UNIVERSITY OF LATVIA
FACULTY OF GEOGRAPHY AND EARTH SCIENCES
DEPARTMENT OF ENVIRONMENTAL SCIENCE

Natālija Suhareva

# TRANSFER OF HAZARDOUS SUBSTANCES THROUGH THE FOOD CHAIN IN COASTAL WATERS OF THE BALTIC SEA

## PIESĀRŅOJOŠO VIELU PĀRNESE BARĪBAS ĶĒDĒ BALTIJAS JŪRAS PIEKRASTES ŪDEŅOS

Doctoral Thesis

Riga, 2023

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DOCTORAL THESIS

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# ABSTRACT

Coastal ecosystems are under threat from persistent organic pollutants and heavy metals, which can accumulate in fish and cause harm to both human health and the environment. Unfortunately, despite various measures at the EU and global level to reduce the leakage of hazardous substances into the environment, the goals set to achieve pollution levels that do not harm the marine ecosystem have not yet been achieved. The proposed explanation was that the concentrations of some target substances have levelled off during the recent years due to their internal circulation in marine ecosystems. Another reason may be the ability of organisms to biotransport hazardous substances both vertically, by absorbing them from the ambient environment or with food and passing them up the food chain, and horizontally, by migrating to habitats in search of better feeding or shelter. Therefore, the aim of this research was to characterise the trophic transfer and other bio-transportation paths of priority hazardous substances, thus understanding the true sources of pollution registered in coastal waters of the Baltic Sea.

The thesis is based on three articles and one submitted manuscript devoted to bio-transportation of mercury, polybrominated diphenyl ethers and polychlorinated biphenyls in order to answer the research question in four principal aspects: (1) pollution transportation vectors from land-based sources to the Baltic Sea, (2) effect of diet composition on uptake of hazardous substances, (3) trophic magnification of hazardous substances in different feeding grounds (4) accumulation of hazardous substances in fish tissues.

The study revealed that concentrations of target pollutants measured in organisms with a high trophic position can be affected by feeding ecology. However, the high mobility of studied species transporting hazardous substances, trophically derived from other feeding grounds with a higher pollution load to the monitored areas, may have a stronger impact. Consequently, it is suggested to treat the sea coast, rich in lagoon-type lakes and river estuaries as a single interconnected ecosystem.



**Keywords:** pollution, hazardous substances, bio-transportation, feeding ecology in a coastal ecosystem, pollution trophic transfer, pollution accumulation, the Baltic Sea, the Eastern Gotland Basin, the Gulf of Riga, mercury, polychlorinated biphenyls, polybrominated diphenyl ethers, European perch.

# CONTENTS

**ABSTRACT** ..... 3

**ABBREVIATIONS** ..... 5

**INTRODUCTION** ..... 6

**1. LITERATURE REVIEW** ..... 12
1.1. Study area ..... 12
1.2. Model organism ..... 14
1.3. Overview of the status of hazardous substances considered in the study ..... 15

**2. MATERIALS AND METHODS** ..... 22
2.1. Sampling and pre-treatment ..... 22
2.2. Laboratory analyses ..... 24
2.3. Data analysis and models ..... 27

**3. RESULTS AND DISCUSSION** ..... 30
3.1. Transfer of HS from land-based sources to the coastal waters of the Baltic Sea ..... 30
3.2. Effect of diet composition on uptake of HS ..... 35
3.3. Trophic magnification in different feeding grounds ..... 44
3.4. Accumulation of HS in fish tissues and factors affecting it ..... 46

**4. CONCLUSIONS** ..... 52

**ACKNOWLEDGEMENTS** ..... 54

**REFERENCES** ..... 55

**APPENDICES** ..... 65
**APPENDIX A**
**ARTICLE I** ..... 65
**APPENDIX B**
**ARTICLE II** ..... 83
**APPENDIX C**
**ARTICLE III** ..... 99
**APPENDIX D**
**MANUSCRIPT IV** ..... 115

# ABBREVIATIONS

| Abbreviation | Meaning |
|---|---|
| **C** | Carbon |
| **Cu** | Copper |
| **DW** | Dry weight |
| **EQS** | Environmental Quality Standard |
| **FK** | Fulton's condition factor |
| **GAM** | Generalised additive model |
| **HELCOM** | The Baltic Marine Environment Protection Commission |
| **Hg** | Mercury |
| **HS** | Hazardous substances |
| **LIAE** | Latvian Institute of Aquatic Ecology |
| **LW** | Lipid weight |
| **N** | Nitrogen |
| **(P)BDE** | (Poly)brominated diphenyl ethers |
| **PCA** | Principal Component Analysis |
| **(P)CB** | (Poly)chlorinated biphenyls |
| **THg** | Total mercury |
| **TL** | Trophic level |
| **TMF** | Trophic magnification factor |
| **TMS** | Trophic magnification slope |
| **WW** | Wet weight |
| **WWTP** | Wastewater treatment plant |
| **Zn** | Zinc |

# INTRODUCTION

The Baltic Sea is highly exposed to many anthropogenic pressures due to a wide range of land-based industrial, agricultural and urban activities, as well as intensive shipping and overfishing. Due to the semi-enclosed shape of the Baltic Sea, and the limited level of water exchange (Håkanson et al., 2003), the named pressures have greater implications. Furthermore, the Baltic Sea is one of the world's largest brackish water reservoirs, resulting in complex biogeochemical interactions (Nekoro, 2013).

Hazardous substances (HS) have been acknowledged as a threat to environmental integrity since the 1970s. Due to this threat, the European Union adopted Directive 76/464/EEC on pollution caused by certain dangerous substances discharged into the aquatic environment (EC, 1976). Some HS (or substance groups) have been included in the list of priority substances – those that present a significant risk to the aquatic environment, and in the list of priority hazardous substances – those which are toxic, persistent, and likely to bioaccumulate (EC, 2013, 2008a, 2000). Also, it is likely that the list of priority substances will be expanded to include contaminants of emerging concern, such as some pharmaceuticals (HELCOM, 2018a). Besides that, the Baltic Marine Environment Protection Commission (HELCOM) has been addressing this issue for almost five decades, so pollution by HS is one of four segments currently targeted by the Baltic Sea Action Plan (BSAP) (HELCOM, 2021). The subject of their interaction with the ecosystem, like sedimentation (Ogrinc et al., 2019; Pazikowska-Sapota, 2006), remobilization (Josefsson et al., 2010), absorption directly from the ambient environment or bioconcentration (van der Velden et al., 2013), bioaccumulation (Borgå, 2013) and biomagnification (Drouillard, 2008), has become an increasingly relevant topic in the last decade (EC, 2019; HELCOM, 2021). Even though various measures have been taken to reduce HS leakage into the environment (EC, 2019, 2013, 2008a, 2008b, 2000; UNEP, 2001) the set environmental goals have not been met yet (HELCOM, 2018b). The conventional explanation would be that concentrations of some target substances have been levelled off in recent years due to their internal circulation within marine ecosystems(European Environment Agency, 2017; HELCOM, 2018a; McLachlan and Undeman, 2020). Slow biodegradability of substances, such as PCBs (McLachlan and Undeman, 2020), is often cited as an explanation for their persistence in the environment even after anthropogenic emissions have been reduced or terminated. Similarly, remobilization of PBDEs by bioturbation (Josefsson et al., 2010) or during storm events (O'Driscoll et al., 2016) from sediments has been referred to as an important secondary pollution source. Nonetheless, the reason may also be attributed to anthropogenic sources that cannot be easily reduced, such as ammunition dumpsites, which have been proven to be a permanent source of mercury (Siedlewicz et al., 2020). Another reason may be the ability of organisms to biotransport hazardous substances both vertically, by absorbing them from the ambient environment or with food and passing them up the food chain, and horizontally, by migrating to habitats in search of better feeding or shelter (Couture and Pyle, 2015). There are many river estuaries and coastal lagoon-type lakes along the Latvian coast of the Baltic Sea. Although individual lakes are generally considered isolated units in ecological research (EC, 2000; UN Environment, 2018), short-distance fish migrations frequently occur between interconnected basins

(Daniels et al., 2008; Landsman et al., 2011; Pfauserová et al., 2022; Schmidt et al., 2020). Therefore, fish mobility between feeding grounds can serve as a vector for the transport of materials (including pollution) within interconnected aquatic environments.

## Motivation and objectives

This study is **motivated** by the lack of knowledge and data regarding the nature of secondary sources of pollution and their interaction with the environment, in the study region. Therefore, this dissertation presents a wide range study exploring concentrations of three groups of HS in aquatic organisms, collected in the Gulf of Riga and the Eastern Gotland Basin of the Baltic Sea. The organisms occupy a variety of trophic positions, including several fish species, crustaceans (amphipods, mysids, other shrimps), bivalves and others.

The main **research questions** studied in the thesis were related to the fate of HS in the coastal food chain of the Baltic Sea, and processes which regulate availability of these substances for trophic transfer and therefore the observed levels in fish of high trophic position. It has been **hypothesized** that, in addition to the pollution load, concentrations of hazardous substances measured in the tissues of fish from high trophic positions may be influenced by the diet in specific feeding grounds and, therefore, the composition of the food chain as a whole. Understanding the measured concentration trends and trophic transfer mechanisms can help to distinguish pollution transfer paths hidden in the ecosystem until now. Thus, it can provide a solid foundation for improvement of monitoring procedures, assessment of pollution levels and advanced comparison of the assessed sites. Therefore, the **goal** of this dissertation was to characterise the trophic transfer and other biotransportation paths of priority hazardous substances in Latvian waters of the Baltic Sea.

To address the research question and reach the set goal, concentrations of Hg, PBDEs and PCBs were measured in tissues of European perch (*Perca fluviatilis*), selected as a model organism, as well as in other organisms of the food chain.

The thesis includes the following **tasks**:

1. To explore HS transportation vectors from land-based sources to the coastal waters of the Baltic Sea;
2. To study the effect of dietary composition on the uptake of HS;
3. To investigate trophic magnification of HS in different feeding grounds;
4. To describe accumulation of HS in fish tissues and assign possible factors affecting it.

## Novelty of the research

Until the study presented in this thesis, no publication or widespread study has been conducted on the concentration of HS in Latvian coastal waters of the Baltic Sea, except the measurement of heavy metals (including mercury) within the framework of the state monitoring program performed by the Latvian Institute for Aquatic Ecology (LIAE). In this thesis, in addition to levels of regionally important priority hazardous substances (Hg, PBDE, PCB) measured in biota, accumulation, trophic magnification and biotransportation of these substances between interconnected reservoirs is discussed as well.

An innovative technique has been developed to normalize contaminant concentrations to age/length of interest, which can be used to offset the accumulation effect and thus increase the number of individuals used for site comparison and pollution monitoring.

In this work, for the first time in the region, a significant amount of data on the signatures of stable carbon and nitrogen isotopes was obtained for three spatially distant marine populations and two freshwater populations of perch, as well as for a wide range of prey organisms (fish, crustaceans, polychaetes, bivalves), relevant and necessary for current and further studies of trophic interactions.

This thesis provides substantial data on feeding ecology and dietary shifts of European perch in the study region, including marine and freshwater habitats.

## Practical applicability and significance of the research

The study brings an important added value to the following contamination assessment and holistic assessment of ecosystem health (HELCOM, 2018b). Also, the results provide important information necessary for improving the procedures for state monitoring of pollution levels and the national assessment of the environmental quality of the Baltic Sea, as well as to enhance environmental management policies.

## Structure of the thesis

The present thesis is based on 3 peer-reviewed articles and one manuscript submitted to a peer-review journal. The articles and the manuscript are devoted to a biotransportation of HS in four principal aspects: domestic pollution and emissions, as well as environmental concentrations (**Article I**), accumulation of pollution with age in different fish species inhabiting the Baltic Sea (**Article II**), bio-transportation of HS from polluted freshwater ecosystems to the marine coast via fish migration (**Article III**), trophic magnification in coastal waters of the Baltic Sea (**Manuscript IV**). The interrelation among the articles and investigated research questions is represented in **Table 1.1**.

*Table 1.1*

**Overview of the topics described in the related scientific papers**

| Task | Scientific article | Hazardous substances have been discussed | | |
|---|---|---|---|---|
| | | Hg | PBDE | PCB |
| Transfer of HS from land-based sources to the coastal waters of the Baltic Sea | I | | | |
| | III | | | |
| | IV | | | |
| Effect of diet composition on uptake of HS | III | | | |
| | IV | | | |
| Trophic magnification of HS in different feeding grounds | IV | | | |
| Accumulation of HS in fish tissues and factors affecting it | II | | | |
| | III | | | |

The structure of this thesis includes an INTRODUCTION, four main chapters and appendices. The BACKGROUND part describes the keystone knowledge about the study area and its habitat characteristics, ecology of the model organism and the overview about of priority HS discussed in this thesis. It is followed by the MATERIALS AND METHODS part, where are shortly described sampling methods and laboratory analyses used, as well as specification of data analysis methods. The RESULTS AND DISCUSSION part encloses the main discoveries of the research, which are described in detail in the enclosed scientific articles (**Appendices A, B, C, D**), compares them with other studies presented in the field, and discusses limitations recognized in the process. CONCLUSION gives a short summary regarding reached goals set for this thesis and gives an outlook on the perspectives developed during the research.

The thesis consists of 64 pages and is supplemented with 24 figures, 7 table and 4 appendices (A, B, C, D).

## Author's contribution

N. Suhareva contributed in conceptualization and structuring of the study, did entire data analysis and developed data transformation techniques used in the research, participated in sampling campaigns, majority of sample pre-treatment events and investigation of ecological features of the studied species. She is a main author for the majority of presented papers, under supervision of *Dr.geogr.* Juris Aigars. Detailed author's contribution in preparation of the scientific papers is listed below:

**Article I:** Conceptualization 10%; Methodology 10%; Experimental work 10%; Data analysis 100%; Writing (original draft preparation, review, editing) 40%; Visualization 100%.

**Article II:** Conceptualization 50%; Methodology 70%; Experimental work 10%; Data analysis 100%; Writing (original draft preparation, review, editing) 60%; Visualization 100%.

**Article III:** Conceptualization 100%; Methodology 80%; Experimental work 50%; Data analysis 100%; Writing (original draft preparation, review, editing) 60%; Visualization 100%.

**Manuscript IV:** Conceptualization 100%; Methodology 80%; Experimental work 50%; Data analysis 100%; Writing (original draft preparation, review, editing) 60%; Visualization 100%.

## Approbation of the results

The results of the study are published in 3 articles included in the Web of Science and/or Scopus database, manuscript the fourth article has been submitted to peer-review journal. The results of the research work were reported at 5 international conferences and 3 national conferences. The research results have been partly published in a book about the studies carried out during the research program VPP EVIDEnT - The value and dynamic of Latvia's ecosystems under changing climate: "Latvijas ekosistēmu dinamika klimata ietekmē", redacted by Juris Aigars. The gained knowledge was used to prepare a research project proposal for The Open Tender for 2022 Fundamental and Applied Research Projects announced by the Latvian Council of Science.

## Scientific publications related to the thesis

**Article I:** Aigars, J., **Suhareva, N**., Poikane, R. (2017) Distribution of Polybrominated Diphenyl Ethers in Sewage Sludge, Sediments, and Fish from Latvia. *Environments*, 4(1). https://doi.org/10.3390/environments4010012.

**Article II: Suhareva, N**., Aigars, J., Poikane, R., Jansons, M. 2020. Development of fish age normalization technique for pollution assessment of marine ecosystem, based on concentrations of mercury, copper, and zinc in dorsal muscles of fish. *Environmental Monitoring and Assessment* 192(5). https://doi.org/10.1007/s10661-020-08261-x.

**Article III: Suhareva, N.**, Aigars, J., Poikane, R., Tunens, J. (2021) The influence of feeding ecology and location on total mercury concentrations in Eurasian perch (Perca fluviatilis). *Environmental Sciences Europe*, 33(1), 82. https://doi.org/10.1186/s12302-021-00523-w.

**Manuscript IV: Suhareva, N**., Aigars, J., Poikāne, R., Heredia, N.A., Tunēns, J., Baraškova, Ļ. The trophic transfer of hazardous substances from the perspective of feeding ecology.

## Other scientific publications

Barone, M., **Suhareva, N.**, Aigars, J., Putna-Nimane, I., Dimante-Deimantovica, I. (2023) Dataset on microplastic concentrations, characteristics, and chemical composition in the marine surface waters of Latvia – the Eastern Gotland basin and the Gulf of Riga. *Data in Brief*, 47. https://doi.org/10.1016/j.dib.2023.108992.

Strode, E., Barda, I., **Suhareva, N.**, Kolesova, N., Turja, R., Lehtonen, K. K. (2023) Influence of Environmental Variables on Biochemical Biomarkers in the Amphipod *Monoporeia affinis* from the Gulf of Riga (Baltic Sea). Water, 15(2):248. https://doi.org/10.3390/w15020248.

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Dimante-Deimantovica, I., **Suhareva, N**., Barone, M., Putna-Nimane, I., Aigars, J. (2022) Hide-and-seek: Threshold values and contribution towards better understanding of recovery rate in microplastic research. MethodsX, 9, 101603. https://doi.org/10.1016/j.mex.2021.101603.

Aigars, J., Barone, M., **Suhareva, N**., Putna-Nimane, I., Deimantovica-Dimante, I. (2021) Occurrence and spatial distribution of microplastics in the surface waters of the Baltic Sea and the Gulf of Riga. Marine Pollution Bulletin, 172, 112860. https://doi.org/10.1016/j.marpolbul.2021.112860.

Damanik-Ambarita, M. N., Boets, P., Nguyen, T. H. T., Eurie Forio M. A., Everaert, G., Lock, K., Musonge, P. S., **Suhareva, N**., Bennetsen, E., Gobeyn, S., Ho, L., Dominguez-Granda, L., Goethals, L. M. P. (2018) Impact assessment of local land use on ecological water quality of the Guayas river basin (Ecuador). Ecological Informatics, 48: 226–237. https://doi.org/10.1016/j.ecoinf.2018.08.009.

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Damanik-Ambarita, M. N., Lock, K., Boets, P., Everaert, G., Nguyen, T. H. T., Eurie Forio M. A., Musonge, P. S., **Suhareva, N**., Bennetsen, E., Landuyta, D., Dominguez-Granda, L., Goethals, L. M. P. (2016) Ecological water quality analysis of the Guayas river basin (Ecuador) based on macroinvertebrates indices. Limnologica, 57: 27–59. https://doi.org/10.1016/j.limno.2016.01.001.

Damanik-Ambarita, M. N., Everaert, G., Eurie Forio M. A., Nguyen, T. H. T., Lock, K., Musonge, P. S., **Suhareva, N**., Dominguez-Granda, L., Bennetsen, E., Boets, P., Goethals, L. M. P. (2016) Generalized linear models to identify key hydromorphological and chemical variables determining the occurrence of macroinvertebrates in the Guayas river basin (Ecuador). Water, 8(7). https://doi.org/10.3390/w8070297.

### Reports presented in international conferences

**Suhareva, N.,** Aigars, J., Poikane, R., Tunens, J. 2022. The influence of feeding ecology and location on total mercury concentrations in European perch (Perca fluviatilis) from the Baltic Sea and the Gulf of Riga. *4th ICES/PICES Early Career Scientist Conference 2022 "Ocean sciences for the future we want"*, St. John's, Newfoundland, Canada.

**Suhareva, N.,** Aigars, J., Poikane, R., Jansons. 2020. Development of fish age - metal concentration technique for assessment of pollution level in the Baltic sea and the Gulf of Riga. *The 78th International Conference of The University of Latvia.*

**Suhareva, N.,** Aigars, J., Poikane. 2017. Differences in variations of heavy metal concentrations in muscle tissues of representative fish species of the Baltic Sea and the Gulf of Riga. *3rd ICES/PICES Early Career Scientist Conference 2017 "Climate, Oceans and Society: Challenges and Opportunities"*, Busan, South Korea.

**Suhareva, N.,** Aigars, J., Poikane. Polybrominated diphenyl ethers – distribution and source tracking. *8th International scientific conference of Daugavpils University*, Daugavpils, Latvia.

**Suhareva, N.,** Aigars, J., Poikane, R., Jansons. 2016. Variation of mercury concentrations in different fish species of the gulf of Riga and the Baltic sea. *18th International conference on heavy metals in the environment ICHMET*, Ghent, Belgium.

***Reports presented in national conferences***

**Suhareva, N.,** Aigars, J., Poikane. 2022. Piesārņojošas vielas un savienojumi Baltijas jūrā, to ietekme uz dzīviem organismiem, sastopamība pārtikā. Jūras diena 2022. *Jūras diena 2022. Zināšanu uzlabošana jūras vides aizsardzībai*, Vides aizsardzības un reģionālās attīstības ministrija, Rīga, Latvija.

**Suhareva, N.,** Aigars, J., Poikane. 2017. Concentrations of heavy metals in representative fish species of the Gulf of Riga and the Baltic sea. *75th Conference of University of Latvia*, Riga, Latvia.

Tunēns, J., **Suhareva, N**., Poikāne, R. 2017. Baltijas jūras un Rīgas līča barības tīklu trofisko pozīciju noteikšana, izmantojot stabilo izotopu attiecību analīzi. *VPP EVIDEnT 3. zinātniskā konference*, Rīga, Latvija.

# 1. LITERATURE REVIEW

## 1.1. Study area

The **Baltic Sea** (**Figure 1.1**) is an epicontinental shallow sea, water exchange with the North Sea and Atlantic Ocean is slow and limited by narrow connection via Danish straits (Håkanson et al., 2003). The sea is nutrient rich which is due to both post-glacial land uplift (Håkanson et al., 2003) and extensive discharge from agricultural areas in the surrounding countries (HELCOM, 2018b, 2010a). It is confirmed that the basin drainage area is under serious anthropogenic stress (HELCOM, 2021) due to the dense population of 90 million people and intense industrialization (Nekoro, 2013). Water salinity in the sampling area of the Baltic Sea (the **Eastern Gotland Basin**) is approximately 7 PSU (Feistel et al., 2010). The **Gulf of Riga** is a semi-enclosed bay connected to the central Baltic Sea via Irbe Strait. Water salinity in the gulf varies between 4 and 7 PSU (practical salinity unit) with even lower salinity levels in the river estuaries (Yurkovskis et al., 1993).


*Figure 1.1.* **Map of the Baltic Sea and its catchment area** (Szymczycha et al., 2019)[a]

[a] Reprinted from "*World Seas: an Environmental Evaluation*" by Szymczycha, B., Zaborska, A., Bełdowski, J., Kuliński, K., Beszczyńska-Möller, A., Kędra, M., Pempkowiak, J. "*Chapter 4 The Baltic Sea*", Page No. 86, Copyright (2019), with permission from Elsevier; permission conveyed through Copyright Clearance Center, Inc., License Nr. 5558680343532.

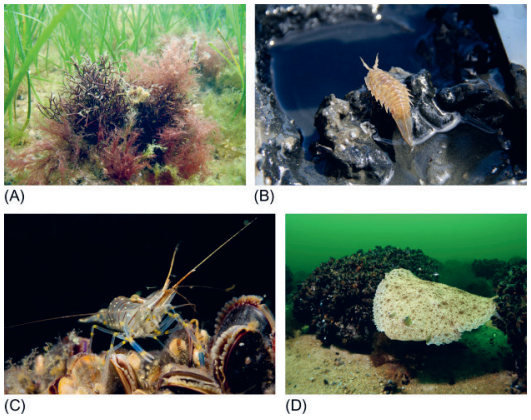


*Figure 1.2.* **Selected examples of the Baltic Sea organisms; A: Furcellaria lumbricalis. Photo: Piotr Bałazy; B: Saduria entomon. Photo: Kajetan Deja; C: Palaemon adspersus and Mytilus edulis. Photo: Piotr Bałazy; D: Scophthalmus maximus. Photo: Piotr Bałazy** (Szymczycha et al., 2019)[b]

There is a wide range of benthic habitats in both areas, ranging from species-rich macroalgae colonies in shallow areas to soft bottom fauna in deeper areas (HELCOM, 2018b). The biological community (**Figure 1.2**) is primarily composed of brown and red seaweeds (on hard substrates), primarily *Fucus vesiculosus* in the Gulf and *Furcellaria lumbricalis* in the sea (HELCOM, 2018b). Depending upon the substrate, polychaete worms and molluscs occupy the benthic habitats, including bivalves the Baltic clam *Macoma balthica* and the blue mussel *Mytilus trossulus*. Among the other species found in the study areas are amphipods (mainly *Monoporeia affinis*), isopods (*Saduria entomon*), mysids, and decapods (*Palaemon adspersus*, *Crangon crangon*). Some of the above organisms are important food sources for benthic fish species, such as turbot *Scophthalmus maximus*, flounder *Platichthys flesus* and round goby *Neogobius melanostomus* (Ustups et al., 2016). Nevertheless, the most widespread predatory fish is the bentho-pelagic fresh-water species *Perca fluviatilis* or European perch (HELCOM, 2012).

The pelagic community is relatively poor, it consists of different phytoplankton species (primary producers), several primary consumers or zooplankton, in particular with a predominance of copepods (e.g. *Acartia* sp, *Pseudocalanus* sp, *Temora* sp), cladocerans and rotifers. Several species of pelagic planktivorous fish depend on zooplankton crustaceans, including sprats (*Sprattus sprattus*) and herring (*Clupea harangus*). Predatory fish (*Gadus morhua*) and top predators, such as seabirds and seals, occupy the highest trophic positions (Håkanson et al., 2003; HELCOM, 2010a).

There are numerous fresh water reservoirs along the Latvian coast, such as estuaries and lagoon-type lakes (Håkanson et al., 2003), which are naturally connected to the coast. Previous studies intensively discussed input of nutrients (Grimvall and

[b] Reprinted from "*World Seas: an Environmental Evaluation*" by Szymczycha, B., Zaborska, A., Bełdowski, J., Kuliński, K., Beszczyńska-Möller, A., Kędra, M., Pempkowiak, J. "*Chapter 4 The Baltic Sea*", Page No. 94, Copyright (2019), with permission from Elsevier; permission conveyed through Copyright Clearance Center, Inc., License Nr. 5558680343532.

Stålnacke, 2001; HELCOM, 2018c; Kuliński et al., 2022) and pollution into the Baltic Sea carried by the riverine inflow (HELCOM, 2021; Kanwischer et al., 2022). However, in such complex interconnected ecosystems, transfer of energy and biomass may also occur through bio-transport by aquatic organisms (Brink et al., 2018). Consequently, contaminants can be transferred from inland freshwater to the coast during fish migration to spawn or reaching marine feeding grounds (Blais et al., 2007; Gerig et al., 2020). The freshwater reservoirs connected to the Eastern Gotland Basin and the Gulf of Riga considered in this study were River Daugava, Lake Ķīšezers and lake Liepājas.

## 1.2. Model organism

Representative fish species of the Baltic Sea were used for different sections of this study, particularly **European perch** (*Perca fluviatilis*), Baltic cod (*Gadus morhua callarias*) and Baltic herring (*Clupea harengus membras*), flounder (*Platichthys flesus*) and round goby (*Neogobius melanostomus*) etc. Although European perch (hereinafter referred to as perch) was selected as the main model organism throughout the study of this thesis. Perch is freshwater species widespread in Latvian freshwater and coastal ecosystems (and elsewhere in Europe and Asia). In both running and standing freshwaters, it is usually a major component of fish communities (**Figure 1.3**), as it is tolerant of high variations of environmental conditions, such as temperature and salinity (Couture and Pyle, 2015).

They may undertake short spawning migrations (Kottelat and Freyhof, 2007), on average 20 km and a maximum observed of 180 km (Järv, 2000). The mobility of adult perch is driven by prey availability as well as physicochemical factors of the surrounding environment (Radabaugh et al., 2010).

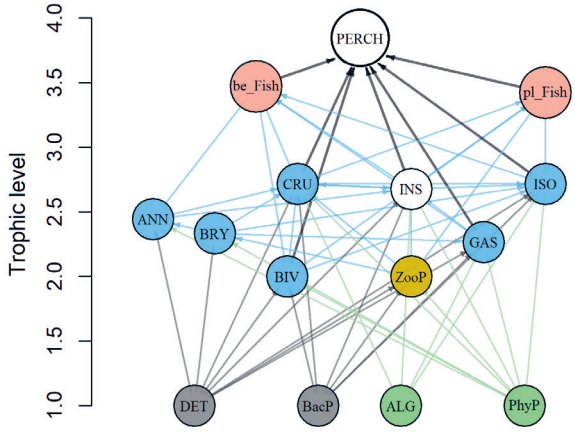


*Figure 1.3.* **Schematic representation of the coastal food web with the European perch (Perca fluviatilis) at the highest trophic position. Elements of the food web are colored according to their trophic guild affiliation, where: DET – Detritus, BacP – Bacteria, ALG – Algae, PhyP – Phytoplankton, ANN – Annelida, BRY – Bryozoa, BIV – Bivalvia, ZooP – Zooplankton, GAS – Gastropoda, CRU – Crustacea, INS – Insecta, ISO – Isopoda, be_Fish – Benthivorous fish, pl_Fish – Planktivorous fish** (by A. Labuce)

In areas with low vegetation cover and limited resources, perch tend to migrate a longer distance than they would in their preferred habitat (Olsson et al., 2007), in exchange for higher metabolic costs (Couture and Pyle, 2015). This species reach maturity between 1–7 years old and 6–18 cm long, and spawns between April and June (in Latvia specifically) (Plikšs and Aleksejevs, 1998). Perch is an opportunistic diurnal feeder that exploits any available prey. Small juveniles and larvae feed primarily on planktonic invertebrates. During the first year, juveniles move close to shores to feed on benthic prey, and larger specimens normally switch to a piscivorous diet (Jacobson et al., 2019; Kottelat and Freyhof, 2007).

Due to their worldwide distribution, these species are ideally suited for scientific purposes ensuring easy comparison. There has been widespread use of perch as a biological matrix for pollution accumulation and ecotoxicology studies (Couture and Pyle, 2015). It has been recognized that the species can be used to monitor hazardous substances and characterise local and regional pollution (HELCOM, 2018a). However, in this and previous studies it was shown that perch's salt tolerance and the relatively low water salinity of the Eastern Gotland Basin and the Gulf of Riga allow the species to migrate from inland lagoon-type lakes and river estuaries to coastal waters (Suhareva et al., 2021). This raises concerns regarding the adequacy of monitoring data and the representativeness of HS levels in specific locations.

## 1.3. Overview of the status of hazardous substances considered in the study

### 1.3.1. Background information

The marine environment in the Baltic Sea faces a significant threat from contamination. The ecosystem continues to be affected by hazardous substances, as evident from the integrated assessment, with notable levels of contamination observed for mercury, polybrominated diphenyl ethers, and cesium-137 (HELCOM, 2018b). Several open sea areas, including Kiel Bay, Eastern Gotland Basin, and Bothnian Bay, stand out with the highest contamination levels among those evaluated. However, areas showing comparatively better conditions are associated with lower confidence in the assessment data (**Figure 1.4**). It is worth noting that the overall contamination status is strongly influenced by the "biota" matrix, which is consistently identified as the most severely affected component (HELCOM, 2018b).

The origin of the pollution in the Baltic Sea can be categorized into point sources, land-based diffuse sources, and atmospheric deposition (**Figure 1.5**). Point sources near the coast and within the catchment area have historically been significant contributors of heavy metals and persistent organic pollutants (POPs) to the Baltic Sea's surface waters (HELCOM, 2004).

A range of contaminants originate from minor industrial sources, agricultural activities involving pesticides, pharmaceuticals, and fertilizers, household use of consumer products, as well as practices such as sludge disposal, dump sites, and waste deposition in landfills. Diffuse emissions, often carried by stormwater and sewage, make their way into the sea. Hazardous substances from atmospheric emissions, including those from traffic, shipping, energy production, waste incineration, and even small-scale household combustion, are significant contributors. These substances disperse throughout the marine environment after being deposited on the sea surface *(HELCOM, 2010b).*

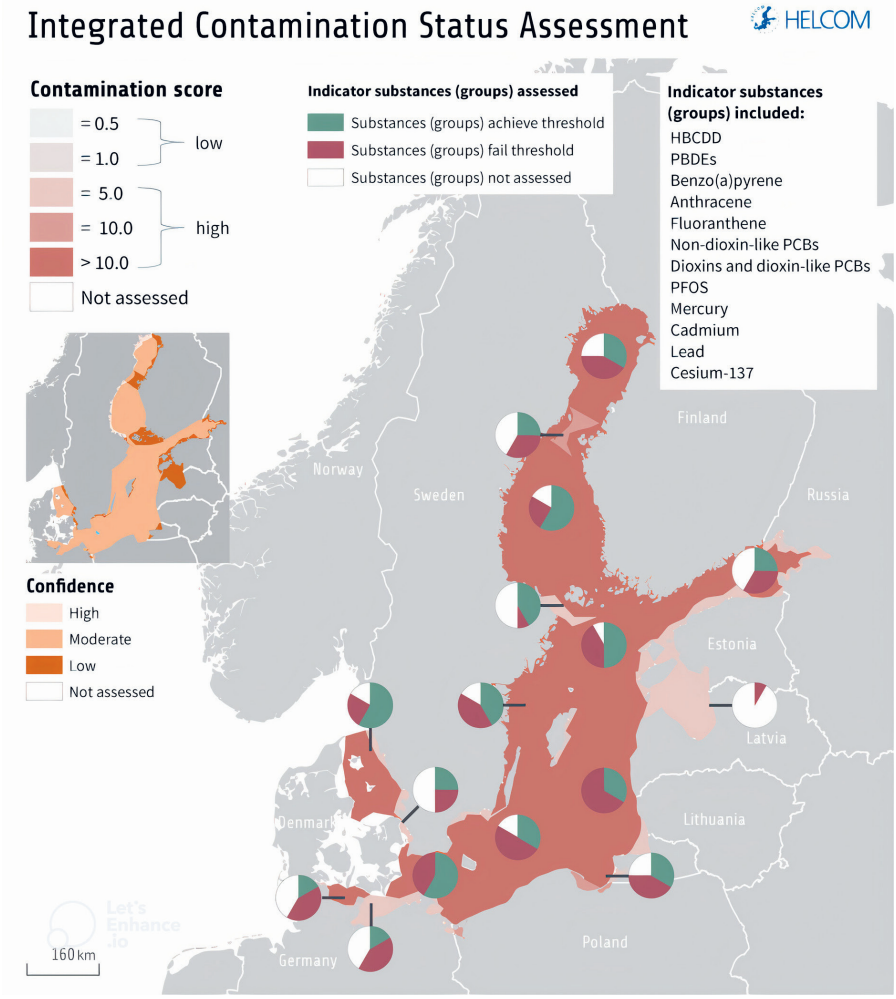


*Figure 1.4.* **The integrated contamination status of the Baltic Sea assessed using the CHASE tool, presented in the latest thematic assessment of hazardous substances (2011–2016)** (HELCOM, 2018b)

In this thesis, concentrations of mercury (THg – total mercury), polybrominated diphenyl ethers (PBDE) and polychlorinated biphenyls (PCB) were measured and investigated. The list of 13 priority hazardous substances and substance groups was first included in the Water Framework Directive 2000/60/EC (EC, 2000) and Directive 2008/105/EC (EC, 2008a) on **environmental quality standards (EQS)**. Later the list was extended up to 21 substances and substance groups in Directive 2013/39/EU (EC, 2013) amending the previous two. Today the problem is also addressed in the Baltic Sea Action Plan – strategic programme of measures and actions for achieving good environmental status of the sea (HELCOM, 2021). According to the directives, chemical pollutants are defined as harmful for surface water if they pose a threat to the aquatic environment, with effects such as acute and chronic toxicity in aquatic organisms,

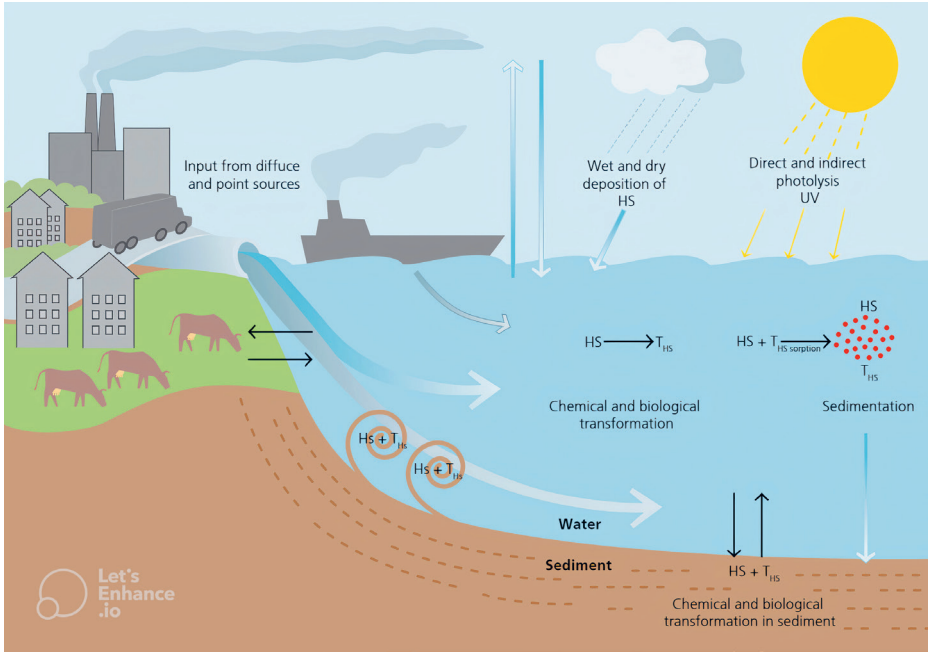


*Figure 1.5.* **Conceptual model of the sources of pollution inputs to the Baltic Sea marine environment and the fate of hazardous substances (HSs) and their transformation products (THS)** (HELCOM, 2010b)

accumulation in the ecosystem and loss of habitats and biodiversity, and also pose a threat to human health. As a matter of priority, causes of pollution should be identified and emissions of pollutants should be dealt with at their source in the most economically and environmentally effective manner (EC, 2000). **Priority hazardous substances** pose a significant risk to or through the aquatic environment at the Union level. Hg, PCBs and PBDEs are classified as persistent pollutants by the Stockholm Convention due to their persistence, ability to bioaccumulate in the environment and toxicity to humans and wildlife (UNEP, 2001).

### 1.3.2. General terminology of the processes

**Bioconcentration** refers to the capacity of an organism to accumulate a particular substance from its surrounding environment. This phenomenon is typically assessed using a quantitative measure known as the bioconcentration factor (BCF). The BCF is calculated by determining the ratio between the concentration of the substance within the organism and its concentration in the ambient environment (Peake et al., 2016). BCF can be calculated by the following equation (Wang, 2016):

$$BCF = \frac{C}{C_w}, \qquad \text{(a)}$$

where $C$ is the contaminant concentrations in the organisms ($\mu g \cdot kg^{-1}$) under equilibrium condition, $C_w$ is the contaminant concentration in the water ($\mu g \cdot L^{-1}$).

