Other aggregative reviews (e.g. Meta-analyses, Critical reviews)

Title
What is the overall PFAS trophic magnification estimate and the key drivers of variability?

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PFOA, PFOS, Food web, Trophic magnification, Biomagnification

Background
Per- and polyfluoroalkyl substances (PFAS) are man-made compounds extensively utilised in various consumer products for over six decades. These substances are detected globally in wildlife, even in remote areas (Muir et al., 2019), and they tend to accumulate and intensify in concentration as they progress through the food chain (Miranda et al., 2022), a phenomenon referred to as biomagnification. The accumulation of substances to hazardous levels in living organisms has become a major concern for biodiversity, ecosystem function, food safety, and human health (Kelly et al., 2007). Biomagnification is often quantified using trophic magnification estimates. The simple log-linear relationship between PFAS concentration and the organisms' trophic level (TL) is widely used to quantify the degree of trophic magnification. However, estimates of PFAS trophic magnification among taxa are highly variable. This variability hinders our ability to generate and develop models to predict the behaviour of fluorinated compounds through the food chain. In this systematic review and meta-analysis, we will compile and query a comprehensive database of studies on PFAS biomagnification in aquatic and terrestrial food webs. We will estimate the overall and compound-specific trophic magnification potential to assess what compounds are more likely to biomagnify in wildlife food webs. Considering the substantial variation in study methodologies, this meta-analysis seeks to combine the results from various studies and quantify the overall trophic magnification estimate and its sources of variability. We will also identify data gaps and methodological and conceptual challenges within the literature and outline suggestions for future research on biomagnification.

Theory of change or causal model
The trophic magnification slope (TMS) quantifies the trophic magnification of pollutants up the food chain (Supplementary Figure 1). It is estimated by: 1) measuring contaminant concentration in organisms from at least three different trophic levels (TLs); 2) using stable nitrogen isotope analysis or dietary data to determine organisms' TLs; 3) regressing the logarithm of contaminant concentration against TLs to fit a linear relationship: $\log10[\text{contaminant}] = TL(b) + a$ where b represents the TMS, and a is the intercept. A TMS > 0 indicates trophic magnification, while a TMS < 0 indicates biodilution (Borgå et al., 2012). If TMS = 0, it suggests that no significant trophic magnification or trophic dilution is occurring. The TMS is a standardised and comparable metric

across different studies, ecosystems and contaminants. The trophic magnification factor (TMF) is the antilogarithm of the TMS and, like the TMS, is frequently employed to quantify the trophic magnification potential of chemical compounds. Due to the word limit count, a more extensive explanation of the conceptual model is provided in Supplementary Information.

Stakeholder engagement
No stakeholders were involved in the formulation of the question and the development of the protocol.

Objectives and review question
Primary review question: To what extent do PFAS biomagnify in wildlife food webs? Secondary review question: Can differences in compound and study properties (i.e., methodological factors) account for variation (heterogeneity) in trophic magnification estimates? Objectives: Our objectives are three-fold: 1. Estimating the overall and compound-specific trophic magnification of PFAS across available evidence. 2. Estimating variability (heterogeneity) in PFAS trophic magnification at the within- and between-study level. 3. Identifying and quantifying sources of variability in trophic magnification estimates, including: a. Methodological factors such as differences in study design. b. Geographic variation across food web locations. c. Food web ecological factors such as complexity and structure. d. Compounds' physicochemical properties. A complete list of moderators, predictions, explanations, and references is provided in Supplementary Table 1. Hypotheses: In this meta-analysis, we will use TMS (i.e., slope) values as an effect size of the trophic magnification estimate. We hypothesise that the overall effect size is > 0, indicating PFAS trophic magnification on average. However, we expect high effect size variability between and within studies and a large proportion of variation explained by sampling variance. We expect different PFAS compounds to biomagnify to different extents because of their carbon chain length, further explaining the variability of the overall effect. The inconsistent research methods across studies may also explain variability. However, we expect a considerable unexplained variance in effect sizes even after accounting for methodological factors due to food webs' complex biological and ecological nature.

Definitions of the question components
We follow the PECO (Population, Exposure, Comparator, Outcome) approach to question formulation. • Population: The population in this study refers to the global aquatic and terrestrial food webs (any biological organism) affected by PFAS contamination. • Exposure: The exposure of interest is the exposure to any PFAS. Thus, PFAS contamination must be present and quantitatively measured in (whole or any part of) organisms within the studied food web. • Comparator: We will compare organisms' PFAS concentration and trophic levels (a relationship measured through the trophic magnification slope (TMS)). We will also quantitatively assess the effects of factors that might influence PFAS trophic magnification. These factors include research methodologies, geographical variables, ecosystem characteristics, and food web composition (Supplementary Table 1). • Outcome: The outcome of interest is the trophic magnification slope (TMS) within a studied food web. The TMS is the slope of the relationship between logarithm-transformed PFAS concentration and trophic levels of biota within a food web (see the "Casual model" section for more details).

