Environmental Evidence

## SYSTEMATIC MAP PROTOCOL

# Evidence of the impacts of pharmaceuticals on aquatic animal behaviour: a systematic map protocol

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## Abstract

**Background:** Globally, there is growing concern over the impacts of pharmaceuticals and drug manufacturing on aquatic animals, and pharmaceuticals are now recognized as contaminants of emerging environmental concern. In recent years, scientists, environmental managers, and policymakers have been interested in using behavioural endpoints for chemical regulation, given their importance for fitness and survival. The body of research on whether and how pharmaceutical exposure alters the behaviour of aquatic animals has grown exponentially, making it difficult to get an overview of the results. With an international spotlight on the management of these environmental threats, synthesizing the currently available data is vital to inform managers and policymakers, as well as highlighting areas where more research is needed. This is a protocol for a systematic evidence map (SEM) and serves as an a priori record of our objectives and methodological decisions. Our objectives are to identify, catalogue, and present primary research articles on the effects of human and veterinary pharmaceuticals on aquatic animal behaviour.

**Methods:** The literature search will be conducted using two electronic databases: Web of Science and Scopus, and we will supplement these searches with additional sources. The search string has been developed using a Population–Exposure–Comparison–Outcome (PECO) framework, to capture articles that used an aquatic organism (P, population) to test the effects of a pharmaceutical (E, exposure) on behaviour (O, outcome). Eligible articles must also have a control group (C, comparison). Articles will be screened in two stages, title and abstract, followed by full-text screening before data extraction. Decision trees have been designed a priori to appraise articles for eligibility at both stages of screening. At both stages, screening each article will be completed by two independent reviewers. Study validity will be appraised but not used as a basis for article inclusion. The information extracted from the eligible articles, along with bibliometric data, will be mapped and displayed. All data associated with this SEM will be publicly available through the Open Science Framework (OSF) and a future project webpage.

**Keywords:** Medicine, Neurotoxicology, Psychoactive, Ecotoxicology, Evidence synthesis, Fitness

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## Background

Pharmaceutical residues are ubiquitous in the environment and have been measured globally on every continent [1, 2]. Pharmaceuticals present a particular concern for aquatic animals, with the discharge of human, veterinary, and livestock wastewater effluents being a primary

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source of contamination. These contaminants of emerging concern can also enter the environment during pharmaceutical manufacturing, through landfill leachates, and run-off from biosolids used in agriculture [2, 3]. Aquatic animals exposed to pharmaceuticals can directly or indirectly bioconcentrate these compounds in their tissues [4, 5]. Understandably, there are now growing calls for the effective management of pharmaceutical pollution in aquatic environments [6, 7]. Yet, for many pharmaceuticals, empirical sublethal ecotoxicology information is lacking, precluding robust ecological risk assessments for aquatic animals [8]. Where ecotoxicity data are available, they are often limited to standard toxicological endpoints (i.e. measured outcomes), such as growth, reproductive output and mortality (reviewed in [8]). It is essential to consider that the effects of pharmaceutical exposure are likely to be subtle, given that pharmaceuticals are typically detected at low concentrations (low ng/L – low μg/L), are specifically designed to have low-dose effects in their target organisms, and many drug targets are conserved across vertebrate taxa [9]. However, this does not discount adverse environmental impacts, as wildlife may experience unintended, therapeutic-like effects from pharmaceutical exposure [10–12]. Consequently, a growing body of research is investigating adverse outcomes of pharmaceutical exposure, specifically sub-lethal effects on processes like endocrine signalling, development, bioenergetics, and behaviour (reviewed in [13–16]).

In recent years, behaviour has re-emerged as a key endpoint of interest for emerging chemicals of environmental concern, including human pharmaceuticals and veterinary medicines [13, 17, 18]. This is because behaviour is a tractable endpoint, as it is a particularly sensitive indicator for measuring contaminant-induced effects on non-target species, especially when compared to standard ecotoxicological endpoints [19, 20]. Behaviour can also bridge the gap between proximate, sub-organismal, individual-level processes, to ultimate, ecologically-relevant, population-level outcomes, which are important for environmental protection goals [16, 21]. However, behaviour is rarely used in a regulatory context [17, 18, 22]. Recent recommendations have highlighted that integrating behavioural endpoints with other adverse outcomes or standard endpoints (e.g., sub-organismal endpoints, growth) and improving the reliability of behavioural studies will help improve its implementation in regulatory settings [17, 22].

