Exposure pathways and bioaccumulation of per- and polyfluoroalkyl substances in freshwater aquatic ecosystems: Key considerations

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Abstract

Due to the bioaccumulative behavior, toxicity, and recalcitrance to degradation, per- and polyfluoroalkyl substances (PFAS) are a focus for many researchers investigating freshwater aquatic ecosystems. PFAS are a diverse set of chemicals that accumulate and transport quite differently in the environment depending on the length of their fluoroalkyl chains and their functional groups. This diversity in PFAS chemical characteristics combined with varying environmental factors also impact the bioaccumulation of these compounds in different organisms. In this review, we evaluate environmental factors (such as organic carbon, proteins, lipids, and dissolved cations) as well as PFAS characteristics (head group, chain-length, and concentration) that contribute to the significant variation seen in the literature of bioaccumulation metrics reported for organisms in aquatic ecosystems. Of the factors evaluated, it was found that PFAS concentration, dissolved organic matter, sediment organic matter, and biotransformation of precursor PFAS tended to significantly impact reported bioaccumulation metrics the most. Based on this review, it is highly suggested that future studies provide sufficient details of important environmental factors, specific organism traits/ behavior, and PFAS concentrations/compounds when reporting on bioaccumulation metrics to further fill data gaps and improve our understanding of PFAS in aquatic ecosystems.

Keywords: per- and polyfluoroalkyl substances; bioaccumulation; trophic transfer; environmental pollution; biotransformation; geochemical factors

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of fluorinated man-made chemicals that are frequently found in the environment (1). PFAS are commonly used in in fluorotelomer coating applications (water, soil, oil, and stain resistant products), fire-fighting response and training, and industrial production (2–5). Products and processes that use PFAS typically do so because of their favorable properties that allow for the production of water-resistant and stain-resistant materials. Release of PFAS to the environment is commonly linked to the use of aqueous film forming foams (AFFF) used in fire-fighting training and military bases, wastewater treatment plants (WWTP) effluents, industrial activity, atmospheric deposition, and biosolids applications (6–11). The diverse set of release points, whether point source or non-point source, makes the issue of environmental PFAS pollution a worldwide challenge.

In the class of PFAS, there is a wide range of chemistries and terminologies associated when describing them. PFAS are defined as fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without bonded H/Cl/Br/I atoms) (12). Perfluoroalkyl acids (PFAAs), which are a subset of PFAS, are often referred to as terminal PFAS since precursor PFAS tend to break down to form them in the environment (13,14). In PFAAs, there are two major classes often reported on in the literature – PFSAs (perfluoroalkylsulfonic acids) and PFCAs (perfluorocarboxylic acids). The major differences within the class of compounds are related to fluorinated chain length and the functional head group due to their implications on their behaviors in the environment (15).

Freshwater aquatic ecosystems can be strong indicators on total environmental health, are heavily relied on by humans, and can be extensively impacted by anthropogenic activities. Rivers, lakes, streams, and other components of freshwater watersheds cover large areas of land and provide a method for pollutants to travel, making them representative of the overall extent of pollution in a region (16,17). Since some PFAS can partition to sediments (18), they may act as sinks that serve as secondary sources for biotic exposure especially when disturbed through dredging, storm events, bioturbation, or any developments (19). Aquatic macroinvertebrates are frequently used in environmental studies for the effects of pollutants on freshwater aquatic ecosystem health, and therefore typically reported on in the literature (20). Higher trophic level organisms, like fish, can be adversely impacted by exposure to PFAS and generally bioaccumulate and biotransform some of these compounds, leading to potential human exposure via fish consumption (21). PFAS release to the environment is concerning due to their ability to bioaccumulate and their toxic effects on biota. When PFAS enter the environment, the accumulation is dependent on the compound characteristics, accumulate rates, and environmental factors. A wide distribution of PFAS compounds has been demonstrated to accumulate in human tissues (22), plant tissues (23), and in other wildlife (24,25) through interaction with specific components such as proteins and lipids.

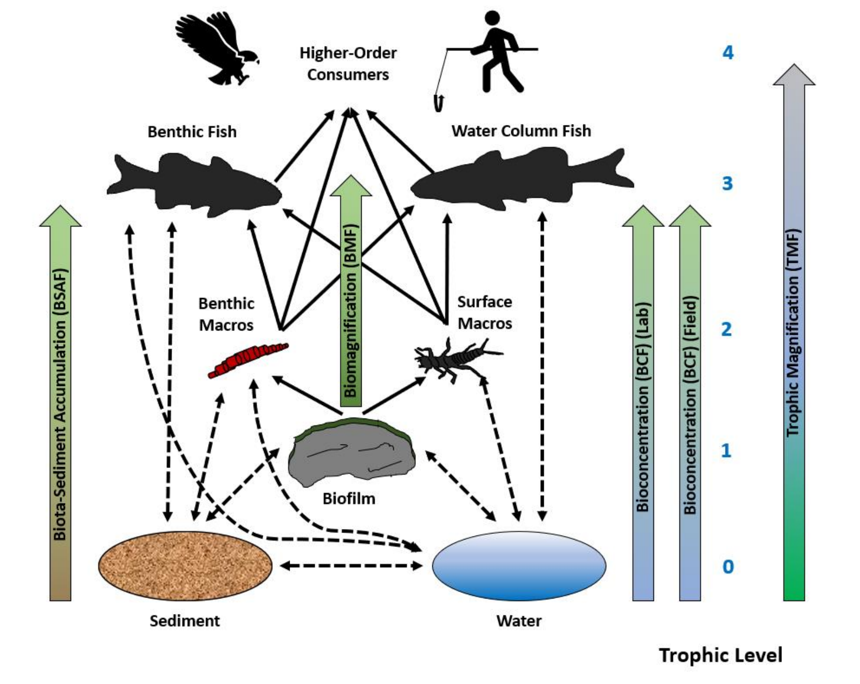

Figure 1. Environmental transfer of organic pollutants throughout aquatic ecosystems and commonly reported bioaccumulation metrics associated.

Before looking at the bioaccumulation and impacts of PFAS contamination on freshwater aquatic ecosystems, it is important to have a general description of what a typical freshwater aquatic ecosystem may look like. Biota in these environments range from simple to complex, have different energy consumption methods, and live in different areas. Primary producers are autotrophic and include biofilms, aquatic plants, and algae and are at the bottom of the trophic level. Herbivorous consumers are heterotrophic and feed on the bottom of the trophic levels, the primary producers. Further up on the trophic levels are the carnivorous consumers, those who feed on animals below them on the food chain. In Figure 1, for example, higher-order consumers such as birds and humans feed on benthic and water column fish that subsequently feed on low level benthic and surface macroinvertebrates, providing a mechanism for trophic transfer of PFAS and other pollutants or nutrients.

Most of the research regarding negative health effects of PFAS exposure has focused mainly on two compounds, perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS), but not on the full range of PFAS compounds that are often released into the environment (25). Concerns with consistency between bioaccumulation metrics reported in the literature include the incorrect interchangeability between some metrics, reporting of specific tissues values instead of whole-body values, and direct comparisons to bioaccumulation of other commonly studied organic pollutants (26). Currently there are limited data on the effects of PFAS related to toxicity at environmentally relevant concentrations in freshwater aquatic environments, with many studies looking at significantly higher concentrations of exposure. Even though toxicity is observed for many organisms at higher than environmentally relevant concentrations, biota is often exposed to non-quantifiable PFAS, precursor PFAS, and a wide range of compounds suggesting that additive negative health effects are possible (27). Some focus, as with other contaminants, has been placed on the PFAS body burden of the impacted organisms, or the total amount of PFAS present in the tissues of the affected biota (28,29).

Table 1. Commonly reported bioaccumulation metrics in aquatic ecosystems: definitions and calculations in biota.

Bioaccumulation Metric	***Definition***	***Animals***	***Plants***	***Implications/Pros/Cons***
Bioconcentration Factor (BCF)*(26)*[a]	The direct uptake of PFAS by an organism from the water column, measured as the ratio of the concentration in an organism to the concentration in the water (measured in the laboratory).	$\frac{[PFAS]_{Tissue}}{[PFAS]_{Water}} (\frac{L}{kg})$	$\frac{[PFAS]_{Plant}}{[PFAS]_{Water}} \left(\frac{L}{kg}\right)$	- Dermal exposure to PFAS - Implies no dietary exposure - Implies no interaction with sediment - Reported in lab studies - Can be used to inform real world but not representative
Bioaccumulation Factor (BAF)*(26)*	The amount of PFAS taken up from bioconcentration plus the contribution of PFAS in the diet of the organisms (measured in the field). Calculation assumes no importance of dietary exposure.	$\frac{[PFAS]_{Tissue}}{[PFAS]_{Water}} (unitless)$	$\frac{[PFAS]_{Plant}}{[PFAS]_{Water}} \left(\frac{L}{kg}\right)$	- Unable to distinguish the exact uptake route (does not account for dietary exposure) - Implies no interaction with sediment - Reported in field studies
Biota Sediment Accumulation Factor (BSAF)*(30)*	A way to present the tendency of a compound present in the sediment to accumulate in an organism.	$\frac{[PFAS]_{Tissue}}{[PFAS]_{sediment}} (unitless)$	Not defined	- Adjust bioaccumulation by the sediment consumption/exposure - Does not account for exposure to the water column
Biomagnification Factor (BMF)*(26,31)*	An increase in tissue concentration as one moves up the food chain based on a predator/prey relationship (measured in the lab or field).	$\frac{[PFAS]_{Tissue}}{[PFAS]_{diet}} (unitless)$	Not applicable	- Lab and field values cannot be directly compared; lab BMF(31) never combines dietary and water exposure while field BMF values may
Trophic Magnification Factor (TMF)*(32)*	An average food web biomagnification factor and is determined empirically using field measurements of contaminant concentrations and relative trophic level.	10^{b} (b = the regression slope from a log-normal relationship between trophic level and log [PFAS]	Not applicable	- Dietary absorption occurs faster than elimination -TMFs > 1 indicate the occurrence of biomagnification – Provides information on change in residues per trophic level

* $[PFAS]_{matrix}$ signifies concentration of PFAS in specified matrix.

