Evidence of the Impacts of Pharmaceuticals on Aquatic Animal Behaviour (EIPAAB): a systematic map and open access database

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60 ecotoxicology, evidence synthesis, fitness, medicine, neurotoxicology, psychoactive

Abstract

Background

The global proliferation of pharmaceutical pollutants in aquatic ecosystems has emerged as a pressing environmental concern. These contaminants—designed to modulate biological functions at minute dosages—pose a unique threat to aquatic organisms, particularly through behavioural alterations. Recent years have seen a surge in scientific interest in the use of behavioural endpoints in chemical risk assessment and regulatory activities, underscoring their importance for fitness and survival. In parallel, research on how pollution, particularly pharmaceuticals, alters the behaviour of aquatic animals appears to have grown rapidly. Despite this, there has been a notable absence of systematic efforts to consolidate and summarise this field of study. To address this gap, our objectives are twofold: first, to systematically identify, catalogue, and synthesise primary research articles on the effects of pharmaceuticals on aquatic animal behaviour; and second, to organise the 'Evidence of the Impacts of Pharmaceuticals on Aquatic Animal Behaviour' (EIPAAB) into a comprehensive open-access database for scientists, policymakers, and environmental managers.

Methods

We systematically searched two electronic databases (Web of Science and Scopus) and supplemented these with additional article sources. The search string followed a Population–Exposure–Comparison–Outcome (PECO) framework to capture articles that used an aquatic organism (population) to test the effects of a pharmaceutical (exposure) on behaviour (outcome). Eligible articles also needed a control group (comparison). Articles were screened in two stages: an initial screening of title and abstract, followed by full-text screening alongside data extraction. Decision trees were designed *a priori* to appraise eligibility at both stages. Information on study validity was collected but not used as a basis for inclusion.

Review findings

We identified and screened 5,988 articles, of which 901 were included in the final EIPAAB database, representing 1,739 species-by-compound combinations. The database includes data collected over 48 years (1974–2022), with most articles having an environmental focus (510) and fewer relating to medical and basic research topics (233 and 158, respectively). The EIPAAB database includes 173 distinct species representing 8 phyla and 21 classes. Ray-finned fishes were by far the most common clade (75% of the evidence base). The database also includes 426 distinct pharmaceutical compounds; the most frequently investigated groups were antidepressants (28%), antiepileptics (11%), and anxiolytics (10%). The impacts of pharmaceuticals on locomotion and boldness/anxiety behaviours were most assessed out of the 10 broad behavioural categories assigned in the database (62 sub-categories in total). Generally, we detected poor reporting and/or compliance with several of our study validity criteria, including the use of experimental blinding, randomisation, reporting of compound details, and experimental treatment concentration verification.

Conclusions:

Our systematic map revealed a rapid increase in this research area over the past 15 years. We highlight multiple areas now suitable for quantitative synthesis and areas where evidence is lacking. We also highlight some obvious pitfalls in method reporting and practice. More detailed reporting would facilitate the use of behavioural endpoints in aquatic toxicology studies, chemical risk assessment, regulatory management activities, and improve the overall replicability of this research area. The EIPAAB database can be used as a tool for closing these knowledge and methodological gaps in the future.

Background

Pharmaceutical residues are ubiquitous in the environment and have been detected 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 source of contamination. These contaminants 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 ecotoxicological 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. morphometric endpoints), such as growth, reproductive output, and mortality (reviewed in [8]). It is essential to consider that the effects of pharmaceutical exposure on aquatic animals 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 or human side 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 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 among 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. survival, growth) and improving the reliability of behavioural studies will help improve the quality of scientific contributions and utility in regulatory settings [17,22].

Alongside the increasing use of 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 are specifically designed to alter behaviour as their primary therapeutic effect (e.g. antidepressants, anxiolytics, antipsychotics), whereas others may inadvertently lead to behavioural changes (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 synthesise the data being produced and identify focus areas, knowledge gaps, and opportunities for future research.

Here, we have conducted systematic mapping to identify, categorise, and visualise research detailing the effects of pharmaceuticals on the behaviour of aquatic animals. Systematic Evidence Maps (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) [33,34]. Importantly, SEMs have recently been identified as an underutilised tool for chemical risk assessment and decision-making because they can provide a comprehensive summary of literature relevant for future policy while also minimising bias [35]. SEMs are especially valuable for connecting

heterogeneous interdisciplinary data, like those used in ecotoxicology and chemical risk assessments, which are beyond the scope, and/or expertise of any one scientist [36]. Therefore, given the rapid expansion of behavioural ecotoxicology and growing interest in behavioural endpoints for chemical risk assessment and management, a SEM is a timely approach for understanding the behavioural effects of pharmaceuticals on aquatic animals.

Objective of the review

Primary objective

We aimed to identify and catalogue evidence on the effects of human and veterinary pharmaceuticals on aquatic organism behaviour and present this evidence in an open-access database. Our SEM has the following elements:

Population: Any aquatic animal that is a metazoan with at least one obligate aquatic phase of its life (e.g. fish, amphibia, 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. We define behaviour as organismal kinematic responses, or lack of kinematic responses (e.g. freezing, bursting), to an internal or external stimulus (e.g. foraging in response to hunger [internal] or food [external] stimuli).

Secondary questions

In addition, our SEM addressed two secondary questions.

(1) Identify knowledge gaps, research priorities, and areas of research that have sufficient data for further synthesis.

193

194 (2) Collate information on additional endpoints (e.g. sub-organismal, reproduction, growth,
195 survival) measured alongside behaviour in each article to facilitate connections across
196 domains that may be useful for future aquatic toxicology studies and environmental
197 management activities.

198

199 Methods

200 The reporting of the methodology followed MeRIT to improve author contributions'
201 granularity and accountability [37]. This systematic map is based on the methods described
202 in the previously published protocol [38]. It follows the reporting standards for Systematic
203 Evidence Syntheses in environmental research (ROSES [39]; see Supplementary File 1). Our
204 SEM has also been pre-registered using the Open Science Framework (OSF) online platform,
205 and the registration is freely available at: https://doi.org/10.17605/OSF.IO/7N92E.

206

207 Deviations from the protocol

208 Several deviations from the original published protocol [38] were made. These deviations
209 are summarised as follows:

210 1. The planned bibliometric analyses and the screening of academic theses were not
211 conducted because of changes to the initial search string during the protocol peer-
212 review process. This resulted in an increase in the total number of search returns
213 and, so too, the total amount of screening effort required for the project. The
214 additional workload meant that some elements of the project had to be reduced or
215 removed.
216 2. In the protocol, full-text screening was to be performed in duplicate. This was also
217 changed as a result of the increased number of search returns (i.e. 1,239 articles
218 underwent full-text screening). Instead, 10% of all articles at the full-text screening

stage (*n* = 127) underwent duplicate screening to estimate the consistency of eligibility decisions and meta-data extraction of the final EIPAAB database (see 'Article screening and eligibility criteria' and Table 1 for details). In addition, every article that was excluded at the full-text screening stage was subsequently cross-screened (i.e. subsequently screened in duplicate).

3. Some questions in the online full-text and data extraction form were removed and/or altered to decrease extraction workload and increase replicability. All changes were made before the full-text screening and data extraction began. The changes made to the data collection form are detailed in Table S1.
4. New authors were recruited to the project, and two original authors withdrew from the project (JTO and GCM). The new authors included were: SO, KNF, LML, KRS, ESJT, and NSH.

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. *Animal that have at least one phase of their life as obligate aquatic (e.g. fish, amphibia, aquatic mammal, aquatic invertebrate)*	Population (P)	Both
Uses a wild type animal *An animal that is not genetically modified*	Population (P)	Full text
Uses at least one pharmaceutical compound *A decision tree will be used to assist screeners in deciding whether a compound qualifies as a pharmaceutical compound (Figure S1)*	Exposure (E)	Both
Has a control group	Comparator (C)	Both

A non-exposed group to which the exposed group is compared and is therefore not a review, meta-analysis, conference proceeding etc		
Measures behaviour *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)*	Outcome (O)	Both
Is in a language in which our review team is proficient: *English, Swedish, Norwegian, Czech, Slovak, Japanese, Polish, Russian*	Language	Both

236

237 ## Search for articles

238 ### Search terms and strings

239 ESM and JMM designed the search string with assistance from ML for WoS and Scopus to
240 reflect our PECO framework. The aquatic organism search terms (i.e. population terms)
241 captured broad taxonomic groups for animals that have at least one phase of their life as
242 obligate aquatic (e.g. fish, amphibia, aquatic mammal, aquatic invertebrate), in addition
243 to the common aquatic model species or any species used in Organization for Economic
244 Cooperation and Development (OECD) Toxicity Testing Guidelines (e.g. guppy, medaka,
245 minnow, cladocerans; both common and genus names). Pharmaceutical compound terms
246 (i.e. exposure terms) included general synonyms for medications and specific
247 pharmaceutical classes (e.g. antidepressants, analgesics). Exposure environment terms
248 covered aspects of the experimental environment and the process of exposing animals to a
249 pharmaceutical (e.g. exposure, treatment, tank). Behaviour terms (i.e. outcome terms)
250 included variants of behaviours that could be measured in aquatic animals (e.g. movement,
251 cognition). No search terms were included addressing the comparator (i.e. a control group)
252 as these terms were unlikely to appear in bibliometric records. We instead covered this in
253 our screening process and eligibility criteria. The full search strings used in both WoS and
254 Scopus are reported in Table S2. The search strings were applied to all keywords, titles, and
255 abstracts in both databases. The searches in WoS and Scopus were initially performed on 17

November 2021 and were subsequently updated on 13 February 2024 to include the rest of articles published 2021 and all of 2022.

Search filters

No filters for language or document type were used in WoS and Scopus. However, only languages with which the co-authors are proficient were included (English, Swedish, Norwegian, Czech, Slovak, Japanese, Polish, Russian). No limit was placed on publication year (except up until 2022).

Search sources

Our map targeted experimental research articles (i.e. no reviews or meta-analyses). We targeted this type of article because we wanted to build a database of articles where a controlled pharmaceutical exposure has been conducted. We searched for articles in two broad-coverage online databases: WoS (Core Collection) and Scopus.

Supplementary searches

We supplemented the database searches in two ways: First, we conducted reference searches of key review articles published on the behavioural effects of pharmaceuticals in aquatic animals. For this, JMM and ESM *a priori* selected six reviews, that focused on the impacts of pharmaceuticals on aquatic organism behaviour (provided in [38]). Second, ESM and the co-author team advertised on social media platforms and mailing lists (e.g. “X” and the Society of Environmental Toxicology and Chemistry Pharmaceuticals Interest Group) that we were seeking articles on this topic (including any well-documented reports from grey literature). Any articles submitted were sent via a simple Google Form to collect basic article information. We did 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]).

