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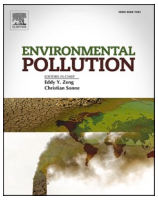

Review

The effects of exposure to environmentally relevant PFAS concentrations for aquatic organisms at different consumer trophic levels: Systematic review and meta-analyses

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ABSTRACT

Per-and polyfluoroalkyl substances (PFAS) is a collective name for approximately 4700 synthetic chemicals ubiquitous in the aquatic environment worldwide. They are used in a wide array of products and are found in living organisms around the world. Some PFAS have been associated with cancer, developmental toxicity, endocrine disruption, and other health effects. Only a fraction of PFAS are currently monitored and regulated and the presence and effects on aquatic organisms of many PFAS are largely unknown. The aim of this study is to investigate the health effects of environmentally relevant concentrations of PFAS on aquatic organisms at different consumer trophic levels through a systematic review and meta-analysis. The main result shows that PFAS in concentrations up to 13.5 μg/L have adverse effects on body size variables for secondary consumers. However, no significant effects on liver or gonad somatic indices and neither on fecundity were found. In addition, the results show that there are large research gaps for PFAS effects on different organisms in aquatic environments at environmentally relevant concentrations. Most studies have been performed on secondary consumers and there is a substantial lack of studies on other consumers in aquatic ecosystems.

1. Introduction

Per-and polyfluoroalkyl substances (PFAS) is a collective name for approximately 4700 anthropogenic compounds (Toward a New Comprehensive Global, 2018) used in a myriad of products, such as nonstick cookware, waterproof clothing, firefighting foams and many more (Glüge et al., 2020). PFAS have been produced and used in industrial and commercial products since the early 1950s. Due to their chemical and thermal stability (Toward a New Comprehensive Global, 2018; Houde et al., 2006) are they very useful in for industrial purpose PFAS are sometimes called "forever chemicals" because they break down very slowly, if at all, in the environment (Cousins et al., 2016). The extensive production and use in combination with low degradation rates has led to PFAS pollution being ubiquitous, in indoor and outdoor environments, accumulated in wildlife (Giesy and Kannan, 2001) as well as in virtually every human (Mamsen et al., 2019; Lau et al., 2004; Olsen et al., 2009). PFAS, especially those with longer carbon chains (e.g. perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA)), have been studied and shown to be persistent in the environment, with bioaccumulation potential, and toxic effects (Olsen et al., 2009; DeWitt et al., 2012; Lindstrom et al., 2011a; White et al., 2011). PFAS have been associated with cancer, developmental toxicity, endocrine disruption, and other health effects (Fenton et al., 2021). A primary concern regarding PFAS revolves around possible adverse health effects in humans and given their persistence, accumulation, and unwanted effects, a few PFAS have to some extent, globally been regulated and restricted (e.g. PFOS and PFOA are listed in the Stockholm convention on Persistent Organic Pollutants for a global restriction (Secretariat, 2017). Although several years have passed since the ban and restrictions of certain PFAS, it has been shown that they still linger in the environment and, even though the banned PFAS are declining in many places, there is no general clear pattern of declining concentrations in abiotic or biotic samples, and aquatic organisms are still being exposed to significant levels (Faxneld et al., 2016; MacGillivray, 2021; Jouanneau et al., 2020; Land et al., 2018; Catherine et al., 2019; Panieri et al., 2022). Furthermore, the need to replace the legacy PFAS (e.g. PFOS and PFOA) has led to an introduction of typically short chain alternatives designed to be less persistent, the so called "emerging variants of PFAS chemicals"

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(e.g. GenX, PFBS, PFBA) (Scheringer et al., 2014; Wang et al., 2013), and there is a lack of research and regulation for these emerging PFAS (Pelch et al., 2019; Awad et al., 2020; Hoppin et al., 2019) and only a small fraction of the total PFAS released in the environment are monitored (Wang et al., 2019). Hence, the presence and effects of most PFAS in the environment are still largely unknown. The current knowledge of health effects suggests toxic effects at high doses (Sinclair et al., 2020). It is still unclear what the effects from PFAS are on different organisms at concentrations currently present in the environment, and if there are cascading or additive effects throughout the food web. The bioaccumulation and elimination rate of PFAS vary among substances, depending on geochemical properties of the matrix, among species and within different species. Within species differences can be due to developmental stage and reproductive status, and behavior in relation to the physiochemical properties of PFAS (Ahrens and Bundschuh, 2014; Savoca and Pace, 2021; Lewis et al., 2022).

PFAS are ubiquitous in the aquatic environment hence posing a risk of negative effects of exposure for aquatic organisms (Giesy and Kannan, 2001; Panieri et al., 2022; Evich et al., 2022). PFAS can enter the aquatic environment from many sources such as direct from the use of fire-fighting foams, atmospheric disposition, landfill leakage discharge from fluorochemicals production or from households and industry through the sewage treatment plants (STPs) (Evich et al., 2022; Bossi et al., 2008). PFAS can be found in very high concentrations in areas with fluorochemical industries. Up to 13.5 μg/L have been detected in surface waters in China (Zhao et al., 2020) and 11 μg/L of PFOA was detected in surface water in Alabama, US (Lindstrom et al., 2011b). Although the levels are not as high as in the vicinity of the industries, PFAS in surface water matrices in Europe, Asia and North America often exceed 70 ng/L[34]and another review found short chain PFAS measured in river, surface and sea water around the world ranging from not detected to 6280 ng/L[35(Li et al., 2020)]. Aquatic organisms are often exposed to a range of different PFAS compounds, as well as other pollutants. Correlations between concentrations of several different PFAS in the tissue and health variables like alterations in liver somatic index and body size has been found in Baltic cod (Schultes et al., 2020) and exposure to concentrations of 10 ng/L 6:2 Cl-PFAES and HFPO-DA and 100 ng/L PFECHS reduced growth of the marine microalgae *Chlorella* sp (Niu et al., 2019). Accumulation of various PFAS has been shown in mussels in screening studies (Hanssen et al., 2019; Liu et al., 2011). Exposure to 10 μg/L PFOS, PFOA, PFNA and PFDA have shown immunotoxic effects in green mussels (Liu et al., 2014). Exposure to 0,1 and 1 μg/L of the PFAS C604, introduced as a PFOS replacer, caused reduced feeding rate changes in gut microbiota in the Manila clam *Ruditapes philippinarum*. Gene expression analysis at transcriptional level also shows that C6O4 caused significant alterations on the expression of genes connected to immune response, apoptosis regulation, nervous system development, lipid metabolism and cell membrane and xenobiotic metabolism (Bernardini et al., 2021). Scientific studies on most PFAS in aquatic environments are however still scarce, and most studies have tested effects on organisms at concentrations in the upper bounds of levels found in the environment (Sinclair et al., 2020). There is a large number of chemicals with little, or no, scientific knowledge about toxicity and environmental risks, hence the field of ecotoxicology is not updated in the same pace as chemicals are put on the market (Kristiansson et al., 2021; Mahoney et al., 2022). There is a growing discussion regarding the possibility of regulation of PFAS as a group, since the time and resources needed to evaluate and monitor all 4700 PFAS individually would be overwhelming (Cousins et al., 2020).

