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Systematic Map Protocol

Title
What are the current gaps and gluts within pesticide research that use zebrafish as a behavioral model?

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Corresponding author's email address
z5393783@ad.unsw.edu.au

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zebrafish, pesticide, behavior, chemical pollution, ecotoxicology

Background
Chemical pollution is among the leading threats to biodiversity across the globe (Landrigan et al., 2018). Although much effort has been done to mitigate the impacts of chemical pollutants, chemicals remain indispensable in many human activities such as the production of pesticides in our agricultural systems. The adverse effects of chemical pesticides do not always result in mortality, particularly at acutely toxic levels. For example, acute levels of pesticide contamination in the environment can disrupt fundamental biological processes such as reproduction (Aulsebrook et al., 2020) and development (Besson et al., 2020). Recently, ecotoxicologists have used behavioral endpoints to measure the impacts of sublethal concentrations of pesticides in the environment. Behavior has become an endpoint of ecological importance because it is extremely sensitive to changes in the environment (Bertram et al., 2022). An important model species of gaining tremendous interest is zebrafish; routinely used because of their rapid development, and their extreme sensitivity to changes in their environment (Bailey, Oliveri and Levin, 2013). For example, zebrafish have been used to explain how glyphosate can influence locomotion and exploration (Bridi et al., 2017). Additionally, zebrafish have been used to explain how pesticides can influence social behaviors with changes in aggression (Lamb, Chia and Johnson, 2020) and shoaling being described (Hawkey et al., 2021). Despite the rapid rise in the literature using zebrafish as a behavioral model, there is little to no consensus as to where the current gaps in our understanding are. Therefore, we propose the first implementation of systematic mapping and bibliometric analysis within the pesticide literature. Recently, systematic mapping and bibliometric analysis has been combined in one novel framework named research weaving (Nakagawa et al., 2019). Using this novel research synthesis technique, we will extenuate the current gaps in knowledge and describe the scientific networks within behavioral ecotoxicology.

Theory of change or causal model
Despite the rapid rise in primary research within behavioral ecotoxicology, implementation of quantitative research synthesis remains limited (Bertram et al., 2022). The lack of research synthesis is particularly concerning as we cannot make widespread conclusions when looking at studies in isolation. To help address the scarcity in research synthesis we propose a systematic map and bibliometric analysis that aims to provide a more nuanced understanding of the impact's

pesticides are having on animal behaviour. To describe the impacts of chemical pesticide we will use an important model species within behavioural ecotoxicology, zebrafish.

Stakeholder engagement
N/A

Objectives and review question
The objective of this systematic map is to identify the current gaps and gluts within pesticide research that uses zebrafish as a behavioral model. Primary questions: 1) What are the current research patterns of existing literature on the impacts of pesticides on zebrafish behavior (i.e., which pesticides have been used and which behaviors have been quantified)? 2) How are authors, studies, and keywords interconnected within behavioral ecotoxicology using zebrafish as a model?

Definitions of the question components
Population: The study must be an empirical study on zebrafish. Exposure: The study must assess the impacts of pesticide exposure on zebrafish. Studies that do not explicitly state pesticide exposure was conducted or provide only generic descriptions of exposure type (e.g., chemical, endocrine disrupting chemicals, polychlorinated biphenyls, or persistent organic pollutants) will be excluded. Comparator: Impacts of pesticides on zebrafish behavior are to be compared to a control group, where zebrafish are not exposed to pesticides. Outcome: A behavioral change in zebrafish behavior due to exposure to a pesticide. Behavioral changes can be in a social or non-social context. Study Type: The systematic map will only include studies that unambiguously focus on the impact of pesticides on zebrafish behavior. Therefore, purely observational studies will be excluded from the systematic map because the effects of pesticides can be confounded by other factors. Timeframe: There is no time restriction for included studies.