Search strategy
The search strategy for this research project will be comprehensive and systematic, aiming to capture both conventionally published scientific literature and grey literature. The sources of articles will include a combination of six databases listed below. Sources to be Searched: The databases will include PubMed, Web of Science (Core Collection), Scopus, and GreenFILE. Grey literature will be searched using OpenGrey and ProQuest. Search Terms and Strings: On 11/03/2024, we tested a pilot search string for all six databases. Our search strings proved sensitive

and comprehensive (for more details, see the “Comprehensiveness of the search” section). Supplementary Table 2 provides each database's pilot search string formula and corresponding result.

Bibliographic databases

The bibliographic databases/platforms to be used in this research project include: 1. PubMed • Web link: https://pubmed.ncbi.nlm.nih.gov/ • Full access via institutional subscription • Type of literature: peer-reviewed articles and books • Language: mainly English 2. Web of Science (Core Collection) • Web link: https://www.webofscience.com/ • Full access via institutional subscription • Type of literature: peer-reviewed articles and books • Language: mainly English 3. Scopus • Web link: https://www.scopus.com/ • Full access via institutional subscription • Type of literature: peer-reviewed articles and books • Language: mainly English 4. GreenFILE • Web link: https://research.ebsco.com/ • Full access via institutional subscription • Type of literature: peer-reviewed articles and books • Language: mainly English 5. Open Grey • Web link: https://www.greynet.org/opengreyrepository.html • Full access via institutional subscription • Type of literature: government reports, conference papers, and theses • Language: mainly English 6. Theses and Dissertations Database (ProQuest) • Web link: https://www.proquest.com/ • Full access via institutional subscription • Type of literature: dissertations and theses • Language: mainly English

Web-based search engines

We will do a brief, focused search on one web-based search engine to find relevant articles on this topic. Our web-based search engine will be Google Scholar, using English search terms. Our search string will be "PFAS trophic magnification bioaccumulation". We will screen the top 100 hits from this search string in Google Scholar, reviewing paper titles and abstracts to determine their relevance. In addition, we will use the reference lists of key reviews (i.e., Ahrens et al., 2014; Franklin, 2016; Lewis et al., 2022; Miranda et al., 2022) to identify additional relevant studies (aka snowballing). We will examine the citations within these key papers to find other relevant literature that may not have been identified through the initial search strategy. The iterative process of expanding the search based on references will help us identify a broader range of evidence and improve the overall rigour and completeness of the systematic review.

Organisational websites

None.

Comprehensiveness of the search

We ensured a comprehensive search strategy by evaluating the sensitivity of our search strategy. To do so, we independently assembled a list of benchmark articles and calculated the recall rate of our search strings (percentage retrieval). Specifically, we used the 25 studies included in the most recent literature review of PFAS trophic magnification conducted by Miranda et al. (2022) as a benchmark. Our search string (see ‘Search strategy’ section) was tested against the benchmark studies on the Scopus database on 12/03/2024. The search string retrieved all 25 bibliometric records (100% benchmarking accuracy). Supplementary Table 3 provides the results of the retrieval of benchmark studies.

Search update

Given the aim of publishing this review within 18 months from the initial search, we think additional searches would provide minimal benefit. If we encounter a significant delay in completing data synthesis and analysis within the intended timeframe, adding an updated search up to 3 months before submission for publication will be considered. This would incorporate only very recent, applicable studies to maintain the currency of evidence covered in the final manuscript. If published within one year, we expect an updated search would yield few or no additional studies.

Screening strategy
Steps of screening studies for eligibility will include: 1. Initial Screening: We will screen the titles and abstracts of the identified studies against our inclusion criteria (see ‘Eligibility criteria’ section). Two reviewers will independently perform the initial screening to minimise bias. In case of discrepancies between the reviewers, a discussion will be held to reach a consensus. A third reviewer will be involved if a consensus cannot be reached. 2. Full-text Screening: We will retrieve the full text of the articles that passed the initial screening. We will screen the full texts of the identified studies against our inclusion criteria (see ‘Eligibility criteria’ section). Two reviewers will independently screen 10% of the full texts and discuss conflicts until a 100% agreement is reached. Then, one of these two reviewers will conduct the full-text screening for all identified studies.

