determined the pollutant classes according to Orr et al. (2024), with some modifications. Hydrocarbons were classified as “other chemicals” according to Orr et al. (2024). However, due to the frequency of hydrocarbons in the dataset, I also made a class called “hydrocarbons” to distinguish these stressors from “others”. In addition, stressors that combined two different types of pollutant classes (for example, the combination of “heavy metals” and “microplastics”) and more, I classified them as “composite stressors”. For the origin of animals used in the experiment, I categorised them as “Field” (animals collected from the wild) or “Lab” (animals that originated from laboratory conditions). Animals collected from outdoor rearing facilities and farms were also considered “Field”. I placed species from brackish or estuarine environments in either “Freshwater” or “Marine” depending on their experimental conditions. For example, if the authors stated they used “seawater” I categorised it as marine, and if they stated, “tap water”, “river water” and similar phrasing, I considered it “Freshwater”. Furthermore, insects such as *Chironomids* were considered “Freshwater” although they have adult life stages in the terrestrial realm. They were categorised as freshwater because they were exposed to the pollutant during their aquatic (larval) life stage. The studies on *Chironomids* were all emergence studies where the sexes were recorded, and sex-specific effects therein, once they emerged from their aquatic habitats (See Castro et al., 2022; Ferreira-Junior et al., 2017; Jesus et al., 2022; Rodrigues et al., 2015).

I extracted coordinates from each study based on the origin of the animals used in the experiments. If provided by the authors, I extracted the coordinates directly from the text to find the origin of the animals. However, if only a location without coordinates were available, I chose a random coordinate from that location. If there was no information on the origin, I used the primary authors' affiliation for coordinates. If stated in the text that the origin of the animals was already described in a previous study, I checked the citation provided by the authors until I found the origin.

To quantify the effect of sex, I extracted data from text, tables and figures directly. Whenever data was not reported in text or tables, I used plot digitizer to extract data from the plot itself (https://plotdigitizer.sourceforge.net), which has been applied in a previous meta-analysis (See

Smale et al., 2019). I also extracted any relevant data from supplementary material when applicable, as some data were not extractable in the articles themselves.

To address research questions 1 and 2, I performed a meta-analysis and extracted quantitative data. I collected numerical data to calculate effect sizes for survival, LC_{50} and sex ratio. To address research questions 3 and 4, I performed a narrative and semi-quantitative review. For sex-specific mechanistic and underlying effects I collected qualitative data. By “mechanisms” and “underlying effects” I refer to different mechanisms such as gene expressions, hormones, enzymes, GSI (Gonado-Somatic Indices), HSI (Hepato-Somatic Indices), K-factor (condition factor, length-weight factor), MXR (multixenobiotic resistance activity) and bioamplification factor (bioamplification of pollutant). Explanations behind all the different mechanism categoriesare provided by the “Readme” (Appendix 4). I recorded “Yes” in the dataset if a statistically significant difference between the sexes occurred due to the experiment, and “No” if there was no difference between the sexes. If the same study provided more than one parameter for a mechanism category (such as multiple gene expressions) and sex-specific effects, I summarised the findings for all parameters into one. For example, I recorded the findings in gene expression from a study as “Yes” if one of the genes tested reported a significant difference between the sexes, even if other gene expressions were classified as “No” (Table 1). I only included results from histology when this was reported by statistics and supporting figures. To clarify, a single study may report several different mechanistic categories, thus some studies were more represented in the dataset. To address differences between realms, animal groups, and get overall results, I looked at each study in the mechanistic categories, and if they had recorded “Yes” in one of the mechanisms studied I recorded the study as “Yes” for overall effect on whether this specific study had detected an effect of sex (Table 1).

Table 1. *An example dataset showing how the different mechanisms were categorised. Study_ID with the same first number (2.1 and 2.2) are studies that both derived from the same article. The colour (light green) is the colour coding for the different mechanisms. This table is only meant for illustrative purposes, and do not show any of the real data used in this study.*

Study_ID	Habitats	Groups	Gene_exp_all	Gene_expression_1	Gene_expression_2	Body_weight	Effect_of_sex
1	Freshwater	Mollusc	Yes	Yes			Yes
2.1	Marine	Crustacean	Yes	Yes	No		Yes
2.2	Marine	Crustacean	Yes	No	Yes		Yes
3	Freshwater	Fish				Yes	Yes
4	Freshwater	Fish				No	No
5	Terrestrial	Insect	Yes	Yes			Yes

2.4. Data Analysis

2.4.1. Effect Size Calculations

I calculated effect sizes using log risk ratio (LogRR) for the survival, LC_{50} and sex ratio. LogRR is a common metric in ecological meta-analysis and the effect size compares the mean of two groups (Hedges et al., 1999; Lajeunesse, 2011; Viechtbauer, W., 2010). To calculate LogRR of survival, I first calculated the difference between males and females in the control and experimental groups using this equation (Equation 1):

$$Control\ difference\ (\bar{x}_C) = (\bar{x}_{MC}/\bar{x}_{FC}),\ Experiment\ difference\ (\bar{x}_E) = (\bar{x}_{ME}/\bar{x}_{FE}) \tag{1}$$

Where $(\bar{x}_{MC})$ refers to the mean in control male survival and $(\bar{x}_{FC})$ refers to the mean in control female survival. $(\bar{x}_{ME})$ refers to the mean in experiment male survival and $(\bar{x}_{FE})$ refers to the mean in experiment female survival.

Then I followed this equation for the effect sizes for survival, and for the sex ratios (Equation 2):

$$LogRR = \ln(\bar{x}_E/\bar{x}_C) \tag{2}$$

LogRR is the effect size which uses the natural logarithmic proportional change in the mean of the experiment $(\bar{x}_E)$ and control $(\bar{x}_C)$ groups for the calculation of survival and sex ratio. This effect size shows the relative changes between experimental and control groups.

I estimated the sampling variance of each effect size for survival and for the sex ratios following this equation (Equation 3):

$$\sigma^2(LogRR) = \frac{(SD_C)^2}{N_C\bar{x}_C^2} + \frac{(SD_E)^2}{N_E\bar{x}_E^2} \tag{3}$$

Where SD and N are given by the standard deviation and sample size for the control and experimental groups (Lajeunesse, 2011). To calculate the sampling variance of survival I used equation 3 to estimate variance for both females and males separately. Then I added the variance of females and males, and divided this variance by 2 to find the mean variance of female and male survival.

I followed this equation for the effect sizes of female and male LC_{50} (Equation 4):

$$LogRR = \ln(\bar{x}_M / \bar{x}_F) \tag{4}$$

LogRR is the effect size which uses the natural logarithmic proportional change in the mean of the male ($\bar{x}_M$) and female ($\bar{x}_F$) LC_{50} values. This effect size shows the relative changes between male and female LC_{50} values.

I estimated the sampling variance of each effect size for LC_{50} as follows (Equation 5):

$$\sigma^2(LogRR) = \frac{(SD_M)^2}{N_M \bar{x}_M^2} + \frac{(SD_F)^2}{N_F \bar{x}_F^2} \tag{5}$$

Where SD and N are given by the standard deviation and sample size for the male and female groups (Lajeunesse, 2011).

All the authors reported information on sample sizes. However, standard deviation (SD) was not available for all studies. Whenever standard error or 95% Confidence Interval (CI) was available, I converted these to SD before calculating the variance following the methods by Higgins et al. (2024). I contacted seven authors for information on SD in March 2025 (Calhôa et al., 2012; Castro et al., 2022; Ferreira-Junior et al., 2017; Ji et al., 2013; Rodrigues et al., 2015; Takai et al., 2020; Ye et al., 2018). As of April 2025, two authors responded to provide clarifications for SD (Ji et al., 2013; Takai et al., 2020). The survival data from Ji et al. (2013) had no replicates, therefore no SD and no variance, thus I could not include the data on survival from this article in the random-effects model for survival. For the studies from the remaining five authors, I imputed the missing variance data, using mean imputation. I imputed this by using the average variance of the calculated variances from the other studies for each meta-analytical model separately (survival, LC_{50}, and sex ratio; Higgins et al., 2024; Nakagawa, 2015).

2.4.2. Data Analysis

I performed statistical analysis and visualisation using R (Version 2023.06.1; R Core Team, 2023), and the figures were mainly produced using *ggplot2* (Wickham, 2016). For the meta-analysis, I produced forest plots for the survival, LC_{50}, and sex ratios. Raw data used for the forest plots are provided by the appendices (Appendix 5). I performed random-effects models as they are more applicable in ecology (Senior et al., 2016), using the *metafor* package and *rmv* function (Viechtbauer, W., 2010). I used the alpha level of significance at $p < 0.05$ for the meta-analysis. For the systematic review of the mechanistic and underlying effects between the sexes, I used graphical comparisons categorised by realms and animal groups. Finally, I produced a figure describing the differences in sex-specific effects across the mechanistic categories, comparing the different realms (freshwater, marine and terrestrial) and animal groups. RStudio code and additional sources that aided in data analysis are available in the appendices (Appendix 6).

2.4.3. Sensitivity Analysis and Publication Bias

To perform sensitivity analysis and check for publication bias, I did three types of analyses. I did a visual assessment of the funnel plots produced by each model, followed by Egger's regression test for data asymmetry using a mixed-effects meta-regression model. For Egger's regression, I used the alpha level of significance at $p < 0.1$ (Egger et al., 1997). Finally, I checked for influence of studies performing influential case diagnostics using the *influence* function (Viechtbauer, W., 2010).

3. Results

3.1. Description of Studies

Most of the studies included in the dataset were from the Global North (57%, *n* = 81) and China (35%, *n* = 49), with most of the studies clustering around Europe and East Asia (Fig. 2a). Only a few studies originated from the Global South (9%, *n* = 12). The majority of the studies were from the Northern hemisphere at mid latitudes (Fig. 2b). The most represented habitat was freshwater (56%, *n* = 80), followed by marine (37%, *n* = 53) and lastly terrestrial ecosystems (6%, *n* = 9).

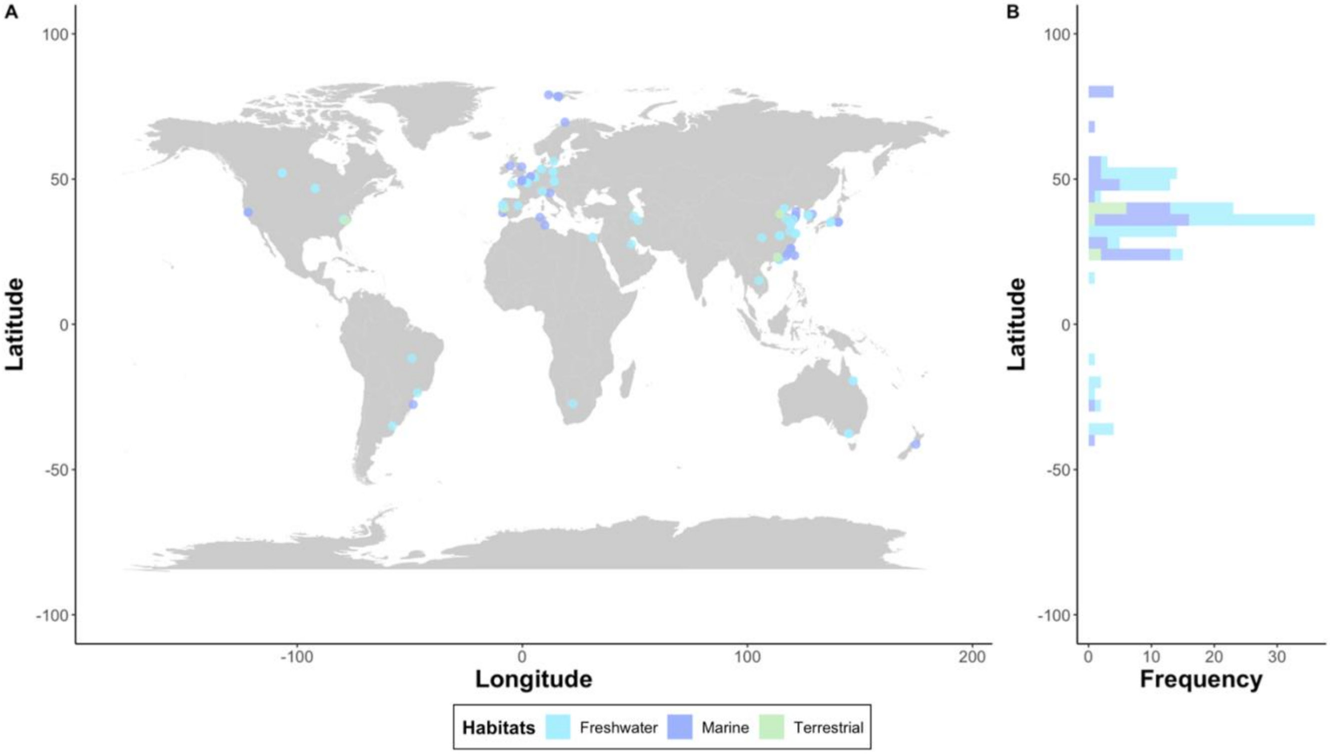

Figure 2. *World map depicting all the studies from freshwater, marine and terrestrial realms (n = 142) (**A**). The frequency of studies from the different latitudes is shown by realm (**B**).*

From the dataset, I identified nine animal groups where fish were the most common (52%, *n* = 74), followed by crustaceans (25%, *n* = 35) and molluscs (11%, *n* = 16). Less common animal groups were insects (6%, *n* = 8), amphibians (*n* = 4), reptiles (*n* = 2), mammals (*n* = 1), echinoderms (*n* = 1) and annelids (*n* = 1; Fig. 3a). Fish species originating from the lab were the most represented in the dataset (47%, *n* = 67; Fig. 3b). Overall, the dataset shows a large overrepresentation of animals originating from laboratories (73%, *n* = 104), and less animals from field origins (27%, *n* = 38). The animal groups with the largest fraction of field origins were echinoderms, molluscs and crustaceans at 100%, 94% and 43% field origins respectively. The most common species in the dataset were *Danio rerio* (20%, *n* = 28), *Oryzias melastigma* (9%, *n* = 12), *Oryzias latipes* (6%, *n* = 8) and *Eurytemora affinis* (5%, *n* = 7). Furthermore, the most common genera were *Mytilus* spp. (5%, *n* = 7), *Chironomus* spp. (4%, *n* = 6) and *Gammarus* spp. (4%, *n* = 5; Appendix 4).

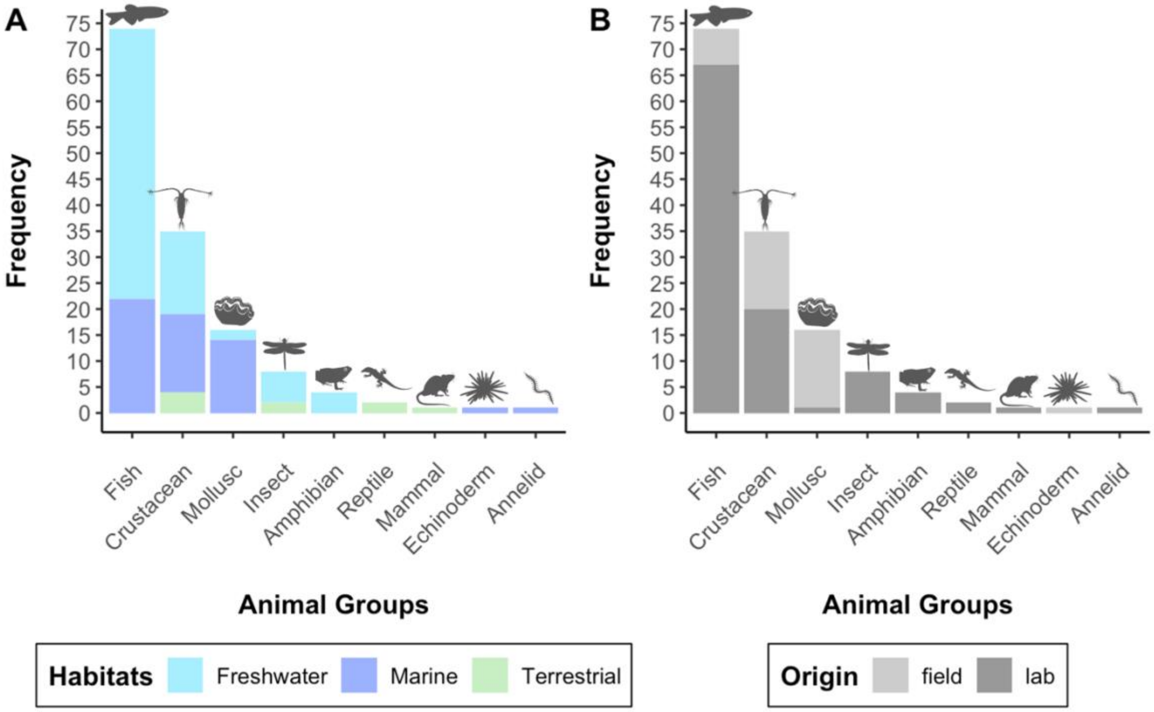

Figure 3. *Stacked bar plots showing animal groups across habitats (n = 142) (**A**) and origins (n = 142) (**B**). Animal icons obtained from PhyloPic (https://www.phylopic.org) CC0 1.0**.***

I identified 16 different classes, and 114 unique identities of chemical pollutants across the dataset of 142 studies. Pharmaceuticals were the most tested class of chemical pollutants (*n* = 28), followed by chemical pollutants classified as "others" (*n* = 20), then hydrocarbons (*n* = 16), pesticides (*n* = 15) and flame retardants (*n* = 14; Table 2).

Table 2. *Classes of chemical pollutants with number of pollutants in brackets for each class, and number of the same identity in brackets for each chemical identity. A comma (,) differentiates the different pollutants from each other whereas the plus sign (+) indicates two or more pollutants being added together in a "mix".*

Class	**Identity**
Antibiotics (1)	Triclocarban
Antifoulants (6)	3,3′-Diindolylmethane, 4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one (3), Butenolide (2)
Biocide (3)	Tributyltin (3)
Composite stressor (9)	Megestrol acetate + 17α-ethinylestradiol, 17α -ethinylestradiol + Sodium lauryl sulfate, Zinc Chloride + Polystyrene microplastics beads, Halogenated Organic Pollutants, Treated effluent water, Polluted site resuspended sediment, Less polluted site resuspended sediment, Oil sands process-affected water, Diluted leachate

Flame retardants (14)	2,2',4,4'-Tetrabromodiphenyl ether (3), 2,2',4,4',5-Pentabromdiphenyl ether (2), Tris(1,3-dichloro-2-propyl) phosphate, Polybrominated diphenyl ethers, 1,3-dichloro 2-propyl phosphate, Decabromodiphenyl ethane, Trichloropropyl phosphate, 1,2,5,6-tetrabromocyclooctane, Bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate + 2-ethylhexyl-2,3,4,5 tetrabromobenzoate, 2,4,6-Tribromophenol, Polybrominated diphenyl ethers mixture
Fungicide (1)	Vinclozolin
Heavy metals (12)	Lead, Nickel, Nickel + Lead, Cadmium (2), Copper, Zinc Chloride (2), Mercury chloride, $Cd(Cys)_2$, $Cd(NO_3)_2$, Lead + Arsenic + Cadmium
Herbicide (2)	2,4-dichlorophenol, Roundup
Hydrocarbons (16)	Benzo[a]pyrene (4), Hydrocarbon mixture (water soluble fraction), Phenanthrene (2), Mechanically dispersed oil, Chemically dispersed oil, Burned oil residues, Kobbe crude oil, Marine diesel, Pyrene + phenanthrene + fluoranthene, Pyrolitic, Heavy fuel oil, Light crude oil
Microplastics (8)	Polyethylene microplastics, Polyethylene spheres, Polyethylene fibers, Polyester fibers, Virgin polystyrene microplastics, Polystyrene microplastics beads, Virgin polyethylene pre-production pellets, Polyethylene marine plastic
Nanoparticles (3)	Polystyrene nanoplastics, Plain polystyrene nanoplastic fragments, Gold nanoparticles
Others (20)	Methyldihydrotestosterone, Nonylphenol (3), Cotton fibers, Perfluorobutanesulfonate, Benzotriazole, Polychlorinated biphenyls 153, Bisphenol A (3), Polychlorinated biphenyls mix, 3,3′,4,4′,5-pentachlorobiphenyl, Bisphenol S, Perfluorooctane sulfonate, Perfluorooctanoic acid, Perchlorate, Seleno-L-methionine (2), Potassium cyanide
Personal care products (2)	Sodium lauryl sulfate, Methylparaben
Pesticide (15)	Atrazine, 1S-cis-bifenthrin, 1R-cis-bifenthrin, Pentachlorophenol, Esfenvalerate, Glyphosate, Diazinon (2), Dichloro-diphenyl-trichloroethane, Organochlorine pesticide mixture, Endosulfan, Glufosinate-ammonium, L-Glufosinate-ammonium, Pendimethalin, Glyphosate
Pharmaceuticals (28)	Fluoxetine, Methamphetamine (2), Tramadol, Ethinylestradiol, Tamoxifen, Flutamide, Megestrol acetate, Sulfamethazine, 17α-Ethynylestradiol (6), Acetylsalicylic acid, Diclofenac, Ibuprofen (2), Mefenamic acid, Naproxen, 17β-Estradiol, Levonorgestrel, Fadrozole, 17β-trenbolone (2), Fluoride, Estrogenic compounds mix
Plasticisers (2)	Di(2-ethylhexyl) phthalate (2)

All studies except one were carried out under controlled laboratory conditions. One study was carried out in a laboratory experiment for 30 days, followed by 135 days in the field (See Appendix 4). Across the dataset, exposure duration ranged from 1-274 days, with mean exposure duration being 44.18 days. Methods of exposure were via water (*n* = 104), food (*n* = 27), sediment (*n* = 9) and injection (*n* = 2). The number of different concentration levels for the chemical pollutants ranged from 1-8, with the mean number of concentration levels being 2.28 (Appendix 4).

