BOREAL ENVIRONMENT RESEARCH 31: 101–110
ISSN 1797-2469 (online)

Helsinki 21 September 2026

Benthic-pelagic coupling as a suggested mechanism for PFAS transfer across trophic levels in the northern Baltic Sea

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Received 19 Feb. 2026, final version received 3 Aug. 2026, accepted 3 Aug. 2026

Lehtiniemi M., Näkki P., Tirroniemi J. & Setälä O. 2026: Benthic-pelagic coupling as a suggested mechanism for PFAS transfer across trophic levels in the northern Baltic Sea. *Boreal Env. Res.* 31: 101–110.

The per- and polyfluoroalkyl substances (PFAS) are persistent pollutants consisting of over 4700 fluorinated compounds used in various applications. Global PFAS pollution has exceeded safe limits, affecting rainwater, surface water, and soil. PFAS contamination is emerging as a significant concern also in the Baltic Sea, which is already highly polluted due to various anthropogenic pressures and has characteristics making it vulnerable to pollution. This study aimed to assess PFAS concentrations in lower trophic levels, specifically zooplankton and mysid shrimps, in the Bothnian Sea and the Gulf of Finland, the northern Baltic Sea. The study showed that PFAS were present in all meso- and macrozooplankton samples analysed. Both legacy and alternate PFAS compounds were found in the samples. Results also showed that perfluorooctane sulfonate (PFOS) was the most abundant of the PFAS compounds found. Higher concentrations were found in bentho-pelagic mysids compared to copepods in the Bothnian Sea indicating the importance of habitat in PFAS accumulation in meso- and macrozooplankton. We discuss how variation in PFAS concentrations among different crustacean plankton prey could affect biomagnification in fish, particularly herring.

Introduction

The planetary boundaries framework (Rockström *et al.* 2009) presents safe limits for human pressures on the nine critical processes which together maintain a stable and resilient life on earth. It has recently been proposed that the per- and polyfluoroalkyl substances (PFAS) would form a separate planetary boundary, which has already been exceeded (Cousins *et al.* 2022). The reasoning being that PFAS pollution at the global scale is diffuse and has led to levels of PFAS substances universally high above the guideline levels set for rainwater, surface and soil (Cousins *et al.* 2022).

PFAS comprise more than 4700 fluorinated substances commonly used in coating applications, fire-fighting foams, and industrial manufacturing (Glüge *et al.* 2020). They can enter the environment via multiple sources and routes, including e.g. fire extinguishing foam, industrial processes, landfill leakage, riverine inflow, wastewater treatment plants, and atmospheric deposition (Glüge *et al.* 2020, Filipovic *et al.* 2013). PFAS are resistant to environmental and metabolic degradation, and globally spread in

Editor in charge of this article: Johanna Mattila
https://doi.org/10.60910/ber2026.0y8p-3e73

all environmental matrices (Wang *et al.* 2013, Cousins *et al.* 2020, 2022).

Although the production of some perfluoroalkyl acid substances (PFAAs) have been banned and their use thus decreased, they are commonly detected in marine environment in water (Takazawa *et al.* 2009), sediment (Wan *et al.* 2017), and biota (Giesy and Kannan 2001, Fair *et al.* 2019) because of their high persistence. Water-soluble PFAAs may migrate even to the most remote areas via ocean currents and aerosols (Giesy and Kannan 2001, Sha *et al.* 2021). Many PFAAs are also toxic and bioaccumulative; hence, some of them, such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been internationally restricted under the Stockholm Convention (2001).

The Baltic Sea has often been referred to as the most polluted sea in the world, as a consequence of its long history of pollution starting from the very beginning of industrialization in the late 19th century (HELCOM 2010). The semi-enclosed Baltic Sea has many characteristics that make it vulnerable to pollution: it has a long water residence time (~30 years), more than 85 million people living in its vast drainage area, and a relatively species-poor community adapted to brackish water environment (HELCOM 2010). Currently, the most severe environmental threat to the Baltic Sea ecosystem is considered to be eutrophication due to excess nutrient loading, but in the past decades the area was seriously polluted by e.g. polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT), which impaired the reproduction of seals and white-tailed eagles (*Haliaeetus albicilla*) before efficient measures were taken to combat the pollution sources (Sonne *et al.* 2020). Lately, attention has been drawn to emerging environmental contaminants, especially PFAS, which are widely detected in the surface waters, sediments, and biota of the Baltic Sea (e.g. Gebbink *et al.* 2016, Joerss *et al.* 2019, Soerensen *et al.* 2024).