**Bioaccumulation** arises from an intricate equilibrium between the uptake and elimination mechanisms of chemicals within organisms. The degree of bioaccumulation directly influences the manifestation of toxic effects (Chojnacka and Mikulewicz, 2014). In order to quantify bioaccumulation, the Bioaccumulation factor (*BAF*) is calculated as a ratio between the tissue concentrations ($C_b$) and the environmental concentrations ($C_{env}$) (Borgå, 2013):

$$BAF = \frac{C_b}{C_{env}}, \tag{b}$$

**Biomagnification** denotes a circumstance in which the concentration of a chemical substance within an organism surpasses the concentration present in its food, primarily resulting from dietary exposure. The term "food web biomagnification" is employed to characterize the trophic enrichment of contaminants within food webs. It encompasses the progressive amplification of chemical concentrations as the trophic level of animals ascends (Drouillard, 2008). This phenomenon signifies the augmentation of chemical substances along the food chain, culminating in higher concentrations in organisms occupying higher trophic positions. Biomagnification factor (BMF) is the ratio of concentration in the organism (consumer) to the organism diet (Verma et al., 2023):

$$BMF = \frac{C_{consumer\ organism}}{C_{prey\ organism}}, \tag{c}$$

**Trophic Magnification Factors** (TMFs) have emerged as a robust approach to quantifying biomagnification, offering improved reliability. TMFs serve as an estimate of the average transfer of a chemical substance from the diet to consumers within food webs. Distinguishing themselves from biomagnification factors, which pertain to specific species and exhibit considerable variability across predator-prey pairings, TMFs provide a more comprehensive perspective. TMFs are determined by calculating the slope of a regression line that relates the concentration of the chemical substance to the trophic level of organisms within the food web. The trophic level of organisms can be ascertained using stable nitrogen isotope ratios (δ15N) (Borgå et al., 2012). By leveraging δ15N values, which serve as an indicator of an organism's position within the food chain, TMFs enable a quantitative assessment of biomagnification dynamics.

### 1.3.3. Mercury (Hg)

Mercury (Hg) is a toxic, highly bioaccumulative heavy metal that enters the Baltic Sea ecosystem both via atmospheric deposition and waterborne sources. Various forms of Hg exist, including elemental Hg, inorganic salts (relatively low toxicity), and organic forms (more toxic and bioaccumulative). Organisms in aquatic environments are highly exposed to dissolved methylmercury [$(CH3Hg)^+$] (MeHg), produced via biomethylation process (Barkay and Wagner-Döbler, 2005). Previous studies suggested that Hg is often absorbed from the ambient environment by organisms of low trophic levels, however it is easily transferred and biomagnified through a trophic chain through food. Furthermore, MeHg fraction in aquatic organisms increase from primary producers to fish (Wiener and Spry, 1996).

Wildlife and humans are particularly vulnerable to MeHg due to its neurotoxic properties, adverse effect on the genome, as well as due to the reduction of reproductive success resulting from maternally derived mercury at embryonic and larval stages (de Almeida Rodrigues et al., 2019).

Based on the latest assessment presented by (HELCOM, 2018a), Hg emissions into the Baltic Sea ranged between 4.8 and 5.0 tonnes per year between 2012 and 2014. There were 70% of inputs from atmospheric deposition, 26% from river runoff, and 4% from point sources, although point sources and river runoff were the leading sources in the previous decade (Gusev, 2009; Knuuttila, 2009). Air emissions from power plants and industries accounted for 71% of anthropogenic Hg emissions, while waterborne emissions mainly came from impurities released during the processing of materials (metallurgical activities, agriculture, etc).

HELCOM (2018a) reports that atmospheric mercury depositions to the Baltic Sea have decreased since the 1990s. While the legislation to reduce mercury input into the Baltic Sea is in place, according to the latest published assessment (HELCOM, 2018d), fish muscle concentrations exceeded the threshold level in almost all monitored sub-basins.

### 1.3.4. Polybrominated diphenyl ethers (PBDE)

A group of brominated flame retardants known as polybrominated diphenyl ethers (PBDEs) have been used in many materials including plastics, foams, and textiles since the 1970s (Abbasi, 2015). Production and use of PBDEs are currently restricted at the EU level as well as globally. There are 209 possible congeners, varying in degree of bromination (number of brome atoms), up to deca-BDEs. PBDEs are toxic, hydrophobic and persistent substances. It is likely that PBDEs emitted into the environment are transported to sediments and soil (Palm et al., 2002), especially those with a higher bromination level. Heavy PBDEs in the air are debrominated directly by photolysis (Schenker et al., 2008; Söderström et al., 2004). Debromination of deca-BDE is thought to produce 3-20% of lower-brominated PBDEs found in the environment (Schenker et al., 2008; Söderström et al., 2004; Undeman and Johansson, 2020). Biomagnification of PBDEs in food chains is commonly observed, although it declines with bromination degree (**Figure 1.6**) and dietary absorption efficiency decreases with hydrophobic properties (Kierkegaard, 2007; La Guardia et al., 2007).

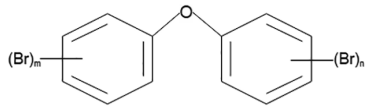


*Figure 1.6.* **The general molecular structure of polybrominated diphenyl ethers (PBDEs), where the congeners can have from 1 to 10 bromine atoms attached to the two phenyl rings connected by an ether bridge** (Ohoro et al., 2021)

The number of studies evaluating PBDE emissions is limited (Andersson, 2012; Björklund et al., 2012; Morf et al., 2008; Undeman and Johansson, 2020). Andersson (2012) estimated yearly penta-BDE emissions to European agricultural soil via sludge (400–1500 kg), to indoor air from PUR foam and electronic appliances (in total 9600 kg), to air from steel production (3-33 kg) and accidental fires in e-waste recycling facilities (0.2–500 kg), and to surface water from WWTP effluents (<1–216 kg). According to the model, atmospheric deposition contributed to 63-87% of total inland surface

water emissions in Europe (Andersson, 2012), indicating that air emissions are more important (>70%) than those from WWTP effluents (30%). Likewise, inland surface water emissions of 60–1800 kg, agricultural soil emissions of 3500–11000 kg, as well as atmospheric emissions of 6–150 kg deca-BDE were also estimated.

According to the latest assessment by (HELCOM, 2018b), all monitoring stations in the Baltic Sea exceed the Environmental Quality Standard (EQS) threshold value for biota (BDE 28, 47, 99, 100, 153 and 154). However, there are no exceedances of secondary thresholds set for sediment concentrations in the basins where sediment data are available (HELCOM, 2018b).

PBDE concentrations have been reported in herring muscle tissue since 2008 by the Swedish environmental monitoring program. They indicated that, in general, the concentration of the measured PBDEs decreased between 2008 and 2017, with a significant 6–10% annual decrease in concentration at 5 of the monitored sites (Hellström, 2016) for BDE 47 and BDE 99 (the predominant penta-BDEs). Similarly, they reported (Fredricsson et al., 2018) that atmospheric BDE 47, 99, 100 depositions at three Swedish monitoring sites declined between 2001 and 2017.

### 1.3.5. Polychlorinated biphenyls (PCB)

Polychlorinated biphenyls (PCBs) were used as insulating fluids in electrical transformers, capacitors in fluorescent light fixtures, plasticizers, flame retardants in building materials, and as combustion products. They were first manufactured in the 1930s and reached their peak production rates 40 years later (McLachlan and Undeman, 2020).

Based on chlorination degree and position (**Figure 1.7**), there are 209 possible congeners (McLachlan and Undeman, 2020). PCBs are persistent, toxic substances (some congeners have dioxin-like toxicity) with lipophilic and hydrophobic properties that tend to increase with the chlorination degree. By disrupting the endocrine, reproductive, and immune systems, PCBs pose an important threat to marine environments and humans, causing cancer and cognitive disabilities (Buha Djordjevic et al., 2020; Ngoubeyou et al., 2022).

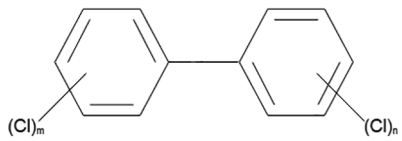


*Figure 1.7.* **The general molecular structure of polychlorinated biphenyls (PCBs) where the congeners can have from 1 to 10 bromine atoms attached to a pair of two linked phenyl rings** (McFarland and Clarke, 1989)

The main path of PCBs entering the Baltic Sea is atmospheric deposition, including remobilization from urban environments (Harner et al., 2004), as well as sedimentation, where hydrophobic PCBs bind to organic matter. The co-operative programme for monitoring and evaluation of the long-range transmission of air pollutants in Europe (EMEP) estimated that in the region 26% of PCB 153 (the modelled congener) comes

from primary emissions, 6% from cross-border sources, and 68% from reemissions (McLachlan and Undeman, 2020). In contrast, riverine PCB inputs have been estimated to be two orders of magnitude lower than atmospheric PCB depositions (Wiberg and Josefsson, 2009). Airborne PCB input is also supported by uniform PCB concentrations (normalized to organic carbon) found in sediments across the Baltic Sea (Axelman et al., 2001; Jonsson, 2000). Even though (Wiberg and Josefsson, 2009) claimed that coastal waters are influenced equally by sediment emission and atmospheric deposition.

According to multiple studies, PCBs have decreased by an average of 5% per year in biota (fish muscles) and sediments for the last 2–3 decades (Airaksinen et al., 2014; Sobek et al., 2015), while atmospheric deposition has decreased by 61% between 1990 and 2016 (McLachlan and Undeman, 2020).

The latest core holisstic assessment (HELCOM, 2018b) of dioxin-like PCBs, together with furans and dioxins against EQS showed that in most compartments of the Baltic Sea the concentrations in biota were below the threshold value (except Bothnian Bay and coastal water of the Gulf of Finland and Kattegat). In spite of this, the group of substances is still considered as one of the most problematic pollutants in the Baltic Sea.

# 2. MATERIALS AND METHODS

## 2.1. Sampling and pre-treatment

### 2.1.1. Sampling procedures

The sampling campaigns took place in the Baltic Sea basin and its catchment area, including several categories of sampling sites (**Figure 2.1**). Pelagic stations were located in a pelagic zone of the Eastern Gotland Basin: P1–P7, and a pelagic zone of the Gulf of Riga P8-P9. The coastal stations were in a coastal zone of the Gulf of Riga: C1 – Salacgrīva, C2 – Daugavgrīva, and a coastal zone of the Eastern Gotland Basin: C3 – Irbe, C4 – Jūrmalciems. Additionally, samples of water, sediments and biota were collected at the 16 freshwater stations: F1 – River Salaca; F2 – Lake Dūņezers; F3 – River Gauja; F4 – Lake Ķīšezers; F5 – River Daugava; F6 – Lake Liepājas, F7 – River Mazsalaca, F8 – River Pedele, F9 – Lake Albus, F1 – Lake Burtnieks, F11 – Lake Murāts, F12 – Lake Juveris, F13 – Lake Lizdoles, F14 – Lake Trikātas, F15 – Lake Alauksts, F16 – Lake Limbažu. Sewage sludge was collected at 8 wastewater treatment plants (WWTP): Rīga WWTP, Daugavpils WWTP, Liepāja WWTP, Ventspils WWTP, Rēzelne WWTP, Valmiera WWTP, Saldus WWTP and Dobele WWTP.

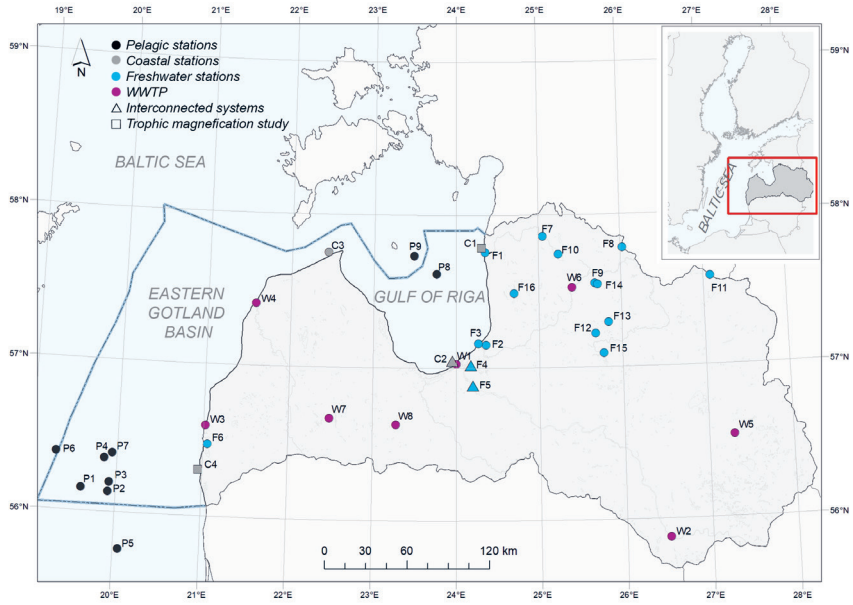


*Figure 2.1.* **Map of sampling sites in the Baltic Sea: pelagic zone of the Eastern Gotland Basin (black circles): P1–P7; pelagic zone of the Gulf of Riga (black symbols): P8–P9; coastal zone of the Gulf of Riga (grey symbols): C1–C2; coastal zone of the Eastern Gotland Basin (grey symbols): C3–C4 (Jūrmalciems); freshwater stations (blue symbols): F1–F16; wastewater treatment plants (purple symbols): W1–W8; triangle symbols indicate interconnected ecosystem, and square symbols indicate sites were trophic magnification study was performed** (by S. Spruka)

The entire sampling campaign took place in several approaches: between 2010 and 2012 (Aigars et al., 2017), in 2015 (Suhareva et al., 2020), in 2017 (Suhareva et al., 2021) and from 2018 to 2021 (Appendix D). In total, over 2000 perch samples and in total about 800 samples of other organisms were collected during the sampling period.

Perch and other fish species were collected at coastal (less than 1 nautical mile from coastline) waters by means of scientific Coastal Survey multimesh gillnets (Nippon Verkko Oy, Finland) with nine 5-m-long panels of different mesh size (mesh size ranging between 10 and 60 mm). In specific cases perch individuals were sampled by means of fish hooks, the sampling was performed by duly authorised personnel, possessing valid fishing permits. Samples of pelagic fish, such as cod and herring were gathered by trawling in the open sea area. Crayfish were caught by a two-ring drop net with the diameter of 80 cm and the mesh size 20 mm. Nektobenthic and pelagic invertebrates and fish (as well as juveniles) were caught by means of beach seine (hand-made: the wing length 10 m and mesh size of 10 mm knot to knot, the depth of 1.5 m, and mesh size of the cod end of 5 mm). Soft bottom benthic organisms were sampled by van Veen Grab (25 kg, Hydrobios, Germany) or by Petite Ponar Grab (Wildco, USA). After sampling all caught organisms were immediately sorted by species, placed in plastic containers and/or pre-cleaned glass jars (certified by Cole-Palmer, USA) and frozen at -20 °C till further.

### 2.1.2. Pre-treatment procedures

The total length and weight of fish were determined by measuring board (accuracy ± 1 mm) and technical scale KERN FCE3K1N (KERN & SOHN GmbH, Germany; accuracy ± 1 g), thereafter, dorsal muscles and otoliths were dissected (**Figure 2.2**).

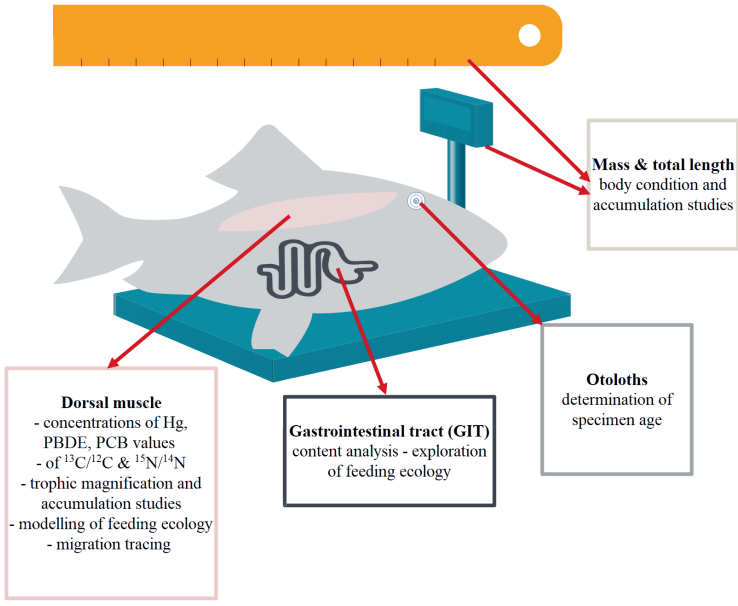


*Figure 2.2.* **Scheme of dissection of perch tissues and their purpose in the study**

In case of perch, the digestive tract was carefully removed for the following analysis of gastrointestinal tract (GIT) content. The dorsal muscle was placed in an individual plastic container for further analysis of total mercury (THg), cupper (Cu) and (Zn) content and analysis of stable isotope ratios of nitrogen (N) and carbon (C). Perch prey organisms and small fish were analysed as a whole body matrix. For PBDE and PCB analyses perch specimens were sorted by length into five groups (<130 mm, 130–159 mm, 160–189 mm, 190–219 mm and ≥220 113 mm), then another part of dorsal muscle was added to a pooled sample by length in pre-cleaned glass jars (certified Cole-Palmer, USA) and frozen at temperature –20 °C. For the trophic magnification study, besides perch also pooled samples of prey organisms (whole body matrix) were collected and frozen at temperature –20 °C, particularly, decapods (*Crangon crangon*, *Palaemon adspersus*), mysids (*Mysidae* sp.), round goby, herring juveniles, smelt juveniles (*Osmerus eperlanus*), lesser sandeel (*Ammodytes tobianus*), flounder, bleak (*Alburnus alburnus*) and stickleback (*Gasterosteus aculeatus*). For the further analysis of THg/Cu/Zn content and stable isotopes of N and C, the samples of fish tissues and prey organisms were dried in a freeze-dryer (LYOVAC GT 2-E, STERIS GmbH, Germany), water content was recorded. Dry samples were homogenised by knife mill (IKA A11 basic, IKA-WERKE GmbH & CO.KG, Germany) or agate pestle and then stored in a desiccator in the dark at room temperature (+20 °C).

## 2.2. Laboratory analyses

### 2.2.1. Biological analyses

The **age determination** technique used in this study was based on the study of seasonal growth patterns found in fish otoliths. Thus, zones of fast and slow growth were counted, delimited by distinct rings within the otoliths. Observations were carried out at low magnification using an Olympus SZX-ILLD2-200 microscope.

**Digestive tract content analysis** was performed as described in Manko (2016) with few modifications. The extracted gastrointestinal tract (GIT) was defrosted at room temperature. Thereafter, the length of stomach, intestine and whole GIT, were measured on a measuring board to the nearest mm mark. Next, the stomach and intestine were separated by making a cut at the pylorus, just before the pyloric caeca. To remove any moisture, the GIT parts were placed between two sheets of tissue paper and gently dried. The masses of the stomach, intestine, and the entire GIT were then measured using an analytical balance, with the measurements recorded to the nearest 0.001 g. The stomach and intestines were placed in separate Petri dishes and carefully opened using a scalpel, ensuring the contents were not damaged. Once opened, the contents were either flushed out with a gentle stream of tap water from a wash bottle or pushed out with a blunt probe. Subsequently, the samples were examined under a stereomicroscope (Leica MEB126, Leica Microsystems, Singapore). Any indigestible contents found in the intestines, such as fish otoliths and bones, mollusc shells, polychaete bristles, and chitinous insect and crustacean parts, were identified to the lowest possible taxonomic level. However, due to the digestion process and the presence of mucosa, the intestinal contents were not weighed. The stomach contents, taxonomically separated, along with the empty stomach and intestines, were once again dried by blotting and weighed with a precision of 0.001 g.

### 2.2.2. Chemical analyses

**Concentrations of total mercury (THg)** in the dorsal muscles of fish and other organisms were determined at the accredited laboratory of the Latvian Institute of Aquatic Ecology (Daugavpils University) using direct combustion Hg analyser (Teledyne Leeman labs, "Hydra IIc", (Mason, Ohio, USA) following US EPA Method 7473 (U.S. EPA, 1998).

THg concentrations were measured in every perch specimen and all groups of other organisms collected at the sampling stations (**Table 2.1**). Prior to the **analysis of Zn and Cu** content (are not hazardous substances, but were used as additional variables in the accumulation study described in Suhareva et al. (2020)), the tissue samples have been mineralized in microwave oven "Mars 5" by supra pure concentrated nitric acid, according to method US EPA 3052 "Microwave assisted acid digestion of siliceous and organically based matrices" (U.S. EPA, 1996). Concentrations of Cu and Zn were measured according to "Flame Atomic Absorption Spectrometry Method" (US EPA 7000B), using "VARIAN Spectra AA 800" atomic absorption spectrometer (U.S. EPA, 2007a).

**Concentrations PBDE and PCB** in biota were measured in accredited external laboratory on a commercial basis – analytical laboratory "ALS Czech Republic" (Prague, Czech Republic, accreditation certificate Nr. 610/2017 valid until 28.02.2022.). PBDE were analysed according to the local accredited method CZ_SOP_D06_06_177 based on modified US EPA 1614 method (U.S.EPA, 2007b). The method included the following BDEs: 28, 47, 99, 100, 153, 154, 183, 209. PCB were analysed according to the local accredited method CZ_SOP_D06_06_173 based on methods US EPA 1668A (U.S.EPA, 2003) and ČSN EN 16190 (CEN, 2018). Following congeners were pooled together in group ΣPCBs, regardless of Toxic Equivalent (TEQ) scheme: dioxin-like PCBs 77, 81, 105, 114, 118, 123, 126, 156, 167, 169, 189, as well as indicator PCBs 28, 52, 101, 138, 153, 180. In order to enable use of different organisms in the same trophic transfer analysis, PBDE and PCB concentrations were normalised to 5% lipid content, as recommended by (EC, Directorate-General for Environment, 2015).

Sample preparation, clean-up and quantification of PBDEs in sewage sludge, sediments and water samples are described in the Aigars et al., (2017).

*Table 2.1*

**An overview of the organisms examined in this study**

| Study site | | Organism | $\delta^{15}N$ \| $\delta^{13}C$ | THg | Cu \| Zn | PCBs | PBDEs |
|---|---|---|---|---|---|---|---|
| *C1*[1,2], *C4*[1,2] | Invertebrates | Polychaeta | X | X | | | |
| *C1*[1,2], *C4*[1,2] | | Oligochaeta | X | X | | | |
| *F4–5*[1] | | *Chirinomidae* larva | X | | | | |
| *C1*[1,2], *C4*[1,2] | | Cardiida *(M. balthica)* | X | X | | | |
| *C1*[1,2], *C4*[1,2] | | Isopoda *(Idotea sp.)* | X | X | | | |
| *C1*[1,2], *C2*[1], *C4*[1,2], *F4–5*[1] | | Amphipoda | X | X | | | |
| *C1*[1,2,4,5], *C2*[1], *C4*[1,2,4,5] | | Mysida | X | X | | X | X |
| *C1*[1,2], *C4*[1,2,4,5] | | Decapoda *(C. crangon)* | X | X | | X | X |
| *C1*[1,2], *C4*[1,2,4,5] | | Decapoda *(P. elegans)* | X | X | | X | X |
| *F5*[1] | | *O. limosus* | X | | | | |
| ***C1***[1,2,3,4,5], ***C2***[1,2,3], ***C3***[2,3], ***C4***[1,2,3,4,5], ***F1–5***[5], ***F6***[4,5], ***F10–14***[5], ***F16***[5] | | ***P. fluviatilis*** | **X** | **X** | **X** | **X** | **X** |

| Study site | | Organism | $\delta^{15}N$ \| $\delta^{13}C$ | THg | Cu \| Zn | PCBs | PBDEs |
|---|---|---|---|---|---|---|---|
| *F4*[1] | Vertebrates | *P. fluviatilis* (juv) | X | | | | |
| *C1*[1,2,4,5], *C4*[1,2] | | *G. aculeatus* | X | X | | X | X |
| *C1*[1,2,4,5], *C4*[1,2] | | *N. melanostomus* (≤50 mm) | X | | | X | X |
| *C1*[1,2,3,4,5], *C2*[1], *C4*[1,2] | | *N. melanostomus* (100-120 mm) | X | X | X | X | X |
| *C1–4*[2,3] | | *N. melanostomus* (>120 mm) | | X | X | | |
| *C1*[1,2], *C2*[1], *C4*[1,2,4,5] | | *A. tobianus* | X | X | | X | X |
| *C1*[1,2], *C4*[1,2,4,5] | | *O. eperlanus* (juv) | X | X | | X | X |
| *C1*[1,2], *C2*[1], *C4*[1,2,4,5] | | *C. harengus membras* (juv) | X | X | | X | X |
| *P6–9*[2,3] | | *C. harengus membras* | | X | X | | |
| *C1*[1,2], *C4*[1,2,4,5] | | *P. flesus* (juv) | X | X | | X | X |
| *C1–4*[2,3] | | *P. flesus* | | X | X | | |
| *C1*[1,2,4,5], *C4*[1,2] | | *A. alburnus* | X | | | X | X |
| *F4–5*[1] | | *G. cernua* | | X | | | |
| *P1–7*[2,3] | | *G. morhua* | | X | X | | |

At the indexed study sites were measured:
[1] C and N stable isotopes (from where were calculated δ15N and δ13C);
[2] mercury (THg) concentration in dorsal muscles or whole body (section 2.1);
[3] cupper (Cu) and zinc (Zn) in in dorsal muscles;
[4] concentration of polybrominated diphenyl ethers (PBDEs) in dorsal muscles or whole body (section 2.1);
[5] concentration of polychlorinated biphenyls (PBCs) in dorsal muscles or whole body (section 2.1).

The determination of the dry-matter content in fish muscle, sediment, and sludge samples was performed using a gravimetric method. For this analysis, separate wet subsamples were subjected to drying at a controlled temperature of 105 ± 5 °C until a constant dry weight was attained.

The quantification of total carbon content in the sediments followed the ISO method 10694:1995, titled "Soil quality—Determination of organic and total carbon after dry combustion (elementary analysis)". A 2 mg dry weight (DW) sub-sample was utilized for carbon measurement using the Vario EL III CHNOS Elemental Analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany).

To determine the lipid content, a 20 g DW sub-sample underwent Soxhlet extraction with a mixture of dichloromethane and n-hexane in a 1 : 1 ratio (v/v). The extraction process was conducted for 16 hours, after which the extracts were transferred to pre-weighed round-bottom flasks for solvent evaporation utilizing a rotary evaporator operating at a temperature below 30 °C. The gravimetric method was then employed to determine the lipid content.

**Analysis of stable isotopes** and calculation of C and N isotope ratios was performed for the majority of caught organisms. The 2 mg per samples of dried tissues were analysed in the Laboratory of Analytical Chemistry at Faculty of Chemistry, University of Latvia by elemental analyser (EuroEA-3024, EuroVector S.p.A, Italy)

coupled with continuous flow stable isotope ratio mass spectrometer (Nu-HORIZON, Nu Instruments Ltd., UK).

Isotope values were reported relative to Vienna Pee Dee Belemnite with a lithium carbonate anchor (VPDB-LSVEC) for carbon isotope value $\delta^{13}C$ and to atmospheric nitrogen (air) for nitrogen isotope value $\delta^{15}N$.

Stable isotope values were denoted as parts per thousand (‰) deviation from the standard, as follows (Kumar, 2011):

$$\delta X\ (‰ = \frac{R_{sample}}{R_{standard}} - 1) \times 1000 \quad (1)$$

where δX is $\delta^{13}C$ or $\delta^{15}N$ and the R ratio is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

## 2.3. Data analysis and models

### 2.3.1. General information

Data exploration and statistical analyses were performed using R software for Windows of different releases from 3.3.2 to 4.2.1. Artworks were produced by means of R and Wondershare EdrawMax softwares. Various statistical methods and diagnostic tools were implemented to distinguish possible relations and associations: regression models, Principal component analysis (PCA), correlation analyses, parametric and non-parametric tests, such as one-way analysis of variance (ANOVA), Tukey's HSD test, Wilcoxon-Mann-Whitney rank sum test and Kruskal-Wallis one-way analysis of variance, as described in the Appendices A, B, C and D. The verified relationships were considered statistically significant at $p < 0.05$.

### 2.3.2. Normalization of concentration to the specific age of specimen

To improve comparability of metal concentrations (Hg, Cu, Zn) between different age groups and among the sampling sites C1, C2, C3, C4 for coastal fish species and P1-P9 for pelagic fish species, the measured concentrations of metals were **normalised to correspond to a 2-year-old specimen** of the same fish species according to the procedures described in Suhareva et al. (2020). The concentrations were normalised to the selected age group according to the following computed equation:

$$[ME]_{norm} = [ME]_{msrd} \cdot e^{K_{sp}(A_{norm} - A_{msrd})}, \quad (2)$$

where $[ME]_{norm}$ is the normalised concentration at selected age; $[ME]_{msrd}$ is the measured concentration at current age; $A_{msrd}$ is the current age; $A_{norm}$ is the selected normalisation age; and $K_{sp}$ is species-specific coefficient.

### 2.3.2. Estimation of feeding grounds and perch subpopulations in the interlinked ecosystem

Ward's minimum variance **Clustering analysis** was performed for identification of perch subgroups with agglomeration of objects based on variables $\delta^{13}C$, $\delta^{15}N$ and sampling location in the interlinked ecosystem of three waterbodies (C2, F4 and F5). The objective of this analysis was to partition subgroups of perch based on spatial factors

such as sampling location and geographical markers inferred from stable isotope values. The determination of the optimal number of clusters was accomplished using the Silhouette Widths method (Borcard et al., 2011).

The clustering analysis was followed by the **Bayesian mixing model SIAR** (Phillips et al., 2014) in order to quantify the main diet contributors in the computed subgroups of perch. The mixing model was applied to both perch subgrous: those which correspond to specific sampling location and a mixed group including the specimens with the isotopic values which cannot be attributed to any of the station (as described in Suhareva et al., 2021).

### 2.3.3. Prediction of Hg concentration based on the feeding ecology

The contributions of specific prey in the perch diet at different feeding grounds were subsequently employed as independent variables to predict the concentrations of Hg measured in perch tissues. Smoothing function of Generalized Additive Models (GAM) was used to cover a slightly non-linear relationship of log-transformed THg concentration and length of perch specimens, thus allowing more sensitive evaluation of dietary shifts. Prior to the modeling process, the data exploration protocol recommended by Zuur et al. (2010) was implemented. Subsequently, the models obtained were validated according to the guidelines proposed Zuur and Ieno (2016). This validation included assessing homogeneity, independence, influential observations, normality, and the goodness-of-fit of the estimated values. The Akaike Information Criterion (AIC) (Zuur et al., 2009) was utilized to compare the obtained Generalized Additive Models (GAMs) and determine the best fit for the data. This analysis aimed to identify the feeding sources and other concurrent factors that impact the accumulation of Hg in consumer tissues. Due to the collinearity observed among certain variables, such as Crustacean and *Neogobius melanostomus* (correlation coefficient of –0.8), Crustacean and *Neomysis integer* (correlation coefficient of –0.7), *Gymnocephalus cernua* and *N. integer* (correlation coefficient of –0.7), *G. cernua* and *N. melanostomus* (correlation coefficient of –0.7), *N. melanostomus* and *N. integer* (correlation coefficient of –0.7), three distinct models (A, B, and C) were constructed. Each model comprises a combination of non-collinear variables, ensuring that all selected food items, identified as sources in the SIAR model mentioned earlier, are included. *Ammodytes tobianus* was excluded from the models due to its high covariance with *C. harengus membras* (correlation coefficient of 1.0), suggesting a similar effect on Hg uptake. The following three models were selected:

$$Hg\ concentration_{ij} \sim Gaussian(\mu_{ij})$$
$$E(Hg\ concentration_{ij}) = \mu_{ij}$$

Model A:

$$\log(\mu_{ij}) = Intercept + \delta^{15}N_{ij} + Crustacea_{ij} + Chironomidae\ larva_{ij} + P.\ fluviatilis\ juvenile_{ij} + G.\ cernua_{ij} + O.\ limosus_{ij} + C.\ harengus_{ij} + s(Length)_{ij} + fSeason \quad (3)$$

Model B:

$$\log(\mu_{ij}) = Intercept + \delta^{15}N_{ij} + Chironomidae\ larva_{ij} + P.\ fluviatilis\ juvenile_{ij} + O.\ limosus_{ij} + C.\ harengus_{ij} + N.\ melanostomus_{ij} + s(Length)_{ij} + fSeason \quad (4)$$

Model C:

$$\log(\mu_{ij}) = Intercept + \delta^{15}N_{ij} + Chironomidae\ larva_{ij} + P.\ fluviatilis\ juvenile_{ij} + O.\ limosus_{ij} + C.\ harengus_{ij} + N.\ integer_{ij} + s(Length)_{ij} + fSeason \quad (5)$$
$$\alpha_i \sim N(0, \sigma^2_{Nest})$$

### 2.3.4 Trophic magnification study

The **trophic level (TL)** of organisms was calculated as follows (Fisk et al., 2001; Post, 2002):

$$TL = \frac{\delta^{15} N_{sample} - \delta^{15} N_{base}}{3.4} + 2, \quad (6)$$

where $\delta^{15}N_{sample}$ is the $\delta^{15}N$ value of the organism of interest, $\delta^{15}N_{base}$ is the $\delta^{15}N$ value of a primary consumer *M. balthica*, which was used as a base organism in both sampling locations, 3.4 is the average increment of $\delta^{15}N$ per trophic level (Nfon et al., 2008; Post, 2002) and 2 is the TL value of the primary consumer.

**Trophic magnification factor (TMF)** represents the average diet-to-consumer transfer of a chemical through food webs (Borgå et al., 2012). TMF was calculated based on the species included into the station-specific diet of perch and perch itself. The food web TMF was computed from the coefficient *b* or slope of the following equation (Fisk et al., 2001; Muto et al., 2014):

$$LOG(HS) = a + b \times \delta^{15}N, \quad (7)$$

where $TMF = 10^b$.

In addition to the common method of trophic transfer analysis, where the trophic magnification factor (TMF) and the trophic magnification slope (TMS) were calculated for prey separately ($TMF_{prey}$) and for perch occupying trophic positions from 2.9 to 4.1 ($TMF_{consumer}$).

For the better comparability of Hg, PBDE and PCB levels among the sampling sites (Appendix D), measured concentrations were first log-transformed to get the linear relationship between concentration and TL, and then each measurement was normalised to correspond to a trophic level (TL) of 2 for prey organisms and to a TL of 3.5 for perch, as shown by (EC, Directorate-General for Environment, 2015) for estimation of $EQS_{biota}$ for other trophic levels than 4.

### 2.3.5. Comparison of perch populations

Fulton's condition factor (FK) was calculated from fish total length (L) and weight (W) using equation (Ogle, 2016):

$$FK = \frac{L}{W^3} \times 100, \quad (8)$$

To examine statistically significant differences among perch populations, the Kruskal-Wallis one-way analysis of variance, and Dunn's Kruskal-Wallis Multiple Comparisons integrated into fish condition analysis, as recommended by (Ogle, 2016), were performed using the R package FSA. Gabelhouse lengths representing different proportional size distributions (substock = 0, stock = 130, quality = 200, preferred = 250, memorable = 300, trophy = 380) were computed using the psdVal function for "White perch." These length ranges closely matched those observed in the European perch captured during the current study (Ogle, 2016).

# 3. RESULTS AND DISCUSSION

## 3.1. Transfer of HS from land-based sources to the coastal waters of the Baltic Sea

There are several HS transfer vectors from land-based sources to marine environment (**Figure 3.1**). The atmospheric transfer and freshwater loading have been studied extensively in the past. At the same time, transfer of hazardous substances by biological organisms has been understudied, whilst focussing narrowly on anadromous species (Gerig et al., 2020). Thus, in this thesis study of transfer of hazardous substances from land-based sources to marine environment has been undertaken in two consecutive steps (Aigars et al., 2017 and Appendix D).

As the first step polybrominated flame retardants (PBDE) were used to study transfer of hazardous substances from wastewater treatment plants to aquatic environment with subsequent assimilation in biological organisms (perch) and accumulation in sediments.

As previously described, the PBDE is a family of brominated organic chemicals, containing 209 mono- to deca- congeners with varying number of bromine atoms per molecule. For the scientific purposes and pollution monitoring concentrations of eight PBDE congeners (28, 47, 99, 100, 153, 154, 183, 209) are widely measured, sum of the first six of which are used for the assessment of the environmental quality. According to the previous studies (Burreau et al., 2004; Mizukawa et al., 2009), number of bromine atoms in PBDE classes affects its biomagnification potential, thus: penta-BDE show maximum biomagnification, whereas hexa- to hepta-BDE, exhibit lower biomagnification properties, and large octa- to deca-BDE were suggested to be unable to biomagnify.

Swedish environmental monitoring reported (Fredricsson et al., 2018) that the dominant congeners constantly found in atmospheric deposition were BDE 47, 99, 100, at ranges from 0.037 to 0.73 ng·m$^{-2}$ per day (monitored since 2001), followed by 85, 153 and 154, at ranges from 0.02 to 0.85 ng·m$^{-2}$ per day (monitored since 2013), while BDE

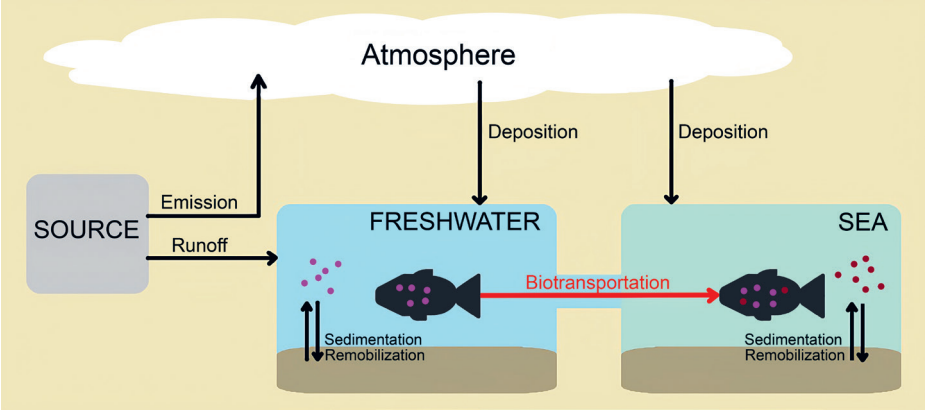


*Figure 3.1.* **Transfer vectors of hazardous substances from inland-based source to marine ecosystem**

209 was only sporadically observed although in high range of concentrations from <0.3 up to 3.3 ng·m$^{-2}$ per day (monitored since 2009). However, they did not find BDE 209 presence in air samples in 2017 opposite to other new flame retardants that have been introduced to the market as an alternative to the phased out PBDEs.

The air transport is reflected in concentrations found in pelagic fishes, such as herring (*C. harengus membras*). So, in this study, as well as in report published by the Swedish environmental monitoring program (Undeman and Johansson, 2020), the most dominant congener in herring was BDE 47. In this study second most dominant congeners were BDE 154 and 100, while in the Swedish environmental monitoring program it was BDE 99. The observed congener composition in offshore organisms was reflected also in sediments, e.g., (Josefsson and Apler, 2019) reported that in off-shore sediments of the Baltic Sea BDE 47, 85 and 99 were dominating congeners, whereas the BDE 209 was below detection limit of 0.3 μg·kg$^{-1}$ WW, in all studied stations. On the other hand, high concentrations of BDE 209 were found in sewage sludge of Latvian WWTPs (Aigars et al., 2017), as well as Olofsson et al. (2012) showed rapidly increasing trend of this congener in Swedish WWTPs between 2004 and 2012, although in later years it has levelled off (Haglund, 2019). From the above, with a certain grade of speculation, it can be assumed that in our region, among other PBDE congeners, BDE 209 is less transported via atmospheric deposition and more likely leaks into the environment through sewage sludge and waste deposition. Whereas other widespread congeners, such as BDE 47, 99, 100 are delivered by atmospheric deposition both above the sea and land. The BDE 209, as well as 47 and 99, were the most dominant congeners in a tested sewage sludge from 8 wastewater treatment plants across Latvia (Aigars et al., 2017), thus indicating a clear pollution footprint permanently leaking from diffuse pollution sources.