Alongside the increasing use of, and attention on, behavioural endpoints in ecotoxicology, there has been growing awareness that pharmaceuticals specifically designed to modify behaviour are present in the aquatic environment and the tissues of aquatic animals (e.g. antidepressants, anxiolytics, antipsychotics [23–27]).

Indeed, many pharmaceuticals have the potential to modify or disrupt animal behaviour, or may change behaviour as side-effects differing from their principal course of treatment (e.g. analgesics, hormone therapies) [8, 13]. Widespread environmental contamination with behaviour-modifying drugs, together with increased recognition of behaviour as a sensitive endpoint for ecotoxicology, has culminated in an exponential growth of research focused on the behavioural effects of a multitude of pharmaceuticals on aquatic organisms (e.g. [28–32]). For this rapidly expanding field, it is now essential that we synthesize the evidence being produced and identify where the field has focused, knowledge gaps, and opportunities for future research.

We will use systematic mapping and bibliometric analysis to identify, categorize, and visualize research detailing the effects of pharmaceuticals on the behaviour of aquatic animals. Systematic evidence maps (SEMs) are an effective tool to collate evidence whilst minimizing bias for broad research topics [33, 34]. More specifically, SEMs help to identify research trends, show knowledge gaps where further primary research is needed, and specify areas with enough data for targeted evidence synthesis approaches (i.e. systematic review, meta-analysis). Importantly, SEMs have recently been identified as an underutilized tool for chemical risk assessment and decision-making, because they can provide a comprehensive summary of literature relevant for future policy while also minimizing bias [35]. SEMs are especially valuable for connecting heterogeneous interdisciplinary data, like that used in the ecotoxicological sciences and chemical risk assessments, which are beyond the scope, and/or expertise, of any one scientist [36]. When SEMs are then combined with bibliometric analyses—for example, citations and co-author networks—one can gain a deeper understanding of research progress in a field (i.e. a "research weaving" approach [34]). Specifically, this technique can identify particularly influential articles, interconnected research fields, and any research biases stemming from such articles or connections [34]. Therefore, given the rapid expansion of behavioural ecotoxicology and growing interest in behavioural endpoints for regulatory risk assessments, a SEM is incredibly timely for understanding the behavioural effects of pharmaceuticals on aquatic animals.

We will create a SEM, with complimentary bibliometric analyses, and a searchable database of extracted data on the effects of pharmaceuticals on the behaviour of aquatic animals. In doing so, we will accomplish the following objectives.

## Objective of this review

### Primary question

What evidence exists on the effects of human and veterinary pharmaceuticals on aquatic organism behaviour?

### Population

Any aquatic animal. That is, a metazoan with at least one obligate aquatic phase of its life (e.g. fish, amphibian, aquatic mammal, aquatic invertebrate).

### Exposure

A human or veterinary pharmaceutical compound.

### Comparator

A control (i.e. unexposed) or solvent control group of animals.

### Outcome

A behavioural trait. Here we define behaviour as an organism's kinematic response, or lack of kinematic response (e.g. freezing, resting), to an internal or external stimulus (e.g. foraging in response to hunger [internal] or food [external] stimuli).

### Secondary objectives

In addition, the proposed SEM will also address three secondary questions.

(1) Use the evidence map to identify knowledge and methodological gaps, research priorities, and areas of research that have sufficient data for further synthesis.

(2) Collate information on additional treatments (e.g. temperature, noise) or endpoints (e.g. sub-organismal, reproduction, growth, survival) measured alongside behaviour in each article to facilitate connections across domains that may be useful for future regulatory decision making.

(3) Identify influential articles and research group clusters working in this field using bibliometrics (article citations and article co-authorship networks), highlighting areas and/or researcher groups that could benefit from further cross-talk.