3.2. Sex-Specific Sensitivity of Survival and LC_{50}

I conducted a random-effects model on the difference in survival between females and males. The pooled effect size comparing survival between the sexes with exposure to chemical pollutants was negative (LogRR = -0.40, 95% CI [-0.76, -0.04], $k = 6$), meaning females depicted higher survival than males with exposure to chemical pollutants ($z = -2.18$, $p = 0.03$; Fig. 4; Appendix 7). However, there was considerable statistical heterogeneity among the studies ($Q = 498.37$, df = 5, $I^2 = 99\%$, $\tau^2 = 0.20$). Four of the studies favoured female survival, with terrestrial crustaceans having the highest effect sizes, followed by marine crustaceans. One study did not favour survival for any sex (on a marine fish), whereas one study (on a marine fish) favoured male survival.

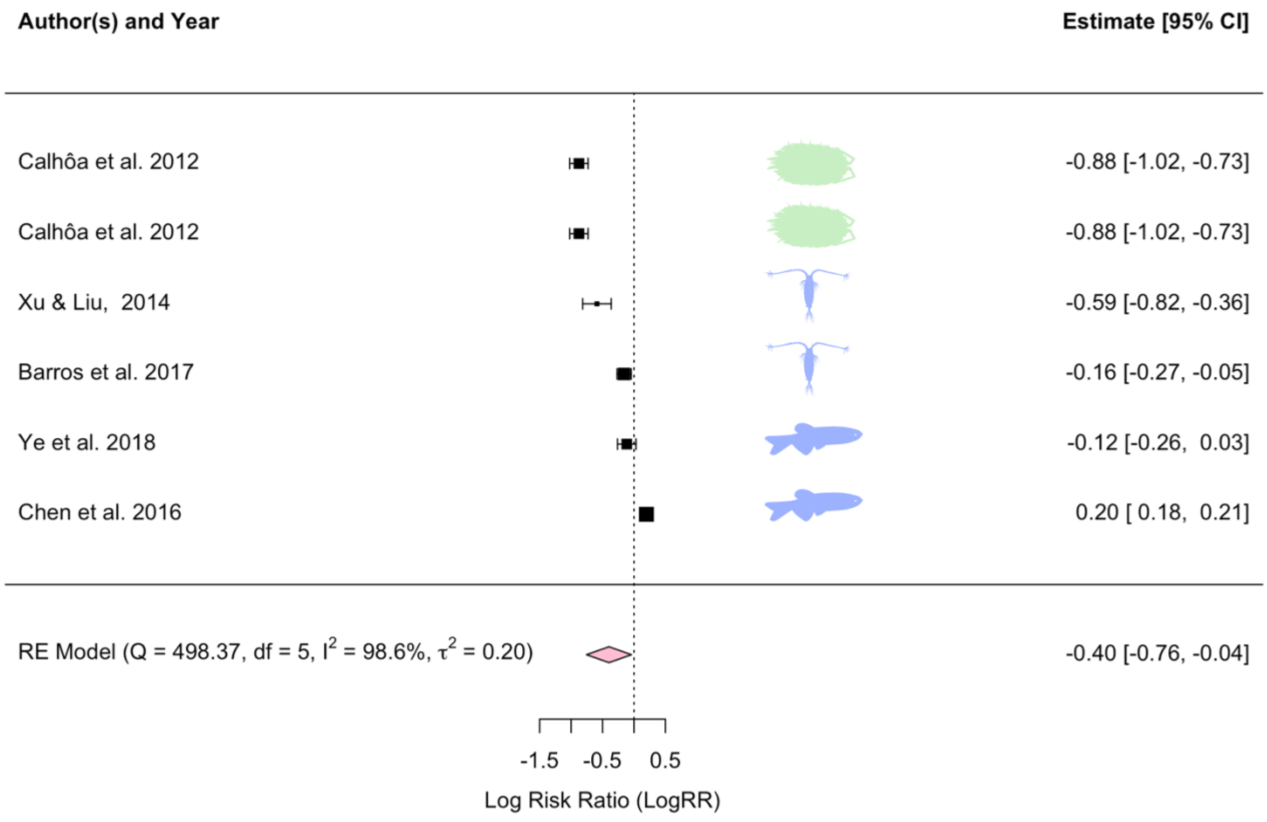

Figure 4. *Forest plot depicting the difference in survival of female and male animals following chemical exposure (k = 6), using a random-effects model. Negative values indicate female survival is favoured, whereas positive values show male survival is favoured. Each square is the effect size in LogRR for each individual study, with the bars around the mean showing 95% Confidence Intervals, and the size of the squares representing the weight of the studies. The diamond at the bottom of the plot is showing the pooled effect size across all studies. Data for variance was imputed using mean imputation for Ye et al. (2018) and Calhôa et al. (2012) (n = 3). Animal icons obtained from PhyloPic (https://www.phylopic.org) CC0 1.0.*

I conducted a random-effects model on the difference in LC_{50} between females and males. The pooled effect size comparing female and male LC_{50} with exposure to chemical pollutants was negative (LogRR = -0.19, 95% CI [-0.32, -0.05], k = 5), with males being significantly more sensitive to chemical pollutants than females (z = -2.62, p = 0.01; Fig. 5; Appendix 7). Moreover, there was substantial statistical heterogeneity among the studies (Q = 19.66, df = 4, I^2 = 79%, τ^2 = 0.02). Two datapoints were mainly driving the overall effect towards favouring females, as they did not cross 0 with the 95% CIs. One study had no effect of sex, with females and males tolerating the same pollutant concentration. All the studies involved crustaceans from marine and freshwater realms.

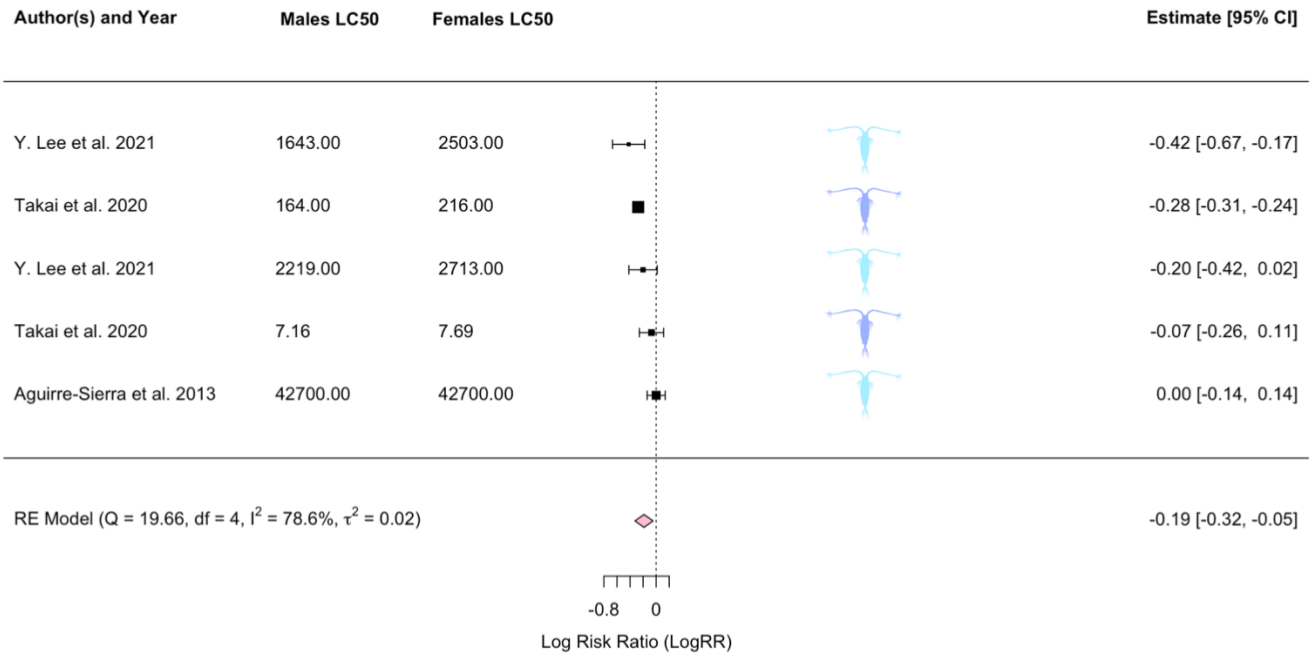

Figure 5. *Forest plot showing effect sizes of female and male LC_{50}, using a random-effects model. Raw LC_{50} values (µg/L) for both sexes are depicted as "Males LC50" and "Females LC50". Negative values indicate females tolerate higher concentrations of pollutants, whereas positive values show males tolerate higher concentrations of pollutants. Each square is the effect size in LogRR for each individual study with the bars around the mean showing 95% Confidence Intervals, and the size of the squares representing the weight of the studies. The diamond at the bottom of the plot is showing the pooled effect size across all studies. The study from Aguirre-Sierra et al. (2013) pooled the responses for LC_{50} between the sexes due to no effect of sex as reported by the authors. Animal icons obtained from PhyloPic (https://www.phylopic.org) CC0 1.0.*

3.3. Sex Ratio Changes in Response to Chemical Pollutants

I conducted a random-effects model on the difference in sex ratios between control and experimental groups. The pooled effect size of sex ratio changes with exposure to chemical pollutants was zero (LogRR = -0.00, 95% CI [-0.29, 0.28], *k* = 13), not significantly different compared to control (z = -0.03, p = 0.98; Fig. X; Appendix 7). Furthermore, there was considerable statistical heterogeneity among the studies (Q = 33169.00, df = 12, I^2 = 100%, τ^2 = 0.27). However, the pooled effect size may be misleading, since five studies resulted in significantly more females, and five studies resulted in significantly more males, whereas 3 studies resulted in no changes to sex ratio following chemical exposure.

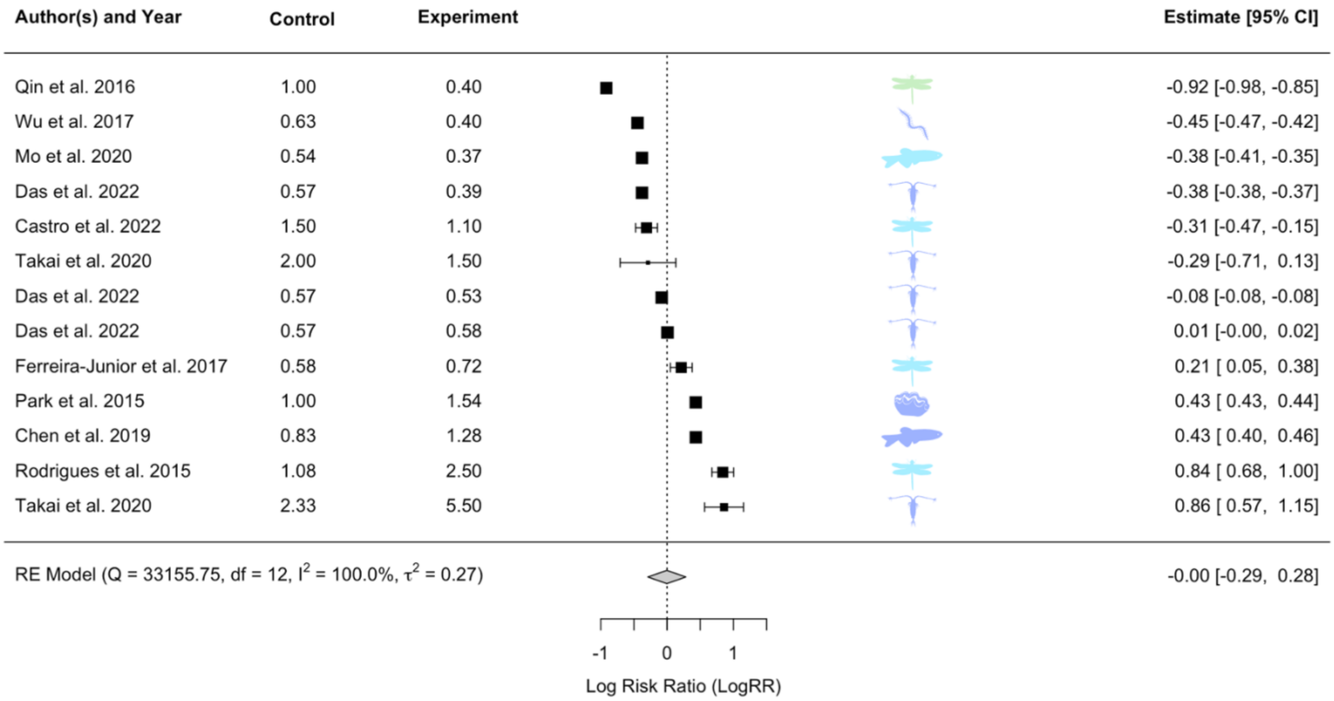

Figure 6. *Forest plot showing control and experiment sex ratios, using a random-effects model. Raw sex ratio data for both sexes are depicted in ratio (males/females) for both "Control" and "Experimental" groups. Each square is the effect size in LogRR for each individual study with the bars around the mean showing 95% Confidence Intervals, and the size of the squares representing the weight of the studies. The diamond at the bottom of the plot is showing the pooled effect size across all studies. Data for variance was imputed using mean imputation for the studies from Castro et al. (2022), Ferreira-Junior et al. (2017) and Rodrigues et al. (2013) (n = 3). Animal icons obtained from PhyloPic (https://www.phylopic.org). CC0 1.0.*

3.4. Sensitivity Analysis and Publication Bias

Visual assessments of the funnel plots indicated data asymmetry for survival and sex ratios, whereas the data on LC_{50} indicated less data asymmetry. Egger's regression test indicated that neither LC_{50} nor sex ratios had presence of data asymmetry and publication bias, confirming none or less bias present. However, the data on survival indicated data asymmetry, confirming presence of bias ($p = 0.07$; Appendix 8). Finally, for survival and sex ratios, there was not a single study with a large influence. However, for LC_{50}, study 5 had a large influence marked by the red dot in the plot (Appendix 8).

3.5. Mechanisms Underlying Sex-Specific Vulnerabilities to Chemical Pollutants

3.5.1. Sex-Specific Effects in Freshwater Species

Gene expressions were the most studied mechanism for the freshwater fish species exposed to chemical pollutants, wherein 92% of combined gene expressions from each study showed sex-specific effects (n = 25). Hormones (n = 17), GSI (n = 16) and K-factor (n = 14) were the next commonly studied mechanisms with sex-specific effects found at 82%, 31% and 21% respectively. Additional mechanistic categories including more than five studies were accumulation of pollutants (n = 6), histology (n = 8), proteins (n = 5), enzymes (n = 7), reactive oxygen species (n = 5), body weight (n = 12), HSI (n = 11) and body length (n = 11) with sex-specific effects found at 67%, 63%, 60%, 54%, 40%, 33%, 27% and 9%, respectively (Fig. 8). Although less studied, behaviour showed an effect of sex at 100% (n = 3). Overall, the results suggest that sex-specific effects for freshwater fish species were more commonly found in physiological mechanisms (genes, hormones and accumulation of pollutants) rather than morphological mechanisms (body length, body weight and K-factor). However, body weight had a higher frequency of sex-specific effects compared to body length (Fig. 7).

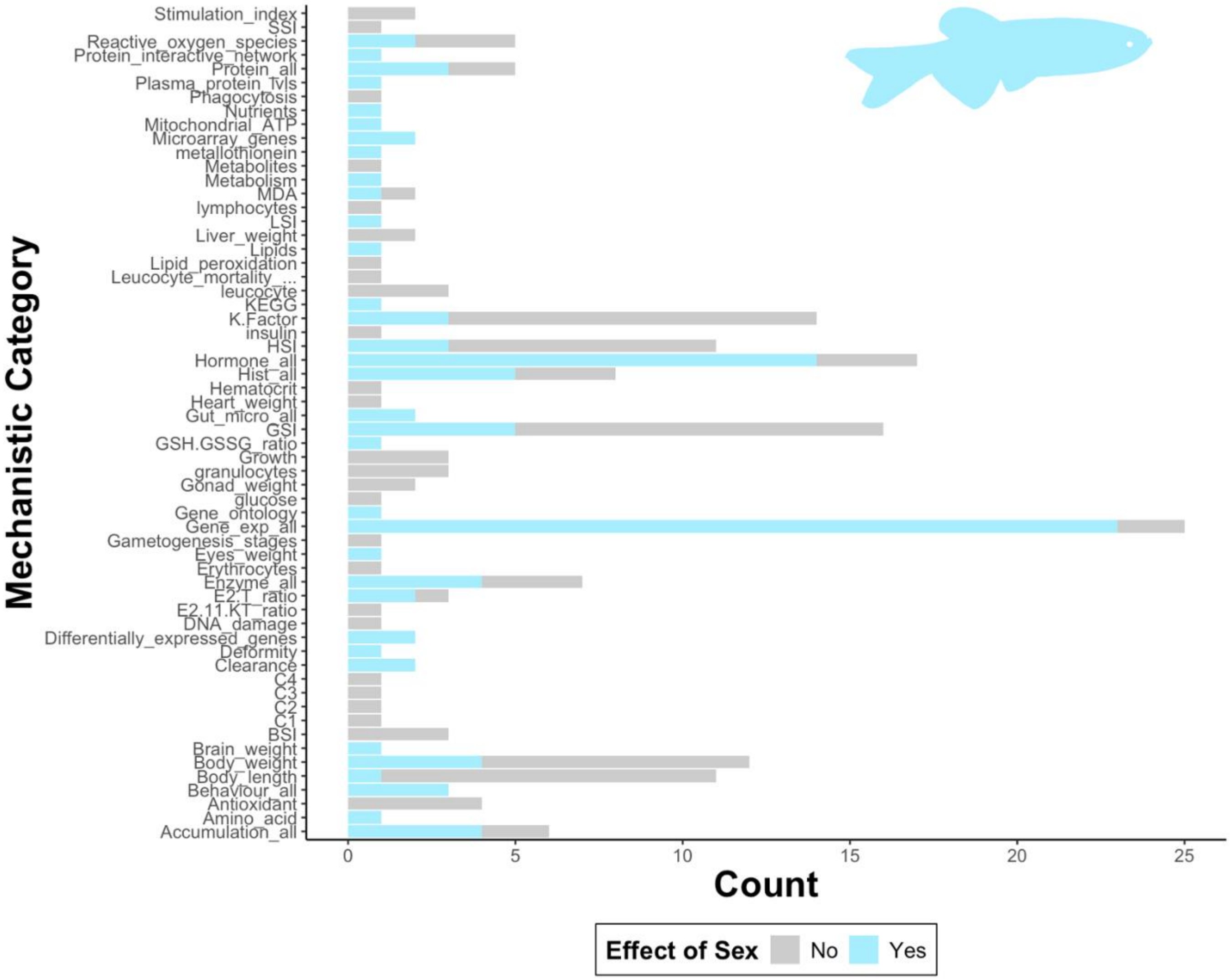

Figure 7. *Stacked bar plot showing sex-specific mechanistic effects for freshwater fish species. Explanations for each mechanistic category is found in the "Readme" (Appendix. 4). Animal icon obtained from PhyloPic (https://www.phylopic.org) CC0 1.0.*

Gene expressions depicted 100% sex-specific effects for the freshwater crustaceans, insects, molluscs and amphibians (Fig. 8). For freshwater crustaceans enzymes (*n* = 7), accumulation of pollutants (*n* = 6) and behaviour (*n* = 6) showed sex-specific effects at 100%, 67% and 33%, respectively. Sex-specific effects were found in hormones and proteins, while no sex-specific effects were found in body weight and body length (Fig. 8a). For freshwater insects' sex-specific effects were low in emergence time (*n* = 6) and body weight (*n* = 4) at 33% and 25% (*n* = 4), respectively (Fig. 8b). For freshwater molluscs enzymes had 50% (*n* = 2) sex-specific effects, while the other mechanisms (MXR, hormones, DNA fragmentation and accumulation of pollutants) showed no effect of sex aside from gene expressions (Fig. 8c). Overall, the results suggest freshwater crustaceans and amphibians may be more sensitive to sex-specific effects than molluscs and insects, however, the data is limited (Fig. 8).