Therefore, in this case BDE 209 can serve as a local pollution biotransportation tracer, found in perch caught both in freshwater reservoirs and marine coastal waters. Furthermore, the concentrations of BDE 209 in sediments were below LOD (0.2 ng·g$^{-1}$ DW) in all samples. At the same time, the concentrations of the second (BDE 47) and the third (BDE 99), the most dominant congeners found in fish tissues, were the most prevalent in the sediments (**Figure 3.2**).

PBDE concentrations, especially BDE 209, were relatively low in comparison to previously reported values (Chen et al., 2009; Song et al., 2004; Zhou et al., 2012). It seems that, at present, the observed differences of PBDE congener concentrations in lake and river sediments, can be attributed to differences in sediment properties. For example, sediments with a low content of dry matter and relatively high content of organic carbon, showed substantially higher concentrations of PBDEs than those with a high dry matter content of sediments (**Figure 3.3**). The fate of other congeners, such as BDE 85, 153 and 154 seems to be region specific and can be affected by hidden processes such as debromination of larger BDEs and change of congener proportions.

The above mentioned is in line with results presented in Aigars et al. (2017), e.g., the concentrations of BDE 209, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183 in perch muscles, caught at 10 different inland reservoirs (**Figure 3.4**) constituted on average 53%, 18.6%, 7%, 4.9%, 1.5%, 3.2%, and < 1%, respectively, of the total. Similarly, the most dominant congeners found in perch tissues caught both in coastal waters of Salacgrīva and Jūrmalciems (**Figure 2.1**: C1 and C4) were BDE 209 and 47, although BDE 99 has relinquished its third place to BDE 154 (Appendix D). The high

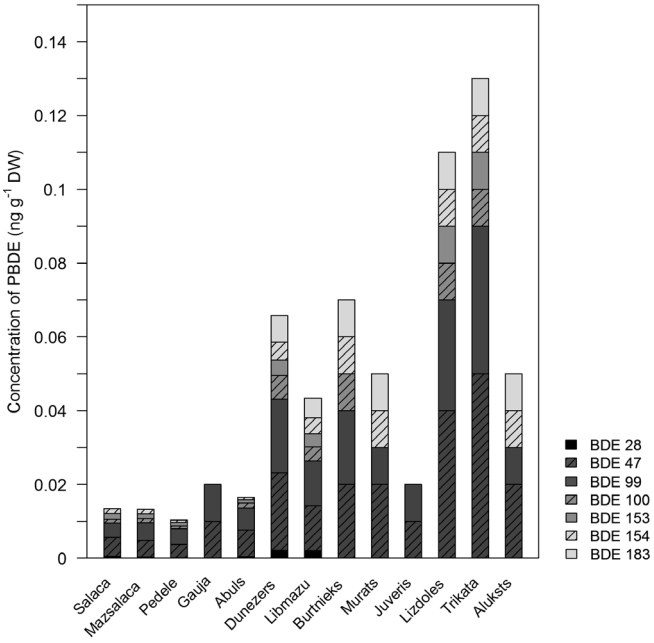


*Figure 3.2.* **Concentrations of PBDEs in sediments from rivers and lakes in Latvia** (Aigars et al., 2017)

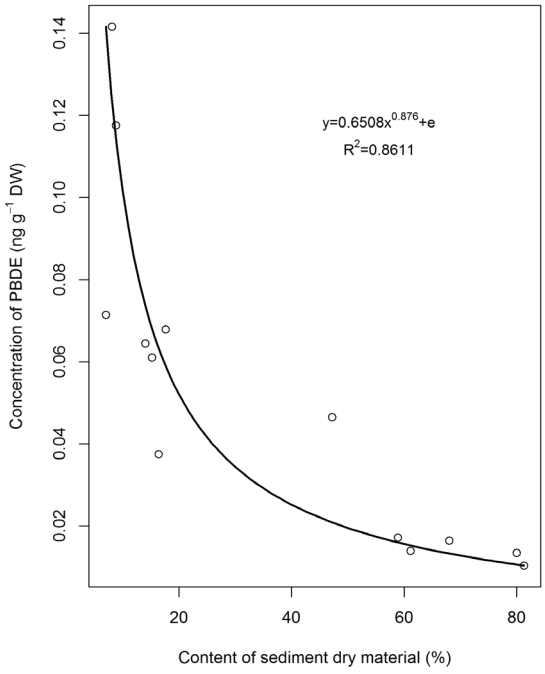


*Figure 3.3.* **Non-linear regression showing dependency of Σ7PBDE concentration from the sediment content of dry matter in lakes and rivers of Latvia** (Aigars et al., 2017)

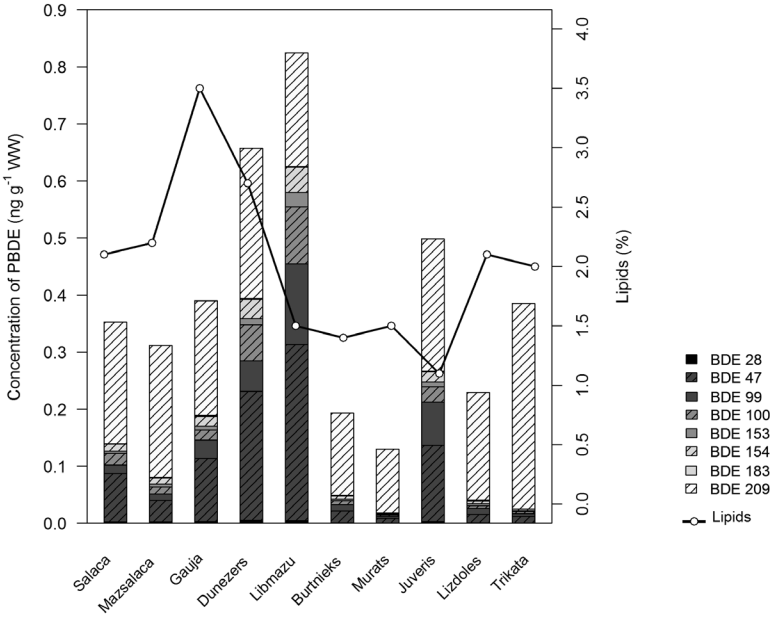


*Figure 3.4.* **Concentrations of PBDEs and lipid content in tissues of European perch (Perca fluviatilis) from rivers and lakes of Latvia** (Aigars et al., 2017)

concentration level of BDE 209 in the fish tissues, despite the large molecular size, was consistent with previous observations published by Mackintosh et al. (2015) and Shanmuganathan et al. (2011). Therefore, biotransport with fish migration may be one of the BDE 209 vectors that deliver the substance to coastal waters.

Another evidence of pollution biotransport is the decrease in concentrations with increasing trophic level observed for almost all measured congeners of both PBDEs and PCBs in the five size groups of perch from Jurmalciems (Appendix D). The relatively high ΣPCBs concentration measured in smaller perch (TL ≤ 3.6) from Jūrmalciems, can be related to the early life stages, which perch has likely spent in a nursing ground with higher levels of PCBs loads. This finding is discussed in detail in **Section 3.3**, devoted to trophic magnification in the coastal waters of the sea.

To further expand study of transfer of hazardous substances stable isotopes of carbon and nitrogen were used to describe range and level of perch mobility in the interconnected system lake-river-coastal waters (**Figure 2.1**: triangle symbols F4, F5, C2). The study presented in Suhareva et al. (2021) was conducted on base of three different ecosystems of fully conjoined reservoirs: river Daugava – lake Ķīšezers – coastal zone of the Gulf of Riga. Stable isotopic signals of C and N, obtained from metabolically active tissues (such as muscles) provide dietary and source information for up to several weeks (Hobson, 1999). This approach allowed to assign perch individuals to spatially different sampling sites and therefore allowed identification of recent arrivals, i.e. individuals that fed and accumulated isotopes and Hg in areas different from where they were caught: individuals caught in the bay, demonstrated the isotope signals of the lake and the river, and vice versa (**Figure 3.5 B** and **C**).

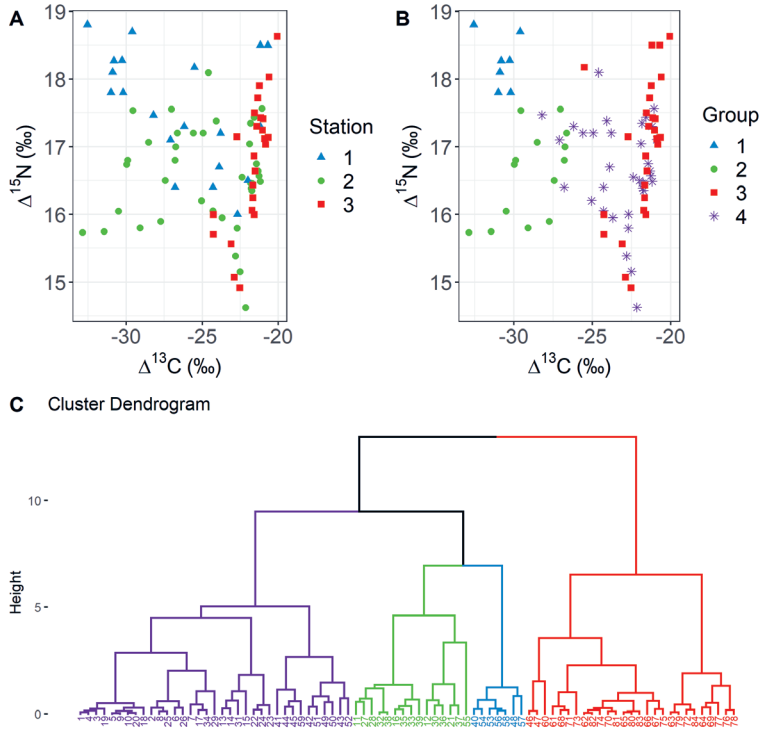


*Figure 3.5.* **Scatterplots of the measured of stable isotope values (δ13C and δ15N): A distribution between the sampling stations; B distribution between the groups assigned by means of the clustering analysis; C cluster dendrogram indicating new grouping of the sampled perch, where blue—group 1 (F5), green—group 2 (F4), red—group 3 (C2), purple—the mixed group 4 (potential recent arrivals, isotope values cannot be attributed to any of sampling stations)** (Suhareva et al., 2021)

Further in the text, the group of mobile perches is referred to as a "mixed group". Since perch in the Gulf of Riga lack suitable spawning and nursing grounds, the mixed group probably migrated to the Gulf of Riga from freshwater reservoirs, as observed elsewhere by Järv (2000). This finding is consistent with the behavioural patterns of perch, such as seasonal distribution and movement between habitats (Couture and Pyle, 2015).

Upon comparing THg concentrations and the distribution of individual's length among the sampling stations and the new group designated via cluster analysis, it was observed that the highest THg concentrations (209.4 ± 14.0 μg·kg$^{-1}$ WW) were exhibited by perch from the River Daugava, while the lowest mean concentration of THg was found in the Gulf (114 ± 10.3 μg·kg$^{-1}$ WW) (**Figure 3.6 A**). The mixed group's THg concentrations showed a high degree of variation (ranging from 71.8 to 298.5 μg·kg$^{-1}$ WW), with a mean concentration of 155.9 ± 9.1 μg·kg$^{-1}$ WW, which was found to be just in between of the concentrations observed in the Gulf and the River. It was noted that the observed differences could not be attributed to non-homogeneous distribution of specimen size since, in contrast to the concentration levels, the highest mean length of perch was found in group Daugavgrīva, while the lowest mean length was found in Ķīšezers (**Figure 3.6 B**).

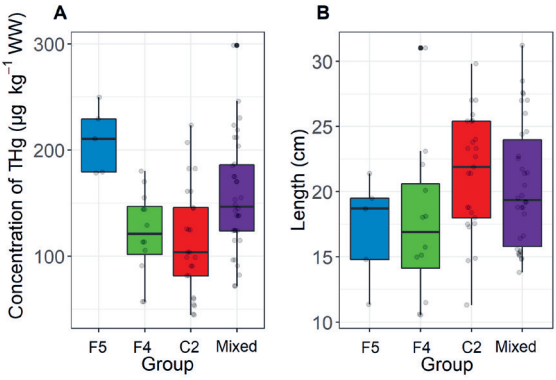


*Figure 3.6.* **A: Variation of total mercury (THg) concentrations (µg kg–1 wet weight) among the four groups of perch, 3 of them are assigned to the specific feeding ground, and one is the mixed group; B: Variation of perch length among the four assigned groups** (modified from Suhareva et al., 2021)

Therefore, the geographical differences in THg concentration were mainly observed because background concentrations of Hg are substantially higher in the inland water bodies than in the Gulf of Riga. Accordingly, it can be assumed with some confidence that the observed interannual differences in THg values measured within the framework of the national monitoring program (LIAE database) in the transitional waters of the Gulf (from 30 µg·kg$^{-1}$ WW in 2019 to 103 µg·kg$^{-1}$ WW in 2015) can mainly be explained by the difference in the ratio between the recent arrival from neighbouring freshwater reservoirs and individuals that fed in the area for longer periods of time. Furthermore, the seasonal factor produced by all three GAM models, e.g. higher THg concentrations were associated with spring sampling, can be related to recent migration from inland waters to the marine coast. The effect of dietary ecology on HS concentrations measured in coastal marine fish is discussed in **Section 3.2**.

## 3.2. Effect of diet composition on uptake of HS

### 3.2.1. Effect of diet composition change by the feeding ground (brackish and freshwater) in an interconnected ecosystem

Although mercury is absorbed by fish through the body surface and gills, the main route of exposure is through feeding (Polak-Juszczak, 2018). Perch are shown to be omnivorous in their early years, while adults are mostly piscivorous (Jacobson et al., 2019). Due to their assumed stationary nature once they reach the feeding grounds in coastal waters (Gerlach et al., 2001), perch have been considered to be a representative organism for characterising pollution by HS, providing a biologically relevant context. Therefore, understanding how feeding ecology at the visited or inhabited feeding grounds can affect the uptake of Hg and potentially other hazardous substances is crucial. The previously discussed case study of the interconnected ecosystem of three waterbodies (River Daugava, Lake Ķīšezers, and transitional water of the Gulf of Riga at Daugavgrīva) sheds light on how prey availability at different feeding grounds

can change the rates of Hg uptake. In order to address the question, stomach content analysis, analysis of stable isotopes of N and C, Bayesian mixing model SIAR and regression models were implemented to each perch specimen caught at these water bodies.

The predominant prey at sampling stations Daugava and Ķīšezers were amphipods. Juvenile perch and *Chironomidae* larvae were the second most frequently consumed prey organisms, while *O. limosus* and *G. cernua* were mainly found only in the digestive tract of perch from station Daugava. On the other hand, *N. integer* was the most frequent prey in station Daugavgrīva, and it was also found in a quarter of perch stomachs from freshwater station Ķīšezers. The second most common prey in station Daugavgrīva was *N. melanostomus*, which was found in one third of perch stomachs. *A. tobianus* and *C. harengus membras* were found in only a few stomachs of perch caught at Daugavgrīva.

The use of stable isotopes of N and C in fish soft tissues is a common method to study food webs and migration in aquatic ecosystems (Huxham et al., 2007; MacKenzie et al., 2011; Richert et al., 2015). As discussed in **Section 3.1**, the $^{13}C/^{12}C$ carbon stable isotope ratios have been used to track the movements of migratory species between coastal and pelagic ecosystems based on changes in dietary preferences during migration (Mizutani et al., 1990; Smith et al., 1996). This is possible due to the clear isotopic differences between $^{13}C$-depleted freshwater and $^{13}C$-enriched marine food webs. On the other hand, changes in nitrogen isotope ratios $^{15}N/^{14}N$ have been utilised to differentiate trophic levels in freshwater and marine environments (McCormack et al., 2019; Post, 2002). To determine the most frequently consumed prey and identify general food categories present in the stomach (Manko, 2016), the information on feeding ecology gained from the analysis of C and N isotopes and perch stomach content analysis was incorporated into the Bayesian mixing model SIAR (**Figure 3.7**).

According to the model, amphipods, *G. cernua*, *Chironomidae* larvae and *O. limosus* were the main food sources for perch at station Daugava. The feeding base at station Ķīšezers mainly consisted of amphipods, *G. cernua*, *Chironomidae larva*e and juvenile perch while the *N. integer*, *N. melanostomus*, *A. tobianus*, *C. harengus membras* and amphipods again were main food items of perch at station Daugavgrīva. In order to reflect the spatial distribution of the available diet in the interconnected system, the following representative food items were selected: *O. limosus* from station Daugava, juvenile perch from station Ķīšezers, and *N. integer* and *N. melanostomus* from station Daugavgrīva. The four sources formed an appropriate mixing polygon which covered the vast majority of data points of the mixed group (**Figure 3.7 D**). The proportion of specific prey in the perch diet was then used for regression analysis to find diet items that potentially promote or reduce Hg uptake.

In order to avoid covariance of variable food sources, three generalized additive models with different prey combinations were selected and interpreted (**Table 3.1**). The models identified that *C. harengus membras* and amphipods (mostly *Gammarus* sp.) had the most significant mitigating effect on THg concentration. The remaining food items were secondarily significant, with different directions of the effect and slope values. *Chironomidae* larvae, *O. limosus*, perch juvenile, and *G. cernua* contributed to the Hg uptake by perch, while *N. melanostomus* and *N. integer* exhibited a neutral influence on the THg concentration measured in consumer perch.

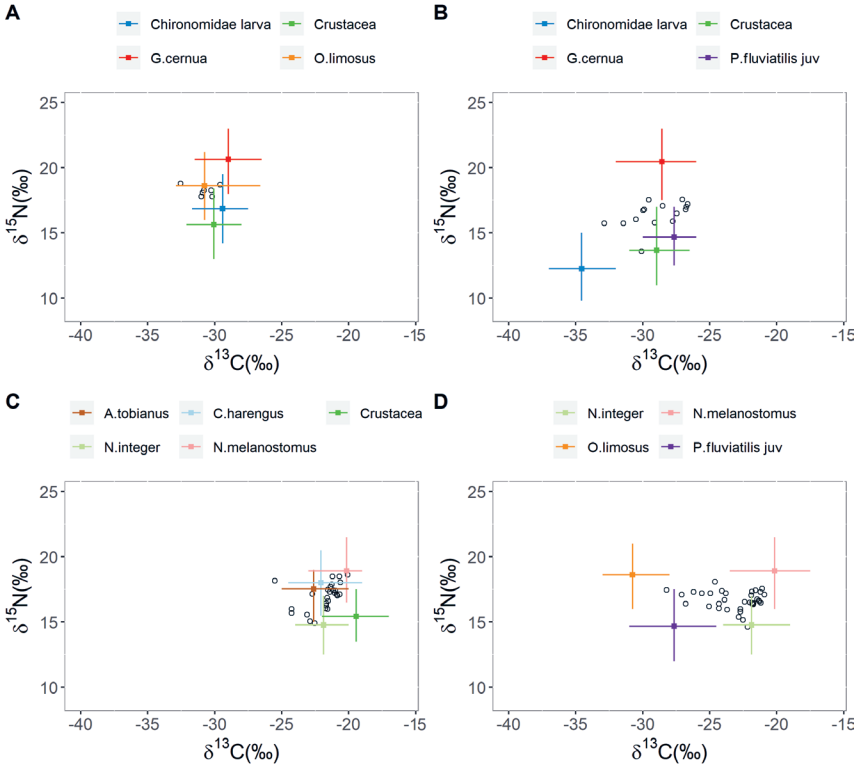


*Figure 3.7.* **Results of SIAR Bayesian mixing model. A: site F5, B: site F4, C: site C3, D: the mixed group; black circles—perch specimens (consumers)** (Suhareva et al., 2021)

Based on the above, a Gulf associated diet containing less contaminated food sources can successfully replace more contaminated freshwater prey and thus reduce mercury accumulation in fish tissue. This explains the significant differences in the levels of THg measured in groups that were associated with stations using cluster analysis (**Section 3.1**). The study also showed that the intensity of Hg uptake by consumers is related not only to the trophic position of prey represented by $\delta^{15}N$ values, but rather to feeding ecology (e.g., the scavenger *O. limosus*). Thus, in addition to accurately identifying food sources, information about background concentrations at a site is also important to understand accumulation trends and transport pathways. Furthermore, according to Jones et al. (2013), fish biometrics modelling is of high significance when designing any monitoring program focused on seafood safety to avoid misinterpretation of spatial and temporal trends.

The limitation of this study is that the well-known opportunistic feeding behaviour of perch (Couture and Pyle, 2015) suggests that they will inevitably switch to other taxa if availability of previously consumed taxa becomes limited, or if a more profitable source of energy appears. For example, the round goby (*N. melanostomus*, invasive in the Baltic Sea) has become a heavily consumed perch prey in recent years (Almqvist et al., 2010; Puntila et al., 2018). Another weak point to be considered is that the isotopic signal changes faster than the level of accumulated Hg (Bradley et al., 2017; Van

Walleghem et al., 2013). This can result in a recently arrived perch showing isotope signals from a new feeding site after some time, while prior to capture the mercury levels have not been adjusted, thus representing the level of contamination accumulated in another reservoir (as discussed in **Section 3.4**). More regular sampling could improve the design and provide more accurate information on the effect of perch motility on measured Hg concentrations.

*Table 3.1.*

**Estimated regression parameters (intercept and slope values) for the Gaussian GAM presented in Eqs. 3, 4 and 5** (Suhareva et al., 2021)

| | |
|---|---|
| **Model A** | |
| log (μij) = | |
| AUTUMN | 0.536 + 0.092 × δ15N$^{ij}$ - 0.460 × *Crustacea*$^{ij}$ + 0.460 × *Chironomidae larva*$^{ij}$ + 0.098 × Perch juvenile$^{ij}$ + 0.120 × *G.cernua*$^{ij}$ + 0.142 × *O.limosus*$^{ij}$ - 0.349 × *C.harengus*$^{ij}$ + s(Length)$^{ij}$ |
| SPRING | 0.642+ 0.092 × δ15N$^{ij}$ - 0.460 × *Crustacea*$^{ij}$ + 0.460 × *Chironomidae larva*$^{ij}$ + 0.098 × Perch juvenile$^{ij}$ + 0.120 × *G.cernua*$^{ij}$ + 0.142 × *O.limosus*$^{ij}$ - 0.349 × *C.harengus*$^{ij}$ + s(Length)$^{ij}$ |
| **Model B** | |
| log (μij) = | |
| AUTUMN | 0.514 + 0.090 × δ15N$^{ij}$ + 0.299 × *Chironomidae larva*$^{ij}$ + 0.186 × Perch juvenile$^{ij}$ + 0.174 × *O.limosus*$^{ij}$ - 0.484× *C.harengus*$^{ij}$ + 0.079 × *N.melanostomus*$^{ij}$+ s(Length)$^{ij}$ |
| SPRING | 0.628 + 0.090 × δ15N$^{ij}$ + 0.299 × *Chironomidae larva*$^{ij}$ + 0.186 × Perch juvenile$^{ij}$ + 0.174 × *O.limosus*$^{ij}$ - 0.484 × *C.harengus*$^{ij}$ + 0.079 × *N.melanostomus*$^{ij}$ + s(Length)$^{ij}$ |
| **Model C** | |
| log (μij) = | |
| AUTUMN | 0.529 + 0.091 × δ15N$^{ij}$ + 0.233 × *Chironomidae larva*$^{ij}$ + 0.167 × Perch juvenile$^{ij}$ + 0.184 × *O.limosus*$^{ij}$ - 0.501 × *C.harengus*$^{ij}$ + 0.023 × *N.integer*$^{ij}$+ s(Length)$^{ij}$ |
| SPRING | 0.649 + 0.091 × δ15N$^{ij}$ + 0.233 × *Chironomidae larva*$^{ij}$ + 0.167 × Perch juvenile$^{ij}$ + 0.184 × *O.limosus*$^{ij}$ - 0.501 × *C.harengus*$^{ij}$ + 0.023 × *N.integer*$^{ij}$+ s(Length)$^{ij}$ |

Standard errors, t-values and p-values can be found in Suhareva et al., (2021), Additional file 1: Table S5

### 3.2.2. Influence of prey proportions in different feeding grounds in the coastal waters of the East Gotland Basin and the Gulf of Riga

In order to evaluate the influence of feeding ecology on uptake of hazardous substances, concentrations of THg, PBDE and PCB were explored in perch muscle tissues and its prey items collected from two distant feeding grounds: coastal waters of the Gulf of Riga (station Salacgrīva) and the Eastern Gotland Basin (station Jūrmalciems).

During the study period perch individuals were collected from Salacgrīva and Jūrmalciems (**Table 2.1**). The size range of the selected specimens was 73–353 mm (mean size = 157 ± 38 mm) at Salacgrīva, and 85-358 mm (mean size = 181 ± 49 mm) at Jūrmalciems. The trophic level of perch varied from 2.5 to 4.0 at Salacgrīva and from 2.4 to 4.1 at Jūrmalciems. The prey used for the analysis occupied a broad trophic niche, with TL values of 1.9–3.7 at both sites.

First, perch populations from both sites were compared by body condition factor and trophic level increase with growing. Based on Fulton's factor values (**Figure 3.8 A**), it was observed that the body condition index of perch caught at Jūrmalciems was significantly higher than that of specimens from Salacgrīva, indicating more favourable nutritional conditions (Datta et al., 2013; Ramos et al., 2013). This suggests that there may be different food reserves and dietary compositions affected by prey availability. Additionally, only Jūrmalciems catches had a maximum length group "memorable" (> 300 mm). Furthermore, the lower trophic level occupied by specimens caught at Jūrmalciems compared to those of the same total body length from Salacgrīva (**Figure 3.8 B**) provides further evidence suggesting variable perch ecology among the different habitats.

The growth patterns reflected by the body condition factor are influenced by numerous biotic and abiotic factors such as water quality, parasitic infections, and metabolic rate (Cren, 1951; Datta et al., 2013; Famoofo and Abdul, 2020). Among the factors that

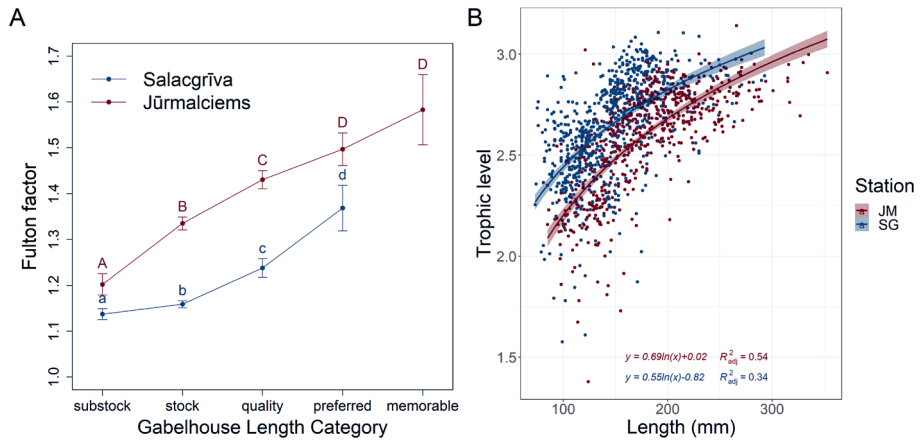


*Figure 3.8.* **A: Fulton factor as a fish condition metric for perch caught in coastal waters of the Gulf of Riga (Salacgrīva) and the Baltic Sea (Jūrmalciems). Letters A–D and a-d indicate statistically significant differences in Fulton's factor among Gabelhouse length categories. B: Relation between trophic level of caught perch and the total body length (mm), JM – Jūrmalciems, SG – Salacgrīva (Appendix D)**

could be responsible for differences in fish body condition between locations are feeding ecology, differences between littoral and pelagic feeding grounds, trade-offs in foraging efficiency, individual body morphology, and variations in migratory strategy (Chapman et al., 2015; Hansson et al., 2019; Svanback and Eklov, 2004). Additionally, lower salinity environments have been observed to have higher growth rates (Christensen et al., 2021). The European perch is a visual predator (Jacobson et al., 2019). Therefore, the predation success and feeding ecology of perch are dependent on a narrow range of water depths and temperatures (Bergman, 1987; Persson, 1986), vegetation cover (refuge), and turbidity levels (Andersson et al., 2009; Lunt and Smee, 2015). As both study areas have similar hydromorphological characteristics and relatively minor differences in salinity levels, the observed higher values of the body condition factor (Fulton's factor) in Jūrmalciems compared to those in Salacgrīva cannot be attributed to physical or hydromorphological conditions. Therefore, differences in nutrition and feeding ecology should be explored. This was done using analysis of gastrointestinal tract (GIT) content, carried out in almost every individual perch.

Although, at both sites, perch diet consisted of the same principal components, frequency of prey occurrence and variation along the trophic level gradient were substantially different (**Figure 3.9**). Perch diet at Jūrmalciems showed substantial differences between the lower and higher trophic levels. In the lower trophic levels, the diet was almost homogeneously distributed among mysids, decapods, *Gobiidae* and *Gasterosteidae*, and in the higher trophic levels, the most frequent prey items were decapods and *Gobiidae*. Other prey groups, such as amphipods, polychaetes, isopods and small fish (*Ammodytidae*, *Osmeridae*, *Pleuronectidae*) were present in very small quantities regardless of the consumer trophic levels. In the GIT of perch from Salacgrīva, the most abundant prey were amphipods, followed by *Gobiidae* and mysid. Decapods, isopods, polychaetes and insects occurred in 3.9 to 8.3% of analysed fish. Small fish, including *Gasterosteidae*, *Ammodytidae*, *Osmeridae*, *Pleuronectidae* and others were present in less than 1%.

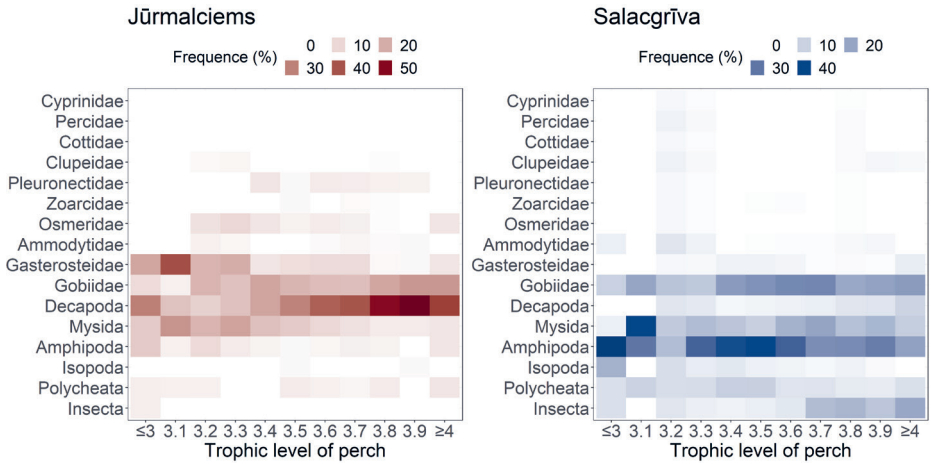


*Figure 3.9.* **Occurrence of the most common prey found in stomachs of perch caught at coastal waters of the Gulf of Riga (Salacgrīva) and the Baltic Sea (Jūrmalciems); values are available in Appendix D, Table S4**

The GIT content analysis revealed that at both sites perch mostly follows benthic diet, with some exceptions. Therefore, the differences in the body condition factor are due to the predominance of decapods (*Crangon crangon* and *Palaemon adspersus*) in the diet of perch at Jūrmalciems compared to amphipods in the diet of Salacgrīva perch. The differences in diet are due to the higher availability of decapods in Jūrmalciems creates more favourable feeding conditions as described by Ray (2005), rather than lower competition for food with other species as described by Martino and Able (2003).

In order to compare concentration levels of Hg, ΣPBDEs and ΣPCBs at both sites, together with the measured concentrations, the concentrations in the prey were normalised to the trophic level of 2 ($HS_{TL2}$) and set as the baseline concentration of the site, while the concentration in the perch were normalised to the trophic level of 3.5 ($HS_{TL3.5}$) and was assigned as a pollution level in predatory fish.

The normalised $THg_{TL2}$ concentration indicated that at the prey level environmental concentrations of THg in Salacgrīva and Jūrmalciems are similar (**Table 3.2**). Opposite to prey, $THg_{TL3.5}$ levels in Jūrmalciems were significantly higher than in Salacgrīva.

*Table 3.2*

**Descriptors of trophic magnification calculated from Hg, PCB and PBDE concentrations in prey and consumer organisms** (from Appendix D)

| | | Jūrmalciems | Salacgrīva | Statistically significant difference between sampling sites |
|---|---|---|---|---|
| **Hg** | **TL2** | 25.4 ± 2.2 μg·kg$^{-1}$ DW | 33.9 ± 3.0 μg·kg$^{-1}$ DW | p = 0.069 |
| | **TL3.5** | 338.3 ± 37.6 μg/kg DW | 225.7 ± 6.2 μg/kg DW | p < 0.001 |
| | **$TMF_{consum}$** | 9.8 | 4.7 | Slope: p < 0.001<br>Intercept: p < 0.001 |
| | **$TMF_{prey}$** | 1.9 | 1.7 | Slope: p = 0.718<br>Intercept: p = 0.018 |
| **PCB** | **TL2** | 0.31 ± 0.066 μg·kg$^{-1}$LW | 0.35 ± 0.11 μg·kg$^{-1}$ LW | p = 1.000 |
| | **TL3.5** | 3.67 ± 1.13 μg·kg$^{-1}$LW | 1.46 ± 0.20 μg·kg$^{-1}$ LW | p = 0.005 |
| | **$TMF_{consum}$** | 0.3 | 1.9 | Slope: p = 0.029<br>Intercept: p = 0.003 |
| | **$TMF_{prey}$** | 1.4 | 1.4 | Slope: p = 0.979<br>Intercept: p = 0.225 |
| **PBDE** | **TL2** | 0.063 ± 0.016 μg·kg$^{-1}$ LW | 0.12 ± 0.06 μg·kg$^{-1}$ LW | p = 0.715 |
| | **TL3.5** | 0.63 ± 0.058 μg·kg$^{-1}$ LW | 1.40 ± 0.38 μg·kg$^{-1}$ LW | p = 0.029 |
| | **$TMF_{consum}$** | 0.1 | 8.1 | Slope: p = 0.040<br>Intercept: p = 0.560 |
| | **$TMF_{prey}$** | 1.4 | 1.2 | Slope: p = 0.928<br>Intercept: p = 0.684 |

$TL_2$ – concentration normalized to trophic level 2;
$TL_{3.5}$ – concentration normalized to trophic level 3.5;
$TMF_{consum}$ – trophic magnification factor calculated on perch data only;
$TMF_{prey}$ – trophic magnification factor calculated on prey data only.

The study discovered conflicting patterns of Hg trophic magnification, namely the concentration at lower trophic levels of the benthic food web does not always correspond to the same relative pollution level at higher levels of the trophic chain. Furthermore, this uncertainty does not appear to have an easy solution as Hg concentrations in sediments do not always reflect potential of Hg bioaccumulation (Xu et al., 2019), despite serving as a significant Hg reservoir (Chen et al., 2012; Gworek et al., 2016; Jędruch et al., 2019). However, the higher normalized concentration of Hg in perch from Jūrmalciems is accompanied by a markedly higher consumption of decapods in this feeding area compared to Salacgrīva. This suggests that the higher proportion of more polluted decapods in the diet of perch from Jūrmalciems (**Table 3.4**) compared to less polluted amphipods (**Table 3.3**), as it is at Salacgrīva, may serve as a significant channel for the transfer of mercury in the perch food chain in the studied feeding areas.

*Table 3.3*

**Concentrations of THg, PCB and PBDE in perch and its prey collected at Salacgrīva (C1)**

| Study site | | Organism | $\delta^{15}N \pm SE$ (‰) | TL ± SE | THg ± SE (μg·kg$^{-1}$DW) | ΣPCBs (μg·kg$^{-1}$LW) | ΣPBDEs (μg·kg$^{-1}$LW) |
|---|---|---|---|---|---|---|---|
| C1 | *Consumer* | *P. fluviatilis* | 16.5 ± 0.0 (12.9-18.1) | 3.6 ± 0.0 (2.5-4.0) | 236.4 ± 5.5 (82.6-1455.5) | 2.15 ± 0.19 (1.53-2.69) | 0.64 ± 0.13 (0.42-1.12) |
| | Prey invertebrates | Polychaeta | 14.7 ± 1.0 | 3.0 ± 0.3 | 81.7 ± 9.5 | | |
| | | Oligochaeta | 10.9 ± 0.0 | 1.9 ± 0.0 | 83.5 ± 0 | | |
| | | Cardiida *(M. balthica)* | 11.2 ± 0.1 | 2.0 ± 0.0 | 112.7 ± 19.7 | | |
| | | Isopoda *(Idotea sp.)* | 11.7 ± 0.0 | 2.2 ± 0.0 | 91.5 ± 0.0 | | |
| | | Amphipoda | 11.2 ± 0.2 | 2.0 ± 0.1 | 52.9 ± 11.5 | | |
| | | Mysida | 13.0 ± 0.2 | 2.5 ± 0.1 | 80.0 ± 9.2 | 0.70 | 0.22 |
| | | Decapoda *(C. crangon)* | 14.3 ± 0.5 | 2.9 ± 0.1 | 79.2 ± 2.8 | | |
| | | Decapoda *(P. elegans)* | 13.2 ± 0.4 | 2.6 ± 0.1 | 101.5 ± 2.0 | | |
| | Prey vertebrates | *G. aculeatus* | 13.9 ± 0.0 | 2.8 ± 0.0 | 157 ± 0 | 1.73 | 0.24 |
| | | *N. melanostomus* (≤50 mm) | 12.1 ± 0.0 | 2.3 ± 0.0 | NA | 1.95 | 0.77 |
| | | *N. melanostomus* (100-120 mm) | 14.0 ± 0.7 | 2.8 ± 0.2 | 117.4 ± 24.2 | 2.03 | 0.97 |
| | | *A. tobianus* | 13.5 ± 0.4 | 2.7 ± 0.1 | 128.6 ± 54.8 | | |
| | | *O. eperlanus* (juv) | 16.2 ± 0.0 | 3.5 ± 0.0 | 54.9 ± 0.0 | | |
| | | *C. harengus membras* (juv) | 15.2 ± 0.0 | 3.2 ± 0.0 | 97.7 ± 0.0 | | |
| | | *P. flesus (juv)* | 15.6 ± 0.0 | 3.3 ± 0.0 | 109.3 ± 0.0 | | |
| | | *A. alburnus* | 12.9 ± 0.0 | 2.5 ± 0.0 | NA | 2.06 | 0.52 |

THg concentrations per perch individual and concentrations for PCB and PBDE congeners are available in Appendix D, Tables S3a and S3b.