Eligibility criteria
All studies included in this systematic review and meta-analysis should be consistent with the PECO elements of our research question (refer to section 7.1, "Definitions of the question components"). Furthermore, the following eligibility criteria apply: • Study Design: Any study design, such as field-based observational and mesocosms-based studies, is eligible. Studies must estimate and provide the trophic magnification slope or the trophic magnification factor or any measure that can be used to calculate the trophic magnification slope. • Other restrictions: o Time range: No restrictions. o Languages: Only studies published in English or a language spoken by any of the authors (i.e., Italian, Japanese, Polish, Russian, Traditional and Simplified Chinese, French, Portuguese, Spanish) will be eligible due to language constraints. Due to the word limit count, eligibility criteria for title and abstract screening and full-text screening are provided in the attached Supplementary Information.

Consistency checking
Two reviewers will independently screen bibliographic records at the title plus abstract level. Before one reviewer conducts the full-text screening, two reviewers will independently screen 10% of the full texts and discuss conflicts until a 100% agreement is reached.

Reporting screening outcomes
The outcomes of the screening process will be reported as follows: 1. PRISMA Diagram: A PRISMA-EcoEvo (The Preferred Reporting Items for Systematic reviews and Meta-Analyses in ecology and evolutionary biology; O’Dea et al., 2021) flow diagram will visually represent the screening process. This diagram will show the number of articles identified, included and excluded at each stage of the screening process. 2. List of Eligible Articles: A comprehensive list of all eligible articles that have been included in the review will be provided. This list will include the citation for each article, and a brief summary of the study design, ecosystem studied, PFAS compounds examined, and the reported trophic magnification factors. 3. List of Excluded Full-Text Articles with Reasons for Exclusion: A list of all full-text articles excluded during the screening process will be provided, along with the main reasons for their exclusion (e.g., the study design not meeting the eligibility criteria, the article not being available in English, or the article not providing sufficient data on the outcome of interest).

Study validity assessment
We will attempt to evaluate the internal validity of included studies, commonly called “risk of bias” (RoB) in systematic reviews. We will use a modified version of SYRCLE’s risk of bias tool for animal studies (Hooijmans et al., 2014). Supplementary Table 4 shows our proposed SYRCLE’s adapted version and describes each of the five items it includes. Our aim in developing this tool was to identify studies with significant issues related to selection bias, performance bias, measurement bias, reporting bias, and funding bias. Studies displaying a high risk of bias in any of these areas will

undergo sensitivity analysis for further evaluation. Supplementary Figure 4 provides an example of a high risk of reporting bias for an eligible study for quantitative analysis.

Consistency checking

A consistency-checking procedure will be implemented to ensure the consistency and reliability of the study validity assessment. This involves having one reviewer appraise a set of studies, which will constitute 10% of the total studies included in the meta-analysis. A second reviewer will cross-check the set of studies. Discrepancies in the scores will be discussed in a consensus meeting between the two reviewers. If a consensus cannot be reached, a third reviewer will be consulted to make the final decision. This process ensures that the appraisal is not only consistent but also transparent and reproducible. The consistency checking procedure will be conducted before the full appraisal process begins. This allows any disagreements or misunderstandings about the appraisal criteria to be addressed early on, ensuring a smoother and more efficient appraisal process.

Data extraction strategy

Due to the word limit count, the data extraction strategy for this meta-analysis is extensively described in Supplementary Information.

Meta-data extraction and coding strategy

We will extract relevant data from the included studies according to the strategy described in this section. The strategy will consist of creating various data tables that will be coded in a relational database. Each data table will include information on similar aspects of the study (e.g., study details, PFAS details, food web details, etc) and will be characterised by primary and foreign keys. A primary key will ensure that data in the specific column is unique. A foreign key is a column or group of columns in a relational database table that links data in two tables. Keys uniquely identify a record in the relational database table. The data tables will be as follows: • Study_data: it will include (meta-)data on the included studies in the meta-analysis. • Appraisal_data: it will include (meta-)data on the characteristics of included studies that will be assessed for risk of bias (see the “Study validity assessment” section). • FoodWeb_data: it will include (meta-)data on the food webs investigated in the included studies. • TMS_data: it will include trophic magnification slopes and related (meta-)data. Supplementary Table 5 lists all meta-data for extraction and provides their description. An additional data table (i.e., PFAS_info) will include information on individual compounds investigated in the included studies (Supplementary Table 6). This additional table will not contain data extracted from the included studies; it will include general data on compound characteristics extracted from external sources (the data source will always be recorded and provided using a comment field).