Search strategy
The methodology for the proposed systematic map will follow an established approach (James, Randall and Haddaway, 2016). The literature search will use three bibliometric databases: Scopus, PubMed, and Web of Science to search for relevant studies. In addition, grey literature will be searched using the web-based search engine, BASE. An example string has been provided for Scopus in 8.1. From each database search, we will export citation information, bibliographic information, and abstracts. Additionally, relevant studies will be searched for using a backward/forward citation search. All literature searches will be combined, and duplicate studies will be removed in R. The deduplicated data file will then be uploaded to Rayyan (Ouzzani et al., 2016) for title and abstract screening. A total of 2706 studies were found across all literature search methods (i.e., bibliometric search, grey literature search, and forward/backward citation search). After deduplication, there was a total of 1788 unique studies to proceed to title and abstract screening.

Bibliographic databases
A search string has been designed for the Scopus database and tested on 9/8/2022. The additional database search strings (i.e., Web of Science and Pubmed) are provided in additional file “zf_sm_scoping_strategy”. (TITLE-ABS-KEY ((pesticide. OR insecticide. OR herbicide. OR rodenticide. OR bactericide. OR fungicide. OR larvicide. OR miticide. OR carbamate. OR organophosphate. OR organochlorine. OR pyrethroid OR chlorpyrifo. OR deltamethrin OR atrazine OR glyphosate OR fipronil OR diazinon OR permethrin OR tebuconazole OR ddt OR dichlorodiphenyltrichloroethylene OR dde OR dichlorodiphenyldichloroethylene OR endosulfan OR imidaclorpid OR propiconazole OR paraquat OR rotenone OR strobilurin OR bifenthrin OR triadimefon OR propiconazole OR difenoconazole OR acetohlor OR fenvalerate) AND (zebrafish OR "danio rerio" OR d.rerio OR zfish) AND (behav* OR exploration OR avoidance OR socia* OR bold

OR boldness OR aggress* OR personality OR plasticity OR locomotion OR avoidance OR "circadian rhythm" OR seizure OR seizures OR predator OR rest OR wake OR escape OR shoal* OR movement OR lateralization OR foraging OR anxiety OR courtship OR mating OR learning))) To develop the search string terms we completed a series of discussions and pilot searches. We created the search terms from pesticides and behaviors found in the benchmark studies and from additional reading. We deemed the search string as final once all 10 benchmark studies were found and each reviewer was satisfied with the terms. Once the final search string was developed on Scopus, we modified the Boolean operators for each of the bibliometric databases.

Web-based search engines
The scientific grey literature search engine BASE was used to search for relevant studies within the grey literature. The following search string found 38 potential studies: zebrafish AND behav* AND pesticid* doctype:(14 18*)

Organisational websites
N/A

Comprehensiveness of the search
To test the comprehensiveness of the search we used an established method (Livoreil et al., 2017). Ten relevant studies were collected from Google Scholar (see additional file “zf_sm_benchmark”) and were used as a benchmark set of studies to assess the comprehensiveness of the Scopus search string. When articles were absent from the search, we added additional search terms in subsequent searches until each benchmark study was found. The final search string for Scopus found all of the ten benchmark studies.

Search update
If the time exceeds two years between the search and publication submission an update on search strings will be performed.

Screening strategy
The systematic reviewing tool Rayyan (Ouzzani et al., 2016) will be used to screen search strings from all literature searches. The screening will be completed in a two-step process, firstly on titles and abstracts, and secondly on full texts. Each publication will be evaluated against a set of predefined selection criteria. During the abstract screening, if a reviewer is unsure that a publication fulfills the selection criteria the study will proceed to full-text screening. During the full-text screening, the specific reason for the rejection must be recorded. The rejected studies at the full-text screening stage will then be provided as supplementary material. If the full text of a publication is unavailable the study will be rejected. Each of the full-text screening criteria must be fulfilled for a publication to be included in the systematic map.

Eligibility criteria
Eligible Population: We will include studies that investigate juvenile, adolescent, and adult life stages in zebrafish (i.e., non-larval and non-embryonic life stages). Eligible Exposure: The study must assess the impact of at least one pesticide. Some common groups include organochlorines, organophosphates, carbamates, and neonicotinoids (note pesticides are not limited to these groups). Eligible Comparator: The impact of chemical pesticides must be compared to a control group that has no pesticide exposure. In addition, a study can assess the correlation between pesticide exposure and behavioural change. Eligible Outcomes: The study must assess a behavioral change in response to chemical pesticide exposure. Behavioral changes can be in a social or non-social context. Study Type: The study must be a primary empirical study on zebrafish, where zebrafish are exposed to specified pesticides only. Therefore, experimental studies will be included in the systematic map.