The aim of this study is to systematically review the current knowledge of how PFAS affects aquatic organisms at different consumer trophic levels. The objective is to collect data from exposure studies published in scientific journals and reports for all different PFAS and review the impacts of PFAS at concentrations and conditions found in the environment for different physiological endpoints such as growth rate, reproduction, and morphological and physiological malfunctions. Following the systematic review several meta-analyses will be used to assess the strength of evidence regarding PFAS effects on aquatic organisms exposed to a concentration range of 0–13.5 μg/L. In addition to earlier reviews, our findings will offer estimates of effect sizes, and will provide estimates of heterogeneity among studies.

2. Methods

We have analyzed the current scientific literature following a modified version of the Protocol, Search, Appraisal, Synthesis, Analysis and Reporting (PSALSAR) framework for conducting a systematic review and meta-analyses in environmental science (Savoca and Pace, 2021).

2.1. Search strategy

This systematic review and meta-analysis were conducted in accordance with PRISMA (Preferred Reporting Items for Systematic Review and Meta analyses) guidelines (Lewis et al., 2022). Relevant publications were searched in ProQuest, Scopus, GreenFILE, Web of Science, Science Direct up until November 2021. Grey literature was searched on DiVA and other platforms or organizational websites that host practitioner-generated research. DiVA is an institutional repository for research publications and student theses from 50 universities and research institutions in Sweden. The specific research question formulated according to the PICO (Population, Intervention, Comparator, Outcome) framework (Mengist et al., 2020) was "What are the effects on aquatic organisms at different trophic levels exposed to different PFAS at environmentally relevant concentrations?" Using the PICO framework, we defined key concept terms for the research question (Table 1). For each key concept term, we formed a list of search terms (Table S1), that were combined into Boolean search strings (Tables S2 and S3).

2.2. Selection criteria

After removing duplicates, articles were screened for title, abstract and full text eligibility. The final inclusion criteria used were: (1) full text (2) written in English (3) aquatic organisms (4) exposure to PFAS for longer than 96 h (5) exposure to PFAS below 13.5 μg/L or 1.67 μg/g (6) a control group not exposed to PFAS (7) outcomes measure including health effects (8) an experimental or observational study design. See Table S4 for more information regarding the inclusion criteria.

The range of concentration 0–13.5 μg/L was determined based on a primary search of the scientific literature of recorded PFAS concentrations in water and in tissue of aquatic organisms in samples not measured directly at a point source (e.g. outlet of fluorochemistry industry or drainage from firefighting foam use). In 2020 Zhao et al. detected ionic PFAS compounds, Σi-PFAS, concentrations with the range from 10.5 to 13.5 μg/L the Bohai Sea estuaries (Zhao et al., 2020). Penland et al., 2020 found highest value of 1,67 μg/g PFAS concentrations in tissue of aquatic organisms representing the cutoff value for studies with food-borne exposure (Penland et al., 2020).

2.3. Data extraction

Data extracted from each study included authors, year, country,

Table 1
Research question concept terms according to the adapted PICO framework.

Concept	Key concept terms
Population	aquatic organisms: primary consumers (c1), secondary consumers (c2), tertiary consumers (c3)
Intervention	exposure to PFAS at environmentally relevant concentrations
Comparator	non-exposed aquatic organisms as c1, c2, c3
Outcome	health effects

study type, species, exposure type, specific PFAS, concentrations, and statistical result for endpoints used as response variables. Responses extracted were morphometric, morphologic, reproductive, and behavioral endpoints. Specifically, length and weight, hepatosomatic index (HSI), gonadosomatic index (GSI), brainsomatic index (BSI), egg production, swimming activity, swimming distance in light and dark periods, burst activity in light and dark periods, and startle response in light and dark periods.

Publications with exposures to more than one PFAS were described as mixed. The three trophic levels, primary (c1), secondary (c2) and tertiary consumers (c3), were determined with help of FishBase (Froese and Pauly, 2021), and publications describing the diet of aquatic organisms. For more information of how the trophic levels were defined see supplementary information. Mean values, standard deviation and/or standard error of the mean and sample size were extracted from each study from text, figures and tables. Data was extracted from plots with the help of WebPlotDigitizer version 4.5[48] (Ankit, 2021). When needed authors were contacted for request of data (Table S5).

2.4. Quality assessment

The risk of bias of each study was assessed using the critical appraisal tool from the Collaboration for Environmental Evidence (CEE) (Konno and BarbaraPullin Andrew, 2020). The ARRIVE guidelines (Percie du Sert et al., 2020), a checklist for reporting in animal research, were used to answer the questions in each domain in the critical appraisal tool. O'connor and Sargeant (O'Connor and Sargeant, 2014) served as a guide for a transparent and systematic approach to critical appraisal. It also provides guidance on where in the manuscript a critical appraiser might expect to find the information needed to assess each bias domain. The ARRIVE Guideline states the best ways to report on animal research also with examples of non-biased and biased reporting from the literature. (See Table S8 for an example of a quality assessment). For the quasi-experimental studies, the Joanna Briggs Institute Critical Appraisal tool for non-randomized experimental studies (Tufanaru et al., 2020) was used.