[a] Some studies do not limit BCF calculations to only lab studies and incorrectly report field measurements using BCF.

PFAS have been demonstrated to be present throughout almost all ecosystems; some PFAS have greater potential to bioaccumulate and cause negative toxic effects such as higher mortality and growth inhibition, however, many commonly detected compounds have low acute toxicities (6). Unfortunately, when bioaccumulation metrics (Table 1) are reported in the literature, there can be considerable variability. Specifically, BMF and TMF from field measurements can range over several orders of magnitude (33). Regarding this, the review conducted here looks to investigate potential environmental factors (biological, physical, chemical, geological) that have been affecting the discrepancy in reported values (34) whether it be non-steady state systems, organic matter, precursor PFAS, dissolved proteins, or other factors. The work presented here aims to look at specifically which factors impact accumulation of PFAS the greatest based on where the biota lives in the aquatic environment, how accumulation may differ between rivers and lakes, organism behavior (feeding or dwelling), and which trophic level they belong to. Based on the findings, suggestions will be made for what considerations and what data should be collected while conducting PFAS bioaccumulation research to minimize variability in reported metrics in freshwater aquatic ecosystems. The present review investigates the exposure pathways and demonstrates environmental factors that impact bioaccumulation of PFAS in freshwater aquatic ecosystems. By compiling reported values for various bioaccumulation metrics in the literature, an overview of significant environmental factors and data gaps on what impacts bioaccumulation are presented.

2. PFAS Occurrence in Freshwater Aquatic Ecosystem Biota

PFAS have been found throughout freshwater aquatic ecosystems (6,35,36), due to contamination of waters, sediments, and soils, in a wide range of macroinvertebrate species (37,38), and fish (39). PFAS are a diverse set of chemicals that are often characterized by the specific head group or the chain length. Table 2 represents the groupings relevant to the literature review of the reported compounds and specific information on the number of carbons and chain length. Due to the biomagnification potential of PFAS, trophic transfer is of great concern regarding environmental health, and the structure of the literature review will focus on various trophic levels, starting at the bottom, as well as other fate and transport PFAS processes.

Table 2. Important PFAS compounds presented in the literature and relevant groupings.

PFAS Grouping	PFAS Compound	Number of Carbons	Chain Length Classification
Perfluorocarboxylic Acids (PFCAs)	PFBA	4	Short
	PFPeA	5	Short
	PFHxA	6	Short
	PFHpA	7	Long
	PFOA	8	Long
$\boldsymbol{C_nF_{2n+1}CO_2H}$	PFNA	9	Long
	PFDA	10	Long
	PFUnDA	11	Long
	PFDoDA	12	Long
	PFTrDA	13	Long
	PFTeDA	14	Long
Perfluorosulfonic Acids (PFSAs)	PFBS	4	Short
	PFHxS	5	Short
$\boldsymbol{C_nF_{2n+1}SO_3H}$	PFHpS	6	Long
	PFOS	8	Long
	PFDS	10	Long
Precursor PFAS	6:2 FtS	8	Long
$\boldsymbol{C_nF_{2n+1}X}$	PFOSA	8	Long
***X = various headgroups**	EtFOSA	10	Long

2.1 Biofilms

Biofilms, algal or microbial dominated, play a key role in the transfer of organic pollutants to higher trophic level organisms by acting as a food source to many low level aquatic macroinvertebrates (40). To look at PFAS bioaccumulation in freshwater aquatic ecosystems, it is important to look at all trophic levels starting at microbes, biofilms, and algae. Biofilms are ever present in freshwater systems and may be a potential pathway for bioaccumulation and transfer of contaminants to higher trophic levels such as fish and macroinvertebrates. Biofilms are mainly comprised of adherent microorganisms embedded with extracellular polymeric substances (EPS) composed of polysaccharides, proteins, lipids, and DNA (41,42). Specifically, the proteins and lipid components of the EPS have the potential to play a major role in PFAS bioaccumulation since PFAS have been shown to have high affinities to these materials in the environment (43,44). Biofilm bioaccumulation and trophic magnification of other contaminants has been illustrated in freshwater systems previously, e.g., mercury and herbicides (45,46). However, other research has shown that passive exposure to organic pollutants (water column exposure) can be the dominant route of bioaccumulation for organisms that consume biofilms (47). While exposure and bioaccumulation routes in biofilms has been studied for other organic contaminants in these ecosystems, research involving PFAS bioaccumulation is still limited. PFAS contamination in biofilms is key to understand and requires more research since understanding the lowest trophic level bioaccumulation processes are vital to understanding the whole freshwater aquatic ecosystem dynamics.

To our knowledge, only a few studies have looked at and reported on PFAS transfer to biofilms in a freshwater aquatic ecosystem. In these systems, PFAS may be adsorbed at the biofilm surface, incorporated within the EPS, or accumulated intracellularly (48). In a field study by Munoz et al., there were high detection frequencies of long chain PFAS (80-100%) in biofilm samples, with the branched isomer of PFOS dominating the PFAS contamination (53-92%) (49). The components of the biofilms can vary, which will have impacts on the bioaccumulation of

PFAS since some have lipophilic and proteinophilic tendencies (50–52). Specifically in bacteria, PFAS can partitioning intracellularly, as well as to membranes composed of phospholipid liposomes and bilayers, with a positive correlation to the number of fluorinated carbons (43). Additionally, this group found that PFSAs incorporated at greater rates than PFCAs. In another field study with low background concentrations, biofilms were found to accumulate a wide range of PFAS (10 of the 14 studied), with planktonic algae accumulating little comparatively (53). This suggests that algae may play a role in bioaccumulation of PFAS, but when comparing planktonic algae to biofilms the EPS and surface heterogeneity may also play a major role.

These studies, while limited, indicate that biofilms may strongly contribute to PFAS bioavailability to its consumers. Key takeaways from the literature to date is that biofilms are quite heterogeneous and the EPS, specifically protein and lipid content, may play a major role in PFAS bioaccumulation and subsequent trophic transfer. BCFs were found to be concentration dependent, where BCFs decrease as PFAS concentrations in their corresponding water increases (49). Apparent knowledge gaps related to PFAS bioaccumulation in biofilms is how the heterogeneity of the biofilms can be characterized to better predict accumulation, separation between algae and bacteria bioaccumulation, and lab-scale studies to report on bioaccumulation metrics more accurately. To date, only concentrations of PFAS are reported in algae and biofilms, but various metrics (Table 1) are needed to better understand the role they have in PFAS transfer to higher trophic levels.

2.2 Aquatic Plants

Aquatic plants play a key role in freshwater aquatic ecosystems as food sources, nutrient recycling, and as protection. As primary producers, plants capture and recycle carbon, nutrients, and vitamins, engineer soil/sediment stability, and flow conditions at the sediment-water interface. They also contribute particulate and dissolved organic carbon to the surrounding ecosystem, supplying nutrients and energy to their consumers. For contaminated waters and sediments, aquatic plants may also serve as a potential tool used for phytoremediation (54). Therefore, understanding bioaccumulation and tissue distribution of PFAS within the plant body is important for evaluating the environmental risk.

Demonstrated factors affecting PFAS bioaccumulation into aquatic plants include PFAS characteristics, the plant's environment, and organic matter content of the sediment. PFAS chain length has been shown to impact its accumulation in aquatic plants, where BCF values positively correlate with increasing PFCA chain lengths (C4-C13) and PFSA chain lengths (C4,C6,C8) in laboratory controlled mesocosms (55). To our knowledge, only a few studies have looked at PFAS accumulation in freshwater aquatic plants in the field (56–58). The most recent work (56), used a constructed wetland planted with *Typha angustifolia*, and was spiked with a range of eight PFAS. A major result from this is that short-chain PFAS were shown to be translocated from roots to shoots (i.e., the emerged portion) of the plant, while long-chain compounds were highest in roots. Additionally, a significant positive correlation between BAF (whole plant) and PFAS chain length was observed. In field studies, Wang et al., reported on BAF values for 12 separate PFAS with findings indicating that as perfluoroalkyl chain length increased, BAF values increased among four submerged plant species (58) but this trend did not continue in the six emergent plant species studied (57) in the same study area (Table S2, Supplementary Materials). As suggested by the authors, the difference in the trends may be a result of different aquatic plant age (the dynamic instantaneous values and the PFAS tissue concentrations not closely related to the water in the sampling site), suggesting that the main route of exposure is through the water column and not uptake from the sediment. However, in terrestrial plants, opposite trends were observed than the results from the field studies, and BAFs have been demonstrated to be negatively correlated with PFAS chain length (23,59,60). This negative correlation in terrestrial plants has been proposed to be reversed, if plants are cultured with hydroponics instead of soil, due to preferential binding of long-chain PFAS to soil organic matter (61). The reason for this, is that the trend may not be entirely related to the environment the plant is in, but rather related to the difference in PFAS behavior between solids and water. In water PFAS may be entirely soluble while in soils and sediments, PFAS can adsorb and have reduced bioavailability, especially long-chain PFAS. The functional group of the PFAS has a significant impact on how aquatic plants bioaccumulate PFAS. Several studies have observed that for a given perfluorinated carbon chain length, PFSAs exhibited higher bioaccumulative potential than PFCAs in aquatic plants (55,57,58). Longer chain PFAS and PFSAs have exhibited high affinities to soil substrates (56).

