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REGISTERED REPORT STAGE 1: STUDY DESIGN

Profiling research on PFAS in wildlife: Protocol of a systematic evidence map and bibliometric analysis

Catharina Vendl[1,2] | Matthew D. Taylor[1,2,3,4] | Jennifer Braeunig[4] | Matthew J. Gibson[5] | Daniel Hesselson[6] | Graham Gregory Neely[6,7,8,9] | Malgorzata Lagisz[1,2] | Shinichi Nakagawa[1,2]

[1] Evolution and Ecology Research Centre, University of New South Wales Sydney, Sydney, New South Wales, Australia

[2] School of Biology, Earth and Environmental Sciences, University of New South Wales Sydney, Sydney, New South Wales, Australia

[3] Port Stephens Fisheries Institute, New South Wales Department of Primary Industries, Nelson Bay, New South Wales, Australia

[4] Queensland Alliance for Environmental Health Sciences, Faculty of Health and Behavioural Sciences, The University of Queensland, Brisbane, Queensland, Australia

[5] School of Computer Science and Engineering, University of New South Wales Sydney, Sydney, New South Wales, Australia

[6] Centenary Institute, University of Sydney, Sydney, New South Wales, Australia

[7] Dr. John and Anne Chong Lab for Functional Genomics, University of Sydney, Sydney, New South Wales, Australia

[8] Charles Perkins Centre, University of Sydney, Sydney, New South Wales, Australia

[9] School of Life and Environmental Sciences, University of Sydney, Sydney, New South Wales, Australia

Correspondence
Catharina Vendl and Shinichi Nakagawa, Evolution and Ecology Research Centre, University of New South Wales Sydney, Sydney NSW 2052, Australia.
Email: c.vendl@unsw.edu.au and s.nakagawa@unsw.edu.au

Malgorzata Lagisz and Shinichi Nakagawa share senior authorship.

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Abstract

1. Per- and polyfluoroalkyl substances (PFAS) are a large group of manufactured chemicals. Since the beginning of their commercial production in the 1950s, PFAS have not only found their way into numerous industrial and commercial applications, but also into the bloodstream of much of the human population, the natural environment and wildlife. Exposure to high levels of PFAS poses a health risk for humans and animals, and may exacerbate the effects of other anthropogenic impacts faced by wildlife species. To gain a comprehensive overview of the abundance and distribution of PFAS research on wildlife species, and to better understand the drivers of this research, we will collate the available literature into a systematic evidence map and perform bibliometric analyses. The systematic mapping will present the distribution of research evidence that exists on PFAS in wildlife. The bibliometric analysis will provide an insight into the historical trends, interdisciplinarity, connectedness and the impact of the individual papers.
2. We will conduct a systematic literature search on Scopus, Web of Science and 10 other databases using predefined search strings. We will screen title, abstract and keywords first. We will then screen full-text papers. Two reviewers will be involved in the screening process. We will only consider publications in English, peer-reviewed articles, preprints and theses. We will include papers reporting

concentrations of any of 34 main PFAS types (based on a previous study) in wild animals. We will assess all publications included in the systematic map for predetermined indicators of quality and potential study-level biases. In addition, we will use bibliometric records from Scopus to perform impact and network analyses. We will present the results using a narrative summary, tables and colour-coded maps, bar and network plots. Results and associated database will be available on a dedicated freely accessible website.

3. This study will provide critical insight into the gaps and clusters of the literature with regard to the PFAS concentrations in wildlife. Our study will inform and direct future research efforts to fill the gaps revealed.

KEYWORDS

evidence synthesis, persistent organic pollutants, PFOA, PFOS, research synthesis, science mapping, systematic review

1 | INTRODUCTION

1.1 | Background

Per- and polyfluoroalkyl substances (PFAS, also spelled PFASs) are a group of 5000–10,000 organic chemicals commonly used in numerous industrial and commercial applications worldwide (USEPA, 2018). PFAS are exclusively synthetic, and thus do not naturally occur in the environment (Wang et al., 2017). They are water and oil repellent and have a high heat resistance. These chemical properties have made them common additives to many different products. Some of the best-known and widely distributed applications are the fluoropolymer Teflon, the stain-resistant coating Scotchguard and aqueous film-forming foam (AFFF) (Kissa, 2001; Paul et al., 2009; Prevedouros et al., 2006; Wang et al., 2017). The popularity of PFAS, along with their extreme persistence and high mobility, contributes to their ubiquitous distribution throughout the environment. PFAS not only accumulate in the environment, but also bind to human and animal blood proteins (Giesy & Kannan, 2002; Kannan, 2011; Kannan et al., 2004; Olsen et al., 2003). Some studies have also presented evidence for a link between PFAS exposure and health impacts in humans (Kirk et al., 2018) and wildlife (Ishibashi et al., 2008; Kannan et al., 2006).

PFAS can be divided into long-chain and short-chain substances. Perfluoroalkyl carboxylic acids (PFCA) – with seven or more fully fluorinated carbon atoms ($CnF2n + 1COOH$; $n \geq 7$; e.g. PFOA) – and perfluoroalkane sulfonic acids (PFSA) – with six or more ($CnF2n + 1SO3H$; $n \geq 6$; e.g. PFHxS) – are considered long-chain PFAS and tend to accumulate in biota and the environment more than their short-chain counterparts (see Table 1 for a list and abbreviations of common PFAS) (Buck et al., 2011; OECD, 2013; Wang et al., 2015). In addition, PFSAs accumulate to a larger extent than PFCAs of the same perfluoroalkyl chain length. This is thought to be due to their ability to bind to serum proteins more strongly (Conder et al., 2008; Ng & Hungerbühler, 2013).

Figure 1 shows a short timeline of important events in PFAS-related history of production, use and legal restrictions, since the discovery of these chemicals. While the U.S.-based company DuPont accidentally developed the first PFAS compound in 1938 (Lyons, 1994), the company 3M, also U.S. based, grew into the biggest PFAS producer worldwide and started the commercial manufacturing process of PFOA, PFOS and many other PFAS in the 1950s (3M Company, 2020). Since then, PFOS and PFOA have become the most produced, distributed and researched members of the PFAS family (USEPA, 2016a, 2016b). One of the main applications of PFAS is in AFFF products which included a wide range of different PFAS as active ingredients, including PFOS, PFOA and PFHxS. As early as 1968, research results suggested that PFAS accumulated in the human bloodstream (Taves, 1968). Belisle (1981), Ubel et al. (1980) and Yamamoto et al. (1989) eventually confirmed Taves' (1968) suspicion. Nevertheless, it took until the early 2000s before a large number of studies left no doubt that PFAS had not only made it into the human body, but also into wildlife (Giesy & Kannan, 2002), the oceans (Yamashita et al., 2005) and drinking water (Exner & Färber, 2006). The unique chemical properties of PFAS prevented an earlier detection in the environment, as measurements required specific and particularly sensitive analytical methods that were beyond the capabilities of most laboratories until recent times (Giesy & Kannan, 2002).