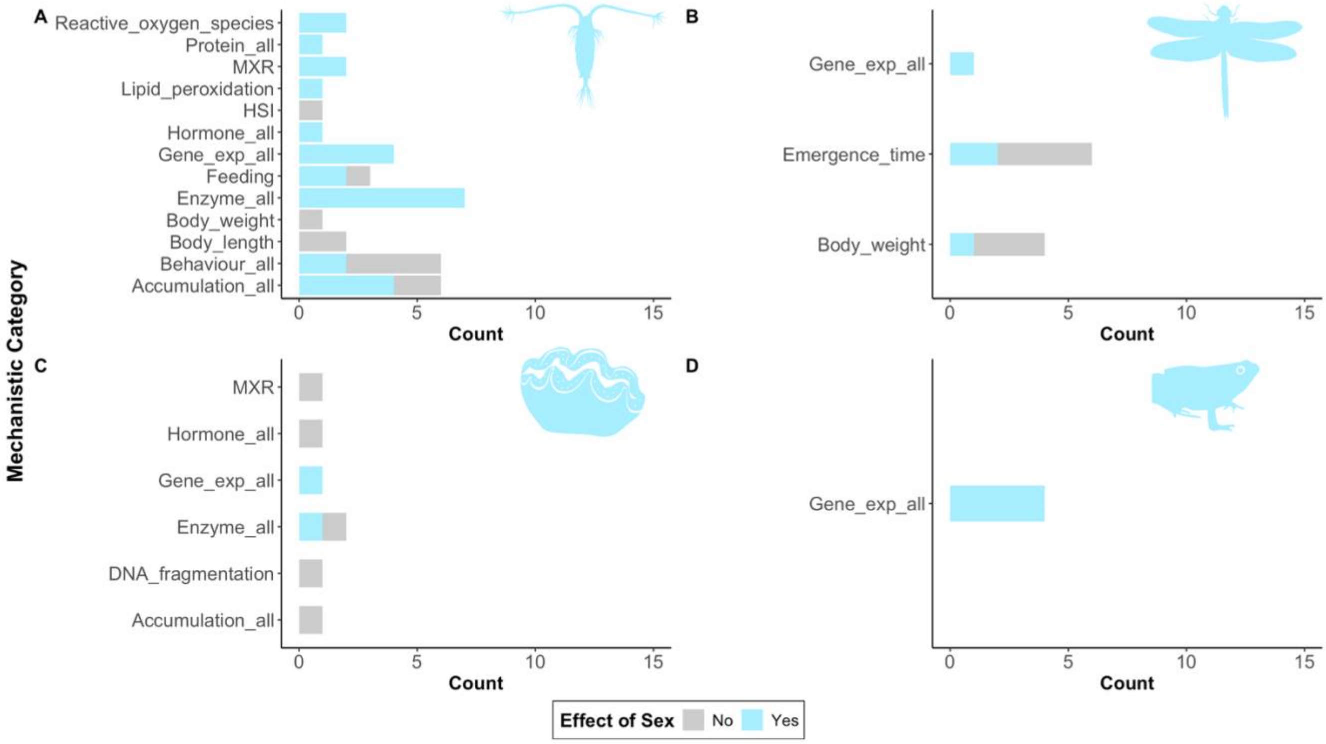

Figure 8. *Stacked bar plots showing sex-specific mechanistic effects for freshwater crustaceans (**A**), insects (**B**), molluscs (**C**) and amphibians (**D**). Explanations for each mechanistic category is found in the "Readme" (Appendix. 4). Animal icons obtained from PhyloPic (https://www.phylopic.org) CC0 1.0.*

3.5.2. Sex-Specific Effects in Marine Species

Gene expressions and GSI were the most studied mechanisms for the marine fish species exposed to chemical pollutants. For the combined gene expressions from each study, 100% showed sex-specific effects (*n* = 10), and for GSI, 40% showed sex-specific effects (*n* = 10). Additional mechanistic categories including more than five studies were hormones (*n* = 6) enzymes (*n* = 8), HSI (*n* = 9), body weight (*n* = 8), K-factor (*n* = 9) and body length (*n* = 6), which showcased sex-specific effects at 83%, 75%, 33%, 13%, 11% and 0%, respectively (Fig. 9a). Similar to the freshwater fish species, the results indicated marine fishes were more sensitive to sex-specific effects at a physiological rather than morphological levels.

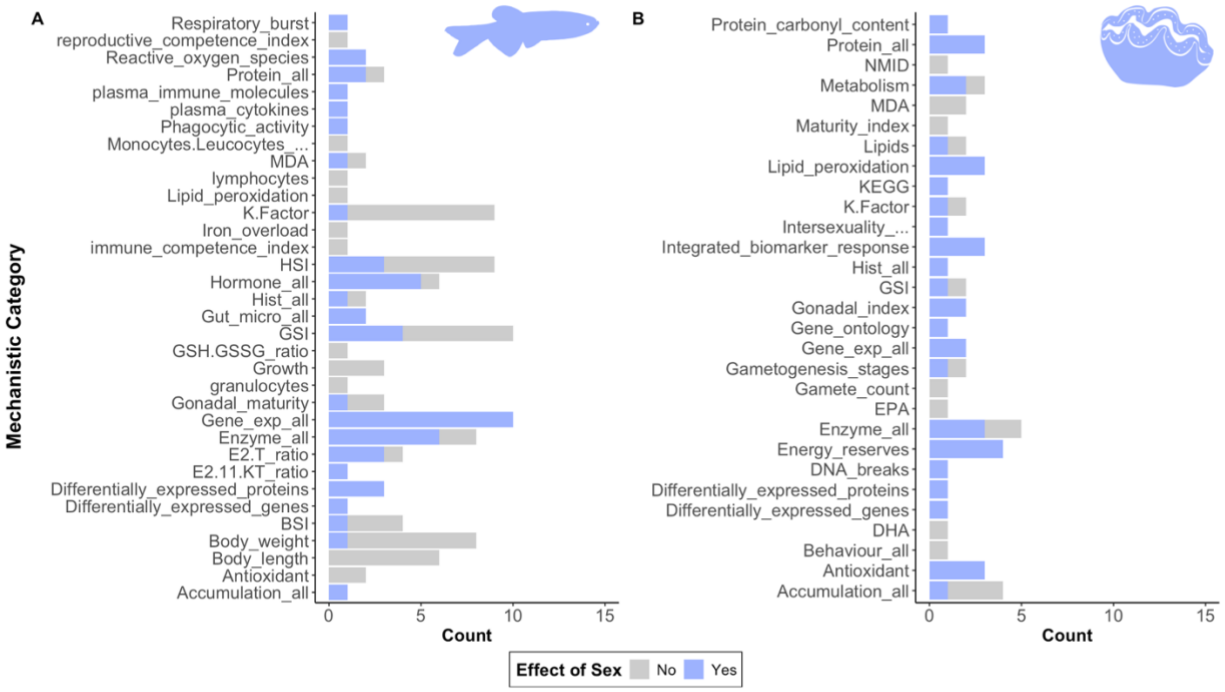

Figure 9. *Stacked bar plots showing sex-specific mechanistic effects for marine fish (**A**) and molluscs (**B**). Explanations for each mechanistic category is found in the "Readme" (Appendix. 4). Animal icons obtained from PhyloPic (https://www.phylopic.org) CC0 1.0.*

For marine crustaceans, behaviour was the most studied mechanistic category, and it showed sex-specific effects at 83% (*n* = 6), which showed markedly higher sex-specific effects for behaviour in marine crustaceans than freshwater crustaceans. Furthermore, body length had no effect of sex, and enzymes had effect of sex for the marine crustaceans (Fig. 10a). Marine molluscs had 100% effect of sex on gene expressions (*n* = 2), proteins (*n* = 3) and antioxidants (*n* = 3), whereas enzymes showed 60% sex-specific effects (*n* = 5). Accumulation of pollutants (*n* = 4) had a low sex-specific effect at 25% (Fig. 9b). With the limited data available, the results suggest marine molluscs may be more sensitive to sex-specific effects than freshwater molluscs. For marine echinoderms, body weight was not affected by sex, however, pollutant burden showed sex-specific effects (Fig. 10b).

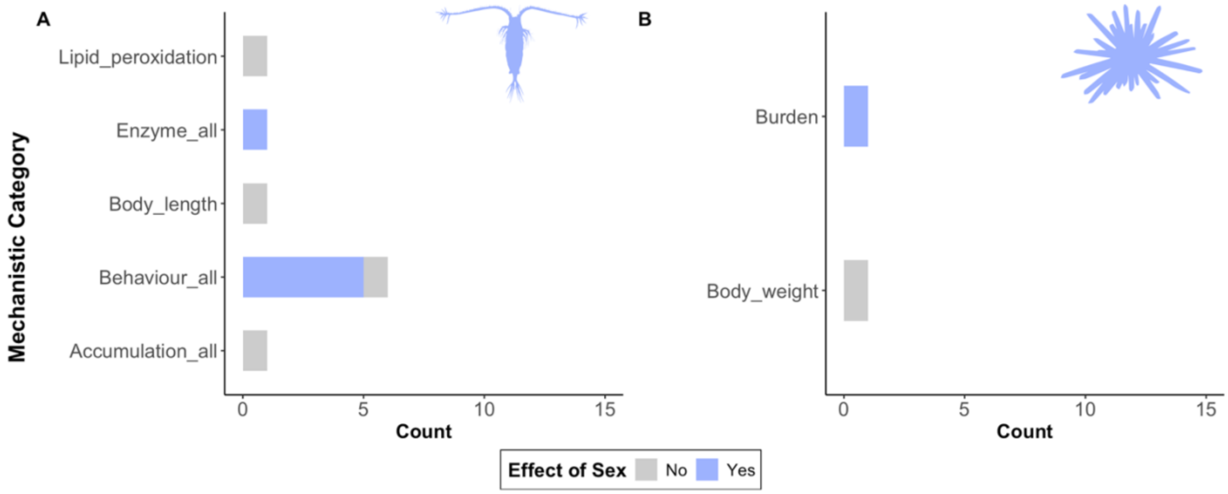

Figure 10. *Stacked bar plots showing sex-specific mechanistic effects for marine crustaceans (**A**) and echinoderms (**B**). Explanations for each mechanistic category is found in the "Readme" (Appendix. 4). Animal icons obtained from PhyloPic (https://www.phylopic.org) CC0 1.0.*

3.5.3. Sex-Specific Effects in Terrestrial Species

For terrestrial crustaceans growth (*n* = 2) had no sex-specific effects, while body weight had low effect of sex at 25% (Fig. 11a, *n* = 4). Terrestrial insects showed sex-specific effects at all mechanisms studied, which was at 100% for both body weight (*n* = 2) and bioamplification factor (*n* = 1), and at 50% for accumulation (Fig. 11b, *n* = 2). The results suggest terrestrial insects may be more affected by sex-specific effects following chemical exposure in body weight compared to freshwater insects, however, the data was limited. There was one mammal in the dataset, *Rattus norvegicus*, and only p-glycoprotein transport as the mechanistic response, and it showed no effect of sex (Fig. 11c). For the terrestrial reptiles' enzymes, behaviour and body weight had sex-specific effects, while there was no effect of sex on body length (Fig. 11d). Due to the limited data available, there was no pattern indicating one animal group may be more sensitive to sex-specific effects than others for the terrestrial species.

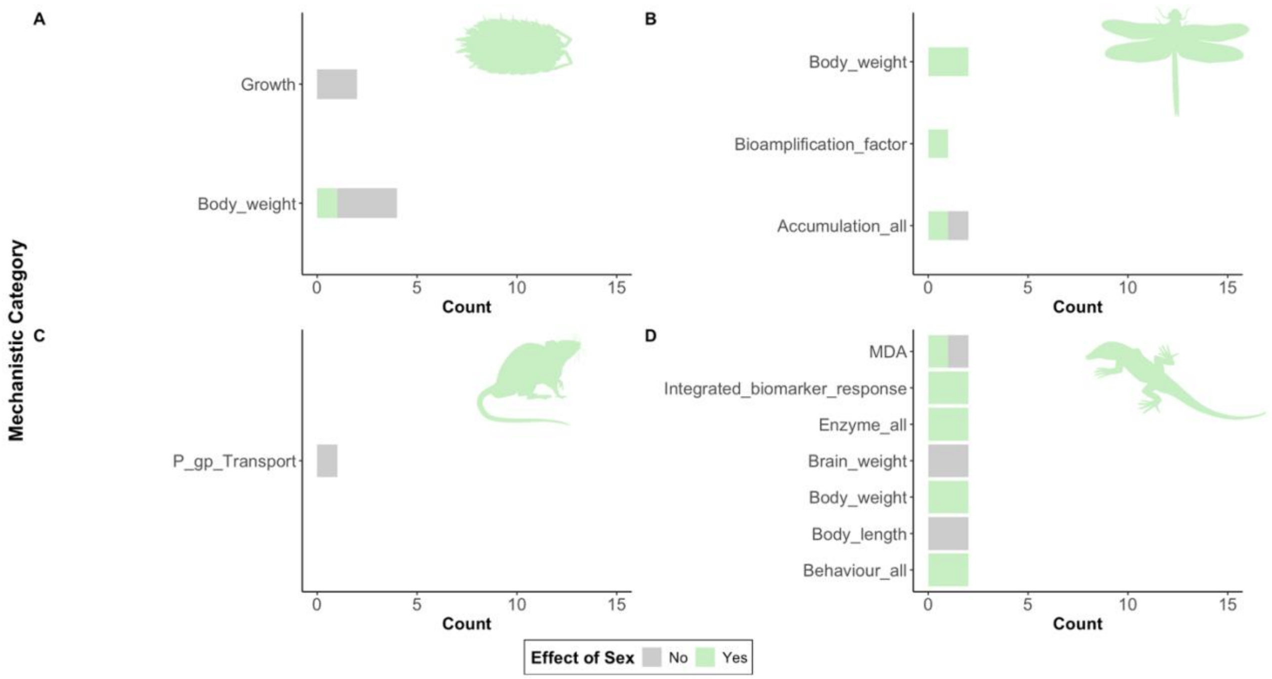

Figure 11. *Stacked bar plots showing sex-specific mechanistic effects for terrestrial crustaceans (**A**), insects (**B**), mammals (**C**) and reptiles (**D**). Explanations for each mechanistic category is found in the "Readme" (Appendix. 4). Animal icons obtained from PhyloPic (https://www.phylopic.org). CC0 1.0.*

3.6. Sex-Specific Sensitivities among Freshwater, Marine and Terrestrial Species

Across all studies, when I combined all the results from the mechanistic categories, sex-specific effects due to chemical exposures were found in 84% of studies (*n* = 131). When grouped by realm sex-specific differences were detected in 89% of studies in marine species (*n* = 44), in 85% of studies in freshwater species (*n* = 78) and in 56% of studies in terrestrial species (*n* = 9; Fig. 12). When grouped by animals I found sex-specific differences in 89% of fish species (*n* = 74), 81% for molluscs (*n* = 16), 75% for insects (*n* = 8) and 72% for crustaceans (*n* = 25). Furthermore, sex-specific effects were detected in 100% of studies for amphibians (*n* = 4), reptiles (*n* = 2) and echinoderms (*n* = 1). No effect of sex was detected in mammals (*n* = 1; Fig. 12). Across all species and realms, the results indicated that the type of mechanistic response investigated in each study may have influenced the magnitude of sex-specific effects. Physiological mechanisms, such as gene expressions, hormones and enzymes, showed higher frequency of sex-specific effects, while morphological mechanisms such as body weight and body length showed lower frequency of sex-specific effects. Therefore, the results were also influenced by the type of mechanistic response studied for the different animal groups and realms.

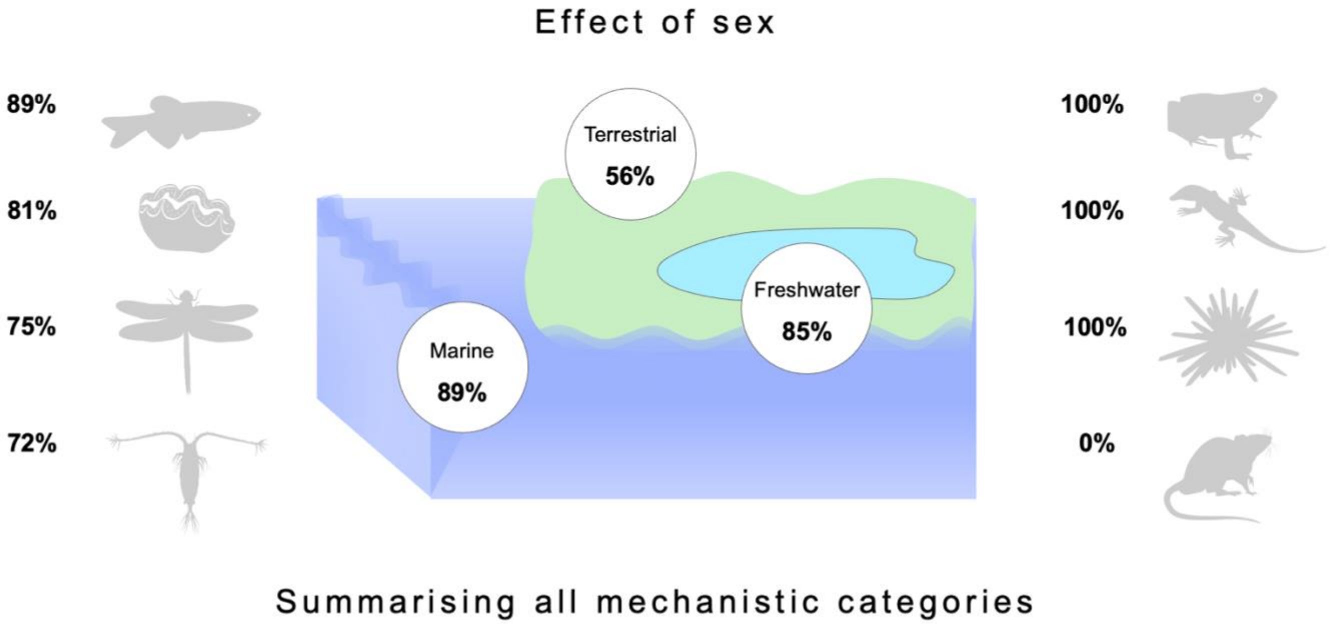

Figure 12. *Graphical summary of underlying mechanisms (mechanistic categories) explaining the different sensitivities between females and males in the animal kingdom due to chemical exposure. The percentages on the image show the frequency of an effect of sex detected due to chemical exposure (% of "Yes" responses in the dataset, n = 131). Animal icons obtained from PhyloPic (https://www.phylopic.org). CC0 1.0.*

4. Discussion

In this study, I summarised findings on sex-specific sensitivities in species from different phyla and realms to chemical pollution from 142 experimental studies. Firstly, I highlighted potential knowledge gaps within this field by investigating the diversity of species and chemical pollutants, as well as which realms and latitudes were the most represented in the dataset. Secondly, I quantified the differences between females and males in responses to survival, LC_{50} and sex ratios following chemical exposure. Finally, I investigated the underlying mechanisms underpinning the sex-specific sensitivities to chemical pollution, and explored whether freshwater, marine and terrestrial species had different sex-specific sensitivities. This study confirms that females and males may react differently due to chemical exposure across multiple animal groups from freshwater, marine and terrestrial realms.

4.1. Sex-Specific Sensitivities in Survival and LC_{50}

Overall, females depicted significantly higher survival than males with exposure to chemical pollutants, with this pattern being evidenced in the crustacean species (Fig. 4). The higher survival of females was mainly driven by three datapoints from the dataset. The terrestrial crustacean *Porcellio dilatatus* females had comparatively higher survival with exposure to two different cadmium species ($Cd(Cys)_2$ and $Cd(NO_3)_2$) than the females (Calhôa et al., 2012). Additionally, females also showed higher survival than males in the marine copepod *Sinocalanus tenellus* with exposure to dichloro-diphenyl-trichloroethane (Xu & Liu, 2014). Conversely, males had higher survival than females in the marine fish *Oryzias melastigma* exposed to 3,3′-Diindolylmethane (Chen et al., 2016).

For the LC_{50}, males were significantly more sensitive to chemical pollutants than females, wherein two datapoints were mainly driving this pattern (Fig. 5). The highest effect size was found in the freshwater crustacean *Daphnia magna* with exposure to zinc chloride and polystyrene microplastic beads (Lee et al., 2021), followed by the second highest effect size in the marine copepod *Tigriopus japonicus* when exposed to diazinon (Takai et al., 2020). Conversely, the freshwater crustacean *Austropotamobius pallipes* exposed to fluoride showed no effect of sex (Aguirre-Sierra et al., 2013).

Males may have lower survival and be more sensitive to chemical pollutants than females in instances where males are the heterogametic sex (males with XY chromosomes). On the Y chromosome, there may be deleterious mutations, which can potentially influence male survival. (Pipoly et al., 2015). Furthermore, for some species, females can also transfer pollutants through their offspring, for instance in eggs (Huang et al., 2020; McClellan-Green et al., 2007; Wesner et al., 2017; Wu et al., 2013), thereby potentially reducing pollutant loads for females. However, data on LC_{50} were conducted in short timespans (max 96hrs; See Appendix 4), therefore, additional mechanisms may explain why one sex can tolerate a higher pollutant concentration. Indeed, mechanisms identified from this study showed an effect of sex across multiple different types of mechanisms (See Fig. 7). Furthermore, another sex analysis study found evidence that survival in females exposed to pollutants are higher in insects and crustaceans than male survival (McClellan-Green et al., 2007). Additionally, males may have lower immunocompetence than females (Kurtz & Sauer, 2001).

The global pattern detected from this study suggested females had higher survival with exposure to chemical pollutants and tolerated a higher concentration of pollutants than males. However, the effect of sex depends on the chemical pollutant itself, exposure concentration, and duration, as well as the sensitivity of the exposed species in question (Michelangeli et al., 2022). Indeed, the results from this study suggested that the type of chemical pollutant influences the magnitude of effect sizes for the same species, thus different pollutants may exert varying degrees of sex-specific effects (Fig. 5). For the marine copepod *T. japonicus* it had a higher effect size when exposed to diazinon compared to tributyltin (Takai et al., 2020), while the freshwater crustacean *D. magna* had a higher effect size when exposed to zinc chloride and polystyrene microplastics beads, compared to exposure to zinc chloride only (Lee et al., 2021). Therefore, future studies should explore why some pollutants may magnify the effect of sex in the same species.