2.5. Meta-analysis

Data was converted to single units of measurement. Length to millimeters (mm) and weight to grams (g). Micrograms per liter (μg/L) was used as unit for PFAS concentration in water. PFAS concentration expressed as nano molars (nM) or micro molars (μM) were transformed using their molecular weight calculated with the help of the Molecular Weight Calculator (Lenntech, 2021) and then converted using Omicalculator – Molarity calculator (Bogna and Filip, 2021) to μg/L. Publications investigating foodborne exposure to PFAS (Jantzen et al., 2017; Martin et al., 2003) were excluded from the analysis due to the different exposure scenario and units (μg/g). Most original studies reported mean and Standard Error of Mean (SEM) for exposure and control groups. SEM was transformed to Standard Deviation (SD) extracting the sample size and using the formula: $SD = SEM \times \sqrt{N}$.

2.6. Statistical analysis

Meta-analyses were performed using R version 4.1.2[57] (R Core Team, 2021), RStudio version 2021.9.1.372 (RStudio Team, 2021), and package metaphor (Viechtbauer, 2010) and dplyr (Wickham et al., 2021). Extracted response data was used to calculate standardized mean difference (SMD) ± 95% CI, between the mean of the PFAS exposed group and the control group for each study. SMD was calculated with the *escalc* function in the metaphor package (Viechtbauer, 2010) as the difference of the means from the exposure and the control group divided by the pooled standard deviation of the two groups. Using SMD as effect size is indicative of how much the intervention affects the average of the response outcome from the exposure group compared to the control group (Higgins et al., 2019).

For the meta-analyses included in this study we either fitted a random effects model, or a mixed-effects model with study used as an additional random effect to control for correlative study effects since some studies were represented more than once in some of the data set. The pooled estimate in all fitted models is the SMD of all studies included in the model. A negative SMD indicates a negative effect of PFAS exposure for the endpoint(s) analyzed. According to Cohen SMDs between 0 and -0.5 are regarded as small negative effects, and below −0.5 as moderate to large negative effects (Cohen, 1988) (See Table 2).

Meta-analyses were performed both as omnibus tests for all organisms, endpoints and PFAS, but also for subsets of organisms at different trophic levels, PFAS, endpoints and sex of organism. The individual PFAS analyzed were PFOS, PFOA, PFNA, PFBS, and PFAS mixtures. PFAS mixtures included both the above mentioned PFAS and a few other substances.

3. Results

3.1. Systematic review

The search resulted in 11857 peer review scientific publications and 1026 grey literature publications. After removing duplicates, 9511 records remained. During title and abstract screening 9341 publications were excluded and only 170 met the inclusion criteria. After full text reading, 62 publications met the inclusion criteria and were included in the critical appraisal and further selection for meta-analyses (Fig. 1, Table S6).

All quasi-experimental publications were included for further analysis. The final list of the 61 publications included in the systematic review can be found in Table S9. From the 61 included publications, 53 are randomized control trials (RCTs) and 8 are quasi-experimental field studies. The RCTs have control and PFAS exposed groups for subsequent comparison and the quasi-experimental publications compare groups of organisms from PFAS polluted with non-polluted areas. The risk of bias for the 53 RCT publications was evaluated at a low-medium-high scale, with 15 publications classified as high risk and the rest as medium. (Table S7, Table S8).

In the publications investigating PFAS within the concentration range metabolic and reproductive endpoints are disproportionately more researched when compared to effects related to vision, oxidative toxicity, development, endocrine, immunotoxicity and genotoxicity (Table 3.). Study organisms in RCTs are fish, crustacean, amphibians, mollusks and urchins, with fish in the trophic level c2 being the most common (Table 4.). Altogether, of the studies included in this review 25 different PFAS have been investigated separately, a few studies exposed organisms to combinations of several compounds (see Table S10). In RCTs using mixture exposure 2–9 PFAS were investigated. In field studies 2 to 24 PFAS were investigated (Table S10).

3.2. Meta-analysis

From the 61 included articles in the systematic review, 26 RCTs and 4 quasi-experimental studies (Tables S12 and S13) were included in the meta-analyses. The final data frame encompasses 225 separate effect

Table 2
Interpretation of standardized mean difference (SMD) values according to Cohen, 1988) to represent the following effects of exposure.

Estimate	Effect of exposure
0	no effect
0 – (−)0.2	very small negative effect
< (−)0.2– (−)0.5	Small negative effect
< (−)0.5 – (−)0.8	Moderate negative effect
< (−)0.8	Large negative effect

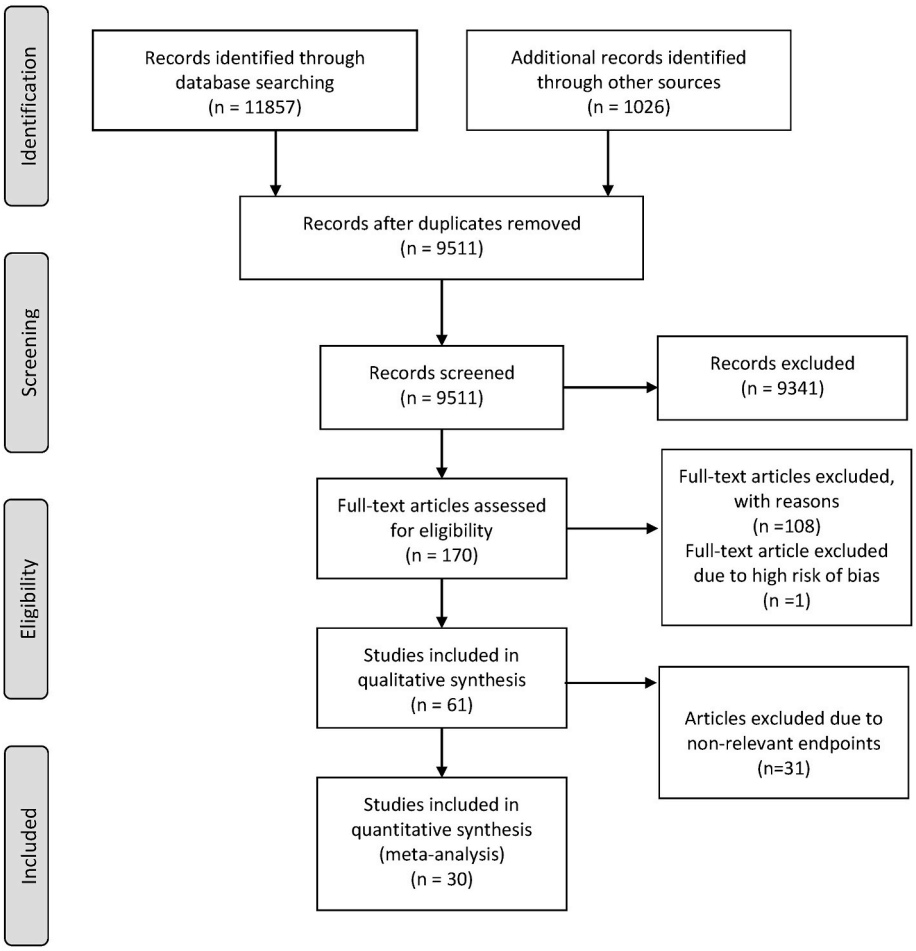

Fig. 1. Flow chart of the screening process used to exclude or retain studies for the systematic review and meta-analyses of PFAS effects on aquatioc organism.