*Table 3.4*

**Concentrations of THg, PCB and PBDE in perch and its prey collected at Jūrmalciems (C4)**

| Study site | | Organism | $\delta^{15}N \pm SE$ (‰) | TL ± SE | THg ± SE (μg·kg$^{-1}$DW) | ΣPCBs (μg·kg$^{-1}$LW) | ΣPBDEs (μg·kg$^{-1}$LW) |
|---|---|---|---|---|---|---|---|
| C4 | *Consumer* | *P. fluviatilis* | 13.6 ± 0.0 (9.4–15.4) | 3.6 ± 0.0 (2.4–4.1) | 342.9 ± 8.2 (46.9–1110.3) | 4.19 ± 0.48 (3.19–5.89) | 0.53 ± 0.10 (0.4–0.83) |
| | Prey invertebrates | Polychaeta | 13.9 ± 0.0 | 3.7 ± 0.0 | 92.3 ± 0.0 | | |
| | | Oligochaeta | 8.5 ± 0.2 | 2.1 ± 0.1 | 43.5 ± 0.0 | | |
| | | Cardiida *(M. balthica)* | 8.1 ± 0.0 | 2.0 ± 0.0 | 106.0 ± 0.0 | | |
| | | Isopoda *(Idotea* sp.*)* | 8.1 ± 0.5 | 2.0 ± 0.1 | 23.1 ± 2.9 | | |
| | | Amphipoda | 8.2 ± 0.2 | 2.0 ± 0.1 | 36.6 ± 1.0 | | |
| | | Mysida | 10.3 ± 0.0 | 2.6 ± 0.0 | 61.6 ± 2.4 | | |
| | | Decapoda *(C. crangon)* | 11.3 ± 0.4 | 2.9 ± 0.1 | 80.7 ± 21.4 | 3.48 | 0.20 |
| | | Decapoda *(P. elegans)* | 10.4 ± 0.1 | 2.7 ± 0.0 | 71.9 ± 17.6 | 2.92 | 0.27 |
| | Prey vertebrates | *G. aculeatus* | 9.8 ± 0.3 | 2.5 ± 0.1 | 88.0 ± 2.0 | | |
| | | *N. melanostomus* (100–120 mm) | 10.2 ± 0.0 | 2.6 ± 0.0 | 126.2 ± 0.0 | 3.98 | 0.67 |
| | | *A. tobianus* | 12.4 ± 0.0 | 3.2 ± 0.0 | 37.7 ± 0.0 | 1.70 | 0.54 |
| | | *O. eperlanus* (juv) | 13.3 ± 0.0 | 3.5 ± 0.0 | 23.0 ± 0.0 | 7.68 | 1.28 |
| | | *C. harengus membras* (juv) | 10.5 ± 0.0 | 2.7 ± 0.0 | 32.5 ± 0.0 | 1.17 | 0.36 |
| | | *P. flesus, S. maximus* (juv) | 11.7 ± 0.2 | 3.0 ± 9.1 | 68.0 ± 28 | 6.44 | 0.67 |

THg concentrations per perch individual and concentrations for PCB and PBDE congeners are available in Appendix D, Table S3a and S3b

The method for THg analysis allows to measure concentration in each individual fish and in almost all prey taken, regardless of the size of the sample. Thus, the procedure insures high resolution of data ability to trace Hg uptake along the trophic scale. Conversely, the detection of PBDEs and PCBs requires a certain sample weight, which often forces researchers to pool many individuals into a pooled sample, thereby obtaining a concentration that represents the average of the pool and precluding detailed analysis of trophic transport.

Nevertheless, this study established that, similarly to Hg, although concentrations of ΣPBDEs and ΣPCBs in prey were statistically similar at Jūrmalciems and Salacgrīva. Whereas, the normalised concentrations in perch differed significantly (**Table 3.2**). Comparing PBDE levels in decapods from Jūrmalciems and mysids from Salacgrīva, as well as round gobies from both locations, it can be seen that only the latter are slightly higher in Jūrmalciems. At the same time, the concentration of PCBs in these prey organisms is significantly higher in Jūrmalciems, which is consistent with the higher

normalized concentrations of PCBs in perch in this area. At the same time, normalized concentrations do not reflect a decrease in concentration with an increase in the trophic level of perch, which is highly important. Therefore, in this case, the study of trophic magnification may serve as better approach to explain concentration variations, as it is presented in the next section (see **Section 3.3**).

## 3.3. Trophic magnification in different feeding grounds

In most of the samples, the concentrations of PBDEs and PCBs exceeded the level of detection and quantification, which made it possible to study the trophic magnification in Jūrmalciems and Salacgrīva. Where necessary, concentrations were estimated by calculating the mean between the limit of detection and the limit of quantification, or the limit of detection and zero. Trophic magnification of HS at both locations was expressed using: trophic magnification factor ($TMF_{prey}$) calculated for prey items only, and $TMF_{consumer}$ calculated for perch only (**Table 3.2**).

There was no significant difference between the sites in trophic magnification of ΣPCBs and ΣPBDEs among prey ($TMF_{prey}$). In contrast, the trophic magnification trends in five size groups of perch (<130, 130–159, 160–189, 190–219, and ≥220 mm) were different. Moreover, ΣPCBs and ΣPBDEs exhibited a positive (TMF>1) and negative (TMF<1) $TMF_{consumer}$ at Salacgrīva and Jūrmalciems, respectively. Therefore, the higher concentration of $\Sigma PCB_{TL3.5}$ (see **Section 3.2.2**) may be the result of high concentrations in small perch individuals.

As it was stated earlier (see **Section 3.1**), the relatively high ΣPCBs concentrations in smaller individuals (TL ≤ 3.6), may be treated as a legacy of nursing ground where levels of available PCBs are higher than in coastal waters in the study area. Although there is not enough evidence to avoid speculation and indicate exactly where perch caught in coastal waters spent their early stages of life, circumstantial information supports this assumption. For instance, a pooled sample of perch from Lake Liepājas (**Figure 2.1**: F6), a freshwater body near the Jūrmalciems sampling site, showed substantially higher ΣPCBs concentrations compared to perch from both Jūrmalciems and Salacgrīva. Furthermore, juvenile smelt (*O. eperlanus*) caught in Jūrmalciems can be used as a proxy for concentration comparisons. Anadromous smelt typically inhabit marine coastal zones, spawn in freshwater bodies, spend the summer season in larval stage, and migrate to the sea in autumn (Plikšs and Aleksejevs, 1998). The ΣPCBs concentrations measured in the pooled sample of smelt juveniles were higher than those in any other prey analysed. Previous studies have also reported high PCB concentrations in European eels (*Anquilla anquilla*) caught in Lake Liepājas (Zacs et al., 2016).

The varying bioaccumulation and trophic magnification potential among different congeners of PCB and PBDE are widely discussed. The fate of persistent organic pollutants is dependent on the physical-chemical properties of substances, such as hydrophobicity and molecular size (Nfon et al., 2008; Olsson et al., 2000; Russell et al., 1999). As it has been mentioned in **Section 3.1**, it is acknowledged that the higher the hydrophobic properties, the higher the biomagnification and, therefore, the trophic magnification potential (Burreau et al., 2004). So, penta-BDE exhibits maximum biomagnification, whereas hexa- to hepta-BDE, as well as hexa-PCB, exhibit lower biomagnification properties, whereas it is suggested that large octa- to deca-BDE were suggested to be unable

to biomagnify (Burreau et al., 2004; Mizukawa et al., 2013). Therefore, trophic magnification can vary significantly among PCB and PBDE congeners (**Figure 3.10**).

In general, it was found that the magnitude and direction of the lipid-normalised trophic magnification slope (TMS) for both PCBs and PBDSs were found to be inconsistent between prey and perch. Moreover, TMS directions in perch were not covariant between the studied sites. Taken together, this indicates that the trophic magnification observed in this study is due to a combination of more complex factors than the hydrophobic properties of the substance itself.

The highest concentrations among PBDEs in all analysed organisms during this study were detected for (tetra-) BDE 47 and (deca-) BDE 209 at both sampling sites, which agrees with PBDE concentrations detected in perch from inland freshwater bodies (Aigars et al., 2017). Opposite to the expected trends, BDE 47 exhibited moderate or no trophic magnification, while BDE 209 showed very high TMS among both perch and their prey at Salacgrīva. Like it was reported by Kierkegaard et al. (1999) and Burreau et al. (2004), this finding confirms the bioavailability of BDE 209 and the evidence that the congener can magnify in a trophic chain despite its large molecular size. Moreover, the penta-PCB congeners with the highest potential for biomagnification, which also showed the highest concentration detected at both sites (CB 105, CB 118 and CB 156), did not show noticeable signs of trophic magnification, as evidenced by the negligible TMSs.

In the case of THg, despite similar $TMF_{prey}$ and $THg_{TL2}$ between the sites, as discussed in **Section 3.3.2**, perch exhibited significant trophic magnification at both feeding grounds, resulting in higher $THg_{TL3.5}$ in perch from Jūrmalciems (Appendix D). Magnification rates among prey that are similar across sampling sites can be explained by the fact that the feeding ecology at lower trophic levels is more linear and consists

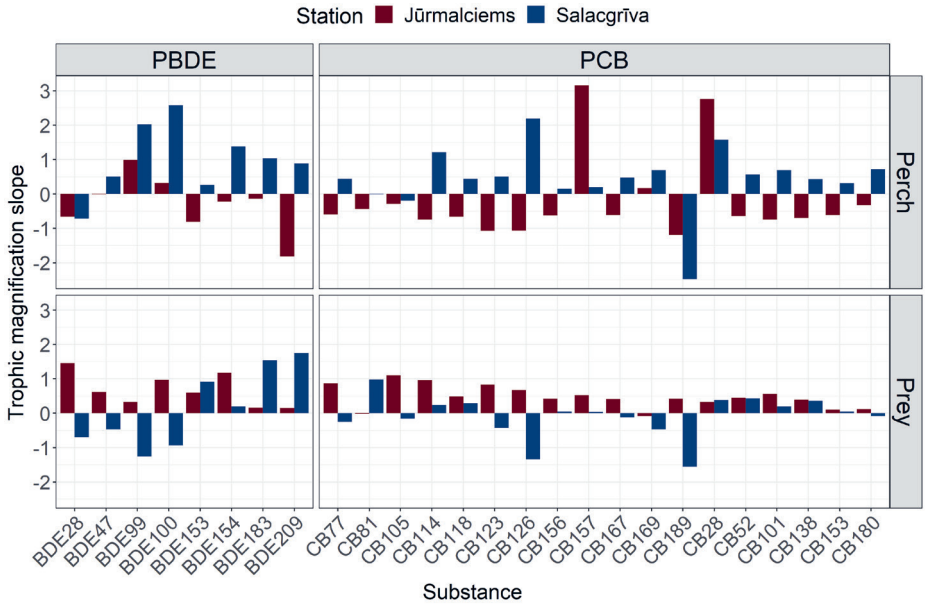


*Figure 3.10.* **Trophic magnification slopes calculated for log-concentration of PCB and PBDE, normalized to 5% lipid content** (from Appendix D)

of narrower food sources compared to higher trophic levels, resulting in a more even transport of mercury. This study did not consider the absorption of metyl-Hg species via the respiratory surfaces (Gray, 2002) in benthic organisms such as crustaceans, polychaetes, and bivalves, which could potentially provide additional information on the origin of Hg concentrations in these organisms.

As it has been mentioned (see **Section 3.2**), the opportunistic feeding ecology of perch can be highly affected by prey availability and habitat features (see above), therefore trophic transfer of Hg can follow numerous scenarios and dietary paths. The more rapid trophic magnification of THg in perch from Jūrmalciems is likely due to a strongly pronounced dietary shift from the least polluted Amphipoda and Mysida to prey with higher THg concentrations, specifically Decapoda and *N. melanostomus*, which was not observed in Salacgrīva. Elevated levels of Hg available in the coastal habitats and its biomagnification may result due to a range of factors, including low rates of primary and secondary production, hydrological connection to methylation sites (Ward et al., 2010), larger amount of nutrients (Kidd et al., 2012) which probably correlates with inflow of terrigenous organic matter (Jędruch et al., 2019) via coastal erosion and river runoff (Hilgendag et al., 2022; Jędruch et al., 2019) and finally bioavailability of Hg species (Jędruch et al., 2019; Kidd et al., 2012).

The results show that the trophic magnification of THg is site-specific due to the unique prey base with varying levels of contamination and availability. This finding is also supported by previous studies on trophic magnification and biomagnification of Hg (Fioramonti et al., 2022; Hilgendag et al., 2022; Jędruch et al., 2019; Ward et al., 2010).

## 3.4. Accumulation of HS in fish tissues and factors affecting it

### 3.4.1. Concentration-to-age normalisation

Accumulation of hazardous substances in fish tissues has been widely discussed in the scientific society (Łuczyńska and Tońska, 2006; Olsson et al., 2000; Polak-Juszczak, 2012, 2009; Vuorinen et al., 1998). Bioaccumulation is the result of a dynamic equilibrium between uptake and excretion of substances (Chojnacka and Mikulewicz, 2014), and therefore is an important process in biogeochemical cycling of any ecosystem. Due to that, it was essential to examine and describe the process taking place in the coastal waters of the studied area.

In order to explore age-related accumulation patterns depending on fish species and location the concentrations of Hg and two more metals (Cu and Zn) were measured in tissues of three coastal fish species: European perch (benthopelagic species), round goby (benthic species) and flounder (benthic species); and as a reference, two pelagic fish species: Baltic herring and Baltic cod; collected at four coastal stations and pelagically by scientific trawling in the Gulf of Riga and the Eastern Gotland Basin (**Figure 2.1**: C1–C4, P1–P9). Regression analyses and F test of the overall significance affirmed that all accumulation curves can be successfully described by means of exponential function, as it was previously suggested by Conti and Iacobucci (2008) and Brown and Depledge (1998) (**Figure 3.11**).

Based on the empirical observations, it was concluded that the exponential degree *b* (further in the text to as $K_{sp}$ – species specific coefficient) represents the accumulation speed depending on dietary ecology and contaminant properties, and it is relatively stable among species representatives, while the base coefficient *a* differs between stations

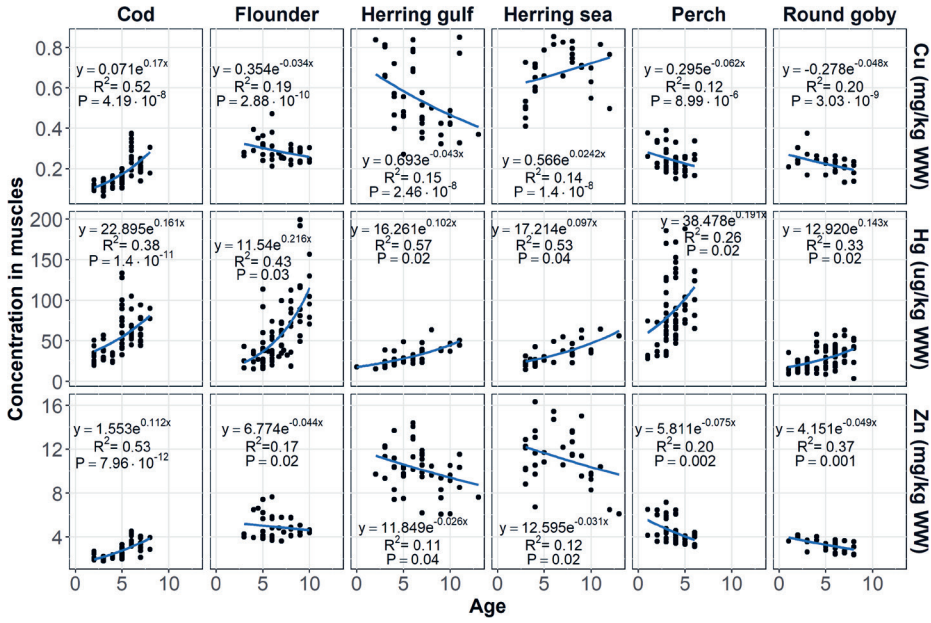


*Figure 3.11.* **Variation of copper (Cu), mercury (Hg), and zinc (Zn) wet weight (WW) concentrations with age, in dorsal muscles of cod, flounder, herring, perch, and round goby** (Suhareva et al., 2020)

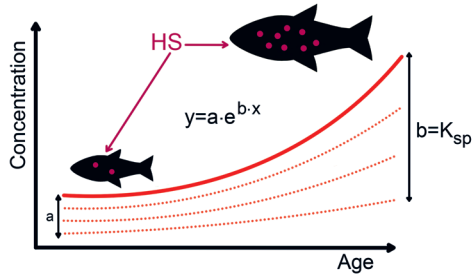


*Figure 3.12.* **Accumulation of hazardous substances (HS) with fish ageing following exponential function, where coefficient *a* characterises the feeding ground (pollution level and/or food availability) and base *b* characterises the pollutant accumulation rate by an organism**

and contains information about site-specific features such as food availability and local pollution levels (**Figure 3.12**).

The correlation between concentrations of bioaccumulative substances in fish tissues and fish body size presents a challenge when comparing concentration levels encountered at different locations or time periods because it degrades the outcome of statistical tests due to the variable age composition of the fish sample pool. Sample collection via gill nets and trawling is weakly controlled, therefore there is possible a situation, with high scatter of specimen age/size groups, which disables homogeneity of samples (**Figure 3.13**).

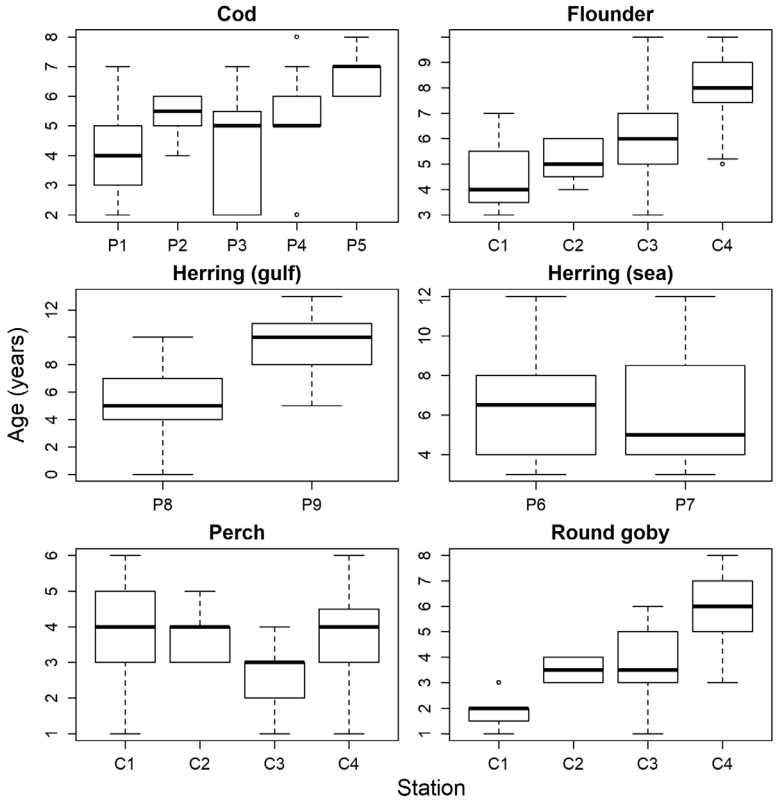


*Figure 3.13.* **Distribution of age groups among the sampling stations shown by five fish species: cod (G. morhua), flounder (P. flesus), herring C. harengus membras , perch (P. fluviatilis), round goby (N. melanostomus)** (Suhareva et al., 2020)

In order to enhance the comparability of metal concentrations among various age groups and sampling locations, normalising the measured concentrations of metals to correspond with a chosen age group was deemed necessary. The normalisation method was based on the equation for establishing an equivalently protective EQS for another biota taxon taking account trophic level, proposed by Guidance Document No. 32 on Biota Monitoring (the Implementation of $EQS_{biota}$) under the Water Framework Directive (EC, Directorate-General for Environment, 2015). After species-specific coefficient $K_{sp}$ was computed from the exponential base *b*, and site-specific characteristic was computed from exponential coefficient *a* (see Suhareva et al., 2020), the observed concentrations of metals were normalised to 2-years-old age group, according to the Equation 2 (see **Materials and Methods section**).

The normalisation by fish age allowed to smooth off significant differences among stations for several species and metals. As presented in **Table 3.5**, normalisation was observed to have an effect in 11 out of 18 cases. For perch, the effect of normalisation was minimal, as the age distribution of perch specimens was similar in all stations. The implementation of the normalisation technique considerably reduced the influence of bioaccumulation with fish ageing, similarly to that reported by Åkerblom et al. (2014),

*Table 3.5*

**Statistically significant differences in mean metal concentrations between sampling stations before and after the normalization** (Suhareva et al., 2020)

| | After normalization | | | Before normalization | | |
|---|---|---|---|---|---|---|
| | Hg | Cu | Zn | Hg | Cu | Zn |
| **Cod** | **p-value** | **p-value** | **p-value** | **p-value** | **p-value** | **p-value** |
| P2-P1 | 1.000 | 0.700 | 0.923 | 0.854 | *0.041* | 0.111 |
| P3-P1 | *0.044* | 1.000 | 0.877 | *0.037* | 1.000 | 0.932 |
| P4-P1 | 1.000 | 1.000 | 0.993 | 0.976 | 0.720 | 0.402 |
| P5-P1 | 1.000 | 0.928 | 0.955 | 0.153 | *<0.001* | *<0.001* |
| P3-P2 | 0.509 | 0.971 | 1.000 | 0.981 | 0.227 | 0.902 |
| P4-P2 | 1.000 | 0.975 | 1.000 | 1.000 | 0.987 | 1.000 |
| P5-P2 | 1.000 | 1.000 | 1.000 | 0.999 | 0.989 | 0.958 |
| P4-P3 | 0.421 | 1.000 | 1.000 | 0.931 | 0.937 | 0.992 |
| P5-P3 | 0.147 | 0.999 | 1.000 | 1.000 | *0.005* | 0.095 |
| P5-P4 | 1.000 | 0.999 | 1.000 | 0.990 | 0.501 | 0.825 |
| **Flounder** | **p-value** | **p-value** | **p-value** | **p-value** | **p-value** | **p-value** |
| C2-C1 | 1.000 | 0.044 | *<0.001* | 1.000 | 0.060 | *<0.001* |
| C3-C1 | 1.000 | 0.996 | *0.151* | 0.783 | 1.000 | 0.256 |
| C4-C1 | 1.000 | 0.957 | 0.056 | *0.028* | 1.000 | 0.118 |
| C3-C2 | 1.000 | 0.067 | *<0.001* | 0.916 | *0.004* | *<0.001* |
| C4-C2 | 1.000 | 0.176 | *<0.001* | 0.109 | *0.006* | *<0.001* |
| C4-C3 | 1.000 | 1.000 | 0.997 | 0.071 | 1.000 | 1.000 |
| **Herring gulf** | **p-value** | **p-value** | **p-value** | **p-value** | **p-value** | **p-value** |
| P9-P8 | 0.673 | 0.998 | 0.933 | <0.001 | 0.272 | 0.881 |
| **Herring sea** | **p-value** | **p-value** | **p-value** | **p-value** | **p-value** | **p-value** |
| P7-P6 | 0.906 | 0.280 | 0.770 | 0.936 | 0.143 | 0.902 |
| **Perch** | **p-value** | **p-value** | **p-value** | **p-value** | **p-value** | **p-value** |
| C2-C1 | *<0.001* | 0.099 | *0.016* | *<0.001* | 0.149 | *0.004* |
| C3-C1 | 1.000 | *0.034* | *<0.001* | 0.795 | *0.001* | *<0.001* |
| C4-C1 | 0.256 | 0.091 | 0.656 | 0.333 | 0.142 | 0.433 |
| C3-C2 | *<0.001* | *<0.001* | *0.031* | *<0.001* | *<0.001* | *<0.001* |
| C4-C2 | *0.001* | 1.000 | 0.522 | *0.008* | 1.000 | 0.430 |
| C4-C3 | 0.774 | *<0.001* | *<0.001* | *0.010* | *<0.001* | *<0.001* |
| **Round goby** | **p-value** | **p-value** | **p-value** | **p-value** | **p-value** | **p-value** |
| C2-C1 | 0.829 | 0.596 | 1.000 | 0.408 | 0.881 | 0.998 |
| C3-C1 | 0.347 | 0.851 | 0.920 | 1.000 | 1.000 | 0.996 |
| C4-C1 | 1.000 | 1.000 | 0.937 | *<0.001* | 0.133 | *0.002* |
| C3-C2 | 0.241 | 0.997 | 0.983 | 0.301 | 0.860 | 1.000 |
| C4-C2 | 0.875 | 0.314 | 0.990 | 1.000 | *0.030* | 0.340 |
| C4-C3 | 0.062 | 0.480 | 0.196 | *<0.001* | 0.281 | 0.055 |

p values below significance level $\alpha = 0.05$ are indicated by italic

thereby enabling estimation of the importance for other limiting factors, which might be dietary preferences or local environmental concentrations of heavy metals. Consequently, based on the normalised THg concentrations in perch, it was possible to confirm that the coastal area represented by station Daugavgrīva (C2) was significantly more polluted than other coastal areas (**Table 3.5**). The elevated THg concentration at this coastal station was probably caused by excessive contamination due to the proximity of three river estuaries: Daugava, Lielupe, and Gauja.

Fish age usually correlates well with fish size and weight. However, this rule is not absolute since depending on feeding conditions, fishes of the same age might substantially differ in size and weight. Therefore, Principal Component Analysis (PCA) was performed for each fish species separately (**Figure 3.14**) to assess the level of covariance. The PCA results exhibited strong positive correlation between age, length, and weight of specimens for all species, except Gulf herring and round goby, where the correlation is slightly lower, but still positive. To minimise unclearness of further results of the normalisation procedure, for this study, fish age was selected as an independent variable, although similarly length, weight and trophic level can be chosen as appropriate metrics for such an approach.

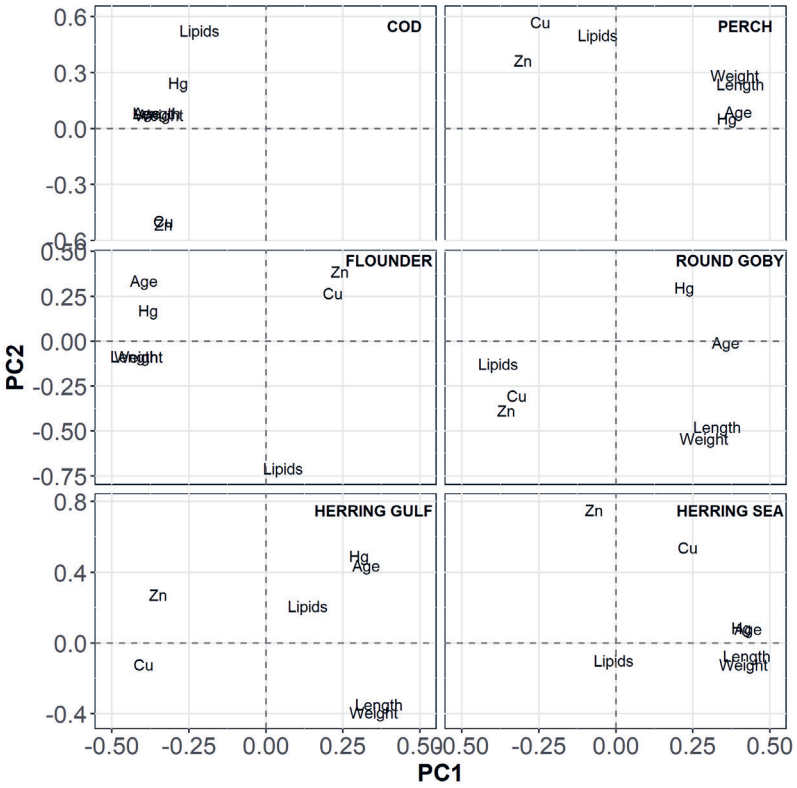


*Figure 3.14.* **Correlation between the variables (age, length, weight, content of lipids, muscle concentration of mercury (Hg), copper (Cu), and zinc (Zn) and the first two principal components (PC1 and PC2), performed on species-specific sub-sets of data (cod, flounder, herring, perch, round goby) and entire data set** (Suhareva et al., 2020)

### 3.4.2. Accumulation with perch age in conjoined ecosystems

Significant levels of hazardous substances tend to accumulate in the tissues of adult perch due to their high trophic position. By accounting for the migratory characteristics of this species, it is possible to determine the origin of the analysed samples and trace the chain of mercury uptake by different populations. Based on the case study presented in Suhareva et al. (2021) (**Figure 2.1**: F4, F5, C2), patterns of Hg bioaccumulation were compared between different perch populations in an interconnected aquatic system, as well as the effect of perch motility on the rate of accumulation.

The THg accumulation curves in relation to the length of individuals provided detailed information about the specific uptake tendencies. Regression analysis showed that the accumulation slopes were statistically similar among the groups (coefficient values from 0.015 to 0.029). Considering the information provided by accumulation curve (see **Section 3.4.1**), the results suggest that functional processes responsible for Hg accumulation (e.g., fish biometrics), Hg bioavailability, and chemical composition of Hg substances (Rainbow and Wang, 2005) are quite similar, irrespective of the origin of the specimen or the local feeding base. Simultaneously, intercepts of the curves varied substantially (coefficient values from 1.4 for group 3 to 2.1 for group 1), thus confirming high variation of background Hg concentrations between the studied sites, as stated above (**Figure 3.15**). The intercept for River Daugava was significantly higher comparing to Lake Ķīšezers, transitional waters in Daugavgrīva, while the intercept for Daugavgrīva was significantly lower than in other groups.

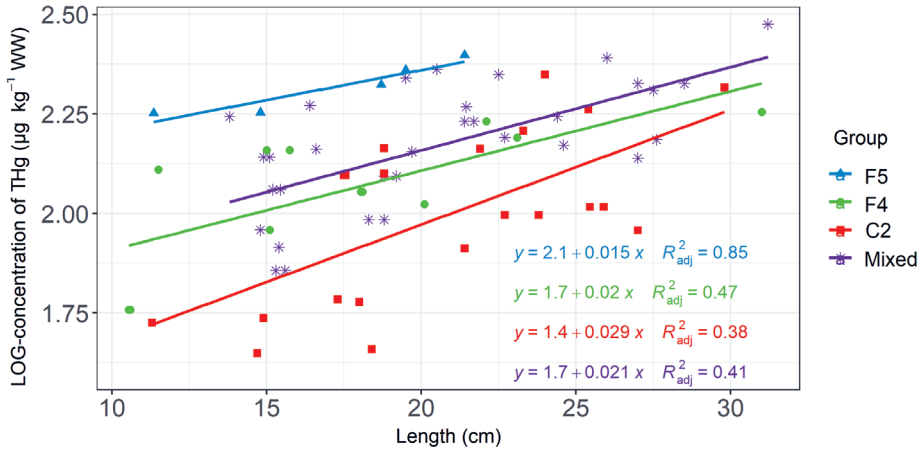


*Figure 3.15.* **THg accumulation curves expressed as linear equations of LOG-transformed THg concentration versus perch total length** (Suhareva et al., 2021)

# 4. CONCLUSIONS

The study showed that in addition to the atmospheric deposition which is recognized as the major transport route of such pollutants as PBDE, PCB and Hg, there is a **noticeable impact of trophic transfer on the concentrations of hazardous substances** measured in perch from the coastal waters of the Eastern Gotland Basin and the Gulf of Riga. As well as it was demonstrated, that environmental concentrations (measured in water and sediments) do not necessarily reflect the same pollution patterns as they are found in aquatic organisms of higher trophic position (in this case predatory fish).

Pollution level measured in predator and its potential prey at different monitored sites of the coastal ecosystem may not be consistent in terms of offering unambiguous and identical information on local pollution level due to **different trophic magnification rates** in omnivorous and piscivorous perch on one hand and its prey organisms which mostly follow simple non-variable diet, on the other.

However, the thesis presented a **concentration normalisation approach which allows to level up measured concentrations to the selected age** (length or trophic level) of the studied species, thus avoiding high variation of concentrations acquired during the life cycle of the model organism. The normalization method is intended to facilitate pollution monitoring by expanding the variability of samples suitable for comparative analysis. In addition, it can help identify individuals with prohibitively high concentrations of hazardous substances, thereby shedding light on the true level of pollution for a particular location.

In the thesis, accumulation trends of heavy metals in different fish species of Latvian part of the Gulf of Riga and the Easter Gotland Basin of the Baltic Sea were presented for the first time. It was demonstrated that **the accumulation curve contains information about species-specific features, such as feeding ecology or metabolic capacities**, which can probably be better explained with extended study of bioenergetics, and location-specific characteristics, such as pollution level and food availability.

It was found that even in a fully interconnected ecosystem consisting of different water bodies, the **uniqueness of the food base on the feeding area can affect the accumulation rate** of hazardous substances in fish tissues. This turns out to be a serious problem due to low salinity of the brackish Baltic Sea, and even more complicated in transitional waters of the Gulf of Riga, where some salinity tolerant freshwater species such as European perch can easily reside. It was successfully proven that perch are highly mobile species, which migrate between connected water bodies in both directions: to the sea and vice versa to inland reservoirs, regardless of their life stage. Moreover, the study revealed that the time spent **in heavily polluted nursery ground can highly affect concentrations found in the individuals of the smallest length groups**, which consequently may change entire trophic magnification and accumulation trend after the specimen migrates to a less polluted aquatic system.

Two key findings were discovered from the analysis of trophic magnification, apart from the high variation among the individual congeners of PBDE and PCB. First**, the magnitude and direction of TMS for specific congeners may not match between the prey and perch**, indicating uneven trophic magnification at the individual

substance level in the trophic chain. Second, the **directions of TMS in perch may not coincide between the studied sites**, which further support the previous suggestion regarding heavily polluted inland reservoirs where perch may spend its early life stage. The above suggests, that in case of PCB and PBDE, high mobility of perch, as well as relatively close accessibility of heavily polluted feeding grounds at some stage or along the annual migration paths, plays more important role in accumulation of these substances than trophic magnification from specific diet in the coastal waters of the catchment area.

**The main conclusion of this thesis is that the concentrations of targeted pollutants measured in organisms of high trophic position in coastal waters of the Eastern Gotland Basin and the Gulf of Riga can be affected by feeding ecology. However, the high mobility of coastal fish species may have a greater impact as they are able to biotransport hazardous substances trophically derived from other feeding grounds with higher pollution loads.**

The sea coast of Latvia is rich in lagoon type lakes (Lake Papes, Lake Liepājas, Lake Engures, Lake Babītes, Lake Ķīšezers, Lake Dzirnezers) and river estuaries (Venta, Lielupe, Daugava, Gauja, Salaca, etc.). Therefore, it can be assumed that the complex ecosystem should be considered and treated as a whole. Otherwise, in addition to the European perch, which is widely used as a matrix for pollution monitoring in the coastal zone of the Baltic Sea, other fish species could be considered in order to improve pollution assessment procedures. For example, the invasive round goby (*Neogobius melanostomus*) is a suitable candidate due to its narrow prey base (unpublished LIAE data on GIT content analysis) and wide distribution along the coast.

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# APPENDICES

## APPENDIX A

## ARTICLE I

Aigars, J., **Suhareva, N.**, Poikane, R.

**DISTRIBUTION OF POLYBROMINATED DIPHENYL ETHERS IN SEWAGE SLUDGE, SEDIMENTS, AND FISH FROM LATVIA**

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# Distribution of Polybrominated Diphenyl Ethers in Sewage Sludge, Sediments, and Fish from Latvia

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**Abstract:** The polybrominated diphenyl ethers (PBDEs) are bioaccumulative, persistent, and toxic. They have a high risk of emission into the environment via volatile losses and diffuse sources, such as commercial product disposal or the use of sewage sludge. The PBDEs' congeners were analyzed in municipal waste water treatment plant (WWTP) sludge, river and lake water, sediment, and fish samples, to investigate the concentrations in urban and natural locations. The sum of eight PBDE congener ($\sum_8$PBDE 28, 47, 99, 100, 153, 154, 183, 209) concentrations in WWTP sludge varied from 78 ng·g$^{-1}$ DW, to 714 ng·g$^{-1}$ DW. The BDE 209 constituted up to 93%–98% of $\sum_8$PBDE. In water, the concentrations of all of the measured PBDE congeners were below the limit of detection. Similarly, the concentration of BDE 209 in the sediments was below the limit of detection in all samples. The sum of seven PBDE congener concentrations in the sediments varied from 0.01 to 0.13 ng·g$^{-1}$ DW. The sum of eight PBDE congener concentrations in fish (European perch) tissues varied from 0.13 to 0.82 ng·g$^{-1}$ WW. As was recorded for the WWTP sludge, the BDE 209 was the dominant congener, constituting 24%–93% of $\sum_8$PBDE. The sum of seven PBDE congener concentrations, excluding BDE 209, as well as the concentrations of BDE 209 that were measured in WWTP sludge, exhibited a weak negative correlation (Pearson's $r = -0.56$, $p = 0.1509$ and $r = -0.48$, $p = 0.2256$, respectively) with the content of dry matter in the sludge. The sum of seven PBDE congener concentrations measured in sediments exhibited a strong negative correlation (Pearson's $r = -0.82$, $p = 0.0006$) with the content of dry matter in the sediments, and a strong positive correlation (Pearson's $r = 0.68$, $p = 0.0109$) with the total carbon content. The obtained results indicated that the fine-grained WWTP sludge particles, with a larger relative surface area, adsorbed BDE 209 the most effectively. This finding was supported by the relatively low environmental concentrations of PBDE congeners, especially BDE 209, which can be explained by the lack of using sewage sludge in agricultural application in Latvia. Furthermore, it seems that, at present, the observed differences in the PBDE congener concentrations in sediments can be attributed to differences in the physical-chemical properties of sediments.

**Keywords:** PBDEs; sewage sludge; sediments; fish; distribution

## 1. Introduction

The polybrominated diphenyl ethers (PBDEs) are halogenated compounds, which were listed as persistent organic pollutants (POPs) by the Stockholm Convention in 2009 [1]. The lipophilic, bioaccumulative, and toxic nature of these compounds [2], combined with the diversity of sources and transport mechanisms [3,4], have been causing significant public concern during the last decades. Three commercial mixtures (penta-, octa-, and deca-BDE), introduced in the 1970s, have been widely used as additive flame retardants in various industrial applications, such as plastics, textiles, electronics, building materials, furniture, and other products for manufacturing [3,5,6]. However, they have a high risk of emission into the environment [7]. Due to volatile losses and discharges during

the constant use and recycling of the products containing these compounds, the diffuse sources of PBDEs, represented by commercial product disposal, the use of sewage sludge, agricultural run-off, and domestic wastewater [8,9], become even more significant than the point sources [10], imposing an additional environmental concern.

The first recorded detection of the presence of PBDEs in the environment was published by Andersson and Blomqvist, in 1981 [11]. Since then, PBDEs have been found in various environmental and biological samples across the globe [4,12–15]. Notably, the contamination of PBDEs was even reported in places with no local point source or industrial production [16,17]. PBDEs tightly bind to solid particles due to their properties [14], however, they can be transferred to the air due to volatilization [10], and transported over large distances. In the environment, PBDEs are transferred to aquatic systems, accumulated in sediments and biota [3,10,13], and are eventually biomagnified in top predators [18–20], to the point at which they can be transferred to humans through the consumption of contaminated food sources [2]. The transport mechanism described above is supported by environmental studies, which report on the increased levels of tetra- to deca-BDE isomers found in marine mammals, bird eggs, and human tissues [5,14]. According to several recent studies, the toxic effect caused by PBDEs can be observed as suppression of the immune system, reproductive dysfunction, endocrine disruption, change of thyroid hormone levels [5,8,21–23], damage of liver and kidney morphology, and fetal toxicity/teratogenicity cases [24–29]. Due to the toxicological effects, the production of PBDE congeners (penta- and octa-BDE) and their commercial availability were banned in the European Union [30–32]. These restrictions promoted a general decrease of PBDE concentrations in soils within Europe [33]. In addition, a decline of penta- and octa-mix PBDE concentrations has been observed in sewage sludge during recent years [34]. At the same time, concentrations of BDE 209 in sewage sludge, have exhibited a clear increase between 2004 and 2010 [34]. This fact poses a serious environmental concern, since current evidence suggests that some aquatic organisms, including fish, have a capacity to de-brominate BDE 209 to lower-brominated congeners [35,36], which have higher mobility and toxicological properties. Furthermore, it has been demonstrated that benthic fauna is able to re-mobilize buried PBDEs from sediments [37], and consequently, sediments become an important secondary source of these compounds.

The aim of this study was to evaluate the concentration levels and composition of PBDE congeners in the water, fish tissues, and sediments, as well as in the sludge collected from different WWTPs in Latvia.

## 2. Materials and Methods

Sewage sludge samples from effluent, after the dewatering step, were collected from eight urban WWTPs (Table 1), located in the biggest cities and smaller towns of Latvia. The WWTPs were selected based on a range of city sizes and the type of effluents treated by WWTPs.