In the early 2000s, it also became evident that PFAS had indeed a compromising effect on human and animal health (Hekster et al., 2003; OECD, 2002). In the light of such findings, in 2002, the company 3M voluntarily phased out most of its production of long-chain PFAS substances, including PFHxS, PFOS, PFOA and FOSA (Martin et al., 2010). However, the worldwide production of other PFAS, such as PFUnDA, that were of lesser public concern increased (Bodin et al., 2016). In the meantime, national and international initiatives began attempts to restrict production and use of the most common long-chain PFAS globally. Among the most extensive programmes was the 2010/2015 PFOA Stewardship Program, initiated by the U.S. Environmental Protection

TABLE 1 Types of PFAS included in the systematic map (Pelch et al., 2019). PFAS are listed in their acidic form

Name of PFAs group	Abbreviation	Full name	CAS Registry No.
Perfluoroalkyl carboxylic acids (PFCA)	PFBA	Perfluorobutanoic/perfluorobutyric acid	375-22-4
	PFPeA	Perfluoro-n-pentanoic acid	2706-90-3
	PFHxA	Perfluorohexanoic acid	307-24-4
	PFHpA	Perfluoroheptanoic acid	375-85-9
	PFOA	Perfluorooctanoic acid	335-67-1Perfluorooctanoic acid 95% | 335-67-1 Perfluorooctanoic acid 95% | 335-67-1
	PFNA	Perfluorononanoic acid	375-95-1
	PFDA/PFDeA/PFDcA	Perfluorodecanoic acid	335-76-2
	PFUnDA/PFUnA/PFUA/PFUdA	Perfluoroundecanoic acid	2058-94-8
	PFDoA/PFDoDA	Perfluorododecanoic acid	307-55-1
	PFTrDA/PFTriDA/PFTrA	Perfluorotridecanoic acid	72629-94-8
	PFTA/PFTeDA	Perfluorotetradecanoic acid	376-06-7
Perfluoroalkane sulfonic acids (PFSA)	PFBS/PFBuS	Perfluorobutane sulfonic acid	375-73-5
	PFPeS	Perfluoropentane sulfonic acid	2706-91-4
	PFHxS	Perfluorohexane sulfonic acid	355-46-4
	PFHpS	Perfluoroheptane sulfonic acid	375-92-8
	PFOS	Perfluorooctane sulfonic acid	1763-23-1
	PFNS	Perfluorononane sulfonic acid	68259-12-1
	PFDS	Perfluorodecane sulfonic acid	335-77-3
	PFECHS	Perfluoroethylcyclohexane sulfonic acid	335-24-0
Polyfluoroalkyl substances derivates	ADONA	4,8-dioxa-3H-perfluorononanoic acid	958445-44-8
Perfluoroalkyl ether sulfonic acids	6:2Cl-PFESA (F-53B)	6:2 Chlorinated polyfluoroalkyl ether sulfonate	73606-19-6
	8:2 Cl-PFESA	8:2 Chlorinated polyfluorinated ether sulfonate	83329-89-9
	Nafion BP2	Nafion Byproduct 2	749836-20-2
Fluorinated polymers	Hydro-Eve	2,2,3,3-Tetrafluoro-3-((1,1,1,2,3,3-hexafluoro-3-(1,2,2,2-tetrafluoroethoxy)propan-2-yl)oxy) propanoic acid	773804-62-9
Perfluoroether alkane carboxylic acids	PFO4DA	Perfluoro-3,5,7,9-tetraoxadecanoic acid	39492-90-5
	PFO5DoDA	Perfluoro-3,5,7,9,11-pentaoxadodecanoic acid	39492-91-6
	HFPO-DA (GenX)	Hexafluoropropylene Oxide (HFPO) Dimer Acid	13252–13–6
	HFPO-TA	Hexafluoropropylene Oxide (HFPO) Trimer Acid	13252-14-7
Fluorotelomer sulfonates (FTSs)	6:2 FTS/FTSA	h,1h,2h,2h-Perfluorooctane sulfonic acid	27619-97-2
	8:2 FTS/FTSA	2-(Perfluorooctyl)ethane-1-sulfonic acid	39108-34-4