This study did find an effect of sex in studies on survival and LC_{50}. For the species and pollutants identified by this study, it is likely an issue if sex is not accounted for as females and males have shown to have different sensitivities depending on species and pollutants. Therefore, ecological risk assessments and environmental regulations should account for sex whenever possible, as pooling the sexes may under- or overestimate risks of chemical pollution in populations (Tannenbaum et al., 2019). However, the confidence intervals were especially broad for the random-effects model on survival. As such, the findings on survival need careful interpretation as the data may have favoured female survival due to chance. However, no freshwater species were tested in the model for survival, and the results on LC_{50} were limited to freshwater and marine crustaceans. Therefore, the pattern of females being favoured in survival and LC_{50} needs to be explored further to see if it might occur in other animal groups and habitats.

4.2. Changing Sex Ratios due to Chemical Pollution

Despite the pooled effect size being non-significant for the sex ratios, the results suggested that sex ratios change in response to chemical pollutants. This was mainly explained by the dataset diverging in either direction, wherein five studies significantly skewed towards more females, and five studies significantly skewed towards more males. Furthermore, three studies did not significantly alter sex ratios following exposure to chemical pollutants (Fig. 6). Therefore, I found no indication chemical pollution generally favours more male- or female-biased sex ratios.

Importantly, this study included species from many phylogenetic groups, which may mask trends if divergent selection pressures exist between groups (Weaving et al., 2023).

The studies that significantly skewed towards more females following chemical exposure were first the terrestrial insect *Spodoptera litura* when exposed to perchlorate (Qin et al., 2016), then the marine annelid *Perinereis nuntia* with exposure to benzo[a]pyrene (Wu et al., 2017), then the freshwater fish *Danio rerio* with exposure to seleno-L-methionine (Mo et al., 2020), followed by the marine copepod *Eurytemora affinis* when exposed to polluted resuspended sediment (Das et al., 2022) and finally the freshwater insect *Chironomus sancticaroli* larvae with exposure to polyethylene microplastics (Castro et al., 2022). The five studies that skewed significantly towards more males due to chemical exposure were the freshwater insect *Chironomus xanthus* larvae with exposure to glyphosate (Ferreira-Junior et al., 2017), the marine mollusc *Gomphina veneriformis* with exposure to tributyltin (Park et al., 2015), the marine fish *Oryzias melastigma* with exposure to perfluorobutane sulfonate (Chen et al., 2019), followed by the freshwater insect *Chironomus riparius* larvae with exposure to esfenvalerate (Rodrigues et al., 2015) and finally the marine copepod *Tigriopus japonicus* with exposure to tributyltin (Takai et al., 2020). Due to the diverse set of species, realms and pollutants there appears to be no clear pattern in which a female-skewed sex ratio is favoured over a male-skewed sex ratio due to chemical pollution and vice versa.

Similar to the findings on LC_{50}, the same species may respond differently due to different chemical pollutants. For instance, in the copepod species *T.japonicus* with exposure to diazinon, the sex ratio skewed slightly towards more females with the confidence interval crossing zero, while the pollutant tributyltin skewed the sex ratio towards more males (Takai et al., 2020). Moreover, neither a mixture of heavy metals (lead + arsenic + cadmium) or exposure to resuspended sediments from a less polluted site (as defined by the authors) influenced the sex ratio in either direction for the copepod *E.affinis*, while exposure to resuspended sediments from a polluted site significantly skewed the sex ratio towards more females (Das et al., 2022). Therefore, future studies should attempt to elucidate which pollutants or pollutant classes exert higher sex-specific sensitivities on the species.

Sex-specific effects due to chemical exposure are likely to have ecological implications once these effects ripple onto the population (McClellan-Green et al., 2007). Indeed, accounting for changes to sex ratios in ecological risk assessments may be essential as chronic exposure to pollutants affecting sex ratios can alter population dynamics, or ultimately lead to the decline in wild populations (Chelyadina & Popov, 2021; Kidd et al., 2007; Le Galliard et al., 2005). Changes to sex ratios can lead to long-term decline in a population, as found by Le Galliard et al. (2005), where a male-biased sex ratio in the common lizards *Lacerta vivipara* resulted in increased male aggression and negative impacts on female survival (Le Galliard et al., 2005). The mollusc *Mytilus galloprovincialis* in the Black Sea has seen a decline in natural populations in recent years, one reason for this can be higher mortality in females when exposed to polluted water, and this sex-specific mortality in turn have likely skewed the sex ratios towards more males since the 2000s (Chelyadina & Popov, 2021). Sex of mussels may be environmentally determined, which could explain why females and not males were more sensitive in *M. galloprovincialis* due to chemical pollution (Chelyadina & Popov, 2021). As such, future research on changes to sex ratios due to chemical pollution should investigate how different sex determination systems of species influence sex-specific sensitivities. Furthermore, one of the studies identified in this analysis on another marine mollusc (*G.veneriformis)* the sex ratio skewed towards more males with exposure to tributyltin (Park et al., 2015). Whether exposure to chemical pollutants in marine molluscs may skew towards more males was a coincidence or biologically relevant should be explored further. However, as aforementioned, sex-specific sensitivities may depend on the chemical pollutant itself and the pollutant tributyltin also skewed towards more males in the copepod *T.japonicus* (Takai et al., 2020).

4.3. Sex-Specific Sensitivities in Underlying Mechanisms

The main underlying mechanism that highlighted sex-specific vulnerabilities to stressors was gene expressions across freshwater and marine species. I found gene expressions to range from 92-100% from sex-specific effects. Another review found gene expressions at 95% sex-specific differences in biological processes in marine biological systems. Although the authors did not investigate effects due to chemical pollutants their finding on gene expressions is similar to this analysis (Gissi, Schiebinger, Santoleri, et al., 2023). Moreover, sexual dimorphism in gene expressions is prevalent across animal groups; thus, controlling for sex in gene expression studies is essential (Ellegren & Parsch, 2007). However, none of the studies identified from this analysis checked sex-specific effects on gene expressions in terrestrial species.

Other mechanisms with high sex-specific effects across animal groups and realms were hormones and enzymes. Due to the limited data available on terrestrial species there was no clear indication of which underlying mechanisms depicted the most sex-specific vulnerabilities to stressors. Behaviour depicted variable sex-specific effects and was high in freshwater fish, marine crustaceans and terrestrial reptiles, and lower in freshwater crustaceans. The glufosinate-ammonium herbicide exposed to lizard *Eremias argus* revealed males to be more sensitive than females with implications on neurotoxic behaviour and locomotor performance (Zhang et al., 2019). In addition, female fish *Poecilia reticulata* showed different response in behaviour with exposure to 17β-trenbolone, whereas male fish did not (Tomkins et al., 2016). Different responses between the sexes in underlying mechanisms such as behaviour may influence fitness by for example impacting mate choice. This in turn can also have consequences for population dynamics (Candolin & Wong, 2019).

Among the mechanisms with the least sex-specific effects were morphological characteristics such as body weight and body length. However, a review on sex-specific differences in biological processes in marine biological systems found morphology to have an effect of sex at 89% (Gissi, Schiebinger, Santoleri, et al., 2023). Interestingly, a study on clownfish investigating gene expressions and histological changes during sex change detected a change on a genetic level in the brain after two weeks, while histological changes were observed three to four weeks later (Casas et al., 2016). Therefore, one cannot rule out the possibility that differences between females and males may first be detected on a genetic level with exposure to chemical pollutants. The mean exposure duration for the experiments included in this analysis was 44, therefore, the findings were limited to effects occurring around this timescale. It may have been due to the duration of the experiments that changes on a morphological level were, in several cases, not detected. Furthermore, the methods applied in this study for the mechanistic categories were limited to a semi-quantitative review of detecting differences between the sexes with a simple “Yes” and “No”. In several cases, “No” was noted when there was not an effect of the pollutant itself on the control versus experimental treatments. For example, there was no effect on body weight in the experiment, therefore no effect of sex in *Chironomus riparius* with exposure to esfenvalerate (Rodrigues et al., 2015). Similarly, no effect was detected in body length for *Oryzias melastigma* exposed to 3,3′-Diindolylmethane (Chen et al., 2016).

4.4. Are There Differences Between Realms?

Across all mechanistic categories, I found an effect of sex in 89% of studies on marine species, 85% of studies on freshwater species and 56% of studies on terrestrial species (Fig. 12). As such, sex-specific effects among mechanistic categories with exposure to chemical pollutants were similar in marine and freshwater species. Although the results suggested less sex-specific effects for the terrestrial species in all mechanistic categories summarised the sample size was smaller than for the other two realms (*n* = 9), thus it is difficult to conclude that the results are accurate. Furthermore, the results from the random-effects models on survival and sex ratio changes showed terrestrial species with high sex-specific effects following exposure to chemical pollutants (See Fig. 4, Fig. 6; Calhôa et al., 2012; Qin et al., 2016). Additionally, a meta-analysis on ectotherms found wild-caught terrestrial females to have a higher cold tolerance plasticity than males, with this sexual dimorphism not being present in aquatic ectotherms (Pottier et al., 2021). Therefore, I have limited evidence to support that terrestrial species may be less sensitive to chemical pollutants despite the lower percentage in the mechanistic categories.

Nevertheless, different realms are likely to experience different environmental variabilities, selection pressures and adaptations (Jackson et al., 2016). Thus, understanding sex-specific vulnerabilities and potential differences between realms may improve our understanding of ecological impacts following chemical exposure. Therefore, whether differences in sex-specific sensititvities between realms exists or not, it needs to be explored further. Finally, due to chemical pollution being ubiquitous (Bernhardt et al., 2017), and due to the connectivity between ecosystems and realms (Richmond et al., 2018; Yamamuro et al., 2019; Wesner et al., 2017) more knowledge on sex-specific differences in terrestrial species is needed to gain a broader understanding of this topic.

4.4. Wider Implications of Findings and Limitations

Most studies identified from this study were from the Global North (57%) and China (35%), which is similar to a recent review on multiple stressors in freshwater ecosystems that also found most studies to be from the Global North and China (Orr et al., 2024). However, identified regions of concern by pesticide risk, water scarcity and biodiversity are commonly located in the Global South, such as South Africa, South America, India, China, and Mexico (Tang et al., 2021). Moreover, the risk of pharmaceutical pollution in the world's rivers is higher in the Global South (Wilkinson et al., 2022). Furthermore, many pollutants such as plastics are transported to more remote regions, such as the Arctic and deep-sea habitats (Bergmann et al., 2022). The studies included in this analysis could not sufficiently capture sex-specific sensitivities to pollutants in regions beyond the Global North and China. Therefore, more research effort on chemical pollutants is needed in the Global South and beyond.

Moreover, fish, crustaceans and molluscs were the most represented animal groups in this study at 52%, 25% and 11%, respectively. These findings are comparable to other reviews where fish and crustaceans were more represented in studies of freshwater and marine ecosystems (Gissi, Schiebinger, Santoleri, et al., 2023; Orr et al, 2024). However, this study reported sex-specific differences in more animal groups than is commonly studied, thus including less-studied species in future studies will improve our knowledge on this topic. Furthermore, this study had animals of laboratory origins, representing 73% of the dataset. This is a concern as another meta-analysis found differences between female and male thermal acclimation capacity to be more variable in wild animals than in laboratory animals (Pottier et al., 2021).

While I found an effect of sex, there are a few limitations to the findings from this study worth mentioning. Although it vastly limited screening time, I used sex-specific search strings, namely “female” and “male”, to obtain the articles. It is not unlikely that studies that have investigated sex-specific differences have done so due to *a priori* reasoning or knowledge (Ellis et al., 2017). Similarly, Gissi, Schiebinger, Santoleri, et al. (2023) found a high effect of sex and the authors used sex-specific search strings such as “sex-specific”, “sex analysis” and “sex based”. Moreover, multiple species show more sex allocation than females and males. The findings herein were limited to females and males, but many different sex allocations and life-history strategies (such as sex change and hermaphroditism) related to sex are relevant in understanding how chemical pollution may influence natural populations (Ellis et al., 2017; Yusa, 2007).

Furthermore, the methodology applied to the random-effects models had a few limitations, and the results showed a presence of bias in the sensitivity analysis. All the random-effects models applied to the findings on survival, LC_{50}, and sex ratios had high and significant statistical heterogeneity (Deeks et al., 2024; Senior et al., 2016). However, this is common in ecological meta-analysis (Senior et al., 2016) and is expected when there is a wide global dataset from different animal groups and realms (Jackson et al., 2016). Publication bias refers to the likelihood of significant and “positive” results being published over non-significant and “negative” results (Egger et al., 1997). The sensitivity analysis confirmed presence of bias in the survival and sex ratio data. Therefore, there may have been publication bias or data irregularities present in the models, especially for the survival data due to the finding from Egger's regression (Appendix 8). However, sources of asymmetry in funnel plots could also be due to true heterogeneity, wherein there are actual differences in underlying risk between species (Egger et al., 1997). For the LC_{50} data, study 5 had a large influence. Concerningly, the data from this study was pooled, as the authors found no significant effect of sex (Aguirre-Sierra et al., 2013; Appendix 8). As such, I could not calculate a potential true effect size for female and male LC_{50} for this study. Indeed, some researchers argue that the lack of evidence of sex-specific differences does not provide a good reason for excluding or pooling the sexes in data analysis (Miguel-Aliaga, 2022; Tannenbaum et al., 2019).

I encountered several obstacles that made the synthesis of the data and meta-analysis performed in this study more challenging. The main challenge encountered in this study was the lack of standard deviation (SD) data reported by the authors. To address this issue, I extracted the SD from the error bars in the figures using plot digitizer (https://plotdigitizer.sourceforge.net), which may have resulted in less precise data. Moreover, I opted to impute the missing variance data through mean imputation whenever I could not extract SD data. However, imputation methods would never replace the true contribution of the missing variance data, and it makes assumptions about unknown statistics, which result in bias in parameter estimates (Higgins et al., 2024; Nakagawa, 2015). Concerningly, the data on survival also had a higher proportion of imputed variance data compared to the data on sex ratio, whereas LC_{50} had no imputed data. Nevertheless, I opted for a method that minimised loss of relevant information and reduction in statistical power (Nakagawa, 2015). Overall, I suggest future experimental studies in ecotoxicology and beyond to provide access and transparency of data whenever possible and follow FAIR (Findability, Accessibility, Interoperability, and Reusability) data principles (Wilkinson et al., 2022).

This study did not include multiple stressors and interactive stressor studies. However, chemical contaminants in the environment commonly occur in conjunction with other chemicals and in mixtures (Sigmund et al., 2023). While this analysis did include some studies on chemical mixtures, there are other drivers to biodiversity loss that co-occur with chemical pollution. Crucial next steps on sex-specific differences in the animal kingdom ought to include the effects on other drivers of biodiversity loss such as climate change, land/sea use change, and direct exploitations of species (Jaureguiberry et al., 2022). For instance, male and female copepod *Calanus finmarchicus* showed different sensitivities with exposure to pyrene, but also with elevated temperatures (Dinh et al. 2019). Accounting for additional stressors is important as multiple stressors can increase sensitivities of species and exasperate sex-specfic effects to chemical pollutants (Delnat et al., 2019). With growing demand for new technologies, and increased usage of pharmaceuticals in the years to come, chemical pollutants are likely to increase and continue to have an impact on ecosystems and the environment (aus der Beek et al., 2016; Hou et al., 2025). Therefore, understanding how biological attributes, such as sex influences sensitivities to stressors, will potentially improve our predictions on the impacts of chemical pollutants in aquatic and terrestrial ecosystems.

5. Conclusion

The results from this study demonstrated that females and males have different sensitivities to chemical pollutants. Females depicted higher survival, and tolerated higher concentrations of pollutants than males. Importantly, sex ratios may skew towards either more females or males following chemical exposure. Furthermore, the sexes showed different responses in several underlying mechanisms such as gene expressions, hormones, enzymes and behaviour with exposure to chemical pollutants. The results indicated that gene expressions had high sexual dimorphism in responses following chemical pollution. As such, I recommend research on gene expressions to account for sex. The findings from this study suggest that pooling the sexes in ecotoxicological studies may confound the results, also because stress responses that alter sex ratios are likely to have long-term consequences for population fitness (Chelyadina & Popov, 2021; Kidd et al., 2007; Le Galliard et al., 2005; Tannenbaum et al., 2019). I suggest that sex-specific vulnerability to pollutants should be included in ecotoxicological studies whenever possible to improve ecological risk assessments. Nevertheless, the findings from this study were mainly limited to freshwater and marine realms. Furthermore, most of the data presented in this study were from the Global North and China. For future directions on this topic a more diverse and representative set of species, habitats and locations may further enhance our knowledge on how to safeguard species and populations exposed to chemical pollutants.

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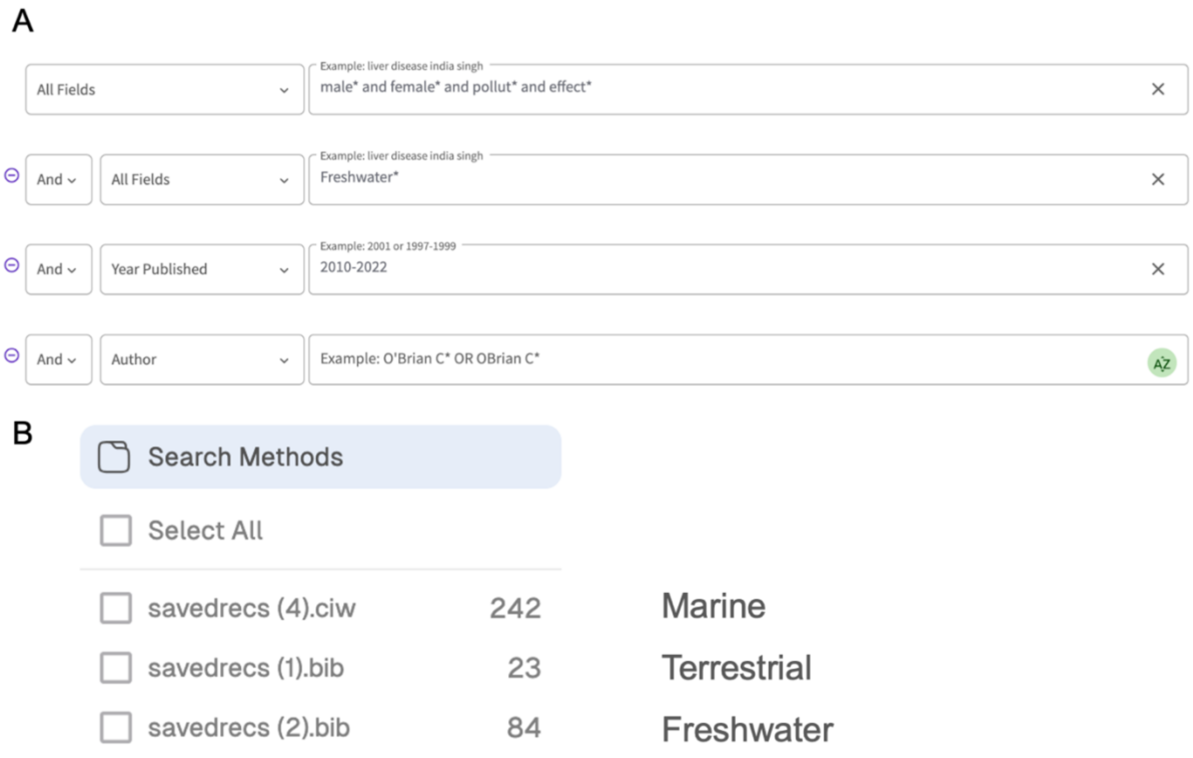
7. Appendices

Appendix 1. Showing search string and number of records for each realm



All Fields: male* and female* and pollut* and effect*

And – All Fields: Freshwater*

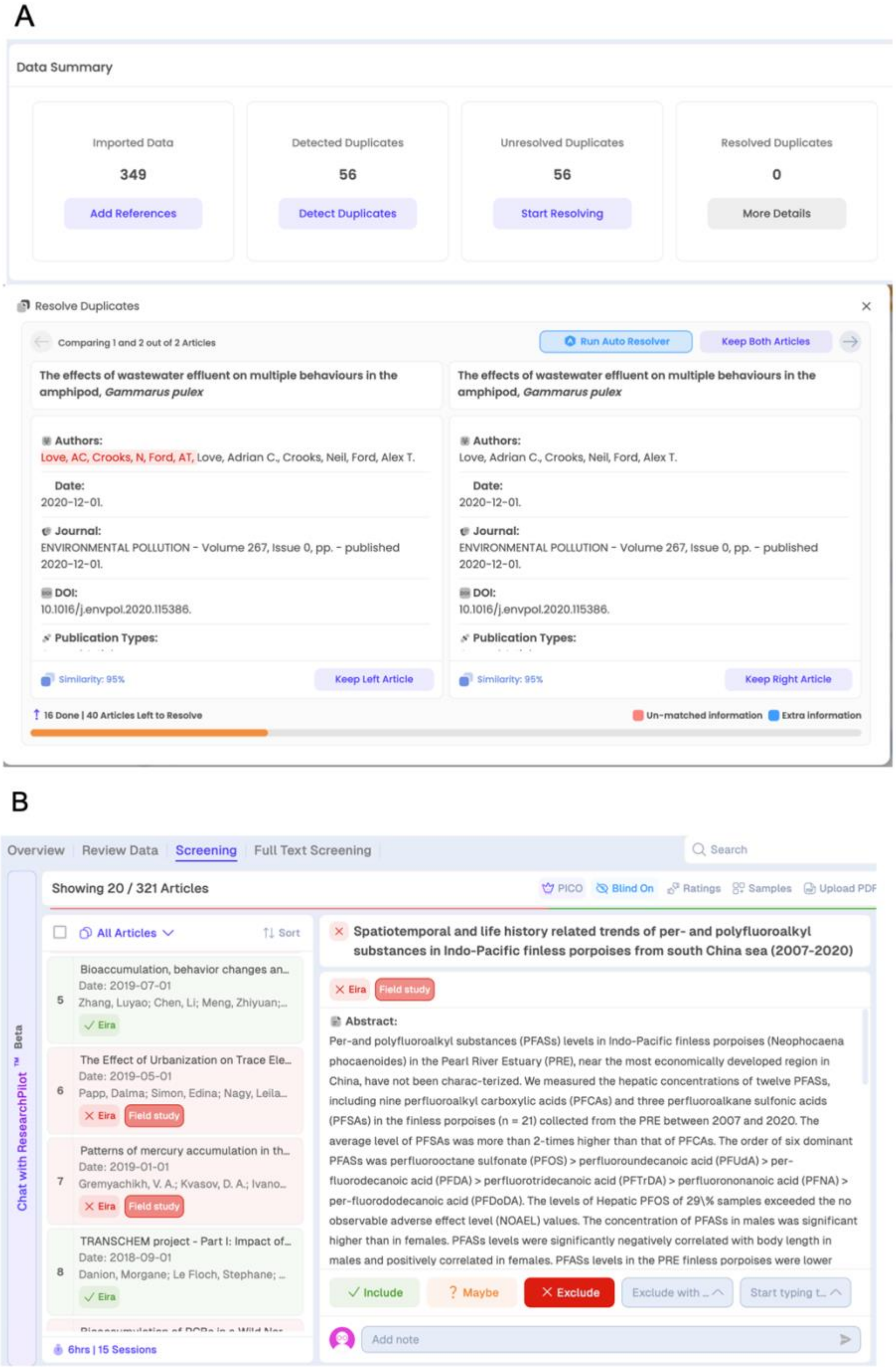
And – Year Published: 2010-2022

And – Author

☐ savedrecs (4).ciw	242	Marine
☐ savedrecs (1).bib	23	Terrestrial
☐ savedrecs (2).bib	84	Freshwater