## Methods

This protocol was created following the reporting standards for Systematic Evidence Syntheses in environmental research (ROSES [37]; see Additional file 3). Our SEM has been registered using the Open Science Framework (OSF) online platform, and the registration is freely available at https://doi.org/10.17605/OSF.IO/7N92E.

### Searching for articles

Our map targets experimental research articles (i.e. not reviews, meta-analyses). We are targeting this type of article because we want to build a database where a controlled pharmaceutical exposure has been conducted. We will search for articles in two broad-coverage online databases: Web of Science (Core Collection) and Scopus. No limit will be placed on publication year. We will supplement these searches in three ways. First, we will perform an addition search for academic theses on this topic using the Bielefeld Academic Search Engine (BASE; see below for search string details). Second, we will conduct

**Table 1** Eligibility criteria associated question element (i.e. PECO element or other criteria such as language) and the screening stage at which it applies, title and abstract, full text or both

| Eligibility criteria | Question element | Screening stage |
|---|---|---|
| Uses an aquatic animal<br> Animal that have at least one phase of their life as obligate aquatic (e.g. fish, amphibian, aquatic mammal, aquatic invertebrate) | Population (P) | Both |
| Uses a wild type animal<br> An animal that is not genetically modified | Population (P) | Full text |
| Uses at least one pharmaceutical compound<br> A decision tree will be used to assist screeners in deciding whether a compound qualifies as a pharmaceutical compound (Additional file 1: Figure S1) | Exposure (E) | Both |
| Has a control group<br> A non-exposed group to which the exposed group is compared and is therefore not a review, meta-analysis, conference proceeding etc | Comparator (C) | Both |
| Measures behaviour<br> An organism's kinematic response, or lack of kinematic response (e.g. freezing, resting), to an internal or external stimulus (e.g. foraging in response to hunger [internal] or food [external] stimuli [40]) | Outcome (O) | Both |
| Is in a language in which our review team is proficient<br> English, Swedish, Norwegian, Czech, Slovak, Japanese, Polish, Russian | Language | Both |

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reference searches of key review articles published on the behavioural effects of pharmaceuticals in aquatic animals. For this, we have a priori selected six reviews, which specifically address the impacts of pharmaceuticals on aquatic organism behaviour (see Additional file 1: Table S1). Lastly, we will advertise on social media platforms and mailing lists (e.g. Twitter and SETAC Pharmaceuticals in the Environment Interest Group) that we are seeking articles on this topic (including any well-documented reports from grey literature). We do not expect a large grey literature outside of academic or government scientific research sources because aquatic environmental risk assessments conducted for the approval of new pharmaceuticals do not include animal behaviour as an endpoint (discussed in [8, 17]). A search update will be performed if the original search was conducted more than two years prior to review completion.

We designed the search string for Web of Science and Scopus to reflect a PECO framework [38]. Although great care has been taken to create a sensitive and effective search string, it is important to highlight that no search list is exhaustive, comprehensive and completely free of familiarity bias. The aquatic organism search terms (i.e. population terms) captured broad taxonomic groups for animals that have at least one phase of their life as obligate aquatic (e.g. fish, amphibian, aquatic mammal, aquatic invertebrate), in addition to the common aquatic model species or any species used in Organization for Economic Cooperation and Development (OECD) Toxicity Testing Guidelines (e.g. guppy, medaka, minnow, daphnia; both common and genus names). Pharmaceutical compound terms (i.e. exposure terms) included general synonyms for medications and specific pharmaceutical classes (e.g. antidepressants, analgesics). Exposure environment terms detailed aspects of the experimental environment and the process of exposing animals to a pharmaceutical (e.g. exposure, treatment, tank). Behaviour terms (i.e. outcome terms) included variants of behaviours that could be measured in aquatic animal (e.g. movement, cognition). No search terms were included addressing the comparator (i.e. a control group) as these terms are unlikely to appear in bibliometric records. We will instead cover this in our screening process and eligibility criteria. See Additional file 1: Table S2, which shows the full search strings to be used in both Web of Science and Scopus. The search string will be applied to author keywords, titles, and abstracts in both databases. No filters for language or document type will be used. However, only languages with which the co-authors are proficient will be included (English, Swedish, Norwegian, Czech, Slovak, Japanese, Polish, Russian).