Estimating comprehensiveness of the search

The details of how we estimated search comprehensiveness and sensitivity are detailed in the published protocol [38]. Briefly, we tested the sensitivity using 83 benchmark articles that were expected to be captured by the search string. Our search string recovered 95% of the benchmark articles.

De-duplication of results

Search returns from WoS, Scopus, and the additional sources were combined and duplicates were removed in Mendeley Desktop Software (Mendeley Ltd.) before being imported to Rayyan [40], a software designed for article screening. Any remaining duplicates were identified in Rayyan and removed before starting title and abstract screening.

Article screening and study-eligibility criteria

Articles were included at the title and abstract screening stage based on five eligibility criteria (listed in Table 1). Title and abstract screening was performed using Rayyan, and was completed in duplicate by two independent reviewers randomly assigned to each article (12,094 total screenings [including duplicates]; JMM 27%, ESM 27%, KNF 12%, JS 12%, JAB 12%, DC 12%, IYL 12%, HT 12%, MM 12%, JTO 12%*, LML 12%, MGB 12%, SO 11%, KRS 11%, GCM 9%*; **left the project after title and abstract screening*). Both reviewers had to agree for the article to be included before moving to the full-text screening and data extraction stage. A list of all title and abstract screening decisions and reasons for exclusion are reported in Supplementary File 2. The full-text screening was completed using Qualtrics Survey Software

306 (Qualtrics, Provo, UT) alongside data extraction. The inclusion decision at the full-text
307 screening stage was based on six eligibility criteria (listed in Table 1). Full-text screening
308 and data extraction were randomly assigned to screeners (1381 total screenings; JMM 10%,
309 ESM 8%, NSH 8%, ESJT 8%, MM 7%, JAB 7%, KNF 7%, LML 7% SO 7%, DC 6%, IYL 6%, KRS 6%, HT
310 6%, JS 6%, MGB 3%, ML <1%), as described above, a subset of full-text screening and data-
311 extraction was performed in duplicate (10%, *n* = 127 selected at random). This subset of
312 duplicate screened articles was used for consistency checks to estimate article inclusion
313 decision alignment. For the 127 articles screened in duplicate, there were 18 disagreements,
314 predominantly resulting from issues assessing the compound eligibility (see Supplementary
315 File 3 for a list of disagreements). In total, 10% of all duplicate-screened articles were
316 excluded incorrectly, while 4% were included incorrectly. As a result of a higher-than-
317 desired false exclusion rate, all articles that had been designated as 'excluded' were
318 subsequently cross-screened (by JMM and ESM). After cross-screening, 10% of articles that
319 were initially 'excluded', were subsequently changed to 'include' (38 of 373). Due to the
320 large number of articles considered in the systematic map, it was not feasible to cross-check
321 all 'included' articles at the full-text stage. Thus, we acknowledge a possible 4% false
322 inclusion rate in the project, which would result in approximately 50 articles being
323 incorrectly included in the final database. We highlight that the broader trends and field-
324 related insights gained from the EIPAAB database are likely robust to this small number of
325 false inclusions. Articles that were allocated as 'discuss' under the eligibility question
326 (indicating extractor uncertainty) were also cross-screened, and a final inclusion/exclusion
327 decision was made (by JMM). A list of all articles excluded at the full-text screening stage
328 and the reason for exclusion is reported in Supplementary File 4. For both screening stages,
329 screeners were not assigned articles in which they were listed as authors.
330

Study-validity assessment

We collected information on study validity from all included articles during data extraction; however, articles were not excluded from the SEM based on any validity criteria. We collected information on study validity guided by the Criteria for Reporting and Evaluating Ecotoxicity Data (CRED [41]), extracting information directly relating to 10 of the 20 CRED reliability criteria. Specifically, we extracted information relating to Criteria 1 ("Is a guideline method [or modified guideline] used"), Criteria 2 ("Is the test performed under GLP conditions"), Criteria 3 ("[A]re validity criteria fulfilled [control survival, growth]"), Criteria 5 ("Is the test substance identified with name or CAS number…"), Criteria 6 ("Is the purity of the test substance reported…"), Criteria 8 ("Are the organisms well described…"), Criteria 9 ("Are the test organisms from a trustworthy source…"), Criteria 11 ("Is the experimental system appropriate for the test organism…"), Criteria 14 ("Is the exposure duration defined"), Criteria 15 ("Are chemical analyses adequate to verify concentrations of the test substance…"). For a list of which metadata corresponded to each of the CRED criteria, and details on why some of the criteria were not considered, see Table S3 (also detailed in Supplementary File 6 'READ-ME'). In addition, we collected 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.

In total, we had 19 metadata questions relating to study validity (detailed in Supplementary File 6 'READ-ME'); we documented study validity via the CRED reliability guidance and the above additional questions for three reasons. First, behavioural studies in ecotoxicology have been criticised [42,43] for not following standardised 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 behaviour-focused studies (e.g. not reporting CAS identifiers, not

reporting water quality parameters). Second, scoring behaviour blind to treatment is a standard protocol in behavioural ecology to reduce experimental bias; however, this method may be less prominent for researchers outside of behavioural ecology. Thus, we wanted to identify the number of articles taking this key methodological consideration into account. Third, we included study validity descriptors to improve the utility of the EIPAAB database for future users.

Data-coding strategy

Data extraction protocol

All articles were assigned a numeric 'article ID' that identified the article throughout the title and abstract screening, full-text screening, and the data extraction process. For full-text screening and data extraction, the screening team was assigned a list of articles which contained the article ID, article title, year of publication, journal, and authors (as a CSV file). The screeners used this document to search for and download the articles. The data extraction was coded using an online form (Qualtrics Survey Software; designed by ESM and JMM with input from all co-authors). Before the allocation of full-text articles, all screeners were first trained using a pilot screening with 10 randomly selected articles. This was done to clarify uncertainty for extractors, and to test the efficacy and functionality of the full-text screening and data collection form (as reported in [38]). The article metadata were extracted in the following survey sections (full survey structure supplied in Supplementary File 5):

1. Details about the screener and article: information on the screener and the article being extracted (e.g. screener initials, article ID, DOI).
2. Inclusion criteria: data on the inclusion criteria (see Table 1). If the reviewer chooses to exclude the article, they skip the remaining data extraction.
3. Study species: data on the aquatic organism(s) studied (e.g. species name, animal source, sex, life stage).

4. Pharmaceutical compound(s): 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: data on which behaviours were measured. Behaviours are first categorised into 10 broad categories (e.g. movement/activity, aggression, foraging, boldness; see Table S4 for full list) and then into more specific subcategories (2–12 per parent category; 62 total), to extract more detail on how the behaviour was measured (e.g. within movement/activity: normal locomotor activity, abnormal movements, dispersal/migration; see Table S4 for full list and definitions).
6. Connecting across biological scales: data on whether the article also measured any sub-organismal traits (e.g. hormone concentrations, mRNA transcription) and/or endpoints capturing growth, reproduction, or survival. We included these questions to increase the utility of the EIPAAB database.
7. Validity: data describing the study validity (see 'Study validity assessment' for further details).
8. Research motivation: the primary scientific motivation of the article was allocated to environmental (i.e. focus on predicting/measuring the effects of environmental pollution on wildlife; ecotoxicology), medical (focus on improving human or veterinary medical practice), or basic research (focus on understanding biological phenomena or methodological development with no overt applicational claims for medical or ecotoxicological purposes).

Data processing

The data collected by the online survey form were downloaded as CSV files and imported into R (version 4.2.3, in the R studio environment, Build 463; [44]) for data processing (by JMM). Errors with DOI and 'article ID' (i.e. unique project allocated IDs) were identified by cross-referencing titles, DOIs, and article IDs with the article allocation list given to

411 extractors. The database was then re-shaped to a long format, where each article was given
412 a row for each tested chemical and each tested species, in other words, a row for each
413 unique species-by-compound combination. Compound names and species names were then
414 assessed for possible synonyms or typographical errors. For compounds, this was done by
415 searching compound names in the PubChem database [45], and collating PubChem CID,
416 PubChem name, CAS, and synonyms (Python script by JMM is provided on Github;
417 https://github.com/JakeMartinResearch). These identifier metadata were then used to
418 evaluate possible synonyms or typographical errors in the database (e.g. different compound
419 names that shared a CAS number). For species, this was done using the National Centre for
420 Biotechnology Information (NCBI) Taxonomy database [46]), with each species name
421 searched, and the taxonomy ID, current taxonomic name, and full lineage collated; these
422 species metadata were used to evaluate possible synonyms or typographical errors in the
423 database. For articles that had multiple species, the compound and behaviour data were
424 cross-checked to make sure that the answers given by extractors applied to all species, if
425 they did not, they were adjusted. This was necessary as the survey form did not allow
426 extractors to give separate answers for different species within the same article. All survey
427 questions with an 'Other' option to provide a free-text based alternate response (e.g. study
428 motivation, behavioural classification, methods used to score behaviour; see survey form
429 linked as Supplementary File 5) were then assessed by JMM and, where appropriate, were
430 re-assigned to existing categories or were grouped into new categories (see Table S4–5 list
431 of new categories).

432 Consistency estimates

433 In total, there were 84 duplicate screened articles included, which represented 305 rows of
434 data (i.e. each unique species-by-compound combination). To estimate the consistency of
435 metadata extraction, JMM calculated the alignment between each survey question within
436 each unique species-by-compound combination. When the answer from extractors matched

exactly, the data were assigned a '1', if it did not match they were assigned a '0'. The median consistency across all metadata was 94.8% ± 8.8%, ranging from 60.8–100% (a list of consistency for all metadata is reported in Table S6). Data that were implicitly consistent (e.g. article ID, DOI, species name, compound name) or not consistent (e.g. screener name), were not included in estimates of the median consistency. As a result of some of the specific behavioural classifications having low consistency (median 95.8%, range 67.6–99.3%; see Table S6), a Boolean value (1 or 0) for categorisation only at the broadest level of the behavioural class was created, which had higher consistency (median 98.6%, range 75.6–99.3%; see Table S6). The reason for low consistency for some of the metadata extraction is discussed below in the limitations section. We have opted to maintain all metadata in the database regardless of estimated extraction consistency, but we suggest that those using the EIPAAB database check the level of consistency for the metadata they plan to use, and decide whether it is appropriate for their individual usage.