Figure A1. *Screenshot from WoS showing the search string for "Freshwater*", wherein the same search string was applied for the other two realms: "Marine*" and "Terrestrial*" (**A**). Screenshot from Rayyan AI showing uploaded records from each search to the software from each realm; Marine (n = 242), Terrestrial (n = 23) and Freshwater (n = 84) (**B**).*

Appendix 2. Showing how Rayyan AI was applied to this study



Data Summary

Imported Data: 349

Detected Duplicates: 56

Unresolved Duplicates: 56

Resolved Duplicates: 0

Resolve Duplicates

Comparing 1 and 2 out of 2 Articles

The effects of wastewater effluent on multiple behaviours in the amphipod, *Gammarus pulex*	The effects of wastewater effluent on multiple behaviours in the amphipod, *Gammarus pulex*
Authors: Love, AC, Crooks, N, Ford, AT, Love, Adrian C., Crooks, Neil, Ford, Alex T.	**Authors:** Love, Adrian C., Crooks, Neil, Ford, Alex T.
Date: 2020-12-01.	**Date:** 2020-12-01.
Journal: ENVIRONMENTAL POLLUTION - Volume 267, Issue 0, pp. - published 2020-12-01.	**Journal:** ENVIRONMENTAL POLLUTION - Volume 267, Issue 0, pp. - published 2020-12-01.
DOI: 10.1016/j.envpol.2020.115386.	**DOI:** 10.1016/j.envpol.2020.115386.
Publication Types:	**Publication Types:**
Similarity: 95%	Similarity: 95%

16 Done | 40 Articles Left to Resolve

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Showing 20 / 321 Articles

5 Bioaccumulation, behavior changes an...
Date: 2019-07-01
Zhang, Luyao; Chen, Li; Meng, Zhiyuan;...
✓ Eira

6 The Effect of Urbanization on Trace Ele...
Date: 2019-05-01
Papp, Dalma; Simon, Edina; Nagy, Leila...
✕ Eira Field study

7 Patterns of mercury accumulation in th...
Date: 2019-01-01
Gremyachikh, V. A.; Kvasov, D. A.; Ivano...
✕ Eira Field study

8 TRANSCHEM project - Part I: Impact of...
Date: 2018-09-01
Danion, Morgane; Le Floch, Stephane; ...
✓ Eira

6hrs | 15 Sessions

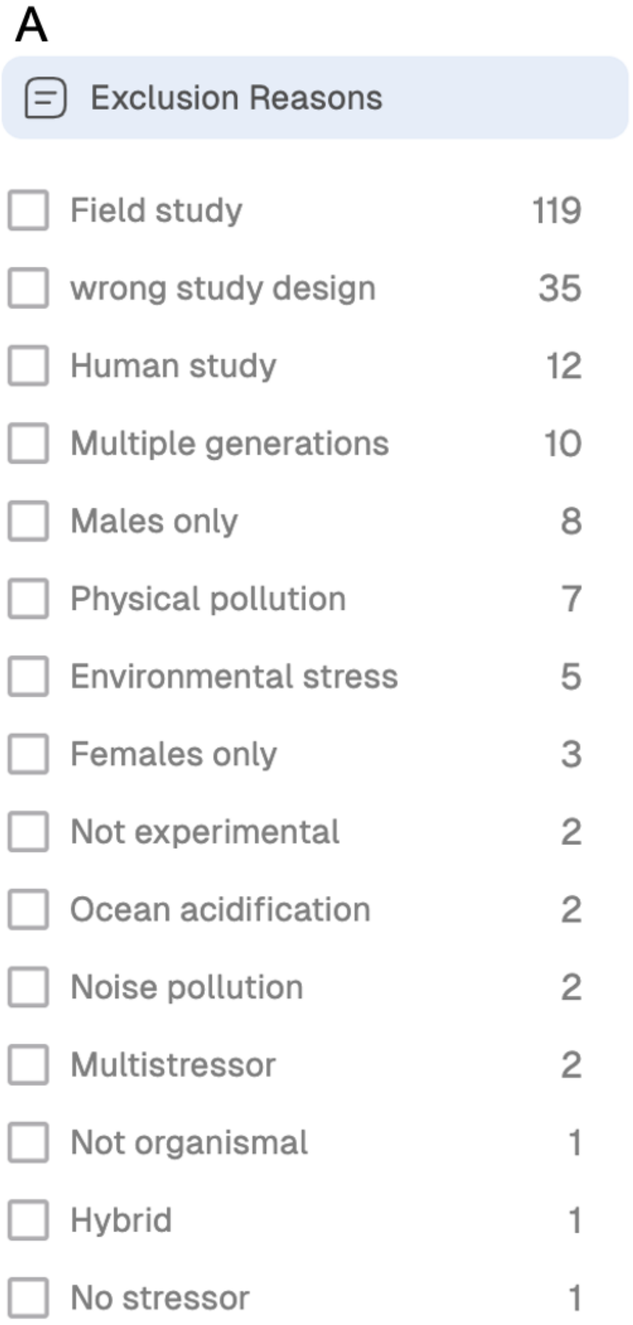
✕ Spatiotemporal and life history related trends of per- and polyfluoroalkyl substances in Indo-Pacific finless porpoises from south China sea (2007-2020)

✕ Eira Field study

Abstract:
Per-and polyfluoroalkyl substances (PFASs) levels in Indo-Pacific finless porpoises (Neophocaena phocaenoides) in the Pearl River Estuary (PRE), near the most economically developed region in China, have not been charac-terized. We measured the hepatic concentrations of twelve PFASs, including nine perfluoroalkyl carboxylic acids (PFCAs) and three perfluoroalkane sulfonic acids (PFSAs) in the finless porpoises (n = 21) collected from the PRE between 2007 and 2020. The average level of PFSAs was more than 2-times higher than that of PFCAs. The order of six dominant PFASs was perfluorooctane sulfonate (PFOS) > perfluoroundecanoic acid (PFUdA) > per-fluorodecanoic acid (PFDA) > perfluorotridecanoic acid (PFTrDA) > perfluorononanoic acid (PFNA) > per-fluorododecanoic acid (PFDoDA). The levels of Hepatic PFOS of 29\% samples exceeded the no observable adverse effect level (NOAEL) values. The concentration of PFASs in males was significant higher than in females. PFASs levels were significantly negatively correlated with body length in males and positively correlated in females. PFASs levels in the PRE finless porpoises were lower

Figure A2. *Screenshot from Rayyan AI showing number of duplicates (n = 56) and the process of removal of duplicates wherein both duplicates (two of the same article) were placed side by side and giving the user the option to “Keep left article” or “Keep right article” (**A**). Screenshot from Rayyan AI showing the screening process at title and abstract stage wherein the user can choose to “Include” or “Exclude” an article (**B**).*

Appendix 3. Showing exclusion reasons during screening



Exclusion Reasons	
☐ Field study	119
☐ wrong study design	35
☐ Human study	12
☐ Multiple generations	10
☐ Males only	8
☐ Physical pollution	7
☐ Environmental stress	5
☐ Females only	3
☐ Not experimental	2
☐ Ocean acidification	2
☐ Noise pollution	2
☐ Multistressor	2
☐ Not organismal	1
☐ Hybrid	1
☐ No stressor	1

B

Exclusion Reasons
Not female, male
not female male
males only
Not relevant
females only
multiple stressors?
not relevant
males only
Wrong study design
Not relevant
Multiple stressors
males only
wrong study design
wrong study design
not experimental
not female, male
Duplicate
Not female, male
MDPI
not diff between male and female response
presence of "Mixed sex", "abnormal male"
presence of "Mixed sex", "abnormal male"
Multiple generations
Sex not reported
multistressor
not female, male
not female, male
wrong study design
multistressor
males only
not female, male
not female, male
not female, male
females only
not female, male
Not relevant
Not stressor study

Figure A3. *Screenshot from Rayyan AI showing the Exclusion Reasons of articles during the title and abstract screening (**A**). Table showing Exclusion Reasons during full-text screening (**B**). Although only providing one reason* *per article for exclusion that does not mean several exclusion reasons were applicable for each article. Nevertheless, one exclusion reason was chosen per article.*

Appendix 4. Information on the studies included in this analysis. Contains details on the **"Readme"** – which explains how to interpret the data collected and how data was collected. It also contains the **"List of studies included in this analysis"** – which provides access to all the studies included in this analysis. Finally, it contains **"Access to articles included"** – which contains doi (online access to article), citation of all the articles that contained information on all the studies included in this analysis.

Readme - how to interpret the data collected and how data was collected.

Colour coding in the document	
Aquamarine	Sex-specific more "lethal" effects; Studies included in the meta-analysis (forest plots) such as survival, sex-ratio and LC_{50}; data provided numerically
Light green	Sex-specific mechanistic effects; Studies included in the narrative and semi-quantitative systematic review; data provided as "Yes/No", if there was an effect of sex
Cornflower blue	Sex-specific colour coding for whenever there was sex-xpecific data recorded; cornflower blue = males
Pink	Sex-specific colour coding for whenever there was sex-xpecific data recorded; pink = females

Name of category	**Explainations**
Study_ID	Unique ID of the study
Doi	Online access to article
Citation	Intext citation APA
Habitats	Freshwater; Marine; Terrestrial
Reasons	Reasons for not including an article in the analyses (Multiple stressors; Multiple generations; Wrong study desgin; Females only; Males only etc)

Year_published	Year the study was published
Country	Where the speciems used in the experiment were collected; brackets for when two countries are relevant (one where the experiment was conducted, one where the animal was from)
Latitude	Lat in decimal, where the study was conducted
Longtitude	Long in decimal, where the study was conducted
Genus_species	Name of species in genus and species used in the study
Origin	Where the speciems used in the experiment originated; lab; field
Groups	Type of animal used in the study; Crustacean, Fish; Mollusc
Pollutant class	Type of pollutant such as pesticides, heavy metals, flame retardants etc
Pollutants	Type of specific pollutant exposed to the specimen used in this study; Atrazine; Benzo[a]pyrene; Phenanthrene etc.
Concentration_highest (µg /L)	The highest concentration used in the study, which is the concentration I used to compare females and males
Concentrations (1,2,3,4,5,6,7,8) (µg /L or mg/kg)	How much of the pollutant is being used in the study, unit: µg/L or mg/kg, numbers indicating different concentration levels used in the experiment
Nr_of_concentrations	How many different concentrations are being used in each study
Exposure duration (days)	How long the specimen in the study have been exposed to the pollutant measured in days, only considering the final day when the results of males and females were reported
Exposure methods	Water (µg/L); food; sediment; injection (mg/kg). The medium in which the organism was exposed to the pollutant
Ratio male : female (control)	Ratio of male and female in the control group
Ratio male : female (experiment)	Ratio of male and female in the experimental group (highest concentration as explained above)

% Survival males (control)	Survival of control males exposed to the experiment measured in percentage (%)
% Survival females (control)	Survival of control females exposed to the experiment measured in percentage (%)
% Survival difference (control)	Difference in survival between control females and males measured in percentage (%)
% Survival males (experiment)	Survival of control males exposed to the experiment measured in percentage (%)
% Survival females (experiment)	Survival of control females exposed to the experiment measured in percentage (%)
% Survival difference (experiment)	Difference in survival between experiment females and males measured in percentage (%)
LC50 males	Concentration at which 50% of males die given a specific time (24 hrs, 48 hrs etc)
LC50 females	Concentration at which 50% of females die given a specific time (24 hrs, 48 hrs etc)
LC10 males	Concentration at which 10% of males die given a specific time (24 hrs, 48 hrs etc)
LC10 females	Concentration at which 10% of females die given a specific time (24 hrs, 48 hrs etc)
LC20 males	Concentration at which 20% of males die given a specific time (24 hrs, 48 hrs etc)
LC20 females	Concentration at which 20% of females die given a specific time (24 hrs, 48 hrs etc)
LC25 males	Concentration at which 25% of males die given a specific time (24 hrs, 48 hrs etc)
LC25 females	Concentration at which 25% of females die given a specific time (24 hrs, 48 hrs etc)
NOEC males	Concentration at which there is no observable effect on males (no observed effect concentration)
NOEC females	Concentration at which there is no observable effect on females (no observed effect concentration)
LT50 males	median lethal time (LT50), (time until death) after exposure of the animal to the pollutant on males
LT50 females	median lethal time (LT50), (time until death) after exposure of the animal to the pollutant on females
LOAEL males	Concentration at which there is the lowest observed adverse effect level on males

LOAEL females	Concentration at which there is the lowest observed adverse effect level on females
EC50 males	Concentration at which there is the median effective concentrations (half maximal effective concentration) on males
EC50 females	Concentration at which there is the median effective concentrations (half maximal effective concentration) on females
E2/T ratio	Difference between males and females hormonal E2/T (estrogen/testosterone) ratio response to stressor (Yes/No)
E2/11-KT ratio	Difference between males and females hormonal E2/11-KT (estrogen/11-Ketotestosterone) ratio response to stressor (Yes/No)
Intersexuality (%)	From the study: "Intersexuality was determined based on the definitive presence of male germ cells in females, and oocytes in males" (Park et al., 2015)
Reactive_oxygen_species	Difference between males and females reactive oxygen species response to stressor (Yes/No)
Protein carbonyl contents (1,2,3,4,5)	Difference between males and females protein carbonyl content response to stressor (Yes/No)
Nutrients	Difference between males and females nutrient content response to stressor (Yes/No)
Gene_exp_all	Difference between males and females gene response to stressor (Yes/No), Combining information from all genes. Yes = if a difference between sexes occurs in one or more genes. No = if no differences between the sexes is detected at all in any genes
Gene expression (1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18)	Difference between males and females gene expression response to stressor (Yes/No), numbers indicating different genes or gene groups accounted for in the experiment
Microarray_genes	Difference between males and females microarray gene response to stressor (Yes/No)
Gene_ontology	Difference between males and females gene ontology (function of genes) response to stressor (Yes/No)

KEGG	Difference between males and females Kyoto Encyclopedia of Genes and Genomes (understanding genes + functions) response to stressor (Yes/No)
Differentially_expressed_genes	Difference between males and females differentially expressed genes response to stressor (Yes/No)
Differentially_expressed_proteins	Difference between males and females differentially expressed proteins response to stressor (Yes/No)
Protein_interactive_network	Difference between males and females differentially protein-protein interaction (network) response to stressor (Yes/No)
Integrated_biomarker_response	Difference between males and females integrated biomarker responses to stressor (Yes/No)
Enzyme_all	Difference between males and females enzyme response to stressor (Yes/No), Combining information from all enzymes. Yes = if a difference between sexes occurs in one or more enzymes. No = if no differences between the sexes is detected at all in any enzymes
Enzyme (1,2,3,4,5,6)	Difference between males and females enzyme response to stressor (Yes/No), numbers indicating different enzymes accounted for in the experiment
Hormone_all	Difference between males and females hormone response to stressor (Yes/No), Combining information from all hormones. Yes = if a difference between sexes occurs in one or more hormone. No = if no differences between the sexes is detected at all in any hormones
Hormone (1,2,3,4,5,6)	Difference between males and females hormone response to stressor (Yes/No), numbers indicating different hormones accounted for in the experiment
Protein_all	Difference between males and females protein response to stressor (Yes/No), Combining information from all proteins. Yes = if a difference between sexes occurs in one or more proteins. No = if no differences between the sexes is detected at all in any proteins

Protein (1,2,3,4,5,6)	Difference between males and females protein response to stressor (Yes/No)
C1	Difference between males and females enzymatic activity of mitochondrial complex 1 response to stressor (Yes/No)
C2	Difference between males and females enzymatic activity of mitochondrial complex 2 response to stressor (Yes/No)
C3	Difference between males and females enzymatic activity of mitochondrial complex 3 response to stressor (Yes/No)
C4	Difference between males and females enzymatic activity of mitochondrial complex 4 response to stressor (Yes/No)
Amino_acids	Difference between males and females amino acids response to stressor (Yes/No)
Lipids	Difference between males and females lipids response to stressor (Yes/No)
Lipid_peroxidation	Difference between males and females lipid peroxidation response to stressor (Yes/No)
NMID	Difference between males and females non-methylene-interrupted dienoic fatty acids response to stressor (Yes/No)
EPA	Difference between males and females eicosapentaenoic acid response to stressor (Yes/No)
DHA	Difference between males and females docosahexaenoic acid response to stressor (Yes/No)
MDA	Difference between males and females Malondialdehyde response to stressor (Yes/No)
Behaviour_all	Difference between males and females behaviour response to stressor (Yes/No), Combining information from all behaviours. Yes = if a difference between sexes occurs in one or more behaviour. No = if no differences between the sexes is detected at all in any behaviours
Behaviour (1,2,3,4,5)	Difference between males and females behaviour response to stressor (Yes/No), numbers indicating different behaviours accounted for in the experiment

Gut_micro_all	Difference between males and females gut microbiome response to stressor (Yes/No), Combining information from all gut microbiomes. Yes = if a difference between sexes occurs in one or more gut microbiome. No = if no differences between the sexes is detected at all in any gut microbiomes
Gut microbiome (1,2,3,4,5,6,7,8)	Difference between males and females gut microbiome response to stressor (Yes/No), numbers indicating different gut microbiomes accounted for in the experiment
Accumulation_all	Difference between males and females accumulation response to stressor (Yes/No), Combining information from all accumulations. Yes = if a difference between sexes occurs in one or more accumulation. No = if no differences between the sexes is detected at all in any accumulations
Accumulation (1,2,3,4,5)	Difference between males and females accumulation response to stressor (Yes/No), numbers indicating different accumulations accounted for in the experiment
Burden	Difference between males and females pollutant burden response to stressor (Yes/No)
Clearance	Difference between males and females clearance of pollutant response to stressor (Yes/No)
Bioamplification_factor	Difference between males and females bioamplification of pollutant response to stressor (Yes/No)
Body_weight	Difference between males and females body weigth response to stressor (Yes/No)
Body_length	Difference between males and females body length response to stressor (Yes/No)
Brain_weight	Difference between males and females brain weigth response to stressor (Yes/No)
Gonad_weight	Difference between males and females gonad weigth response to stressor (Yes/No)
Liver_weight	Difference between males and females liver weigth response to stressor (Yes/No)

Eyes_weight	Difference between males and females eyes weigth response to stressor (Yes/No)
Heart_weight	Difference between males and females heart weigth response to stressor (Yes/No)
Growth	Difference between males and females growth response to stressor (Yes/No)
Deformity	Difference between males and females deformity response to stressor (Yes/No)
MXR	Difference between males and females multixenobiotic resistance activity response to stressor (Yes/No)
Metabolites	Difference between males and females metabolites response to stressor (Yes/No)
Metabolism	Difference between males and females metabolism response to stressor (Yes/No)
Energy_reserves	Difference between males and females energy reserves response to stressor (Yes/No)
glucose	Difference between males and females glucose response to stressor (Yes/No)
insulin	Difference between males and females insulin response to stressor (Yes/No)
Mitochondrial_ATP	Difference between males and females mitochondrial adenosine triphosphate (ATP) response to stressor (Yes/No)
DNA_fragmentation	Difference between males and females deoxyribonucleic acid (DNA) fragmentation response to stressor (Yes/No)
DNA_damage	Difference between males and females DNA damage response to stressor (Yes/No)
Emergence_time	Difference between males and females emergence time response to stressor (Yes/No)
K-factor	Difference between males and females condition factor (K-factor) response to stressor (Yes/No)
BSI	Difference between males and females Brain-Somatic Indices (BSI) response to stressor (Yes/No)
LSI	Difference between males and females Liver-Somatic Indices (LSI) response to stressor (Yes/No)