Chemical structure, accumulation kinetics, and environmental conditions (e.g. salinity and pH) all influence the extent to which these compounds accumulate in biota (Lewis *et al.* 2022, Keller *et al.* 2024). Further, the size, growth rate, protein content of the organism, its feeding behaviour and living habitat as well as the elimination rate of the compound, all influence the potential bioaccumulation of PFAS in biota (Chojnacka and Mikulewicz 2024). Trophic transfer and biomagnification studies verified the bioaccumulation of long-chain PFAAs in the food webs in the South China Sea, but no or very little bioaccumulation of the short-chain PFAAs (Du *et al.* 2021, Diao *et al.* 2022). Similar findings have been reported for freshwater food webs in St. Lawrence river in Canada (Munoz *et al.* 2022) and in a terrestrial food web from invertebrates to Cooper's hawks in Vancouver, Canada (Fremlin *et al.* 2023). However, several studies have shown that PFOA, which is a long-chain (C8) compound did not biomagnify or it may even biodilute along the food chain (Du *et al.* 2021, Munoz *et al.* 2022, Fremlin *et al.* 2023, Ren *et al.* 2023). On the other hand, Kelly *et al.* (2009) found no biomagnification of various PFAS compounds in a piscivorous food web while biomagnification was shown in a marine mammalian food web of the Canadian Arctic due to slow elimination of PFAS in air breathing animals but efficient respiratory elimination in water respiring animals. Studies in aquatic environments have also shown the importance of the benthic environment as a potential source for the contaminants for the food chains based on organisms living and feeding in or on the sediment (Ren *et al.* 2023, Yun *et al.* 2023).

In the study of Kumar *et al.* (2022) PFAA compounds were found from 15 fish species in the Baltic Sea (e.g. herring, sprat, smelt, salmon). A recent monitoring study observed total concentrations of PFAS in the Baltic herring (*Clupea harengus membras*) that exceed the maximum levels set by the EU for the Bothnian Sea, located in the northern Baltic Sea (Suomi *et al.* 2024). For smelt (*Osmerus eperlanus*) and sprat (*Sprattus sprattus*), PFAS concentrations remained below the EU limits. However, PFAS concentrations in smelt were higher, even several-fold, compared with those in herring (Kumar *et al.* 2022; Suomi *et al.* 2024). Nevertheless, the measured PFAS concentrations in smelt remain below the EU limit,