Additional metadata to increase usability

To aid in cross-article comparison and to increase the usability of the database, the following additional information was added to the EIPAAB database:

1. Standardised concentrations were added to the database which converted the original concentration units reported by the authors to one of six standardised units (original units and values were also maintained). Specifically, the following conversions were made: mass/volume measures to ug/L, volume/volume measures to uL/L, mass/mass measures to ug/g, mole units to uM, molarity (mole/volume) units to uM/L, and dimensionless units of concentration to ppm.
2. Compounds were assigned to a therapeutic classification system, specifically the Anatomical Therapeutic Chemical (ATC) classification tree (hereafter ATC; [47]). The ATC classifies active ingredients of drugs according to the organ or system on which they act and their therapeutic, pharmacological, and chemical properties. The ATC

classification was selected as it is widely used, covers many compounds in the EIPAAB database (305 of 426 compounds), and has a simple classification structure. For compounds that returned multiple ATC classification trees, the trees were collated. ATCs were pulled from PubChem by JMM, by searching each compound name, extracting the resulting PubChem substance ID (up to 150), and searching classification information for each SID (Python scripts by JMM are provided on Github; https://github.com/JakeMartinResearch). In addition to the full classification tree (as a semicolon-separated list), the classifications are also provided at each level of the tree separately (e.g. 5 ATC classification levels) to make the data more accessible (see Supplementary File 6 for details).

3. Additional species metadata were added to the EIPAAB database from the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species [48]. Specifically, JMM and MRM searched each species name in the IUCN Red list, and for those with an associated IUCN Red List report, the IUCN report DOI, IUCN Status, IUCN report publication year, geographic range, population trend, habitat type, and movement patterns were collated (see Supplementary File 6 for details of each data type).

4. Additional bibliometric metadata from WoS and Scopus were collected by JMM (05/07/2024), using a search of the full DOIs list across both online databases (*n* = 894), or by searching the title if the article did not have a DOI (*n* = 7). A total of 879 articles were located on WoS, and the extracted metadata included: journal abbreviation (ISO), author keywords, unique WoS ID, WoS Categories, WoS Research Areas, number of cited references, and number of times the article was cited (across all databases). A total of 888 articles were located on Scopus, and the extracted metadata included: journal abbreviation, author keywords, Scopus EID, and number of times the article was cited.

Data–mapping method

We summarise the available research at three levels: (1) the article level, represented as 'article_id' in the database; (2) the population level, represented as 'unique_population_id' (i.e. article id + species name); and (3) the species-by-compound level, represented as 'unique_row_id' in the EIPAAB database (article id + species name + compound name). The level at which our summaries were made depended on the level at which those metadata were extracted and/or applied to the article. For example, metadata like the publication year, conflict statements, and water quality were extracted and summarised at the article level (n = 901). Metadata like species life stage, sex, and source were extracted and summarised at the population level (i.e. unique_population_id; n = 935), because a single article can have multiple species. Metadata like exposure duration, exposure concentration, and category of behaviours measured were extracted and summarised at the species-by-compound level (i.e. unique_row_id; n = 1,739) because in cases where multiple species were used, different exposures and behaviours can be, and were, assessed. The level at which metadata were extracted is listed within Supplementary File 6, and how this was applied to summarise the data is illustrated in Supplementary File 7 (i.e. R script). We also performed many of our summaries with respect to the motivation for the study. During metadata extraction, we categorised each article based on its primary motivation, as either environmental (i.e. focus on predicting/measuring the effects of environmental pollution on wildlife; ecotoxicology), medical (focus on improving human or veterinary medical practice), or basic research (focus on understanding biological phenomena or methodological development with no overt applicational claims for medical or ecotoxicological purposes). We did so because we predicted the motivation of the research to strongly influence many aspects of the study design, such that some of our summary data would be insightful only if applied within a given study motivation. For example, we would expect the applied doses to be very different in an environmentally motivated study compared to a medically motivated study. All data summary methods are explained in detail

516 in Supplementary File 7, which is also designed to act as a starting point for anyone who
517 wishes to use the EIPAAB database for their own projects.
518

519 Results

520

521 Overview of the evidence base and temporal trends

522 In total, 901 articles—representing 1,739 unique species-by-compound combinations—were
523 included in the final EIPAAB database. After collating articles from all sources and de-
524 duplication, we screened a total of 5,988 unique articles for possible inclusion in the
525 systematic map and database (Fig 1). In brief, 4,739 articles were excluded after title and
526 abstract screening, 338 articles were excluded during full-text screening and data
527 extraction, and 10 articles were unretrievable for full-text screening (overall inclusion rate
528 of 21%; Fig 1). Most articles were excluded at the full-text screening stage for not having a
529 compound of interest (i.e. exposure: *n* = 174; Fig 1) or for not measuring a behaviour (i.e.
530 outcome: *n* = 119; Fig 1).

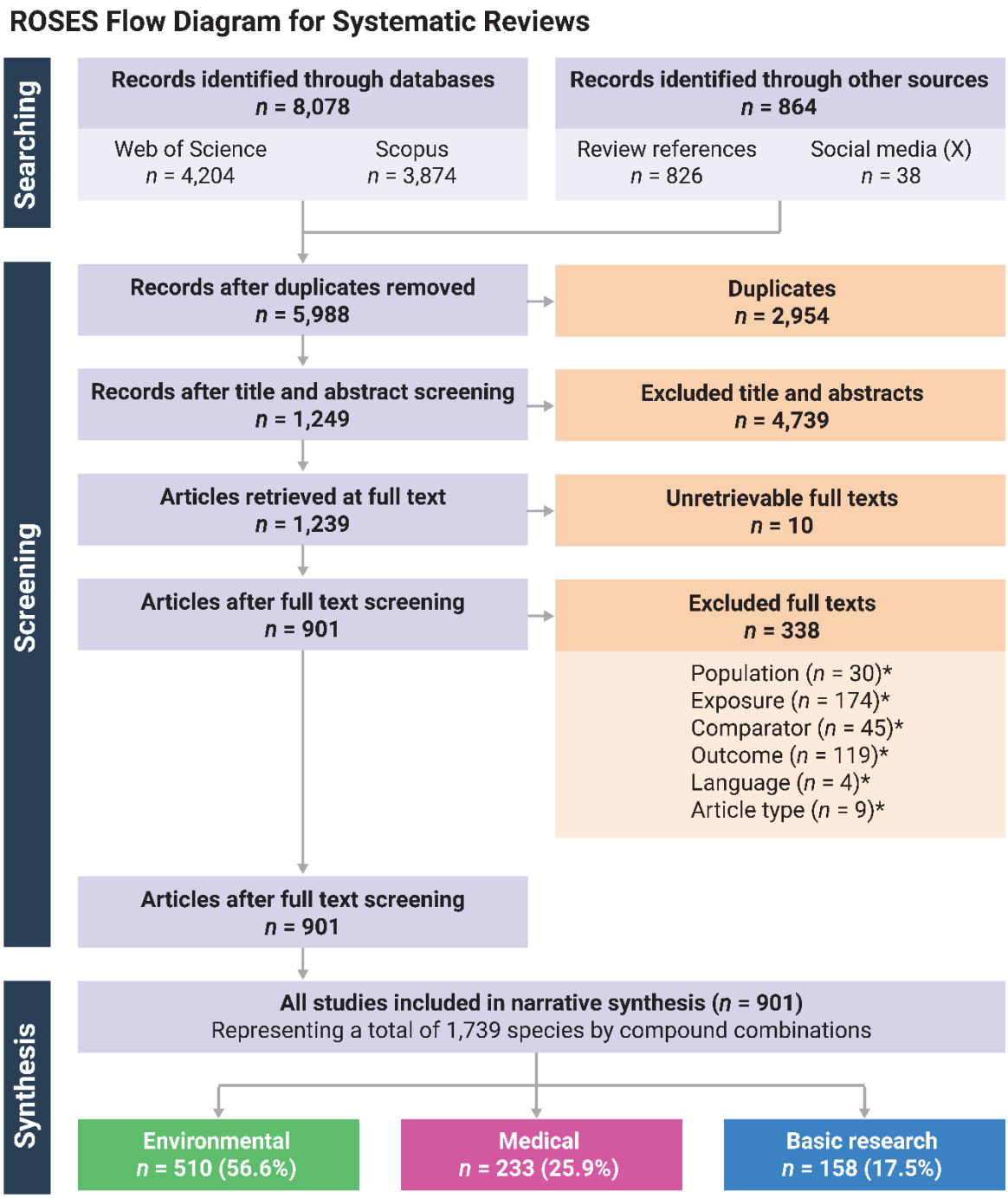
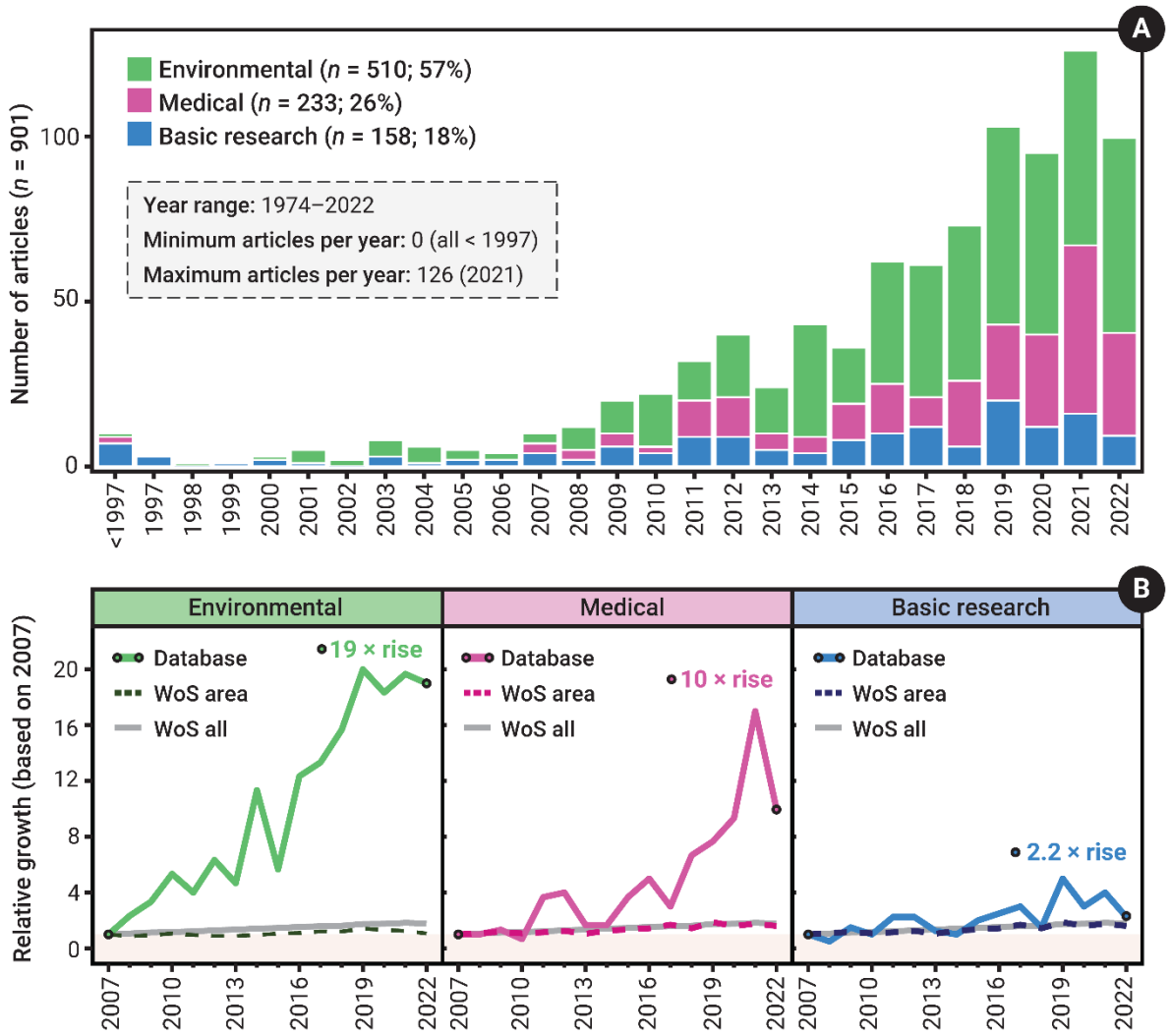