**Table 1.** General information on city-size, waste water type, and the amount treated by WWTP.

| WWTP | Population, Inhabitants | Treatment, $m^3$/Day | Waste Water Type |
|---|---|---|---|
| Riga WWTP | 696,593 | 350,000 | Municipal and industrial waste water |
| Daugavpils WWTP | 96,028 | 12,600 | Municipal and manufacturing waste water, rain water |
| Liepaja WWTP | 78,413 | 18,400 | Municipal waste water, manufacturing and industrial waste water |
| Ventspils WWTP | 40,057 | 19,200 | Municipal and manufacturing waste water |
| Rezekne WWTP | 31,591 | 5600 | Municipal waste water and bio-toilets, manufacturing and industrial waste water |
| Valmiera WWTP | 25,318 | 5000 | Municipal waste water |
| Saldus WWTP | 11,625 | 2900 | Municipal waste water only |
| Dobele WWTP | 10,231 | 3500 | Industrial and municipal waste water |

The water and sediment samples were collected from sampling locations of five rivers and eight lakes (Table 2). Samples of fish dorsal muscles were collected from the same sampling sites as the sediment samples (Table 2), however, the biomass was only sufficient for analysis purposesin 10 cases out of the 13 sampled biota European perch (*Perca fluviatilis*),. The selection of the sampling sites was based on previously reported cases of trans-boundary impact, and the human influence on urban, agricultural, or industrial land use location. The selection of European perch (*Perca fluviatilis*) for the biota matrix was based on its trophic level (predator), and the high abundance of the species occurring in both fresh and brackish waters of the region.

**Table 2.** Description of the investigated water bodies and sediment characteristics.

| Name | Type | Coordinates WGS84 | Mean Depth, m | Length, km | Surface Area, ha | Biota Sampled | Sediments DM (%) | Sediments OC (%) |
|---|---|---|---|---|---|---|---|---|
| Salaca | river | 57.756183, 24.351069 | 0.15 | 95 | NA | Yes | 61.1 | 1.88 |
| Mazsalaca | river | 57.857745, 25.051484 | 0.15 | 95 | NA | Yes | 80.0 | 0.17 |
| Pedele | river | 57.778913, 26.024400 | 0.3 | 31 | NA | No | 81.3 | 0.16 |
| Gauja | river | 57.160162, 24.265724 | 2.0 | 452 | NA | Yes | 68.0 | 0.47 |
| Abuls | river | 57.549172, 25.680512 | 0.4 | 52 | NA | No | 58.9 | 1.92 |
| Dūņezers | lake | 57.150275, 24.358443 | 1.1 | NA | 145.6 | Yes | 17.6 | 18.9 |
| Burtnieks | lake | 57.740413, 25.241186 | 2.9 | NA | 4006.0 | Yes | 14.0 | 10.5 |
| Murāts | lake | 57.575807, 27.085749 | 2.2 | NA | 77.5 | Yes | 15,2 | 14.7 |
| Juveris | lake | 57.218670, 25.676606 | 8.5 | NA | 77.5 | Yes | 16.4 | 11.2 |
| Lizdoles | lake | 57.293444, 25.838392 | 4.4 | NA | 53.9 | Yes | 8.8 | 15.7 |
| Trikātas | lake | 57.541006, 25.714902 | 1.8 | NA | 13.0 | Yes | 8.1 | 10.5 |
| Alauksts | lake | 57.091547, 25.774342 | 3.3 | NA | 774.8 | No | 7.0 | 16.4 |
| Limbažu | lake | 57.486541, 24.699041 | 3.8 | NA | 24.8 | Yes | 47.2 | 15.4 |

NA: not applicable.

### 2.1. Sample Collection

The sewage sludge, water, and sediment samples were placed and kept in previously unused amber-glass sample containers, precleaned and *Certified* to meet US EPA performance-based specification.

The integrated (1 h) sewage sludge samples were collected between June 2010 and February 2011 by the WWTP operational staff in 500 mL containers, and were covered by polytetrafluoroethylene-lined (PTFE) plastic screw-caps. To avoid the adsorption of PBDEs at the PTFE parts of the screw-cap, the container neck was kept isolated by aluminum folium.

The water and sediment samples were collected between the July and September of 2012. Water was sampled by a Van Dorn water sampler 0.3–0.5 m below the water surface, and immediately upon sampling, the water was transferred to prepared glass jars. Sediment samples were collected with a hand-operated Wildco Ponar or VanVeen bottom grab sampler. Sampling was performed at five to seven points around the observation site. All sub-samples were mixed together and sieved,

to remove particles larger than 2.0 mm. Sieved sediment samples were then transferred to prepared glass jars.

European perch (*Perca fluviatilis*) individuals were sampled by means of fish hooks. Sampling was performed by dully authorized personnel, possessing valid fishing permits. Thereafter, the fish tissues were obtained for analysis, in accordance with animal ethic care guidelines. Every sample collected during the study was directly transported from the sampling site to the laboratory, in a mobile cool box filled with cooling agent cartridges.

The obtained sewage sludge, water, and sediment samples were stored in dark conditions, at a temperature of 4–8 °C, until the chemical analysis had been performed. The soft tissues obtained from the fish were stored in a freezer at a temperature of −18 °C, until the chemical analysis had been carried out.

### *2.2. Analytical Procedures*

The pretreatment and analyses of all types of the samples were performed according to the method US EPA 1614, with modification. Water and sewage sludge samples were analyzed by the accredited commercial laboratory, ALS Laboratory Group (Czech Republic), with the estimation of uncertainty for each PBDE congener being equal to 30%. Sediments and fish samples were treated on a commercial basis by the Institute of Food Safety, Animal Health, and Environment–"BIOR" (Latvia), and the recovery range for the PBDEs was 75%–123%. The method of determining the PBDEs in the required matrices was accredited for both engaged laboratories. The limits of quantification were defined on the basis of the blank level (see Supplementary Materials).

#### 2.2.1. Sample Preparation and Clean-Up for PBDEs Analysis

Sample aliquots of sediment (10 ± 2 g) and the fresh dorsal muscle of fish (10 ± 2 g), were spiked with 500 µL of $^{13}C_{12}$-labeled PBDE 138 congener mixture solution, diluted with toluene to a final concentration of 1–5 pg·µL$^{-1}$, before being mixed with 100 g of anhydrous sodium sulfate. After equilibration for 12 h, at UV-protected conditions, e.g., room temperature under an aluminum foil cover, the samples were ground and extracted, using Soxhlet extraction with a dichloromethane/*n*-hexane (1:1, *v*/*v*) mixture for at least 16 h. The extracts were filled into round-bottom flasks and the solvent was removed using a rotary evaporator at <30 °C. The high molecular substances were removed by gel permeation chromatography (GPC). The system was equipped with a glass column (50 × 2.5 cm), filled with 50 g of non-polar divinyl-benzene/styrene copolymer Bio-Beads S-X3 (Bio-Rad, Philadelphia, PA, USA, 3% cross linkage, 40–80 µm bead size, ≤2000 MW limit) stationary phase, and eluted with cyclohexane/ethyl acetate (1:1, *v*/*v*) mobile phase, at a flow rate of 5 mL·min$^{-1}$. The automated GPC program was as follows: dump time 0–19 min, collection time 19–45 min; the collected eluate was concentrated by rotary evaporation at <30 °C. The pre-purified sample extract was placed on top of a glass column (25 × 1.2 cm) filled with 2.5 g of silica gel, containing 50% (*v*/*v*) sulfuric acid for the degradation of remaining lipids. The analytes were eluted with 1.0 mL of toluene, and subsequently, with 25 mL of *n*-hexane. After rotary evaporation to about 150–200 µL, the sample extracts were transferred to 2 mL chromatographic vials, treated with 37 N sulfuric acid (30 µL), and mixed. The mixture was allowed to stand for 20 min, before being centrifuged at 3000 rpm (1508 RCF), in order to separate the acidic and organic layers. The acidic bottom layer was discarded, the organic layer was evaporated, and recovery standard ($^{13}C_{12}$ PBDE 138) solution in *n*-nonane was added until the solution was 50 µL, at which point content of PBDEs was analyzed [38].

#### 2.2.2. Instrumental Analysis and Quantification of PBDEs by GC-HRMS

The instrumental analysis was performed by a Micromass Autospec Premier high resolution mass spectrometer (Milford, CT, USA), coupled with an Agilent 6890 N gas chromatograph (Santa Clara, CA, USA), according to the procedure and GC conditions described by Zacs, et al. [38].

#### 2.2.3. Analysis of Dry Matter Content, Total Carbon, and Lipid Content

The dry-matter content of fish muscle, sediment, and sludge samples, was detected by a gravimetric method, during which separate wet subsamples were dried at 105 ± 5 °C until a constant dry weight was observed.

The total content of carbon in the sediments was determined according to the ISO method 10694:1995, "Soil quality—Determination of organic and total carbon after dry combustion (elementary analysis)". A 2 mg DW sub-sample was used to measure the total content of carbon, and was recorded by a Vario EL III CHNOS Elemental Analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany).

A 20 g DW sub-sample was used in Soxhlet extraction with a dichloromethane and n-hexane (1:1, *v*/*v*) mixture, in order to determine the lipid content. The extraction time was 16 h, after which the extracts were filled into pre-weighted round-bottom flasks for solvent evaporation, by a rotary evaporator at <30 °C. The lipid content was determined gravimetrically.

### *2.3. Data Exploration and Statistical Assessment*

Data exploration and statistical analyses were performed using R software for Windows, Release 3.3.2. The strength of the linear associations between the measured PBDE concentrations, content of dry matter (DM) in sewage sludge, content of dry material (DW), total organic carbon (TOC) in sediments, and content of lipids in fish samples, was investigated by means of the Pearson Correlation Coefficient, with a statistical significance level set at $\alpha = 0.05$. Simple regression models with a single independent parameter were developed, to evaluate the dependence of the concentration of BDE 209 on the content of dry matter in sewage sludge, and the variations in the PBDEs concentrations, depending on the content of dry matter and the total organic carbon in sediment samples. The obtained regression models were evaluated via regression diagnostic tools, including graphical methods and formal statistical tests.

## 3. Results

### *3.1. WWTP Sludge*

The concentrations of PBDE congeners (BDE 28, 47, 99, 100, 153, 154, 183, 209) in WWTPs sludge ranged from 0.1 ng·g$^{-1}$ DW (BDE 28) to 700 ng·g$^{-1}$ DW (BDE 209). Both values were observed at the Valmiera WWTP (Figure 1). The sum of all of the detected congeners ($\sum_8$PBDE) varied substantially among WWTPs, from 78 ng·g$^{-1}$ DW at the Liepaja WWTP, to 714 ng·g$^{-1}$ DW at the Valmiera WWTP. The concentrations of PBDEs in the sewage sludge reported in this study, are at the low end of the wide concentration range reported elsewhere for WWTPs' sludge [6,39,40], and similar to the earlier studies, the concentrations of BDE 209 (on average 296 ng·g$^{-1}$ DW) constituted 89%–98% of the $\sum_8$PBDE concentration registered. The second (BDE 99) and third (BDE 47) most abundant PBDE congeners corresponded to only 0.8%–4.9% and 0.7%–4.4% of the $\sum_8$PBDE, respectively, while the remaining congeners constituted less than 1% of the $\sum_8$PBDE.

The average concentration of the PBDEs, excluding BDE 209, ($\sum_7$PBDE) was 13 ng·g$^{-1}$ DW. The $\sum_7$PBDE, as well as BDE 209, exhibited a weak negative correlation (Pearson's $r = -0.56$, $p = 0.1509$ and $r = -0.48$, $p = 0.2256$, respectively) with the dry-matter content of WWTP sludge (Table 3). The correlation between BDE 209 and the dry-matter content of WWTP sludge is substantially improved (Pearson's $r = -0.98$, $p = 0.0008$) if the data from two WWTPs, Saldus and Rezekne, are not included in the analysis (Table 3). The decision to exclude the outlying observations was based on the statistical diagnosis of linear regression (Figure 2), and a comparison of the significance levels of the accessed interactions ($p$-value = 0.2256 for initial model versus $p$-value = $8.018 \times 10^{-4}$ for the ameliorated model). At the same time, BDE 209 concentration did not exhibit significant correlation with concentrations of other congeners (Table 3).

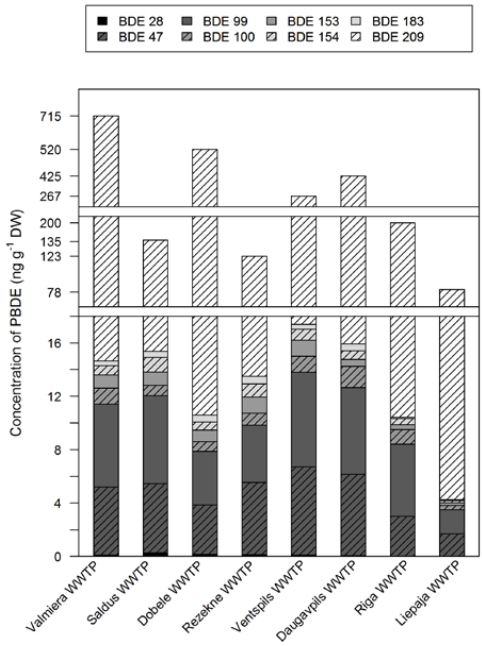


**Figure 1.** Concentrations of PBDEs in sewage sludge from eight urban WWTPs in Latvia.

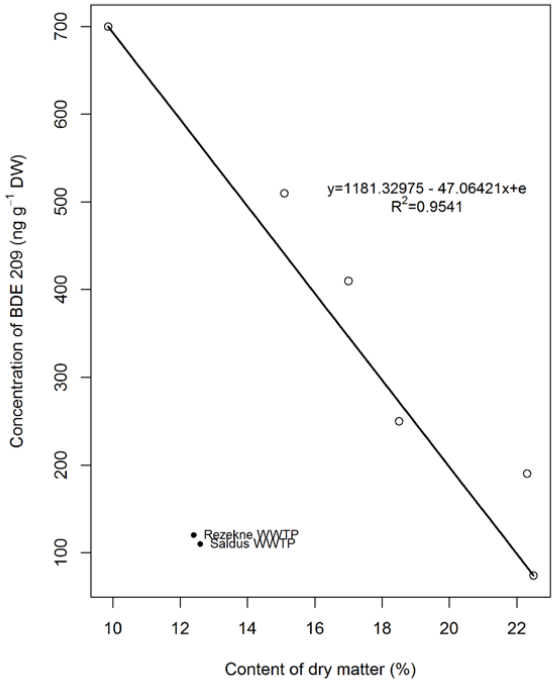


**Figure 2.** Linear regression curve of BDE 209 concentrations with the sewage sludge content of dry matter.

**Table 3.** Pearson correlation coefficients and *p*-values calculated for PBDE concentrations and the content of dry matter analyzed in sewage sludge samples.

| **Sludge** | **BDE 28** | **BDE 47** | **BDE 99** | **BDE 100** | **BDE 153** | **BDE 154** | **BDE 183** | **BDE 209** | **$\sum_7$PBDE** | **BDE 209 $_{6\ WWTPs}$** [1] | **DM** [2] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BDE 28 | 1.00 | −0.50 | −0.20 | −0.86 | 0.31 | 0.63 | 0.14 | −0.44 | −0.32 | 0.25 | −0.39 | |
| BDE 47 | 0.3172 | 1.00 | 0.82 | 0.72 | 0.74 | 0.78 | 0.72 | 0.25 | 0.97 | 0.45 | −0.55 | |
| BDE 99 | 0.7065 | 0.0121 | 1.00 | 0.78 | 0.51 | 0.64 | 0.39 | 0.31 | 0.93 | 0.42 | −0.38 | |
| BDE 100 | 0.0263 | 0.0455 | 0.0217 | 1.00 | 0.20 | 0.28 | 0.35 | 0.47 | 0.75 | 0.42 | −0.19 | |
| BDE 153 | 0.5495 | 0.0370 | 0.2013 | 0.6338 | 1.00 | 0.85 | 0.71 | 0.19 | 0.72 | 0.59 | −0.74 | |
| BDE 154 | 0.1808 | 0.0234 | 0.0887 | 0.4967 | 0.0074 | 1.00 | 0.74 | −0.03 | 0.80 | 0.60 | −0.73 | *R* |
| BDE 183 | 0.7874 | 0.0450 | 0.3415 | 0.3899 | 0.0491 | 0.0356 | 1.00 | 0.31 | 0.65 | 0.69 | −0.73 | |
| BDE 209 | 0.3796 | 0.5518 | 0.4547 | 0.2435 | 0.6458 | 0.9394 | 0.4613 | 1.00 | 0.30 | – | −0.48 | |
| $\sum_7$PBDE | 0.5378 | <0.0001 | 0.0008 | 0.0312 | 0.0449 | 0.0179 | 0.0792 | 0.4693 | 1.00 | 0.50 | −0.56 | |
| BDE 209 $_{6WWTPs}$ [1] | 0.7531 | 0.3667 | 0.4048 | 0.4099 | 0.2187 | 0.2035 | 0.1293 | - | 0.3084 | 1.00 | −0.98 | |
| DM[2] | 0.4495 | 0.1606 | 0.3550 | 0.6557 | 0.0355 | 0.0391 | 0.0417 | 0.2256 | 0.1509 | 0.0008 | 1.00 | |
| | | | | | | *p* | | | | | | |

[1] Concentration of BDE 209, excluding values from Rezekne WWTP and Saldus WWTP; [2] Content of dry matter (%).

### 3.2. Water and Sediments

The concentrations of all measured PBDE congeners, in all of the water samples, were between the limit of detection (LOD) and the limit of quantification (LOQ). The LOD and LOQ of congeners (BDE 28, 47, 99, 100, 153 and 154), for which the sum of the Ecological Quality Standard (EQS) (140 ng·L$^{-1}$) is set in Directive 2013/39/EU for inland surface waters, varied in the samples, between 0.8 and 1.1 ng·L$^{-1}$ for LOD, and 1.7 and 2.3 ng·L$^{-1}$ for LOQ. For these congeners, it is possible to use LOD values to calculate the assumed maximal concentration of 2.3 ng·L$^{-1}$. This estimated concentration is below the EQS that is set in Directive 2013/39/EU. The congeners BDE 183 and 209 are not included in the group of priority PBDEs, for which the EQS is set. However, it is reasonable to evaluate their concentrations in water due to the ability of biological organisms to de-brominate them to lower-brominated congeners. Since the possible concentrations of BDE 183 (below LOQ 0.38 ng·L$^{-1}$) and BDE 209 (below LOQ 2.70 ng·L$^{-1}$) could also be considered as very low, it can be safe to assume that the set EQS would not be exceeded, even if all of the BDE 183 and 209 would be de-brominated.

The concentrations of BDE 209 in sediments were below LOD (0.2 ng·g$^{-1}$ DW) in all samples. The concentrations of other PBDE congeners in sediment samples (Figure 3) varied significantly, from $1.3 \times 10^{-4}$ ng·g$^{-1}$ DW for BDE 28 in the sediments of river Pedele, to 0.052 ng·g$^{-1}$ DW for BDE 47 in the sediments of lake Trikata. Similarly, as is the case for WWTP sludge, the levels of PBDE congeners and the $\sum_7$PBDE (range 0.01–0.13 ng·g$^{-1}$ DW, on average 0.05 ng·g$^{-1}$ DW) detected in the sediments, were on the low end of the range of values observed elsewhere [10,41,42].

The $\sum_7$PBDE exhibited non-linear relations (Figure 4) and a significant (Pearson's $r = -0.82$, $p = 0.0006$) negative correlation with the content of dry matter, and a positive (Pearson's $r = 0.68$, $p = 0.0107$) nonlinear correlation with the total content of carbon in the sediments (Table 4).

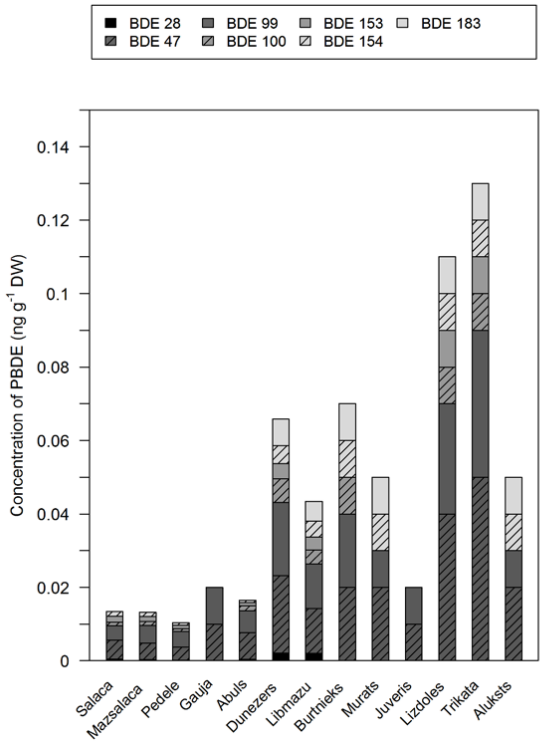


**Figure 3.** Concentrations of PBDEs in sediments from rivers and lakes in Latvia.

**Table 4.** Pearson correlation coefficients and *p*-values calculated for PBDE concentrations, the content of dry material, and the total organic carbon analyzed in sediment samples.

| **Sediments** | **BDE 28** | **BDE 47** | **BDE 99** | **BDE 100** | **BDE 153** | **BDE 154** | **BDE 183** | **DW [1]** | **∑$_7$PBDE** | **TOC [2]** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BDE 28 | 1.00 | −0.15 | −0.04 | 0.10 | 0.12 | −0.16 | −0.01 | 0.07 | −0.07 | 0.36 | |
| BDE 47 | 0.6352 | 1.00 | 0.96 | 0.77 | 0.82 | 0.78 | 0.79 | −0.74 | 0.98 | 0.57 | |
| BDE 99 | 0.9065 | <0.0001 | 1.00 | 0.88 | 0.84 | 0.67 | 0.70 | −0.67 | 0.94 | 0.51 | |
| BDE 100 | 0.7358 | 0.0021 | <0.0001 | 1.00 | 0.75 | 0.61 | 0.63 | −0.52 | 0.77 | 0.42 | |
| BDE 153 | 0.6871 | 0.0006 | 0.0003 | 0.0034 | 1.00 | 0.43 | 0.44 | −0.37 | 0.78 | 0.34 | |
| BDE 154 | 0.6048 | 0.0015 | 0.0128 | 0.0274 | 0.1440 | 1.00 | 0.98 | −0.80 | 0.83 | 0.70 | *R* |
| BDE 183 | 0.9677 | 0.0013 | 0.0083 | 0.0201 | 0.1279 | <0.0001 | 1.00 | −0.84 | 0.85 | 0.80 | |
| DW [1] | 0.8080 | 0.0036 | 0.0122 | 0.0682 | 0.2174 | 0.0011 | 0.0004 | 1.00 | −0.82 | −0.86 | |
| ∑$_7$PBDE | 0.8104 | <0.0001 | <0.0001 | 0.0022 | 0.0017 | 0.0005 | 0.0003 | 0.0006 | 1.00 | 0.68 | |
| TOC [2] | 0.2335 | 0.0426 | 0.0778 | 0.1580 | 0.2519 | 0.0077 | 0.0011 | 0.0002 | 0.0107 | 1.00 | |
| | | | | | *p* | | | | | | |

[1] Content of dry material (%); [2] Content of total organic carbon (%).

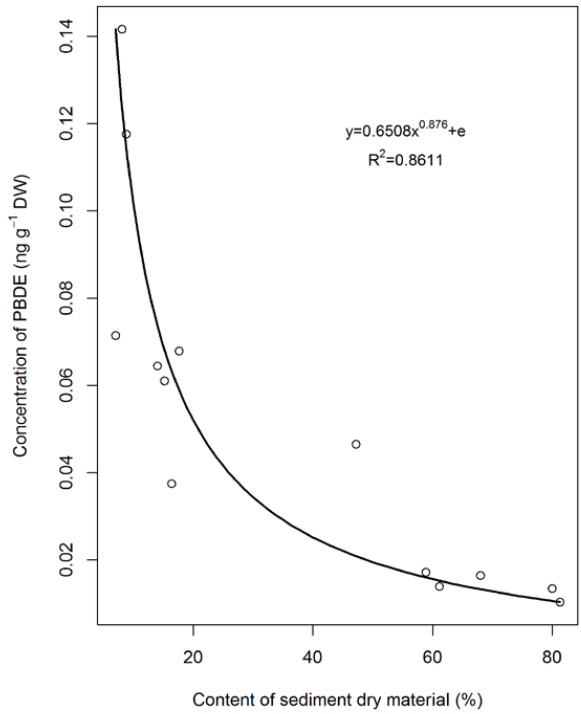


**Figure 4.** Non-linear regression showing dependency of $\sum_7$PBDE concentration from the sediment content of dry matter in lakes and rivers of Latvia.

### *3.3. Fish*

The PBDE congener concentration range (Figure 5) in analyzed fish tissues ranged from $3 \times 10^{-4}$ ng·g$^{-1}$ WW (BDE 28 in lake Murats), to 0.36 ng·g$^{-1}$ WW (BDE 209 in lake Trikata). Similar to sewage sludge, BDE 209 was a dominant congener, constituting 24%–93% (53% on average) of the $\sum$PBDE$_8$.

The concentrations of BDE 47, BDE 99, BDE 100, BDE 153, and BDE 154, constituted 18.6%, 7%, 4.9%, 1.5%, and 3.2%, respectively, of the total, while other PBDE congeners represented less than 1% of the total amount. The accumulation of BDE 209 in the fish tissues observed in this study, is consistent with previous observations [43,44]. It is important to note that the sum of the concentrations of the congener numbers 28, 47, 99, 100, 153, and 154 (range from 0.017 to 0.624 ng·g$^{-1}$ WW), exceeded the EQS (0.0085 ng·g$^{-1}$ WW) set for biota in Directive 2013/39/EU, in all of the rivers and lakes targeted by this study.

The majority of the investigated PBDE congeners demonstrated a good correlation among themselves, except for the concentrations of BDE 209 and BDE 183, which did not correlate to any of the other congeners (Table 5). The established lipophilic properties of PBDEs [2] have been previously used to explain the accumulation of PBDEs in fish tissues, corresponding to their lipid content [45]. However, in our study, only the concentrations of BDE 183 exhibited a medium-strong correlation (Table 5) with the lipid content of fish tissues (Pearson's $r = 0.46$, $p = 0.1803$). The lipid content of the fish tissues observed in this study was between 1.1% and 3.5%. This is at the low end of the lipid content range presented in previous studies [44]. Therefore, we have to assume that, in our study area, factors other than the lipid content could also be important. For example, Bertelsen et al. [46] suggested that proteins and other non-lipid components have a large influence on the bioaccumulation of PBDEs,

if the lipid content is low. However, since we did not measure proteins or other non-lipid parameters in our experiment, we have no observational data to support the conclusion on the importance of proteins, or other components, on the accumulation of PBDEs.

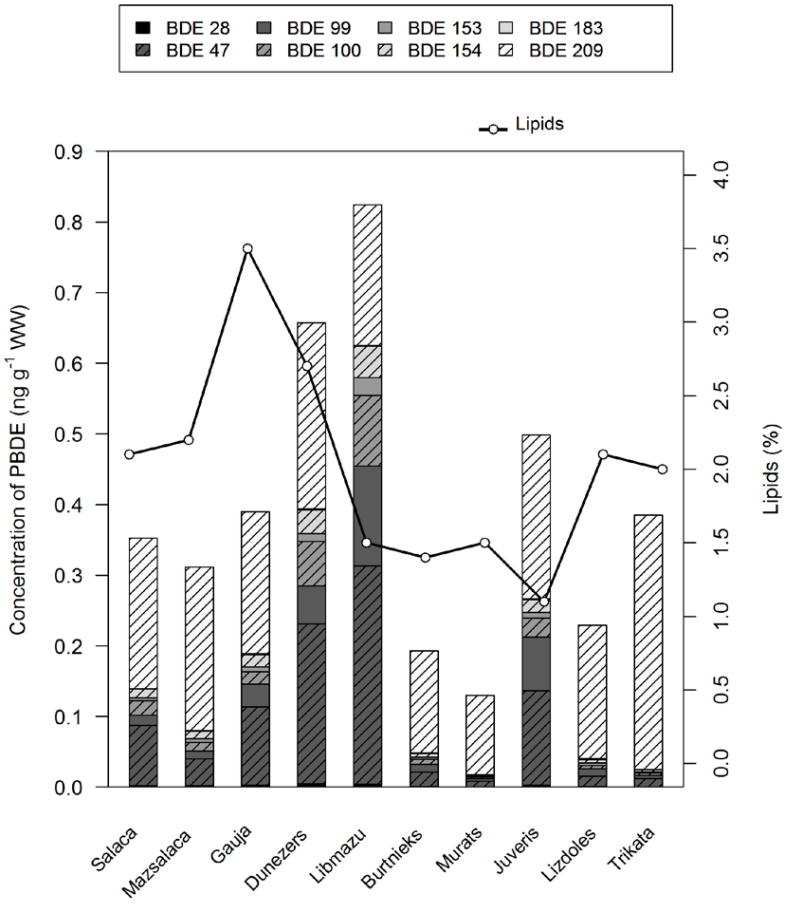


**Figure 5.** Concentrations of PBDEs and lipid content in tissues of European perch (*Perca fluviatilis*) from rivers and lakes of Latvia.

**Table 5.** Pearson correlation coefficients and *p*-values calculated for PBDE concentrations and the content of lipids analyzed in tissues of European perch (*Perca fluviatilis*).

| Fish | BDE 28 | BDE 47 | BDE 99 | BDE 100 | BDE 153 | BDE 154 | BDE 183 | BDE 209 | $\sum_7$PBDE | Lipids (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BDE 28 | 1.00 | 0.96 | 0.80 | 0.91 | 0.84 | 0.97 | 0.62 | 0.15 | 0.93 | 0.24 | |
| BDE 47 | <0.0001 | 1.00 | 0.92 | 0.98 | 0.94 | 0.99 | 0.52 | 0.11 | 0.99 | 0.06 | |
| BDE 99 | 0.0060 | 0.0002 | 1.00 | 0.92 | 0.96 | 0.90 | 0.40 | 0.03 | 0.95 | −0.22 | |
| BDE 100 | 0.0003 | <0.0001 | 0.0002 | 1.00 | 0.97 | 0.98 | 0.45 | 0.08 | 0.98 | −0.03 | |
| BDE 153 | 0.0024 | <0.0001 | <0.0001 | <0.0001 | 1.00 | 0.95 | 0.44 | 0.02 | 0.97 | −0.09 | |
| BDE 154 | <0.0001 | <0.0001 | 0.0003 | <0.0001 | <0.0001 | 1.00 | 0.55 | 0.11 | 0.99 | 0.10 | *R* |
| BDE 183 | 0.0574 | 0.1271 | 0.2558 | 0.1921 | 0.2017 | 0.0995 | 1.00 | 0.00 | 0.49 | 0.46 | |
| BDE 209 | 0.6694 | 0.7670 | 0.9373 | 0.8186 | 0.9489 | 0.7712 | 0.9963 | 1.00 | 0.09 | 0.23 | |
| $\sum_7$PBDE | 0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.1497 | 0.8146 | 1.00 | −0.01 | |
| Lipids (%) | 0.4965 | 0.8612 | 0.5478 | 0.9336 | 0.8135 | 0.7747 | 0.1803 | 0.5147 | 0.9729 | 1.00 | |
| *p* | | | | | | | | | | | |

## 4. Discussion

The largest variations for a single congener in the WWTPs, were observed for BDE 209, as has also been observed in previous studies [6], while the levels of other congeners exhibited lower levels of variation. The observed variability in the BDE 209 levels could not be attributed to the city size, or the capacity of the WWTP, which is in agreement with conclusions from earlier studies [47]. At the same time, the approach for explaining the variance in BDE 209 levels, in relation to the different effluent types, e.g., industrial versus domestic [6], cannot be used in this study, since domestic sources of waste water dominated over industrial sources, in all cases. We cannot exclude that, in cities, such as Valmiera or Dobele, where comparatively high levels of BDE 209 in WWTP sludge were observed, the elevated BDE 209 input was generated by industrial objects. However, to the best of our knowledge, this is not the case. So, it is plausible to assume that the generation of the BDE 209 load per inhabitant, could be rather similar in all observed cities, but the capacity of WWTP to trap BDE 209, is different, depending on sludge properties. This assumption is supported by the observed negative correlation ($r = -0.48$, $p = 0.2256$) between the content of dry matter and the BDE 209 concentration (Table 3), which clearly demonstrates that fine-grained sludge particles, with a larger relative surface area, adsorb BDE 209 much more effectively than relatively coarser-grained sludge particles (Figure 2). At the same time, it should be recognized that this correlation is built on a limited number of observation points, and two cases clearly diverge from the obtained linear regression curve (Figure 2). Therefore, prior to drawing a final conclusion, the factors that can possibly affect the effectiveness of BDE 209 retention in WWTP sludge, should be tested in a more detailed study.

It should be noted that the effectiveness of BDE 209 removal by WWTP sludge, poses a certain problem in relation to the widely practiced land-application of sludge in agriculture [6,47]. As has been previously shown, terrestrial plants can de-brominate BDE 209 to more mobile and toxic congeners [48,49]. Furthermore, substantial amounts of sludge applied to land are bound to enter aquatic environments, due to soil erosion. Although it can be expected that sludge particles will rapidly settle on the water basin floor, the ability of benthic animals to re-mobilize brominated flame retardants from sediments, can create a secondary pollution source [37]. Thereafter, the re-mobilized BDE 209 can be de-brominated by aquatic biota [35,36]. Since the fine-grained WWTP sludge, exhibiting a high capacity to accumulate BDE 209, would be best suited for land-application purposes, it is very likely that substantial amounts of BDE 209 might be introduced into the environment as a result of poorly evaluated WWTP sludge management actions.

The environmental concentrations of PBDE congeners observed in this study, especially BDE 209, are relatively low in comparison to previously reported values [10,41,42]. This can most likely be explained by the lack of using sewage sludge in agricultural applications in Latvia. Furthermore, it seems that, at present, the observed differences of PBDE congener concentrations in lake and river sediments, can be attributed to differences in sediment properties. For example, the sediments that have a low content of dry matter, and a relatively high content of organic carbon, exhibit substantially higher concentrations of PBDEs than those with a high content of sediment dry matter (Figure 4).

## 5. Conclusions

Although, relatively low environmental concentrations were indicated during the study, the risk of future concentration increase in the environment exists since WWTP sludge can be considered as a resource of monetary benefit and due to continuous investments in WWTP sector there is steady growing pressure on WWTP sludge utilization capacity. Therefore, it is necessary to invest particular attention to WWTP sludge utilization management practices and if necessary to modify them basing recommendations on further more detailed study about sewage sludge retention properties and affecting factors.

**Supplementary Materials:** The following are available online at www.mdpi.com/2076-3298/4/1/12/s1, Table S1: Concentrations of PBDEs ($ng \cdot g^{-1}$) and content of dry matter (DM) in sewage sludge, Table S2: Concentrations

of PBDEs ($ng \cdot g^{-1}$), content of dry material (DW) and total organic carbon (TOC) in sediments, Table S3: Concentrations of PBDEs ($ng \cdot g^{-1}$) and content of lipids in tissues of European perch (*Perca fluviatilis*), Table S4: Limits of detection of PBDE concentration in water samples.

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**Author Contributions:** Juris Aigars analyzed the data. Juris Aigars and Natalija Suhareva were involved in writing the paper. Rita Poikane designed the experiments, performed the sampling campaign, and was involved in sample pretreatment and data interpretation.

**Conflicts of Interest:** The authors declare no conflict of interest.

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## APPENDIX B

## ARTICLE II

**Suhareva, N.,** Aigars, J., Poikane, R., Jansons, M.

**DEVELOPMENT OF FISH AGE NORMALIZATION TECHNIQUE FOR POLLUTION ASSESSMENT OF MARINE ECOSYSTEM, BASED ON CONCENTRATIONS OF MERCURY, COPPER, AND ZINC IN DORSAL MUSCLES OF FISH**

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# Development of fish age normalization technique for pollution assessment of marine ecosystem, based on concentrations of mercury, copper, and zinc in dorsal muscles of fish

**Natalija Suhareva · Juris Aigars · Rita Poikane · Mintauts Jansons**

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**Abstract** Correlation between metal concentrations in fish tissues and fish body size poses certain challenge when comparing concentration levels encountered at different locations or time periods by degrading performance of statistical tests due to variable age composition of fish sample pool. In order to overcome this, the concentrations of Hg, Cu, and Zn, measured in tissues of five fish species, were normalized to selected age group. Computed species-specific equations, based on empirically obtained exponential relationship, provided accurate estimates of the normalized concentrations under the conditions of substantial metal and fish age covariation. Obtained normalized and measured concentrations were then compared among sampling stations by means of commonly used analysis of variance (ANOVA) in combination with Tuckey's HSD test, where 11 out of 18 considered cases showed significant smoothing of the observed differences. The applied method worked well in the case of locally distributed coastal species populations where transformed data allowed clearer separation of spatial areas exhibiting different levels of pollution. At the same time, application of the method on pelagic fish species was less successful due to high mobility of specimens and mixed impact on the population originating from variable pollution levels at different areas of the entire migration region; therefore, attribution of a sample pool to a specific catchment area can cause a bias in assessment results.

**Keywords** Trace metals · Baltic Sea · Gulf of Riga · Fish age · Normalization of concentration · Pollution assessment

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s10661-020-08261-x) contains supplementary material, which is available to authorized users.

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## Introduction

Heavy metals that originate both from natural and anthropogenic sources enter marine ecosystem via river runoff and atmospheric deposition. Once introduced into the marine ecosystem, the heavy metals are redistributed through the water column (Pohl et al. 2006) and subsequently bioaccumulate in biota (El-Moselhy et al. 2014; Velusamy et al. 2014). Since the metals are not biodegradable, they can be removed from marine ecosystem only by burial in sediments or lateral transport to adjacent water body. As has been demonstrated by Pohl et al. (2006) in the brackish semi-

enclosed Baltic Sea, the accumulation in sediments is the only noteworthy sink of heavy metals due to long water residence time. Consequently, heavy metals that are particle reactive, like Pb and Zn, have very low residence time, while nutrients like Cd and Cu have a residence time of several decades primarily due to their coupling to biological processes (Pohl et al. 2006). Special attention has been dedicated to mercury since exposure to high levels of mercury has led to various health disorders in the past (Gibb and O'Leary 2014; Turaga et al. 2013). Fish is a dominant source of mercury to birds and mammals feeding on it (Evers et al. 2008) and is considered a serious exposure route to human as well (NFA 2012). To assess pollution levels and trends, the heavy metal content in fish is usually analyzed in environmental monitoring programs. Therefore, the fish species and size are factors which are frequently considered in monitoring program designs. Correlation between fish body size and concentration of contaminant is a common phenomenon (Moriarty 1988; Sonesten 2003). The contaminant concentrations positively correlate also with trophic level (Magalhães et al. 2007), although trophic magnification can be dependent on environmental factors (Lavoie et al. 2013). At the same time, the concentrations in fish tissues of similar trophic levels can differ since they are determined by water chemistry, occurrence depth, and concentration of contaminant in food source (Magalhães et al. 2007). The correlation problem is often circumvented by use of fish within narrow size or age range (Sonesten 2003; Åkerblom et al. 2012) or by means of regression procedures (Lindqvist et al. 1991; Harris and Bodaly 1998). In order to conduct cross-species comparison, empirically supported transfer function has been used in freshwater ecosystem (Åkerblom et al. 2014).