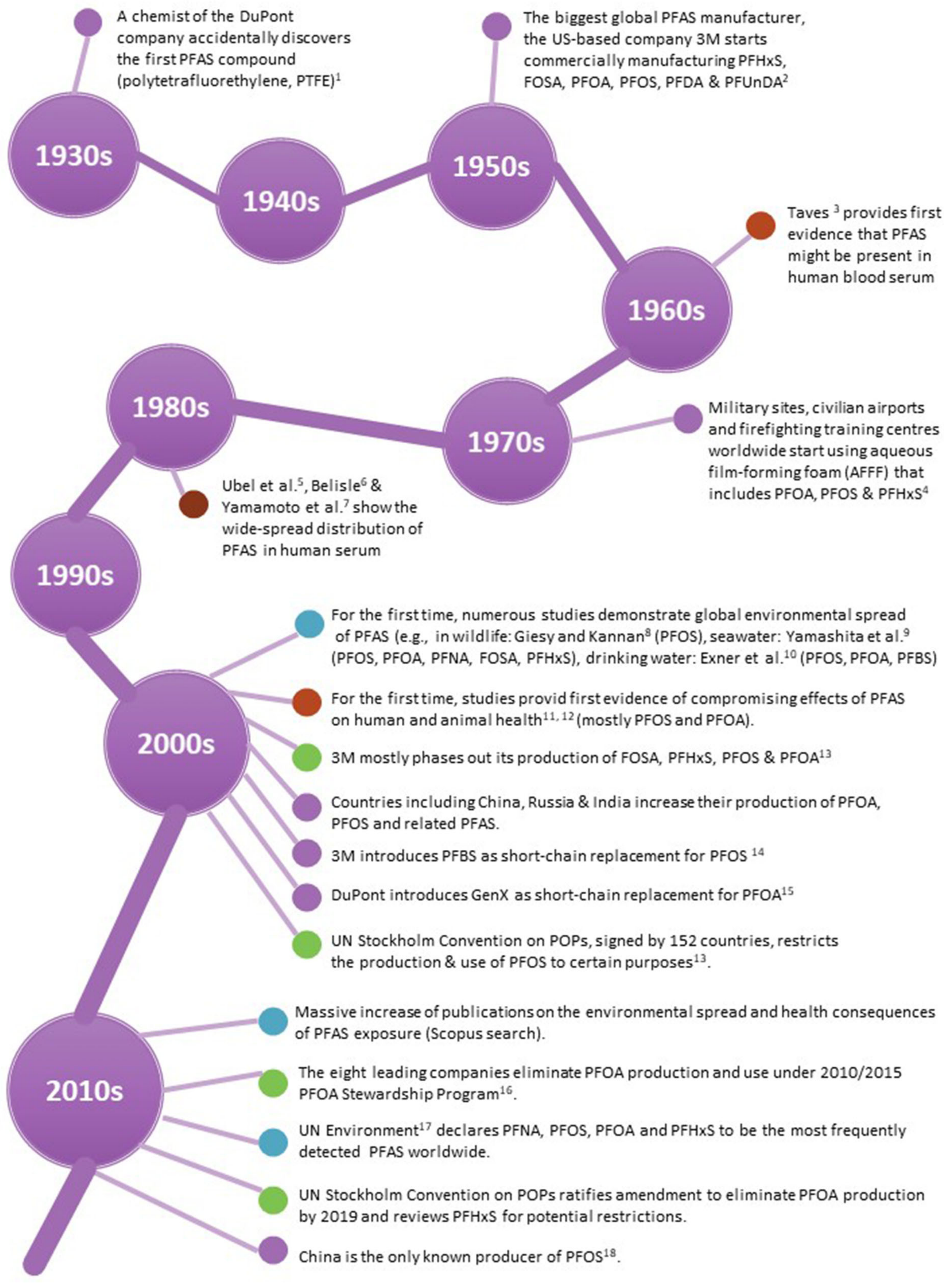

FIGURE 1 Short historic timeline of selected PFAS-related events including introduction, usage and legal restrictions. Large circles represent decades and smaller circles selected key events in PFAS history by category: purple – PFAS use; brown – PFAS presence in animals in humans; blue – PFAS environmental spread; green – attempts to restrict PFAS production and use. References: 1 – Lyons (1994); 2 – 3M Company (2020); 3 – Taves (1968); 4 – Ubel et al. (1980); 5 – Belisle (1981); 6 – Yamamoto et al. (1989); 7 – Giesy and Kannan (2001); 8 – Yamashita et al. (2005); 9 – Exner & Färber (2006); 10 – OECD (2002); 11 – Hekster et al. (2003); 12 – Martin et al. (2010); 13 – Olsen et al. (2007); 14 – Ahearn (2019); 15 – USEPA (2020); 16 – UN Environment (2018); 17 – Worldbank (2017)

Agency in 2006, that aimed to eliminate PFOA emissions and production by the eight leading U.S. manufacturers by 2015 (USEPA, 2020). Furthermore, the UN Stockholm Convention on Persistent Organic Pollutants (POPs) was signed by 152 countries in 2000, and vowed to strictly limit the use of PFOS to certain purposes (Martin et al., 2010). However, the list of these exempted purposes included most of the common usages, such as photoimaging, firefighting foams, insect baits, metal plating and surface treatment of leather (Martin et al., 2010). Moreover, the speed of the implementation of the Stockholm Convention differed significantly across countries.

PFAS substances have truly earned their infamous reputation as 'forever chemicals'. However, questions remain as to whether conventions and restrictions are actually reducing PFAS burdens in the humans, animals and the environment, and if so, how long it takes for the effects of restrictions to be detectable. In 2018, Land et al. (2018) published a large systematic review on PFAS concentrations in humans and showed that exposures to PFOS, PFOA and PFHxS were in decline in North America and Europe, potentially reflecting the impacts of legislated restrictions towards some types of PFAS. On the other hand, in China people are increasingly exposed to PFAS, such as PFOS and PFOA, which is presumably due to the recent local peak in production (Concawe, 2016).

PFAS contamination affects not only humans, but also non-human biota. Wildlife is constantly exposed to contaminants in the natural environment. While PFAS burdens in wildlife are expected to reflect those of their habitat, there is some uncertainty in these patterns (compared to patterns in humans). Depending on the geographical region and species, longitudinal studies have provided conflicting reports on trends in PFAS presence in wildlife and the natural environment over the past 20 years (Jouanneau et al., 2020; Rigét et al., 2013; Shaw et al., 2009).

PFAS concentrations in wildlife are also relevant in other ways than just reflecting the contamination of our natural environment. Many wildlife species, particularly fish, are an essential part of the diet of people in many different cultures (Thompson et al., 2011). The assessment of PFAS concentrations in such species is therefore of relevance to public health. Finally, assessing PFAS burdens in wildlife also serves the purpose of conservation management, especially for those species that have already been impacted by anthropogenic threats, such as loss of habitat and climate change. Exposure to ubiquitous PFAS in the environment could be another potential driver of population decline and extinction (Ishibashi et al., 2008; Kannan et al., 2006).

The plethora of published evidence on PFAS in wildlife makes it difficult at times to gain an overview of what is known and what is missing. One way to provide a comprehensive overview of the available evidence is systematic mapping. This technique collates and presents the available evidence in a structured and compressed way and, thus, allows an insight into the gluts and gaps of evidence in the research field. In addition, it can represent a reflection of PFAS history in wildlife, for example by presenting the types of PFAS investigated at a certain time (associated with the introduction or ban of certain PFAS).

A relatively new approach in addition to systematic mapping is bibliometric analysis; the combined approach is referred to as 'research weaving' (Nakagawa et al., 2019). Bibliometric analysis reveals the patterns of collaborations among researchers and their countries and finds the connections among the variety of scientific disciplines involved in the research. It also demonstrates how the individual papers are connected by citations and authors, and which publications have the most influence on the field in terms of their number of citations. Furthermore, bibliometric analysis also has the capability of revealing patterns of PFAS history in wildlife (e.g. trends in year of publication, keywords and disciplines). In summary, bibliometric analysis lifts systematic mapping to a new level by providing additional insights on how research is conducted, and therefore reveals and visualizes the 'infrastructure' of a research area.