Consistency checking

A consistency-checking procedure will be implemented to ensure the reliability of the data extraction process. This involves having one reviewer extract data from the identified studies and having a second reviewer cross-check 10% of the studies. Discrepancies in the extracted data will be discussed in a consensus meeting between the two reviewers. A third reviewer will be consulted to decide if a consensus cannot be reached. The consistency checking procedure will be conducted before the full-text data extraction process begins. This allows any disagreements or misunderstandings about the data extraction criteria to be addressed early on, ensuring a smoother and more efficient data extraction process.

Potential effect modifiers/reasons for heterogeneity

A list of effect modifiers with descriptions and references is presented in Table 1 (see the “Objective and review questions” section).

Type of synthesis
A quantitative synthesis combined with a succinct narrative synthesis.

Narrative synthesis methods
We will support our quantitative synthesis with a succinct narrative synthesis of findings.

Quantitative synthesis methods
We will employ multilevel meta-analytic and meta-regression models accounting for the non-independence due to clustering variables. We will build an intercept-only meta-analytic model with the trophic magnification slope (TMS) of the linear regression between logarithmic PFAS concentrations and trophic levels. This model will aim to assess the overall trophic magnification of PFAS, its level of variability within and between studies, and the trophic magnification potential of individual PFAS compounds. The meta-analytic model will account for multiple sources of statistical non-independence as random factors, including the presence of multiple effect sizes from the same studies (1), food webs (2), compounds (3), and the impact of effect size identity (4). We will estimate the variance components of the random effects using the restricted maximum likelihood (REML) method. In addition to presenting variance components, we will assess the magnitude of relative heterogeneity among studies and species employing the multilevel version of I2 statistics, proposed by Nakagawa and Santos (2012) (cf. Higgins et al. (2003) and available in the orchaRd package under the function i2_ml). We will fit multiple moderators (see Supplementary Table 1) as fixed effects to quantify their moderating impact on PFAS biomagnification estimates among the included studies. Then, we will fit a multilevel multi-moderator model to estimate the combined effect of moderators on the outcome variable while controlling for the random effects. Finally, we will assess the significance of moderators in explaining heterogeneity through model selection and (multi)model inference (Anderson & Burnham, 2004; Yang et al., 2022).

Qualitative synthesis methods
We do not plan to conduct a formal qualitative synthesis. However, we will use mapping methods of evidence to categorise the studies' content, and we will discuss and graphically illustrate the results of the study assessment.

Other synthesis methods
None.

Assessment of risk of publication bias
We will examine the possible influence of publication bias on the meta-analytic estimates using graphical representations (Funnel plot) and statistical tests (Egger's Regression Test). Funnel plots are a common tool for visually assessing publication bias. In the absence of publication bias, the plot should resemble a symmetrical inverted funnel. Asymmetry can suggest publication bias, although it can also be due to other factors such as poor methodological quality or true heterogeneity. We will use Egger's Regression Test as a statistical test for funnel plot asymmetry (Egger et al., 1997).

Knowledge gap identification strategy
We will evaluate the availability and quality of data, particularly about specific PFAS compounds, geographical regions, or trophic levels. We will identify areas where data are scarce or absent. Moreover, we will evaluate the methodologies used in existing studies, including the types of tissue analysed, trophic level calculation methods, and other research approaches to identify methodological challenges and limitations.

Demonstrating procedural independence
We will ensure procedural independence by working according to the following methodological

points: • Recusal of Authors: Team members who have authored articles to be considered within the review will voluntarily recuse themselves from any discussions, decisions, or critical appraisals related to their own work. They will not participate in the evaluation of their own publications. • Implement a Blind Review Process: We will utilise a blinded review process where the names and decisions for inclusion of the reviewers are concealed during the initial screening and appraisal phases. • Document Decisions and Rationale: All decisions related to the inclusion or exclusion of articles and their critical appraisal will be thoroughly documented. This includes justifications for inclusion/exclusion and critical appraisal assessments.

Competing interests

No competing interests to disclose.

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Author's contributions

Protocol Conceptualisation: LR Pilot Data curation: LR Pilot Formal analysis: LR Funding acquisition: SN, LR Methodology: LR, ML, SN Project administration: LR Supervision: ML, SN, MDT Writing – Research Protocol Original Draft: LR, ML, SN, MDT Writing – Research Protocol Review and Editing: all authors

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References

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