The ability to compare concentrations across fish species using fish of various sizes is essential also for marine ecosystems. Fish species (herring, sprat, cod, perch, flounder, plaice, turbot) representing different trophic levels, feeding habits, and geographical distribution have been previously used for studies of heavy metal bioaccumulation (Vuorinen et al. 1998; Polak-Juszczak 2009, 2012; Boalt et al. 2014) and in assessments of status of marine environment (HELCOM 2010). Since due to sampling constrains quite often specimens of narrow age range are not sufficiently numerous to comprise adequate sample pool, the weight and size of fish used in the analyses exhibit substantial spatial and temporal variability (Vuorinen et al. 1998), what most likely has been causing large variability in measured concentration levels of heavy metals (HELCOM 2002). Thus, long-term data rows of annual concentration levels are required to detect temporal changes with desired level of confidence. However, according to Directives 2000/60/EC (Water framework directive) (EC 2000) and 2008/56/EK (Marine strategy framework directive) (EC 2008), assessment of marine waters should be carried out by comparing current 6-year period with the previous one. Therefore, an approach to decrease variability of data used in the analyses is needed.

The aim of this study was to test whether normalization to arbitrary fish size could be used to decrease the level of concentration variation caused by variations of individual fish size in sample.

## Material and methods

### Sampling

The study was performed in the Baltic Sea – Eastern Gotland Basin and the Gulf of Riga. The Gulf of Riga is a semi-enclosed bay connected to the central Baltic Sea via Irbe Strait. Water salinity in the gulf varies between 4 and 7 PSU (practical salinity unit) with even lower salinity levels in the river estuaries (Yurkovskis et al. 1993). Water salinity in the sampling area of the Baltic Sea basin is approximately 7 PSU (Feistel et al. 2010).

The fish samples were gathered during 2015 in frame of scientific fishing cruises with the aim of gathering specimens of representative species from coastal to open sea areas. Flounder and round goby samples were collected from May to August; perch samples were collected from May to September; cod samples were collected during the second week of March; sea herring samples were collected in May, and gulf herring samples were collected in the beginning of August. The samples of flounder (*Platichthys flesus*), perch (*Perca fluviatilis*), and round goby (*Neogobius melanostomus*) were collected by means of scientific gill nets "Nordic" and capron gill nets at coastal (less than 1 nautical mile from coastline) stations C1, C2, C3, and C4 (Fig. 1). Samples of cod (*Gadus morhua*) (stations: P1, P2, P3, P4, and P5), sea herring (*Clupea harengus*) (stations: P6, P7), and gulf herring (*Clupea harengus*) (stations: P8, P9) were gathered by trawling in open sea area (Fig. 1). In total, 393 fish specimens were gathered: 66 cod, 73

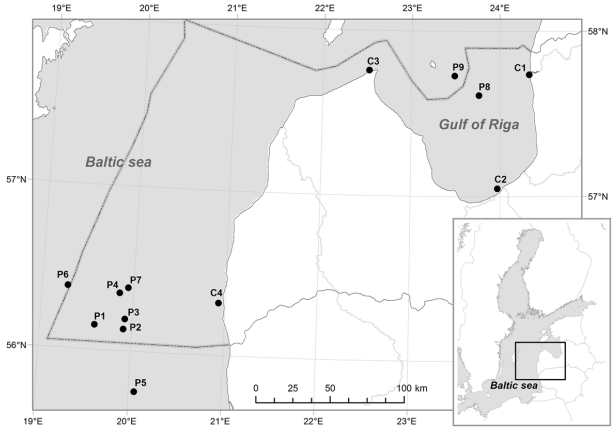


**Fig. 1** Map of sampling stations in the study

flounder, 39 gulf herring, 45 sea herring, 67 perch, and 90 round goby samples.

Sample pretreatment

The length and weight of the whole fish was determined immediately after sampling. Then the dorsal muscle of fish and otoliths were extracted. The dorsal muscle was placed into plastic container and frozen at − 18 °C. Thereafter, the samples of muscle tissues were dried in vacuum freeze dryer until sample weight loss stopped and homogenized by knife mill. Measured content of water in the samples was between 72 and 87%. Plastic containers with dried tissue samples were stored in desiccator in dark at room temperature (+ 20 °C) until further analyses. After extraction, the otoliths were washed by clean water, air dried, and kept in the dark at room temperature (+ 20 °C) until further analyses.

Laboratory analyses

The concentration of mercury in dorsal muscle was determined according to US EPA method 245.6 "Determination of Mercury in Tissues by Cold Vapor Atomic Absorption Spectrometer," using "VARIAN Spectra AA 880" atomic absorption spectrometer equipped with cold vapor generation block VGA77 (US EPA 1991). Approximately 0.5–0.7 g of dry tissue sample was digested with mixture of 2 mL concentrated nitric and 8 mL concentrated sulfuric acid for 16 h at room temperature until the tissue is completely dissolved and then followed by oxidation with 20 mL 5% $KMnO_4$ and 8 mL 5% $K_2S_2O_8$ solution for 90 min at 30 ± 1 °C in water bath with temperature control Julabo SW22. Calibration standards and blank were prepared fresh for each new series of measurements and treated in the same manner as samples. The excess of KMnO4 was reduced with 6 mL 12% hydroxylamine hydrochloride solution. All samples were diluted with Milli-Q grade water to 100 mL total volume. Mercury was released from digested sample solution by 10% stannous chloride solution in the continuous flow Hg vapor generator. Mercury working standards 1.0 to 10 μg $L^{-1}$ were made diluting mercury stock solution of 1000 mg Hg $L^{-1}$. Mercury calibration curve was constructed by plotting the absorbances of standards versus mercury concentration in working standard solutions until the analytical working curve remain linear at wavelength 253.7 nm.

According to our long-term studies, for determination of Zn and Cu, the tissue samples have been mineralized in microwave oven "Mars 5" by suprapure concentrated nitric acid, as it is described in method US EPA 3052 "Microwave assisted acid digestion of siliceous and organically based matrices" (US EPA 1996). Concentration of Cu and Zn in digested samples was measured according to "Flame Atomic Absorption Spectrometry Method" (US EPA 7000B), using "VARIAN Spectra AA 800" atomic absorption spectrometer (US EPA 2007). Zn working standards 0.25 to 2.5 mg $L^{-1}$ were made by diluting Zn stock solution

1000.3 ± 3 mg Zn $L^{-1}$, and Cu working standards 10 to 1000 μg $L^{-1}$ were made by diluting Cu stock solution 999.8 ± 3.2 mg Cu $L^{-1}$.

The analyses of reference material of mussel tissue ERM-CE278k (certified by Institute for Reference Materials and Measurements at Joint Research Centre, European Commission, Geel, Belgium) were performed to prove accuracy of determination (Online Resource). Our results were in good agreement with the certified values given for the reference materials, and our laboratory is accredited according ISO17025 (International Organization for Standardization 2017) and takes part regularly and successfully in the QUASIMEME laboratory performance studies for quality assurance. Presented analytical methods are used in our laboratory for routine analyses since 2000. Numerous data of regular calibration plot construction prior every measurement series and analysis of reference materials were used for calculations of the analytical method detection limit (DL) and expanded uncertainty (U) (Magnusson et al. 2012) (Online Resource). Detection limit for the method for Hg determination in biota samples was 0.050 mg $kg^{-1}$ dry weight, U ± 25%; for Zn – DL was 10.0 mg $kg^{-1}$ dry weight, U ± 12.5%; and for Cu – DL was 43.0 mg $kg^{-1}$ dry weight; U ± 25%.

The fish age determination method was based on seasonal growth patterns in fish ear bones (otolites), i.e., on differentiation of fast and slowly grown zones (rings) in otoliths with low-power microscope Olympus SZX-ILLD2-200.

### Data analyses and statistical assessment

Potential outliers were identified by means of calculation of interquartile range. Five major outliers were omitted, whereas minor outliers did not show any significant influence, therefore were included into the data set. Linear regression models were used to check data for a good fit and descriptiveness by means of regression data analysis and particularly *F* test of the overall significance (selected value of significance level was α 0.05).

Concentration of metals, as well as relative fish length, age, weight, and content of lipids, showed non-normal distribution; therefore, Log10-transformation was necessary to satisfy the assumptions of normality required by statistical methods.

Principal component analysis (PCA) was used to identify main patterns and correlations between different variables, also to reduce variance in multi-dimensional data, retaining the most explanatory information. During the analysis, selected value of significance level α was 0.05.

Estimated (normalized from a generated data set) and generated concentrations at 2-year-old specimens were compared by means of Wilcoxon-Mann-Whitney rank sum test and Spearman rank correlation.

One-way analysis of variance (ANOVA) was implemented to assess the difference between sampling stations to test an influence of sampling locations on concentrations of heavy metals.

Tukey's HSD test was implemented to evaluate statistical significance of differences of heavy metals concentrations between sampling stations, before and after normalization procedure (selected value of significance level α was 0.05).

Data exploration, artworks, and statistical analyses were performed using R software for Windows, release 3.6.1.

## Results

### Distribution of fish age groups

Distribution of fish age groups exhibited substantial variability within and between sampling stations. Age of cod varied from 2 to 8 years (Table 1). Overall, the highest median age of cod was found at station P5, and the lowest at station P1 (Fig. 2). The age of herring specimens varied from 0 to 13 years (Table 1). The age group distribution varied substantially even between stations that were spatially very close exhibiting overall age median range from 4 to 10 years (Fig. 2). No spatial pattern of herring age group distribution could be distinguished from the available data. The age of flounder specimens varied from 3 to 10 years (Table 1). The flounder age groups exhibited clear spatial pattern where significantly older specimens (median age 8) were caught at coastal stations of the Eastern Baltic Proper, while median age of specimens caught at the coastal stations of the Gulf of Riga was only 4 and 5 years (Fig. 2). Similarly, the age distribution of round goby also showed a clear spatial gradient with oldest specimens occurring at stations along the coast of Eastern Baltic Proper (median age 6 years), while specimens caught in the Gulf of Riga were substantially younger (median age 2 and 3.5 years) (Fig. 2). The age of perch specimens varied from 1 to 6 years (Table 1). Although, perch specimen age distribution varied among stations

**Table 1** Sample size, age range, and mean wet weight (WW) concentration of mercury (Hg), copper (Cu), and zinc (Zn)

| | | Age (years) | | | Hg (μg kg$^{-1}$ WW) | | Cu (mg kg$^{-1}$ WW) | | Zn (mg kg$^{-1}$ WW) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample size | Range | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Cod | 66 | 2–8 | 4.92 | 1.66 | 55.05 | 25.17 | 0.18 | 0.07 | 2.78 | 0.74 |
| Flounder | 73 | 3–10 | 6.7 | 1.89 | 59.52 | 39.31 | 0.29 | 0.05 | 4.85 | 1.03 |
| Herring (gulf) | 39 | 0–13 | 6.29 | 2.92 | 33.17 | 12.70 | 0 51 | 0.17 | 9.57 | 2.32 |
| Herring (sea) | 45 | 3–12 | 6.32 | 2.91 | 33.92 | 13.69 | 0.67 | 0.12 | 11.36 | 2.49 |
| Perch | 67 | 1–6 | 3.66 | 1.23 | 86.14 | 40.15 | 0.24 | 0.06 | 4.48 | 1.04 |
| Round goby | 90 | 1–8 | 4.57 | 2.03 | 24.56 | 13.37 | 0.22 | 0.05 | 3.25 | 0.55 |

(median age from 3 to 4 years) (Fig. 2), no distinct spatial pattern could be identified.

## Exploration of concentration data

Concentration of Cu varied from 0.06 mg kg$^{-1}$ in wet weight (WW) in cod to 0.85 mg kg$^{-1}$ WW in herring. Age-dependent Cu concentration increase could be observed only in cod, whereas Cu concentration in gulf and sea herring was irregularly variating throughout all age groups, and other species exhibited concentration decrease with aging (Fig. 3).

Concentrations of Hg varied between 3.4 μg kg$^{-1}$ WW in round goby and 199.4 μg kg$^{-1}$ WW in flounder. Age-dependent Hg accumulation trend was positive for all fish species. However, it should be noted that slopes of observed trends were species-related (Fig. 3).

Concentrations of Zn ranged from 1.8 mg kg$^{-1}$ WW in cod to 16.3 mg kg$^{-1}$ WW in sea herring. Cod and flounder exhibited slight increase of zinc content with age, whereas in perch and round goby, the concentrations

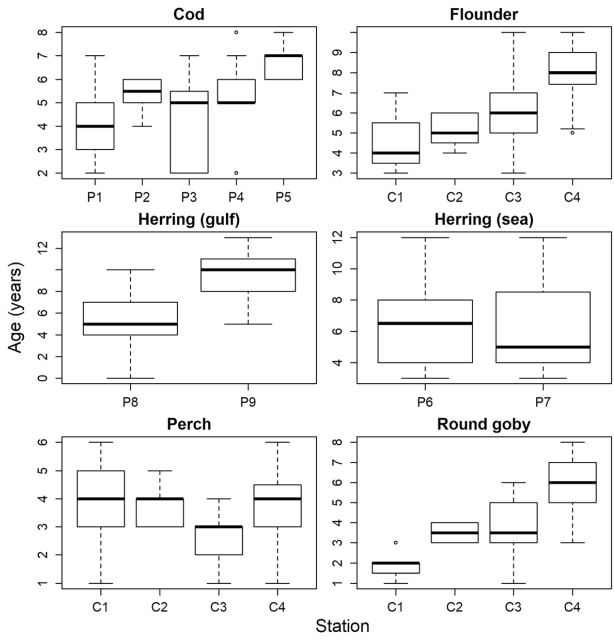


**Fig. 2** Distribution of fish age groups among sampling stations

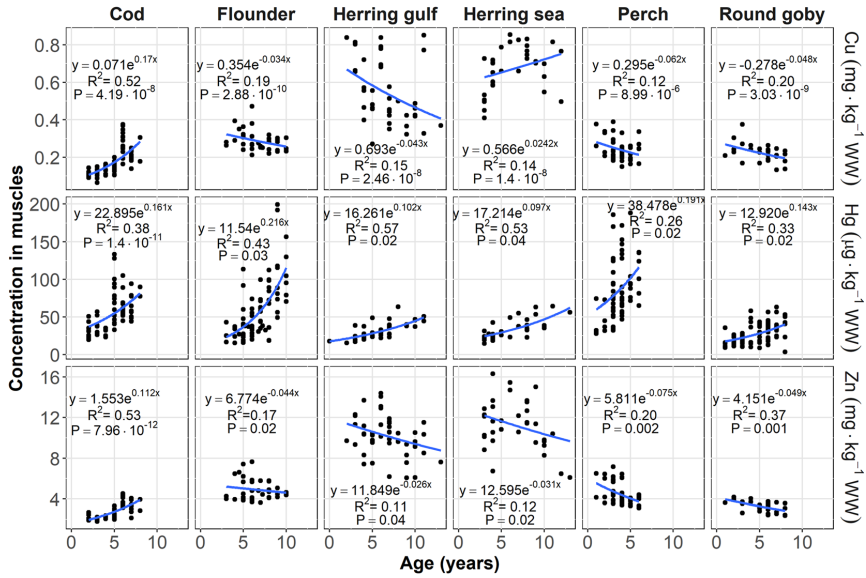


**Fig. 3** Variation of copper (Cu), mercury (Hg), and zinc (Zn) wet weight (WW) concentrations with age, in dorsal muscles of cod, flounder, herring, perch, and round goby

showed a negative trend (Fig. 3). Zn concentration in herring was notably higher than in other fish species; in addition, the concentration showed nonlinear decrease at elder age groups (after 5 years old threshold).

## Principal component analysis

Fish age usually correlates well with fish size and weight. However, this rule is not absolute since depending on feeding conditions, fishes of the same age might substantially differ in size and weight. Therefore, principle component analysis (PCA) was performed for each fish species separately (Fig. 4) to assess the level of covariance. The PCA results exhibited strong positive correlation between age, length, and weight of specimens for all species, except gulf herring and round goby, where the correlation is slightly lower, but still positive.

As can be seen, the principle component 1 (PC1) explains majority of age variations (Table 2). It can be observed that concentration of Hg is strongly positively correlated with fish age in flounder, herring, and perch and is positively correlated in round goby and cod, whereas concentration of Cu is positively correlated with age in cod and sea herring, strongly negatively correlated in round goby and gulf herring, and weakly negatively correlated in flounder and perch. Concentration of Zn exhibits positive correlation with age in cod and sea herring; negative correlation in perch, flounder, and gulf herring; and strong negative correlation in round goby. Content of lipids showed positive correlation with age in cod and strong negative correlation in round goby and has no significant correlation in flounder, herring, and perch.

## ANOVA

Since it is assumed that variation of metal concentrations in fish tissues reflects pollution status of respective area, the variance analysis ANOVA was performed (where sampling station was set as independent variable, while concentration of metal-dependent variable). The variation analysis of the untransformed data set (Table 3) exhibited significant difference between sampling stations for 14 of 18 considered cases except concentration of Hg in sea herring ($p = 0.622$), concentration of Cu in gulf herring ($p = 0.082$), and Zn concentration in both gulf and sea herring ($p = 0.476$ and $p = 0.511$, respectively). The correlation of fish age and metal concentration suggests that observed significant differences among stations are at least partly determined by differences in fish age structure what

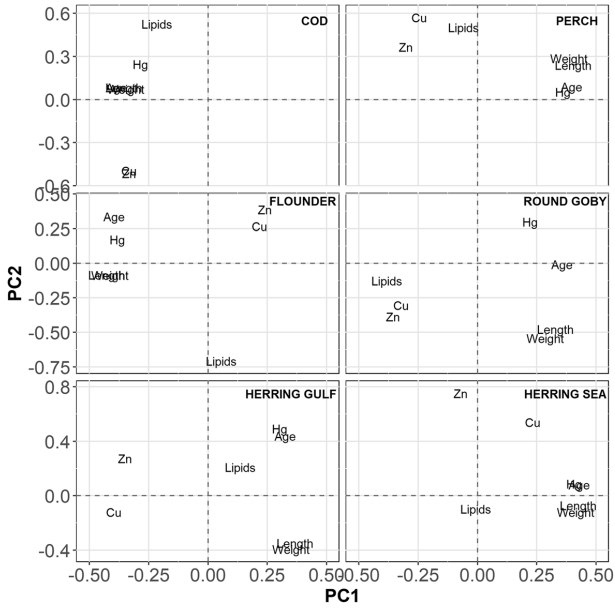


**Fig. 4** Correlation between the variables (age, length, weight, content of lipids, muscle concentration of mercury (Hg), copper (Cu), and zinc (Zn) and the first two principal components (PC1 and PC2), performed on species-specific sub-sets of data (cod, flounder, herring, perch, round goby) and entire data set

makes comparison of pollution levels at different areas questionable. Therefore, we proceeded with normalization of concentrations that would exclude age factor from analyses of spatial distribution of metal concentrations.

## Normalization of concentrations: technique

Based on the obtained PCA results (Fig. 4), it was assumed that fish age, length, and weight are presenting similar (covariant) information; therefore, to minimize unclearness of further results of the normalization procedure, fish age was selected as independent variable.

To improve comparability of metal concentrations between different age groups and among the sampling sites, measured concentrations of metals were normalized to correspond to a 2-year-old specimen of the same species. The normalization procedure was based on assumption that accumulation of metal by organism follows an exponential function, as it was suggested by Conti and Iacobucci (2008) and Brown and Depledge (1998), differing by coefficients and bases:

$$f(x) = a \cdot c^x = a \cdot \left(e^b\right)^x = a \cdot e^{b \cdot x},$$

where $a$ is a coefficient of the function and $b$ is a base; wherein exponential functions with small values of absolute $b$ $(-1 < b < 1)$ can describe an accumulation/release curves which are visually similar to linear relationship. Thereafter, we assumed that base $b$ expresses accumulation speed depending on feeding preferences and stays unchanged among one species, whereas coefficient $a$ differs between stations and is probably related to feeding conditions and food availability.

**Table 2** Importance of principle components (PC) and its sum via proportion of variance (%) in principle component analysis (PCA)

| | Cod | Flounder | Herring gulf | Herring sea | Perch | Round goby |
|---|---|---|---|---|---|---|
| PC1 (%) | 65.91 | 52.10 | 51.16 | 50.13 | 50.32 | 54.05 |
| PC2 (%) | 15.53 | 19.16 | 17.01 | 20.20 | 16.69 | 19.55 |
| SUM (%) | 81.44 | 71.26 | 68.17 | 70.33 | 67.01 | 73.60 |

**Table 3** Statistically significant differences in measured concentrations of mercury (Hg), copper (Cu), and zinc (Zn) between sampling stations (ANOVA)

| | Hg | | | Cu | | | Zn | | |
|---|---|---|---|---|---|---|---|---|---|
| | Df | F | *p* value | Df | F | *p* value | Df | F | *p* value |
| Cod | 4 | 2.943 | 0.027 | 4 | 6.900 | < 0.001 | 4 | 5.707 | < 0.001 |
| Flounder | 3 | 4.835 | 0.004 | 3 | 5.933 | 0.005 | 3 | 15.478 | < 0.001 |
| Herring gulf | 1 | 18.585 | <0.001 | 1 | 3.197 | 0.082* | 1 | 0.519 | 0.476* |
| Herring sea | 1 | 0.2479 | 0.622* | 1 | 4.462 | 0.043 | 1 | 0.442 | 0.511* |
| Perch | 3 | 14.542 | <0.001 | 3 | 20.718 | < 0.001 | 3 | 24.174 | < 0.001 |
| Round goby | 3 | 16.042 | <0.001 | 3 | 6.386 | 0.002 | 3 | 9.276 | < 0.001 |

*$p$ value > 0.05 no significant difference between sampling stations

Since the real data obtained during sampling campaign do not reflect concentration level at any other age except the measured one, the only possibility to track the tendency along aging was to generate theoretical data set with known concentration level at every age of every specimen. The generated data set included 45 specimens and calculated concentration for every specimen at age from 1 to 9. Different accumulation equations for each specimen were calculated adding a random positive or negative number ($r$, in our case $-\frac{1}{2}a < r < \frac{1}{2}a$) to the coefficient $a$:

$$y = (a + r) \cdot e^{b \cdot x}.$$

Then, a data set of 45 samples was obtained by random selection of only one age and concentration ($y_{obs}$) for every specimen, except age 2, which was nominated as the normalization goal. The 45 samples were plotted in a scatterplot (concentration versus age), and then exponential trendline and mutual standard equation were computed. Standard equation allowed to calculate fixed concentration value ($y_{st}$) at every age group between 1 and 9. From the above, any ($y_{obs}$) relates to ($y_{st}$) at the same age as:

$$y_{st} - y_{obs} = R,$$

where $R$ is a difference between the observed and standardized concentration, and

$$y_{obs} = \left(a_{st} \cdot e^{b \cdot x}\right) - R,$$

$$a_{obs} \cdot e^{b \cdot x} = \left(a_{st} \cdot e^{b \cdot x}\right) - R,$$

$$a_{obs} = \frac{\left(a_{st} \cdot e^{b \cdot x}\right) - R}{e^{b \cdot x}}.$$

After species-specific coefficient $b$ was empirically obtained, the generated concentrations were normalized to one age group (2-year-old) according to the following equation:

$$y_{norm} = a_{obs} \cdot e^{b \cdot 2},$$

$$y_{norm} = \frac{\left[\left(a_{st} \cdot e^{b \cdot x}\right) - R\right] \cdot e^{b \cdot 2}}{e^{b \cdot x}},$$

$$y_{norm} = y_{obs} \cdot e^{b(2 - x_{obs})}.$$

Estimated (normalized from the generated data set) and generated concentrations at 2-year-old specimens were compared by means of Wilcoxon-Mann-Whitney rank sum test due to insufficient normality of data distribution. The $p$ value = 0.2958 driven by the test does not allow to reject the hypothesis that calculated and generated concentrations are statistically equal. Spearman rank correlation (concentration of 2-year-old specimen ~ estimated concentration at 2-year-old specimen) showed that the relationship explains 97% of variation. Therefore, it was admitted that the normalization technique works satisfactorily, despite to little deviation of calculated values shown at Fig. 5, and can be implemented as an assistant tool.

Normalization of concentrations: implementation

First, concentration of metals was log-transformed with natural base $e$, in order to obtain a linear

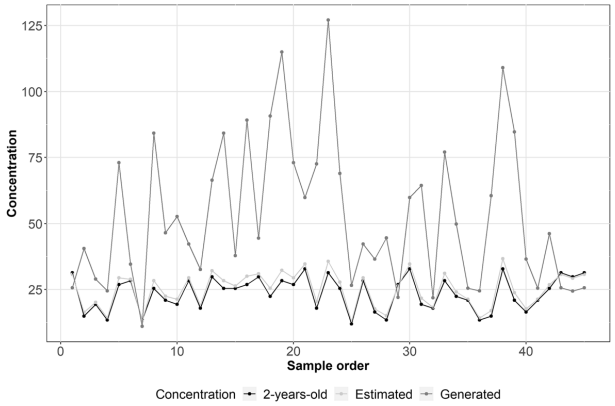


**Fig. 5** Comparison of generated (dark gray, for different age groups between 1 and 9; black, for 2-year-old age group) concentrations versus estimated concentrations at 2-year-old specimens obtained via normalization technique (light gray)

dependence on age according to the following equations:

$$\ln(y) = \ln\left(a \cdot e^{b \cdot x}\right);$$

$$\ln(y) = \ln(a) + \ln\left(e^{b \cdot x}\right);$$

$$y' = a' + b \cdot x.$$

Then linear regression of accumulation (or release) was computed for every particular case, 18 equations in total (6 species and 3 metals). The obtained regression models were checked by means of $F$ test of overall significance for reliability of a good fit and the relationship between $y'$ and $x$ (Fig. 3). Herring data was split into two sub-groups: the sea and the gulf herring, to achieve the satisfying regression models.

Applying $e$ to both sides of the linear equation yields, the linear regression was converted back to an exponential dependence. After species-specific coefficient $K_{sp}$ was empirically obtained from base $b$, and observed concentrations of metals were normalized to one age group according to the following equation:

$$[ME]_{norm} = [ME]_{msrd} \cdot e^{K_{sp}(A_{norm} - A_{msrd})}$$

where $[ME]_{norm}$ is the normalized concentration at selected age; $[ME]_{msrd}$ is the measured concentration at current age; $A_{msrd}$ is the current age; $A_{norm}$ is the selected normalization age; and $K_{sp}$ is species-specific coefficient.

## Comparative analysis based on Tukey's HSD test

The normalization by fish age allowed us to smooth out significant differences among stations for several species and metals, like cod, flounder, gulf herring, and round goby in case of Cu, flounder gulf herring and round goby in case of Hg, and cod and round goby in case of Zn (Table 4). In total, 11 cases out of 18 considered ones showed the evident effect of normalization on significance of differences among stations (Table 4).

For perch, the normalization effect was very low, but since age distribution of perch specimens in all stations was fairly similar (Fig. 2), it was expected that effect of normalization will be minimal. At the same time, the normalization allowed us to conclude that in perch, the increased accumulation of metals like Cu or Zn and Hg occurs at different sampling stations – C3 and C2, respectively – regardless of age (Figs. 2 and 6, Table 4). Less so for round goby where age distribution clearly differed among stations (Fig. 2) and so more convincing effect of normalization could have been expected (Fig. 6). Most likely, this is due to demonstrated lower impact of fish age on concentration level (Fig. 3).

In case of sea herring, it was demonstrated that the normalization has no effect on significantly indifferent measured concentrations (Fig. 6, Table 4), which can serve as an additional evidence of the validity of the technique.

From the above, it was possible to conclude that for all metals, in majority of cases, there have been significant differences in concentration levels among stations that were caused by differences in fish age structure.

**Table 4** Statistically significant differences in mean metal concentrations between sampling stations (Tukey's HSD test), before and after the normalization technique was applied

| | After normalization | | | Before normalization | | |
|---|---|---|---|---|---|---|
| | Hg | Cu | Zn | Hg | Cu | Zn |
| Cod | *p* value | *p* value | *p* value | *p* value | *p* value | *p* value |
| P2-P1 | 1.000 | 0.700 | 0.923 | 0.854 | *0.041* | 0.111 |
| P3-P1 | *0.044* | 1.000 | 0.877 | *0.037* | 1.000 | 0.932 |
| P4-P1 | 1.000 | 1.000 | 0.993 | 0.976 | 0.720 | 0.402 |
| P5-P1 | 1.000 | 0.928 | 0.955 | 0.153 | *< 0.001* | *< 0.001* |
| P3-P2 | 0.509 | 0.971 | 1.000 | 0.981 | 0.227 | 0.902 |
| P4-P2 | 1.000 | 0.975 | 1.000 | 1.000 | 0.987 | 1.000 |
| P5-P2 | 1.000 | 1.000 | 1.000 | 0.999 | 0.989 | 0.958 |
| P4-P3 | 0.421 | 1.000 | 1.000 | 0.931 | 0.937 | 0.992 |
| P5-P3 | 0.147 | 0.999 | 1.000 | 1.000 | *0.005* | 0.095 |
| P5-P4 | 1.000 | 0.999 | 1.000 | 0.990 | 0.501 | 0.825 |
| Flounder | *p* value | *p* value | *p* value | *p* value | *p* value | *p* value |
| C2-C1 | 1.000 | 0.044 | *< 0.001* | 1.000 | 0.060 | *< 0.001* |
| C3-C1 | 1.000 | 0.996 | *0.151* | 0.783 | 1.000 | 0.256 |
| C4-C1 | 1.000 | 0.957 | 0.056 | *0.028* | 1.000 | 0.118 |
| C3-C2 | 1.000 | 0.067 | *< 0.001* | 0.916 | *0.004* | *< 0.001* |
| C4-C2 | 1.000 | 0.176 | *< 0.001* | 0.109 | *0.006* | *< 0.001* |
| C4-C3 | 1.000 | 1.000 | 0.997 | 0.071 | 1.000 | 1.000 |
| Herring gulf | *p* value | *p* value | *p* value | *p* value | *p* value | *p* value |
| P9-P8 | 0.673 | 0.998 | 0.933 | < 0.001 | 0.272 | 0.881 |
| Herring sea | *p* value | *p* value | *p* value | *p* value | *p* value | *p* value |
| P7-P6 | 0.906 | 0.280 | 0.770 | 0.936 | 0.143 | 0.902 |
| Perch | p value | p value | p value | p value | p value | p value |
| C2-C1 | *< 0.001* | 0.099 | *0.016* | *< 0.001* | 0.149 | *0.004* |
| C3-C1 | 1.000 | *0.034* | *< 0.001* | 0.795 | *0.001* | *< 0.001* |
| C4-C1 | 0.256 | 0.091 | 0.656 | 0.333 | 0.142 | 0.433 |
| C3-C2 | *< 0.001* | *< 0.001* | *0.031* | *< 0.001* | *< 0.001* | *< 0.001* |
| C4-C2 | *0.001* | 1.000 | 0.522 | *0.008* | 1.000 | 0.430 |
| C4-C3 | 0.774 | *< 0.001* | *< 0.001* | *0.010* | *< 0.001* | *< 0.001* |
| Round goby | *p* value | *p* value | *p* value | *p* value | *p* value | *p* value |
| C2-C1 | 0.829 | 0.596 | 1.000 | 0.408 | 0.881 | 0.998 |
| C3-C1 | 0.347 | 0.851 | 0.920 | 1.000 | 1.000 | 0.996 |
| C4-C1 | 1.000 | 1.000 | 0.937 | *< 0.001* | 0.133 | *0.002* |
| C3-C2 | 0.241 | 0.997 | 0.983 | 0.301 | 0.860 | 1.000 |
| C4-C2 | 0.875 | 0.314 | 0.990 | 1.000 | *0.030* | 0.340 |
| C4-C3 | 0.062 | 0.480 | 0.196 | *< 0.001* | 0.281 | 0.055 |

*p* values below significance level $\alpha = 0.05$ are indicated by italic

## Discussion

Similarly to earlier studies (Olsson 1976; Luczynska and Tonska 2006; Polak-Juszczak 2009, 2012), the results of this study demonstrated positive correlation between concentration of Hg in muscle and fish age and mostly negative correlation between fish age and concentrations of Cu and Zn. As has been observed previously (Vuorinen et al. 1998), fish age distribution in sample varied substantially. Due to this, the fish age

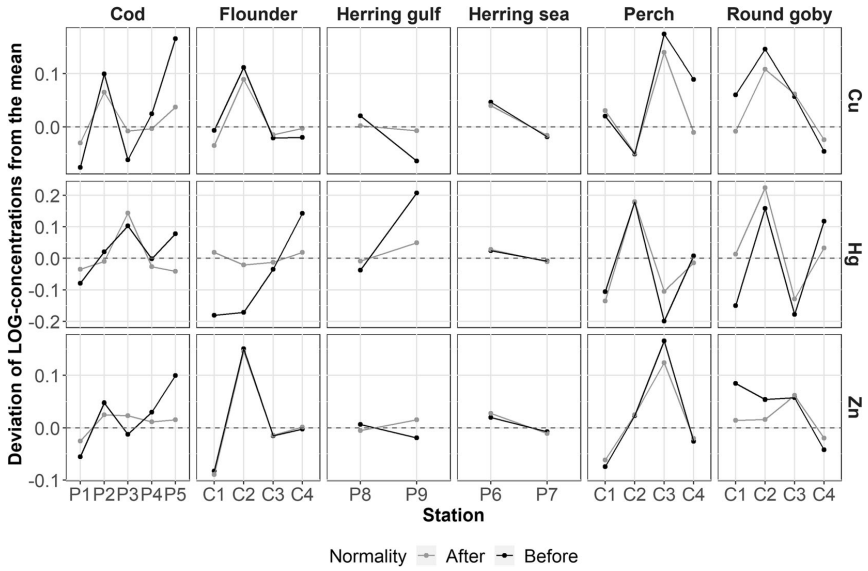


**Fig. 6** Deviation of Log-transformed measured and normalized mercury (Hg), copper (Cu), and zinc (Zn) concentrations from mean values at specific sampling stations and species

specific bioaccumulation of heavy metals caused substantial difficulties during assessment of contamination level at different sampling stations. Similarly to that reported by Åkerblom et al. (2014), the implemented normalization technique considerably eliminated influence of the fish age specific bioaccumulation, thereby allowing estimation of importance of other limiting factors, such as dietary preferences or local environmental concentrations of heavy metals. Consequently, based on concentration levels measured in perch, we were able to confirm that coastal area represented by station C2 (Fig. 1) is significantly more polluted than other coastal areas (Table 4). It can be assumed that elevated Hg concentration at coastal station C2 was caused by excessive contamination due to proximity of three river estuaries: Daugava, Lielupe, and Gauja, where perch act as good indicator of local contamination due to its high trophic position and predatory dietary habits (Tomczak et al. 2009).

The appliance of normalization to pelagic fish species (cod) was not equally successful most likely due to migratory characteristics of fish. For example, the only statistically different measured and normalized concentrations of Hg in cod were observed at station P3 that is in close proximity to stations P1 and P2. Sonesten (2003) concluded that the standardization of the Hg concentration to an arbitrary fish size is still dependent on the fish size distribution in the sample. Therefore, it can be argued that the observed differences among stations are likely caused by high variability of age groups at station P3 (Fig. 2), and therefore, significant differences between stations could be attributed to sample pool inhomogeneity at station P3 in relation to other sampling stations. However, the outlying average concentration level of Hg at station P3 (Fig. 6) is mostly due to several 5- and 6-year-old specimens exhibiting concentration levels that more than twice exceeded those observed for other specimens of the same age. This strongly suggests that the observed differences are caused by cod specimens migrated to this area from another one. At the same time, normalization successfully eliminated differences between gulf herring stations P8 and P9 (Fig. 6, Table 4) caused by several older specimens with relatively very high Hg content encountered at station P9.

Concentrations of Cu and Zn measured in coastal fish species seem to be highly dependent on spatially different specificity of feeding habitat and diet composition. Benthivorous flounder and benthopelagic perch exhibited elevated concentrations of Cu and Zn at different sampling stations (C2 and C3, respectively) (Fig. 6).

Sandy sediments dominate at both stations; however, the station C2 receives significant amount of detritus originating from adjacent rivers that in turn promotes rich biological diversity of benthic organisms, while at station C3, biodiversity of benthic organisms is much poorer, and bivalves are predominant species (National monitoring data). Everaarts et al. (1993) have reported that content of Cu and Zn in zooplankton is several times higher than in benthic organisms and that accumulation level of Cu is substantially higher in epibenthic shrimps than in bivalves. Therefore, it can be assumed that the comparatively higher concentrations of Cu and Zn in flounder at station C2 are due to more variable diet enabled by high diversity of benthic food sources. At the same time, the low variability of benthic food sources at station C3 caused benthopelagic perch to shift diet from mixed pelagic-benthic to predominately pelagic. So the proportion of organisms with relatively high content of Cu and Zn in their ration increased resulting in elevated concentrations of Cu and Zn in their tissues.

## Conclusion

Implementation of species-specific regressions for the normalization method described in this study was performed with excellent results in case of coastal fish species allowing us to exclude high variability of the initial data caused by differences in fish age structure of sample pool, thereby avoiding ambiguous results of following statistical analyses. At the same time, the normalization method was less successfully applied to pelagic fish species, for which a common feature is a long-distance migration of specimens. In this case, additional data treatment, like careful preliminary data exploration ensuring homogeneous distribution of age groups among a data pool, should be considered as a viable option.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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## APPENDIX C

## ARTICLE III

**Suhareva, N.,** Aigars, J., Poikane, R., Tunens, J.

**THE INFLUENCE OF FEEDING ECOLOGY AND LOCATION ON TOTAL MERCURY CONCENTRATIONS IN EURASIAN PERCH (*Perca fuviatilis*)**

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Environmental Sciences Europe

RESEARCH

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# The influence of feeding ecology and location on total mercury concentrations in Eurasian perch (*Perca fluviatilis*)

Natalija Suhareva[1*], Juris Aigars[1], Rita Poikāne[1] and Juris Tunens[1,2]

## Abstract

**Background:** Eurasian perch (*Perca fluviatilis*) is an ecologically significant fish species in the Baltic Sea and has been recognized as a suitable organism to measure concentrations of mercury (Hg) contamination. The adult species occupy a high trophic position; therefore, significant levels of the hazardous substances tend to bioaccumulate in their tissues. However, the ability of the species to inhabit a wide range of feeding ground raises concerns about the adequacy of monitoring data in relation to the representativeness of measured levels of Hg at specific locations. Accounting for the migratory characteristics of this species can shed light on the origin of the analyzed specimens and thus trace Hg uptake chain. Perch samples and potential perch prey were collected at three remote stations in a fully interlinked system river–lake–coastal/transitional waters of the Gulf of Riga. Total mercury (THg) concentration and stable isotope ratios were measured in each sampled item. The perch data were divided into three subgroups associated with specific feeding grounds and one mixed group. A Bayesian mixing model was implemented to quantify the feeding preferences of each group, and based on the results, influence of each food source on Hg uptake by perch was modeled by means of Gaussian GAM model.

**Results:** Calculated carbon and nitrogen stable isotope values demonstrated clear evidence of perch specimens migrating between the sampling stations. Substantial proportion of specimens sampled in river and lake stations had isotopic signals consistent with feeding in the gulf. The group of perch associated with feeding in the river grounds exhibited the highest THg concentrations with mean value of 209 μg $kg^{-1}$ wet weight. The food items *C. harengus membras* and Crustacean showed significant mitigating effects on THg concentration. The rest of the food items showed a secondary influence on the variation of THg concentration.

**Conclusions:** The study clearly showed that the high mobility of perch along associated aquatic systems has a noticeable effect on Hg concentrations measured in the fish. Therefore, trophic position and isotopic signatures, along with identification of the food sources, can serve as important supplementary tools for more accurate data interpretation of Hg accumulation.