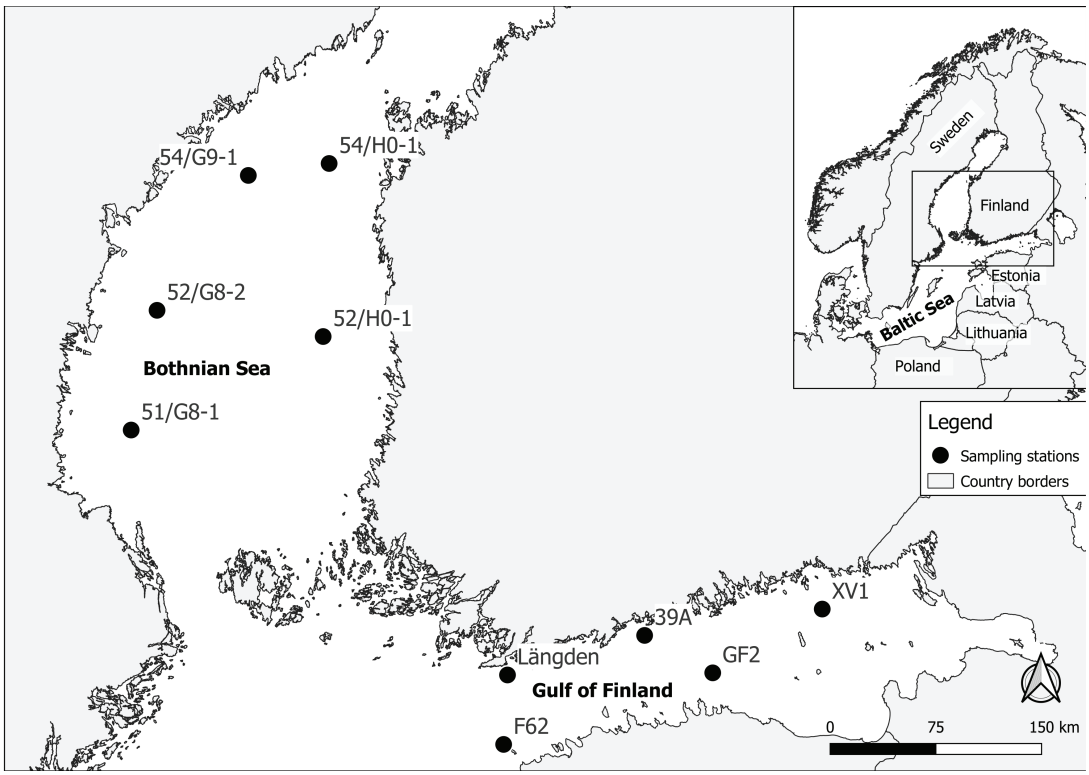

Fig. 1. Sampling stations in the Bothnian Sea and in the Gulf of Finland, northern Baltic Sea.

as the permissible level for this species is higher than the limit applied to herring. Furthermore, the concentrations of PFAS in herring have been considerably increasing from 2009 to 2023 in the Bothnian Sea, but not in other parts of the Baltic Sea, such as in the Gulf of Finland (Suomi *et al.* 2024). The reasons behind the spatiotemporal and species-specific trends are unclear, but one hypothesis is that the food sources of fish may explain some of the variation (Suomi *et al.* 2024).

Baltic herring and smelt are selective predators changing their prey as they grow and across seasons. Smaller fish are planktivores feeding on mesozooplankton while for larger herring and smelt macrocrustacean prey living close to sea floor or in the sediment (mainly mysids and amphipods) become an important food source especially during autumn and winter (Möllmann *et al.* 2004, Taal *et al.* 2014). Mysids and amphipods serve as important links between the benthic and pelagic food webs in the Baltic Sea as a large share of their energy originates from the benthic realm. Their energy is transferred to higher trophic levels in the pelagic food web through consumption by herring (Kiljunen *et al.* 2020). On the other hand, sprat feed only in the pelagial on zooplankton (Möllmann *et al.* 2004).

At present, no data are available on the PFAS concentrations in the food sources of planktivorous fish (herring, sprat, smelt) to assess the importance of food source in the accumulation of PFAS in fish in the northern Baltic Sea. Thus, the aim of this study was to gain knowledge on the PFAS concentrations in the lower trophic levels in the Bothnian Sea and Gulf of Finland. We hypothesized that detectable concentrations of PFAS would be found in the studied taxa, and that concentrations would be higher in the secondary consumers (mysid shrimps) feeding both close to the bottom on detritus and benthic invertebrates and in the pelagial on phyto- and zooplankton (Viherluoto *et al.* 2000) compared to primary consumers feeding solely in the pelagial on phytoplank-

ton (mesozooplankton). However, various other factors like feeding rate, growth and protein content may influence the PFAS concentrations of the studied biota (Chojnacka and Mikulewicz 2024), not only the habitat in which the animals feed.

Material and methods

Sampling

Sampling was carried out at two areas in the northern Baltic Sea during the cruises of the R/V Aranda. Salinities varied between 5 and 8 from the surface to the bottom waters. Both cruises were conducted after the growing season of zooplankton making the communities sampled comparable. The sampling in the Bothnian Sea (Fig. 1) was carried out at five sampling stations in September 2024, consisting of 1) macrozooplankton (*Mysis* spp.) and 2) mesozooplankton (*Limnocalanus macrurus* copepods). A large zooplankton net (opening 1 m, mesh size 500 µm) was lowered close to the bottom and towed slowly to the surface. Sampling was performed during nighttime to catch vertically migrating mysids. The contents of the net were poured on a 100 µm sieve, rinsed to a glass petri dish and zooplankton individuals were picked by species to precleaned glass vials with caps without teflon liners where they were frozen at –20°C. From the Gulf of Finland, mesozooplankton was collected from five sampling stations in January 2025 (Fig. 1). A 100 µm WP-2 zooplankton net was lowered close to the bottom and towed slowly to the surface. The contents were further concentrated on a 20 µm sieve and scooped with a small metal spoon to precleaned glass vials where they were frozen at –20°C. Because a smaller mesh-size net and sieve were used in the Gulf of Finland, smaller taxa were included in the PFAS analyses compared with the Bothnian Sea samples. This is a limitation of this study, and therefore the cross-areal comparison results should be taken as indicative. A subsample of the zooplankton community from each sampling station was microscopically qualitatively inspected (Leica DMIL LED, 50× magnification) to identify the most common species in the community. The mesozooplankton communities at the sampling stations in the Gulf of Finland were composed of a diverse group of calanoid copepods (*Eurytemora affinis, Pseudocalanus elongatus, Limnocalanus macrurus, Temora longicornis* and *Centropages typicus*), bivalve larvae and rotifers (*Synchaeta baltica, Keratella quadrata)*.