The search string for BASE to target academic theses was simplified due to the structure of this search engine and the broader writing style used in thesis abstracts. Here, we again will search for terms following a PECO structure (see Additional file 1: Table S2 for the full search string. This will be done for all search fields ("Entire Document") with document type "Thesis" (document type 18). Again, no filters for language will be used, but only theses in a language in which our co-authors are proficient can be included.

## Estimating the comprehensiveness of the search

The search strings for Web of Science, Scopus, and BASE are presented in Additional file 1: Table S2. We tested the sensitivity of our search string for Web of Science and Scopus using a set of 83 benchmark articles that we would a priori expect our search string to capture. These 83 benchmark articles were selected from a pool of 130 articles sourced from a personal Mendeley library. Specifically, the 130 articles were exported from E.S. McCallum's personal document library. The 130 articles were screened for titles and abstracts (following the eligibility criteria in Table 1) and included 83 relevant articles. We searched for these 83 articles (benchmark articles, a validation set) in the returns from our search string and recovered 79 of the 83 articles. We therefore recovered 95% of the benchmark articles. Two of the four missing articles were not indexed by Web of Science or Scopus, and the remaining two articles did not contain the correct combinations of terms to be identified by our string. However, it is possible that we will recover these articles via our additional sources (i.e., key review article reference list checking, soliciting for articles on social media). Due to logistical restraints, we accept 95% sensitivity, and should more resources become available to the project later we can strive to attain 100% sensitivity.

We compiled a benchmark list of 12 theses (validation set) to test the sensitivity of our search for BASE. These 12 theses were selected from our personal document libraries and included any relevant theses completed by the authors. Eight of the 12 theses were captured in the returns of our search string. However, two of the four missing theses were not indexed in BASE and were removed. We therefore recovered 80% of the possible benchmark theses.

See Additional file 2 for a history of the tested search strings and lists of the benchmark articles/theses.

## Article screening and study eligibility criteria
### Screening process and eligibility criteria

First, search returns from Web of Science, Scopus, and additional sources will be combined and duplicates will

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be identified in Mendeley Desktop Software (Mendeley Ltd.) and removed before being imported to Rayyan [39]. The remaining duplicates will be identified in Rayyan and removed before starting title and abstract screening.

Articles will be included at the title and abstract screening stage based on five eligibility criteria (listed in Table 1). Title and abstract screening will be performed by two independent reviewers randomly assigned to each article (e.g. JMM, ESM, MGB, JAB, DC, IYL, MM, JTO, JS, and HT). However, reviewers will not be assigned articles in which they are listed as an author (despite some of the reviewers co-authoring articles, there will be enough reviewers for this to be achievable). Both reviewers must decide to exclude the article for it to be excluded from moving to the full-text screening and data extraction stage. Any disagreement will be discussed by the reviewers until a consensus can be reached. If a consensus cannot be reached, a third reviewer will be the deciding party. Last, the full-text screening will be completed using Qualtrics Survey Software (Qualtrics, Provo, UT) alongside data extraction. The inclusion decision for full text screening stage will be based on six eligibility criteria (listed in Table 1). The initial section of the survey contains questions that will determine the article's inclusion. Full-text screening and data extraction will again be performed by two independent reviewers randomly assigned to each article. Both reviewers must unanimously decide to exclude the article for it to be excluded from the map (with discrepancies being handled as described above for title and abstract screening). We will provide a list of all excluded articles at the full-text screening stage with reasoning for why they were excluded.

### *Study validity assessment*
We will collect information on study validity from all included articles during data extraction but the articles will not be excluded from the SEM based on these descriptors. We will collect information on study validity guided by the Criteria for Reporting and Evaluating Ecotoxicity Data (CRED [41]), specifically focusing on the first 16 of the 20 CRED reliability criteria. We do not appraise the final four reliability criteria on statistical analysis as these are not relevant to the SEM. We do not appraise the 13 relevance criteria as they require the reviewer to have specific knowledge on a given species, compound, or environmental conditions, and judge their appropriateness, which is not possible given the broad scope and high number of expected articles for this SEM. For a list of which questions in the data extraction form correspond to each of the CRED questions, see Additional file 1: Table S3. In addition, we will collect the following study validity data not specific to ecotoxicity data:

(1) Whether animals were randomly assigned to treatment groups.
(2) Whether behaviour was scored blind to treatment.
(3) How behaviour was scored (e.g. manual versus automated).
(4) If any conflicts of interest were stated.
(5) If any commercial funding was provided for the study.
(6) If the data and/or code associated with the study are available for download.