Timeframe: There is no limit on timeframe. Language: The study will be limited to studies published in English.

Consistency checking
To check the consistency of screening results all studies will be independently screened by at least two reviewers. Any conflicts between reviewer screening results will be resolved through discussion, if the conflict persists a third reviewer will be consulted. Consistency between reviewers will be determined by the percentage of conflicts in screening results. Pilot screening has determined the consistency of screening outcomes for both title and abstract, and full-text screening. A total of 100 studies were included in two separate pilots to determine conflict rates between reviewers. Pilot screening of title and abstracts determined a conflict rate of 1% between KM and ML in the first pilot, and a conflict rate of 4% between KM and MS in the second the pilot. The total number of studies that fulfilled the title and abstract screening within the two pilots was 43 (39 “maybe” and 4 “Yes”). Moreover, full-text screening determined a conflict rate of 0% between KM and ML, and 5% between KM and MS, in the first and second pilots respectively. All conflicts between reviewers were resolved through discussion. A total number of 11 studies successfully fulfilled the full-text screening criteria across the 200 total studies within the two completed pilots. Therefore, pilot screening provides an estimation of 85- 102 studies that will fulfill full-text screening and be included in the systematic map.

Reporting screening outcomes
Each study rejected at full-text screening will be recorded with the reason for rejection. In addition, a ROSES flow diagram will be used to present the number of studies assessed and rejected at each screening stage. The ROSES file will be uploaded and will be provided within the supplementary material. Any deviations from the ROSES flow diagram will be discussed in the systematic map.

Study validity assessment
No study validity assessment will be completed.

Consistency checking
N/A

Data coding strategy
Each publication that fulfills each of the inclusion criteria in the screening process will proceed to data extraction. The systematic map will use the predefined extraction protocol provided in the additional file “zf_sm_data_coding_strategy”. The extraction protocol has been iteratively developed to ensure each of the questions and objectives of the project is fulfilled (section 7).

Meta-data to be coded
The meta-data to be coded can be found in the additional file “zf_sm_data_coding_strategy”.

Consistency checking
The role of each reviewer in data extraction has been established through prior testing. A random subset of 20% of studies will undergo duplicate data extraction. Any disagreements between reviewer extraction outcomes will be resolved through discussion. If disagreements persist a third reviewer will mitigate discussion until a resolution is established. If conflict rates between extraction outcomes are over 10%, an additional 10% of studies will be extracted in duplicate.

Type of mapping
We will produce a written report to accompany the database (systematic map) to document the methodology, results, and discussion. The data collected, meta-data and code will be provided in the

supplement. Additionally, we will provide details on each of the literature searches and screening criteria needed to repeat the study.

Narrative synthesis methods
Within the formal systematic map, we will provide a detailed methodology for each step of screening, extraction, and analysis. We will identify and discuss the gaps (topics where a paucity of research exists) and gluts (topics where plenty of research exists) in current ecotoxicological research on zebrafish behavior. This will enable us to provide recommendations of topics in which primary research is needed, due to gaps, or secondary research is needed, due to gluts. Lastly, we will discuss the results of the bibliometric analysis (i.e., explaining the relationships between authors, disciplines, and countries).

Knowledge gap identification strategy
The distribution of extracted data will elicit gaps and gluts in behavioral ecotoxicological research on zebrafish. Potential gaps will be elicited by topics that lack studies, whilst potential clusters will be elicited by topics that have many studies (e.g., types of pesticides, types of behaviors, or combinations thereof). Identifying the potential gaps and gluts of research will provide important scope for future primary and secondary research. In addition to identifying the gaps and gluts of research, the proposed project will identify relationships between keywords, disciplines, countries, and authors. To elicit the relationships between keywords, disciplines, countries, and authors, we intend to use a series of bibliometric analysis and draw a series of bibliometric networks using the R package bibliometrix (Aria and Cuccurullo, 2017) . Identifying key relationships will allow us to present the structure of research and quantify emerging research trends within the literature.