SSI	Difference between males and females Spleno-Somatic Indices (SSI) response to stressor (Yes/No)
HSI	Difference between males and females Hepato-Somatic Indices (HSI) response to stressor (Yes/No)
GSI	Difference between males and females Gonado-Somatic Indices (GSI) response to stressor (Yes/No)
Gametogenesis_stages	Difference between males and females gametogenesis stage response to stressor (Yes/No)
Gonadal_index	Difference between males and females gonadal index response to stressor (Yes/No)
Gonadal_maturity	Difference between males and females gonadal maturity response to stressor (Yes/No)
Maturity_index	Difference between males and females maturity index response to stressor (Yes/No) See Mezghani-Chaari et al. (2017)
Gamete_count	Difference between males and females gamete count response to stressor (Yes/No)
GSH:GSSG_ratio	Difference between males and females glutathione to glutathione disulfide ratio response to stressor (Yes/No)
metallothionein	Difference between males and females metallothionein response to stressor (Yes/No)
Hist_all	Difference between males and females histology response to stressor (Yes/No), Combining information from all histology parameters. Yes = if a difference between sexes occurs in one or more histology parameter. No = if no differences between the sexes is detected at all in any histological parameters
Histology (1,2,3,4,5)	Difference between males and females histology response to stressor (Yes/No), numbers indicating different histological parameters accounted for in the experiment
DNA_breaks	Difference between males and females DNA breaks response to stressor (Yes/No)

Antioxidant	Difference between males and females antioxidant response to stressor (Yes/No)
Plasma_protein_lvls	Difference between males and females plasma protein level response to stressor (Yes/No)
Monocytes/Leucocytes_(%)	Difference between males and females Monocytes/Leucocytes percentage response to stressor (Yes/No)
Respiratory_burst	Difference between males and females respiratory burst response to stressor (Yes/No)
Phagocytic_activity	Difference between males and females phagocytic activity response to stressor (Yes/No)
plasma_immune_molecules	Difference between males and females plasma immune molecules response to stressor (Yes/No)
plasma_cytokines	Difference between males and females plasma cytocines response to stressor (Yes/No)
lymphocytes	Difference between males and females lymphocytes in blood response to stressor (Yes/No)
granulocytes	Difference between males and females granulocytes in blood response to stressor (Yes/No)
leucocyte	Difference between males and females leucocyte in blood response to stressor (Yes/No)
Phagocytosis	Difference between males and females phagocytosis response to stressor (Yes/No)
Leucocyte_mortality_(%)	Difference between males and females leucocyte mortality in percentage response to stressor (Yes/No)
Hematocrit	Difference between males and females hematocrit response to stressor (Yes/No)
Erythrocytes	Difference between males and females erythrocytes response to stressor (Yes/No)
Stimulation_index	Difference between males and females stimulation index (relative luminescence units (RLU)area stimulated / RLUarea unstimulated) response to stressor (Yes/No) (Bunge et al., 2022)

Iron_overload	Difference between males and females iron overload response to stressor (Yes/No)
immune_competence_index	Difference between males and females immune competence index response to stressor (Yes/No). From the auhtors Ye et al. (2018): "The immune competence index was calculated based on the survival curve date obtained in host-resistance assay for determination of the hazard ratio (HR), using the Cox Proportional Hazard model"
reproductive_competence_index	Difference between males and females immune competence index response to stressor (Yes/No). From the authors Ye et al. (2018): "The Reproductive competence index (RCI) of EE2-exposed male fish was calculated based on fertilization success and hatching success. The RCI of EE2-exposed female fish was calculated based on fecundity and hatching success"
Feeding	Difference in feeding between females and males response to stressor (Yes/No)
P_gp_Transport	Difference between males and females P-Glycoprotein transport response to stressor (Yes/No)
Notes	Notes on potentially useful information for analysis or if there´s anything uncertain etc

List of studies included in this analysis – provides access to all the studies included in this analysis.

Table A1. *General information from all the studies used to check for diversity of species, animal groups, countries, and locations*

Study_ID	Habitats	Year_published	Country	Latitude	Longitude	Genus_species	Origin	Groups
9	Freshwater	2020	Canada	52.13339	-106.63135	*Pimephales promelas*	lab	Fish
11	Freshwater	2013	Argentina	-34.961111	-57.777778	*Macrobrachium borellii*	field	Crustacean
14	Freshwater	2014	Belgium	50.583333	5.583333	*Gammarus pulex*	field	Crustacean
14.2	Freshwater	2014	Belgium	50.583333	5.583333	*Gammarus pulex*	field	Crustacean
15	Freshwater	2021	South Africa	-27.35206582	22.50386447	*Branchipodopsis wolfi*	field	Crustacean
16	Freshwater	2022	Thailand	15.0727	105.21948	*Hyriopsis bialata*	field	Mollusc
18	Freshwater	2020	Czech Republic	49.16056	14.17366	*Procambarus clarkii*	lab	Crustacean
18.2	Freshwater	2020	Czech Republic	49.16056	14.17366	*Procambarus clarkii*	lab	Crustacean
19	Freshwater	2022	Brazil	-23.55577	-46.63955	*Chironomus sancticaroli*	lab	Insect
20.1	Freshwater	2015	Belgium	50.57779	5.5706	*Gammarus pulex*	field	Crustacean
20.2	Freshwater	2015	Belgium	50.57779	5.5706	*Gammarus pulex*	field	Crustacean
21.1	Freshwater	2019	China	39.87977	116.40266	*Danio rerio*	lab	Fish
21.2	Freshwater	2019	China	39.87977	116.40266	*Danio rerio*	lab	Fish
23	Freshwater	2014	China	29.81959	106.42662	*Gobiocypris rarus*	lab	Fish

24	Freshwater	2010	China	36.26444	120.03353	*Cyprinus carpio*	lab	Fish
26	Freshwater	2021	China	31.14978	121.12426	*Macrobrachium nipponense*	lab	Crustacean
27	Freshwater	2015	China	30.545533	114.355786	*Danio rerio*	lab	Fish
28.1	Freshwater	2022	Portugal	40.63057	-8.65746	*Chironomus riparius*	lab	Insect
28.2	Freshwater	2022	Portugal	40.63057	-8.65746	*Chironomus riparius*	lab	Insect
28.3	Freshwater	2022	Portugal	40.63057	-8.65746	*Chironomus riparius*	lab	Insect
31	Freshwater	2012	China	30.54	114.36	*Danio rerio*	lab	Fish
33	Freshwater	2015	Portugal	40.63394081	-8.659315603	*Chironomus riparius*	lab	Insect
34	Freshwater	2020	Brazil	27.616081	48.502119	*Macrobrachium potiun*	field	Crustacean
35.1	Freshwater	2010	Germany	52.44887	13.64758	*Xenopus laevis*	lab	Amphibian
35.2	Freshwater	2010	Germany	52.44887	13.64758	*Xenopus laevis*	lab	Amphibian
35.3	Freshwater	2010	Germany	52.44887	13.64758	*Xenopus laevis*	lab	Amphibian
35.4	Freshwater	2010	Germany	52.44887	13.64758	*Xenopus laevis*	lab	Amphibian
38.1	Freshwater	2016	China	30.54605558	114.3556573	*Danio rerio*	lab	Fish
38.2	Freshwater	2016	China	30.54605558	114.3556573	*Danio rerio*	lab	Fish
38.3	Freshwater	2016	China	30.54605558	114.3556573	*Danio rerio*	lab	Fish
39	Freshwater	2018	China	30.54605558	114.3556573	*Danio rerio*	lab	Fish
41	Marine	2016	Hong Kong	22.33777	114.17262	*Oryzias melastigma*	lab	Fish
42	Freshwater	2015	Hong Kong	22.33777	114.17262	*Danio rerio*	lab	Fish
43	Marine	2023	China	35.41645	119.52719	*Mytilus galloprovincialis*	field	Mollusc
46	Marine	2023	Northern Ireland	54.53333	-5.48333	*Pagurus bernhardus*	field	Crustacean
47	Freshwater	2022	USA	36.00142	-78.93822	*Oryzias latipes*	lab	Fish
49.1	Marine	2022	Portugal	40.63706	-8.68569	*Mytilus galloprovincialis*	field	Mollusc
49.2	Marine	2022	Portugal	40.63706	-8.68569	*Mytilus galloprovincialis*	field	Mollusc
49.3	Marine	2022	Portugal	40.63706	-8.68569	*Mytilus galloprovincialis*	field	Mollusc
51	Freshwater	2022	Iran	37.27126	49.59207	*Carassius auratus*	lab	Fish
52.1	Freshwater	2022	Germany	53.477028	8.519139	*Gasterosteus aculeatus*	lab	Fish
52.2	Freshwater	2022	Germany	53.477028	8.519139	*Gasterosteus aculeatus*	lab	Fish
53.1	Marine	2021	China	26.07637	119.26481	*Oryzias melastigma*	lab	Fish
53.2	Marine	2021	China	26.07637	119.26481	*Oryzias melastigma*	lab	Fish

55	Marine	2021	UK	54.21666	-0.26666	*Mytilus edulis*	field	Mollusc
56	Marine	2021	China	36.11403	120.5428	*Chlamys farreri*	field	Mollusc
57	Marine	2021	China	36.16068	120.4975	*Oryzias melastigma*	lab	Fish
58	Marine	2021	China	24.43415	118.09422	*Oryzias melastigma*	lab	Fish
59	Marine	2020	China	37.46352	121.44801	*Mytilus galloprovincialis*	field	Mollusc
60	Freshwater	2020	China	32.05603	118.76373	*Oryzias latipes*	lab	Fish
61	Marine	2020	China	38.866667	121.55	*Ligia cinerascens*	field	Crustacean
63	Freshwater	2020	Iran	35.72345	51.38397	*Salmo trutta caspius*	lab	Fish
64	Marine	2020	Italy	45.2355	12.2702	*Mytilus galloprovincialis*	field	Mollusc
65.1	Marine	2020	Japan	35.1851	140.37714	*Tigriopus japonicus*	lab	Crustacean
65.2	Marine	2020	Japan	35.1851	140.37714	*Tigriopus japonicus*	lab	Crustacean
65.3	Marine	2020	Japan	35.1851	140.37714	*Tigriopus japonicus*	lab	Crustacean
65.4	Marine	2020	Japan	35.1851	140.37714	*Tigriopus japonicus*	lab	Crustacean
67	Marine	2019	Hong Kong	22.3193	114.16936	*Oryzias melastigma*	lab	Fish
68	Marine	2019	China	23.46545	117.13677	*Chlamys nobilis*	field	Mollusc
69	Marine	2019	China	37.51714	121.35853	*Hippocampus erectus*	lab	Fish
70	Marine	2018	Hong Kong	22.33703	114.17272	*Oryzias melastigma*	lab	Fish
71.1	Marine	2018	Norway	78.4334	15.88484	*Boreogadus saida*	field	Fish
71.2	Marine	2018	Norway	78.4334	15.88484	*Boreogadus saida*	field	Fish
71.3	Marine	2018	Norway	78.4334	15.88484	*Boreogadus saida*	field	Fish
73	Marine	2018	Algeria	36.833333	7.833333	*Donax trunculus*	field	Mollusc
74	Marine	2018	New Zealand	-41.2882	174.8326	*Evechinus chloroticus*	field	Echinoderm
75	Marine	2017	Tunisia	34.10984	9.98283	*Ruditapes decussatus*	field	Mollusc
77	Marine	2017	France	49.48277	0.45218	*Eurytemora affinis*	field	Crustacean
78	Marine	2017	China	26.0742	119.29647	*Perinereis nuntia*	lab	Annelid
79	Freshwater	2017	Australia	-19.438317	146.950303	*Poecilia reticulata*	lab	Fish
81	Marine	2017	Portugal	38.45	-8.716667	*Gammarus locusta*	lab	Crustacean
82	Marine	2016	Norway	79	11.66666	*Boreogadus saida*	field	Fish
83	Marine	2016	Norway	69.584333	18.92835	*Chlamys islandica*	field	Mollusc
84	Marine	2016	Hong Kong	22.33777	114.17262	*Oryzias melastigma*	lab	Fish
85.1	Marine	2017	Belgium	50.82121	3.57662	*Eurytemora affinis*	field	Crustacean

85.2	Marine	2017	Belgium	50.82121	3.57662	*Eurytemora affinis*	field	Crustacean
86	Marine	2015	Brazil	-27.59996	-48.51941	*Crassostrea brasiliana*	lab	Mollusc
89	Freshwater	2015	USA	46.78667	-92.10048	*Oryzias latipes*	lab	Fish
90.1	Marine	2015	Hong Kong	22.33777	114.17262	*Oryzias melastigma*	lab	Fish
90.2	Marine	2015	Hong Kong	22.33777	114.17262	*Oryzias melastigma*	lab	Fish
91	Marine	2015	South Korea	37.9075	128.819167	*Gomphina veneriformis*	field	Mollusc
92	Freshwater	2015	USA	46.83857	-92.00328	*Oryzias latipes*	lab	Fish
94.1	Marine	2014	Hong Kong	22.33777	114.17262	*Oryzias melastigma*	lab	Fish
94.2	Marine	2014	Hong Kong	22.33777	114.17262	*Oryzias melastigma*	lab	Fish
95	Marine	2014	Taiwan	23.69781	120.96051	*Oryzias melastigma*	lab	Fish
96	Marine	2014	China	35.93141	120.00908	*Sinocalanus tenellus*	field	Crustacean
97.1	Freshwater	2013	South Korea	37.46486	126.95472	*Danio rerio*	lab	Fish
97.2	Freshwater	2013	South Korea	37.46486	126.95472	*Danio rerio*	lab	Fish
97.3	Freshwater	2013	South Korea	37.46486	126.95472	*Danio rerio*	lab	Fish
97.4	Freshwater	2013	South Korea	37.46486	126.95472	*Danio rerio*	lab	Fish
97.5	Freshwater	2013	South Korea	37.46486	126.95472	*Danio rerio*	lab	Fish
97.6	Freshwater	2013	South Korea	37.46486	126.95472	*Danio rerio*	lab	Fish
98.1	Marine	2013	China	36.77644	121.15846	*Cynoglossus semilaevis*	lab	Fish
98.2	Marine	2013	China	36.77644	121.15846	*Cynoglossus semilaevis*	lab	Fish
99	Marine	2011	France	49.473056	0.464167	*Eurytemora affinis*	field	Crustacean
101.1	Freshwater	2011	Japan	35.15385	136.96824	*Oryzias latipes*	lab	Fish
101.2	Freshwater	2011	Japan	35.15385	136.96824	*Oryzias latipes*	lab	Fish
102	Freshwater	2023	China	31.29067	121.56133	*Danio rerio*	lab	Fish
104.1	Freshwater	2021	USA (South Korea)	36.09597	-79.43716	*Daphnia magna*	lab	Crustacean
104.2	Freshwater	2021	USA (South Korea)	36.09597	-79.43716	*Daphnia magna*	lab	Crustacean
104.3	Freshwater	2021	USA (South Korea)	36.09597	-79.43716	*Daphnia magna*	lab	Crustacean
104.4	Freshwater	2021	USA (South Korea)	36.09597	-79.43716	*Daphnia magna*	lab	Crustacean
105	Freshwater	2021	USA (South Korea)	45.77593	8.81504	*Danio rerio*	lab	Fish

106	Terrestrial	2019	USA	35.77958	-78.63817	*Rattus norvegicus*	lab	Mammal
107	Freshwater	2017	China	30.54531	114.35577	*Danio rerio*	lab	Fish
108	Freshwater	2017	Portugal	41.17739	-8.7009	*Danio rerio*	lab	Fish
110.1	Freshwater	2014	France	48.70184	2.13407	*Danio rerio*	lab	Fish
110.2	Freshwater	2014	France	48.70184	2.13407	*Danio rerio*	lab	Fish
110.3	Freshwater	2014	France	48.70184	2.13407	*Danio rerio*	lab	Fish
111.1	Marine	2014	USA	38.52922	-121.78276	*Oryzias latipes*	lab	Fish
111.2	Marine	2014	USA	38.52922	-121.78276	*Oryzias latipes*	lab	Fish
112.1	Freshwater	2014	Australia	-37.64618	144.99802	*Melanotaenia fluviatilis*	lab	Fish
112.2	Freshwater	2014	Australia	-37.64618	144.99802	*Melanotaenia fluviatilis*	lab	Fish
115	Freshwater	2012	China	36.19393	117.11937	*Danio rerio*	lab	Fish
116.1	Freshwater	2011	China	34.60586	119.22185	*Oreochromis niloticus*	lab	Fish
116.2	Freshwater	2011	China	34.60586	119.22185	*Oreochromis niloticus*	lab	Fish
118	Freshwater	2011	Hong Kong	22.26002	114.19013	*Danio rerio*	lab	Fish
119	Terrestrial	2020	China	23.15334	113.3467	*Bombyx mori*	lab	Insect
120.1	Terrestrial	2019	China	38.03598	114.46979	*Eremias argus*	lab	Reptile
120.2	Terrestrial	2019	China	38.03598	114.46979	*Eremias argus*	lab	Reptile
121	Freshwater	2018	France	48.38143	-4.6204	*Oncorhynchus mykiss*	lab	Fish
122	Terrestrial	2016	China	23.16034	113.35875	*Spodoptera litura*	lab	Insect
124.1	Terrestrial	2012	Portugal	40.6405	-8.65375	*Porcellio dilatatus*	lab	Crustacean
124.2	Terrestrial	2012	Portugal	40.6405	-8.65375	*Porcellio dilatatus*	lab	Crustacean
125.1	Terrestrial	2010	Portugal	39.96933	-8.03488	*Porcellio scaber*	lab	Crustacean
125.2	Terrestrial	2010	Portugal	39.96933	-8.03488	*Porcellio scaber*	lab	Crustacean
126	Freshwater	2023	China	30.50721	114.35197	*Danio rerio*	lab	Fish
131	Freshwater	2020	China	30.47066	114.35675	*Danio rerio*	lab	Fish
132	Freshwater	2019	China	30.59275	114.30525	*Procambarus clarkii*	lab	Crustacean
134	Freshwater	2017	Brazil	-11.74647	-49.04942	*Chironomus xanthus*	lab	Insect
135	Freshwater	2016	Australia	-19.438317	146.950303	*Poecilia reticulata*	field	Fish
139	Freshwater	2013	Spain	40.9002	-1.91376	*Austropotamobius pallipes*	field	Crustacean
140	Freshwater	2013	Australia	-37.63463	144.89392	*Gambusia holbrooki*	field	Fish
141	Freshwater	2013	Egypt	30.01903	31.22007	*Clarias gariepinus*	field	Fish

143	Freshwater	2017	China	36.05829	120.351	*Carassius auratus*	lab	Fish
145.1	Freshwater	2012	Portugal	41.13878	-8.61637	*Danio rerio*	lab	Fish
145.2	Freshwater	2012	Portugal	41.13878	-8.61637	*Danio rerio*	lab	Fish
146	Freshwater	2022	Sweden	56.1125	13.909722	*Anodonta anatina*	field	Mollusc
148.1	Marine	2022	France	49.433333	-0.266667	*Eurytemora affinis*	lab	Crustacean
148.2	Marine	2022	France	49.433333	-0.266667	*Eurytemora affinis*	lab	Crustacean
148.3	Marine	2022	France	49.433333	-0.266667	*Eurytemora affinis*	lab	Crustacean
149	Freshwater	2012	Canada	52.13339	-106.63135	*Pimephales promelas*	lab	Fish

Table A2. *Information from all the studies on the classes of chemical pollutants, chemical pollutant identity, highest concentration (applied to this analysis), number of concentrations, exposure duration, exposure methods and notes*

Study_ID	**Chemical_class**	**Pollutants**	**Concentration_highest (µg /L) or (mg/kg)**	**Nr_of_concentrations**	**Exposure_duration (days)**	**Exposure_methods**	**Notes**
9	Hydrocarbons	Benzo[a]pyrene	12	2	4	water	
11	Hydrocarbons	Hydrocarbon mixture (water soluble fraction)	600	2	7	water	
14	Flame retardants	BDE-47	1	2	4	water	
14.2	Flame retardants	BDE-99	1	2	4	water	
15	Pharmaceuticals	Fluoxetine	0.5	2	19	water	

16	Pesticide	Atrazine	2000	2	28	water	
18	Pharmaceuticals	methamphetamine	1	2	7	water	
18.2	Pharmaceuticals	tramadol	1	2	7	water	
19	Microplastics	Polyethylene microplastics	320 000	2	40	water	
20.1	Flame retardants	BDE-47	0.1	2	4	water	
20.2	Flame retardants	BDE-99	0.1	2	4	water	
21.1	Pesticide	1S-cis-bifenthrin	0.5	2	60	water	
21.2	Pesticide	1R-cis-bifenthrin	0.5	2	60	water	
23	Pesticide	Pentachlorophenol	160	2	28	water	
24	Hydrocarbons	Phenanthrene	2500	2	3	water	
26	Nanoparticles	Polystyrene nanoplastics	40 000	2	28	water	
27	Flame retardants	Tris(1,3-dichloro-2-propyl) phosphate	100	2	182	water	
28.1	Heavy metals	Lead	75 000	2	28	sediment	
28.2	Heavy metals	Nickel	75 000	2	28	sediment	
28.3	Heavy metals	Nickel + Lead	150 000	2	28	sediment	
31	Herbicide	2,4-dichlorophenol	300	2	21	water	
33	Pesticide	Esfenvalerate	1.4	2	28	water	
34	Herbicide	Roundup	280	2	14	water	
35.1	Pharmaceuticals	Ethinylestradiol	2.96	2	28	water	
35.2	Pharmaceuticals	Tamoxifen	3.72	2	28	water	
35.3	Others	Methyldihydrotestosterone	3.05	2	28	water	
35.4	Pharmaceuticals	Flutamide	2.76	2	28	water	
38.1	Pharmaceuticals	Megestrol acetate	0.33	2	21	water	
38.2	Pharmaceuticals	17α-ethinylestradiol	0.01	2	21	water	
38.3	Composite stressor	Megestrol acetate + 17α-ethinylestradiol	0.34	2	21	water	
39	Flame retardants	Polybrominated diphenyl ethers	0.005	1	7	water	
41	Antifoulants	3,3′-Diindolylmethane	8.46	2	28	water	
42	Flame retardants	1,3-dichloro 2-propyl phosphate	100	2	183	water	Exposure at embryo stage
43	Flame retardants	decabromodiphenyl ethane	500	2	30	water	
46	Microplastics	Polyethylene spheres	500000	2	5	water	