The average wet weight of the samples was 8.79, 7.21, 5.72 g for mysids, *Limnocalanus* and zooplankton community samples, respectively The samples were stored at –20 °C until freeze-dried (Christ Beta 2-8 LD plus) in the laboratory. Main drying lasted 24 hours in –44 °C and 0.08 mbar followed by final drying of 2 hours in –76 °C and 0.0010 mbar. Dried samples were grinded into fine powder by pressing with a glass rod cleaned with acetone and hexane (≥ 99.8%, ≥ 98%). The average dry weight of the samples was 1.08, 0.65 and 0.14 g for mysids, *Limnocalanus* and zooplankton community samples, respectively.

Analysis of PFAS

Further pre-treatment of samples and PFAS analyses were conducted at the Finnish Institute of Health and Welfare according to their own method (YKAT MO20, accredited to serum and fish samples by the Finnish Accreditation Service FINAS) based on a liquid chromatograph coupled to a triple quadrupole mass spectrometer (LC-MS/MS). The analyses targeted certain perfluoroalkyl carboxylic acids (PFCA) and perfluoroalkyl sulfonates (PFSA) belonging to the PFAAs (Table 1). Perfluoroalkyl compounds were extracted from the sample into acetonitrile, and the extract was purified on a solid phase column. The sample was analyzed by liquid chromatography-mass spectrometry (LC: Dionex Ultimate 3000 RS, MS/MS: Thermo TSQ Quantum Discovery max). The compounds were separated on a reversed phase column (XBridge C18 3.5 µm, length 30 mm, id 2.1 mm) and quantified using authentic reference compounds and labeled internal standards. The limit of quantification (LOQ) was 0.16–0.42 ng g^{-1} sample.

In further data analysis, PFAS concentrations below LOQ were assumed to be zero. Statistical analyses were performed with IBM SPSS Statistics. Non-parametric tests were done due to heterogeneous variances of the data. A significance level of 0.05 was used, and the results are given as mean and standard deviation.

Results and discussion

Spatial differences in PFAS concentrations

Per- and polyfluoroalkyl substances (PFAS) were found present in all the macro- and mesozooplankton community samples (Fig. 2, Table S1 in Supplementary Information; SI). Differences were found between the sampling areas in the mesozooplankton trophic level, the Gulf of Finland community samples having significantly higher (Mann-Whitney U-test: z: 475.5; p = 0,000) total concentration of PFAS (28.82 ± 16.3 ng g^{-1} dw) than *L. macrurus* samples from the Bothnian Sea (5.83 ± 3.4 ng g^{-1} dw). PFOS was the dominating substance in zooplankton of both areas as shown also for zooplankton in Lake Erie, US (Ren *et al.* 2023). Although the Stockholm Convention on Persistent Organic Pollutants (2001) started to restrict production and use of PFOS in 2009, it is still widely found in the environment and known to cause deleterious effects at cell and organ level as well as hamper growth, impair immune functioning and reproduction in vertebrates including fish and humans (e.g. Hagenaars *et al.* 2008, ATSDR 2015). PFOS has also been shown to cause deteriorated growth and reproduction in zooplankton and to be 2.5 times more toxic to zooplankters than PFOA (review by Ma *et al.* 2022). PFOA and perfluorononanoic acid (PFNA) were markedly present in the Bothnian Sea samples while in the Gulf of Finland samples PFOA and PFNA were found in similar concentrations to several other PFAS compounds.