Demonstrating procedural independence
Authors will not screen or extract any publication in which they are authors, or they assisted in the publication process.

Competing interests
The authors have no competing interest to declare.

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Author’s contributions
The research aims, objectives, and conceptualization were completed by KM under the supervision of SN and ML. Each author contributed to the creation of search strings. KM, MS, and ML completed the pilot screening. Whilst KM and SN developed the coding strategy. KM wrote the first draft of the protocol and lead revisions. All authors contributed to the revisions of the protocol.

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References
Aria, M. and Cuccurullo, C. (2017) ‘bibliometrix : An R-tool for comprehensive science mapping analysis’, Journal of Informetrics, 11(4), pp. 959–975. Available at: https://doi.org/10.1016/j.joi.2017.08.007. Aulsebrook, L.C. et al. (2020) ‘Reproduction in a polluted world: implications for wildlife’, p. 11. Bailey, J., Oliveri, A. and Levin, E.D. (2013) ‘Zebrafish model systems for developmental neurobehavioral toxicology’, Birth Defects Research Part C - Embryo Today: Reviews, 99(1), pp. 14–23. Available at: https://doi.org/10.1002/bdrc.21027. Bertram, M.G. et al. (2022) ‘Frontiers in quantifying wildlife behavioural responses to chemical pollution’, Biological

Reviews, p. brv.12844. Available at: https://doi.org/10.1111/brv.12844. Besson, M. et al. (2020) ‘Anthropogenic stressors impact fish sensory development and survival via thyroid disruption’, Nature Communications, 11(1), p. 3614. Available at: https://doi.org/10.1038/s41467-020-17450-8. Bridi, D. et al. (2017) ‘Glyphosate and Roundup® alter morphology and behavior in zebrafish’, Toxicology, 392, pp. 32–39. Available at: https://doi.org/10.1016/j.tox.2017.10.007. Hawkey, A.B. et al. (2021) ‘Neurobehavioral anomalies in zebrafish after sequential exposures to DDT and chlorpyrifos in adulthood: Do multiple exposures interact?’, Neurotoxicology and Teratology, 87. Available at: https://doi.org/10.1016/j.ntt.2021.106985. James, K.L., Randall, N.P. and Haddaway, N.R. (2016) ‘A methodology for systematic mapping in environmental sciences’, Environmental Evidence, 5(1), p. 7. Available at: https://doi.org/10.1186/s13750-016-0059-6. Lamb, S.D., Chia, J.H.Z. and Johnson, S.L. (2020) ‘Paternal exposure to a common herbicide alters the behavior and serotonergic system of zebrafish offspring’, PLoS ONE, 15(4). Available at: https://doi.org/10.1371/journal.pone.0228357. Landrigan, P.J. et al. (2018) ‘The Lancet Commission on pollution and health’, The Lancet, 391(10119), pp. 462–512. Available at: https://doi.org/10.1016/S0140-6736(17)32345-0. Livoreil, B. et al. (2017) ‘Systematic searching for environmental evidence using multiple tools and sources’, Environmental Evidence, 6(1), p. 23. Available at: https://doi.org/10.1186/s13750-017-0099-6. Nakagawa, S. et al. (2019) ‘Research Weaving: Visualizing the Future of Research Synthesis’, Trends in Ecology & Evolution, 34(3), pp. 224–238. Available at: https://doi.org/10.1016/j.tree.2018.11.007. Ouzzani, M. et al. (2016) ‘Rayyan—a web and mobile app for systematic reviews’, Systematic Reviews, 5(1), p. 210. Available at: https://doi.org/10.1186/s13643-016-0384-4.

Authors and Affiliations

Name	Country	Affiliation
Manuela Santana	Brazil	Marine Ecology Laboratory, Center for Marine Studies, Federal University of Paraná, Paraná, Brazil
Shinichi Nakagawa	Australia	Ecology and Evolution Research Centre, University of New South Wales, Sydney, Australia
Kyle Morrison	***Australia***	***Ecology and Evolution Research Centre, University of New South Wales, Sydney, Australia***
Malgorzata Lagisz	Australia	Ecology and Evolution Research Centre, University of New South Wales, Sydney, Australia

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