47	Microplastics	Polyethylene fibers	364.14	2	21	food	Exposure to stress not continuously
49.1	Pharmaceuticals	17 alpha-ethinylestradiol	0.125	2	28	water	
49.2	Personal care products	Sodium Lauryl Sulfate	4000	2	28	water	
49.3	Composite stressor	17 alpha-ethinylestradiol + Sodium lauryl sulfate	4125	2	28	water	
51	Others	Nonylphenol	251	2	25	water	
52.1	Microplastics	polyester fibers	2000	2	63	food	
52.2	Others	cotton fibers	2000	2	63	food	
53.1	Pharmaceuticals	Sulfamethazine	5000	2	30	food	
53.2	Nanoparticles	Plain polystyrene (PS) nanoplastic fragments	5000	2	30	food	
55	Plasticisers	Di-2-ethylhexyl phthalate	50	2	7	water	Has a second stressor; temperature
56	Hydrocarbons	benzo[a]pyrene	3.8	2	24	water	
57	Microplastics	virgin polystyrene microplastics	200	2	150	water	
58	Plasticisers	di(2-ethylhexyl) phthalate	10	2	1	water	
59	Flame retardants	Trichloropropyl phosphate	32.76	2	42	water	
60	Pharmaceuticals	Methamphetamine	100	2	90	water	
61	Heavy metals	Cadmium	100	2	28	water	
63	Others	Nonylphenol	100	2	21	water	
64	Pesticide	Glyphosate	0	2	21	water	
65.1	Biocide	Tributyltin	24.4	2	2	water	
65.2	Pesticide	Diazinon	326	2	2	water	
65.3	Biocide	Tributyltin	3.9	2	16	water	
65.4	Pesticide	Diazinon	73.6	2	16	water	
67	Others	Perfluorobutanesulfonate	9.5	2	182	water	
68	Others	benzotriazole	1000	2	60	water	
69	Hydrocarbons	benzo[a]pyrene	50	2	7	water	
70	Pharmaceuticals	17α-Ethynylestradiol	0.113	2	21	water	survival data reported after a "bacterial challenge"
71.1	Hydrocarbons	mechanically dispersed oil	67 000	2	213	water	Exposure for 2 days, then measurement of effect at 7 months
71.2	Hydrocarbons	chemically dispersed oil	67 000	2	213	water	
71.3	Hydrocarbons	burned oil residues	67 000	2	213	water	
73	Heavy metals	Cadmium	2.02	2	4	water	Concern: Concentration for males (2.02) and

							females (1.6) different
74	Heavy metals	Copper	25	2	25	water	
75	Pharmaceuticals	17α-Ethynylestradiol	0.04	1	165	water	1-month laboratory exposure, 4-month transplantation in situ (Tunisia).
77	Nanoparticles	Gold nanoparticles	11.4	2	1	water	
78	Hydrocarbons	benzo[a]pyrene	25	2	60	water	
79	Pharmaceuticals	17α-ethinylestradiol	0.037	2	21	water	
81	Antibiotics	Triclocarban	2.5	2	60	water	
82	Hydrocarbons	Kobbe crude oil	1.14	2	217	food	
83	Hydrocarbons	marine diesel	6370	2	7	water	
84	Antifoulants	4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one	9.86	2	28	water	
85.1	Hydrocarbons	Pyrene + phenanthrene + fluoranthene	0.116	2	1	food	
85.2	Others	polychlorinated biphenyls 153	0.216	2	1	food	
86	Hydrocarbons	Phenanthrene	1000	2	10	water	
89	Flame retardants	1,2,5,6-tetrabromocyclooctane	1000	2	21	food	
90.1	Antifoulants	4,5-dichloro-2-n-octyl-4-isothiazolin-3-one	2.55	1	28	water	
90.2	Antifoulants	Butenolide	2.31	1	28	water	
91	Biocide	Tributyltin	0.8	2	252	water	
92	Flame retardants	bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate + 2-ethylhexyl-2,3,4,5 tetrabromobenzoate (TBPH + TBB)	2896	2	21	food	
94.1	Antifoulants	Butenolide	2.3	2	28	water	
94.2	Antifoulants	4,5-dichloro-2-n-octyl-4-isothiazolin-3-one	2.5	2	28	water	
95	Flame retardants	PBDE-47	high dose	1	21	food	Concern: couldn’t find precise pollutant concentration
96	Pesticide	dichloro-diphenyl-trichloroethane	0.25	2	31	water	
97.1	Pharmaceuticals	acetylsalicylic acid	100	2	14	water	
97.2	Pharmaceuticals	diclofenac	100	2	14	water	
97.3	Pharmaceuticals	ibuprofen	100	2	14	water	
97.4	Pharmaceuticals	mefenamic acid	100	2	14	water	
97.5	Pharmaceuticals	naproxen	100	2	14	water	

97.6	Pharmaceuticals	ibuprofen	10	2	14	water	
98.1	Pharmaceuticals	17β-Estradiol	10	2	10	injection	
98.2	Others	Bisphenol A	250	2	10	injection	
99	Others	Nonylphenol	2	2	1	water	
101.1	Others	Polychlorinated biphenyls mix	33	2	21	food	
101.2	Others	3,3′,4,4′,5-pentachlorobiphenyl	0.33	2	21	food	
102	Others	Bisphenol S	100	2	35	water	
104.1	Heavy metals	Zinc Chloride	1000	2	1	water	Concern: Males (750) and females (1000) different concentrations
104.2	Microplastics	Polystyrene microplastics beads	1000	1	1	water	
104.3	Heavy metals	Zinc Chloride	10 000	2	4	water	
104.4	Composite stressor	Zinc Chloride + Polystyrene microplastics beads	20000	1	1	water	
105	Pesticide	Organochlorine pesticide mixture	25	2	84	water	
106	Flame retardants	2,4,6-Tribromophenol	1.654	2	1	food	
107	Heavy metals	Mercury chloride	30	2	30	water	
108	Pharmaceuticals	levonorgestrel	1	2	21	water	Second stressor; warming
110.1	Hydrocarbons	pyrolitic	18.15	2	274	food	Concentration of the different hydrocarbons hard to decipher
110.2	Hydrocarbons	heavy fuel oil	4.66	2	274	food	
110.3	Hydrocarbons	light crude oil	20	2	274	food	
111.1	Microplastics	virgin polyethylene pre-production pellets	8	2	61	food	
111.2	Microplastics	polyethylene marine plastic	8	2	61	food	
112.1	Others	Bisphenol A	500	2	4	water	
112.2	Pharmaceuticals	Fadrozole	50	2	4	water	
115	Pesticide	Endosulfan	10	2	28	water	
116.1	Others	perfluorooctane sulfonate	10 000	2	6	food	
116.2	Others	perfluorooctanoic acid	10 000	2	6	food	
118	Flame retardants	Polybrominated diphenyl ethers mixture (DE-71)	50	2	120	water	
119	Composite stressor	Halogenated Organic Pollutants	1.1378	1	10	food	Attempted to add all the conc of "spiked leaves from S2"
120.1	Pesticide	Glufosinate-ammonium	20	1	60	sediment	

120.2	Pesticide	L-Glufosinate-ammonium	20	1	60	sediment	
121	Pesticide	Pendimethalin	0.1	2	152	water	males and females sampled from S1 to S5' were pooled since no sex variation
122	Others	Perchlorate	200	2	45	food	
124.1	Heavy metals	Cd(Cys)2	744	1	82	food	
124.2	Heavy metals	Cd(NO3)2	355	1	82	food	
125.1	Others	Bisphenol A	300	2	112	sediment	
125.2	Fungicide	Vinclozolin	300	2	112	sediment	
126	Personal care products	Methylparaben	10	2	28	water	
131	Others	Seleno-L-methionine	60	2	90	food	
132	Others	Seleno-L-methionine	60	2	60	food	
134	Pesticide	Glyphosate	12 060	2	10	water	
135	Pharmaceuticals	17β-trenbolone	0.004	2	21	water	
139	Pharmaceuticals	Fluoride	96 000	2	8	water	females and males grouped together since they found no sex effecrt
140	Pharmaceuticals	17β-trenbolone	0.006	2	21	water	
141	Others	potassium cyanide	50	2	28	water	
143	Composite stressor	Diluted leachate	183 780	2	21	water	
145.1	Pharmaceuticals	17α-ethinylestradiol	0.1	2	21	water	
145.2	Pharmaceuticals	Estrogenic compounds mix	1.74	2	21	water	
146	Composite stressor	Treated effluent water	432 598.80	1	4	water	
148.1	Heavy metals	Lead + Arsenic + Cadmium	97.09	1	4	water	
148.2	Composite stressor	Polluted site resuspended sediment	400 000.00	1	4	sediment	
148.3	Composite stressor	Less polluted site resuspended sediment	400 000.00	1	4	sediment	
149	Composite stressor	Oil sands process-affected water	19700	2	7	water	

Access to articles included – contains doi (online access to article) and citation of all the articles that contained information on all the studies included in this analysis

Table A3. *Information on how to access the studies used in this analysis such as "doi" and "citation"*

Study ID	**Doi**	**Citation**
9	10.1016/j.chemosphere.2020.126461	DeBofsky et al., 2020
11	10.1016/j.cbpc.2013.03.006	Pasquevich et al., 2013
14	10.1016/j.envpol.2014.04.017	Gismondi & Thomé, 2014
15	10.1016/j.aquatox.2021.105877	Thoré et al., 2021
16	10.1016/j.envpol.2022.119710	Nuchan et al., 2022
18	https://doi.org/10.1016/j.scitotenv.2019.135138	Guo et al., 2020
19	10.1007/s10646-022-02536-4	Castro et al., 2022
20.1	10.1007/s10646-015-1438-4	Horion et al., 2015
21.1	10.1016/j.envpol.2019.04.089	Xiang et al., 2019
23	10.1007/s10695-013-9887-2	X. Zhang et al., 2014
24	https://doi.org/10.1080/15533171003766550	Z.-X. Han et al., 2010
26	10.1016/j.chemosphere.2021.130827	Y. Li et al., 2021
27	10.1016/j.aquatox.2015.01.014	Q. Wang, Lam, Han, et al., 2015
28.1	10.1016/j.aquatox.2022.106347	Jesus et al., 2022
31	10.1016/j.aquatox.2011.11.006	Ma et al., 2012
33	10.1016/j.envpol.2015.09.035	Rodrigues et al., 2015
34	10.1007/s11356-020-11025-2	de Melo et al., 2020
35.1	10.1016/j.ygcen.2010.04.012	Massari et al., 2010
38.1	10.1016/j.envpol.2016.03.031	Hua et al., 2016
39	10.1016/j.envpol.2018.04.062	Chen et al., 2018
41	10.1021/acs.chemrestox.6b00074	Chen et al., 2016
42	10.1016/j.aquatox.2014.11.001	Q. Wang, Lam, Man, et al., 2015
43	10.1016/j.scitotenv.2022.160724	S. Wang et al., 2023
46	10.1016/j.scitotenv.2022.158576	McDaid et al., 2023

47	10.1002/etc.5456	DiBona et al., 2022
49.1	10.1016/j.jhazmat.2021.128058.	Lopes et al., 2022
51	10.1016/j.cbpc.2022.109270	Ardeshir et al., 2022
52.1	10.1016/j.scitotenv.2022.153077	Bunge et al., 2022
53.1	10.1016/j.ecoenv.2021.112820	Y. T. Zhang et al., 2021
55	10.1016/j.marpolbul.2021.112624	Mincarelli et al., 2021
56	10.1016/j.envpol.2021.117084	Yang et al., 2021
57	10.1016/j.jhazmat.2021.125537	Wang et al., 2021
58	10.1016/j.chemosphere.2020.129053.	Yin et al., 2021
59	10.1016/j.envpol.2020.115537.	Zhong et al., 2020
60	10.1016/j.watres.2020.116164.	Z. Wang et al., 2020
61	10.1080/17451000.2020.1805631	Sun et al., 2020
63	10.1016/j.cbpc.2020.108756.	Shirdel et al., 2020
64	10.1007/s11356-020-08985-w	Fabrello et al., 2020
65.1	https://doi.org/10.5109/4103893	Takai et al., 2020
67	10.1021/acs.est.9b01711.	Chen et al., 2019
68	10.1007/s11356-019-04201-6.	He et al., 2019
69	10.1016/j.fsi.2018.12.068.	Jiang et al., 2019
70	10.1016/j.aquatox.2018.07.019.	Ye et al., 2018
71.1	10.1016/j.marenvres.2018.09.005.	Bender et al., 2018
73	10.1007/s11356-017-9031-4.	Merad et al., 2018
74	10.1016/j.marenvres.2018.01.020.	Phillips & Rouchon, 2018
75	10.1007/s11356-017-0146-4.	Mezghani-Chaari et al., 2017
77	10.1016/j.envpol.2017.04.084.	Michalec et al., 2017
78	10.1007/s00343-017-6024-6.	Wu et al., 2017
79	10.1016/j.aquatox.2017.02.016.	Saaristo et al., 2017
81	10.1016/j.ecoenv.2016.10.013.	Barros et al., 2017
82	10.1016/j.aquatox.2016.10.005.	Bender et al., 2016
83	10.1007/s11356-016-6572-x.	Geraudie et al., 2016

84	10.1021/acs.est.6b01137.	Chen, Ye, et al., 2016
85.1	10.1016/j.aquatox.2015.08.010.	Michalec et al., 2017
86	10.1016/j.aquatox.2015.10.011.	dos Reis et al., 2015
89	10.1016/j.aquatox.2015.01.018.	Saunders, Podaima, Wiseman, et al., 2015
90.1	10.1021/es5046748.	Chen et al., 2015
91	10.1080/19768354.2014.995225.	Park et al., 2015
92	10.1016/j.aquatox.2014.10.019.	Saunders, Podaima, Codling, et al., 2015
94.1	10.1016/j.aquatox.2014.01.023.	Chen et al., 2014
95	10.1016/j.aquatox.2013.12.009.	Deane et al., 2014
96	https://doi.org/10.1007/s13131-014-0524-4	Xu & Liu, 2014
97.1	10.1016/j.jhazmat.2013.03.036.	Ji et al., 2013
98.1	10.1007/s00343-013-2232-x.	F. Li et al., 2013
99	10.1016/j.aquatox.2010.12.017.	Cailleaud et al., 2011
101.1	10.1016/j.marpolbul.2011.01.015.	Nakayama et al., 2011
102	10.1016/j.envpol.2022.120820	Z. Li et al., 2023
104.1	10.1016/j.jhazmat.2021.126652	Y. Lee et al., 2021
105	10.1016/j.jhazmat.2021.125956	H. Lee et al., 2021
106	10.1093/toxsci/kfz155.	Trexler et al., 2019
107	10.1016/j.chemosphere.2017.08.148.	Q.-L. Chen et al., 2017
108	10.1016/j.envpol.2017.05.090.	Cardoso et al., 2017
110.1	10.1007/s11356-014-2629-x.	Vignet et al., 2014
111.1	10.1016/j.scitotenv.2014.06.051.	Rochman et al., 2014
112.1	10.1007/s00244-014-0047-1.	Shanthanagouda et al., 2014
115	10.1007/s10646-012-0907-2.	Shao et al., 2012

116.1	10.5897/AJB11.1767	Z.-X. Han et al., 2011
118	10.1016/j.marpolbul.2011.04.001.	X. B. Han et al., 2011
119	10.1021/acs.est.9b07585.	Huang et al., 2020
120.1	10.1016/j.chemosphere.2019.04.007.	L. Zhang et al., 2019
121	10.1016/j.aquatox.2018.07.002.	Danion et al., 2018
122	10.1007/s11356-016-6124-4.	Qin et al., 2016
124.1	10.1007/s10646-011-0762-6.	Calhôa et al., 2012
125.1	10.1007/s00244-010-9474-9.	Lemos et al., 2010
126	10.1016/j.jes.2022.07.012.	Hu et al., 2023
131	10.1016/j.chemosphere.2020.127455.	Mo et al., 2020
132	10.1016/j.envpol.2019.07.082.	Mo et al., 2019
134	10.1007/s11270-017-3536-9.	Ferreira-Junior et al., 2017
135	10.1016/j.aquatox.2015.09.019.	Tomkins et al., 2016
139	10.1007/s00244-013-9892-6.	Aguirre-Sierra et al., 2013
140	10.1371/journal.pone.0062782.	Saaristo et al., 2013
141	10.1016/j.ecoenv.2013.01.006.	Authman et al., 2013
143	10.1016/j.ecoenv.2016.09.022	Gong et al., 2017
145.1	10.1016/j.envpol.2012.01.018	Urbatzka et al., 2012
146	10.1007/s11356-021-15633-4	Ekelund Ugge et al., 2022
148.1	10.1016/j.marpolbul.2022.113854	Das et al., 2022
149	10.1021/es3019258	Y. He et al., 2012

Appendix 5. Raw data used for producing the random-effects models and forest plots

Table A4. *Raw data used for the random-effects models on survival between females and males. Pink refers to data on females, and cornflower blue to data on males*

study_ID	sample_size_ control_f	sample_size_control_m	sample_size_exp_f	sample_size_exp_m	mean_control_f (%)	mean_control_m (%)	mean_exp_f (%)	mean_exp_m (%)	sd_f_control	sd_m_control	sd_f_exp	sd_m_exp	LogRR (yi)	LogRR_var (vi)	Notes
41	60	60	60	60	100	100	82.1	100	0	0	6.24	0	0.19723217	0.00004814	Data in table, converted SEM to SD
70	42	42	42	42	50.49	92.83	38.84	63.64	NA	NA	NA	NA	-0.115203	0.005569211	No SD reported - mean imputation
81	40	40	40	40	100	100	96	82	6	17	27.16	31.6	-0.1576289	0.003263109	Used plot digitizer for SD
96	30	30	30	30	73	67	53	27	10	11.2	11.73	22.72	-0.5886882	0.013396384	Used plot digitizer for SD
97.1	4	4	4	4	100	100	75	100	0	0	0	0	0.28768207	0	SD is 0 due to no replicates
97.2	4	4	4	4	100	100	100	75	0	0	0	0	-0.2876821	0	SD is 0 due to no replicates
97.3	4	4	4	4	100	100	100	100	0	0	0	0	0	0	SD is 0 due to no replicates
97.4	4	4	4	4	100	100	100	75	0	0	0	0	-0.2876821	0	SD is 0 due to no replicates
97.5	4	4	4	4	100	100	75	75	0	0	0	0	0	0	SD is 0 due to no replicates
124.1	10	10	10	10	100	80	30	10	NA	NA	NA	NA	-0.8754687	0.005569211	No SD reported - mean imputation
124.2	10	10	10	10	100	80	90	30	NA	NA	NA	NA	-0.8754687	0.005569211	No SD reported - mean imputation

Table A5. *Raw data used for the random-effects models on LC$_{50}$ between females and males. Pink refers to data on females, and cornflower blue to data on males*

Study_ID	**sample_size_f**	**sample_size_m**	**mean_exp_f**	**mean_exp_m**	**sd_f**	**sd_m**	**LogRR (yi)**	**LogRR_var (vi)**
65.1	8	8	7.69	7.16	1.39	1.41	-0.071410803	0.008991071
65.2	8	8	216.00	164.00	1.79	8.97	-0.27541198	0.000382537
104.3	10	10	2713.00	2219.00	587.68	610.07	-0.20099839	0.012250863
104.4	10	10	2503.00	1643.00	682.83	477.14	-0.420966173	0.015875868
139	5	5	42700.00	42700.00	4794.45	4794.45	0	0.005042921

Table A6. *Raw data used for the random-effects models on sex ratio in the control versus experimental groups.*

study_ID	**sample_size_control**	**sample_size_exp**	**mean_control**	**mean_exp**	**SD_control**	**SD_exp**	**LogRR (yi)**	**LogRR_var (vi)**	**Notes**
19	60	60	1.50	1.10	NA	NA	-0.310154928	0.007012731	No info on SD - used imputation
33	35	35	1.08	2.50	NA	NA	0.839329691	0.007012731	No info on SD - used imputation
65.3	20	20	2.33	5.50	1.44	1.41	0.858879825	0.022482235	Got SD from author
65.4	20	20	2.00	1.50	1.38	1.00	-0.287682072	0.045923835	Got SD from author
67	450	450	0.83	1.28	0.15	0.32	0.433189656	0.000216033	Used plot digitizer for SD - fig reporting SEM
78	150	150	0.63	0.40	0.07	0.06	-0.446287103	0.000200384	Used plot digitizer for SD
91	186	150	1.00	1.54	0.04	0.04	0.431782416	0.000011694	
122	50	50	1.00	0.40	0.04	0.09	-0.916290732	0.00104125	Used plot digitizer for SD
131	300	300	0.54	0.37	0.09	0.07	-0.378066134	0.000211901	SD reported in Table S3
134	25	25	0.58	0.72	NA	NA	0.213110601	0.007012731	No info on SD - used imputation
148.1	334	158	0.57	0.58	0.01	0.04	0.006968669	0.000026265	Used plot digitizer for SD
148.2	334	233	0.57	0.39	0.01	0.02	-0.377877152	0.000011301	Used plot digitizer for SD
148.3	334	281	0.57	0.53	0.01	0.01	-0.081369345	0.000002414	Used plot digitizer for SD