Especially for such a politically charged field as PFAS pollution, the state of research infrastructure can be crucial in providing true advancement in knowledge and consequently sensible policymaking. The battlefield of PFAS pollution includes many opposing stakeholders, such as PFAS producing companies, policy makers, environmental NGOs and the general public. Each of them comes with their own set of interests. Therefore, it is crucial to closely monitor PFAS research for bias. One of the potential signals of bias can be a lack of collaboration and connection to other institutions and countries. The other is lack of transparency in published studies, for example, when raw data and analysis code are not publicly shared, hindering replication and reuse of the research. Although reviews exist on PFAS concentrations or even time trends of PFAS burdens in wildlife (Houde et al., 2011; Land et al., 2018), no endeavour as comprehensive as this study has been initiated and explicitly aimed at mapping and assessing research on PFAS burdens in wildlife species.

1.2 | Objectives

We aim to perform a comprehensive overview of the existing state of knowledge on the abundance and distribution of PFAS in wildlife species, as well as an overview of the interdisciplinarity, connectedness and influence of studies included in this analysis. Therefore, we will collate the available literature into a systematic evidence map and perform a bibliometric analysis. Eventually, our work will streamline and inform future research and policymaking. We will address two main questions:

1. Mapping: What evidence exists on PFAS in wildlife?

The systematic evidence map will not only reveal patterns and relationships in the existing data, but also identify knowledge gaps. Our main research questions will explore 'when and where the papers were published', 'what the recent trends in publications numbers were' and 'what (e.g. types of PFAS, species), where (habitat, location) and when was tested'. In addition, we will explore whether included studies exclusively investigate PFAS burdens in wildlife or whether they also link those burdens to functional aspects, for example reproduction, metabolism rate and so forth. With this work, we will create a body of information to complement the systematic map of Pelch et al. (2019), who aimed to synthesize the health effects of PFAS in people. For this aim, we will use the following information from all included studies: study species, focus on PFAS or POPs in general, years of sample collection, habitat and biogeographical region of study species and type of PFAS investigated.

1. Bibliometrics: How interdisciplinary, connected and diversely represented across institutions and countries are the papers in the research field?

We will perform a bibliometric analysis to gain an understanding of the interdisciplinarity, connectedness and citation rate of the studies on PFAS in wildlife. For this purpose, we will use the following data: publication time and place, co-authorships, country affiliations, word frequencies and number of citations and papers cited, for all articles indexed in the Scopus database. The data on co-authorships will provide an overview of the level of collaborations between authors, institutions and countries. Word frequencies will show the range of disciplines involved and the clustering of concepts in the research field. The number of citations will give an idea about the impact the individual studies have on the research field and how this impact is distributed.

2 | METHODS

This protocol has been prepared in accordance PRISMA-P (Moher et al., 2015). The PRISMA-P checklist is attached as an additional file 1. We registered the project on osf.io (osf.io/gnt2y). Our methods were developed and tested by performing a pilot search, pilot data extraction and preliminary analyses on the data for the systematic map (described below).

2.1 | Eligibility criteria

To be eligible for the inclusion in the systematic evidence map, studies need to fulfil the following requirements, stated in PECO format:

- Population: wild or feral animal species that are not kept in captivity or under otherwise controlled conditions.
- Exposure: natural PFAS exposure from the environment (i.e. controlled PFAS exposure in the lab or field is not eligible).
- Comparator: this element does not apply as eligible studies are observational.
- Outcome: the papers should investigate the concentration of one or several of 34 types of PFAS of emerging importance (Kirk et al., 2018; Pelch et al., 2019) listed in Table 1. Concentration of PFAS should be measured on whole animals or their parts or products (e.g. eggs, muscle tissue, blood, feathers, liver) that were not processed for consumption (e.g. cooked, smoked).

In addition, eligible studies must conform with the following criteria (also presented as decision trees A, B and C; Figure 2):

1. Publication type: the studies have to be journal articles, preprints or theses.
2. Year: any year of publication (includes preprints and theses).
3. Language: full text is in English.
4. Availability: full text version available for examination.
5. Type of literature: primary (empirical) literature. In addition to primary literature, we will also collect secondary literature that focuses on PFAS concentrations in wildlife, for performing backward and forward reference searches and for providing context to the included primary studies.

2.2 | Information sources

For the systematic evidence map, we will identify the relevant peer-reviewed published literature by searching the interdisciplinary broad-coverage electronic databases Scopus and Web of Science Core Collection. We will also search Agricola, Science Direct and ECOTOX Knowledgebase for relevant publications. We will also include grey literature (theses and reports) in our search, using BASE, OpenGrey, Ebsco and the Australian Policy Observatory, as well as the preprint repositories bioRxiv and OSF. We will also perform backward and forward reference searches from the key secondary publications (reviews) on the topic. We will periodically (every 6 months) update the systematic map until the manuscript is accepted for publication.

2.3 | Search strategy, study selection and data collection process

2.3.1 | Development and piloting

Our search strategy, selection process and data collection process are based on a pilot test. We performed a pilot search (Table S1) in the Scopus database to develop and evaluate our main search strings (Tables S2–S5) and scope the available literature. We randomly selected 100 bibliometric records from our pilot search and screened them according to the eligibility criteria. Two researchers (ML and CV) performed the pilot screening independently using the online software Rayyan QCRI (Ouzzani et al., 2016) to facilitate the process. Firstly, we screened the bibliometric records containing title, abstract and keywords of the studies, using decision tree A (Figure 2). We excluded 55 records that did not fit the initial inclusion criteria. As the second step, we screened full texts of publications that had passed the initial screening step, using decision trees B and C (Figure 2). A total of 29 out of an initial 100 papers passed the second screening step. When the decision of two screeners on the inclusion of publications was not unanimous, we discussed and resolved divergent opinions.