Aquatic plant species and their habitat (submerged, emerged, floating-leaved, or free-floating) impact the extent of PFAS accumulation and which compounds are preferentially accumulated. Submerged and free-floating aquatic plant species were observed to have similar whole-plant BCF values (55,56). The complexity of field conditions leads to many environmental factors that may impact reported value and lead to large discrepancies in reported values. Submerged species exhibited higher bioaccumulation potential of long-chain PFAS in shoots than free-floating species or emerged species (55,58). For example, the BCFs of long-chain PFAS were two to four times higher in shoots of submerged species than free-floating species. This can be explained by their different habitats, where shoots are directly exposed to water or have PFAS translocated to the emerged portions and submerged portions can have roots directly exposed to PFAS in sediments. When specifically looking at what part of the plant accumulates PFAS, long-chain PFAS tend to be retained in roots whereas short-chain PFAS can be easily translocated to shoots (55–58,62) with evidence that the driving for from roots to shoots being transpiration (63). Short-chain PFAS with higher aqueous solubilities than long-chain PFAS have increased aquatic mobility with transpiration while long-chain PFAS tend to accumulate in roots due to their hydrophobicity, large molecular weights, and association with sediments (56,61).

Similarly to biofilms, the accumulation of PFAS in aquatic plants have a demonstrated PFAS concentration dependency, where the BCFs decrease with increasing exposure concentration (56). PFAS association with sediments can also impact PFAS bioaccumulation by reducing bioavailability of PFAS compounds at different rates (long-chain PFAS and PFSAs have greater association with sediment). Finally, the plant environment greatly impacts the mechanism of accumulation and different parts of the plant will have different PFAS distributions and extent of bioaccumulation. Key knowledge gaps regarding PFAS bioaccumulation in aquatic plants is mainly related to determining the major mechanism of bioaccumulation and the most important environmental factors. Aquatic plant habitat (submerged, emerged, floating-leaved, or free-floating) likely have different major bioaccumulation mechanisms, ranging from water-column exposure, root uptake from sediment, and translocation to shoots. Different environmental factors can impact bioaccumulation by reducing bioavailability of PFAS (64) and should be monitored especially in field studies to help determine the most significant ones.

2.3 Macroinvertebrates

The previous two sections focused on lower-level trophic organisms, plants, biofilms, and algae, who are at risk to PFAS bioaccumulation solely through water and sediment exposure. Macroinvertebrates have a greater level of complexity regarding PFAS bioaccumulation since they are heterotrophic and are thus considered the first level of trophic transfer to organisms higher on the food chain. Bioaccumulation of PFAS in macroinvertebrates can be influenced by a variety of factors: PFAS characteristics, organism aspects, and environmental characteristics, with the most important factors described throughout this section. Research focused specifically on accumulation of PFAS in macroinvertebrates is limited to date (low 10s of published studies), especially when considering PFAS exposure through consumption of macroinvertebrates by higher trophic levels may be a major pathway of biomagnification by larger aquatic organisms.

For PFAS characteristics, chain length has been demonstrated to have a significant impact on the bioaccumulation potential in macroinvertebrates. When aquatic earthworms (*Lumbriculus variegatus*), a sediment-dwelling aquatic oligochaete, were exposed to PFAS contaminated field sediments from the carpet industry, PFOS and PFCAs with greater than ten perfluorinated carbons were most prevalent in their tissues (65). Additionally, the uptake rate constants of PFAS have been demonstrated to increase with increasing chain length, likely due to the stronger interactions the long-chain compounds have with the tissues in organisms (66). Looking specifically at BSAF, contradictory results have been reported in the literature where BSAF values have been reported to decrease with increasing chain length (67) as well as increase with increasing chain length (68,69). The most recent studies (68,69) report significant positive correlations between BSAF and increasing PFCA chain length, with the latter study even reporting on a range of organisms not a single species. Prosser et al. showed that out of five PFAS compounds (PFOS, PFOA, PFNA, PFDA, and PFDoDA), PFOS had the highest BSAF values from field collected sediment in *Hexagenia* spp. but was not consistently the highest for *Lumbriculus variegatus* in the same study (70). These findings from Prosser et al. (70) suggest that these trends may vary across species and individual foraging modes, potentially providing an explanation between the differing trends reported by Higgins et al. (67) and the two studies by Bertin et al (71) and Pan et al. (69). Different foraging mode groups for macroinvertebrates are typically divided into grazers, gatherers, filterers, shredders, and predators; all with different diets and PFAS exposure routes. In different foraging modes, the diet changes which alters the PFAS bioaccumulation and the compounds they are exposed to, but the habitat may change as well and they can reside in various parts of the water column, above the sediment, or in the sediment. In addition to chain length and head group, the overall n-octanol/water partitioning coefficients of PFAS should be considered as they have been positively correlated with bioaccumulation of *Daphnia magna* (72).

Macroinvertebrate species that live in the water column, such as *Daphnia magna* and *Chironomus plumosus*, are exposed to PFAS mainly through diet and water, but not directly from sediment, so BCF and BAF (depending on if samples are from laboratory or field) are more relevant when looking at bioaccumulation. Since pelagic organisms do not have exposure from sediment consumption or contact, this limited pathway affects the magnitude of certain PFAS they are exposed to since PFAS partition differently to sediment and water depending on the compound characteristics. On the other hand, macroinvertebrate species that live in sediment, such as *Lumbriculus variegatus* and *Perna viridis* are exposed to PFAS through diet, water, and sediment. Sediment-ingestion rate is a function of organic carbon (OC) content (less feeding with increased OC in sediment), so the exposure of the sediment-dwelling *Lumbriculus variegatus* to PFAS is likely to be impacted by the OC content of the sediment (65,73). *Chironomus riparius*, a sediment-dwelling freshwater macroinvertebrate has been demonstrated to accumulate long-chain PFCAs, PFOS, and PFAS precursors (6:2 FTSA and PFOSA) (71). *Chironomus plumosus* BSAF values have been demonstrated to decrease between 66% - 97% of its original value with a 1.5% amendment of carbonaceous material, showing that sediment remediation efforts could drastically reduce bioaccumulation and that sediment composition has a significant impact on PFAS bioavailability in water (74).

In freshwater aquatic ecosystems, water, natural organic matter (NOM) are important determinants on the fate and transport of nutrients and contaminants. In water, NOM can be broken down into dissolved organic matter (DOM) and particulate organic matter (POM) which is separated by molecular weight of the compounds. In addition to reducing bioavailability of some PFAS, DOM can be microbial activity indicators, show agricultural impacts, and be used as a marker to understand the flow of carbon and nutrients in aquatic food webs (75,76). Increasing protein concentration in water, which can contribute to DOM in water, has a direct effect on the availability of dissolved PFAS to organisms that reside in the water column (77). *Daphnia magna* were exposed to a variety of PFAS compounds with varying levels of dissolved protein (1, 10, and 20 mg/L), to see the effect on bioaccumulation in the organisms (66). Proteins selected were bovine albumin and soy peptone and added directly to water at varying concentrations. At low levels of dissolved organic protein content (0-1 mg/L) the amount of PFAS accumulated was increased compared to the control, but at higher concentrations of dissolved protein the accumulation was significantly reduced. This suggests that the test species, *Daphnia magna*, a filter feeder, has two pathways of exposure (diet and dermal exposure) and that increased protein concentrations in the water sorbs PFAS, reducing bioavailability through dermal exposure. The effect of humic substances and protein compounds (forms of DOM) has been investigated to see the effects it has on bioaccumulation in *Daphnia magna* (78). The four DOM substances (humic acid, fulvic acid, peptone, and albumin) were added at 20 mg/L and resulted in reduced values of BAF. The reduction of BAF values between humic substances (humic acid and fulvic acid) and proteins (peptone and albumin) were not significantly different, suggesting that even though PFAS are proteinophilic, hydrophobic interactions with dissolved organic matter in the environment could have significant impacts on their bioavailability. *Chironomus plumosus* larvae, were demonstrated to bioconcentrate PFAS in a way that was affected by the different types of dissolved organic matter present (77). In this study by Wen et al., the PFAS body burdens decreased with peptone (244.3 g/mol) and humic acid (225.1 g/mol) and increased with fulvic acid (308.2 g/mol) and tannic acid (1,701.19 g/mol) concentrations. The variability seen here demonstrates that the DOM effect on bioconcentration not only depends on the presence or concentration of DOM but can potentially be explained by the molecular weight of the type of DOM. DOM and PFAS can likely form large aggregates and reduce the freely dissolved PFAS concentrations and influence the elimination rates of PFAS in macroinvertebrates.

Dissolved cations can have a significant impact on accumulation of chemicals in macroinvertebrates and other freshwater aquatic organisms. To see the effect of cations on PFAS bioconcentration in *Daphnia magna*, organisms were exposed to various concentrations of Ca^{2+} and Na^{+} along with protein, either bovine albumin or soy peptone (79). In this study, cations concentrations (Ca^{2+} and Na^{+}) had inhibition effects on PFAS bioaccumulation through elevated partitioning of PFAS to the proteins in water and the divalent cation, Ca^{2+}, had a stronger inhibitory effect. This suggests that when proteins are present in natural waters, there should be careful consideration on the concentrations of proteins and associated dissolved cations when assessing bioavailability of PFAS.