The most abundant compounds in *L. macrurus* were PFOS (2.16 ± 1.3 ng g^{-1} dw), PFNA (1.7 ± 0.98 ng g^{-1} dw) and PFOA (1.5 ± 1.3 ng g^{-1} dw). Also, in the Gulf of Finland zooplankton communities the most abundant compound was PFOS (12.94 ± 8.3 ng g^{-1} dw), while the concentrations of other measured compounds were at clearly lower levels e.g. PFNA (2.62 ± 2.2 ng g^{-1} dw), PFOA (2.27 ± 2.6 ng g^{-1} dw) and perfluoroundecanoic acid (PFUnA) (2.44 ± 0.8 ng g^{-1} dw), perfluorohexanesulphonic acid (PFHxS)

Table 1. Analysed PFAS compounds and their estimated measurement uncertainties of the quantitative method for individual PFAS.

Group	Abbreviation	Name	Estimated measurement uncertainty
Perfluoroalkyl carboxylic acids (PFCA)			
	PFHxA	Perfluorohexanoic acid	50 %
	PFHpA	Perfluoroheptanoic acid	30 %
	PFOA	Perfluorooctanoic acid	30 %
	PFNA	Perfluorononanoic acid	30 %
	PFDA	Perfluorodecanoic acid	30 %
	PFUnA	Perfluoroundecanoic acid	30 %
	PFDoA	Perfluorododecanoic acid	30 %
	PFTrA	Perfluorotridecanoic acid	50 %
	PFTeA	Perfluorotetradecanoic acid	40 %
Perfluoroalkyl sulfonates (PFSA)			
	PFHxS	Perfluorohexanesulphonic acid	30 %
	PFHpS	Perfluoroheptanesulfonic acid	40 %
	PFOS	Perfluorooctane sulfonate	30 %
	PFDS	Perfluorodecanesulfonic acid	50 %

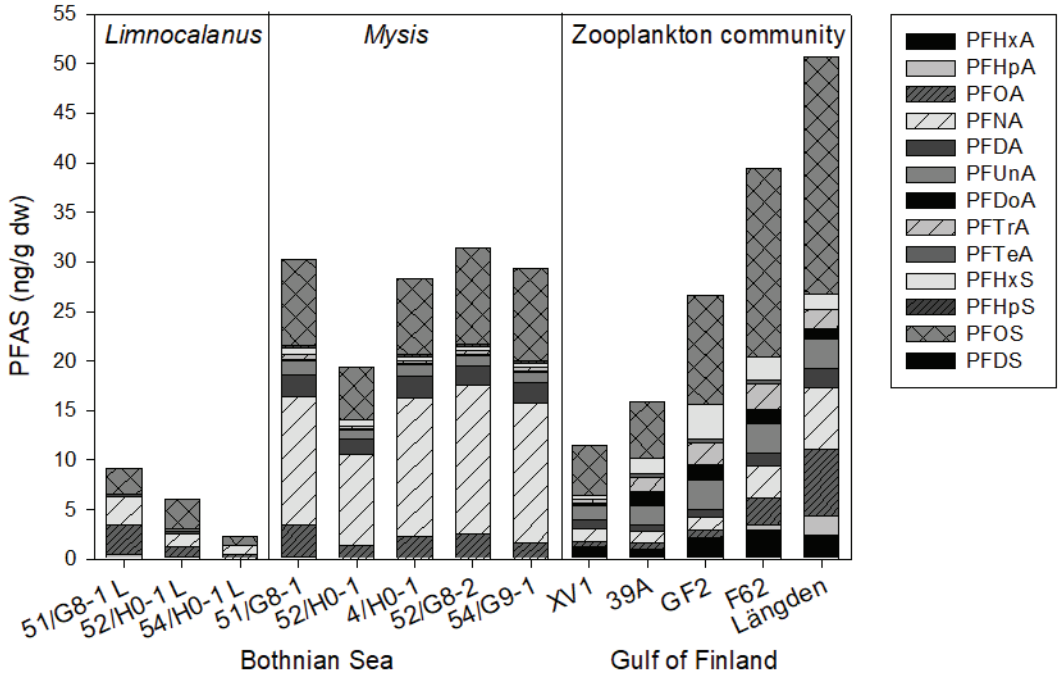

Fig. 2. PFAS concentrations (ng g^{-1} dw) in the taxa collected from the Bothnian Sea and from the Gulf of Finland. Concentrations below the limit of quantification (LOQ) were assumed as zero.