Table 3
Number of published studies of PFAS effects on aquatic organisms focusing on different health realted endpoints.

Endpoint	Number of studies
Reproduction	19
Metabolism	14
Endocrine	5
Development	4
Genetics and genotoxicity	3
Immunotoxicity	3
Microbiome	3
Oxidative toxicity	2
Behavior	1
Vison	1
Various	6

Table 4
The number of published studies of PFAS effects on different organisms among primary consumers (c1), secondary consumers (c2), and tertiary consumers (c3).

	c1	c2	c3
Amphibians	–	1	–
Crustaceans	5	–	–
Fish	1	44	5
Molluscs	8	–	–
Reptiles	–	1	–
Urchins	1	–	–

sizes from 30 studies. One study (Menger et al., 2020) accounts for more than half of the effect sizes. The data frame is based on 16 different species, their distribution across trophic levels is shown in Table 5.

The random effects model output for all publications on all endpoints shows a statistically significant negative effects of PFAS (SMD = −0.321; 95% CI [−0.511 -0.132], $p < 0.001$), but the SMD indicates only a small difference between the exposure and the control groups. See Table 6 for a summary of results. The heterogeneity test results ($Q = 1165.24$, $p < 0.001$) reveal that the study results are not homogenous. The heterogeneity of the studies can be explained by inclusion of different species, concentrations, sex, age groups, and endpoints (Senior et al., 2016). Therefore, to understand the effects of specific PFAS on different endpoints, trophic levels, age and sex of the organism's subgrouping and subsequent analyses were performed.

3.3. Subgroup analyses within trophic levels of all PFAS

The meta-analysis results for the different endpoints after exposures to PFAS (not separated by substance) at different trophic levels, primary consumers (c1), secondary consumers (c2), tertiary consumers (c3), shows that PFAS have a significant negative effect on morphometric endpoints on c1 and c2 organisms of all ages and sex.

The exposure to PFAS on c1 organisms had a significant negative effect on length (Fig. 2, Table 6). There was not enough data to analyze weight or to separate analyses by sex. Analyses of length and weight for female and male secondary consumers were performed separately. Organisms at other trophic levels were not analyzed due to data scarcity.

The results show that PFAS have a significant large negative effect on weight for c2 females (Fig. 3A, Table 6), but with considerable

Table 5
Distribution of aquatic organisms across trophic levels (TL) in the meta-analysis. Trophic levels were determined with help of Fishbase (Froese and Pauly, 2021) and/or diet studies. Common names are shown in brackets.

Primary consumers c1	TL	Secondary consumers c2	TL	Tertiary consumers c3	TL
Daphnia magna (water flea)	<2.8	*Anguilla anguilla* (European eel)	3.6	*Semotilus atromaculatus* (Creek chub)	4.0
Lampsilis siliquoidea (Fatmucket)	<2.8	*Cyprinus carpio* (Common carp)	3.1	*Esox Lucius* (Northern pike)	4.1
Carassius auratus (Goldfish)	2.0	*Danio rerio* (Zebra fish)	3.1		
Luxilus cornutus (Common shiner)	2.8	*Etheostoma nigrum* (Johnny darter)	3.2		
		Lepomis gibbosus (Pumpkinseed)	3.3		
		Melanotaenia fluviatilis (Murray River rainbowfish)	2.9		
		Orizia melastigma (Marine medaka)	3.3		
		Perca flavescens (American yellow Perch)	3.7		
		Rhinichthys atratulus (Blacknose dace)	3.1		
		Xenopus laevis (African clawed frog)	>2.8		

heterogeneity. There was only a small negative effect on weight for c2 males (Fig. 3C, Table 6), but then with insignificant heterogeneity.

The length of males was significantly negatively affected by PFAS with insignificant heterogeneity (Fig. 3D, Table 5D), while PFAS negative effects on females' body length is marginally attributed to chance (Fig. 3B, Table 6). In tertiary consumers (c3) there is only one study included in the meta-analysis, this study shows a non-significant effect on PFAS on the length of adult *Esox Lucius*[65].

3.4. Effects of PFAS exposure on somatic morphological index endpoints

There were no significant PFAS effects on any of the somatic morphological index endpoints HSI, BSI, and GSI in the secondary consumers, c2 (Table 6) when analyzing all PFAS. No analyses on any other trophic level were conducted due to scarcity of data.

3.5. Effects of PFAS exposure on reproductive endpoints

The reproductive endpoint analyzed was egg production per female per day. Yet, again data was not available for primary and tertiary consumers. Thus, the analysis focused only on secondary consumers. PFAS does not have a significant effect on aquatic secondary consumers egg production in the present analysis, but with considerable heterogeneity among the included studies.

3.6. Effects of different PFAS

There was enough data to analyze PFOS, PFOA, PFNA, PFBS and mixtures (Table 7) separately. When analyzed separately for all endpoints, significant small to moderate negative effects were seen for males exposed to PFOS and PFNA. In the Mixture group a small effect was seen for all organisms pooled together and a stronger effect for females when analyzed separately (Table 7).

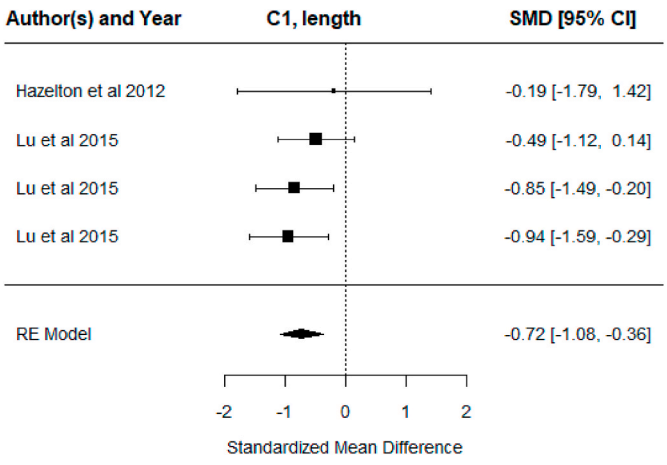

Fig. 2. Forrest plot showing the Standardized Mean Difference (SMD) between PFAS exposure and control for the growth variable length among primary consumers (c1). Whiskers denote ±95% confidence interval. The RE model is a mixed random model with "study" included as random factor to control for the repetitive inclusion of the same study.