Demonstrated environmental factors that impact PFAS bioaccumulation in freshwater aquatic macroinvertebrates include DOM, dissolved cations, sediment composition, and organism behavior. Additionally, like for plants and biofilms, there has been evidence that suggests there are direct correlations between PFAS concentration and uptake rates of long chain PFAAs in freshwater aquatic macroinvertebrates (80,81). BCF (lab studies) and BAF (field studies) could vary greatly based off the concentration dependency (not considering other environmental parameters) and care should be taken when general conclusions are made on the bioaccumulative potential of PFAS since uptake and elimination are non-linear (81). This suggests that for PFAS, BCF and BAF may not be considered a constant across the literature and hence a reason why constants from laboratory and field studies have inconsistencies. Key knowledge gaps regarding PFAS bioaccumulation in freshwater macroinvertebrates is mainly related to the impacts biogeochemical factors have on the reported bioaccumulation metrics (BAF and BCF). Research reported on in this section cover a few important metrics that impact the BCF and BAF reported, but there remain gaps related to other factors such as temperature, clay content, biotransformation, and more. Since a range of environmental factors have been shown to impact bioaccumulation, care should be taken when reporting on BCF and BAF to consider and measure for these, since it can have great impacts on the bioavailability in the system.

2.4 Fish

Higher trophic level aquatic organisms, like fish, frequently have high concentrations of some PFAS detected due to the PFAS ability to bioaccumulate through trophic levels. For example, the BCFs of PFTeDA were observed as high as 30,000 L/kg in the blood and liver of rainbow trout and the BAF of PFOS was 73,612 L/kg in the blood of mandarin fish (82,83). Often higher trophic level consumers in aquatic ecosystems transfer nutrients, and potentially PFAS, up trophic levels to their consumers, such as larger fish, fish-eating birds, mammals, and humans (Figure 1). Previous studies have illustrated that dietary route, particularly fish consumption, is one of the primary pathways for human exposure to PFAS (84–87).

PFAS bioaccumulation in fish was found to be dependent on the foraging mode. Carnivorous fish have been demonstrated to accumulate more PFAS than omnivorous fish; for example, in a study that included 15 PFAS analytes, the total PFAS tissue concentration on average was 272 ng/g and 110 ng/g in carnivorous fish and omnivorous fish, respectively (88). However, total PFAS body burdens may not be the best way to understand total exposure to PFAS since contaminated sites may differ in the type of PFAS and environmental conditions. PFAS partition different depending on their chemical characteristics and related to the environmental context such as sediment characteristics, water quality parameters, and redox potential. Several studies also reported that long-chain PFAS account for a significant proportion in carnivorous fish compared with omnivorous and herbivorous fish, while short-chain PFAS account for a higher proportion in herbivorous and omnivorous fish than carnivorous fish (82,88–90). Liu et al., demonstrated specifically that PFAS body burdens increase up the trophic level, and represent this well in the graphical abstract and subsequent figures in the publication (88). The variation in the type of PFAS accumulated in tissue might be explain by different foraging modes, because herbivorous fish consumed mainly aquatic plants and biofilms, omnivorous fish also consumed a significant portion of aquatic plants where short-chain PFAS dominate, whereas carnivorous fish consumed aquatic organisms where long-chain PFAS dominate (89).

A number of studies have looked at PFAS accumulation in different fish species with all of them showing that PFAS preferentially accumulate in blood and liver tissue, and have moderate accumulation in other tissues, such as kidneys, gills, and intestines, but little accumulation in muscle (28,82,83,88,91). For example, in five different fish species with various foraging modes and habitat uses, total PFAS concentrations were all found highest in liver, followed by stomach, cheek, and lowest in muscle. The compounds at the highest concentrations were different among different tissues, indicating each PFAS compound bioaccumulation was tissue-specific with different accumulation capacities (88). This study reported higher proportions of PFPeA, a short-chain PFCA, in muscle tissues of omnivorous or herbivorous fish studies, suggesting that feeding on plant and algae may be an exposure route to short-chain compounds. Additionally, this might be related to the fact that organic anion transporters and transporting polypeptides which mediate PFAS uptake and elimination can be expressed differently in various tissues (91–94). Previous studies also indicated PFAS not binding with all proteins but some specific proteins related to their specific conformations, such as liver fatty acid-binding protein and serum albumin which could explain high concentrations of PFAS accumulated in liver and blood of fish (50,95–97).

PFAS bioaccumulation in fish exhibited a long-chain preference, especially PFOS which was the predominant which is the predominant compound found in many field studies (28,82,88). In one field study, the four dominant PFAS (PFOS, PFNA, PFUnA, and PFDoDA, all long-chain PFAS) accounted for more than 94% of the total analyzed ten PFAS (C6-C12 PFCAs and C6, C8, C10 PFSAs) (82). It was also found that long-chain PFAS have an inhibitive effect to short-chain PFAS accumulation through competition for binding sites and transporters in zebrafish. The competitive effect was also concentration dependent which suggest that the effect seen was increasingly significant when long-chain PFAS concentrations were higher (91). In particular, researchers found PFAS binding affinities were significantly increased with increasing carbon chain length (50,52,97–99). This competitive effect may also be responsible for the predominance of long-chain PFAS in environmental biota samples.

Generally, PFAS bioaccumulation in fish increased with increasing perfluorinated carbon chain length for PFCAs and PFSAs. This trend observed in Figure 2 is striking but could also be affected by the fact that under natural conditions beyond the organism and water (for BCF) or dietary source (for BAF) that other phases, such as sediments or other non-aqueous phases, could inflate observed bioaccumulation metrics because their presence could lower water concentrations, especially as the perfluoroalkyl chain length increases. It should also be pointed out that BAF values in Figure 2 were collected at different concentrations and that even though bioaccumulation metrics, like BCF and BAF, assume a linear relationship between the concentration of a compound in an organism/tissue and its concentration in the water/dietary source, caution should be used when interpreting data, such as that shown in Fugre 2, because this assumption may not be correct. For PFCAs, some studies had a plateau in BCF or BAF values at the carbon chain length of 11 (82,83,91,100), which may be related to the lower bioavailability of higher molecular weight compounds. Functional groups also have significant influence on the bioaccumulation; for PFAAs, PFSAs showed higher BCFs and BAFs than PFCAs with the same perfluorinated chain length. A proposed reason was that the sulfonic acid group formed three hydrogen bonds with binding sites while carboxylic acid groups only formed two bonds, decreasing the bond strength (51). Ahrens et al compared the BAFs of PFOS, PFNA, and PFOSA (All having eight perfluorinated carbons) in European perch, and their BAFs were 6400 L/kg, 48 L/kg, and 1200 L/kg, respectively (28) suggesting that the functional group can have a significant effect. PFAS bioaccumulation in fish was also found to be concentration dependent. Chen et al exposed zebrafish to both low-dose and high-dose water concentrations and found BCFs in low-dose exposure to be higher than in high-dose conditions (i.e., negatively correlated), especially for long-chain PFAS, consistent in various tissues (100). Therefore, when comparing BCFs and BAFs one needs to consider the PFAS concentration in water. The physiological conditions of fish also have significant effects on bioaccumulation, such as reproductive stages, sex, body weight, and body length (82,90,101,102).

Overall, in fish, environmental factors, developmental stages, foraging mode, trophic level, and living environment have been demonstrated to have significant factors on the bioaccumulation of PFAS. All these factors vary for each fish species and can have direct implications on the bioaccumulation, range of compounds they are exposed to, and the impacts exposure will have during their life spans. Related to PFAS, chain length and functional groups associated with the compounds impact which tissues the compounds partition to in the fish and how fast they are accumulated. Research gaps related to bioaccumulation in fish is related to which environmental factors impact PFAS bioavailability the greatest and which trophic levels are impacted the greatest by PFAS in their diet.