Figure 1. Flow diagram for the SMAP and EIPAAB database, showing the article numbers at each step of the process (i.e. searching, screening, and synthesis). This figure is based on the Reporting Standards for Systematic Evidence Syntheses (ROSES) flow diagram for systematic reviews, version 1.0 [49]. **The total number of articles for each full-text exclusion criterion includes multiple reasons allocated to a single article; we also expected*

that when articles failed to meet multiple exclusion criteria, screeners may not have indicated every reason for exclusion (e.g. if the article was the wrong article type).

Regarding study motivation, 510 articles had an environmental motivation (56.6%), 233 had a medical motivation (25.9%), and 158 had a basic research motivation (17.5%). The included articles date from 1974–2022, with a steep rise in the number of articles around 2007 (Fig 2A). To specifically assess the growth of research on pharmaceutical impacts on animal behaviour, we compared the relative increase in articles over the last 15 years in the systematic map (2007–2022), against that of the most common WoS Research Area, as well as all researcher areas in the WoS Core Collection (i.e. an overall publication trend). This was done for each study motivation separately (see Supplementary File 6 for full details and Supplementary File 8 for the search results). For articles allocated to the environmental study motivation, the most common WoS Research area was 'Environmental Sciences & Ecology' (65% fall within this research area); for those allocated to medical and basic research, it was 'Neurosciences & Neurology' (47% and 39% fall within this research area, respectively). The growth rate of research articles addressing the impacts of pharmaceutical impacts on animal behaviour with an environmental focus far outpaces that of the broader research area of 'Environmental Sciences & Ecology' and the overall publication trend from 2007–2022 (Fig 2B). The growth in research with a medical focus also outpaced the broader research area of 'Neurosciences & Neurology' and overall publication trends, but this was only evident from 2018–2022 (Fig 2B). The growth in research with a basic research focus did not consistently deviate from the broader research area of 'Neurosciences & Neurology' or overall publication trends (Fig 2B).



560

561 **Figure 2.** (A) The total number of articles included in the EIPAAB database by publication
562 year (articles published before 1997 were grouped; total range 1972–2022). Study motivation
563 is represented by the stacked colours within the bar chart (Environmental = green, Medical
564 = pink, Basic research = blue, stacked in that order). (B) The relative growth in the number
565 of articles per year from 2007–2022 based on 2007, as compared to the respective WoS
566 Research area (WoS area: 'Environmental Sciences & Ecology' or 'Neurosciences &
567 Neurology'), and WoS global publication trends (WoS all), for Environmental, Medial and
568 Basic research articles in the database.

569

Mapping characteristics of the population, exposure, and outcomes

Study species (population)

Collectively, the database includes 173 different species from 21 classes (Fig 3A). In terms of taxonomic diversity, 41.0% of the species present in the evidence database belonged to the clades *Actinopterygii* (i.e. ray-finned fishes), 12.1% to *Malacostraca* (i.e. soft-shelled crustaceans), 11.0% to *Gastropoda* (i.e. gastropods), 6.9% to *Amphibia* (i.e. amphibians), and 5.8% to *Branchiopoda* (e.g. fairy shrimp, water fleas)—all other clades represent less than 5% of the total distinct species (Fig 3A). Regarding the representation in the evidence base (i.e. how often they were studied), *Actinopterygii* was by far the most common, representing 75.4% of all data in the database; all other clades represented less than 10% of the data included in the database (Fig 3B). The most common species in the database was the zebrafish, *Danio rerio*, being included in 44.1% of all articles, which is almost a factor of 10 higher than the next most common species, *Daphnia magna* (5.8%; the top 15 most common species shown in Fig 3C). Interestingly, many species were only used in a single article (103/173), with very few being used in more than 5 articles (17 species; Fig S2).

Taxonomic usage and representation also differed by study motivation; compared to medical articles, those with an environmental and basic research motivation showed a more even spread of taxa, although all had a very strong skew towards ray-finned fishes (Fig S3; Figure 3C). Considering the total number of articles identified per study motivation, environmental and basic research included substantially more species than medical research (Environmental = 143:510; Medical = 26:233, Basic research = 43:158, species:articles).

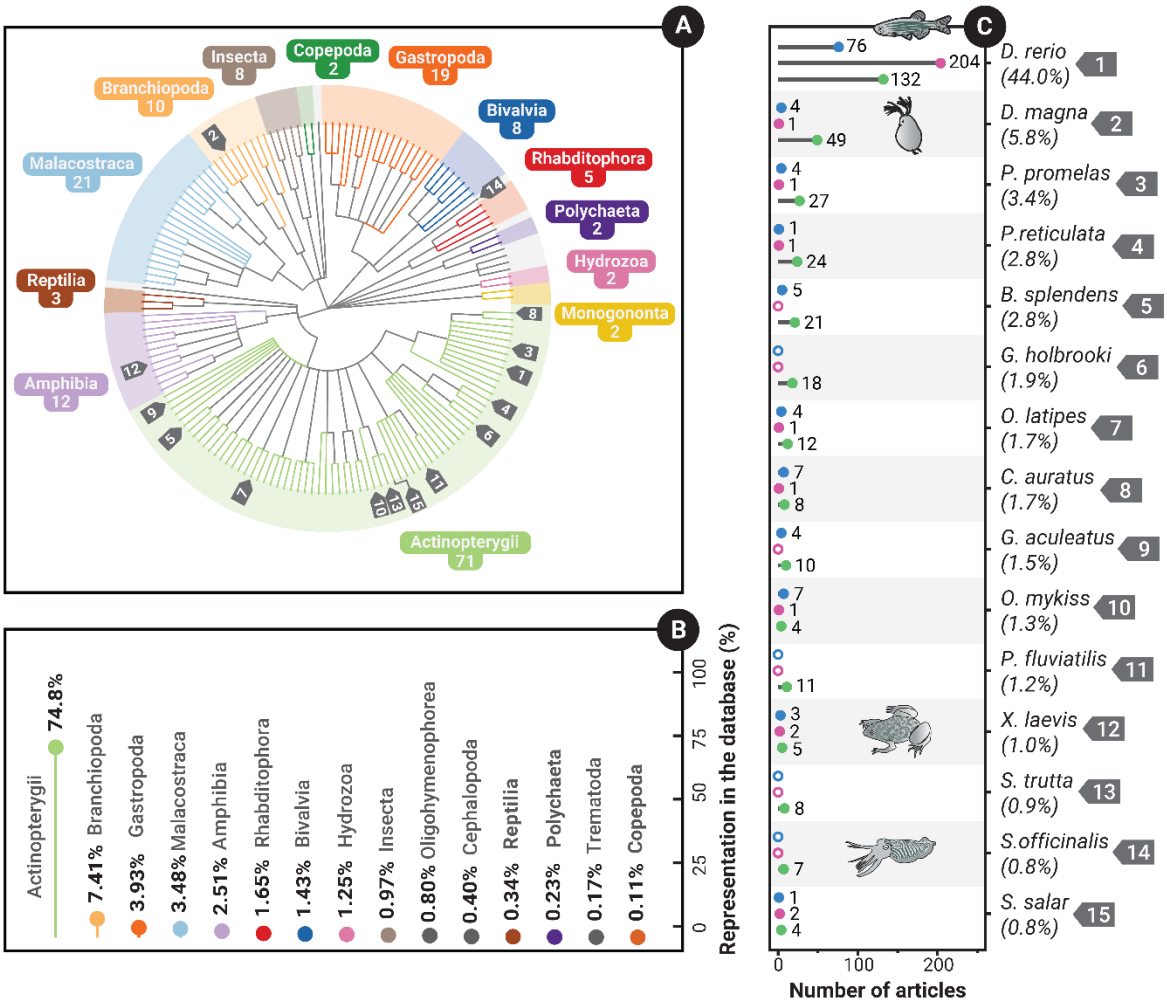


Figure 3. (A) Cladogram showing all species included in the EIPAAB database. All classes with more than one species are shown in distinct colours (those with a single species are light grey). The numbered labels 1–15 represent each of the top 15 species represented in panel C. (B) The 15 most common taxonomic classes in the evidence database. The colours are unique to each phylum and apply across both plots A and B. (C) The 15 most common species used in articles within the evidence database. The percentage value given under the species name is the percentage of total articles, and the counts within the plot are the number of articles for each species by study motivation (Basic research = blue, Medical = pink, Environmental = green, in that order). The open circles are cases of zero articles. The accompanying species images indicate the first occurrence of a distinct taxonomic class in the top 15 species (i.e. Actinopterygii [1st], Branchiopoda [2nd], Amphibia [12th], and Cephalopoda [14th])

There was an overrepresentation for species from freshwater habitats compared to marine (80.4% *versus* 19.6%), although this was less obvious in environmental and basic research (Table S7). There was also an overrepresentation for studying animals at the adult life stage (53.3%), compared to juveniles (14.8%), larvae (26.4%) and embryos/eggs (5.5%). This was broadly consistent across all study motivations, although environmental articles had a more balanced representation of life stages (Table S7). The use of female and male animals, when reported, was roughly equal (44.9% *versus* 55.1%), and this was constant across all study motivations (Table S7). Overall, the most common source of study animal was commercial suppliers/fish farms (38.0%), followed by a lab stock with undisclosed origin (26.6%), collection from the wild (24.4%), lab stock from a commercial supplier (6.9%), and lab stock from a wild population (4.1%). The animal source did, however, vary by study motivation, with environmental articles having the highest representation of wild-collected animals and less sourced from commercial supplies or fish farms (Table S7).

