Cover Page

Title:

A systematic evidence map and bibliometric analysis of the behavioural impacts of pesticide exposure on zebrafish.

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Abstract

Pesticides are indispensable in agriculture and have become ubiquitous in aquatic environments. Pesticides in natural environments can cause many negative impacts on aquatic species, ranging from mortality to sub-lethal physiological and behavioural changes. The complex sub-lethal impacts of pesticides are routinely tested on model species, with zebrafish (*Danio rerio)* being regularly used as a behavioural model. Although behavioural ecotoxicology research using zebrafish is increasing rapidly, we lack quantitative evidence to support which pesticides have been tested and how study designs are carried out. This shortcoming not only limits the deliberate planning for future primary studies to fill the knowledge gaps but also hinders evidence synthesis. To provide quantitative evidence of what pesticides are currently studied and what study designs are used, we combined a systematic evidence map approach and bibliometric analysis. This novel method has been coined research weaving and allows us to elicit gaps and clusters in our evidence base, whilst showing connections between authors and institutions. The methodology can be summarised in five primary steps: literature searching, screening, extraction, data analysis and bibliometric analysis. We identified four areas where research on the sub-lethal effects of pesticide exposure on zebrafish is lacking. First, some widely used pesticides, such as neonicotinoids, are understudied. Second, most studies do not report important elements of the study design, namely the sex and the life-stage of the zebrafish. Third, some behaviours, such as impacts of pesticide exposure on zebrafish cognition, are underexplored. And last, we revealed through the bibliometric analysis that most of the research is conducted in developed countries and there is limited cross country co-authorships. Upon identifying these gaps, we offer solutions for each limitation, emphasizing the importance of diverse global research output and cross-country co-authorships. Our systematic evidence map and bibliometric analysis provide valuable insights for helping to guide future research, which can be used to help support evidence-based policy decisions.

Keywords: fish, agrochemicals, behavior, pollution, toxicology

Introduction

Pesticides are extensively used worldwide for protection against agricultural pests and to control vectors of harmful and potentially fatal diseases (Tang et al., 2021; van den Berg et al., 2021). However, the environmental release of pesticides, together with additives, often results in their buildup within aquatic environments (Stehle and Schulz, 2015; Tang et al., 2021). Aquatic environments are vulnerable to pesticide pollution because they are highly sensitive to environmental changes (Hua and Relyea, 2014; Ippolito et al., 2012). Today, the application of pesticides is considered a significant contributor to biodiversity loss in aquatic ecosystems globally (Beketov et al., 2013; Bernhardt et al., 2017; Pope, 2014; Werner and Young, 2018).

In aquatic ecosystems, it is important to note that not all impacts of pesticide exposure result in mortality at environmentally relevant concentrations (Bertram et al., 2022). As a result, research efforts are intensifying to unravel sublethal impacts, with a particular focus on physiological biomarkers (Santana et al., 2022, 2021), and behavioural characteristics (Shuman-Goodier and Propper, 2016). In fact, animal behaviour can be extremely sensitive to changes in the environment, even at low pollutant concentrations. This is concerning given the role of animal behaviour as an integrative mechanism linking an organism's physiology and environment (Wong and Candolin, 2015). The sensitivity to change, combined with the role of behaviour as a link between physiology and the environment, underscores the importance of animal behaviour as a valuable endpoint to measure in aquatic ecotoxicology (Melvin and Wilson, 2013).

The zebrafish (*Danio rerio*) is the most widely used aquatic model species in the research on the sublethal impacts of pesticides on physiology and animal behaviour (Dai et al., 2014; Teame et al., 2019). This preference stems from several key characteristics of zebrafish, such as ease of manipulation (Avdesh et al., 2012), genetic homology with humans (Howe et al., 2013) and rapid embryonic development (Lawrence et al., 2012; Meyers, 2018). While all life stages of zebrafish offer valuable insight for aquatic ecotoxicology, the investigation of long-term impacts, as well as complex social behaviours such as aggression, requires consideration of post-larvae life stages (i.e., juvenile, adolescent and adult) (Zaluski et al., 2022). The significance of behaviour as a valuable endpoint, combined with the amenability of zebrafish to laboratory experimentation, has spurred an increasing trend of studies leveraging post-larvae zebrafish as a behavioural model in aquatic ecotoxicology (Gonçalves et al., 2020; Tao et al., 2022).

Despite the rapid advancements in post-larvae zebrafish behaviour studies investigating the impact of pesticide exposure, there are significant gaps in our understanding of this research. These gaps seem to arise for three main reasons. First, we do not know which pesticides have been thoroughly investigated and which remain largely disregarded. Second, we do not have a clear picture regarding the use of various study design elements, especially the characteristics of the zebrafish used (e.g., sex and life stage) and the aspects of pesticide application (e.g., dosage, exposure duration, and administration route). Third, we do not know which behavioural endpoints have been inadequately studied in the context of pesticide exposure. This is further complicated by the ambiguities and redundancies of the terminology. This lack of consensus highlights the need for evidence synthesis (i.e., systematic reviews and meta-analysis), which is already a recognized issue in behavioural ecotoxicology (Bertram et al., 2022).

To foster evidence synthesis within the literature, systematic evidence maps, also known as systematic maps (as they will be referred to from hereon), are useful. This is because systematic maps enable reviewers to effectively collate, describe, and catalogue evidence on a given topic (Clapton et al., 2009). Identification of available evidence on a given topic serves multiple purposes: it informs future research (James et al., 2016), supports environmental management strategies (Haddaway et al., 2016), and promotes evidence-based decision-making (Wolffe et al., 2019). These characteristics differ from a systematic review which primarily aims to answer a specific question by combining suitable data from multiple studies (Gough et al., 2012; James et al., 2016). Recently, a methodological advancement proposed the use of bibliometric analysis in combination with systematic mapping, named research weaving (Nakagawa et al., 2019). Research weaving enhances the utility of a systematic map by assessing the connectivity of research, revealing relationships between authors, countries, and institutions (Donthu et al., 2021). By providing insights into collaborative networks, intellectual trends, and research impact within a given research topic or scenario, research weaving can inform future research of gaps and opportunities.