To test the data extraction and coding process for the systematic evidence map, the two researchers (ML and CV) extracted relevant data from 20 included full-text papers using questionnaires implemented in Google Forms. Again, diverging results were discussed and resolved. After the pilot search and data extraction, we adjusted our

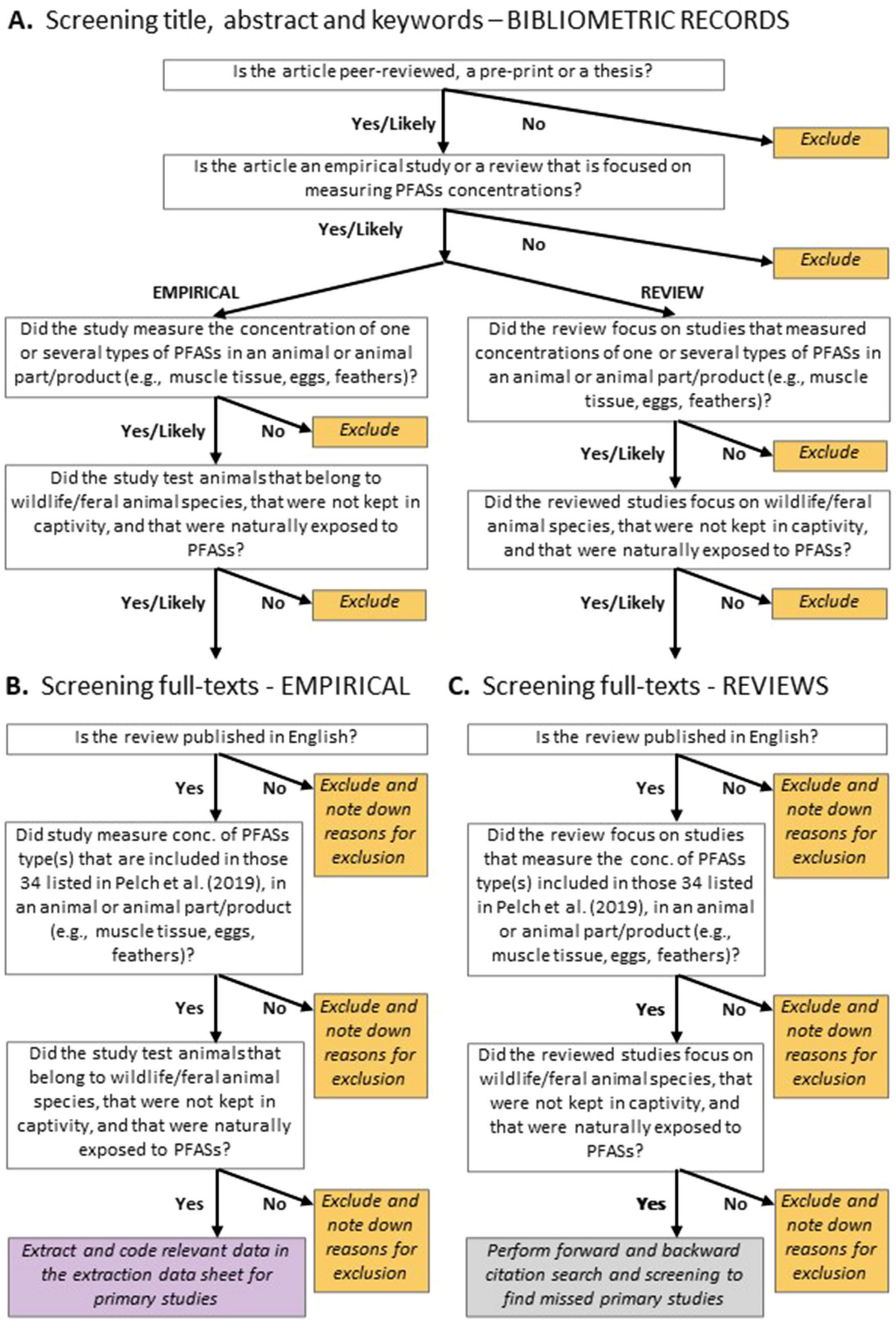

FIGURE 2 Decision tree A for initial screening of bibliometric records; B and C for screening of primary and secondary full-text studies, respectively. Decision tree A: Inclusion criteria for screening title, abstract and keywords of the papers. Decision trees B and C: Inclusion criteria for screening full-text of studies that passed Decision tree A

TABLE 2 Data to be manually extracted for the evidence review map and bibliometric analysis. Each information is allocated to one of the two synthesis objectives: 1. Mapping; 2. Bibliometrics

Objective 1. Systematic mapping – How is PFAS in wildlife – evidence connected?	**Data extraction questions**	**Options of answers [data type]**
Primary_secondary_study	Is the paper primary (empirical) or secondary (review) study?	Primary, Secondary [categorical]
PFAS_focus	Did the study include measurements of PFAS only (vs. other pollutants/POPs)?	Yes, No [categorical]
Year_sampling_start	What was the year of sample collection started? (Earliest year of sample collection for which PFAS concentrations are reported)	Year [continuous]
Year_sampling_end	What was the year of sample collection finished? (Latest year of sample collection for which PFAS concentrations are reported)	Year [continuous]
Species_one_many	Did the study investigate one or multiple species? ('One' if PFAS concentrations are reported for one species only)	One, Multiple [categorical]
Species_scientific_name[a]		Species name, Multiple [categorical]
Habitat	What was the main habitat of studied species?	Aquatic: marine, estuarine, freshwater; Terrestrial: terrestrial inland, terrestrial coastal [categorical]
Sex[a]	What was the sex of studied individuals?	Male, Female, Mixed, Unknown [categorical]
Developmental_stage[a]	What was the developmental stage of study species?	Eggs/early development (e.g. embryo), Juvenile/adult, Unknown [categorical]
Functional_aspects	Did the study investigate functional aspects? (e.g. effects of pollutant burden on growth, reproduction, or immune function; only papers that explicitly attempt to link additional measurements on the individuals to PFAS concentrations will be coded as 'Yes')	Yes, No [categorical]
Biogeographical_region	What was the main biogeographical region of study species?	Tropical, Subtropical, Temperate, Polar [categorical]
PFAS_one_many	Was only one or several types of PFAS investigated?	One, Multiple [categorical]
Tissue_one_many	Was only one or several types of tissue investigated? (e.g. plasma/blood would count as 'One', while whole body homogenate or different tissues in different species as 'Many')	One, Many [categorical]
Objective 2. Bibliometrics – How interdisciplinary, connected and well-cited are the papers in the research field?	**Data extraction questions**	**Options of answers [data type]**
Author_year	What is the name of the first author and year of publication?	Name_year [categorical]
Paper_title	What is the title of the paper?	Title [text]
Publication_year	Which year was the paper published?	Year [continuous]
Journal	Which journal was the paper published in?	Journal name [text]
Country_firstauthor	What was the country the first author is affiliated to?	Country name [text]

[a]Answer can be 'mixed' or 'multiple' – if this answer is selected, multiple categories will be extracted in an additional linked table. All other questions only allow for a singular answer.