Importantly, sex, life stage, or animal source were not obtained from all articles. In some cases, these data were not reported at all, or were not reported in sufficient detail (summary data reported below). The reporting of species-related metadata was considered an aspect of study validity/quality and is discussed in more detail below. With that said, the number of species with missing metadata is also important in interpreting the overall population trends, so this information has been included in the summary table (Table S7). IUCN data was also not available for all species (106 of 173 had IUCN data), which should be considered when interpreting species IUCN red list metadata and habitat data.

Pharmaceutical compounds and exposure (exposure)

Overall, 426 different pharmaceutical compounds were included in the evidence database. The majority of articles used a single compound (*n* = 624, 69.3%), and very few used more than 5 (*n* = 38, 3.9%), with a similar trend in the number of compounds used across study

motivations (Fig S4). We present the compound data in two ways, in terms of the diversity of compounds (irrespective of the number of articles studying them in the EIPAAB database), and their percentage overall representation in the EIPAAB database. In terms of compound diversity—using the WHO Anatomical Therapeutic Chemical (ATC) classification tree—the database includes compounds from all pharmacological groups at the broadest ATC level (14 groups). At this ATC level (i.e. 1st ATC level), the pharmacological group with the most compounds was 'nervous system', with 43% of all classified compounds belonging to this group, followed by 'cardiovascular system' and 'alimentary tract and metabolism' (Fig S5). At the 3rd ATC classification level, antidepressants, antiepileptics, and antipsychotics have the highest number of compounds, at 27, 18, and 11 distinct compounds, respectively (Fig 4A). In terms of overall percentage representation in the EIPAAB database, compounds within the ATC level one group 'nervous system' made up 71.9% of all data, followed by 'genito urinary system and sex hormones' (13.5%) and 'cardiovascular system' (10.6%). At the 3rd ATC level, antidepressants (27.4%), antiepileptics (10.6%), and anxiolytics (9.7%) were the most common (Fig 4A). Overall, the most common compound was fluoxetine (antidepressants), which made up 11.5% of all data in the EIPAAB database (see Fig 4B for the top 10 most common compounds). There were obvious differences in compound use based on study motivation (Fig 4B). For example, 17−alpha−ethinylestradiol (EE2) was the third most common compound overall (63 occurrences), but this was almost entirely driven by environmental research (61 occurrences; Fig 4B). Medical and basic research shared a more similar preference for compounds than they did for environmental research (Fig 4B). It is important to highlight that not all articles had an assigned ATC classification (307 of 428 had an ATC classification; 72%); thus, all summaries based on ATC do not include all available compounds within the database.

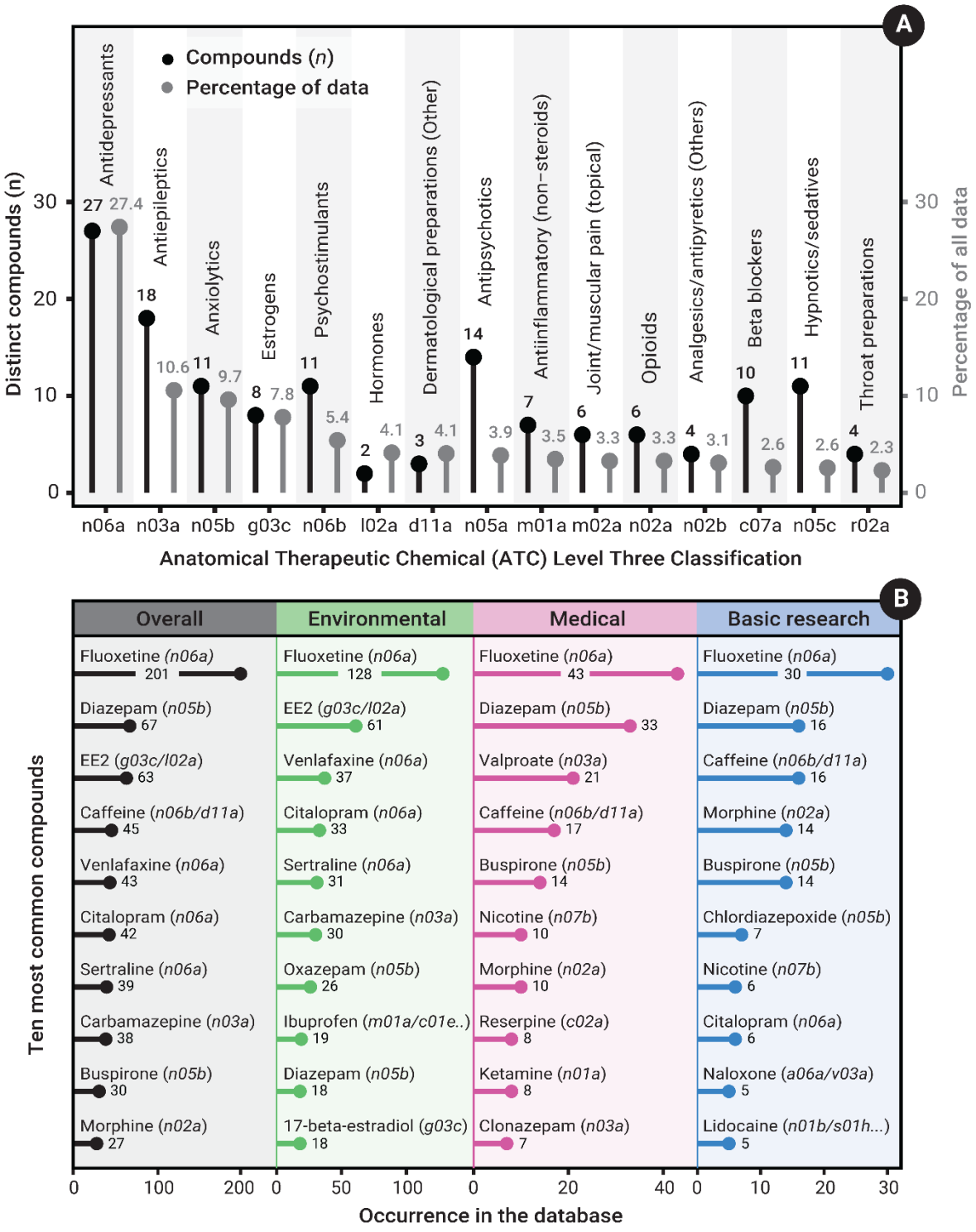


Figure 4. (A) The 15 most common level three ATC pharmacological groups, as shown by the number of distinct compounds within each group (black), and overall percentage of occurrence in the EIPAAB database (grey). The x-axis lists the group's ATC code, while a simplified version of the ATC name is given inside the plot. Note that the total percentage

may exceed 100, as each compound may have multiple classifications. (B) The 10 most common compounds in the database overall and for each study motivation (Environmental, Medical, and Basic Research), the code in brackets following the compound name are the level three ATC pharmacological groups associated with the compound.

Overall, 22.6% of articles included mixture exposures in addition to single compound exposure. The use of mixture exposures differed substantially by study motivation. Specifically, medical articles had a much higher rate of mixture exposure (48.4%) compared to basic (25.4%) and particularly environmental research (12.8%). This is likely a result of medical-based articles investigating potential treatments for various psychological/neurological conditions (e.g. epilepsy), in which a phenotype for the psychological/neurological condition of interest is induced using a compound exposure and another compound is subsequently administered to alleviate the phenotype. Most exposures were solely waterborne (86.8%), as compared to other exposure routes (e.g. injection, dietary; 12.7%) or a combined exposure with multiple routes (0.9%). Exposure duration was most often acute (i.e. < 96 hours), with very few studies using exposure durations over a month (only 8.3%; Fig 6A). However, there were notable differences between the study motivations. Medical and basic research articles typically employed exposures less than 6 hours (61.2 and 76.7%, respectively), and almost never over 3 months (0.6% and 0%, respectively; Fig 5A). On the other hand, environmental articles had more variation in the maximum exposure durations, with the most common being between 3–8 days (26.4%) and more examples of exposures exceeding 3 months (6%; Fig 5A). Further, overall, most studies exposed animals to a single dose of the compound (29.7%), and very few used more than 5 doses (only 9.8%; Fig 5B). For environmental research, there was a more even spread in the percentage of articles that included up to 5 doses (15.3–22.3%; Fig 5B). Broadly speaking, the concentrations used varied substantially, both within and across study motivation (Fig 5C). Generally, environmental studies used much lower concentrations (both the minimum

690 and maximum dose) and had a smaller within-study dose range (Fig 5C). Basic research
691 studies used the highest concentrations and had the highest within-study dose range (Fig
692 5C). With that said, there was still substantial overlap in the concentrations used between
693 study motivations, which could help facilitate across-discipline comparisons (although this
694 should be checked explicitly at the compound level). Almost all exposures were conducted
695 in indoor laboratory settings (99.4%) *versus* in a semi-controlled outdoor environment (0.3%)
696 or in the wild (0.2%).
697