Despite prior reviews in ecotoxicology and on zebrafish (Bertram et al., 2022; Cui et al., 2023; Gonçalves et al., 2020; Tao et al., 2022), we currently lack quantitative evidence on the shortcomings of the current evidence base. To address this shortcoming, this study conducts a systematic map and bibliometric analysis of pesticide exposure studies that use zebrafish as a behavioural model. The objectives of this study can be summarised as follows:

1) To investigate the types of pesticides and pesticide classes, both in terms of the chemical structure and target organism, that have been used in studies examining the effects of pesticide exposure on post-larval zebrafish behaviour.
2) To characterise the study set-ups (e.g., characteristics of pesticides such as concentrations and duration of exposure and characteristics of zebrafish such as life stage of exposure and sex) which have been employed to assess the effects of pesticide exposure on the behaviour of post-larval zebrafish.
3) To identify the extent the specific behaviours have been investigated in pesticide exposure studies that use post-larval zebrafish as a behavioural model.
4) To assess the research contributions of different countries and continents and describe the level of collaboration between authors from different countries.

Taken together, our systematic map and bibliometric analysis will help set the course for future primary research and evidence synthesis in behavioural ecotoxicology. Furthermore, the bibliometric analysis will help promote international communication by quantifying global research efforts and international co-authorships within studies investigating the impacts of pesticide exposure on zebrafish behaviour.

Methodology

This study conducts a systematic map and bibliometric analysis under the research weaving framework (Nakagawa et al., 2019). All relevant details to reproduce the conducted methodology are provided in the *Supplementary File 1.* The systematic map was conducted in line with the Reporting Standards for Systematic Evidence Synthesis (Haddaway et al., 2018; checklist provided in *Supplementary File 2*) and was preregistered on PROCEED (PROCEED-22-00047). Any deviations from the preregistration are noted in *Supplementary File 1, Section 1.1*. The complete data and code are provided in full within an external GitHub repository; see (https://github.com/KyleMorrison99/zebrafishSM). Additionally, a knitted Rmarkdown file can be found as a webpage: https://kylemorrison99.github.io/zebrafishSM/.The reporting of the methodology followed MeRIT to improve author contributions' granularity and accountability (Nakagawa et al., 2023).

Searching for articles

The complete details of the literature search are in *Supplementary File 1, Section 1.2*. ML and KM designed the search strategy. KM conducted main searches for relevant literature on three scientific literature databases: ISI Web of Science Core Collection, PubMed, and Scopus, on 29/09/2022 (accessed through the University of New South Wales, Sydney). To augment the main literature search, KM also conducted a backwards/forwards (snowballing) citation search using four reviews on ecotoxicology and zebrafish (Horzmann and Freeman, 2018; Rennekamp and Peterson, 2015; Saiki et al., 2021; Tao et al., 2022). In addition to searching for published literature, KM searched for relevant grey literature (i.e., unpublished), such as PhD theses, using the Bielefeld Academic Search Engine (BASE) to help account for publication bias. To identify relevant publications in the main search, our search strings consisted of three groups of terms. The first group encompassed terms representing pesticides and incorporated their chemical categories (e.g., organochlorine, organophosphate) and their intended targets (e.g., insecticide, fungicide). The second group incorporated terms associated with zebrafish, such as their scientific name, *Danio rerio*, and abbreviated names, such as “zfish”. The third group of keywords pertained to behaviour, such as exploration, sociality, and locomotion. The complete search strings used for all databases, along with articles used for the backwards/forwards search (i.e., snowballing citation search) can be found in the *Supplementary File 1, Section 1.2 & 1.3*. To ensure our search strategy was sensitive and effective, we utilised a set of ten pre-defined benchmark papers identified independently from the search process (*Supplementary file 1, Section 1.4*). All searches were performed in English. We thus acknowledge that limiting the search to a single language may introduce language bias within our systematic map.

Article Screening

KM, YY, MS, ML and SN conducted a two-step article screening, starting with titles and abstracts and then screening full texts. The screening process was implemented using Rayyan QCRI (Ouzzani et al., 2016). The complete inclusion/exclusion criteria for article screening are provided in the decision trees *(Supplementary File 1, Figures s1 & s2)* and were created following our PECO framework *(Supplementary File 1, Table s1)*. Articles that fulfilled each criteria in both decision trees were included in the systematic map. All literature screening was completed in duplicate by at least two independent reviewers (KM screened 100%, YY duplicate screened 80%, MS screened 10 %, ML 5%, and SN 5%), and conflicts between the screeners were resolved through discussion. If conflicts persisted, a mediator (SN) was involved in the conflict resolution. The initial conflict rates among reviewers' screening decisions were determined through a series of pilot tests and subsequently recorded in the registration (PROCEED-22-00047). We excluded articles with full text in languages other than English.

Data Extraction

A full report on all extracted data and metadata can be found in *Supplementary file 1, Section 1.6*. *W*e manually extracted data from each included study across four themes. All variables extracted are provided in the *Supplementary File 1, Table s4.* First, we extracted bibliometric information such as the author, publication year, DOI, journal, and a unique study ID. Second, we obtained information on the classes of each pesticide used in the included studies, recording the target organism class (e.g., insecticide, herbicide, or fungicide) and chemical structure class (e.g., organochlorine, organophosphate, or carbamate). Third, we recorded details of the study set-up, both for zebrafish characteristics such as the life stages that were exposed to the pesticides, life stages that behaviour was assessed (e.g., juvenile, adolescent, adult), and sexes of the zebrafish that were exposed to pesticides. Then, we extracted pesticide characteristics such as the route of pesticide exposure, number of pesticide dosages, lowest and highest concentrations of pesticide exposure and duration of exposure. Last, we extracted information about the behaviours evaluated during the experiment. To do so, we broadly categorized the behaviour (e.g., aggression or sociality). Then we specified how the behaviour was assessed in the assay (e.g., affiliation with a conspecific model or video, or affiliation with a live conspecific behind a barrier to assess sociality). Classifying specific behavioural assays ensured there was no overlap between behavioural classes. The classification of different behavioural outcomes was derived from (Martin et al., 2021). All data were extracted by KM, and the extraction was cross-checked by YY. Any conflicts between reviewers were resolved with a mediator (SN) present if the conflict persisted.

Bibliometric analysis

KM downloaded all bibliometric information, including country affiliations of the authors directly from Scopus on 03/03/2023 using included paper DOIs. KM completed the bibliometric analysis using VOSviewer (Eck and Waltman, 2010) and used bibliometric coupling for network construction. The units of analysis were document, source, author, organisation, and country. The counting method selected for the bibliometric coupling was full counting (i.e., each bibliometric coupling link has the same weight). For each of the created networks, we filtered for the largest set of connected unit items.