2.5 Comparison of reported BAF values across aquatic organism groupings

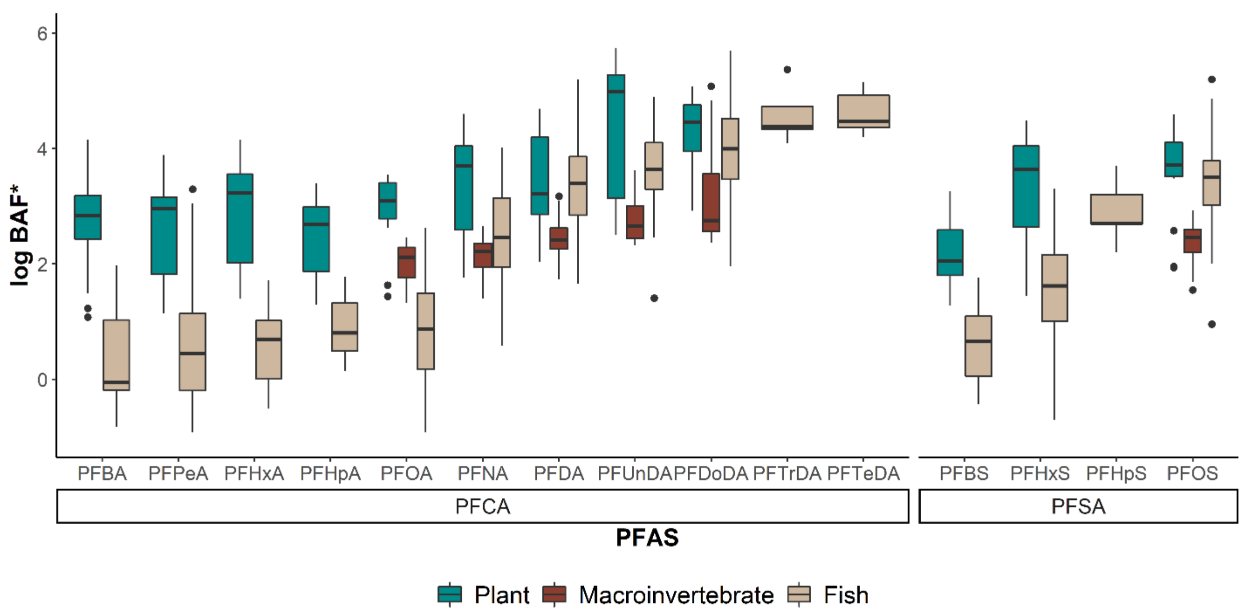

Figure 2. Comparison of log BAF* (combination of reported BAF and BCF values from literature) values across different groupings of aquatic organisms (aquatic plant, macroinvertebrate, fish). In total, data represented in the figure comes from 20 publications that report across 40 different species of freshwater aquatic plants, macroinvertebrates and fish listed in S2. The number of reported values varied between each compound and type of tissue (aquatic plants, macroinvertebrates, or fish), and can be found in the Supplementary Materials.

To look at how PFAS bioaccumulation into tissues varies across trophic levels, reported values were combined across the literature and looked at comparatively. In Figure 2, reported BAF and BCF values were combined from the literature that were calculated the same way, as the concentration of PFAS in tissue divided by the concentration of PFAS in water, allowing insight into how exposure to water impacts bioaccumulation. By looking at these results, Figure 2 suggests that as the perfluoroalkyl chain length increases, the BAFs tend to accumulate similarly, while short-chain PFCAs preferentially accumulate in aquatic plants. Additionally, fish tissue BAF values increase at a much greater rate after PFOA with increasing carbons, while plants and macroinvertebrates tend to increase more linearly. Plants and fish specifically, have a wide range of reported values, suggesting that the species diversity in the studies have a significant impact on PFAS bioaccumulation. Macroinvertebrate studies are limited in the number of studies and the number of PFAS compounds investigated, and more research is needed to be able to make clear connections.

2.6 Biotransformation and metabolism of PFAS and precursor PFAS

PFAAs can be directly released into the environment or be created via transformed precursor PFAS *in vivo*. The presence of precursor PFAS in the environment is of particular concern because some of them are transformed to compounds that are known to toxic and precursor PFAS have unknown toxicities. Even with the phasing out of some PFAAs from production, the diverse and lesser understood range of precursor PFAS that are frequently detected in sediment suggest that ongoing exposure to biota will occur (103). In most bioaccumulation studies of field collected samples, or partitioning studies in aquatic ecosystems, data on PFAS concentrations is presented as PFAA concentration. However, precursors are frequently detected in biota, sediment, and water in environmental matrices, sometimes up to 44% of the extractable organic fluorine (EOF) (104). EOF is a method typically used to present an estimate for the overall exposure to PFAS compounds in the environment, further explained in a human serum study (105). Due to the frequency of biotransformation of precursors and persistence in the environment, total oxidizable precursor (TOP) assays should be done on all environmental samples to realize the full risks of PFAS exposure, fate, and transport in the environment (106). The TOP assay is a method that can quantify precursor PFAS by exposing them to hydroxyl radicals generated by thermolysis of persulfate under basic pH conditions and comparing the PFAA concentrations before and after oxidation (107). In addition, precursor PFAS abiotic degradation to PFAS have been consistently considered negligible, while *in vivo* biotransformation has been observed to be a common pathway to form PFOS (108,109). Researchers have looked at a variety of methods, EOF, TOP assay, *in vivo* biotransformation, but the field is still young, and data are not yet available to properly evaluate different experimental approaches or assess their potential bias.

There have been a number of studies that have detected various precursor PFAS in sediments and soils (110) along with many that directly observed biotransformation in soils (111). Precursor PFAS that have been demonstrated to biotransform in soil to PFAAs have include perfluorooctaneamido quaternary ammonium salt (PFOAAmS) (112), perfluorooctanesulfonoamides (FOSAs) (113,114), fluorotelomer alcohol (FTOH) (115), fluorotelomer thioether amido sulfonate (FtTAoS) (116), and polyfluoroalkyl phosphate esters (PAPs) (111) (Figure S1, Supplementary Materials). The ability of these precursor PFAS to biotransform in soils suggest that similar processes and mechanisms would occurs in sediment in freshwater aquatic ecosystems. Specifically for the precursor EtFOSA, microbial hydrolysis to form PFOS has been proposed as a mechanism to form PFOS directly (117) . Fungal biotransformation of 6:2 FTOH has been demonstrated in a microbial consortia as well, where up to 63% of the initially dosed compound was transformed to either 5:3 fluorotelomer acid, or PFCAs (118). Biota in these habitats will be exposed to both precursor PFAS directly from the sediment as well as PFAA release over time as biotransformation occurs in the sediment. Therefore, PFAS in higher trophic level organisms will be comprised of both PFAA and precursor PFAS, with the potential of biotransformation of precursor PFAS *in vivo*.

Wetland environments in both anoxic and aerobic conditions, specifically related to the microbial population, have demonstrated favorable conditions for the biotransformation of 6:2 FtS. While the transformation was less than 1% mol transformation in the anoxic conditions, in the aerobic conditions there was a shift in the microbial community structure where *Methylcaldum*, a methane-oxidizing bacteria, became the predominant genus and about 12% mol transformation occurred to shorter chain PFCAs and 5:3 FtS acid (119). The same group looked at biotransformation of NEtFOSAA and saw yield of 85.1% mol transformation to PFOS in aerobic conditions, but no transformation anaerobically (119). In river sediments, 6:2 FTOH was dosed at high concentrations and was rapidly biotransformed with a half-life of less than three days into FTOH, 5:3 FtS acid, and PFHxA (114) demonstrating the biotransformation in actual sediment and suggesting that some precursors may be rapidly transformed in the environment compared to other and raises questions on how the redox potential may impact transformation.

Work observing biotransformation of precursors in freshwater macroinvertebrates is limited but there is evidence that suggests it is a common occurrence. In *Lumbriculus variegatus*, aquatic earthworms, exposed to PFAS in field contaminated sediments, a lab study was conducted where NEtFOSAA was observed to transform to PFOS and other PFOS precursors once accumulated in the tissues (67). The only other study to date looking at biotransformation of precursor PFAS in freshwater macroinvertebrates looked at a worm, *Limnodrilus hoffmeisteri*, exposed to 8:2 diPAP, a fluorotelomer phosphate diester, in the sediment and found that the worms accumulated the compound at a greater rate compared to fish species collected, but biotransformed the compound at a lesser rate (120). The authors suggest that the higher bioaccumulation in the worm was related to smaller body sizes, active ingestion of the sediment, and adsorption via skin from sediment pore water. *Eisenia fetida*, a terrestrial macroinvertebrate, has also been demonstrated to biotransform precursor PFAS (121) further suggesting this process may be common across a wide range of macroinvertebrates.

There has been a significant amount of research involving the observation of biotransformation of precursor PFAS during fish metabolism. The ability of fish to be able to biotransform precursor PFAS accumulated likely impacts reported BAF, BCF, and TMF values, because there will be an increased concentration of PFAAs, or some other intermediate, from the initial precursor PFAS, making the partitioning to tissues skew towards higher values. In *Saprus aurata*, a saltwater fish commonly found in Italy that feeds on shellfish and small aquatic plants, it has been shown that 8:2 diPAP introduced in the diet has produced transformed products of 8:2 FTCA, 8:2 FTUCA, 7:2 FTCA, and PFOA as an indirect exposure route (122). In this study, protein rich tissues had the highest concentrations of 8:2 diPAP, while PFOA concentrations occurred at the highest concentrations in the bile and brain. Biotransformation of precursors not only affected the reported accumulation metrics, but also affects where the compounds partition in the body and the subsequent health impacts. In marine settings, it has been demonstrated that higher trophic level organisms have a much higher capacity of transforming PFOSA to PFOS by looking at the contribution to their PFAS profile in their body burdens (123).

Specifically in freshwater, there have only been a few studies of biotransformation of precursor PFAS in fish. An earlier study looked at exposing NEtFOSA to trout liver microsomes *ex* vivo and saw after 30 hours that with increasing incubation times, PFOS and PFOSA were found in increasing concentrations (124). Rainbow trout, *Oncorhynchus mykiss*, were demonstrated to transform PFOSA to PFOS, 10:2 FTOH to 10:2 FTUCA, and 8:2 FTOH to 8:2 FTUCA over 30-day exposure periods (125). For the FTOH exposed tissues, small amounts of PFOA and PFDA were also detected suggesting that transformations to PFAAs for the precursor

FTOH found in the environment is likely a much longer process. PFOSA biotransformation kinetics *in vivo*, and likely other precursors, are affected by the isomeric state; as an example, branched PFOSA isomers are preferentially metabolized over linear PFOSA isomers in common carp (*Cyprinus carpio*) (126). Accumulation and extent of transformation of the precursors 8:2 diPAP is species dependent among benthic organisms. For bioaccumulate kinetics, the highest were observed in order from worms (*Limnodrilius hoffmeisteri*) to common carp (*Cyprinus carpio*) to loach (*Misgurnus anguillicaudatus*), likely related to foraging behavior and location in the water column. However, the rates of transformation of 8:2 diPAP to 8:2 FTUCA and 7:2 FTCA followed a reverse trend, further demonstrating that higher trophic level organisms have greater potential to biotransform precursor PFAS in freshwater aquatic ecosystems. Difference in diet and prey PFAS contamination can also lead to different accumulation profiles and exposures to PFAS. In addition to different PFAS exposed, researchers are able to compare concentration ratios of precursor PFAS to their subsequently transformed PFAA to further understand biotransformation in macroinvertebrate and fish species (38).