(1.84 ± 1.1 ng g^{-1} dw) and perfluorodecanoic acid (PFDA) (1.1 ± 0.5 ng g^{-1} dw) (Fig. 2). New measures have been adopted during the recent years (UNEP 2019, 2022) by amending the list of harmful substances e.g. PFNA, PFOA and PFHxS under the Stockholm Convention (2001) and REACH regulation (EC 2006) to restrict the use of these compounds due to persistence, and their unacceptable risks to human health and the environment. Restrictions are needed as these compounds are still found in sediments and biota, and our results show that there are clear spatial differences in their occurrence which may relate to local hot spots. Higher levels of these compounds and higher variety of different PFAS compounds especially in the western Gulf of Finland zooplankton may indicate the diversity of sources including discharges from both land-based (waste waters, industrial applications, consumer products, fire-fighting foams) and sea-based (antifouling of ships) sources (Glüge *et al.* 2020) as urban coasts of Finland and Estonia are close as well as the very intensive ship traffic passing the area (HELCOM 2025). Junttila *et al.* (2019) stated that part of the PFAS load to the coastal waters of the Baltic Sea comes via atmospheric deposition but a larger part comes via river discharge from land-based sources. They also showed that the significantly highest total PFAS concentrations among all large rivers in Finland were found in surface waters of the River Vantaanjoki (15–75 ng L^{-1}) discharging to the Gulf of Finland which may explain our observations of higher PFAS concentrations of mesozooplankton collected from this study area compared to the Bothnian Sea. It is also an interesting observation that perfluoroheptanoic acid (PFHpA) and perfluorohexanoic acid (PFHxA) were found in zooplankton communities of the Gulf of Finland. These alternate PFAS compounds have shorter half-life concerning environmental degradation than legacy PFAS compounds (e.g. PFOS and PFOA) and should therefore be safer compounds although they can persist for decades or even longer in the environment (Wang *et al.* 2013).

Our results correspond to the range of PFOA concentrations found in oce-

anic zooplankton across the globe (Casal *et al.* 2017); 0.1-43 ng g^{-1} dw, while for PFOS our results from the Gulf of Finland communities (max 24 ng g^{-1} dw) exceed the reported concentrations (0.5-6.7 ng g^{-1} dw). In the Baltic Sea, Gebbink *et al.* (2016) detected average concentrations of 0.11 ng g^{-1} ww of PFOA, < 0.05 ng g^{-1} ww of PFNA, and 0.0014 ng g^{-1} ww of PFDA in zooplankton samples collected in the Baltic Proper. When our results are converted into ng g^{-1} ww according to Kiørboe (2013), who calculated that the dry weight of crustacean zooplankton would be 15–25% of the wet weight, the average concentrations in *L. macrurus* from the Bothnian Sea would be at maximum 0.38 ng g^{-1} ww for PFOA and 0.43 ng g^{-1} ww for PFNA. Similarly, the PFOA and PFNA concentrations in the Gulf of Finland zooplankton communities would be at maximum 0.57 and 0.66 ng g^{-1} ww, respectively. Thus, seemingly the PFAS concentrations at least for these compounds in mesozooplankton are higher in these northern Baltic Sea areas compared with the Baltic Proper zooplankton.

Trophic magnification of PFAS

Comparison between trophic levels (mysid shrimps and their mesozooplankton prey) in the Bothnian Sea samples shows significantly higher total concentrations of PFAS in the macrozooplankton component (mysid shrimps) compared with mesozooplankton (*L. macrurus*) (Mann-Whitney U-test: z: 528,5; p = 0,001). The most abundant PFAS compounds in mysids were the long-chain PFNA (13.04 ± 2.3 ng g^{-1} dw), PFOS (8.14 ± 1.8 ng g^{-1} dw), PFOA (2.24 ± 0.7 ng g^{-1} dw) and PFDA (1.98 ± 0.2 ng g^{-1} dw) (Fig. 2., Table S1 in SI). The higher prevalence of PFDA in mysids which are at the higher trophic level (Kiljunen *et al.* 2020) compared with *L. macrurus* based on stable isotopes may be attributed to the particularly high trophic magnification factor (TMF) of PFDA (Miranda *et al.* 2022). The concentrations of PFOA, on the other hand, are fairly similar within the studied taxa, which supports findings of several earlier studies showing no biomagnification or even biodilution of PFOA along the food chain (Du *et al.* 2021, Munoz *et al.* 2022, Fremlin *et al.* 2023, Ren *et al.* 2023). This might be due to higher water-solubility of PFOA making it potentially less bioaccumulative (Miranda *et al.* 2022).