Data analysis

Except the results from the bibliometric analysis, all descriptive statistics and figures were created in the R Statistical Environment version 4.2.1 (R Core Team, 2022) using RStudio build 576 (RStudio Team, 2022). We used *ggplot2, version 3.4.1* (Wickham, 2016) and *circlize, version 0.4.15* (Gu et al., 2014) for visualizations (KM completed all data analyses and the analysis code was checked by YY).

Results and Discussion

Screening results and time trends

To identify studies on the impacts of pesticide exposure on zebrafish behaviour, we conducted a systematic literature search across multiple scientific literature databases (*see Supplementary File 1, Section 1.2*). Our initial search of the literature yielded a total of 1,788 unique articles. After screening titles, abstracts, and keywords, we excluded 1,646 articles that did not meet our predefined eligibility criteria (*Figure 1)*. We then screened the full texts of the remaining 142 articles. After the full-text screening, we excluded additional 59 articles (*Supplementary File 1, Table s3*). Ultimately, we included 83 articles in the systematic map.

The earliest pertinent study was conducted in 1995 (Steinberg et al., 1995). However, it was only in 2010 that articles investigating the impacts of pesticides on zebrafish behaviour became routinely conducted (*Figure 2)*. Since 2010, the number of articles has generally increased each year, although there have been some years of decrease. In 2021, the highest number of conducted articles (n = 16) was recorded. While the impacts of pesticides on aquatic ecosystems have been recognized for decades, our findings suggest that the use of zebrafish as a model for studying the effects of pesticide exposure has only recently become popular (*Figure 2*).

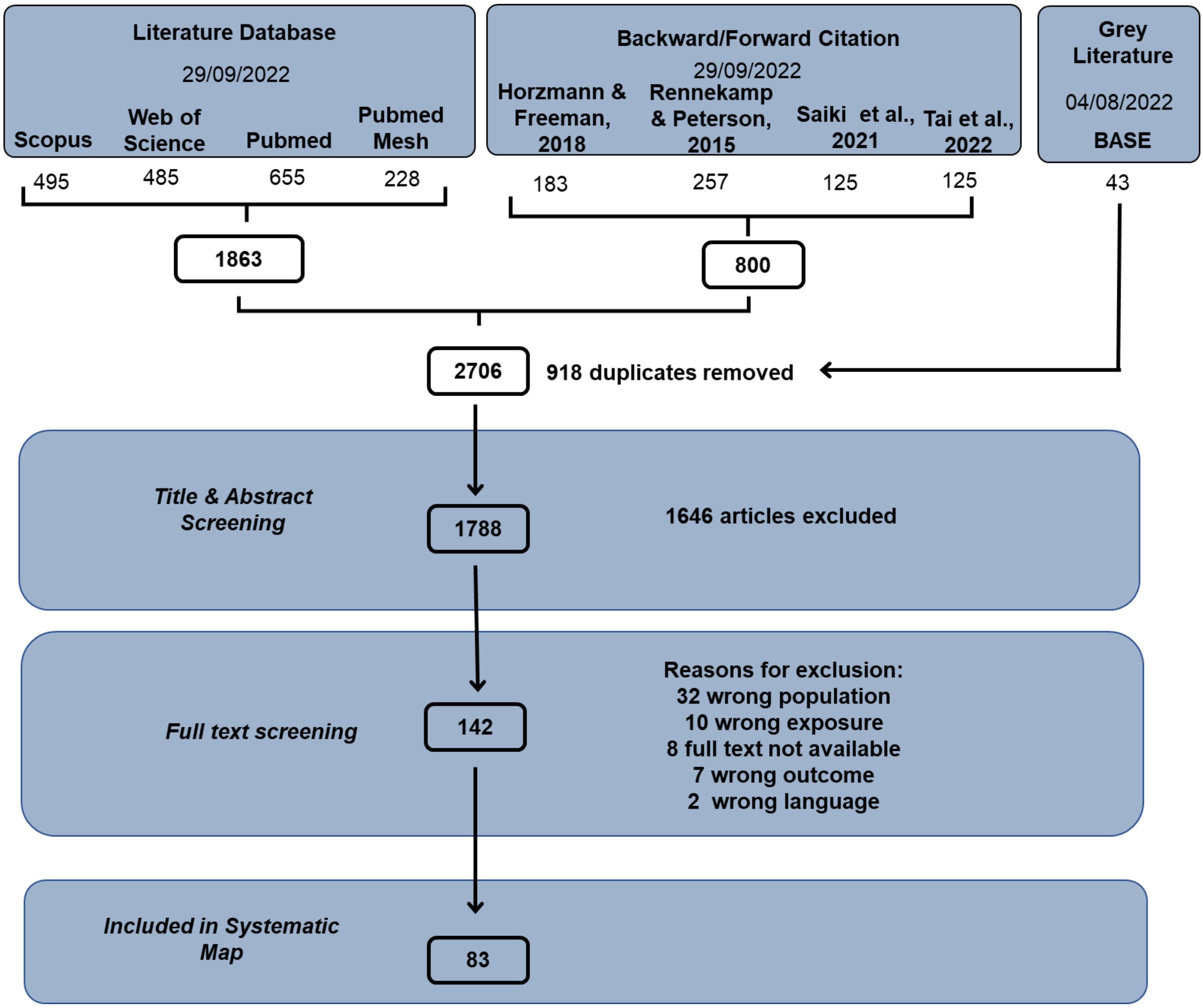

Figure 1- ROSES diagram (Haddaway et al., 2018) showing the number of articles included and excluded during each stage of the screening process.