2.7 Trophic Transfer

As observed with other environmental organic contaminants, biomagnification of PFAS is a common occurrence in many habitats and a reason for high tissue concentrations in upper trophic level organisms. PFAS concentrations in tissues has been shown to be prevalent across all trophic levels in many ecosystems. Ability of PFAS to bioaccumulate in upper trophic levels is affected by their efficient dietary assimilation, partitioning into tissues with high protein content, and PFAA resistance to metabolism and slow elimination rates (127). Many short-chain PFAS do not have bioaccumulative potential, with the exception of some compounds especially PFSAs, and long-chain PFAS are commonly regarded as the most bioaccumulative compounds in the class (128). Trophic magnification factors (TMFs) > 1 provide information on biomagnification (change in residues per trophic level) and have been commonly reported in literature. For example, in an Antarctic ecosystem TMFs for PFOS and PFHxS were reported as >2 in an environment where short-chain PFAS were most commonly detected, suggesting that while at low concentrations biomagnification is still a significant process (129). In France, trophic magnification was studied looking at 16 PFAAs, 12 precursor PFAS, and 2 fluorinated alternatives to long chain PFAS in water, sediments, and biota (including biofilms, invertebrates, and fish) (130) and further evidence for biomagnification of long-chain PFCAs and PFSAs were demonstrated. However, decreases in precursors PFCAs to PFCAs concentration ratios with increasing trophic level suggest a likely contribution from biotransformation. Additionally, TOP assays showed 15-80% of total fluorine in sediments was associated with precursor PFAS and a significant decrease was observed from sediments to invertebrates and fish. In a study in the Lake Ontario food web PFAS bioaccumulation was significantly impacted by highly hydrophobic compounds (PFOS and long-chain PFCAs) and accumulated into the tissues (131). However, results from this study showed that high molecular weight PFCAs did not have increased BMFs or TMFs, suggesting that hydrophobicity does not impact the trophic transfer of PFAS, only binding to tissues. These results from these few studies suggest that concentration of PFAS may play a role on trophic magnification as well as climate, as many of the studies observing trophic magnification take place in temporal or arctic climates.

However, factors discussed previously that affect PFAS biomagnification are often not considered in calculations in the studies presenting TMF values. TMFs reported in literature can range as a factor of approximately 20 and is affected by different ways the metrics are expressed, wrongfully assumed steady-state conditions, feeding ecology, metabolism of precursor compounds, and other environmental factors (132). Recent studies discussed (130,133) have begun to consider transformation of precursor PFAS specifically depending on the trophic levels in which they are accumulated when reporting TMFs.

3. Conclusions: State of Knowledge and Gaps

3.1 Key Considerations for Bioaccumulation of PFAS in Biota

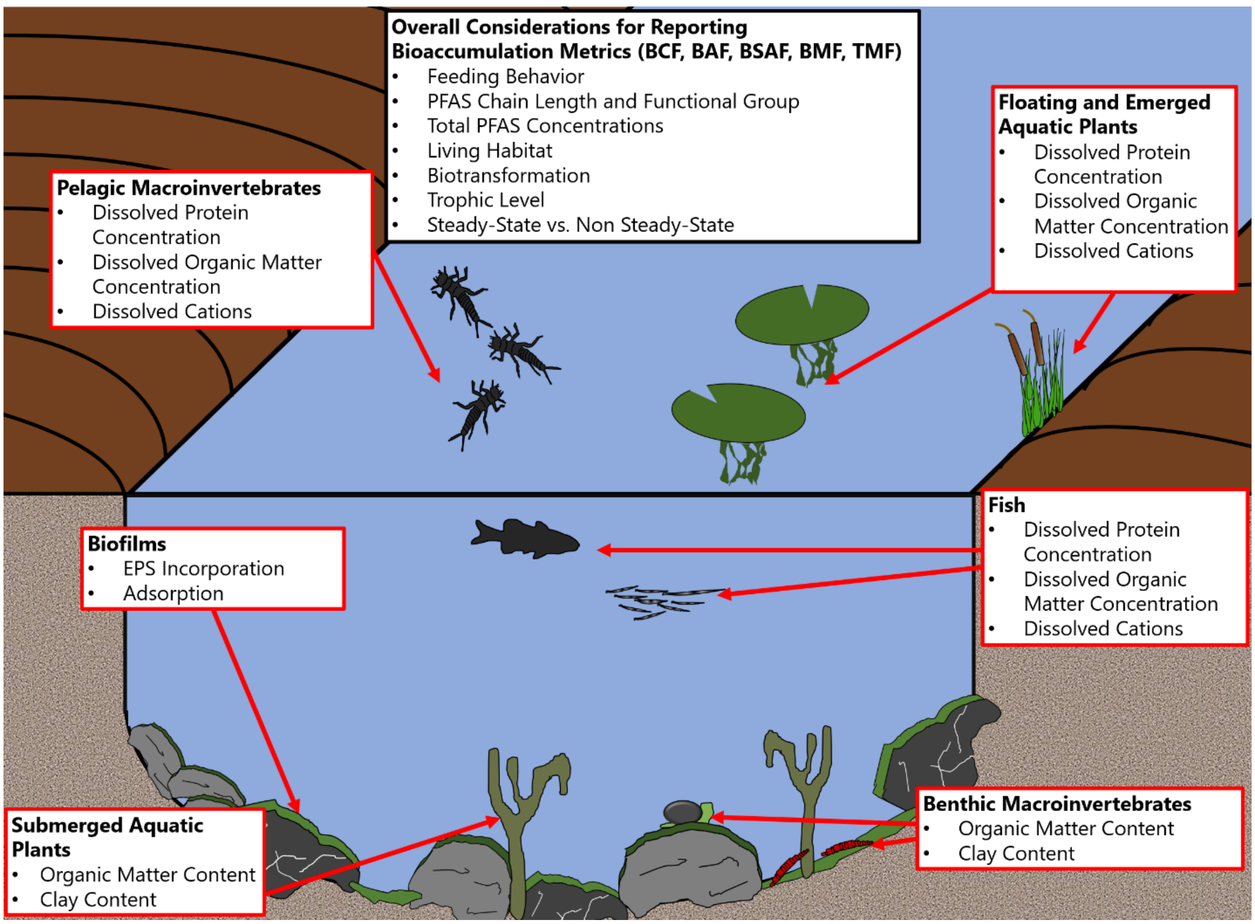

Figure 3. Considerations when reporting on bioaccumulation metrics in literatures and data to collect based on type of organism. Important factors affecting PFAS bioaccumulation based on type of organism.

After reviewing the literature on PFAS bioaccumulation and reported metrics in freshwater aquatic ecosystems, many considerations that should be taken when reporting on bioaccumulation metrics such as BCF, BAF, BSAF, BMF, and TMF became apparent. As discussed previously, reported values for BAF and TMF can range several orders of magnitude and when reporting on these values, care should be taken to bring consistency to the presented values across the literature by using standards for reporting metrics and presentation of key associated data in reported metrics. Using lab generated bioaccumulation metrics, like BCF, researchers cannot recreate the full range of complexity and interactions that occur in the field, but only provide information on specific mechanisms in a highly controlled environment.

Table 3. Key Considerations (PFAS, Biological, and Environmental) for reporting of PFAS bioaccumulation Metrics.

PFAS	Biological	Environmental
• Concentration • Pre-Cursor PFAS • Chain Length • Functional Group	• Foraging Mode • Trophic Level (When applicable) • Dwelling (Benthic/Pelagic)	• Dissolved Organic Carbon • Sediment Organic Carbon • pH • Dissolved Cations

When research is done at any trophic level there are many considerations that can be applied across all levels as seen in Figure 3. Regarding organismal data, descriptions should be included of the selected organisms foraging mode, dwelling in the ecosystem, and trophic level (when applicable). Foraging mode leads to variation in reported values because the reported bioaccumulation metrics for the compounds can vary greatly based on how the organism behaves. Organisms that are near the sediment are likely exposed to more PFAS (specifically longer chain PFAS) and thus presented values should emphasize this information, so it is clear to the reader. The trophic level will likely impact the level of accumulation and biotransformation of precursors which is typically not accounted for in reported values. With field and lab samples and reported bioaccumulation metrics, efforts should be made to confirm that the system is at steady state, and if not, it should be noted since it will lead to significant variation in bioaccumulation metrics.