We are documenting study validity via the CRED reliability guidance and the above additional questions for three reasons. First, behavioural studies in ecotoxicology have been criticized [42, 43] for not following standardized methods or for providing too little data for use in risk assessment procedures. These study validity descriptors will allow us to identify common methodological gaps being overlooked by scientists conducting behavioural-focused studies (e.g. not reporting CAS identifiers, not reporting water quality parameters). Second, scoring behaviour blind to treatment is a standard method in behavioural ecology to reduce the expectancy bias of the researcher on the study outcome. However, this method may not be used by researchers outside of behavioural ecology, and we want to identify the number of articles taking this key methodological consideration into account. Third, we are including the study validity descriptors to improve the utility of the final database for future users. For example, users could filter the database for articles that have received commercial funding or have publicly available data for further analysis.

### Data coding strategy
Following the removal of duplicated articles, all unique articles will be assigned a numeric "Article ID" that will identify the article throughout the title and abstract screening, full-text screening, and the data extraction process. The titles and abstracts for each article will be available directly in Rayyan for the reviewers during the title and abstract screening. For full-text screening and data extraction, all reviewers will download the article, save the file with the Article ID as the filename, read and annotate relevant information using a PDF viewer, and save their annotations for later reference and any necessary discussion (e.g. if discrepancies exist over the extracted data). Reviewers will not contact article's authors if details are missing and we will record instances where key information is missing from the extracted articles. Data generated through the title and abstract screening will be saved as .csv and/or .bib files. Data generated through screening and data extraction with Qualtrics will

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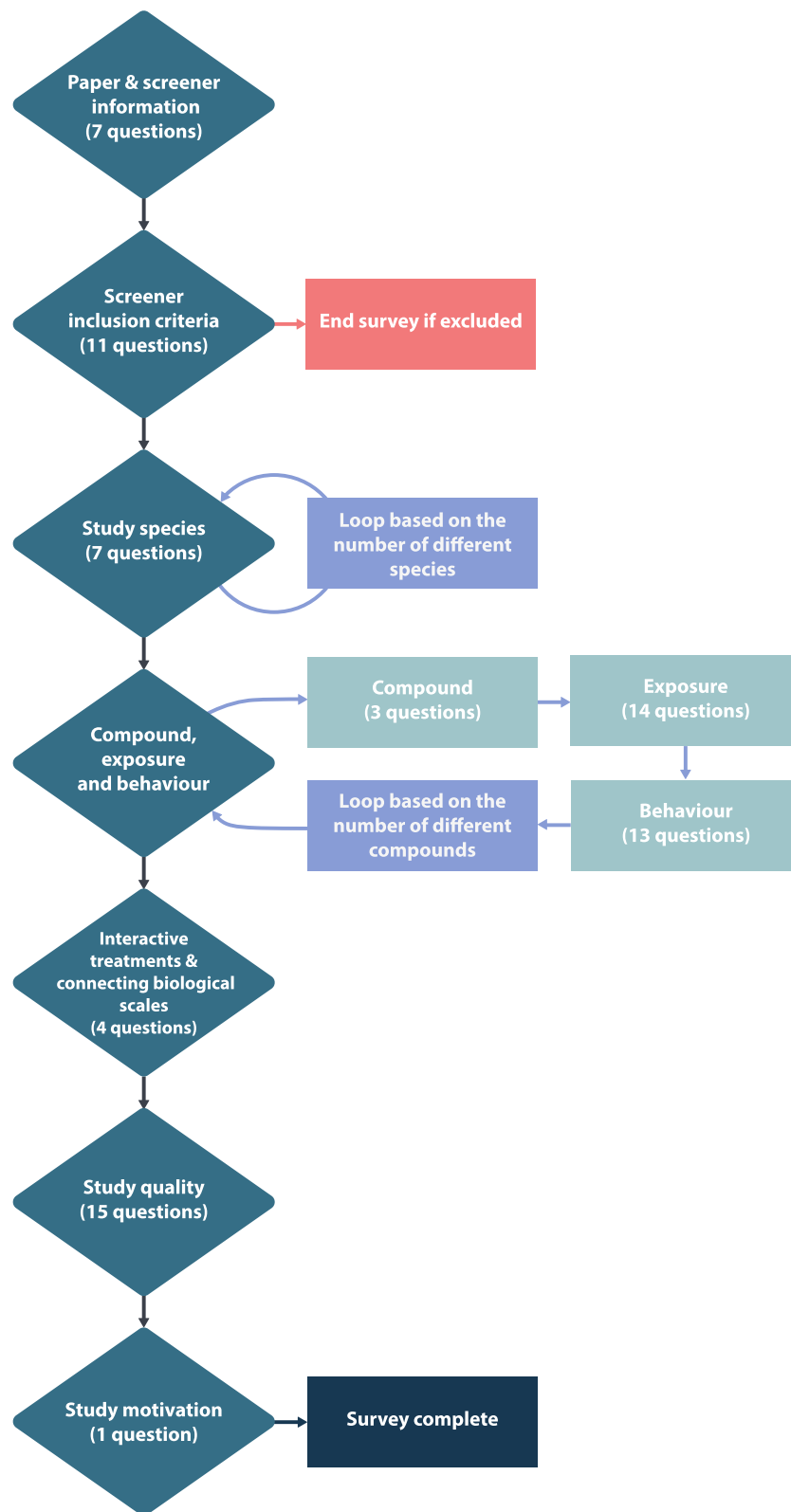
**Fig. 1** Full-text screening and data extraction survey section flow and number of questions in each section. See Additional file 1 for the fully formatted data extraction form