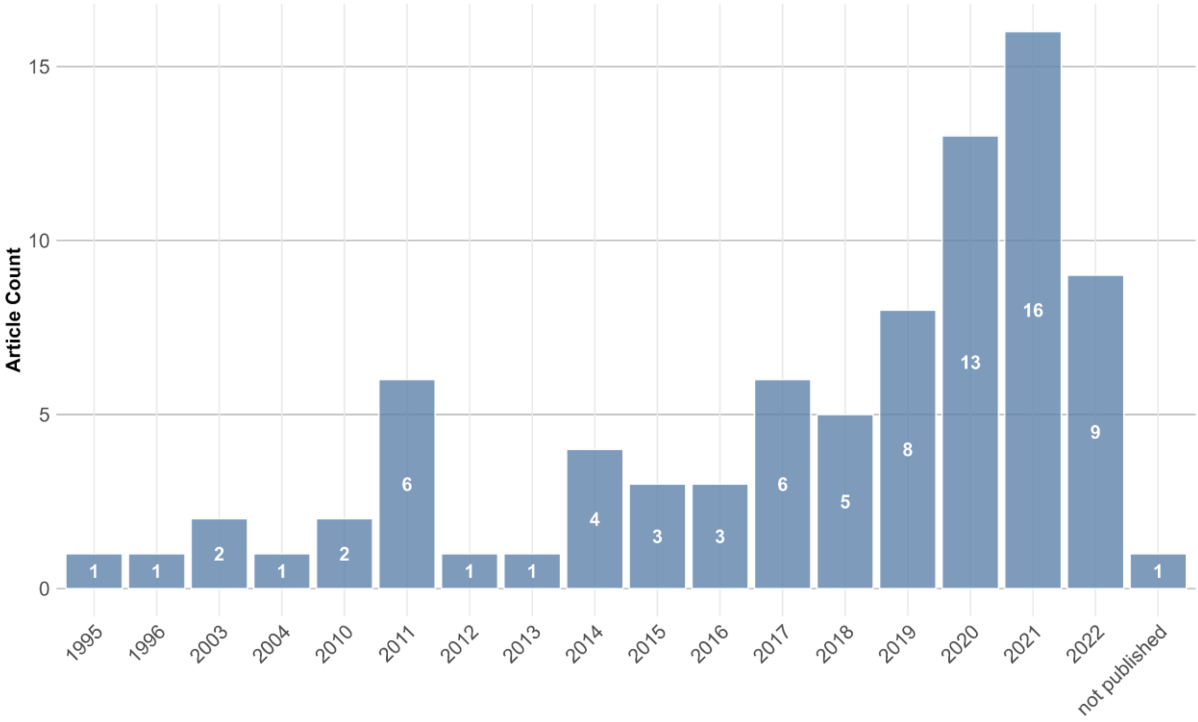

Figure 2– Bar chart showing the annual number of conducted pesticide exposure studies using zebrafish as a behavioural model.

Aim 1: Types of pesticide

The studied pesticide types are not distributed evenly in the literature on the impacts of pesticide exposure on zebrafish behaviour (*Figure 3A).* First, we found the most widely studied pesticides were deltamethrin (n = 11), chlorpyrifos (n = 11), rotenone (n = 10), glyphosate (n = 8) and atrazine (n = 8). Second, we identified that the most frequently studied target class was the insecticides (n = 57), followed by the herbicides (n = 28). And third, the most studied chemical classes found in the literature were the organophosphates (n = 24) and pyrethroids (n = 17).

Numerous pesticides and pesticide classes were understudied or have not been investigated at all. Notably, there is scarce evidence on the impact of neonicotinoids on zebrafish behaviour (Crosby et al., 2015; Guerra et al., 2021) *(Figure 3).* This is concerning given neonicotinoids' status as one of the most commonly used class of pesticides and the mounting evidence that they can indirectly impact vertebrates (Bass et al., 2015; Frank and Tooker, 2020; Hallmann et al., 2014; Li et al., 2020; Lopez-Antia et al., 2013; Yamamuro et al., 2019). The paucity of research on certain chemical classes may limit our understanding of which are likely to affect fish behaviour and, therefore, have downstream effect on aquatic ecosystems. In addition, we observed a clear lack of studies investigating the impacts of pesticide interactions, that is the impacts of multiple pesticides being present simultaneously (n = 2).

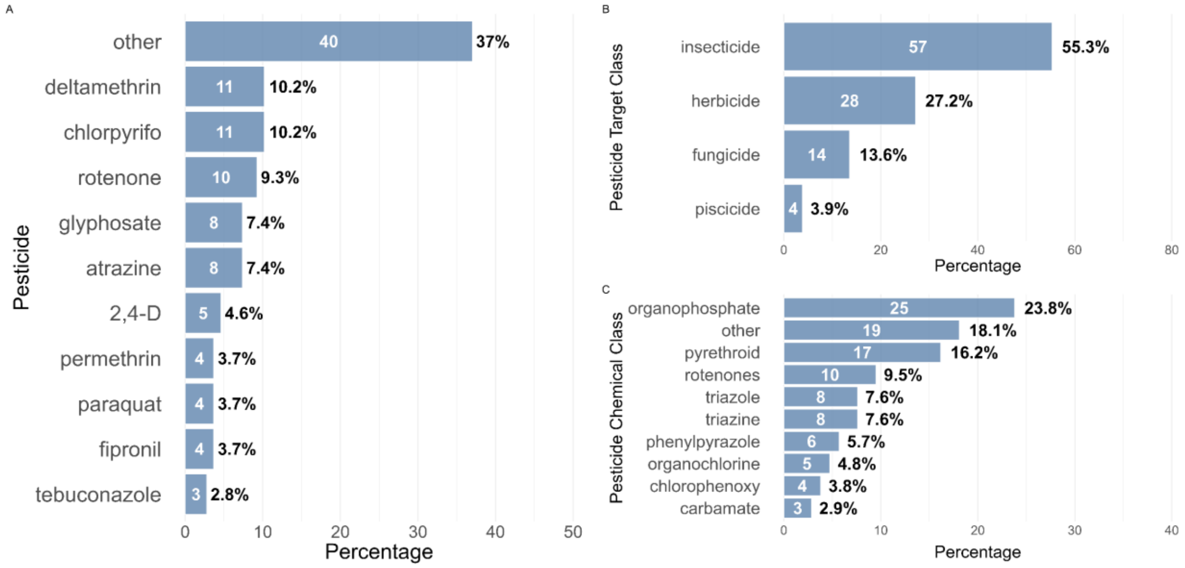

Figure 3 – Bar charts showing the percentages and counts of included studies on the effects of pesticides on zebrafish behaviour (raw count is provided within each bar). Counts are according to A) pesticide types ("other" is a total for all pesticides with a publication count less than or equal to two), B) target organism classes of pesticides, and C) chemical classes

of pesticides ("other" is all pesticide chemical classes with publication count less than or equal to two).