Regarding PFAS data, chain length and functional group impact accumulation at all levels where increased chain length and sulfonate head groups increases bioaccumulation for most trophic levels except floating and emerged aquatic plants. Additionally, a common observance where total PFAS concentration in the exposed water was found to impact bioaccumulation metrics, indicating that reported values will be inconsistent. The literature suggests a negatively correlated relationship between concentration and bioaccumulation where lower concentrations of bioaccumulative PFAS have higher bioaccumulative metric values. However, partitioning metrics, such as BCF and BAF, are based on linear relationships so conclusions on concentration dependence need to consider whether the PFAS are behaving linearly or non-linearly. While most studies report the concentration at which bioaccumulation metrics are measured in the methods or SI section, many do not adequately discuss how the concentration has impacted the final bioaccumulation metric that is calculated.

In the field, reported spatial and temporal trends of PFAS concentrations in tissues could be impacted by biotransformation of precursors and usually only look at PFAAs, which does not represent the full exposure of PFAS in the ecosystem. Emerging analytical methods such as TOF, non-targeted analysis, and the TOP assay should be considered to some perspective on precursors in samples. In addition to environmental factors seen in Figure 3, the calculation of the bioaccumulation metrics can be done in different ways (Table 1), and values used such as tissue concentration in wet or dry weight should be made clear. Environmental characterization of water and sediment should at least include DOC, organic matter content of sediment, and pH, as they have been demonstrated to significantly impact PFAS partitioning.

3.2 Gaps

To date a significant amount of work has been done on characterizing the bioaccumulation of PFAS in freshwater aquatic ecosystems that greatly improves the understanding of the behavior of these compounds in the field. Many different trophic levels have been observed to bioaccumulation PFAS in freshwater aquatic ecosystems and work has been done to look at specific environmental factors that impact bioaccumulation metric values. Many studies present certain factors (dissolved protein, organic matter content, polyvalent cation concentration, etc.) but there is no standard for presenting these in reported values of bioaccumulation metrics. Additionally, the biotransformation of precursors, non-steady state conditions, and the specific organism’s behavior can lead to widely ranging reported values. Trophic transfer and dietary exposure are understood to occur with PFAS, but specific mechanisms and environmental factors that impact it are lesser understood. Overall, advancing analytical methods and approaches, consideration of environmental factors presented in this review, and more holistic characterization of fields conditions is needed to narrow the gap on understanding of PFAS bioaccumulation in freshwater aquatic ecosystems.

4.0 Acknowledgements

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Table 1. Commonly reported bioaccumulation metrics in aquatic ecosystems: definitions and calculations in biota.

Bioaccumulation Metric	***Definition***	***Animals***	***Plants***	***Implications/Pros/Cons***
Bioconcentration Factor (BCF)*(26)*[a]	The direct uptake of PFAS by an organism from the water column, measured as the ratio of the concentration in an organism to the concentration in the water (measured in the laboratory).	$\frac{[PFAS]_{Tissue}}{[PFAS]_{Water}}\left(\frac{L}{kg}\right)$	$\frac{[PFAS]_{Plant}}{[PFAS]_{Water}}\left(\frac{L}{kg}\right)$	- Dermal exposure to PFAS - Implies no dietary exposure - Implies no interaction with sediment - Reported in lab studies - Can be used to inform real world but not representative
Bioaccumulation Factor (BAF)*(26)*	The amount of PFAS taken up from bioconcentration plus the contribution of PFAS in the diet of the organisms (measured in the field). Calculation assumes no importance of dietary exposure	$\frac{[PFAS]_{Tissue}}{[PFAS]_{Water}}\ (unitless)$	$\frac{[PFAS]_{Plant}}{[PFAS]_{Water}}\left(\frac{L}{kg}\right)$	- Unable to distinguish the exact uptake route (does not account for dietary exposure) - Implies no interaction with sediment - Reported in field studies
Biota Sediment Accumulation Factor (BSAF)*(30)*	A way to present the tendency of a compound present in the sediment to accumulate in an organism.	$\frac{[PFAS]_{Tissue}}{[PFAS]_{sediment}}\ (unitless)$	Not defined	- Adjust bioaccumulation by the sediment consumption/exposure - Does not account for exposure to the water column
Biomagnification Factor (BMF)*(26,31)*	An increase in tissue concentration as one moves up the food chain based on a predator/prey relationship (measured in the lab or field).	$\frac{[PFAS]_{Tissue}}{[PFAS]_{diet}}\ (unitless)$	Not applicable	- Lab and field values cannot be directly compared; lab BMF(31) never combines dietary and water exposure while field BMF values may
Trophic Magnification Factor (TMF)*(32)*	An average food web biomagnification factor and is determined empirically using field measurements of contaminant concentrations and relative trophic level.	10^b (b = the regression slope from a log-normal relationship between trophic level and log [PFAS]	Not applicable	- Dietary absorption occurs faster than elimination -TMFs > 1 indicate the occurrence of biomagnification – Provides information on change in residues per trophic level

* $[PFAS]_{matrix}$ signifies concentration of PFAS in specified matrix.
[a] Some studies do not limit BCF calculations to only lab studies and incorrectly report field measurements using BCF.

Table 2. Important PFAS compounds presented in the literature and relevant groupings.

PFAS Grouping	PFAS Compound	Number of Carbons	Chain Length Classification
Perfluorocarboxylic Acids (PFCAs)	PFBA	4	Short
	PFPeA	5	Short
	PFHxA	6	Short
	PFHpA	7	Long
	PFOA	8	Long
$C_nF_{2n+1}CO_2H$	PFNA	9	Long
	PFDA	10	Long
	PFUnDA	11	Long
	PFDoDA	12	Long
	PFTrDA	13	Long
	PFTeDA	14	Long
Perfluorosulfonic Acids (PFSAs)	PFBS	4	Short
	PFHxS	5	Short
$C_nF_{2n+1}SO_3H$	PFHpS	6	Long
	PFOS	8	Long
	PFDS	10	Long
Precursor PFAS	6:2 FtS	8	Long
$C_nF_{2n+1}X$	PFOSA	8	Long
***X = various headgroups**	EtFOSA	10	Long

Table 3. Key Considerations (PFAS, Biological, and Environmental) for reporting of PFAS bioaccumulation Metrics.

PFAS	Biological	Environmental
• Concentration • Pre-Cursor PFAS • Chain Length • Functional Group	• Foraging Mode • Trophic Level (When applicable) • Dwelling (Benthic/Pelagic)	• Dissolved Organic Carbon • Sediment Organic Carbon • pH • Dissolved Cations