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be saved first online in Qualtrics until all extractors have completed this step. Because full-text screening and data extraction happen together, all data will be extracted in duplicate for all articles. We will then export the data in a .csv file and allow extractors to discuss any discrepancies in their extractions. All data files generated throughout the project will be stored in our project's OSF space.

Extracted data will be coded using an online form created with Qualtrics Survey Software. We will code information in the following survey sections (see Fig. 1 for survey section flow):

(1) Details about the screener and article: this section collects information on the screener and the article being extracted (e.g. screener initials, screening date, article title, journal/source, DOI).

(2) Inclusion criteria: this section extracts data on the inclusion criteria (see Table 1). The reviewer will skip the remaining data extraction if they decide to exclude the article.

(3) Study species: this section collects data on the aquatic organism(s) studied (e.g. species name, animal source, sex, life stage).

(4) Pharmaceutical compound(s): this section collects data on the pharmaceutical compound(s) being studied and the exposure environment (e.g. compound name, route of exposure, dosage, exposure duration).

(5) Behavioural endpoints: this section collects data on which behaviours were measured. Behaviours are first categorized broadly (e.g. activity, aggression, foraging, boldness) and then sorted within each category to extract more detail on how the behaviour was measured (e.g. within activity: normal locomotor activity, abnormal movements, dispersal, migration). Extractors always have the option of "Other" to add any additional categories.

(6) Interactive treatments: this section collects data on whether any interactive treatment beyond the pharmaceutical exposure (e.g. light exposure, temperature treatment) was conducted. These were included to increase the utility of the final mapping database.

(7) Connecting across biological scales: this section collects data on whether the article also measured any sub-organismal traits (e.g. hormone concentrations, mRNA transcription) and/or endpoints related to growth, reproduction, or survival. We included these questions to increase the utility of the final database. For example, to connect behavioural endpoints to molecular initiating events and to traditional endpoints currently being used in risk assessments, as per the adverse outcome pathway (AOP) concept [44].

(8) Describing the study validity: this section extracts information on study validity (see Sect. 2.7 for further details).

(9) Research motivation: this question extracts the main scientific motivation of the study (e.g. environmental/toxicological [to understand effects of pollutants on biota], medical [to understand human health/disease, medical models], or biological [to understand fundamental biological processes]). This section was included to identify the number of relevant articles in adjacent research fields that may be currently overlooked in ecotoxicology.