Table 6
Meta-analysis results for the different endpoints after exposures to PFAS (not separated by substance) at different trophic levels, primary consumers (c1), secondary consumers (c2), tertiary consumers (c3). Hepatosomatic Index (HIS), Gonadosomatic Index (GSI) and Brain Somatic Index (BSI).

Trophic level	Endpoint	Sex	SMD [95% CI]	P-value	Cochrans Q (df)	$P_{heterogeneity}$	n[a] (no of studies)
c1, c2, c3	All		−0.321 [-0.511 -0.132]	**<0.001**	1165.24 (224)	<0.001	10240 (30)
c1	Length		−0.723 [-1.084 -0.361]	**<0.0001**	1.53 (3)	0.674	63 (2)
c2	Weight		−0.611 [-0.990 -0.232]	**0.002**	108.67 (17)	<0.0001	412,415(11)
		Female	−1.237 [-2.264; −0.211]	**0.018**	89.83 (8)	<0.0001	177, 180(9)
		Male	−0.358 [-0.621 -0.095]	**0.008**	7.65 (6)	0.265	170(7)
	Length		−0.5921 -0.874 -0.310]	**<0.0001**	352.71 (24)	<0.0001	804,807(11)
		Female	−1.512 [-3.051 -0.027]	0.054	105.97(8)	<0.0001	177,180(9)
		Male	−0.415 [-0.667 -0.163]	**0.001**	8.031(6)	0.236	170(7)
	GSI		−0.124 [-0.487 0.239]	0.053	72.65 (21)	<0.0001	406,409(11)
		Female	−0.201 [-0.546 0.145]	0.255	38.78 (11)	<0.0001	215, 217(10)
		Male	−0.018 [-0.564 0.528]	0.948	33.75 (9)	<0.0001	191, 192 (8)
	HSI		−0.322 [-0.983 0.339]	0.340	166.45 (15)	<0.0001	487,485(8)
		Female	−0.656 [-1.879 0.567]	0.293	31.09(3)	<0.0001	82, 85(4)
		Male	−0.374 [-1.189, 0.440]	0.368	8.47 (2)	0.015	63 (3)
	BSI		−0.386 [-0.970 0.198]	0.195	4.17 (6)	0.653	24 (2)
		Female	−0.344 [-1.086 0.398]	0.364	3.367 (3)	0.338	15 (2)
		Male	–	–	–	–	–
	Egg production		−0.420 [-0.885 -0.045]	0.0766	51.85(7)	<0.0001	275 (6)

[a] When two values are shown, the number of organisms differ between the control group and the exposed group and $n_{control}$, $n_{exposed}$ are stated.