search strings and refined the decision trees and data extraction tables (Tables 2 and 3). Informed by the pilot test, our final search strategy will involve searching a total of 12 online databases. Table S2 lists our validation (benchmarking) set of 10 papers manually collected before performing systematic searches of online databases. Tables S3 and S4 present development and validation of search strings for our two main search databases, Scopus and Web of Science Core Collection, respectively. Table S5 shows our search strategies for the remaining 10 databases. We will not use date, language or subject limits in our searches. One reviewer will screen the entire search results and extract

TABLE 3 Data to be manually extracted as an additional information on study species

Data on study species	General information on study species	Options of answers [data type]
Species_common_name	What is the common name of the study species?	Name [text]
Species_higher_taxon	What is the taxonomic class of study species?	Mammalia, Aves, Actinopterygii, etc. [categorical]
Conservation_status	What is the conservation status of the study species (according to IUCN Red List of Threatened Species, 2020)?	Not evaluated, Data deficient, Least concern, Near threatened, Vulnerable, Endangered, Critically endangered, Extinct in the wild, Extinct [categorical]
Weight	What is the weight (average of male and female) of the study species in kg? (Source of this information to be coded in a dedicated comment field)	Number [continuous]
Charismatic_species	Does the study species belong to one of the 20 highly charismatic species (according to Albert et al. (2018)?	Yes, No [categorical]
General_comments	General comments/notes?	[text]

the data, because the pilot screens for the systematic evidence map showed high consistency between the reviewers (93% for stage one, 89% for stage two of the pilot screening process, and 90% for data extraction). A second reviewer will cross-check a random subset of 10% of records that were identified in the search. If there is disagreement of 5% or more in the screening results for this subset, the second reviewer will double-check the full set. Any conflicts will be discussed and resolved by both reviewers in unison. We will follow the two-step process (firstly, screening of title, abstract and keywords, and secondly, screening of full text), as in the pilot screening, using decision trees A, B and C (Figure 2).

One reviewer will perform data extraction and coding, with the second reviewer cross-checking the data from a random subset of 10% of documents, as in the procedure for the screening of literature. A second reviewer will only double-check all extracted data if the disagreement exceeds 5% of checked data. The two reviewers will discuss all disagreements and solve the issue unanimously, seeking advice from other reviewers if needed.

2.4 | Data management

We will import all literature search results (bibliometric records) to the reference management software Zotero. We will remove duplicate records using Zotero function 'Find Duplicates', based on study title and authors. Following this, we will upload bibliometric records to Rayyan QCRI (Rayyan, 2021) for screening. For studies that were included after initial screening, we will collect full-text studies in Zotero and proceed to full-text screening stage. For studies that were excluded at the full-text screening stage, we will record reasons for exclusion. From included full-text studies, we will collate the extracted data in six interrelated spread sheets (Figure 3; more details below). We will track the numbers of studies retrieved from our literature searches, the numbers screened, and the numbers of papers excluded and included in our systematic review. We use these numbers for creating a workflow diagram based on the PRISMA flowchart (Moher et al., 2015). We will make the collected data available to the public via a dedicated website. Analysis code will be available via GitHub.

2.5 | Data coding strategy

We will perform data extraction from full-text studies using pre-piloted data extraction forms implemented as Google Form questionnaires to collect information summarized in Tables 2 and 3. We will first collate study bibliometric and systematic mapping details. The bibliometric data extracted at this stage will comprise document title, year of publication, country of research institution of first author and study funding sources. The systematic mapping data will include type of PFAS studied, timeframes of sample collection, scientific name of the study species, studied habitat and so forth. If the required information is missing in the publication, we will contact the study authors. The content extracted in Google Forms will be exported into a flat table in .csv format.

We will use the data extraction form presented in Table S6 (related metadata provided in Table 3) to collect additional information regarding the study species, such as common name, conservation status and average weight of adult individuals. This additional table is required, because such information might not be provided in the actual publication itself but will be relevant for the interpretation of the systematic map as a whole. Table S6 will also record the sources the information was obtained from.

We will first scan the included publication itself for the required additional information. If the publication does not provide the required information on the species characteristics, we will refer to the IUCN Red List (IUCN, 2020), the Animal Diversity Web (University of Michigan, Museum of Zoology, 2021) and the AnAge Database of Animal Ageing and Longevity (De Magalhaes & Costa, 2009). Other relevant references for other species-related information categories (e.g. charismatic species as defined in Albert et al., 2018) are stated in Table S3. We will use the R package *rattle* (Williams, 2009) to provide a unique identifier for each study species and to link data stored in different extraction tables.