Aim 2: Study design elements

Exposure characteristics

We quantified four important characteristics of pesticide exposures used in the included studies: 1) dosage, 2) duration, 3) route, and 4) sample size. First, we noticed that the studies used a wide range of pesticide concentrations (e.g., ranging from 4.25 ng/L to 60 mg/L; *Figure 4A*). To make a meaningful impact on policy decisions, studies examining the impacts of pesticide exposure need to incorporate concentrations that hold environmental significance. Determining such ecologically relevant concentrations can be a challenge because "safe levels" of pesticide exposure are determined and regulated by the governmental agencies of each country (Maggi et al., 2019). Further, toxicity and availability are related to the physicochemical properties of the pesticide, making ecologically relevant different per chemical (Van Den Berg et al., 2021b). Thus, we highlight that the broad categories such as the pesticide target class, may not be suitable to determine which concentrations should be selected for exposure studies.

Second, included pesticide exposure studies considered various exposure durations (ranging from 5 minutes to 250 days; *Figure 4B*). Yet, the impacts of various exposure durations remain elusive for understudied pesticides such as the previously highlighted neonicotinoids. It is important to acknowledge the nuanced relationship between pesticide exposure duration, concentration, and toxicity. Chlorpyrifos, for example, have been shown to amplify anxiety-like behaviour in response to acute exposure times (Mena et al., 2022), but diminish such behaviour in response to chronic exposure times (Hawkey et al., 2020). This example demonstrates that the relationship between pesticide exposure time and toxicity is complex and potentially non-linear. This may be due to the underlying mechanisms of uptake, metabolization and excretion (Williams et al., 2000). Hence, to fully understand the effects of pesticides on aquatic ecosystems, it is essential to conduct both acute and chronic exposure experiments.

Third, we found that alternatives to waterborne exposure were scarcely explored, despite evidence suggesting toxicity varies with exposure routes (Huang et al., 2021) (*Figure 4C*). Waterborne exposure was the most prevalent pesticide exposure route (n = 99), and alternative routes, such as diet (n = 6) and intraperitoneal injection (n = 2), were scarcely studied. We highlight that much like the duration of exposure; the route of exposure warrants careful consideration due to variations in water solubility among different chemicals (Finizio et al., 1997). Overall, to effectively inform the dosage, duration and, route in pesticide exposure studies the pharmacokinetics/pharmacodynamics (PK/PD) of each chemical should be investigated and used inform the selected study characteristics (Derendorf and Meibohm, 1999; Meibohm and Derendorf, 1997).

Last, we found that 11 studies failed to report sample sizes, and many studies appeared to be underpowered (Sample size: median = 11.65, 1st quartile = 7.25, 3rd quartile = 20, mean = 16.1, SD = 14.0, *Figure 4D*). The presence of underpowered studies is worrisome, as they are unlikely to detect subtle behavioural changes in response to pesticide exposure, and can lead to inaccurate estimates of true treatment effects (Nakagawa and Foster, 2004). As a result of preferential publication of statistically significant findings, conducted studies with low sample sizes often overestimate effects and induce Type M (magnitude) errors (Button et al., 2013). Our finding that pesticide exposure studies are frequently underpowered is consistent with the observation that underpowered studies are common in other areas of global change research (Yang et al., 2022).

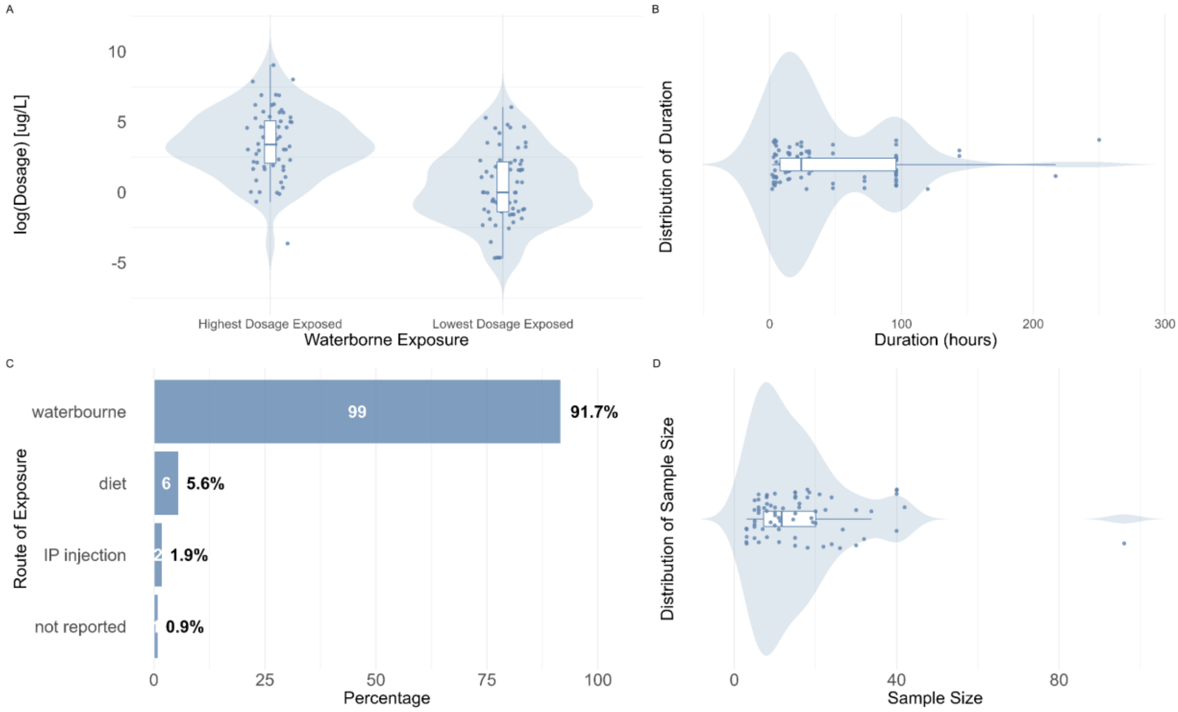

Figure 4 – Summary of characteristics of selected study design elements across included studies. A) a box and violin plot illustrating the distribution of pesticide dosages used in pesticide exposure studies on zebrafish behaviour. Each individual point represents a specific exposure concentration from a given study. The box plot highlights the median value as well as the first and third quartiles, while the violin plot provides a visual representation of the dosage distribution. The dosage is presented on the Log$_{10}$ *scale. The plot shows both the highest and lowest pesticide dosages reported in each study with a waterborne exposure method. B) a box and violin plot illustrating the distribution of pesticide durations used in pesticide exposure studies on zebrafish behaviour. Each individual point represents a specific*

exposure concentration from a given study. The box plot highlights the median value as well as the first and third quartiles, while the violin plot provides a visual representation of the duration distribution. The dosage is presented on the Log_{10} scale. C) a bar plot showing the percentages and counts of studies using each exposure methodology (IP stands for intraperitoneal injection and raw count is provided within each bar) in pesticide exposure studies on zebrafish behaviour. And D) a box and violin plot illustrating the distribution of sample sizes used in pesticides exposure studies on zebrafish. If multiple exposure groups were used in each study, we calculated a mean sample size per study.