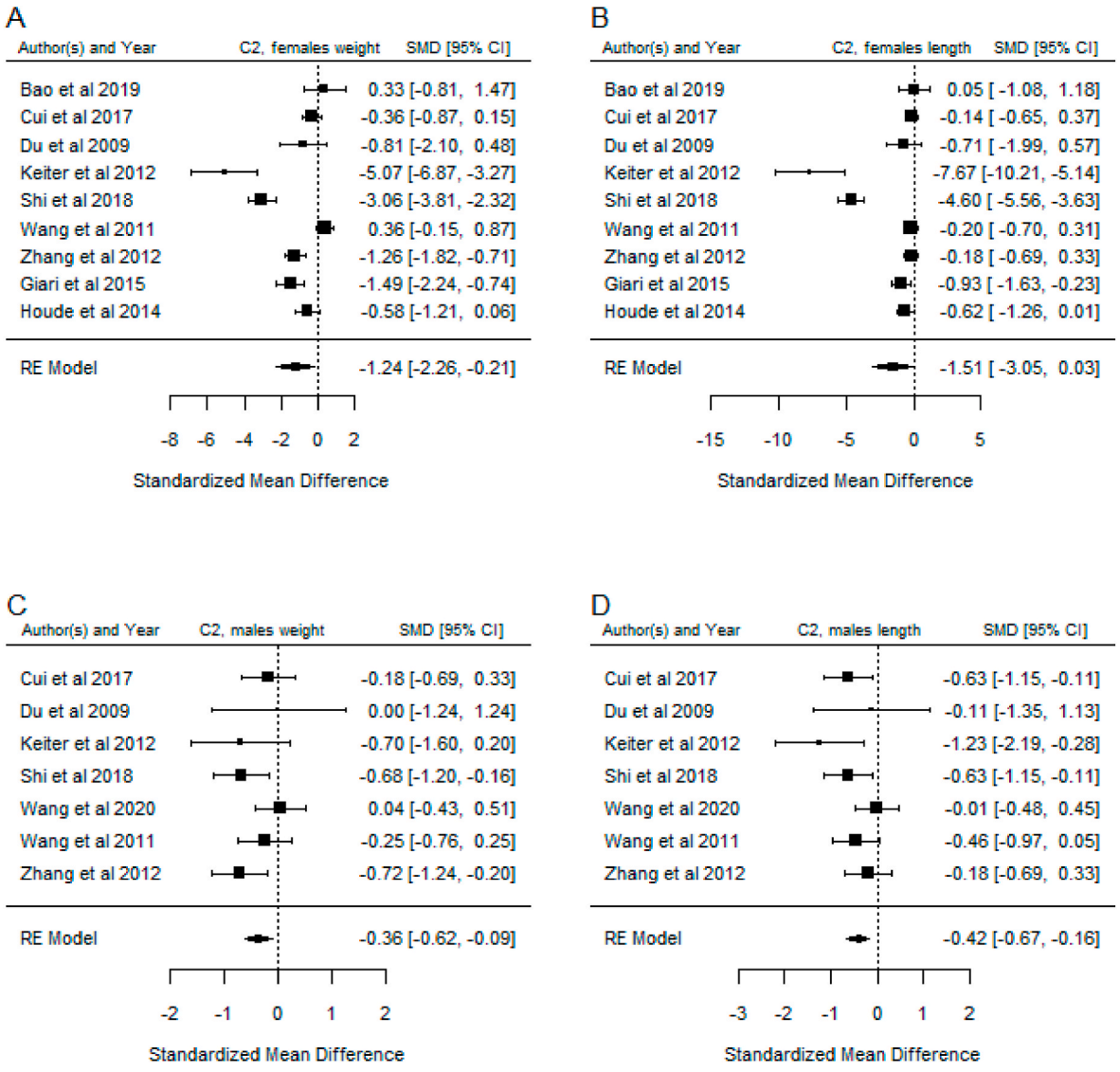

Fig. 3. Forrest plot of the Standardized Mean Difference (SMD) between groups of PFAS exposure and control groups on the weight and length of secondary consumers (c2). A) Female weight B) males weight C) female length D) Males length. Black squares represent the SMD of each endpoint in the different studies. The black diamond represents the average of the SMD. Whiskers denote ±95% confidence interval.

There was data to analyze separate endpoints of the weight and length of organisms exposed to PFOS, revealing significant effects only for the length of the males. The length of organisms exposed to a mixture of PFAS showed a significant moderate negative effect where organisms from three trophic levels were represented. A summary of the statistical analyses of PFAS separately is shown in Table 7.

3.7. Effects of PFAS exposure on behavioral endpoints

Behavioral endpoints were not analyzed statistically due to limited data. Menger et al. (2020) and Dong et al. (2019) were the only studies that investigated behavioral effects of exposure to concentrations of PFAS within the concentration range on first generation aquatic organism. Menger et al. (2020). exposed zebrafish *(Danio rerio)* to several PFAS (PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFBA, PFHxS, PFOS, and 6:2 FTSA) from fertilization to 6 days post fertilization. The endpoints investigated at 1 and 10 μg/L of the 9 different PFAS and a PFAS mix, were swimming distance, burst activity and startle response during light and dark periods. The results show no significant effects from exposure to any of the PFAS at PFAS levels. On the other hand, Dong et al. (2019) exposed adult male *Carassius auratus* to 0.2 and 10 μg/L PFOA for 7 days. Their results show that 10 μg/L PFOA significantly affected swimming activity.

4. Discussion

4.1. Systematic review

The systematic review included 61 quasi-experimental publications where 53 publications were based on are randomized control trials (RCTs) and 8 are quasi-experimental field studies. The RCTs have control and PFAS exposed groups for subsequent comparison and the quasi-experimental publications compare groups of organisms from PFAS polluted with non-polluted areas.

This systematic review reveals that studies investigating PFAS at environmentally relevant concentrations on first generation organisms mainly used metabolic and reproductive endpoints. Other endpoints related to, oxidative toxicity, development, endocrine disorders, immunotoxicity and genotoxicity are less investigated. The least investigated endpoints are related to behavior and vision and it can be argued that many more effects on other physiological systems or organs are still not encompassed by this chart.

Table 7
Meta-analyses for exposure to the different PFAS with enough data to be analyzed separately. Mix refers to experiments with more than two PFAS in exposures or field experiments. HSI refers to Hepatosomatic Index.

Trophic level	PFAS	Endpoint	Sex	SMD [95% CI]	P	Cochrans Q (df)	P[a]	n[b] (n of studies)
c1 c2	PFOS	All	All	−0.318[-0.764 0.127]	0.161	171.41 (38)	<0.001	757, 763 (9)
c2			Female	−1.129 [-2.903 0.646]	0.212	71.56 (15)	<0.001	191 (6)
			Male	−0.427 [0.657–0.197]	**<0.001**	5.75 (7)	0.570	150 (4)
		Weight	All	−0.367 [-0.954 0.221]	0.221	36.12 (8)	<0.001	156 (5)
			Female	−0.880 [-2.384 0.625]	0.251	34.89 (5)	<.001	84 (6)
			Male	−0.264 [-0.586 0.058]	0.10	1.181 (3)	0.758	75 (4)
		Length	All	−0.600 [-1.232 0.031]	0.062	37.84 (8)	<.001	156 (5)
			Female	−1.305 [-3.469 0.859]	0.237	33.84 (5)	<.001	84 (6)
			Male	−0.595 [-0.924 -0.267]	**<0.001**	2.578 (3)	0.461	75 (4)
		HSI	All	0.632 [0.390 0.873]	**<0.001**	34.251(2)	<.001	150,144 (2)
c1-c2	PFOA	All	All	0.101 [-0.245 0.447]	0.568	24.75 (18)	0.132	314, 290 (4)
			Female	–	–	–	–	–
			Male	−0.190 [-0.666 0.287]	0.43	5.74 (4)	0.219	35, 36 (3)
c1 -c2	PFNA	All	All	−0.361 [-1.246 -0.278]	0.05	56.08 (21)	<.001	518,524 (4)
c2			Female	−0.430 [-1.036 0.178]	0.166	12.97 (4)	0.011	126 (2)
			Male	−0.762 [-1.246 -0.278]	**0.002**	5.97 (3)	0.113	120 (2)
c2	PFBS	All	All	−0.061 [-0.403 0.281]	0.727	233.72 (32)	<.001	810,8102 (6)
			Female	−0.220 [-0.732 0.292]	0.400	70.73 (11)	<.001	315 (4)
			Male	−0.036 [-0.415 0.343]	0.852	3.13 (6)	0.792	87 (3)
		Egg prod.	Female	−0.304 [-0.985 0.377]	0.304	0.3329 (4)	<.001	228 (3)
c1 c2 c3	Mix	All	All	−0.433 [-0.807 -0.060]	**0.023**	4.08 (26)	<.001	514, 532 (7)
			Female	−0.671 [-1.106 -0.235]	**0.025**	22.64 (7)	0.002	143, 156 (4)
			Male	–	-	–	–	
		Length	All	−0.692 [-1.043 -0.341]	**<0.001**	3.517 (3)	0.319	65, 69 (4)

[a] p-value for heterogeneity.
[b] Two n-values indicate $n_{control}$, $n_{exposed\ (n\ of\ samples)}$.