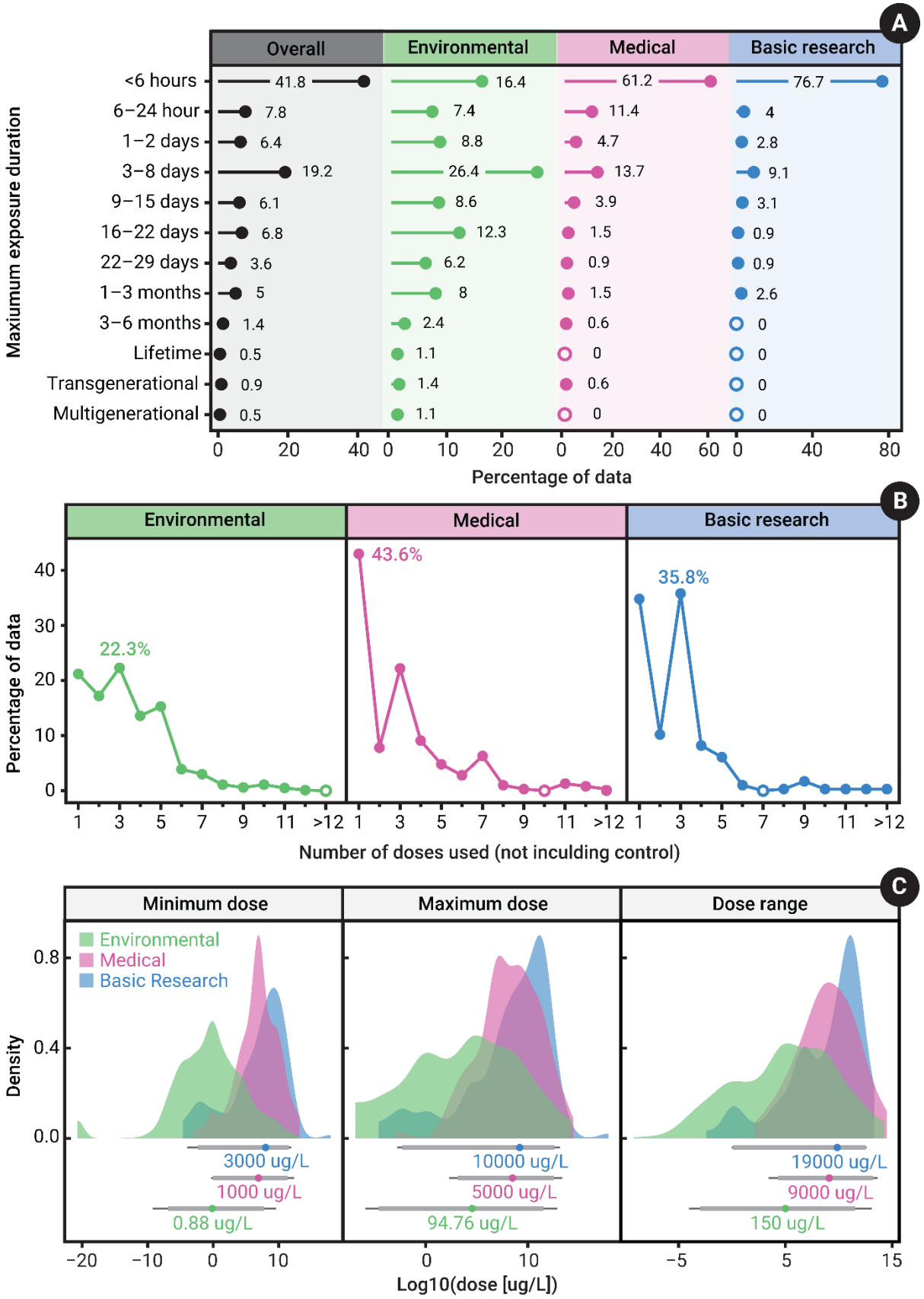


Figure 5. (A) The duration of exposures used by articles in the database. The plot is split by the overall percentage breakdown and those for each study motivation. The percentage

values are calculated within each study motivation. (B) The number of different doses used (excluding the control), as shown by study motivation. The percentage values are calculated within each study motivation. (C) The distribution of minimum and maximum dose used, as well as the within-study dose range (i.e. maximum – minimum). The x-axis (dose µg/L) is plotted on a log10 scale for the density plots and 'eye plots'. The eye plot shows the median, 89, and 95% intervals. The text with the eye plot shows the raw (untransformed) median value and is used to aid in comparisons across study motivations.

Behavioural endpoints (outcome)

We classified behaviour into 10 overarching categories and 62 sub-categories (2–12 sub-categories within each parent category; a full list of sub-categories and descriptions is given in Table S4). The 10 over-arching categories were: (1) movement and locomotion, (2) anxiety and boldness, (3) foraging/feeding, (4) antipredator behaviour, (5) pre-mating and mating behaviour, (6) post-mating behaviour, (7) aggression, (8) sociality, (9) cognition/learning, and (10) other behaviours not categorised (see Table S4 for list). Typically, only one of these behavioural categories was assessed following exposure (69.3%), with few cases assessing more than 3 behavioural categories after exposure (7.8%); this trend was seen within all study motivations. Overall, movement and locomotion behaviours were the most common responses measured (40.4% of all recorded behaviours), followed by boldness and anxiety-related behaviours (23.4%); all other overarching behavioural categories each represented less than 10% of the data. The preference for movement/locomotion and boldness/anxiety-related behaviours was present in all study motivations, the preference for testing the other 7 categories was more variable (Fig 6). Environmental research had a more even spread of research across the 10 behavioural categories (Fig 6). Overall, the behavioural groups that have seen the least research attention are post-mating behaviours (e.g. parental care; <1%), antipredator behaviours (3.5%), and cognition and learning (3.7%). Within this manuscript, we will not detail the

specific breakdown of each behaviour sub-category, but this information is provided for each study motivation in Fig S6.

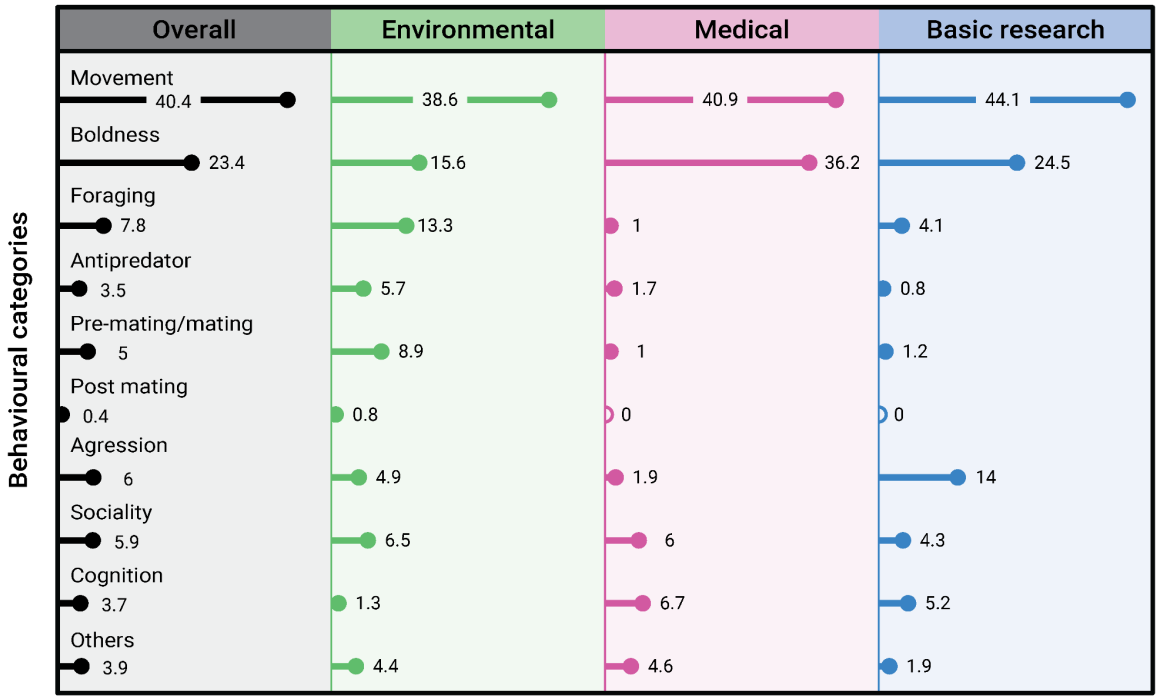


Figure 6. The percentage measurement of different behavioural categories. The plot is split into the overall percentage breakdown and those for each study motivation. For a list of all sub-categories of behaviours and definitions see Table S4 and Figure S7.

Almost all behaviours were assessed in a laboratory setting (99%), with less than 1% of measured behaviour being conducted in an outdoor natural setting (in an open natural setting or restricted natural setting). This almost complete preference for studies in laboratory settings was present regardless of study motivation (98.7%, 99.6%, 99.7%, environmental, medical, and basic research, respectively). Overall, only 22% of behavioural measures were conducted within a social context; in other words, behaviour was rarely tested in a setting in which multiple animals were able to interact freely. Automated behavioural scoring was the most common method for measuring behaviour (e.g. tools like Ethovision, ViewPoint, IDTracker), with 38.9% of articles using an automated quantification

approach, 26.6% manually scoring behaviours from recordings, 21% using an indirect method of counting food consumption (e.g. counting food items remaining), and 8.6% used live scoring (all other methods were used in less than 1% of articles). It is important to highlight that 22.7% of articles (*n* = 221) did not clearly specify the methods used to measure behaviour; the information was considered as one of our validity indicators, and is also presented below in the validity assessment.

Connecting population, exposure, and outcome (PEO)

Considering our population, exposure, and outcome elements (i.e. compounds, species, and behaviours) in combination, we found that most articles addressed the effect of a single pharmaceutical compound on a single species and measured a single behavioural category (41.5% of all articles). The next most common study design was a single pharmaceutical compound, a single species, and two behavioural categories (i.e. 17.7%), all other possible combinations each made up less than 10% of the articles. As a broad overview of the connections between compounds, species, and behaviours and how they varied, we illustrate below the links between the 10 most common phylogenetic clades (class) and each behavioural category, as well as the 10 most common therapeutic groups (ATC level 3; Fig 8). Broadly speaking, for most of the top 10 clades, movement and locomotion are the most frequently measured behaviours, although there are clade-specific differences in the remaining behaviour categories. For example, Actinopterygii has a relatively high contribution to boldness behaviours, while Branchiopoda, Gastropoda, and Bivalvia are seldom used in the investigation of boldness-related behaviour (Fig 7; see link in figure caption for an interactive version of the figure). There is even more variation in selected behavioural endpoints when looking at therapeutic groups. For example, antidepressants (ATC n06a), anxiolytics (ATC n03a), and psychostimulants (ATC n06b) have high relative contributions to measured boldness-related behaviour, while estrogens (ATC g03c) and hormones (l02a) have a high relative contribution to measured pre-mating/mating behaviour

(Fig 7). In the supplementary material, we further illustrate the variability in the relationship between compound, species, and behaviour using fluoxetine, diazepam, and 17-alpha-ethinylestradiol (the three most common compounds) as specific examples (see Fig S7).