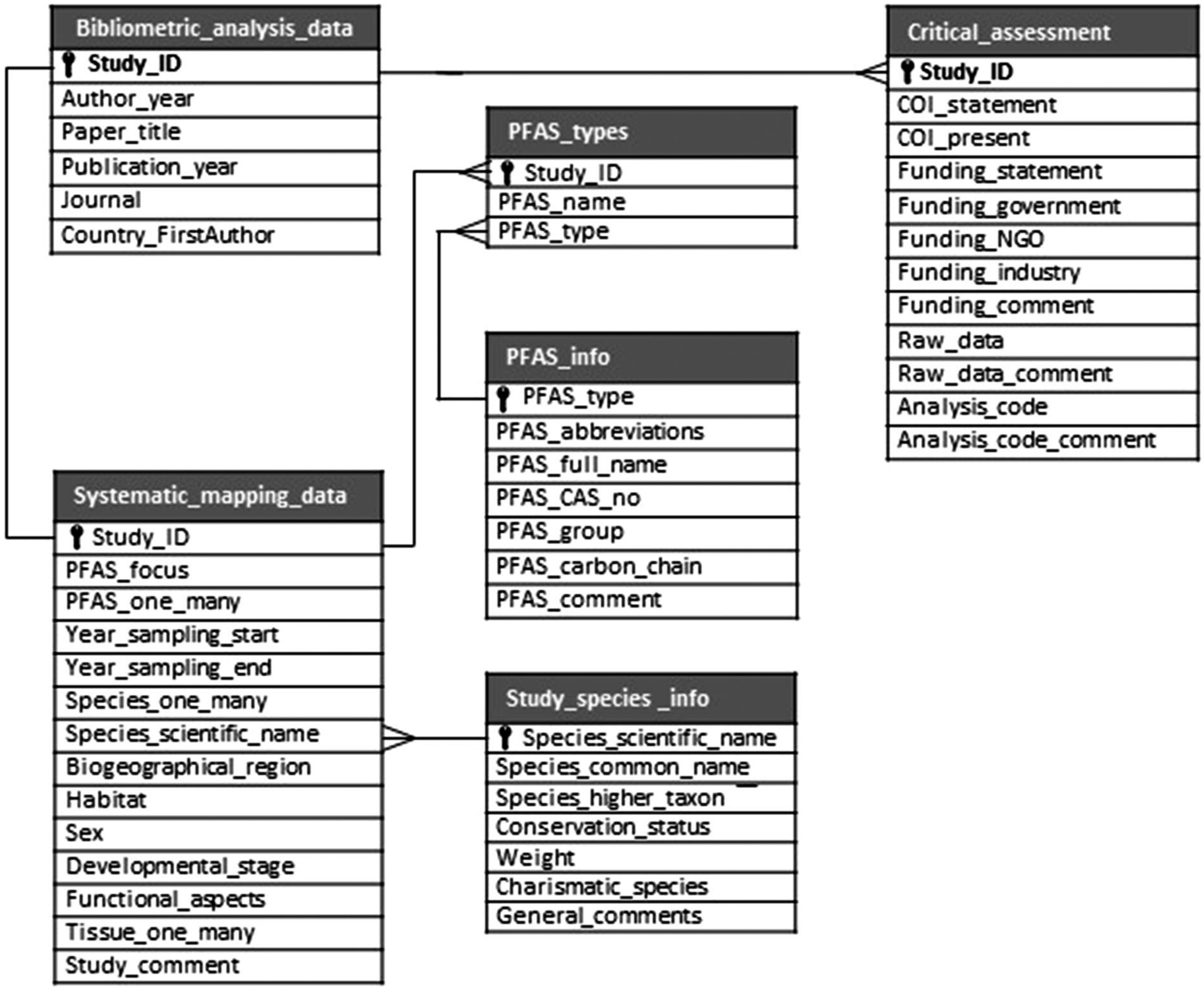

FIGURE 3 Overview and organization of databases of extracted data. Key fields (unique record identifiers) of each table are indicated by a key symbol. Relations between tables are presented using crowfoot notation (single line = one unique value allowed/multiple lines = many values allowed)

2.6 | Critical appraisal

We will check all publications included in the systematic map for the statement of the following information: conflict of interest, funding sources and availability of raw data and analysis code (Table 4), where relevant. This information could be indicative of study quality and potential study-level biases (Bero et al., 2018; Boutron et al., 2019). In addition, the information is easy to extract and comparable across different study types and designs. These extracted variables representing study-level risk of bias will be included in the systematic map results.

2.7 | Data mapping method

To present the extracted data, we will use a combination of tables, plots (e.g. for 'year of publication', 'year of sample collection, 'conservation status [IUCN] of wildlife species') and colour-coded maps (e.g. for 'geographical origin of first author', 'biogeographical regions of tested wildlife species'; as used in Mangano et al., 2017). We will make the systematic map publicly available on a dedicated freely accessible website.

2.8 | Data synthesis criteria and summary measures

We will visualize the systematic map and bibliometric analysis with tables, graphs and a narrative synopsis. Figure 4 shows examples of the visualization of the pilot results.

1. Mapping: What evidence exists on PFAS in wildlife?

We will provide a narrative summary of the systematic evidence map featuring our findings, especially in relation to major events in the history of PFAS (introduction of new types, bans and regulations etc.) (Figure 1). We will discuss the distribution of the studies included in the systematic map by pointing out trends, gaps and gluts. For instance, we will elaborate on trends regarding the countries of affiliation of

TABLE 4 Data to be manually extracted for critical appraisal of included studies. 'Not stated' will be used when there is not explicit statement of COI or funding sources in the publication, as relevant. We will not assess completeness or quality of provided raw data or code

Quality assessment	Data extraction questions	Options of answers [data type]
COI_statement	Did the authors provide a statement of conflict of interest?	Yes, No [categorical]
COI_present	Did the authors have a conflict of interest?	Yes, No, Not stated [categorical]
Funding_statement	Does the study include a statement of funding?	Yes, No [categorical]
Funding_government	Did the study receive funding from a governmental institution?	Yes, No, Not stated [categorical]
Funding_NGO	Did the study receive funding from an NGO?	Yes, No, Not stated [categorical]
Funding_industry	Did the study receive funding from the industry?	Yes, No, Not stated [categorical]
Funding_comment	Is there any general comment on funding?	[text]
Raw_data	Does the publication provide a link to the raw data?	Yes, No [categorical]
Analysis_code	Does the publication provide a link to the analysis code?	Yes, No [categorical]

COI, conflict of interest.

the publications' first authors, providing insight into which countries demonstrate most research activity investigating the issue of PFAS exposure in wildlife. Furthermore, we will discuss which geographical regions the studies mostly focus on and where studies are potentially missing (e.g. here, we expect large focus on polar regions and negligence of the tropics). Moreover, we will assess which types of PFAS are most frequently studied and if the general focus lies more on the exposure to phased-out substances, or whether relevant studies exist on the new generation of PFAS, such as HFPO-DA (GenX) and HFPO-TA (for details on nomenclature, refer to Table 1).

1. Bibliometrics: How interdisciplinary, connected and well-cited are the papers in the research field?

We will visualize the extracted bibliometric data as tables (e.g. top-cited publications and top-producing authors), plots (e.g. counts by year of publication) and colour-coded maps (e.g. country of affiliated institution of first author). Furthermore, we will plot networks of co-authorships, institutions and countries. These data will be automatically extracted from Scopus bibliometrics records of the included studies for which full Scopus records exist. We will process these bibliometric records and perform network analyses using *R* packages such as *bibliometrix* (Aria & Cuccurullo, 2017) and *igraph* R packages (Csardi & Nepusz, 2006) and other relevant stand-alone software packages.

3 | DISCUSSION

The risk of exposure to high levels of PFAS of humans, domestic animals, wildlife and the environment is a major concern worldwide (Exner & Färber, 2006; Hekster et al., 2003). The use of PFAS and subsequent pollution has been ongoing since the mid-20th century (3M Company, 2020). However, the revelation that action should be taken only recently became apparent to legislative bodies and the public eye (Martin et al., 2010; USEPA, 2020). Since then, various research studies has been conducted to trace the extent of PFAS exposure and its consequences (Exner & Färber, 2006; Hekster et al., 2003). Wildlife species worldwide are facing a multitude of anthropogenic threats which has led to a wave of extinction and population declines (IUCN, 2010; Schipper et al., 2008). PFAS exposure adds an additional risk factor to the current situation and should therefore be closely monitored and controlled to minimize its consequences (Ishibashi et al., 2008; Kannan et al., 2006). In addition, PFAS in wildlife poses a threat to public health as it enables PFAS to enter the human food chain (Del Gobbo et al., 2008; Taylor, 2019).