The long-chain compounds, such as PFUnA and perfluorododecanoic acid (PFDoA), have been shown to be predominant in sediments, whereas shorter-chain PFAS are commonly found in surface waters (Xu *et al.* 2014, Goodrow *et al.* 2020). Consistently, PFDoA was only found in mysids, and also PFUnA was present in all mysids but only in one *L. macrurus* sample (station 52/H0-1, Fig. 2). Similarly, PFNA and PFOS concentrations were considerably higher in mysids compared with *L. macrurus*, and while these compounds have high TMFs (Miranda *et al.* 2022), their higher concentrations in mysids may have also been affected by the presence of the substances in the sediments. The solubility of contaminants and following presence in water or accumulation in sediments is of utmost importance in order to understand the differences in the accumulation of substances in different groups of organisms as shown with benthic and pelagic invertebrates in the Arctic (Sørensen *et al.* 2023).

Interestingly, the most abundant PFAS compounds detected in mysids correspond to the most abundant PFAS compounds found in the surface sediments (0–2 cm) of the Bothnian Sea offshore areas (KERTY database, Finnish Environment Institute). At the sampling stations US5B 1, and SR5, (which are the closest PFAS monitoring stations for sediments to the sampling stations of this study) PFOS, PFNA, and PFOA dominate the PFAS compound profile, and have been high during the past 5–10 years (KERTY database). Similarly, in the offshore area of the Bothnian Sea PFDA, PFOA, and PFNA have been observed to be most abundant also in the surface sediments closer to the Swedish coast (Niarchos *et al.* 2024).

Conclusions

The similarity of the most abundant PFAS compounds in mysids and sediments are likely explained by the vertical migration behavior of mysids occurring and feeding at the sea floor

during daytime where they are exposed to PFAS in the surface sediments and in sediment dwelling prey. However, since mysid shrimps in the northern Baltic Sea are known to also feed on mesozooplankton in the pelagial (Viherluoto *et al.* 2000, Kiljunen *et al.* 2020), biomagnification of PFAS to mysid shrimps through predation on zooplankton may also play a role as shown in a freshwater food web in Lake Taihu from zooplankton to shrimps and fish (Xu *et al.* 2014). The dominant PFAS compounds observed in the crustacean taxa sampled in this study seem to correspond to the dominant PFAS compounds in their predators, e.g. herring, smelt and sprat (Kumar *et al.* 2022). Our results provide information on the potential importance of food sources in PFAS biomagnification to certain fish species in the Baltic Sea. As Suomi *et al.* (2024) hypothesized the higher PFAS concentrations in the Bothnian Sea herring and smelt compared to other sampled fish species could be attributed to their diet. The primary food source of large herring and smelt, bentho-pelagic mysid shrimps (Kiljunen *et al.* 2020), could serve as an important link. This is supported by the findings from Lake Erie food web where yellow perch and white perch feeding on both pelagic and benthic prey were shown to have higher PFAS concentrations compared with planktivorous fish (Ren *et al.* 2023). However, there are also other exposure pathways influencing the bioaccumulation and biomagnification of PFAS compounds in fish, e.g., direct uptake from water, wherefore more studies would be needed to verify the main PFAS sources for the observed high concentrations in Baltic herring and smelt.

Acknowledgements: The authors would like to thank Siru Tasala from the Syke Marine Ecology Research Laboratory for identifying the most common taxa in the mesozooplankton community sampled from the Gulf of Finland and Natural Resources Institute Finland (Luke) for access to the BIAS-trawling cruise for sampling. The study utilised R/V Aranda and Syke Marine Ecology Research Laboratory infrastructures as a part of the national FINMARI RI consortium. This research was funded by the Research Council of Finland (PLASTER #3425540). Funder did not have any role in the study design, collection, analysis and interpretation of data, writing of the report and decision to submit the article for publication. Three referees are acknowledged for their valuable comments on the ms.

Supplementary Information: The supplementary information related to this article is available online at: https://doi.org/10.60910/ber2026.0y8p-3e73

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