Among the different trophic groups fish are overrepresented among secondary consumers, mainly due to the fact that the vast majority of aquatic organisms at this tropic level are fish. The primary consumers, including amphibians, urchins, crustaceans, and mollusks are not as well studied as fish. Tertiary consumers (c3) are also almost absent from toxicological studies regarding the effects of PFAS although studies reporting concentrations of PFAS in tertiary consumers are more common. Studies on predatory fish are also indicating a possible biomagnification of PFAS in food webs (Houde et al., 2013; Oakes et al., 2010). Although PFAS has been shown to biomagnify through aquatic food webs the biomagnification process of PFAS in aquatic food is complex with large inter- and intraspecies variability in biological factors (feeding ecology, trophic status, growth) and chemical factors (exposure level, pharmacokinetics, and biotransformation rates), which can greatly affect tissue residue concentrations (Savoca and Pace, 2021; Kelly et al., 2009; Langberg et al., 2020; Loi et al., 2011; Munoz et al., 2017; Fang et al., 2014).

To increase the understanding of biomagnification as well as and elucidate the impact of PFAS on some of the larger aquatic organisms at different trophic levels it is important to include more tertiary consumers from different aquatic food webs in future studies. However, as it is difficult to conduct research on large aquatic organisms, especially in controlled environments field experiments can be a way to fill this research gap. Field experiments can evaluate effects of exposure in higher trophic organisms by correlating PFAS levels in various tissue and physiological effects in organisms sampled in the wild. The many confounding factors in the environment call for replicated studies whereas many variables as possible are controlled for. Furthermore, to evaluate ecological effects of chronic exposures to variable mixtures of PFAS and other contaminants field experiments with replicated contamination gradients are needed as ecological effects are nearly impossible to study in controlled environments. On the contrary, in a controlled experimental environment most variables can be controlled including exposure substances and concentrations and allow for specific studies of individual endpoints. Furthermore, studies of exposure to a mix of different PFAS are also scarce in the literature. Yet, Exposures to PFAS mixtures is unavoidable for aquatic organisms in the wild when surface waters are influenced with PFAS by e.g wastewater inputs (Aro et al., 2021; Undeman et al., 2022), run off from the use of firefighting foams, biosolids or through discharge from fluorochemicals production (Bossi et al., 2008). It shall also be noted that in the environment organisms may be exposed to several hundreds of PFAS together with other contaminants, and it cannot be ruled out that different PFAS have additive or synergistic effects. To elucidate the effects of the mixture of PFAS and to cover both ecological and species-specific effects, an experimental design including both field – and controlled laboratory experiments may be needed.

4.2. Meta-analysis

In our meta-analyses 30 studies were examined to evaluate the effects of PFAS on aquatic organisms distributed over 25 PFAS, 16 aquatic species, 3 trophic levels and 6 endpoints. Despite the heterogeneity among the study-results included in the dataset we found an overall negative effect health effect from PFAS exposure.

The result from each meta-analysis is expressed as the standardized mean difference (SMD) which allows for comparisons across different methods and endpoints but can be challenging to interpret. However, the method from Cohen (1988) facilitates the interpretation of results among meta-analyses (Mahoney et al., 2022). The SMD of the main meta-analysis of PFAS effects indicates only a small difference between the exposure and the control groups, and one study (Menger et al., 2020) account for more than half of the effect sizes used in the data frame. Overall, the results reveal a heterogenous response when assessing the effects of PFAS on aquatic organisms. This may be the result of the effects of PFAS are investigated across several species, life stages, and substances.

For PFOS, PFOA, PFNA, PFBS and mixtures of different PFAS, enough data exists to conduct separate analysis by substance. When analyzed separately for all endpoints, significant small to moderate effects were seen for males exposed to PFOS and PFNA. In the mixture group a small effect was seen for all organisms pooled together, but a stronger effect for females when analyzed separately. Even though these substances are regulated and declining in many sites recent results indicate significant environmental occurrence even after regulations and restrictions. There is also no general clear pattern of declining concentrations in abiotic or

biotic samples, and aquatic organisms are still being exposed to significant levels (Faxneld et al., 2016; MacGillivray, 2021; Jouanneau et al., 2020; Land et al., 2018; Catherine et al., 2019).

4.3. Endpoint analysis

A significant negative effect from PFAS exposure was found for weight and length of both primary and secondary consumers. For primary consumers, the effect on length was analyzed revealing a moderate negative effect on growth, weight was not analyzed due to lack of data. For secondary consumers weight and length were analyzed separately, both with significant negative effects from PFAS exposure for both male and female individuals. The different studies of female individuals were significantly heterogeneous, but the studies including male individual were homogenous. This may be attributed to methodological differences. The studies analyzing c2 males analyzing weight as endpoint included only RCTs, while studies of c2 female's weight included both RCTs and field studies. The exposure to PFAS on c1 organisms of all ages and sex had significant negative effects on length. One caveat for this result is that it is based on only two studies (Hazelton et al., 2012; Lu et al., 2015) with four effect sizes. Furthermore, the results regarding the primary consumers (c1) organisms should be interpreted with large caution since this trophic level only has four representatives with few endpoints, only including weight and length.

No significant effects from PFAS exposure were found for morphological index endpoints among secondary consumers. The data behind these results are rather robust and give a reasonable statistical power for concluding that the effects of PFAS exposure on Gonado-Somatic Index (GSI) and Hepato-Somatic Index (HIS) for secondary consumers is non-significant. The PFAS effect on GSI result is based on 21 effect sizes and 11 publications (Miranda et al., 2020; Bao et al., 2019; Chen et al., 2019; Du et al., 2009; Giari et al., 2015; Giari et al., 2016; Liu et al., 2010; Shi et al., 2018; Tang et al., 2020; Zhang et al., 2016), while data of HSI is based on 15 effect sizes and 8 publications (Oakes et al., 2010; Miranda et al., 2020; Bao et al., 2019; Giari et al., 2015; Giari et al., 2016; Lou et al., 2013; Shi et al., 1987; Shi et al., 2019; Zhang et al., 2012). However, when PFOS was analyzed separately a significant HSI effect was found, in exposed individuals indicating an enlarged liver. This could be a sign of induced fatty liver. Interestingly, a similar pattern has been shown in fish exposed to environmental endocrine disruptors Bisphenol A (BPA), Di(2-ethylhexyl) phthalate (DEHP) and 17α-Etinylestradiol (EE_2) (Huff et al., 2018; Martella et al., 2016). The Brain-Somatic Index (BSI) analysis indicated no negative effect, but it is based only on two studies with seven observations in total, one from Bao et al. (2019) and six from Chen et al. (2018). Thus, some caution is needed when concluding an absence of a negative effect from PFAS on BSI.