**Keywords:** Mercury, Trophic magnification, Stable isotopes of carbon and nitrogen, Feeding ecology, Estuary system, Environmental modeling, Eurasian perch (*Perca fluviatilis*), The Gulf of Riga

## Background

The global mercury (Hg) cycle is dominated by anthropogenic and natural emissions of gaseous substances of Hg to the atmosphere [1]. Simultaneously, the wide use of Hg and its containing products [2] has resulted in more than 3000 localized Hg-contaminated sites worldwide [3]. Furthermore, the improperly disposed industrial,

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household and medical products as well as pesticides used in the past have created legacy Hg contamination sources. Although the sources are not classifiable as Hg-contaminated sites, they are impacting environmental quality of geographically localized water basins they are discharging into.

In order to address local Hg sources and to improve environmental quality, various programs, like river basin management plans in European Union, are being developed and implemented. To assess the effectiveness of implemented measures, Hg levels and their trends are usually analyzed in the frame of environmental monitoring programs. Consequently, environmental research employs a variety of scientific methods [4, 5] in quantitative and qualitative analyses of the Hg in aquatic systems, including selection of a matrix for analysis such as water, sediments and biological material. Eurasian perch (*Perca fluviatilis*) is one of the ecologically significant fish species, along with Baltic herring (*Clupea harengus membras*), cod (*Gadus morhua*) and eelpout (*Zoarces viviparus*), proposed by a regional platform for environmental policy-making—Baltic Marine Environment Protection Commission (HELCOM) as a biological matrix for environmental studies in the Baltic Sea, which reflects local environmental concentrations of hazardous substances [6]. Particularly, a dorsal muscle (fillet) was suggested as an appropriate matrix to measure levels of organic Hg accumulated in fish [6], moreover the tissues were proposed by 'EC regulation 1881/2006' [7] in order to control contamination in fishery products. A fish takes up Hg by absorbing it through the body surface and gills, but a primary source is the diet [8]. Perch is omnivorous in the first years of life, although the adults mostly follow a piscivorous diet [9]. The species occupy a high trophic position; therefore, high concentrations of Hg [10] are commonly found in their tissues. The perch is widespread in freshwater and brackish water ecosystems, but usually are not considered to be an anadromous fish. At the same time, it has been put forward by Järv [11] that the home-range migration (average 20 km, maximum observed 180 km) is a common behavioral feature for perch. The salt tolerance of perch and relatively low water salinity of the Baltic Sea and the Gulf of Riga allows this species to move from inland lakes and rivers to coastal waters. Generally, it has been assumed that once the feeding grounds in the coastal waters have been reached the specimens become reasonably stationary [12], consequently they can be used as a representative organism to characterize concentrations of hazardous substances, such as Hg, providing biologically relevant context for local pollution exposure. However, the high mobility of perch, the ability of the species to inhabit a wide range of feeding ground [9, 11], in addition to relatively high variability of measured concentrations raises concerns about adequacy of monitoring data regarding to the representability of measured levels of hazardous substances, including Hg, at specific locations. These concerns are most pronounced in the cases where different water bodies form an interlinked water system which is fully within the range of perch migration distance, like the river–lake–marine waters system, which is highly common at coastal regions of Latvia due to a number of lagoon type lakes. Consideration of migratory characteristics of the species can shed light on the origin of the analyzed samples, thus filling knowledge gaps on Hg distribution in the interconnected aquatic systems.

Stable isotopes of nitrogen (N) and carbon (C) in soft tissues of fish are commonly used to study food webs and migration in aquatic ecosystems [13–15]. Carbon stable isotope ratios $^{13}C/^{12}C$ in marine and fresh water systems have been used to determine the movements of migratory species between coastal and pelagic ecosystems based on changes in dietary preferences during the migration [16, 17], which is possible due to clear isotopic differences between $^{13}C$-depleted freshwater and $^{13}C$-enriched marine food webs. Changes in nitrogen isotope ratios $^{15}N/^{14}N$ have been used to distinguish between trophic levels in freshwater and marine environments [18, 19].

Stomach content analysis provides important information regarding the recently consumed prey and helps to describe feeding habits of fish. The aim of the analysis is to determinate the most frequently consumed prey and indicate general food categories occurring in the stomach [20].

By employing analysis of C and N isotopes in combination with perch stomach content analysis, the aim of this study was to determine how the fundamentally different food bases affect the uptake of Hg from food chains, as well as whether the perch, caught at a particular location, are representative of specific location, or rather of the entire interconnected coastal–freshwater aquatic system.

## Materials and methods

### Study site description

For this study the fully interlinked system, river Daugava–lake Ķīšezers–coastal area of the Gulf of Riga (Baltic Sea) was chosen (Fig. 1). Sampling station 1, the river Daugava, was selected on the last section of the river between Riga Hydroelectric Power Plant (HEPP) and the estuary Daugavgriva, circa 5 km downstream to the HEPP and 14 and 19 km upstream of the channels connecting lake Ķīšezers and river Daugava. The site can be characterized by rocky sediment type and rapid stream velocity. Sampling station 2, a lagoon type lake Ķīšezers, is connected to river Daugava by two natural channels.

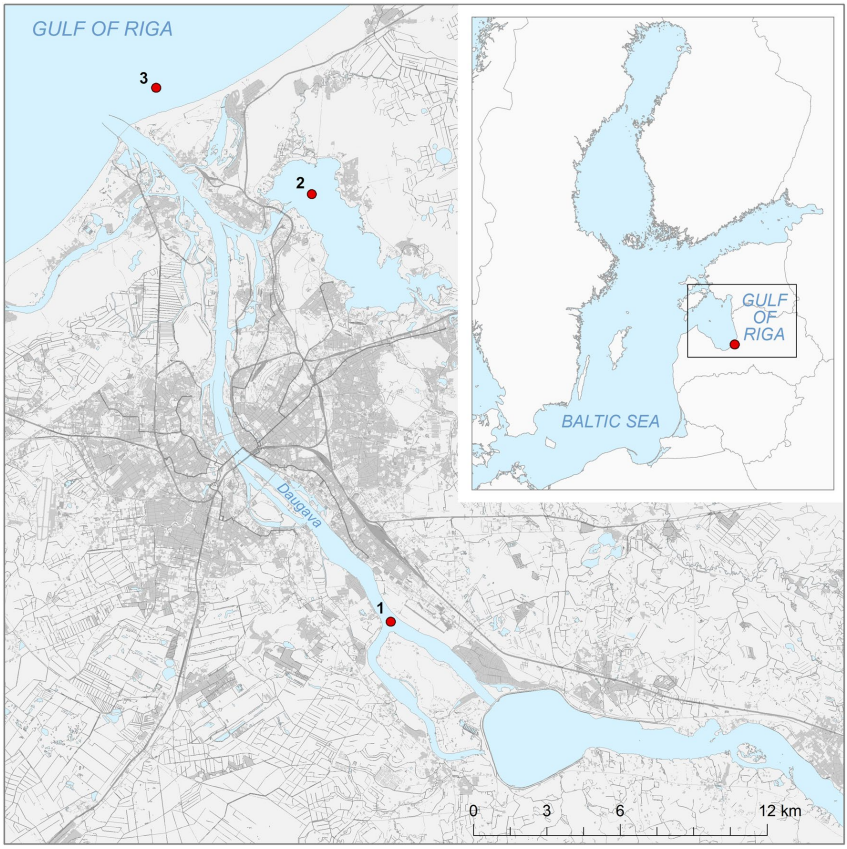


**Fig. 1** Sampling sites: **1**—the Daugava river, **2**—lake Ķīšezers, **3**—the Gulf of Riga

It is rich with aquatic vegetation and represents a stagnant water pool. Sampling station 3, the Gulf of Riga, was selected near the mouth of river Daugava. The location represents a brackish coastal ecosystem with a significant amount of detritus originating from adjacent rivers.

#### Sampling and pre-treatment

The sampling campaigns took place in April and August 2017. Perch and other fish species were collected by means of scientific Coastal Survey multimesh gillnets (Nippon Verkko Oy, Finland) with nine 5-m-long panels of different mesh size (mesh size ranging between 10 and 60 mm). Benthic organisms were sampled by Petite Ponar Grab (Wildco, USA), while zooplankton and fish juveniles were collected by means of beach seine (handmade: the wing length 10 m with the mesh size of 10 mm knot to knot, the depth of 1.5 m, the mesh size of the cod end was of 5 mm). Crayfish were caught by two-ring drop net with the diameter of 80 cm and the mesh size 20 mm. For suspended particulate matter (SPM) surface water samples (up to 5 L) were taken from each sampling site by pre-cleaned plastic bottles. Collected surface water samples were vacuum filtered for at least 30 min on pre-combusted (at 450 °C for 2 h) 24 mm diameter microfiber glass filters (Whatman grade GF/F, pore size 0.7 μm) to collect a sufficient amount of the material.

Only Hg concentration and stable isotope analyses were performed for SPM samples. The data were not incorporated into any of the statistical analyses or the conducted models, instead were later used as a reference point or a proxy of phytoplankton.

Length and weight of the whole fish was determined immediately after sampling by measuring board (accuracy ± 0.1 cm) and technical scale KERN FCE3K1N (KERN & SOHN GmbH, Germany; accuracy ± 1 g), thereafter, dorsal muscles were extracted. The dorsal muscles were placed into a polyethylene container and frozen at temperature − 18 °C. The samples of muscle tissues, zooplankton and benthic organisms were dried in vacuum freeze dryer (LYOVAC GT 2-E, STERIS GmbH, Germany) until sample weight loss stopped and then homogenized by knife mill (IKA A11 basic, IKA—WERKE GmbH &CO.KG, Germany) or agate pestle. Plastic containers with dried tissue samples were stored in desiccator in dark at room temperature (+ 20 °C) until further analyses.

#### Analytical methods

The concentration of total mercury (THg) in the dorsal muscles of fish, other organisms and SPM was determined in laboratory at Latvian Institute of Aquatic Ecology (Daugavpils University) using direct combustion Hg analyser (Teledyne Leeman labs, "Hydra IIc", (Mason, Ohio, USA) following US EPA Method 7473 [21, 22]. The analyses of reference material of mussel tissue ERM-CE278k and aquatic plant ERM-BCR060 (both certified by Institute for Reference Materials and Measurements at Joint Research Centre, European Commission, Geel, Belgium) were performed for calibration verification at the start and to prove accuracy of determination at the end of every batch of 20 samples (Additional file 1: Table S1). Our results were in good agreement with the certified value given for the reference materials—recovery range 100–126% ($n = 24$). Method blank (50 µL of deionized water) and random sample duplicates were also run during each batch. Method blank was less than 25% of the lowest detected THg content in the sample (0.20 ng) and was considered acceptable. Relative percent difference between sample duplicate analyses was 1.5 ± 2% ($n = 22$).

#### Stomach content analysis

Stomach content of the perch was assessed by means of stomach content analysis, using the dissection method suggested by Manko [20]. Full length of uncoiled extracted gastrointestinal tract from esophagus to anus and its parts (stomach and intestine) were measured on a measuring board to the nearest mm, the stomach and intestine was separated, blotted between tissue paper sheets for 1 min to remove excess water, and weighed on electronic balance (LIBROR AEU-210, Shimadzu Corporation, Kyoto, Japan; accuracy ± 0.0001 g) to the nearest 0.001 g. Stomach was opened by making a shallow cut with a scalpel to avoid damage to contents, and washed in a Petri dish with a small amount of water. Intestine contents were removed into a Petri dish by sliding a blunt probe along the length of the segment. Stomach fullness was assessed visually using a scoring system of 1 to 7 (empty to distended/bursting, respectively). Stomach and intestine contents were inspected under a stereomicroscope (Leica MEB126, Leica Microsystems, Singapore). The digestion state of food items in the stomach was visually assessed by a scoring system of 1 to 6 (empty stomach to intact condition, respectively). Food items were identified to species level where possible, and separated into identified taxonomic groups or species, blotted for 1 min between tissue paper sheets, and weighed to the nearest 0.001 g. Intestinal contents were used for identification of undigested food items to the lowest possible taxonomic level and for counting and identification of intestinal parasites.

#### Analysis of stable isotopes

Analysis of stable isotopes and calculation of C and N isotope ratios was performed for all the caught organisms and SPM. Prior to the stable isotope analyses 2 mg per sample of dried tissues were wrapped in a tin cup and analyzed in the Laboratory of Analytical Chemistry at Faculty of Chemistry, University of Latvia by elemental analyser (EuroEA-3024, EuroVector S.p.A, Italy) coupled with continuous flow stable isotope ratio mass spectrometer (Nu-HORIZON, Nu Instruments Ltd., UK). Isotope values were reported relative to Vienna Pee Dee Belemnite with a lithium carbonate anchor (VPDB-LSVEC) for carbon isotope value $\delta^{13}C$ and to atmospheric nitrogen (air) for nitrogen isotope value $\delta^{15}N$. Stable isotope values were denoted as parts per thousand (‰) deviation from the standard, as follows:

$$\delta X\ (‰) = \left(R_{sample}/R_{standard} - 1\right) \times 1000,$$

where δX is $\delta^{13}C$ or $\delta^{15}N$ and the R ratio is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

An internal standard sample (glutamic acid) was used to check reproducibility of the stable isotope ratio determination. The pooled standard deviations were 0.14‰ ($n = 121$) for $\delta^{13}C$ and 0.21‰ ($n = 121$) for $\delta^{15}N$. Reference material L-glutamic acid USGS-40 (Reston Stable Isotope Laboratory of the US Geological Survey, Reston, Virginia, NIST®RM 8573) was used to check accuracy of the stable isotope ratio determination where stable carbon isotopic and nitrogen isotopic compositions with combined uncertainties are $\delta^{13}C_{VPDB\text{-}LSVEC} = -26.39 \pm 0.04‰$ and

$\delta^{15}N_{AIR} = -4.52 \pm 0.06‰$ [23]. Our results for reference standard USGS-40 were $\delta^{13}C = -26.38$, (SD = ±0.03‰, $n = 20$) and $\delta^{15}N = -4.54$ (SD = ±0.08‰, $n = 20$).

#### Data analysis and statistical assessment

Ward's minimum variance Clustering analysis was performed for identification of perch subgroups with agglomeration of objects based on variables $\delta^{13}C$, $\delta^{15}N$ and sampling location. The aim of the analysis was to split spatially subgroups of perch with consideration of sampling location and geographical markers provided by the signals of stable isotope values. Optimal number of clusters was selected according to the Silhouette Widths method [24].

Bayesian mixing model SIAR was chosen for quantification of common diet in the computed subgroups of perch: group 1 (representatives of the station 1), group 2 (representatives of the station 2), group 3 (representatives of the station 3) and group 4 (a mixed group including the specimens with the isotopic values which cannot be attributed to any of the station) [25]. Trophic discrimination factor was approximated for each station separately, based on $\delta^{13}C$ and $\delta^{15}N$ values and trophic levels found in the literature (Additional file 1: Table S2) of every organism sampled. The estimated trophic discrimination factors were 3.37 ± 1.27‰ ($\delta^{15}N$) and 0.36 ± 1.00‰ ($\delta^{13}C$) for station 1, 3.92 ± 1.31‰ ($\delta^{15}N$) and 1.04 ± 1.52‰ ($\delta^{13}C$) for station 2, 3.32 ± 1.14‰ ($\delta^{15}N$) and 0.74 ± 1.21‰ ($\delta^{13}C$) for station 3. Sources for the model were selected based on results of the stomach content analysis and the list of sampled organisms at the study areas. The food items' contribution ratios were extracted from the model and used for the further analysis. Because the system had two isotopes and more than three sources, unique solutions for each fish were replaced by averaged ranges of food items' contribution ratios. The data tables are available in Additional file 1: Table S3.

Trophic magnification factor (TMF) was calculated based on the species included into the station-specific diet of perch and perch itself. The food web TMF was computed from parameter $b$ or slope of the following equation [26, 27]:

$$\log(\mathrm{THg}) = a + b \times \delta^{15}\mathrm{N},$$

where $\mathrm{TMF} = 10^{b}$.

Analysis of covariance (ANCOVA) was implemented to compare obtained trophic magnification regression curves. During the analysis interactions between assigned groups (from cluster analysis) and $\delta^{15}N$ values were evaluated to understand whether the focus on specific diet shows significant difference in slopes of Hg trophic magnification. Two regression models were compared: M1 – LOG THg concentration estimated from the independent variable "$\delta^{15}N$" and independent factor "Group"; M2 – LOG THg concentration estimated from the interrelated variable "$\delta^{15}N$" and factor "Group". "Group" (the dataset was divided into subgroups, based on results of the clustering analysis mentioned above) as an independent factor was considered for identification of differences between intercepts of the trophic magnification regressions.

Smoothing function of Generalized Additive Models (GAM) was used to cover a slightly non-linear relationship of LOG-transformed THg concentration in perch dorsal muscles and length of specimens, thus allowing more sensitive evaluation of effect of dietary preferences. Data exploration protocol recommended by Zuur et al. [28] was applied before the modeling process. The obtained models were validated according to the guide suggested by Zuur & Ieno [29], including check of homogeneity, independence, influential observations, normality and fit of estimated values. Akaike Information Criterion (AIC) [30] was applied to compare the obtained GAMs and determine the best fit for the data, thus identifying feeding sources and other concomitant factors impacting Hg accumulation in consumer tissues. Due to collinearity of some variables, such as Crustacean and *Neogobius melanostomus* (correlation coefficient −0.8), Crustacean and *Neomysis integer* (correlation coefficient −0.7), *Gymnocephalus cernua* and *N. integer* (correlation coefficient −0.7), *G. cernua* and *N. melanostomus* (correlation coefficient −0.7), *N. melanostomus* and *N. integer* (correlation coefficient −0.7), three different models (A, B and C) were performed. Each of the models includes a combination of non-collinear variables, and the three models together contain all the food items selected as sources for the SIAR model mentioned above. *Ammodytes tobianus* was excluded from the models because of high covariance with *C. harengus membras* (correlation coefficient 1.0), thus further it may be considered that the species have similar effect on Hg uptake. The following three models were selected:

$$\text{Hg concentration}_{ij} \sim \text{Gaussian}(\mu_{ij})$$

$$E\left(\text{Hg concentration}_{ij}\right) = \mu_{ij}$$

Model A:

$$\begin{aligned}\log\left(\mu_{\mathrm{ij}}\right) &= \text{Intercept} + \delta^{15}N_{ij} \\ &+ \text{Crustacean}_{ij} \\ &+ \textit{Chironomidae larva}_{ij} \\ &+ \textit{P. fluviatilis juvenile}_{ij} \\ &+ \textit{G. cernua}_{ij} + \textit{O. limosus}_{ij} \\ &+ \textit{C. harengus}_{ij} + s\left(\text{Length}\right)_{ij} \\ &+ f\,\text{Season}.\end{aligned} \tag{1}$$

Model B:

$$\begin{aligned}\log\left(\mu_{\mathrm{ij}}\right) &= \text{Intercept} + \delta^{15}N_{ij} \\ &+ \textit{Chironomidae larva}_{ij} \\ &+ \textit{P. fluviatilis juvenile}_{ij} \\ &+ \textit{O. limosus}_{ij} + \textit{C. harengus}_{ij} \\ &+ \textit{N. melanostomus}_{\mathrm{ij}} + s\left(\text{Length}\right)_{ij} \\ &+ f\,\text{Season}.\end{aligned} \tag{2}$$

Model C:

$$\begin{aligned}\log\left(\mu_{\mathrm{ij}}\right) &= \text{Intercept} + \delta^{15}N_{ij} \\ &+ \textit{Chironomidae larva}_{ij} \\ &+ \textit{P. fluviatilis juvenile}_{ij} \\ &+ \textit{O. limosus}_{ij} + \textit{C. harengus}_{ij} \\ &+ \textit{N. integer}_{\mathrm{ij}} + s\left(\text{Length}\right)_{ij} \\ &+ f\,\text{Season}.\end{aligned} \tag{3}$$

$$\alpha_i \sim N\left(0,\ \sigma^2_{\text{Nest}}\right).$$

The Model C, with the most negative slope coefficient demonstrated by significant food item *C. harengus membras*, was selected as an example for visualization of modeling results. Wilcoxon rank sum exact test was implemented to examine differences in THg concentrations estimated from the Gaussian GAM model. The model was simulated for the scenarios with the maximum and minimum contribution ratio of *C. harengus membras* and continuously ranged from the maximum to minimum consumption (contribution) ratios of the other food items. The range limits at specific sampling stations were the same as computed by the SIAR model mentioned above. The visualization example can be found in Additional file 2.

Relationships tested were considered to be statistically significant for $p < 0.05$, except for GAM outputs, because p-values of estimates included produced by the model are not defining for model interpretation, but indicate secondarily variable. Data exploration, artworks, and statistical analyses were performed using R software for Windows, release 4.0.3 [31].

## Results

### Hg concentrations and stable isotope analysis

The full list of THg concentrations and stable isotope values measured in the SPM and all collected organisms can be found in Additional file 1: Table S4. THg concentrations measured in the dorsal muscles of perch varied notably in all three stations, thus mean concentrations and standard deviations (±SD) were 188.2 ± 42.0, 154.2 ± 71.3 and 110.8 ± 65.1 μg $kg^{-1}$ of wet weight in station 1, 2 and 3, respectively. The ranges of $\delta^{15}N$ values measured in perch were similar at all three sites (between approximately 14 and 18‰). At the same time, $\delta^{13}C$ values showed wide ranges (approximately 12 ‰) for perch individuals caught in stations 1 and 2, covering also isotopic signals associated with the coastal sampling station, and relatively narrow ranges (approximately 4‰) for the individuals from station 3. Similarly, to perch, also ruffe (*Gymnocephalus cernua*) and roach (*Rutilus rutilus*) exhibited high variations of THg concentration and stable isotope values.

### Stomach content analysis

The analysis of stomach content showed that stomach composition of perch significantly differs between freshwater and brackish water habitats (Fig. 2). At sampling stations 1 and 2, the Crustacean (found in 56% and 42% of the analyzed stomachs, respectively) was the

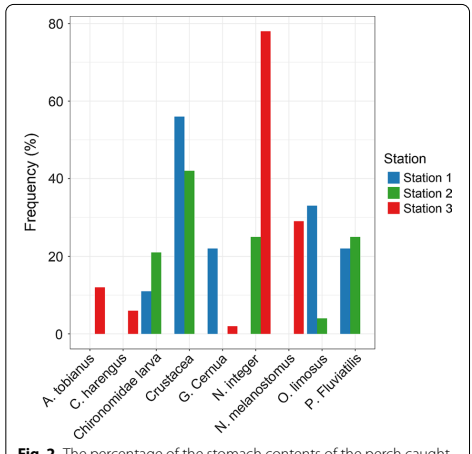


**Fig. 2** The percentage of the stomach contents of the perch caught

predominant prey. Juvenile perch (22% at station 1 and 25% at station 2) and *Chironomidae* larvae (11% at station 1 and 21% at station 2) were the second most frequently consumed prey organisms while *O. limosus* and *G. cernua* were found mainly only in the digestive tract of perch from station 1. At the same time, *N. integer* was the most frequent prey in station 3 (found in 78% of stomachs). *N. integer* was also found in 25% of perch stomachs from freshwater station 2. The *N. melanostomus* was the second most common prey in station 3, where it was found in 29% of perch stomachs. From station 3, the *A. tobianus* and *C. harengus membras* were represented only in 12% and 6% of stomachs, respectively.

**Cluster analysis**

Scatterplot of the calculated stable isotope values $\delta^{13}C$ and $\delta^{15}N$ demonstrated clear evidence that perch specimens migrate between the sampling stations (Fig. 3). Substantial proportion of specimens sampled in stations 1 and 2 had isotopic signals consistent with feeding in the station 3 (Fig. 3A). Consequently, we divided the dataset into four subgroups, according to the three characteristics: sampling place, stable isotope values $\delta^{13}C$, and stable isotope values $\delta^{15}N$ related to the trophic position of an organism (Fig. 3B and C).

The division was done as a cluster analysis based on the linear model criterion of least squares. Three of the subgroups were clearly representing respective sampling stations, while the fourth subgroup was well positioned as the mixed group (group 4) with overlapping

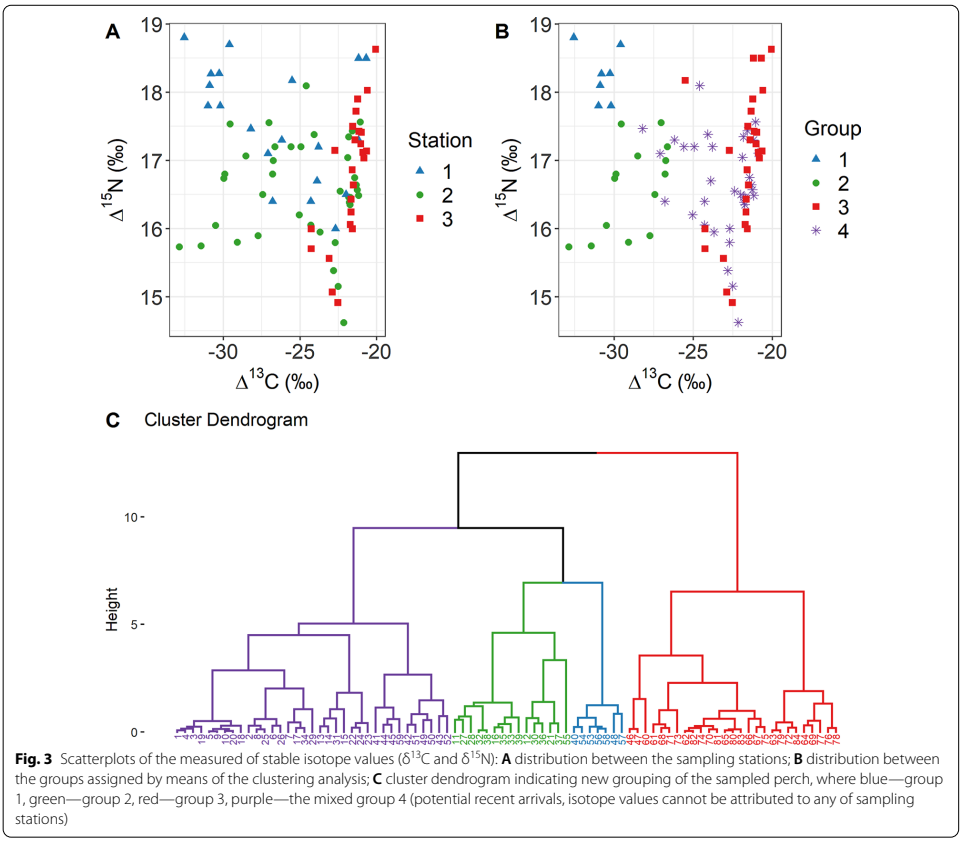


**Fig. 3** Scatterplots of the measured of stable isotope values ($\delta^{13}C$ and $\delta^{15}N$): **A** distribution between the sampling stations; **B** distribution between the groups assigned by means of the clustering analysis; **C** cluster dendrogram indicating new grouping of the sampled perch, where blue—group 1, green—group 2, red—group 3, purple—the mixed group 4 (potential recent arrivals, isotope values cannot be attributed to any of sampling stations)

isotope values, which cannot be associated with any of the three sampling stations. The mixed group was then considered as recent arrivals exhibiting different isotopic values of the station they were collected. The group of recent arrivals provided key information on gradual change of the modeled dietary composition explored by means of the following Bayesian mixing model, thus connecting lake, river and gulf into one ecosystem.

#### Exploration of the identified groups

The data were re-examined comparing THg concentrations and distribution of individual's length among the new groups designated via cluster analysis. Group 1 exhibited the highest THg concentrations (Fig. 4A) while the lowest mean concentration of THg was found in group 3. Opposite to concentration levels, the highest mean length of perch was found in group 3 while the lowest one in group 2. The groups 1 and 4 exhibited the middle values (Fig. 4B). Although the calculated bioaccumulation slopes were quite similar among the groups (coefficient values from 0.015 to 0.029), the intercepts differed noticeably (coefficient values from 1.4 for group 3 to 2.1 for group 1), thus indicating high variation of background Hg concentrations (Fig. 4C).

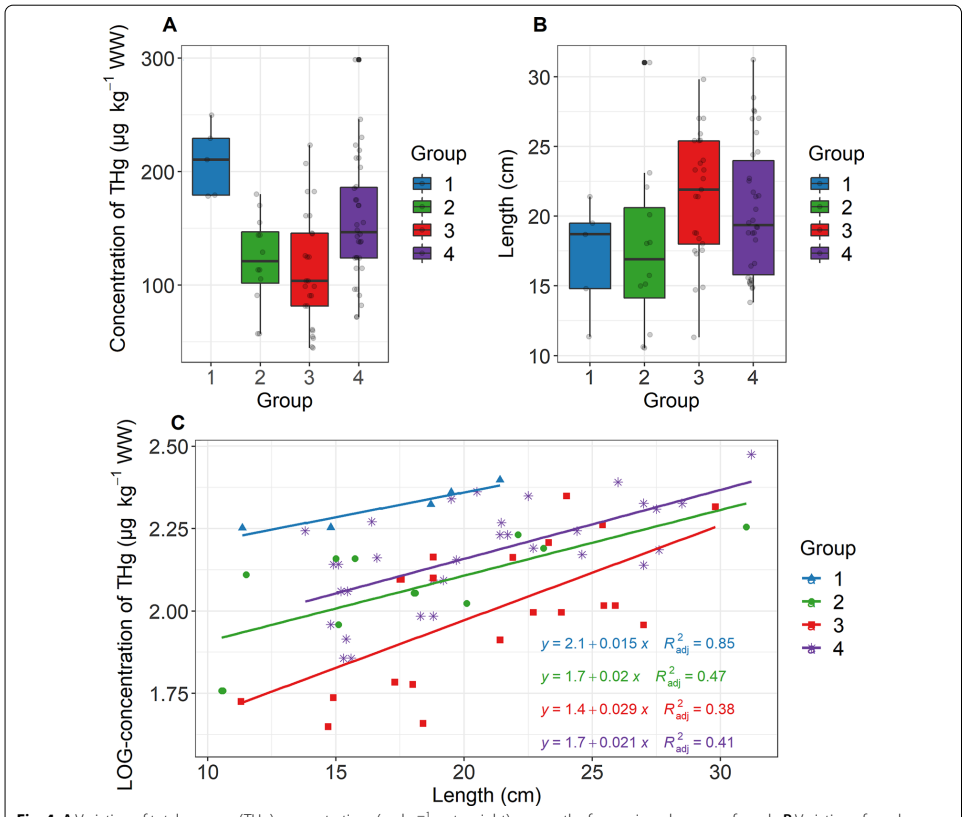


**Fig. 4** **A** Variation of total mercury (THg) concentrations (µg $kg^{-1}$ wet weight) among the four assigned groups of perch. **B** Variation of perch length among the four assigned groups. **C** THg bioaccumulation curves expressed as linear equations of LOG-transformed THg concentration versus perch length

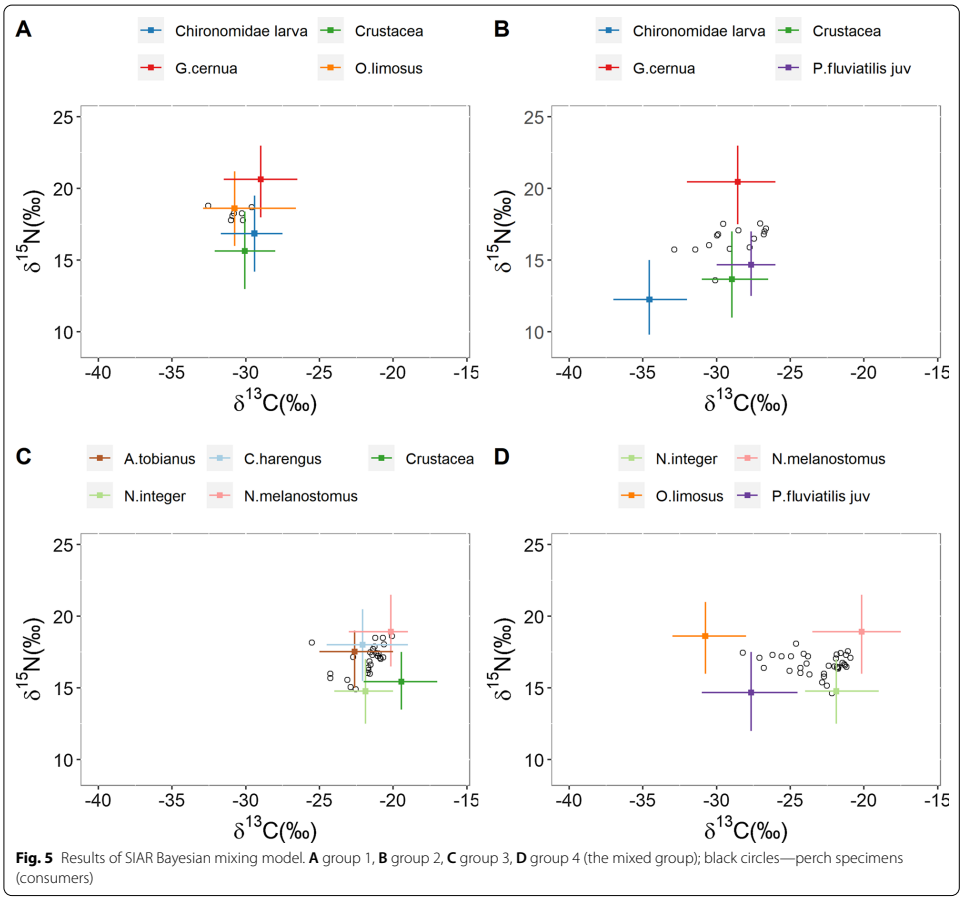


**Fig. 5** Results of SIAR Bayesian mixing model. **A** group 1, **B** group 2, **C** group 3, **D** group 4 (the mixed group); black circles—perch specimens (consumers)

### Quantification of feeding ecology

Feeding ecology of the assigned subgroups were defined by means of Bayesian mixing model SIAR (Fig. 5) based on results of the stomach content analysis of every perch individual representing the corresponding subgroup. The different types of crustaceans, *G. cernua, Chironomidae* larvae and *O. limosus* were defined as the main food sources for perch in station 1. The feeding base in station 2 mainly consisted of crustaceans, *G. cernua, Chironomidae* larvae and juvenile perch while the *N. integer, N. melanostomus, A. tobianus*, *C. harengus membras* and crustaceans were main food items of perch in station 3. In order to reflect the geographical distribution of the available diet in the interconnected system, as food items representative of each sampling stations were selected: *O. limosus* from station 1, juvenile perch from station 2, and *N. integer* and *N. melanostomus* from station 3. The four sources formed an appropriate mixing polygon which covered the vast majority of data points of the mixed group.

### TMF

TMF was calculated for specific food chains, reflecting the localized diet of perch for each identified subgroup. The absolute values of the calculated TMFs were quite similar for the station-assigned groups (1.45, 1.40,

**Table 1** Difference in the trophic magnification curves between the perch groups, compared by means of the variation analysis ANCOVA

| Compared groups | M1: without interaction[1] | | M2: with interaction[2] | | ANOVA: M1 vs M2[3] | |
|---|---|---|---|---|---|---|
| | *F* value | *p*-value | *F* value | *p*-value | *F* value | *p*-value |
| 1. vs 2 | 0.009 | 0.926 | 0.187 | 0.669 | 0.187 | 0.669 |
| 1. vs 3 | **8.569** | **0.005** | 0.011 | 0.916 | 0.011 | 0.916 |
| 1. vs 4 | 0.000 | 0.990 | **13.480** | **< 0.001** | **13.484** | **< 0.001** |
| 2. vs 3 | **9.943** | **0.002** | 0.771 | 0.383 | 0.771 | 0.382 |
| 2. vs 4 | 0.785 | 0.381 | **10.410** | **0.003** | **10.414** | **0.003** |
| 3. vs 4 | **6.859** | **0.011** | **15.110** | **< 0.001** | **15.110** | **< 0.001** |

Bold text indicates significant differences between the curves

[1] The regression model M1 includes independent variable $\delta^{15}N$, independent factor "Group" and dependent variable LOG THg concentrations

[2] The regression model M2 includes interaction between independent variable $\delta^{15}N$ and independent factor "Group" and dependent variable LOG THg concentrations

[3] One-way analysis of variance of the regression models M1 and M2

1.46, respectively), but substantially higher for the mixed group (1.76).

The trophic magnification curve of the mixed group (group 4) was significantly different from the others by a steeper slope (Table 1, M2) and by higher intercept compared to group 3 (Table 1, M1). Meanwhile, groups 1 to 3 had statistically similar slopes, thus indicating similar trophic magnification patterns (Table 1, M2). At the same time, significantly lower intercept of group 3, compared to groups 1 and 2 (Table 1, M1) probably denotes lower Hg background concentrations found at the station.

#### Influence of dietary preferences

Generalized Additive Modeling was implemented to understand how dietary preferences of perch in different feeding grounds affect the Hg uptake. To avoid covariance of food source variables, three validated models with different combinations of food items were selected and interpreted (Table 2). The obtained results indicated seasonality (spring and autumn sampling) as a significant factor affecting measured THg LOG-concentrations, for example, samples collected in spring had higher THg concentrations than the autumn samples (demonstrated by a positive intercept correction for the spring season from 0.106 up to 0.120). The $\delta^{15}N$ values showed a significant relationship with the THg concentration; however, the positive slope coefficient was only 0.09 in the all three models.

The models let us establish that the food item *C. harengus membras* had the most significant mitigating effect

**Table 2** Estimated regression parameters (intercept and slope values) for the Gaussian GAM presented in Eqs. 1, 2 and 3

| | |
|---|---|
| Model A | |
| $\log(\mu_{ij}) =$ | |
| AUTUMN | $0.536 + 0.092 \times \delta^{15}N_{ij} - 0.460 \times Crustacean_{ij} + 0.460 \times Chironomidae\ larva_{ij} + 0.098 \times \text{perch juvenile}_{ij} + 0.120 \times G.\ cernua_{ij} + 0.142 \times O.\ limosus_{ij} - 0.349 \times C.\ harengus_{ij} + s(\text{Length})_{ij}$ |
| SPRING | $0.642 + 0.092 \times \delta^{15}N_{ij} - 0.460 \times Crustacean_{ij} + 0.460 \times Chironomidae\ larva_{ij} + 0.098 \times \text{perch juvenile}_{ij} + 0.120 \times G.\ cernua_{ij} + 0.142 \times O.\ limosus_{ij} - 0.349 \times C.\ harengus_{ij} + s(\text{Length})_{ij}$ |
| Model B | |
| $\log(\mu_{ij}) =$ | |
| AUTUMN | $0.514 + 0.090 \times \delta^{15}N_{ij} + 0.299 \times Chironomidae\ larva_{ij} + 0.186 \times \text{perch juvenile}_{ij} + 0.174 \times O.\ limosus_{ij} - 0.484 \times C.\ harengus_{ij} + 0.079 \times N.\ melanostomus_{ij} + s(\text{Length})_{ij}$ |
| SPRING | $0.628 + 0.090 \times \delta^{15}N_{ij} + 0.299 \times Chironomidae\ larva_{ij} + 0.186 \times \text{perch juvenile}_{ij} + 0.174 \times O.\ limosus_{ij} - 0.484 \times C.\ harengus_{ij} + 0.079 \times N.\ melanostomus_{ij} + s(\text{Length})_{ij}$ |
| Model C | |
| $\log(\mu_{ij}) =$ | |
| AUTUMN | $0.529 + 0.091 \times \delta^{15}N_{ij} + 0.233 \times Chironomidae\ larva_{ij} + 0.167 \times \text{perch juvenile}_{ij} + 0.184 \times O.\ limosus_{ij} - 0.501 \times C.\ harengus_{ij} + 0.023 \times N.\ integer_{ij} + s(\text{Length})_{ij}$ |
| SPRING | $0.649 + 0.091 \times \delta^{15}N_{ij} + 0.233 \times Chironomidae\ larva_{ij} + 0.167 \times \text{perch juvenile}_{ij} + 0.184 \times O.\ limosus_{ij} - 0.501 \times C.\ harengus_{ij} + 0.023 \times N.\ integer_{ij} + s(\text{Length})_{ij}$ |

Standard errors, *t*-values and *p*-values can be found in Additional file 1: Table S5

on THg concentration, with negative slopes ranging from -0.349 to -0.501. Another food item with significant negative slope coefficient (-0.460) was Crustacean. The rest of the food items were secondarily significant for the model ($\alpha > 0.05$), although they had different directions of the influence and slope values. A highly positive effect was observed for *Chironomidae* larvae (slope values from 0.233 to 0.460). *O. limosus* (slope values from 0.143 to 0.184), perch juvenile (slope values from 0.010 to 0.186) and *G. cernua* (slope value 0.120) were other food items that contributed to the uptake of Hg by perch. *N. melanostomus* and *N. integer* exhibited a neutral influence on THg concentration measured in consumer perch, indicating slightly positive slope coefficients of 0.080 and 0.023, respectively.