Appendix 6. Sources that aided in data analysis and access to RStudio code

Sources that aided in data analysis and RStudio code

https://www.sthda.com/

https://r-charts.com/

https://ourcodingclub.github.io/

https://www.campbellcollaboration.org/calculator/

RStudio code

```
# Libraries needed
library(readr)
library(metafor)
library(dplyr)
library(ggplot2)
library(maps)
library(mapdata)
library(ggpubr)
library(tidyr)
library(tidyverse)

# Note: Not showing code for upload of csv. files and minor cleanup of data

# World map code
(Map_thesis_3 <- ggplot() +
  geom_polygon(data = world_map_3, aes(x = long, y = lat, group = group),
         fill = "grey80", size = 0.2) +
  geom_point(data = Map_2, aes(x = Longitude, y = Latitude, color = Habitats),
        size = 2.5, alpha = 0.8) +
  ylim(-100, 100) +
  scale_color_manual(values = c("Freshwater" = "#A7EEFF",
                 "Marine" = "#9EB2FF",
                 "Terrestrial" = "#C8EFC3")) +
  labs(x = "Longitude",
     y = "Latitude",
     color = "Habitats") +
  theme_classic() +
  theme(axis.text.y = element_text(size = 10),
     axis.title.x = element_text(size = 14, face = "bold"),
     axis.title.y = element_text(size = 14, face = "bold"),
     legend.text = element_text(size = 10, face = "bold"),
```

```
          legend.title = element_text(size = 12, face = "bold"),
          legend.background = element_rect(fill='transparent'),
          legend.box.background = element_rect(fill='transparent')))

(Histogram_map <- ggplot(world_map_3, aes(x = Latitude, fill = Habitats)) +
    geom_histogram(binwidth = 4) +
    xlim(-100, 100) +
    labs(y = "Frequency") +
    theme_classic() +
    scale_fill_manual(values = c("#A7EEFF", "#9EB2FF", "#C8EFC3")) +
    coord_flip() +
    theme(axis.title.y = element_blank(),
          axis.title.x = element_text(size = 14, face = "bold"),
          legend.text = element_text(size = 10),
          legend.title = element_text(size = 12, face = "bold"),
          panel.background = element_rect(fill='transparent'),
          plot.background = element_rect(fill='transparent', color=NA),
          panel.grid.major = element_blank(),
          panel.grid.minor = element_blank(),
          legend.background = element_rect(fill='transparent'),
          legend.box.background = element_rect(fill='transparent')))

# Arranging the plots
ggarrange(Map_thesis_3, Histogram_map,
          labels = c("A", "B"),
          common.legend = TRUE, legend = "bottom",
          widths = c(2, 1),
          ncol = 2, nrow = 1, align = "h")

# Organise data for animals, habitat, origins plot
custom_order_animals <- c("Fish", "Crustacean", "Mollusc", "Insect", "Amphibian", "Reptile", "Mammal", "Echinoderm", "Annelid")

habitat_animals_count <- habitat_animals %>%
  count(Groups, Habitats) %>%
  mutate(Groups = factor(Groups, levels = custom_order_animals))

animals_origin_count <- animals_origin %>%
  count(Groups, Origin) %>%
  mutate(Groups = factor(Groups, levels = custom_order_animals))

# Code for animals, habitat, origins plot
```

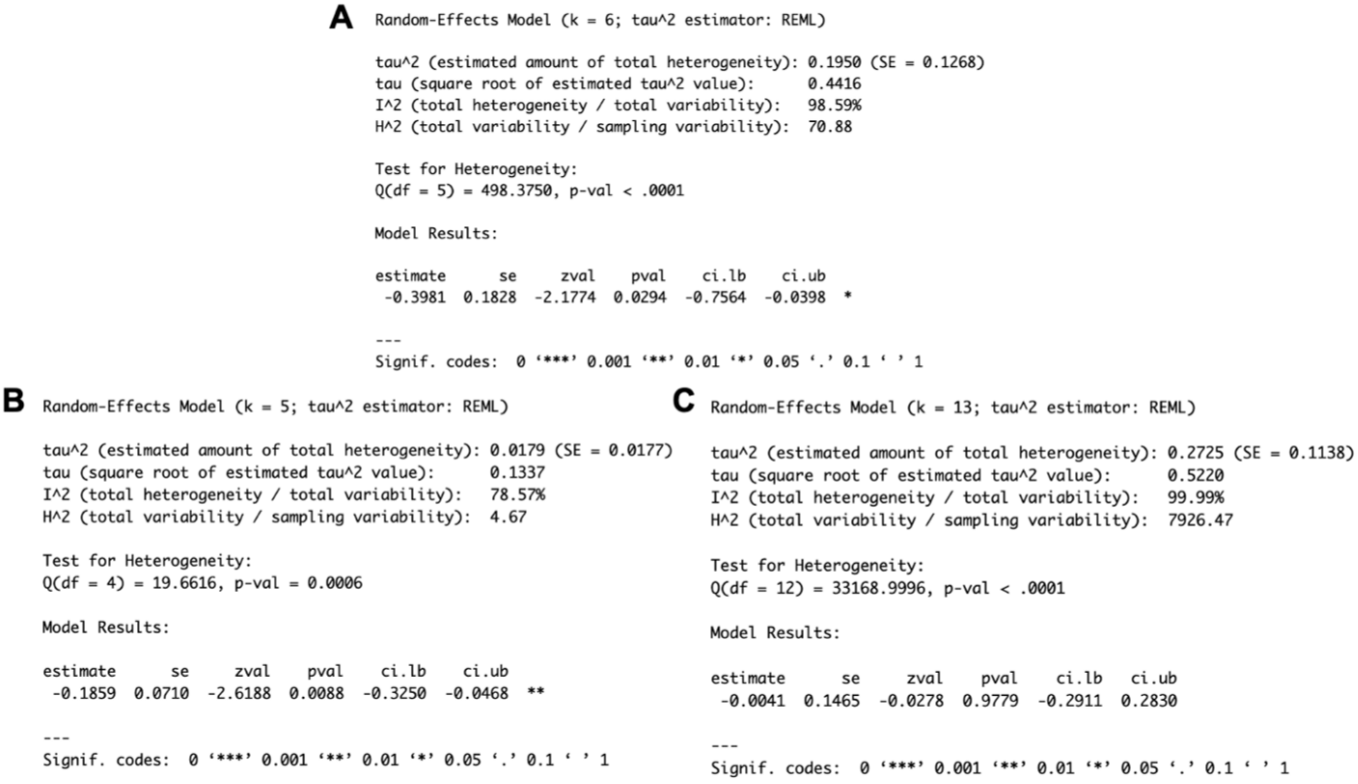
```
animal_habitat <- ggplot(habitat_animals_count, aes(x = Groups, y = n, fill = Habitats)) +
  geom_bar(stat = "identity") +
  labs(x = "Animal groups", y = "Frequency") +
  scale_y_continuous(breaks = seq(0, 90, by = 5)) +
  theme_classic() +
    scale_fill_manual(values = c("#A7EEFF", "#9EB2FF", "#C8EFC3")) +
ylab("Frequency\n") +
xlab("\nAnimal Groups") +
 theme(axis.text.x = element_text(size = 10, angle = 45, hjust = 1, vjust = 1, margin = margin(t = 10)),
        axis.text.y = element_text(size = 10),
        axis.title = element_text(size = 12, face = "bold"),
        legend.text = element_text(size = 10),
        legend.title = element_text(size = 12, face = "bold"),
        panel.background = element_rect(fill = 'transparent'),
        plot.background = element_rect(fill = 'transparent', color = NA),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        legend.background = element_rect(fill = 'transparent'),
        legend.box.background = element_rect(fill = 'transparent'))

animal_origins <- ggplot(animals_origin_count, aes(x = Groups, y = n, fill = Origin)) +
  geom_bar(stat = "identity") +
  labs(x = "Animal groups", y = "Frequency") +
  scale_y_continuous(breaks = seq(0, 90, by = 5)) +
  theme_classic() +
    scale_fill_manual(values = c("grey60", "grey80")) +
ylab("Frequency\n") +
xlab("\nAnimal Groups") +
 theme(axis.text.x = element_text(size = 10, angle = 45, hjust = 1, vjust = 1, margin = margin(t = 10)),
        axis.text.y = element_text(size = 10),
        axis.title = element_text(size = 12, face = "bold"),
        legend.text = element_text(size = 10),
        legend.title = element_text(size = 12, face = "bold"),
        panel.background = element_rect(fill = 'transparent'),
        plot.background = element_rect(fill = 'transparent', color = NA),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        legend.background = element_rect(fill = 'transparent'),
        legend.box.background = element_rect(fill = 'transparent'))

# Arranging the plots
ggarrange(animal_habitat, animal_origins,
```

```
          labels = c("A", "B"),
          legend = "bottom",
          ncol = 2, nrow = 1, align = "h")

# Code for the forest plots on survival. Only showing code for survival as the code for LC50 and sex ratios were almost identical
# Survival removing Ji et al., 2013 as these dont have variance (actually)
read_csv("Survival_both_omitted.csv")
Survival_both_omitted$Author_Year <- paste(Survival_both_omitted$author, Survival_both_omitted$year, sep = " "
# Random-effects meta-analysis
Survival_res_om <- rma(yi, vi, data = Survival_both_omitted, method = "REML", weighted = TRUE)

# Forest plot
forest(Survival_res_om,
       xlim = c(-10, 10),
       cex = 1,
       col = "#FFBDD5",
       xlab = "Log Risk Ratio (LogRR)",
       header = "Author(s) and Year",
       slab = Survival_both_omitted$Author_Year,
       mlab=mlabfun("RE Model", Survival_res_om))

# Results
print(Survival_res_om)

# Sensitivity analysis and publication bias
funnel(Survival_res_om)
confint(Survival_res_om)
inf_survival_om <- influence(Survival_res_om)
print(inf_survival_om)
plot(inf_survival_om)
regtest(Survival_res_om)

# Code showing how I produced the plot for underlying mechanisms for freshwater fish. Near identical codes were applied for all the other realms and species

# Filter data
fresh_fish <- Mechanisms_thesis %>%
  filter(Habitats == "Freshwater", Groups == "Fish")

# Reshape data from wide to long format
fresh_fish_long <- fresh_fish %>%
```

```
  pivot_longer(cols = c(Gene_exp_all, Hormone_all, E2.T_ratio, E2.11.KT_ratio, Intersexuality_..., Reactive_oxygen_species, Protein_carbonyl_content, Nutrients, Microarray_genes, Gene_ontology, KEGG, Differentially_expressed_genes, Differentially_expressed_proteins, Protein_interactive_network, Integrated_biomarker_response, Enzyme_all, Protein_all, C1, C2, C3, C4, Amino_acid, Lipids, Lipid_peroxidation, NMID, EPA, DHA, MDA, Behaviour_all, Gut_micro_all, Accumulation_all, Burden, Clearance, Bioamplification_factor, Body_length, Body_weight, Brain_weight, Gonad_weight, Liver_weight, Eyes_weight, Heart_weight, Growth, Deformity, MXR, Metabolites, Metabolism, Energy_reserves, glucose, insulin, Mitochondrial_ATP, DNA_fragmentation, DNA_damage, DNA_breaks, Emergence_time, K.Factor, BSI, LSI, SSI, HSI, GSI, Gametogenesis_stages, Gamete_count, Gonadal_index, Gonadal_maturity, Maturity_index, GSH.GSSG_ratio, metallothionein, Hist_all, Antioxidant, Plasma_protein_lvls, Monocytes.Leucocytes_..., Respiratory_burst, Phagocytic_activity, plasma_immune_molecules, plasma_cytokines, lymphocytes, granulocytes, leucocyte, Phagocytosis, Leucocyte_mortality_..., Hematocrit, Erythrocytes, Stimulation_index, Iron_overload, immune_competence_index, reproductive_competence_index, Feeding, P_gp_Transport), # Add all relevant columns here
               names_to = "Category",
               values_to = "Response") %>%
  drop_na(Response)

# Count occurrences of "Yes" and "No" for each category
fresh_fish_sum <- fresh_fish_long %>%
  group_by(Category, Response) %>%
  summarise(Count = n(), .groups = "drop")

# Taking out irrelevant rows – did this manually, saw which rows had no data
fresh_fish_sum_1.0 <- fresh_fish_sum[-c(1,4,6,8,10,12,13,16,19,21,22,24,26,28,30,32,33,34,36,37,39,41,42,44,47,48,49,50,53,55,57,58,60,63,64,66,69,71,73,74,75,77,79,82,84,86,89,92,93,94,95,98,100,102,104,106,108,110,113,114,115,117,119,121,123,124,125,127,128,129,131,133,136,137,139,142,143,145,147,149,151,152,154,156,158,160,161,162), ]

# Creating the stacked bar plot for freshwater fish
ultimate_fresh_fish <- ggplot(fresh_fish_sum_1.0, aes(x = Category, y = Count, fill = Response)) +
  geom_bar(stat = "identity", position = "stack") +
  ylim(0, 25) +
  coord_flip() +
  labs(x = "Mechanistic Category", y = "Count") +
  theme_classic() +
  scale_fill_manual(values = c("Yes" = "#A7EEFF", "No" = "grey80"), name = "Effect of Sex") +
  theme(axis.text.y = element_text(size = 10),
        axis.text.x = element_text(size = 10),
        axis.title = element_text(size = 20, face = "bold"),
        legend.text = element_text(size = 12),
        legend.title = element_text(size = 14, face = "bold"),
```

```
        legend.position = "bottom",
        panel.background = element_rect(fill='transparent'),
        plot.background = element_rect(fill='transparent', color=NA),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        legend.background = element_rect(fill='transparent'),
        legend.box.background = element_rect(fill='transparent'))

plot(ultimate_fresh_fish)
```

Appendix 7. Results from the random-effects models



A

```
Random-Effects Model (k = 6; tau^2 estimator: REML)

tau^2 (estimated amount of total heterogeneity): 0.1950 (SE = 0.1268)
tau (square root of estimated tau^2 value):      0.4416
I^2 (total heterogeneity / total variability):   98.59%
H^2 (total variability / sampling variability):  70.88

Test for Heterogeneity:
Q(df = 5) = 498.3750, p-val < .0001

Model Results:

estimate      se     zval    pval    ci.lb    ci.ub
 -0.3981  0.1828  -2.1774  0.0294  -0.7564  -0.0398  *

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

B

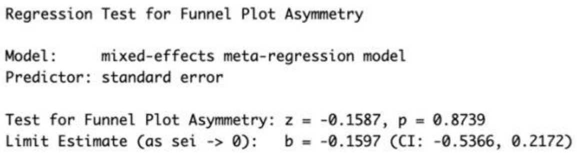
```
Random-Effects Model (k = 5; tau^2 estimator: REML)

tau^2 (estimated amount of total heterogeneity): 0.0179 (SE = 0.0177)
tau (square root of estimated tau^2 value):      0.1337
I^2 (total heterogeneity / total variability):   78.57%
H^2 (total variability / sampling variability):  4.67

Test for Heterogeneity:
Q(df = 4) = 19.6616, p-val = 0.0006

Model Results:

estimate      se     zval    pval    ci.lb    ci.ub
 -0.1859  0.0710  -2.6188  0.0088  -0.3250  -0.0468  **

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

C

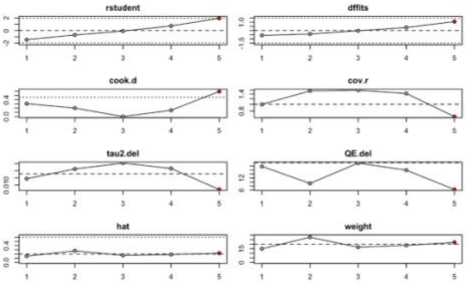
```
Random-Effects Model (k = 13; tau^2 estimator: REML)

tau^2 (estimated amount of total heterogeneity): 0.2725 (SE = 0.1138)
tau (square root of estimated tau^2 value):      0.5220
I^2 (total heterogeneity / total variability):   99.99%
H^2 (total variability / sampling variability):  7926.47

Test for Heterogeneity:
Q(df = 12) = 33168.9996, p-val < .0001

Model Results:

estimate      se     zval    pval    ci.lb   ci.ub
 -0.0041  0.1465  -0.0278  0.9779  -0.2911  0.2830

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

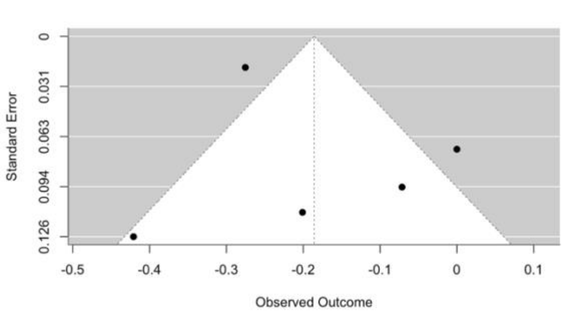
Figure A4. *Results from the random-effects on survival (**A**), LC50 (**B**) and sex ratios (**C**)*

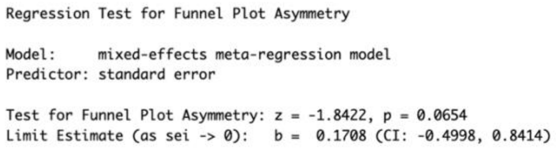
Appendix 8. Sensitivity analysis and publication bias



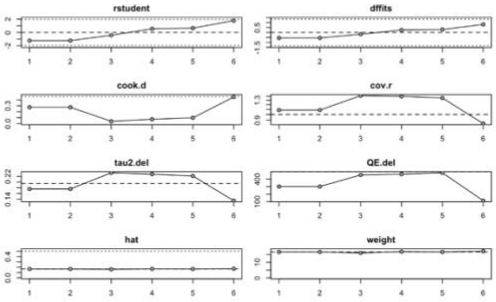
```
Regression Test for Funnel Plot Asymmetry

Model:     mixed-effects meta-regression model
Predictor: standard error

Test for Funnel Plot Asymmetry: z = -0.1587, p = 0.8739
Limit Estimate (as sei -> 0):   b = -0.1597 (CI: -0.5366, 0.2172)
```

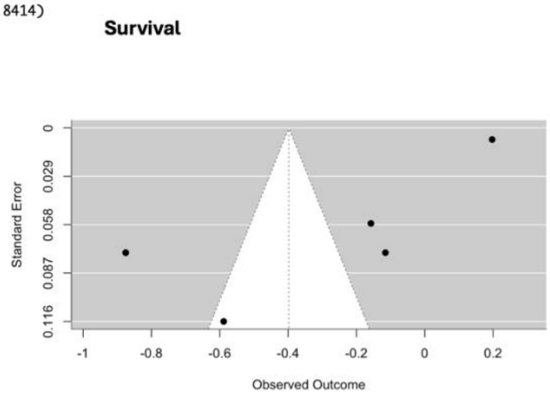


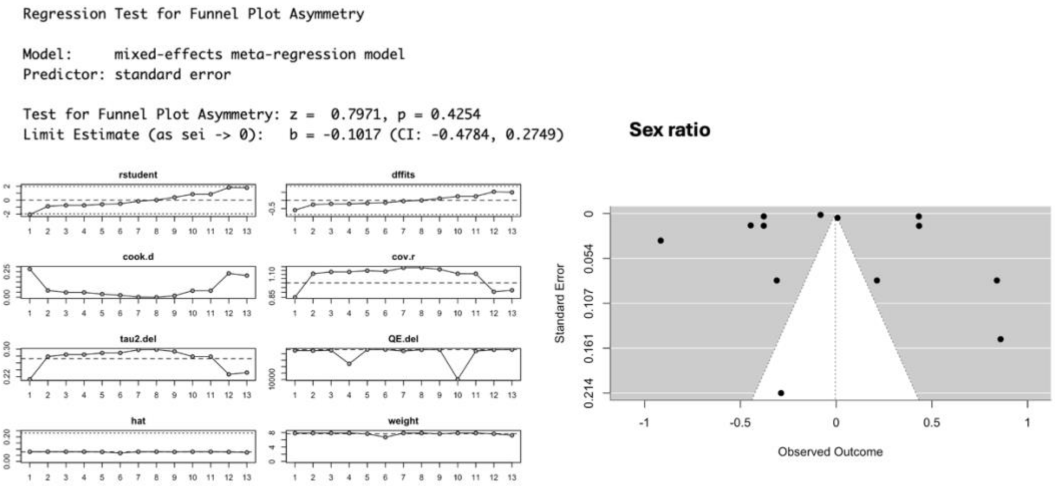




```
Regression Test for Funnel Plot Asymmetry

Model:     mixed-effects meta-regression model
Predictor: standard error

Test for Funnel Plot Asymmetry: z = -1.8422, p = 0.0654
Limit Estimate (as sei -> 0):   b =  0.1708 (CI: -0.4998, 0.8414)
```







```
Regression Test for Funnel Plot Asymmetry

Model:     mixed-effects meta-regression model
Predictor: standard error

Test for Funnel Plot Asymmetry: z =  0.7971, p = 0.4254
Limit Estimate (as sei -> 0):   b = -0.1017 (CI: -0.4784, 0.2749)
```

Sex ratio

Figure A5. *For survival (**top panel**), LC50 (**middle panel**) and sex ratios (**bottom panel**)*

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