Figure 1

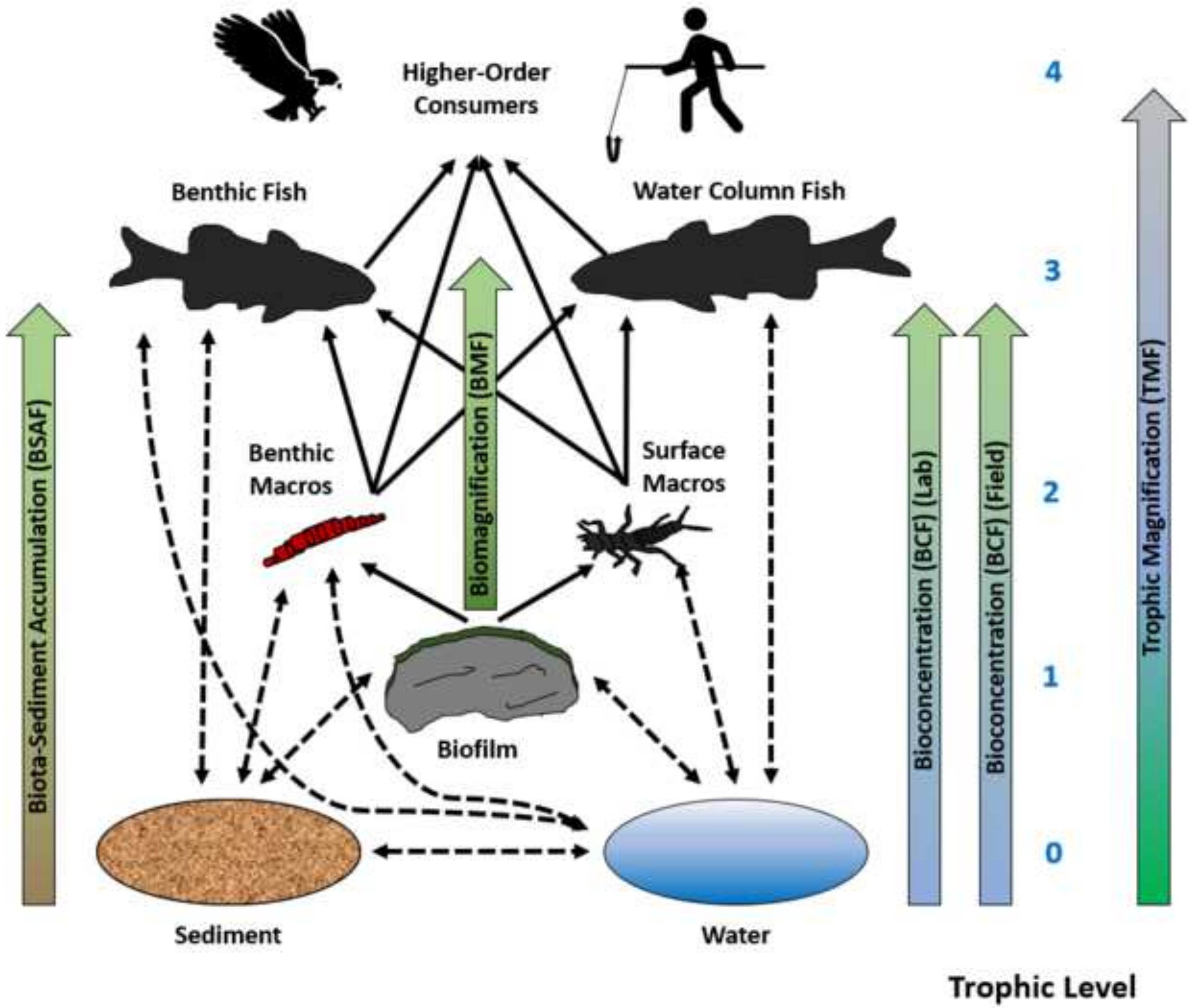
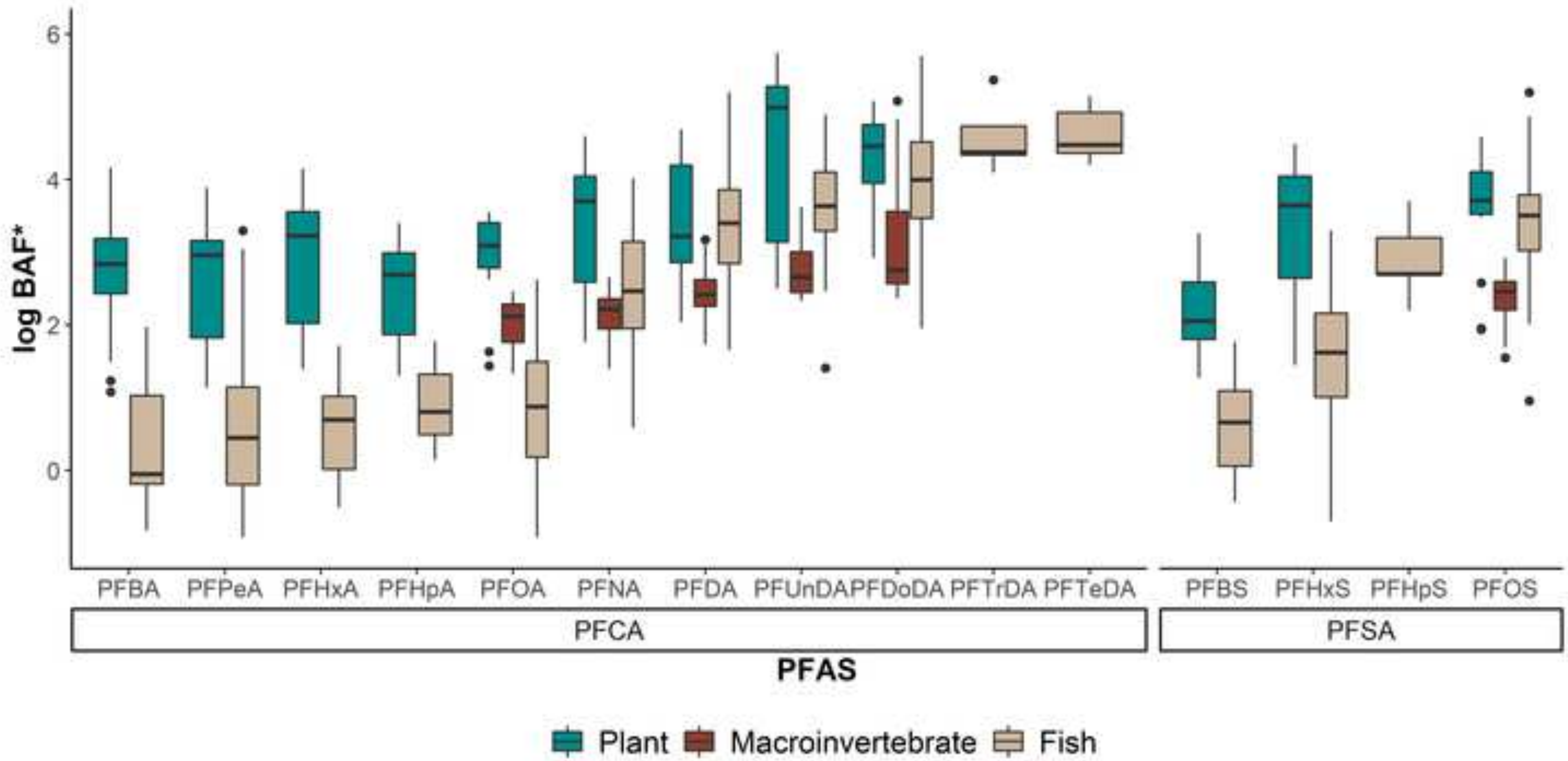
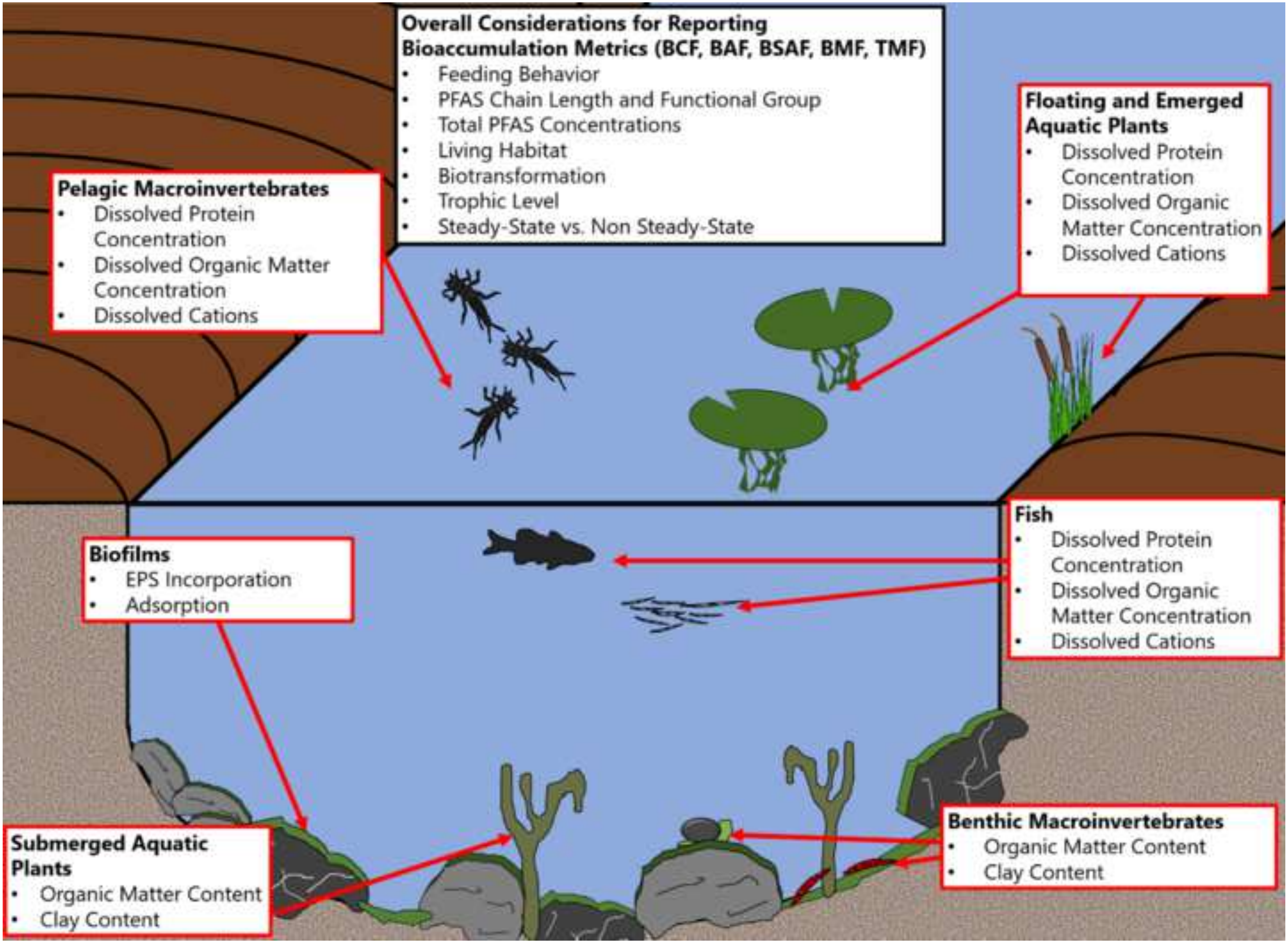

Higher-Order
Consumers
Benthic Fish
Water Column Fish
Benthic
Macros
Surface
Macros
Biofilm
Sediment
Water
Biota-Sediment Accumulation (BSAF)
Biomagnification (BMF)
Bioconcentration (BCF) (Lab)
Bioconcentration (BCF) (Field)
Trophic Magnification (TMF)
4
3
2
1
0
Trophic Level

Figure 2



log BAF*
6
4
2
0
PFBA
PFPeA
PFHxA
PFHpA
PFOA
PFNA
PFDA
PFUnDA
PFDoDA
PFTrDA
PFTeDA
PFBS
PFHxS
PFHpS
PFOS
PFCA
PFSA
PFAS
Plant
Macroinvertebrate
Fish

Figure 3 Click here to access/download;Figure;Fig 3a.jpeg



Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Asa J. Lewis: Conceptualization, Investigation, Writing - Original Draft, Visualization **Xiaoyan Yun:** Conceptualization, Investigation, Writing – Original Draft **Daniel Spooner:** Writing – Review & Editing, Supervision **Marie Kurz:** Writing – Review & Editing, Supervision **Erica R. McKenzie:** Writing – Review & Editing, Supervision **Christopher M. Sales:** Writing – Review & Editing, Supervision