Zebrafish characteristics

We found that the investigations of sex differences in response to pesticide exposure were few and not transparent *(Figure 5A).* It was common that articles omit reporting the sexes of zebrafish exposed to pesticides (n = 33). Furthermore, only a negligible number of articles explored both sexes and incorporated sex differences in their analysis (n = 1). This deficiency in reporting is potentially a harmful oversight, given the evidence that male and female zebrafish respond differently to chemical pollutants (Genario et al., 2020b, 2020a) and evidence that some pesticides are endocrine disruptors (Martyniuk et al., 2020). Ignoring potential sex differences in primary research confines our understanding of how pesticide exposure affects male and female organisms differently. Such oversight could also lead to misleading conclusions about the overall impact of pesticide exposure on a population (Miller et al., 2017). To help improve reporting we recommend the use and development of reporting guidelines in ecotoxicology (Ågerstrand et al., 2011; Hanson et al., 2017; Hitchcock et al., 2018; Moermond et al., 2016).

Similarly, we found that the impacts of early developmental exposure to pesticides on adult zebrafish behaviour were markedly understudied. Most studies focused on the effects of adult zebrafish exposures (n = 69), whilst investigating the impacts of embryonic exposure (n = 8) and larval exposure (n = 2) on post-larval zebrafish behaviour remained scarce *(Figure 5C)*. The paucity of research on the long-term impacts of pesticide exposure during zebrafish early developmental stages is another pressing concern. This is because younger fish display heightened sensitivity to chemical pollutants like Bisphenol A (Wu and Seebacher, 2020) and other endocrine-disrupting chemicals (Tao et al., 2022), which can manifest as altered adult-stage behaviours. In addition, the potential impacts of parental and grandparental exposure on offspring behaviour are largely unexplored (n=2), indicating an opportunity for future research (for more, see the section “Neglected research topics and research opportunities”). Taken together, there is a clear necessity for studies to consider sex and life stage differences when investigating pesticide exposure effects on zebrafish.

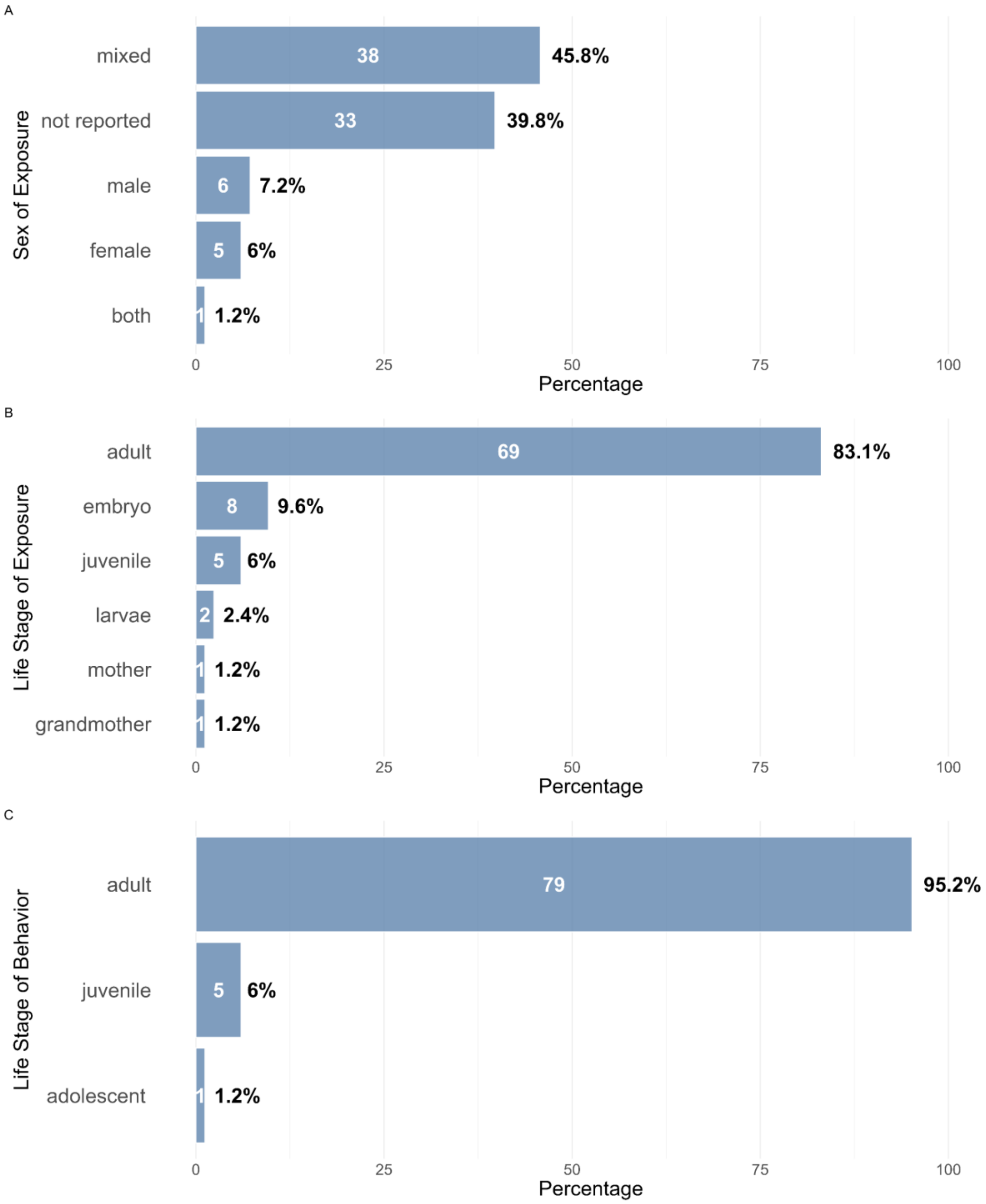

Figure 5 – Summary of selected elements of zebrafish characteristics across included studies (raw count is provided within each bar). Counts are according to A) reported sex of zebrafish exposed to pesticides, B) reported life-stage of zebrafish at which they were exposed to pesticides, and C) reported life-stage of zebrafish at which the behaviour was assessed.