(10) Ease of data extraction: we included this question to value how difficult it was to extract all the necessary details from the article, and how well the rankings correlate among reviewers.

Full-text screening and data extraction survey section flow and number of questions in each section. See "1 Supplementary Materials.pdf" for the fully formatted data extraction form.Please refer to Fig. 1 for survey section flow and see Additional file 1 on Page 7 for the fully formatted and detailed question and answer formats within each section. We will collect data at the study level using Article ID as a consistent identifier through the screening, data extraction, and mapping process. We will record when multiple species, life stages, and/or multiple pharmaceutical compounds are used within each article (via looping questions within a section, see Fig. 1). We use questions with a "select all that apply" to capture if multiple behaviours or other endpoints of interest are measured within each article (see Q23 and Q41 for examples in the fully formatted data collection form in Additional file 1). After data extraction is complete, the coded data will be reviewed by the pair of reviewers handling that article, and any discrepancies in the coded

(See figure on next page.)

**Fig. 2** Example graphs made using simulated data to show possible mapping outputs. **A** Number of published articles plotted by the top five compound classes, and separated by the study's main research motivation. **B** Number of published articles of the top five species used, and separated by the age classes used in the study. **C** Heat map showing the number of published articles plotted by the type of behaviour that was measured against pharmaceutical compound class. **D** Bibliometric results showing the total citations for each article included across time, with colours indicating the pharmaceutical compound class that was studied

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**Fig. 2**  (See legend on previous page.)

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data will be discussed until a consensus can be reached. If a consensus cannot be reached, a third reviewer will be the deciding party. A pilot involving all extractors (JMM, ESM, MGB, IYL, JS, MM, DC, JTO, JAB, and HT) was conducted to test the efficacy and functionality of the full-text screening and data collection form, see Additional file 1, page 6 for details.

### Study mapping and presentation

We will map the extracted data by generating summary statistics, graphs, and visualizations using R [45]. For example, to identify and/or prioritize key knowledge gaps and knowledge clusters, we will report the frequency of articles that test each pharmaceutical compound class on different categories of behaviour, species, life stage, etc. (see Fig. 2 for example static graphs generated with simulated data). Study validity data will be included in the final database, and we will visualize the number of articles using (or not) key best-practice methodological approaches (e.g. blind behavioural scoring). We will also summarize and visualize the number of articles that included additional treatments or endpoints of interest. We will use bibliometric analyses of article citations and article co-authorship to identify influential articles and authors in this research field, as well as the connections among authors. All bibliometric data will be included in the final database.

All data will be hosted on a dedicated and permanent project webpage provided by the Swedish University of Agricultural Sciences (SLU) Sweden (corresponding author's institution). We aim to create interactive graphs to post on our webpage to allow readers to explore the data (e.g. using *ggplot2* [46] and *plotly* [47] functions in R), and to filter and export lists of articles of interest. All code used to generate the graphs (.R or .Rmd files), as well as the raw data (as flat .csv file, long format) and an associated meta-data file defining columns and responses (.pdf file), will be posted on the project webpage and freely downloadable. Finally, we aim to create a searchable interactive database using R Shiny to embed in our project webpage that will allow readers to search and export data of interest.

### Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13750-021-00241-z.

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**Additional file 1**. Supplementary materials, containing supplementary figures, tables, and methods.

**Additional file 2**. Supplementary data file, containing full annotated search strings, search string development, benchmarking, and pilot screening results.

**Additional file 3**. ROSES form.

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### Authors' contributions

Authorship is ordered alphabetically by last name except for the first and last authors. JMM and ESM conceived and designed this study, with guidance on study design from SN and ML. All authors participated in piloting the data extraction process and refining the study design. JMM and ESM wrote this protocol, with input from all authors. JMM, MGB, JAB, DC, IYL, MM, JTO, JS, HT, and ESM will screen and extract the data to build the SEM. All authors read and approved the final manuscript.

### Availability of data and materials

All data associated with the study will be publicly available through the Open Science Framework (OSF; at https://doi.org/10.17605/OSF.IO/7N92E) and a future project webpage.

### Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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