4.4. Analysis of trophic levels

The data of exposure to PFAS within the chosen concentration range of trophic level c3 or other trophic levels than consumers in the aquatic environment are scarce hence no analysis could be conducted. Short time exposures (under 96 h) were not included in this study since most short time exposures include exposure to very high concentrations, that are not considered environmentally relevant in this study. If shorter exposure times within relevant concentrations were included, the analysis could have given a better knowledge of PFAS effects on embryo and larvae stage since development is a sensitive time for exposure, especially at critical windows (Barouki et al., 2012). The two behavioral studies found were not analyzed in the meta-analysis. Overall, the outcomes vary between the two publications. We see a large need for more studies on PFAS effects on behavioral endpoints for first-generation aquatic organisms.

4.5. Concentration of exposure

In the meta-analysis, the study with the lowest concentration of exposure is 0.1 μg/L however no effects are found. The study with lowest concentration to find a significant negative effect of exposure is where significant reduction on length and weight of zebra fish (*Danio rerio*) is found after exposure to 0.6 μg/L PFOS (Zhang et al., 2012). However, only four studies had exposure concentrations under 0.6 μg/L (Dong et al., 2019; Bao et al., 2019; Giari et al., 2016; Lou et al., 2013), hence experiments with lower concentrations are needed. The higher cut-off limit of 13.5 μg/l used in this study is relatively high for studies of PFAS in the environment, but it is not an extreme value (Podder et al., 2021). The highest values are sampled directly in outlets, or in very close proximity, to outlets or run offs from highly PFAS contaminated areas or manufacturers (Cui et al., 2020; Houtz et al., 2013; Heydebreck et al., 2015). The study reporting 13.5 μg/L[32] is from an area impacted by manufacturing, but not sampled directly by an outlet. Furthermore, as the scope of this study is to identify research gaps regarding PFASs studies of different endpoints, organisms, and trophic levels, we aimed for a broad range of concentrations to be able to include as many studies as possible. Also, when studying exposure effects in the environment, it is likely that the additive exposure of all PFAS an organism is exposed to, will be much higher than the instant concentrations during an experiment.

Only two publications investigating foodborne exposure to PFAS were included in the systematic review. However, these were excluded in the meta-analysis due to the different exposure scenario compared with the water borne exposure. One of these studies investigated the effects on Zebrafish (*Danio rerio)* embryo/sac-fry exposed to 2.0 or 0 nM PFOA from 3 to 120 hpf in the water, and the juveniles from the same cohorts were fed spiked food (8 pM) until 6 months (Jantzen et al., 2017). The other study investigated the biomagnification of various PFAS in juvenile Rainbow trout (*Oncorhynchus mykiss*) fed with PFAS spiked food (0.32–1.2 μg/g) (Martin et al., 2003). After chronic exposure to low dose of PFOA exposed Zebrafish significantly altered normal development, survival and fecundity (Jantzen et al., 2017). While dietary exposure did not result in biomagnification of PFAs in juvenile trout. However, no other effects of exposure were explored in juvenile trout and the authors stress that extrapolation of these bioaccumulation parameters to larger fish and homeothermic organisms should not be performed. These results indicate the need for more studies of foodborne exposure including comparative studies between food- and water-borne exposure. Furthermore, the Zebrafish study shows that PFAS may impact wild fish population fitness in watersheds chronically exposed to PFOA. Field-based studies and controlled studies in combination with uptake and accumulation studies are needed to better understand the population effects and in the long run the ecological effects.

4.6. Regulations

PFOS, PFOA, PFNA, PFBS are regulated globally through the Stockholm convention, or listed on the candidate list of Substances of very high concern in EU. And even if regulation could stop the spread and lower adverse health effects, many of the regulated PFAS have been replaced by emerging PFAS that are now found in surface and drinking water resources (Land et al., 2018; Lindstrom et al., 2011b; De Silva et al., 2021). It is evident that both for the PFAS already regulated and for the very large number of not yet regulated substances there is very little or no data either on their environmental distribution or their toxicity. Evaluating all different PFAS separately will be extremely time and resource intensive. Therefore, there is a need for the over 4700 chemicals classified as PFAS to be handled with caution and treated as a group. However, grouping of these chemicals is not straightforward and various grouping strategies have been proposed and evaluated (Cousins et al., 2020).

5. Conclusions

The most studied PFAS are PFOS, PFOA, PFNA, PFBS and mixtures of different PFAS. All substances analyzed show negative effects on several organisms, and especially for female individuals. Even though the most investigated PFAS are regulated, time trends indicate no clear PFAS reduction in environmental occurrence even after implementation of restrictions. Important knowledge gaps identified in this review is the scarcity of studies investigating the effects of PFAS on aquatic primary consumers (c1), and tertiary consumers (c3). Furthermore, many studies expose organisms to concentrations in the high range found in the environment and there is a need to study lower concentrations as well as additive effects.

The meta-analyses show that that PFAS have moderate to high negative effects on morphometric endpoints for c1 and secondary consumers (c2), respectively. PFAS have moderate negative effects on the length and weight organisms within the c2 organisms. These negative effects are significantly affecting weight for females, and both weight and length for males. More data is required for c1 and c3 organisms to determine PFAS effects within these trophic levels.

No effects of PFAS exposure on somatic morphological index endpoints or on egg production in secondary consumers were reported. It is, however, necessary to acquire further data on somatic morphological index endpoints such as BSI at all trophic levels, as well as data on HSI, GSI, and egg production, of primary and tertiary consumers in order to draw definitive conclusions about the effects on these trophic levels. Behavior is also an endpoint category lacking in published studies of PFAS effects.

Credit author statement

Silvia-Maria Banyoi: Conceptualization, Methodology, Formal analysis, Investigation, Writing-Original Draft. Tove Porseryd: Conceptualization, Formal analysis, Investigation, Writing-Original Draft, Writing - Review & Editing, Visualization, Project administration. Josefine Larsson: Conceptualization, Writing - Review & Editing, Visualization. Mats Grahn: Conceptualization, Writing - Review & Editing, Funding acquisition. Patrik Dinnétz: Conceptualization, Methodology, Formal analysis, Writing - Review & Editing, Visualization, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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