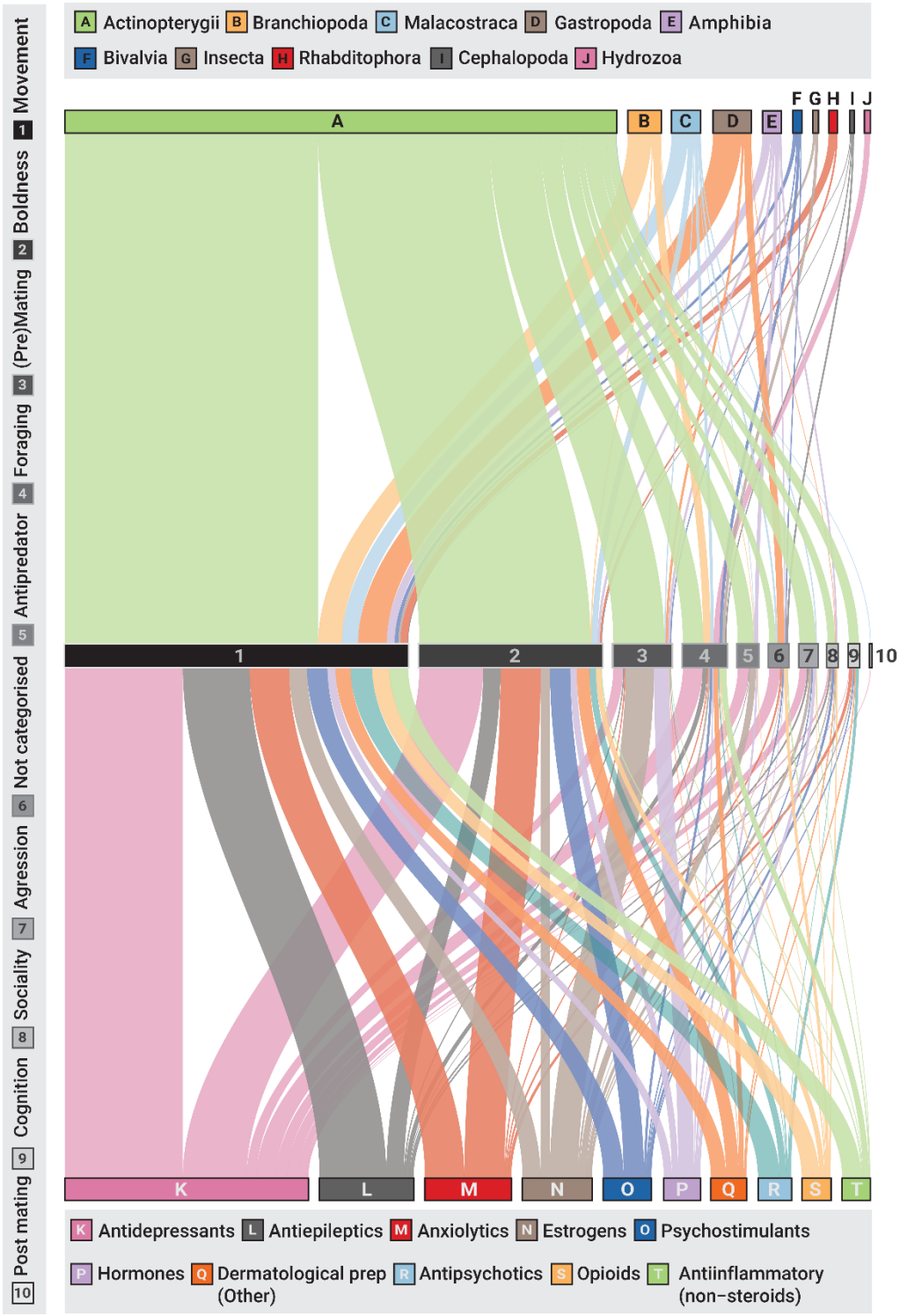


Figure 7. A broad overview of the link between population, exposure and outcome elements. The Sankey plot shows the connection between all behavioural categories (numbered 1–10; represented by the boxes in the middle of the plot), the top 10 most common phylogenetic clades (Class; shown at the top of the plot), and the top 10 therapeutic groups (ATC level 3; shown at the bottom of the plot). The thickness of each band that connects the population to behaviour, or exposure to behaviour element, corresponds to the number of occurrences in the EIPAAB database. An interactive version of the figure is available at https://jakemartinresearch.github.io/EIPAAB-database/

Additional ecotoxicological endpoints

A secondary goal of our SEM was to collate information on additional endpoints (e.g. sub-organismal, reproduction, growth, survival) measured alongside behaviour to facilitate connections across domains that may be useful for future chemical risk assessment and management activities, including potential regulatory decision-making. We found that 51.7% of articles (466/901) also included at least one additional sub-organismal physiological or endocrine endpoint, such as hormone concentrations, biomarker expression, or mRNA transcription. In addition, 39.7% of articles (358/901) measured at least one endpoint that has been used in traditional ecotoxicity testing, such as survival, growth, reproductive output, or developmental abnormalities.

Mapping the quality of the evidence base

Study validity was not used as an inclusion criterion; however, we did extract information about study validity to enrich the database and to identify potential methodological reporting gaps in the evidence base. We extracted information relevant to a subset of study quality information from the CRED reporting guidelines [41] and several additional validity metrics (see Table 2 and Table S3). To highlight key methodological and/or reporting gaps identified: we observed a low percentage of studies employing (or reporting) experimenter

804 blinding during the scoring or analysing of behaviour (17.0%), randomly (or pseudo-
805 randomly) assigning organisms to exposure treatments (40.2%), providing key details about
806 the pharmaceutical compound used in the exposure (e.g. CAS registry number 24.8% or
807 purity 25.4%), employing exposure concentration verification (e.g. water verification 20.6%
808 or tissue verification 8.9%), following any type of guideline (or modified guideline; 15.0%),
809 or performed the test under Good Laboratory Practice (GLP) conditions (0.7%). In the
810 opposite direction, a high percentage of studies reported details related to the source of
811 the animals (84.4%), aspects of animal care and housing (e.g. animal feeding 79.5%; water
812 quality parameters 89.5%; dark-light cycle 83.9%), providing details about exposure duration
813 (minimum exposure duration 94.1%, maximum duration 94.5%), and describing methods for
814 scoring behavioural endpoints (77.3%; although we note lower levels of extractor
815 consistency with some of these metadata; see Table S6).
816 We should highlight that some of the species validity information may be implied or
817 assumed to those with expert knowledge of that species; for example, if a species is
818 hermaphroditic, sex may not have been reported; alternatively, for species that reach
819 adulthood within 14 days, a 14-day exposure may have implied an adult life stage. With that
820 said, we extracted these metadata based on the definitions given by the authors. Where
821 information was not supplied, it was not assumed or inferred by extractors.
822
823 **Table 2**. All extracted information that relates to study validity. If the validity metadata
824 are aligned with a CRED quality criteria [41], the associated CRED number is provided. The
825 percentage of articles meeting the validity criteria is shown overall, and for each study
826 motivation. NA indicated that the criterion was not part of CRED, but an additional criterion
827 we extracted information about.

Validity criteria	CRED	Overall	Environ	Medical	Basic
A guideline or modified guideline was followed	1	15%	21.8%	6.0%	6.4%
The test was performed under Good Laboratory Practice (GLP) conditions	2	0.7%	1.0%	0.4%	0.0%

Survival, growth and/or reproduction of the test organism(s) was reported	3	39.7%	53.5%	26.1%	15.3%
The test substance is identified with a CAS number	5	24.8%	36.7%	10/7%	7.0%
The purity of the test substance was reported	6	25.4%	38.8%	9.4%	5.7%
Organism(s) life stage is known and reported	8	83.4%	82.9%	91.0%	73.5%
Organism(s) sex is known and reported	8	53.5%	49.6%	59.0%	56.8%
Test organism source is reported	9	84.4%	86.9%	78.8%	84.5%
Information provided regarding feeding	11	79.5%	84.3%	68.2%	80.4 %
Information provided regarding water characteristics (e.g. temperature, pH, oxygen content)	11	89.5%	92.7%	88.5%	80.3 %
Information provided regarding light/dark conditions	11	83.9%	84.1%	85.9%	80.3 %
Exposure minimum duration is defined	14	94.1%	95.6%	90.0%	96.7%
Exposure maximum duration is defined	14	94.5%	96.3%	89.8%	97.0%
The concentration of the test substance is verified in the water (waterborne exposures only)	15	20.6%	35.8%	2.5%	2.7%
The concentration of the test substance is verified in the tissue of the organism (waterborne exposures only)	15	8.9%	13.4%	4.2%	4.7%
Employs randomisation (pseudo-randomisation) of treatment allocation	NA	40.2%	44.9%	32.2%	36.7%
Experimental blinding was performed	NA	17.0%	14.7%	18.9%	21.5%
Methods for scoring behavioural endpoints described	NA	77.3%	76.0%	78.5%	79.4%
Conflict of interest statement is made in the article (with or without conflict identified)	NA	54.8%	50.2%	72.1%	44.3%

Limitations of the systematic map

Two potential limitations of the evidence base to consider are the inherent complexity of assigning therapeutic classes to pharmaceuticals and the complexity of defining animal behavioural responses into discrete categories. First, we used Anatomical Therapeutic Chemical (ATC) Classification to group our compounds, which assigns active ingredients of drugs according to the organ or system on which they act and their therapeutic, pharmacological, and chemical properties [47]. However, it is well recognized, even by the World Health Organization (see "Classification Principles & Challenges" [50]), that

837 pharmaceuticals can be prescribed and used for treating non-target illnesses. For example,
838 beta-blockers (a family of blood-pressure regulating drugs) and certain antihistamines (used
839 for treating allergies), can also be prescribed for the treatment of anxiety [51]. As a result
840 of this complexity, we did not independently assign pharmaceuticals without an existing
841 ATC class to their own therapeutic class. Thus, we highlight that 121 drugs (28% of the total
842 database) are not included in summaries made at the pharmacological group level (e.g.
843 Figure 5A). Similarly, it can also be complex to categorise animal behaviour into discrete
844 overarching categories, as behaviour, and how scientists describe it, varies by species.
845 Moreover, behaviour is context-dependent, in that a given behaviour measured in one
846 context could represent a different underlying motivation in another context. For example,
847 affiliation with a group of conspecifics may represent social propensity in one context but
848 antipredator behaviour in another, if a perceived threat is present. We aimed to reduce
849 ambiguity in assigning behaviours to overarching classes (and the sub-categories within each
850 class) by following the author's definition of the behaviour in the article. This could lead to
851 inconsistencies where, for example, an animal solving a maze task could be defined as a
852 measure of “boldness and exploration” in one article, but the same task could be a measure
853 of “cognition” in another article. Moreover, authors can introduce inconsistencies even
854 within articles if they define or refer to behaviours in multiple ways throughout the text.
855 We note in the consistency section above that there was some extractor disagreement in
856 the assignment of behavioural measures to the overarching categories, ranging from 75.5 to
857 99.3% (median 98.6%, see Table S6), as well as the more specific subcategories with a range
858 of 67.6–99.3% (median 95.8%, see Table S6). We believe that this, in part, reflects the
859 inherent difficulty of assigning behavioural classes across a broad range of taxa and study
860 disciplines.
861 We also identified several potential limitations of the review search methods used.
862 Although we included articles written in all languages in which our review team was
863 proficient (8 different languages), the evidence is likely still biased towards research

published in English, because the search strings were written in English, and there is a higher prevalence of English records in the databases used for the search. This is important to highlight as it is well recognized that language can introduce bias in the evidence base [52]. With that said, only 4 articles were excluded from the EIPAAB database at the full-text screening stage based on language. Another potential limitation in the review methods for this map is a limited search of the grey literature. Although we allowed for grey literature to be included from our database searches and we solicited grey literature submissions in our supplementary article search advertising calls, we did not search any grey literature databases and removed the planned screening of academic theses from the map. This decision was taken in part due to time and resources needed to screen the evidence base, but also because screening theses would require further quality checks and detailed deduplication cross-checks to remove duplicated published thesis chapters. We suggest this could be added for subsequent systematic review or meta-analytic projects using this database that have a narrower research scope. Finally, we also screened only a subset of articles at the full-text stage in duplicate, and we have discussed the implications of this above regarding extraction consistency.