Aim 3: Behavioural classes

The behaviours most frequently studied were related to fish activity or movement (n = 49) and boldness or anxiety (n = 34) (*Figure 6*). Other intraspecific and interspecific behaviours, such as sociality (n = 16), aggression (n = 9), and antipredator behaviour (n = 9) were also investigated. These well-studied behaviours provide fertile ground for future meta-analysis, thereby addressing the call for more research synthesis in behavioural ecotoxicology (Bertram et al., 2022). On the other hand, some behaviours, such as changes in cognition in response to pesticide exposure (n = 7), are underexplored and provide avenues for future primary research. Furthermore, we coded specific behavioural assays used within each behavioural class and provided the results in *Supplementary File 1, Figures s8-s13*. A thorough examination of a broad spectrum of individual, interspecific, and intraspecific behaviours could significantly enhance our comprehension of pesticide impacts on animal communities (Saaristo et al., 2018). Future research should also acknowledge that pesticides can influence neural development and function, potentially modifying behaviour through cholinesterase inhibition (Zhang et al., 2002). This consideration is important, given the most common pesticides were organophosphates, which are recognized as anticholinesterase agents (Sandoval-Herrera et al., 2019). In addition, we identified ambiguity in the behavioural endpoints measured by different behavioural assays. For example, locomotion has been assessed with multiple behavioural assays such as within an anxiety or foraging context, leading to different interpretations of this behaviour. Therefore, we emphasize that specific behavioural endpoints can only be measured with specific behavioural assays. Consistent classification of behavioural endpoints will promote more precise communication and allow for more accurate comparisons across behavioural studies. Deepening our understanding of pesticide effects on interspecific and intraspecific interactions, and thus animal communities, will lend critical ecological context to exposure studies. Simultaneously, adopting consistent terminology will foster a consensus that will better position research to inform evidence-based policymaking.

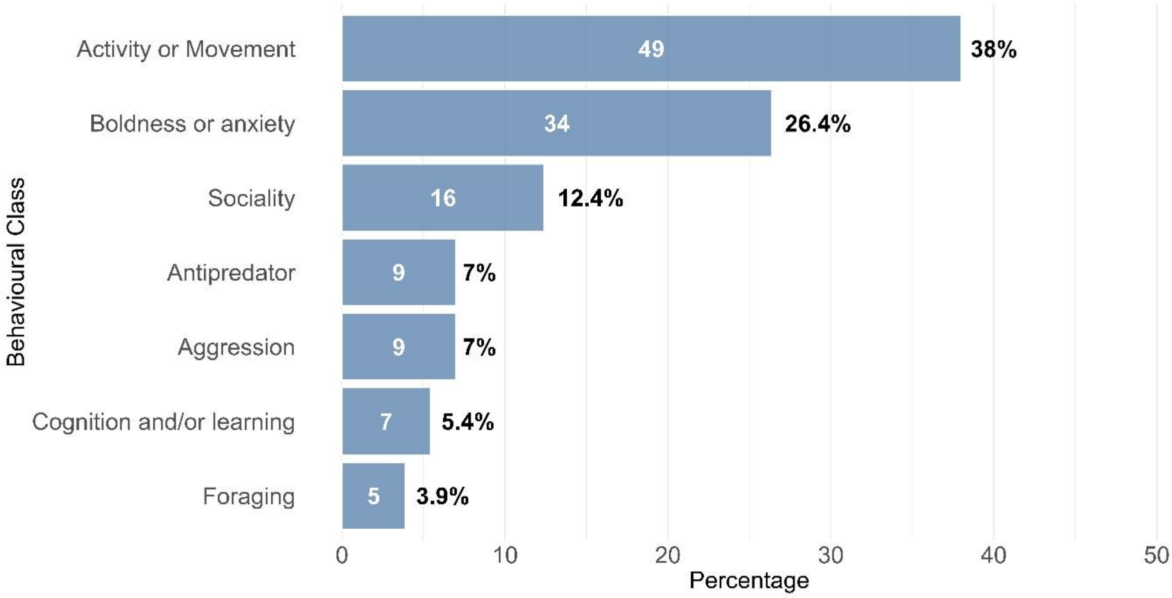

Figure 6 - Bar chart showing the percentages and counts of included papers on the effects of pesticides on zebrafish behaviour across behavioural classes assessed (raw count is provided within each bar).

Aim 4: Research geography and collaborations

We identified global research geography and collaboration efforts in pesticide exposure studies on zebrafish behaviour using bibliometric analysis. Based on the country of the first authors' institutional affiliations, we have found that Brazil (n = 22), China (n = 16), and the USA (n = 11) were the most prolific contributors to the relevant literature (*Figure 7*). In recent years, Brazil has experienced a significant surge in pesticide research, which may be attributed to the recent easing of pesticide bans and regulations in the country (Braga et al., 2020). Our findings also revealed that, at the continent level, South America (n = 24) and Asia (n = 21) were the top contributors to literature investigating the impacts of pesticide exposure on zebrafish behaviour *(Figure 7).* Although some developing countries produced relevant research, overall, few studies originated from developing countries in Africa, Southeast Asia, and Central America. The lack of research observed in developing countries may influence the representation of pesticides, durations and concentrations used in behavioural studies. This is because pesticide regulations are often country- specific, therefore, what pesticides, concentrations, durations are viewed as ecologically relevant may differ between countries. The geographical bias for research conducted in developing countries is likely due to two primary reasons: restricted research funding and limited English proficiency in these regions (Man et al., 2003). We acknowledge that our literature search was limited to English-language articles, which may have accentuated the geographical bias found in our study (Nuñez and Amano, 2021). Several measures could be adopted to ensure the more equitable geographical spread of research in major scientific journals. These include implementing double-blind peer review (Fox et al., 2023), encouraging inclusive collaborations (Guerrero-Medina et al., 2013), and fostering multidimensional mentorship (Davies et al., 2021). Coupled with efforts to promote research in underrepresented regions, these strategies could produce more diverse perspectives and yield a more comprehensive ecotoxicological knowledge.