The aim of our systematic map and bibliometric analysis is to give a critical overview of the studies investigating PFAS concentrations in the wildlife. The systematic map will unfold clusters of knowledge that would not require further research attention and gaps in the aggregation of knowledge that clearly need to be addressed more thoroughly in the future. In addition, the relatively new approach of bibliometric analysis promises a first-time insight into research 'infrastructure'. Unbiased and reliable research needs to be multidisciplinary, highly collaborative and conducted by a diverse array of institutions and countries. Therefore, this planned synthesis of literature will provide guidance and orientation for further research efforts that aim to close existing knowledge gaps and 'infrastructure' issues in this research field of growing importance.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Catharina Vendl, Matthew D. Taylor, Malgorzata Lagisz and Shinichi Nakagawa conceptualized the idea of the study. Catharina Vendl, Malgorzata Lagisz and Shinichi Nakagawa performed formal analysis. Catharina Vendl, Matthew D. Taylor, Jennifer Braeunig, Malgorzata

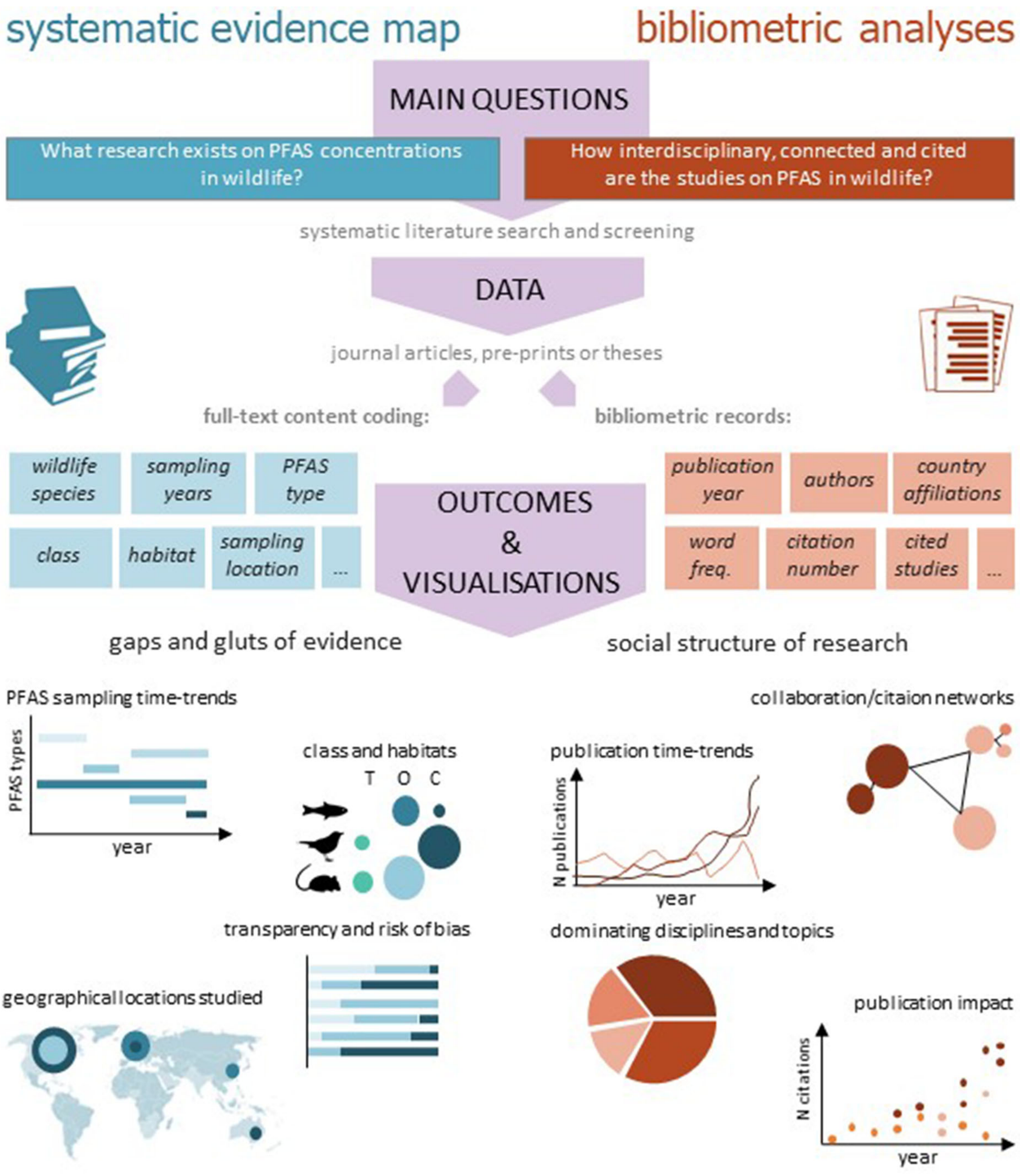

FIGURE 4 Main objectives, data, synthesis approaches (systematic mapping and bibliometric analysis) and expected outcome/visualization types. The plots represent exemplary and simplified visualizations of expected results

Lagisz and Shinichi Nakagawa developed the methodology. Catharina Vendl wrote the original draft. Catharina Vendl, Matthew J. Gibson and Malgorzata Lagisz created the website. Matthew D. Taylor, Jennifer Braeunig, Matthew J. Gibson, Daniel Hesselson, G. Gregory Neely, Malgorzata Lagisz and Shinichi Nakagawa reviewed and edited the manuscript. Malgorzata Lagisz and Shinichi Nakagawa performed supervision. Shinichi Nakagawa acquired funding. Catharina Vendl, Malgorzata Lagisz and Shinichi Nakagawa will be the guarantors of the review.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/2688-8319.12106.

DATA AVAILABILITY STATEMENT

All materials are available within this protocol. During the review, all materials will be made available in a publicly accessible repository at the Open Science Framework.

ORCID

Catharina Vendl https://orcid.org/0000-0001-9870-7765
Shinichi Nakagawa https://orcid.org/0000-0002-7765-5182

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SUPPORTING INFORMATION

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