Conclusion

We sought to systematically synthesise all available Evidence for the Impacts of Pharmaceuticals on Aquatic Animal Behaviour (EIPAAB). We report a considerable amount of research on this topic, with 901 articles—representing 1,739 unique species-by-compound combinations—being included in the EIPAAB database. Broadly, we see that the EIPAAB database would be ideal in supporting future ecotoxicology studies and experiments focusing on animal alternatives, identifying and incorporating evidence from behaviour endpoints into chemical risk assessment and management, to highlight knowledge gaps for future research, and to act as a launching pad for further targeted synthesis with more quantitative

meta-analytical methodologies. The implications of the collated evidence for policy/management and research are discussed below.

Implications for policy and management

Increasingly, behavioural endpoints are being suggested as valuable tools in environmental chemicals assessment and management (including regulatory activities for human and veterinary pharmaceuticals) but are rarely included in such context [17,18]. There are several possible reasons for this, including poor reporting of methodology, using non-standard methods, and limited evidence in an ecotoxicological context of the links between behaviour and adverse outcomes/standard endpoints [53]. The EIPAAB database provides insights into all three of these potential barriers to inclusion in regulation.

Firstly, we have indeed identified several methodological and/or reporting pitfalls. This includes a lack of studies employing (or reporting) experimenter blinding during the scoring/analysing of behaviour, randomly (or pseudo-randomly) assigning organisms to exposure treatments, providing key information about the pharmaceutical compound used in the exposure (e.g. CAS registration number or purity), providing key information about the study organism used in the exposure (e.g. sex), and validating exposure concentration (e.g. water verification or tissue verification). Research on the effects of pharmaceuticals on animal behaviour would benefit from addressing these aspects of methodical reporting and study methodology, many of which require little additional effort from experimenters, and we hope that this review can be a catalyst to improve these aspects in the field. With that said, there are many articles that do not have these identified pitfalls in the evidence base, and if required, those seeking to use this evidence for regulatory purposes (or likewise) could filter the database to help identify those studies that meet relevant criteria. More broadly, the field of behavioural ecotoxicology and toxicology studies with animal alternatives (e.g. fish models) could benefit from the use of data reporting and reliability guidelines specific to behavioural endpoints to increase the likelihood of these studies being

included in future chemical risk assessment and management, such as regulatory processes. A recent set of such guidelines is provided in EthoCRED ([54]), a behavioural endpoint-specific adaptation of the parent CRED guidelines. The use of such guidelines, like EthoCRED, would improve reporting of important methodological information, guide methodological decision-making for future studies, and increase the replicability of the field.

Secondly, the database included a total of 63 different sub-categories of measured behaviours and for which aquatic species they were measured. From these data, we suggest that new standardised ecotoxicity test guidelines that include behaviour could be developed by looking for the most common or most widely applicable testing parameters. As an example, our SEM has revealed a wealth of studies focused on fishes (especially for zebrafish) across multiple behavioural endpoints (particularly movement, anxiety/boldness, and pre-copulatory/copulatory behaviours); by comparing such methods, one could arrive at the most broadly suitable tests. We believe that the next step in achieving this would be a focused review and meta-analysis, looking at the specific methods used for candidate behavioural tests and the nature of the data they provide, followed by multi-lab validity and repeatability tests once a candidate protocol is established.

Thirdly, within the EIPAAB database, we have identified which studies can provide direct links between behaviour and other adverse outcomes/standard endpoints. Specifically, we have identified studies that also measured sub-organismal physiological/endocrine endpoints, as well as which studies assessed more traditional endpoints like animal growth, survival, and/or reproduction alongside behaviour. We see this as a starting point for future work to connect behavioural endpoints to molecular initiating events and to endpoints currently being used in traditional risk assessments, including integration with the adverse outcome pathway (AOP) concept [55]. Specifically, we suggest targeted meta-analytic approaches focusing on articles that have measured behaviour alongside additional morphometric endpoints (sub-organismal, growth, survival,

945 and/or reproduction endpoints), identifying potential correlations in the direction and
946 magnitude of observed effects.
947
948 *Implications for research*
949 Our SEM highlights that this rapidly growing research area has several knowledge clusters
950 appropriate for further quantitative synthesis. Specifically, future meta-analytical work
951 could focus on the behavioural impacts of antidepressants, antiepileptics, or estrogens,
952 particularly for endpoints like locomotion, boldness, and reproductive behaviours. We have
953 also identified that the evidence base is heavily skewed towards research on zebrafish,
954 which is perhaps unsurprising given that the zebrafish is a well-established model in
955 (eco)toxicological, medical, and basic research [56,57]. Therefore, future comparative
956 synthesis across behavioural categories or compounds using zebrafish may offer a suitably
957 homogenous prospect for detailed meta-analysis. Indeed, the available evidence on
958 zebrafish could be a valuable step towards disentangling and identifying quantitative
959 thresholds at which exposure to a given pharmaceutical affects behaviour. For instance,
960 how, and at which exposure concentration, the antidepressant fluoxetine impacts fish
961 behaviour has been disputed in the earlier literature [43].
962 We would also like to highlight gaps in the evidence base that require more primary
963 research. Firstly, there were relatively few studies using wild-caught animals. Wild-caught
964 *versus* lab-reared organisms can differ greatly in their behaviour and underlying physiology
965 traits [58–61], and thus, may also respond differently to pharmaceutical exposure. More
966 research using wild-caught organisms could help identify whether lab-reared model species
967 are equally sensitive to pharmaceutical exposure (e.g. [62]). Additionally, locomotion and
968 boldness were by far the most common behavioural endpoints measured. We argue that
969 measuring contaminant-induced impacts on a more diverse array of behavioural endpoints—
970 particularly those with obvious links to fitness (e.g. pre and post-copulatory, antipredator,
971 and foraging behaviours)—would give a more holistic understanding of potential impacts on

aquatic wildlife. However, we also acknowledge that the most commonly measured behaviours, locomotion and boldness, are often the simplest to measure and offer the highest throughput. There was also a distinct lack of studies measuring behaviour within a social context (e.g. free-swimming groups) and employing exposure durations greater than a week; it is reasonable to assume that for most animals, real-world exposures will occur in social groups (animals rarely, if ever, exist in a social vacuum; [63]), and that many pollutants would have environmental or biological half-lifes exceeding seven days. Thus, future research addressing the impacts of pharmaceutical pollutants on animals under a social context and over chronic time scales would improve our understanding of real-world impacts. Finally, we suggest that research is prioritised on pharmaceutical compounds that are absent or infrequently represented in our database, yet are common in the environment (i.e. what evidence are we currently missing). This could be done by cross-checking the EIPAAB database against recent publications (e.g. [1]) and open databases reporting environmental pharmaceutical concentrations around the world (e.g. AstraZeneca EcoPharmacoVigilance Dashboard [64]; Umwelt Bundesamt "UBA-PHARMS" database [65]; NORMAN EMPODAT chemical occurrence database [66]).

We identified that many of the studies in our database have an environmental motivation; however, we also identified a lot of available research in adjacent fields that focus on medical research questions and basic research questions, particularly with fish models employed as animal alternatives. Future work assessing the bibliometric connections between the fields would be interesting to reveal how much crosstalk (if any) exists via the use of co-author and co-citation networks [34].

We have already pointed out several gaps in study validity that should be considered in future studies and noted that using standard reporting guidelines would increase their utility in regulatory processes. We also advocate that the use of reporting guidelines (e.g. EthoCRED) will more broadly increase the robustness and replicability of studies assessing the effects of pharmaceuticals on aquatic animal behaviour. Importantly, we highlight that

disclosing details about how animals were housed, how they were assigned to treatments, how the behaviour was recorded and scored, and the use of blind scoring, is paramount to increasing transparency and reducing unintended experimenter bias.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: All supplementary files and the EIPAAB database can be accessed from the Open Sciences Framework (OSF) at https://doi.org/10.17605/OSF.IO/ATWY6. Below, we provide a list of all supplementary files and individual links. The R script used to generate the summary statistics and figures presented in this manuscript is available on GitHub (https://github.com/JakeMartinResearch/EIPAAB-database).

Competing interests: The authors declare that they have no competing interests

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Authors' contributions: Authorship is ordered alphabetically by last name except for the first, second, and last authors. We have reported our methods in the manuscript text following the MeRIT guidelines to improve author contributions' granularity and accountability (see main text). Briefly, 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, ESM, KNF, JS, JAB, DC, IYL, HT, MM, LML, MGB, SO, KRS, NSH, ESJT, and ML extracted the data to build the systematic evidence map. JMM, ESM, and MM extracted all supplementary metadata for compounds and species. JMM tidied and analysed the EIPAAB database (writing all R and Python Scripts used during these steps), with input from ESM. JMM and ESM wrote the manuscript with input from all authors. All authors read and approved the final manuscript.

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Additional Files

(1) EIPAAB database: the Evidence of the Impacts of Pharmaceuticals on Aquatic Animal Behaviour (EIPAAB) database created from the systematic review and data extraction described in the manuscript (name: EIPAAB-database.csv; link: https://osf.io/rzwv2 OR https://github.com/JakeMartinResearch/EIPAAB-database)

(2) Supplementary file 1: ROSES Form (name: martin-et-al-supp-file-1-ROSES.xlsx; link: https://osf.io/g2h4x)

(3) Supplementary file 2: Title and abstract screening decisions (name: martin-et-al-supp-file-2-title-abstract-screen-decision.csv; link: https://osf.io/hafs5)

(4) Supplementary file 3: List of eligibility disagreements for duplicate screenings at the full-text screening stage (name: martin-et-al-supp-file-3-eligibility-disagreements.csv; link: https://osf.io/ajbs7)

(5) Supplementary file 4: Full-text screening excluded articles (name: martin-et-al-supp-file-4-full-text-exculded-atricles.csv; link: https://osf.io/3ynrp)

(6) Supplementary file 5: Full-text extraction form from Qualtrics (name: martin-et-al-supp-file-5-full-text-screening-extraction-form.pdf; link: https://osf.io/p57ev)

(7) Supplementary file 6: Read me file (name: READ-ME.csv; link: https://osf.io/8x3rk)

(8) Supplementary file 7: R script used to summarise the EIPAAB Database interactive HTLM (https://jakemartinresearch.github.io/EIPAAB-database/); a static version is also available on OSF (name: martin-et-al-supp-file-7-r-script.Rmd; link: https://osf.io/z5tw4)

(9) Supplementary file 8: The Web of Science annual article counts for each of the most common research categories identified in the database (name: martin-et-al-supp-file-9-wos-research-areas-1992-2022.csv; link: https://osf.io/35hgj)

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