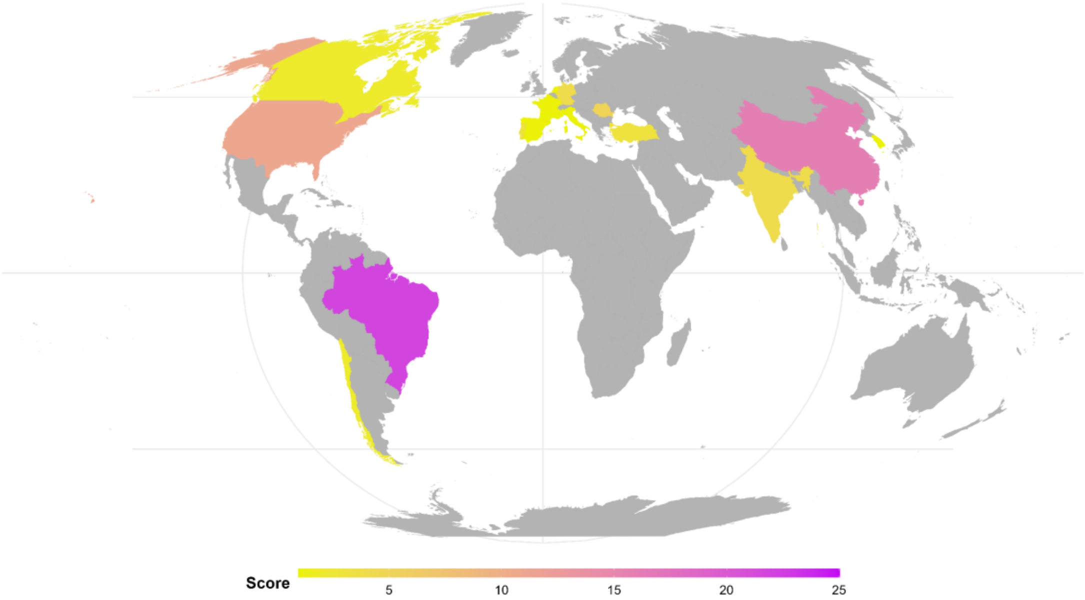

Figure 7 – Heat map of world showing the country-level counts for first authors' country of affiliation of studies investigating the impacts of pesticide exposure on zebrafish behaviour. Grey indicates no publications affiliated with a given country in our data set.

Alongside a limited number of studies in developing countries, we revealed that cross-country co-authorships are limited, as only 25.32% of studies had authors affiliated with multiple countries *(Supplementary File 1, Figure s31 & Figure s32)*. Given the global reach of the pesticide pollution (Tang et al., 2021), international collaboration is critical in crafting effective and balanced policies to mitigate the impact of pesticide pollution. The WTO SPS (Sanitary and Phytosanitary Measures) agreement underscores the importance of international harmonization, which can be further bolstered by fostering cross-country co-authorships (https://www.wto.org/english/tratop_e/sps_e/spsagr_e.htm. Accessed: 30/05/2023). Through such co-authorships, nations can exchange information, resources, and expertise, thereby creating evidence and then policies that are more aptly tailored to specific population's needs.

Neglected research topics and research opportunities

In addition to future research opportunities created by the recognized limitations of the conducted systematic map and bibliometric analysis *(Supplementary File 1, Section 3.1)*, we have identified two key areas of research within pesticide exposure studies on zebrafish which are currently neglected. First, the parental transfer of pesticide exposure effects, that is, how parents' environmental exposure to pesticides can influence the offspring's phenotype (Bonduriansky and Day, 2009) are yet to be fully explored. Among the studies we mapped, only one investigated the transgenerational or intergenerational impacts of pesticide exposure (Blanc et al., 2021). It is important to note that parental effects are not solely driven by maternal exposures, and paternal effects (i.e., those from fathers) also play a substantial role (Rutkowska et al., 2020). This line of research stresses the importance of accounting for sex differences in pesticide exposure experiments. Consequently, we recommend future studies to probe into both matrilineal and patrilineal effects on offspring behaviour to enhance our comprehension of the long-term behavioural implications of pesticide exposure.

Second, the effects of combined pesticide exposures (I.e., mixtures of pesticides) remain largely uncharted. Our map identified only three studies examining combined pesticide exposure (da Costa Chaulet et al., 2019; Robea et al., 2020; Tierney et al., 2011). This gap is significant as chemical interactions are often complex, with impacts that may exceed additive predictions due to synergistic or antagonistic effects (Seebacher, 2022). This reasoning also extends to interactions with other stressors, such as alterations in temperature, light, or noise. Consequently, it is imperative for future environmental research to focus on understanding the collective influence of multiple stressors. By probing these interactive effects, we will achieve a more comprehensive grasp of the unique environmental challenges arising from human activities.

Conclusions

This systematic map and bibliometric analysis summarised the literature on the sub-lethal impacts of pesticide exposure on zebrafish behaviour. The systematic map served as a snapshot of the rapidly expanding literature on the behavioural impacts of pesticide exposure on zebrafish, encompassing many types of pesticides, exposures, behavioural outcomes, and research methodologies. Meanwhile, the bibliometric analysis uncovered that most of the research is conducted in developed countries and international co-authorships remain scarce.

The insights derived from this study signal promising areas for both novel primary research and evidence synthesis. This work provides a comprehensive view of the gaps and clusters of research on the behavioural impacts of pesticide exposure on zebrafish. This can help guide future research to cover a range of ecologically relevant pesticides, concentrations, and durations of exposure, which can be used to help better inform evidence-based policy decisions. By shedding light on the current gaps and trends in this literature, our findings can guide future research endeavours towards a more holistic understanding of pesticide impacts on aquatic ecosystems, in turn, providing an evidence base for evidence-based decision-making.

Declaration of Competing Interests

The authors declare that they have no competing personal relationships or financial interests that could influence the results of this work.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used GPT 4.0 and GPT 3.5, created by OpenAI, and Bard by Google to enhance clarity, readability, and flow of writing. Generative AI was also used to aid in annotating code. After using the tools, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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