## Discussion

The combination of stomach content analysis, as a sort of "snap-shot" of the recently consumed prey, with metabolically active tissues (such as muscles) that provide dietary and source information for up to several weeks [32] were instrumental in sorting out to which geographically distinct sampling area each perch specimen should be assigned. Since perch in the Gulf of Riga (station 3 area) do not have suitable spawning and nursing grounds, the specimen group assigned to that area has probably migrated to the Gulf of Riga from freshwater similarly to that observed elsewhere by Järv [11]. This agrees with behavioral features of perch, like seasonal patterns in their distribution and movement between habitats [33]. The distinct stomach content and characteristic of isotopic signals for this group suggests that once migrated to the coastal waters the perch specimens stay there whether as stable kin-related groups as suggested by Gerlach et al. [12] and Semeniuk et al. [33] or as separate individuals. The approach applied in this study enabled us also to identify recent arrivals, e.g., specimens that have been feeding and accumulating Hg in areas different from where they were caught.

As we successfully demonstrated, the perch specimens in freshwater ecosystems (river and lake stations) have substantially higher THg concentrations. So, with some degree of certainty we can speculate that observed inter-annual differences, from 30 μg $kg^{-1}$ ww in 2019 to 103 μg $kg^{-1}$ ww in 2015, of THg values obtained within national monitoring program (LIAE database) in coastal waters represented by station 3, can mostly be explained by different proportions between recent arrivals from adjacent freshwater basins and specimens that have been feeding in an area for more extended periods of time. Furthermore, the seasonal factor produced by all three GAM models, e.g., higher THg concentrations were associated with spring sampling, and can be clearly related to recent migration from inland waters to the coastal.

Although the concentration of THg in specimens representing freshwater ecosystems is substantially higher than in specimens representing marine coastal waters, the subtraction of values measured in recent arrivals resulted in a slight increase of mean concentration in the coastal group. Most likely, the observed phenomenon is related to significant upward change of median size of perch, and not to the Hg concentration itself. Therefore, it can be argued that comparison of concentration means alone is a poor approach for assessment of Hg contamination.

At the same time, the Hg bioaccumulation curves in relation to the length of individual gave more detailed information about the specific uptake tendencies. The results indicate that functional processes responsible for Hg accumulation (for example fish biometrics), Hg bioavailability and chemical composition of Hg substances [34] are quite similar, regardless of the specimen origin or local feeding base. So, the geographical differences in THg concentration were mainly observed because background concentrations of Hg are substantially higher in the inland water bodies than in the Gulf of Riga. This conclusion is supported by notably higher THg levels in SPM, used as a proxy of phytoplankton, collected in river and lake stations (Additional file 1: Table S4). And, as stated by Kehrig [35], Hg enters the food web at phytoplankton level and is transferred to higher organisms via trophic transfer.

The general structure of the perch diet was quite similar among the studied areas, e.g., mostly several types of crustaceans, *Chironomidae* larvae and small fish. However, *C. harengus membras* present only in the gulf station exhibited noticeable reduction properties of Hg uptake, which explains the substantial differences in the levels of THg measured in the station-associated groups indicated by the clustering analysis. Moreover, according to the study, the trophic position of prey alone (in our case, $\delta^{15}N$ values) cannot be associated with the intensity of Hg uptake by consumers. For example, *Chironomidae* larvae ($\delta^{15}N$ 8.3 to 13.4 ‰) and Crustacean ($\delta^{15}N$ 7.0–12.9‰) within the comparable maximum consumption ratio exhibited the opposite modeled effects on the estimated THg concentration in perch tissues, and *N. integer* ($\delta^{15}N$ 11.4‰) despite the twofold maximum consumption ratio showed a neutral impact. Similarly, higher THg concentration rates cannot be associated with the trophic position of prey within the same feeding ground, which was well demonstrated by *Chironomidae* larvae and *G. cernua* ($\delta^{15}N$ 15.7 to 18.5‰), where the prey with lower $\delta^{15}N$ values had stronger correlation with high THg concentrations estimated from the model. In the case

discussed above *Chironomidae* larvae showed substantially higher THg concentration values than Crustacean *(Amphipoda) and N. integer*, therefore we suggest that consumption ratio has to be discussed in conjunction with Hg concentrations measured in prey. Thus, besides precise determination of the food sources for better tracing of metal accumulation suggested by Le Croizier et al. [36], information on background concentrations at the site is important in our study as well. In addition, Jones et al. [37] suggests that, to avoid misinterpretation of spatial and temporal trends, fish biometrics modeling is of high significance when designing any monitoring program focused on seafood safety.

We propose that for future perspectives, besides Hg, other trace metals such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni) and zinc (Zn) can be investigated with the supplementary tool presented in this article. Although due to limits of models' sensitivity the effectiveness may vary depending on intensity of trophic magnification and/or biodilution slopes [38, 39], we assume that if TMF is close to 1, the tool may not be able to distinguish the difference in concentration of the contaminant between stations. The recent trophic magnification and biomagnification studies in the Baltic Sea indicated that Ni, Zn, Pb, Cd tend to be effectively biodiluted with increasing trophic level (TMF < 1), As, Cr and Cu showed no significant relationship with trophic levels (TMF = 1), while Hg trophically magnified (TMF > 1) [38]. In contrast to this, the global meta-analytical study conducted by Sun et al. [39] suggests that in other regions Pb and Zn also show trophic magnification tendencies [39].

The limitation of this study is that we presented a complete picture only from a single year perspective. We can of course speculate that the site-specific food items defined in this study will influence perch THg concentrations at an equal level also during following years. However, the well-known opportunistic feeding behavior of perch [33] suggests that they will inevitably switch to other taxa if availability of previously consumed taxa becomes limited, or if a more profitable source of energy appears, similarly as round goby (*Neogobius melanostomus*, invasive in the Baltic Sea) became a highly consumed prey for perch in recent years [40, 41]. Another weak point to be considered is that the isotopic signal changes faster than the level of accumulated Hg [24, 42]. So, the specimens, that at the onset of a feeding period have spent sufficient time in one area to equilibrate Hg concentration with the level characteristic for that area and then migrates to another area and have time to change isotopic signals before they are caught, might not have sufficient time to adjust also Hg concentrations. This could be improved by more regular sampling, which would give more precise information about influence of perch mobility on measured Hg concentrations. Also, comparison with another distant Gulf of Riga station which is insignificantly affected by the large freshwater ecosystems, could be a useful adjunct for the further studies.

In the case of this study, use of $\delta^{15}N$ and $\delta^{13}C$ values fulfilled our requirements regarding differentiation of the feeding grounds, examination of feeding ecology and thus trophic magnification of Hg. Although, when the object of interest is a higher resolution of the movement of the fish [43] or if $\delta^{13}C$ alone fails to distinguish spatial differences between habits or sources [44, 45], other isotopes such as sulphur ($^{34}S/^{32}S$) can be applied especially in estuary systems with a certain salinity gradient [44, 46, 47].

## Conclusions

The study showed that the THg concentrations measured in associated aquatic systems may be affected by high mobility of perch, which could be an issue for consideration during Hg contamination monitoring events. The recent arrivals can change distribution of Hg concentrations measured at specific locations, especially more contaminated inland individuals can noticeably rise concentration values in the coastal areas close to estuaries. The home-range migration habits of fish species selected as a biological matrix for investigation of the environmental Hg concentrations and possibly other hazardous substances, are an important feature which has to be discussed more carefully for accurate conclusions on the environmental status of the studied areas. We highly recommend implementation of chemical markers, such as stable isotopes, for identification of mobile specimens when designing monitoring programs focused on Hg and other hazardous substances. All the three sampling stations showed considerable trophic magnification of Hg through the food chain. Therefore, we concluded that different feeding grounds in the frame of one interconnected system may have specific features, such as higher or lower TMF and also unique food items, such as freshwater *O. limosus* and marine species *C. harengus membras* or *N. melanostomus*. The model showed that trophic position of prey is not decisive regarding Hg accumulation rates, although Hg concentration measured in prey in conjunction with its consumption ratio serves as good explainer of the measured concentrations of the hazardous substance.

#### Abbreviations

AAS: Atomic absorption spectrophotometry; AIC: Akaike information criterion; ANCOVA: Analysis of covariance; C: Carbon; $^{13}C/^{12}C$: Carbon stable isotope ratio; $\delta^{13}C$: Carbon isotope value; GAM: Generalized additive model; GF/F:

Glass fiber filter; HELCOM: The Baltic Marine Environment Protection Commission; HEPP: Hydroelectric power plant; Hg: Mercury; LIAE: Latvian Institute of Aquatic Ecology; LOG: Logarithmic; N: Nitrogen; $^{15}N/^{14}N$: Nitrogen stable isotope ratio; $\delta^{15}N$: Nitrogen isotope value; SD: Standard deviation; SIAR: Package "Stable Isotope Analysis in R"; SPM: Suspended particulate matter; THg: Total mercury; US EPA: United States Environmental Protection Agency.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-021-00523-w.

**Additional file 1: Table S1.** Quality assurance with biota reference material ERM-CE278k and ERM-BB422 within current study; **Table S2.** Trophic levels found in the available literature; **Table S3.** Computed ratios of source contribution in the final mixture (perch dorsal muscles), data obtained by means of Bayesian mixing model SIAR; **Table S4.** List of collected organisms and measured total mercury (THg) concentration, carbon and nitrogen isotopic values; **Table S5.** Estimated regression parameters (intercept and slope values), standard errors, *t*-values and *P*-values for the Gaussian GAM presented in Eqs. (1, 2, 3).

**Additional file 2.** Visualization of mercury (Hg) concentrations estimated for the Gaussian GAM for the maximum and minimum possible contribution of Clupea harengus membras to diet of perch.

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**Authors' contributions**
NS: conceptualization, sampling, investigation, sample treatment, data interpretation and visualization, writing of and drafting the original version. JA: conceptualization, data interpretation, writing—review and editing; RP: sampling site selection, experimental analysis, writing—review and editing. JT: sampling, experimental analysis, editing and reviewing. All authors read and approved the final manuscript.

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**Availability of data and materials**
The datasets obtained and analyzed in the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

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## Publisher's Note

## APPENDIX D

## MANUSCRIPT IV

**Suhareva, N.,** Aigars, J., Poikāne, R., Heredia, N.A., Tunēns, J., Baraškova, Ļ.

**THE TROPHIC TRANSFER OF HAZARDOUS SUBSTANCES FROM THE PERSPECTIVE OF FEEDING ECOLOGY**

Manuscript
Supplementary materials can be found here:
https://www.dropbox.com/scl/fi/qfbt94ivjfof824sxljss/Supplementary-materials.xlsx?dl=0&rlkey=qhhnkrzp39trbuqai993mnqok

# Trophic transfer of hazardous substances in the Baltic Sea from the perspective of feeding ecology of the European perch

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**Keywords: trophic magnification, feeding ecology, marine pollution, mercury, PBDE, PCB, Baltic Sea, Gulf of Riga**

## Abstract

Marine ecosystems face a significant ecological challenge due to the introduction and circulation of hazardous substances. While the main external sources are well documented, we still lack an understanding of their internal circulation in the ecosystem, including the mechanism of transfer of pollutants through the food chain. To address these gaps, the present study investigated the transfer of mercury, ΣPBDEs, ΣPCBs and their congeners via trophic transfer by combining consumer dietary characteristics and concentrations of these pollutants using European perch (*Perca fluviatilis*) and its prey as model organisms.

Trophic magnification factors for perch populations and their potential prey varied by trophic level ranges from 1.7 to 9.8 for total mercury, 0.3 to 1.9 for ΣPCBs, and 0.1 to 8.1 for ΣPBDEs. In distant sampling sites, PCBs and PBDEs did not follow the same trophic magnification slopes. The diet shifts between Decapoda, Amphipoda and Gobiidae at different life stages have the capacity to affect concentration levels of hazardous substances in perch tissues. Accordingly, we concluded that the main drivers changing trophic magnification among the sites, apart from pollution load, should be feeding ground characteristics like food availability and dietary composition unique for a particular habitat.

## 1 Introduction

The problem posed by hazardous substances (HS) to environmental integrity has been acknowledged since the 1970's. In order to prevent this threat, the European Union adopted Directive 76/464/EEC (EC, 1976). Thereafter, based on results of scientific studies, main principles, such as sedimentation (Pazikowska-Sapota, 2006; Ogrinc et al., 2019), re-mobilization (Josefsson et al., 2010), absorption directly from the ambient environment or bio-concentration (van der Velden et al., 2013), bio-accumulation (Borgå, 2013) and bio-magnification (Drouillard, 2008), were formulated and action programs targeted at improvement of environment health were elaborated. However, although wide range of actions aimed to decrease leaking of HS into the environment that have been implemented (EC, 2000, 2008a, 2008b, 2013, 2019; UNEP, 2001) the set goals have not been achieved as concentrations of some target substances, like dioxins (McLachlan and Undeman, 2020) or mercury and cadmium (European Environment Agency, 2017; HELCOM, 2018a), have been leveling out in recent years.

This emphasizes the importance of internal circulation of hazardous substances within marine ecosystems. Therefore, the interaction between HS and the ecosystem (Grower et al., 2016; Wenning & Martello, 2014), uptake by marine organisms (Nfon et al., 2008; Mizukawa et al., 2013), and its effects on these organisms and on the ecosystem as a whole became an important research topic (European Environment Agency, 2019; HELCOM, 2021). Quite often, slow biodegradability, like in case of PCBs (McLachlan and Undeman, 2020), is used to explain the tendency of substances to circulate in the environment even after the anthropogenic emissions were terminated or limited. However, the ability of organisms to uptake hazardous substances and pass them up the food chain, often magnifying the concentration at higher trophic levels, is also of significant importance to explain persistence of the number of substances in the marine environment.

There are a number of different monitoring approaches that are presently used to measure concentration levels of HS in various matrices such as biota (fish tissues, mussels), sediments and water (EC Directorate-General for Environment, 2011; Zampoukas et al., 2011; EC, Directorate-General for Environment, 2015). Due to often lipophilic characteristics of persistent organic pollutants, such as PCB and PBDE (Connell, 1997), they are hardly detectable in water (EC, 2013). Unfortunately, obtained concentrations of target pollutants in fish tissues provide no information regarding the trophic pathway of the substances. Furthermore, the best practices of research on trophic magnification (Kidd et al., 2019), suggest measuring substance concentration in the same type of tissue (muscles, liver, whole body, etc.), which is not always possible due to practical considerations. Consequently, uncertainty increases when pollution levels are compared between distant locations without taking into account the trophic path from prey to consumer.

In this study we attempted to trace the path of selected HS from prey to consumer, hypothesizing that different feeding ecology due to varying food availability can change rates of uptake of selected substances and consequently their accumulation. We also hypothesize that in some occasions, locations could only "appear" to be more polluted because of differences in the dietary composition of the monitored species.

The European perch *Perca fluviatilis*, the most widespread predator-fish in the Baltic Sea coastal region, was used as the model organism for this study. Gastrointestinal tract content analysis and analysis of stable isotopes of nitrogen were performed for almost each specimen caught during the study. Concentrations of HS, such as mercury (Hg), polybrominated diphenyl ethers (PBDE) and polychlorinated biphenyls (PCB) were measured in muscle tissues of perch as well as in different species used as prey by perch.

## 2 Materials and Methods

### 2.1 Study area

Two distinctly different coastal areas, Salacgrīva and Jūrmalciems (Figure 1) were selected for study purposes. The study area of Salacgrīva is representative of the Gulf of Riga, which is a semi-enclosed bay connected to the central Baltic Sea via the narrow and shallow Irbe Strait and the Väinameri Sea. Water salinity in the gulf varies between 4 and 7 PSU (practical salinity unit) with even lower salinity levels in the river estuaries (Yurkovskis et al., 1993). The study area of Jūrmalciems is representative of the Eastern Gotland Basin of the Baltic Sea coastal region. The water salinity in the sampling area is approximately 7 PSU (Feistel et al., 2010). The Baltic Sea is an epicontinental shallow sea. Water exchange with the North Sea and Atlantic Ocean is slow and limited by narrow connection via Danish straits (Håkanson et al., 2003). The Baltic Sea is nutrient rich, due to extensive discharge from agricultural areas in the surrounding countries (HELCOM, 2010, 2018b), and the drainage basin is

under serious anthropogenic stress (HELCOM, 2021) due to the dense population and intense industrialization (Nekoro, 2013).

The benthic habitats in both areas vary between species-rich macroalgae colonies in shallow areas, to soft bottom fauna in the deeper areas (HELCOM, 2018b). Biological community, depending on the bottom type, mainly consists of brown and red seaweeds (on hard substrates), predominantly *Fucus vesiculosus* in Salacgrīva and *Furcellaria lumbricalis* in Jūrmalciems (HELCOM, 2018b). The benthic habitats are occupied by polychaete worms and mollusks, including the bivalves the Baltic clam *Macoma balthica* or blue mussel *Mytilus trossulus*, depending on substrate. Other species present in the study areas include crustaceans, such as amphipods (mainly *Monoporeia affinis*), isopods (*Saduria entomon*), mysids and decapods (*Palaemon elegans*, *Crangon crangon*). Some of the above organisms are important food sources for benthic fish species, such as turbot *Scophthalmus maximus*, flounder *Platichthys flesus* and round goby *Neogobius melanostomus* (Ustups et al., 2016). Nevertheless, the most widespread predatory fish is the bentho-pelagic fresh-water species *P. fluviatilis* (HELCOM, 2012). Both locations are naturally connected with freshwater ecosystems: Salacgrīva with River Salaca, and Jūrmalciems is located between freshwater outlets of Lake Liepājas and the Curonian Lagoon.

### 2.2 Sampling and sample pretreatment

The entire sampling campaign took place between 2018 – 2021. Perch and other fish species were collected in coastal (<1.8 km from coastline) waters once per month during the summer season from May to September, by means of scientific Coastal Survey multimesh gillnets (Nippon Verkko Oy, Finland) with nine 5-m-long panels of different mesh sizes (mesh size ranging between 10 and 60 mm). Nektobenthic and pelagic invertebrates and fish (as well as juveniles) were caught by means of beach seine (hand-made: the wing length 10 m and mesh size of 10 mm knot to knot, the depth of 1.5 m, and mesh size of the cod end of 5 mm) monthly in June, July, August 2018 and 2019 and May 2021. Soft bottom benthic organisms were sampled by van Veen Grab (25 kg, Hydrobios, Germany) in August 2018 and 2019 and May 2021.

After sampling all caught organisms were immediately sorted by species, placed in plastic containers and/or pre-cleaned glass jars (certified by Cole-Palmer, USA) and frozen at -20ºC till further processing.

The total length and weight of fish were determined by measuring board (accuracy ±1 mm) and technical scale KERN FCE3K1N (KERN & SOHN GmbH, Germany; accuracy ±1 g). Perch specimens were sorted by length into five groups (<130 mm, 130-159 mm, 160-189 mm, 190-219 mm and ≥220 mm), thereafter, dorsal muscles and digestive tract were dissected. One part of dorsal muscle was placed in an individual plastic container for further analysis of total mercury (THg) content and analysis of stable isotope ratios of nitrogen (N), another part of dorsal muscle was added to a pooled sample by length in pre-cleaned glass jars (certified Cole-Palmer, USA) for PBDE and PCB analyses and frozen at temperature -20°C

For the further analysis of THg content and stable isotopes of N, the samples of fish tissues and prey organisms were dried in a freeze dryer (LYOVAC GT 2-E, STERIS GmbH, Germany), water content was recorded. Dry samples were homogenized by knife mill (IKA A11 basic, IKA-WERKE GmbH & CO.KG, Germany) or agate pestle and then stored in a desiccator in the dark at room temperature (+20°C).

### 2.3 Analytical analyses

### 2.3.1 Detection of THg

Concentration of THg in the dorsal muscles of perch and whole body of other organisms was determined at the accredited laboratory of the Latvian Institute of Aquatic Ecology, agency of Daugavpils University (LHEI) using a direct combustion Hg analyzer (Teledyne Leeman labs, "Hydra IIc", USA) following US EPA Method 7473 (U.S. EPA, 1998). THg concentrations were measured in every perch specimen and in every sample of other organisms pooled by a taxonomic group at the same location. Analytical quality was controlled by measurements of blank, Hg standard solutions made diluting Hg stock solution 1000 mg Hg L-1 (Roti®Star Hg standards for AAS in 10% $HNO_3$, traceable to SRM from NIST, CARL ROTH, Karlsruhe, Germany), reference materials of mussel tissue ERM-CE278k and fish muscle ERM-BB422 (both certified by Institute for Reference Materials and Measurements at Joint Research Centre, European Commission, Geel, Belgium) and random sample duplicates. These measurements were performed for calibration verification at the start and to prove accuracy of determination at the end of every batch of 20 samples (Table S1). Relative percent difference between sample duplicate analyses was 1.2 $\pm$ 1.2% (n=134). The laboratory regularly and successfully takes part in the Quality Assurance of Information for Marine Environmental Monitoring in Europe (QUASIMEME) studies for quality assurance. All Hg concentrationsare expressed as content in dry weight (DW) to ensure comparability among organisms.

### 2.3.2 Detection of PBDE and PCB

Concentrations of polybrominated diphenyl ethers (PBDE) and polychlorinated biphenyls (PCB) were measured in accredited external laboratory on a commercial basis – analytical laboratory "ALS Czech Republic" (Prague, Czech Republic, testing laboratory No. 1163, accreditation certificate Nr. 610/2017 valid until 28.02.2022.). Before delivery pooled samples in pre-cleaned glass jars were frozen at -20ºC, packed in a cool box with cooling elements and delivered to the laboratory within 24 h for further analyses. The temperature of samples was measured immediately after delivery, in all batches it was below +4ºC. PBDE were analyzed according to the local accredited method CZ_SOP_D06_06_177 "Determination of selected brominated flame retardants (BFR) by isotope dilution method using HRGC-HRMS and calculation of brominated flame retardants sums from measured values" based on modified US EPA 1614 method (U.S.EPA, 2007). The method included the following BDEs No.: 28, 47, 99, 100, 153, 154, 183, 209. For trophic transfer analysis the concentration data BDE congeners were also pooled together in group ΣPBDE. PCB were analyzed according to the local accredited method CZ_SOP_D06_06_173 "Determination of polychlorinated biphenyls by isotope dilution method using HRGC-HRMS and calculation of PCB sums and TEQ parameter from measured values" based on methods US EPA 1668A (U.S.EPA, 2003) and ČSN EN 16190 (CEN, 2018). For this study concentration data of the following congeners was pooled together in group ΣPCB, regardless of Toxic Equivalent (TEQ) scheme: dioxin-like PCB No. 77, 81, 105, 114, 118, 123, 126, 156, 167, 169, 189, as well as indicator PCB No. 28, 52, 101, 138, 153, 180. Among prey organisms collected at Salacgrīva, PBDE and PCB concentrations were measured in two size groups (≤50 mm and 100-120 mm) *N. melanostomus*, Mysida, *Gasterosteus aculeatus* and *Alburnus alburnus*, and in the following organisms collected at Jūrmalciems: *C. crangon* and *P. elegans*, juvenile *Ammodytes tobianus*, aggregated sample of juvenile *P. flesus* and *S. maximus*, juvenile *Clupea harengus membras*, juvenile *N. melanostomus*, and *Osmerus eperlanus*. In order to enable use of different organisms in the same trophic transfer analysis, PBDE and PCB concentrations were normalized to 5% lipid content.

### 2.3.3 Analysis of stable isotopes

Analysis of stable isotopes and calculation of N isotope ratios was performed for all organisms used in this study. Prior to the stable isotope analyses, 2 mg per sample of dried tissues were wrapped in a tin cup and analyzed in the Laboratory of Analytical Chemistry at Faculty of Chemistry, University of

Latvia by elemental analyzer (EuroEA-3024, EuroVector S.p.A, Italy) coupled with continuous flow stable isotope ratio mass spectrometer (Nu-HORIZON, Nu Instruments Ltd., UK). Nitrogen isotope $\delta^{15}N$ values were reported relative to atmospheric nitrogen (air). Stable isotope values were denoted as parts per thousand (‰) deviation from the standard, as follows:

$$\delta^{15}N\ (‰) = \left(\frac{^{15}N}{^{14}N_{sample}} \Big/ \frac{^{15}N}{^{14}N_{standard}} - 1\right) \times 1000.$$

An internal standard sample of glutamic acid and international reference material L-glutamic acid USGS-40 (Reston Stable Isotope Laboratory of the U.S. Geological Survey, Reston, Virginia, NIST®RM 8573) were used for stable isotope ratio measurement quality control (Table S2).

### 2.4 Analysis of digestive tract

Digestive tract content analysis was performed as described in Manko (2016) with few modifications. The extracted gastrointestinal tract (GIT) was defrosted at room temperature. Thereafter, the length of stomach, intestine and whole GIT, were measured on a measuring board to the nearest mm mark and the stomach and intestine were separated by a cut at the pylorus just before the pyloric caeca. The GIT parts were then put between two tissue paper sheets, blotted dry, and the mass of stomach, intestine and whole GIT were weighted on an analytical balance to the nearest 0.001 g.

Stomach and intestines were put in separate Petri dishes and carefully cut open with a scalpel without damaging the contents. Once open, contents were flushed out with a gentle stream of tap water via a wash bottle or pushed out with a blunt probe. Samples were then inspected under a stereomicroscope (Leica MEB126, Leica Microsystems, Singapore). When encountered, the indigestible intestinal contents (e.g., fish otoliths and bones, mollusc shells, polychaete bristles and chitinous insect and crustacean parts) were also identified to the lowest possible taxonomic level, however due to their state of digestion and the presence of mucosa, intestinal contents were not weighted. The taxonomically separated stomach contents and the empty stomach and intestines were again blotted dry and weighted with precision of 0.001 g.

### 2.5 Data analysis

Trophic level (TL) of organisms was calculated as follows (Fisk et al., 2001; Post, 2002):

$$TL = \frac{\delta^{15}N_{sample} - \delta^{15}N_{base}}{3.4} + 2,$$

where $\delta^{15}N_{sanple}$ is the $\delta^{15}N$ value of the organism of interest, $\delta^{15}N_{base}$ is the $\delta^{15}N$ value of a primary consumer *M. balthica*, which was used as a base organism in both sampling locations, 3.4 is the average increment of $\delta^{15}N$ per trophic level (Post, 2002; Nfon et al., 2008) and 2 is the TL value of the primary consumer.

In addition to the common method of trophic transfer analysis, where the trophic magnification factor (TMF) and the trophic magnification slope (TMS) are calculated for the entire data set, including consumer and potential prey, the explanatory variables were calculated for prey separately ($TMF_{prey}$) and for perch occupying trophic positions from 2.9 to 4.0 in Salacgrīva and from 3.0 to 4.1 in Jūrmalciems ($TMF_{consumer}$), in order to determine how the trophic magnification changes as a consumer moves from lower to higher trophic levels.

The food web TMF was computed from parameter b, or slope, of the following equation (Fisk et al., 2001; Muto et al., 2014):

$$LOG_{10}(THg) = a + b \times TL,$$

where TMF= $10^b$. A positive slope (b > 0) indicates a biological amplification, whereas a negative slope (b < 0) suggests a biological dilution. Consequently, TMS is the coefficient b in the equation.

Oligochaeta and Polychaeta were excluded from the trophic magnification curveas the former was rarely found in perchdiets and the latter, and due to has a known tendency to have relatively high $\delta^{15}N$ values compared to their true trophic level (Hilgendag et al., 2022). *M. balthica* was used only for the calculation of the trophic level of the studied organisms. *O. eperlanus* was excluded from the trophic magnification analysis due to freshwater origin. To avoid the influence of pollution from the adjacent freshwater origin, the TMF of THg was calculated only for specimens of perch that exhibited clear marine signals of δ13C, according to Suhareva et al. (2021).

To improve comparability of Hg, PBDE and PCB pollution levels among the sampling sites, measured concentrations were first log-transformed to get the linear relationship between concentration and TL, and then each measurement was arithmetically normalized to correspond to a trophic level (TL) of 2 for prey organisms and to a TL of 3.5 for perch, thus increasing the data pool (Suhareva et al., 2020) and statistical power.

Concentrations of contaminants were expressed as mean and standard error (mean±SE), whereas prey occurrence frequency in perch stomach was given as mean and standard deviation (mean±SD).

Fulton's condition factor (FK) was calculated from fish total length (L) and weight (W) using equation:

$$FK = \frac{L}{W^3} \times 100.$$

Statistically significant differences between perch populations were checked by means of Kruskal-Wallis one-way analysis of variance and Dunn's Kruskal-Wallis Multiple Comparisons integrated into fish condition analysis suggested by (Ogle, 2016) and performed using R package *FSA*. Gabelhouse lengths of proportional size distribution (substock = 0, stock = 130, quality = 200, preferred = 250, memorable = 300, trophy = 380) were computed using function *psdVal* for “White perch”, which exhibited the closest length ranges to the European perch caught during this study (Ogle, 2016).

Data exploration and statistical analyses were performed using R software for Windows, Release 4.2.1. Differences between trophic magnification curves and concentration levels were tested by means of linear regression analysis and Kruskal–Wallis one-way analysis of variance. The verified relationships were considered statistically significant at $p < 0.05$.

## 3 Results

### 3.1 Description of collected organisms

During the study period a total of 1214 and 541 individuals of perch were collected from Salacgrīva and Jūrmalciems, respectively (Table 1). The size range of the selected specimens was 73-293 mm (mean size = 157±38 mm) for Salacgrīva, and 85-358 mm (mean size = 181±49 mm) for Jūrmalciems. The trophic level of perch varied from 2.5 to 4.0 in Salacgrīva and from 2.4 to 4.1 in Jūrmalciems. The

prey used for the analysis occupied a broad trophic niche, exhibiting TL values of 1.9-3.7 at both sites (Table 1).

### 3.2 Concentrations of selected pollutants

At both sites, the THg concentrations in perch followed an expected pattern, e.g., concentrations increased with increase in specimen length and varied substantially at both sites (Table 1 and 2; Table S3a). Generally, observed concentrations of THg were noticeably lower in specimens obtained in Jūrmalciems than in specimens obtained in Salacgrīva. Similarly, concentrations of THg in perch prey items generally exhibited lower concentrations in Jūrmalciems (Table 2) than in Salacgrīva (Table 1). Furthermore, the concentration pattern varied between prey items gathered in Salacgrīva and Jūrmalciems. So, the lowest concentrations of THg were observed in Amphipoda in Salacgrīva and in juvenile *O. eperlanus and* Isopoda in Jūrmalciems . The highest concentrations were also observed in different organisms, e.g., *G. aculeatus* in Salacgrīva and juvenile *N. melanostomus* in Jūrmalciems.

Concentrations of PCB and PBDE were only measured in a few selected groups of prey organisms due to insufficient mass of pooled samples for analyses. The PCB concentrations in perch muscle tissue showed a wide range of values, with higher mean concentration in perch caught at Jūrmalciems station (Tables 1, 2, S3b). In prey the lowest ΣPCBs concentrations were observed in Mysida and highest in juvenile *P. flesus* and juvenile *O. eperlanus* substantially exceeding concentrations observed in perch. The highest concentration levels were found for congeners CB105, CB118 and CB156 (Table S3b).

The PBDE concentrations in perch tissues were in more or less similar ranges across the sampling sites (Table 1 and 2, S3b). The lowest concentrations of ΣPBDE were found in Decapoda (*C. crangon*) and highest in *N. melanostomus* and juvenile *O. Eperlanus*. The highest concentration level was found for congener BDE47 (Table S3b).

Prior to further data analysis, we compared body condition of perch between sites. According to Fulton's factor values (Figure 2A), morphometric index of perch caught at Jūrmalciems was substantially higher ($p<0.001$) than it was for specimens from Salacgrīva, suggesting more favorable nutrition condition (Datta et al., 2013; Ramos et al., 2013), thus this can be an evidence of different food reserves and therefore dietary composition affected by prey availability. Moreover, the maximum length group "memorable" (> 300 mm) was met only in Jūrmalciems catches. Lastly, the lower ($p<0.001$) trophic level occupied by specimens caught at Jūrmalciems compared to those of the same total body length from Salacgrīva (Figure 2B) adds to the evidence suggesting variable perch ecology among the different habitats.

### 3.3 Feeding ecology

Although, at both Jūrmalciems and Salacgrīva, perch diet consisted of the same principal components, frequency proportions of prey occurrence and variation along trophic gradient were substantially different (Figure 3). Perch diet at Jūrmalciems showed principal differences between lower and higher trophic levels (3-3.4 and 3.5-4). In lower trophic levels the diet was almost homogeneously distributed among Mysida (on average 19.0 ± 5.2%), Decapoda (18.6 ± 7.7%), Gobiidae (13.5 ± 7.3%) and Gasterosteidae (21.4 ± 12.4%), and in the higher trophic levels, the most frequent prey items were Decapoda (42.8 ± 9.5%) and Gobiidae (20.3 ± 4.2%). Other prey groups, such as Amphipoda, Polychaeta, Isopoda and small fish (Ammodytidae, Osmeridae, Pleuronectidae) were present in very small quantities (in average 2.2 ± 1.7%) regardless of the consumer trophic levels. In perch stomachs from Salacgrīva, the most abundant prey was Amphipoda (on average 30.6 ± 8.9%), followed by Gobiidae (19.7 ± 5.5%) and mysid (15.7 ± 9.1%). Decapoda occurred on average in 3.9 ± 2.9%

analyzed fish, similar to the other less consumed prey: Isopoda (4.2 ± 4.9%), Polychaeta (7.5 ± 2.1%) and Insecta (8.3 ±6.4). Small fish, including Gasterosteidae, Ammodytidae, Osmeridae, Pleuronectidae and other were presented in less than 1%.

### 3.4 Trophic magnification of hazardous substances

Trophic magnification of hazardous substances at both locations was expressed using five main descriptors: base HS concentration normalized to the trophic level of 2 ($HS_{TL2}$) calculated only for prey data; HS concentration normalized to the trophic level of 3.5 ($HS_{TL3.5}$) calculated only for perch data; trophic magnification factor for the entire food chain (TMF); trophic magnification factor ($TMF_{prey}$) calculated for prey items only, and $TMF_{consumer}$ calculated for perch only (Table 3).

TMF and $TMF_{consumer}$ of THg were significantly higher in Jūrmalciems, than in Salacgrīva, indicating a more rapid Hg uptake rate. Whereas $TMF_{prey}$ did not differ significantly among the sites. Despite the lack of statistical evidence for the normalized $THg_{TL2}$ concentration, the difference in the intercepts of the trophic magnification regression suggested that at the prey level environmental concentrations of THg in Salacgrīva are higher than in Jūrmalciems. Simultaneously, opposite to prey, $THg_{TL3.5}$ levels in Jūrmalciems were significantly higher than in Salacgrīva.

Normalized $PBDE_{TL2}$ and $PCB_{TL2}$ were found at the same level in prey from both Jūrmalciems and Salacgrīva (Table 3). Similarly, trophic magnification among prey ($TMF_{prey}$) and of the entire food chain (TMF) did not differ significantly between the sites. Therefore, the gaps in concentration data among the prey organisms can be filled assuming that the trophic magnification of ΣPCBs and ΣPBDEs follows the same statistically insignificant ($p=0.303$ and $p=0.860$, for ΣPCBs and ΣPBDEs, respectively) trophic magnification slope (Figure 4).

The trophic magnification trends for PBDE and PCB in five size groups of perch (<130, 130-159, 160-189, 190-219, and ≥220 mm) were different from those for prey items. Simultaneously, the $TMF_{consumer}$ of PCB and PBDE was positive (TMF>1) at Salacgrīva and negative (TMF<1) at Jūrmalciems. Although, PBDE concentration rapidly increased in Salacgrīva perch starting at TL = 3.8. $PCB_{TL3.5}$ concentration was significantly lower at Salacgrīva than at Jūrmalciems, and opposite $PBDE_{TL3.5}$ was higher in the gulf. So forPCB, higher normalized concentration in perch at Jūrmalciems does not reflect the occurring release of the accumulated substance in the older specimens.

Trophic magnification slopes (TMS) of PBDE and PCB congeners varied substantially both in Jūrmalciems and Salacgrīva (Figure 5). In general, the majority of congeners displayed opposite magnification slopes between the two locations. However, certain congeners exhibited noteworthy trends within each location.

Among PCBs, CB189 had the strongest negative slope in perch from Salacgrīva (TMS = -2.5), which was also supported by a negative TMS in perch from Jūrmalciems (TMS = -1.2). The TMS of CB189 in prey from Salacgrīva displayed similar tendencies to these observed in perch samples from the same location (TMS = -1.6), although the TMS of this congener in prey from Jūrmalciems had an opposite direction and was weaker (TMS = 0.4).

The strongest positive TMS among PCB congeners was exhibited by CB157 in perch from Jūrmalciems (TMS = 3.15), although it was not as strong in perch from Salacgrīva (TMS = 0.2). CB28 also had a strong positive TMS, with similar trends observed in perch from both Jūrmalciems (TMS = 2.8) and Salacgrīva (TMS = 1.6). In prey organisms, CB28 and CB157 displayed very weak positive TMS in both Jūrmalciems and Salacgrīva (TMS = 0.3 and 0.4, and TMS = 0.5 and <0.1, respectively).

Regarding PBDEs, the strongest positive TMS in perch was observed in BDE99 and BDE100, where it was equal to 2.0 and 2.6 in Salacgrīva and 1.0 and 0.3 in Jūrmalciems. The strongest negative TMS was found for BDE209 in perch from Jūrmalciems (TMS = -1.8), although it was not supported by the TMS in Salacgrīva, where it was positive (TMS = 0.9). Similar tendencies were not observed for TMS of PBDEs in prey.

### 3.5 Correlation with dietary composition

Spearman's rank correlation analysis between logTHg concentration in perch muscles and the frequency of occurrence of Decapoda in their stomachs showed a strong positive correlation in Jūrmalciems ($r = 0.9$, $p < 0.001$) and a moderately positive correlation when data from both Jūrmalciems and Salacgrīva were pooled together ($r = 0.53$, $p = 0.011$). In addition, the frequency of occurrence of Mysida in perch diets exhibited a strong negative correlation with THg concentration in Jūrmalciems ($r = -0.88$, $p < 0.001$) and a moderately negative correlation in pooled data from the both sites ($r = -0.56$, $p = 0.007$). The separate correlation analysis for Salacgrīva data showed a strong positive correlation between THg concentration in perch and the frequency of occurrence of insects in fish stomachs ($r = 0.78$, $p = 0.004$), and a negative correlation with the frequency of occurrence of Polychaeta and Isopoda ($r = -0.66$, $p = 0.026$ and $r = -0.66$, $p = 0.028$, respectively).

Spearman's rank correlation analysis was also conducted for PCB and PBDE congeners, BDE99, CB189, and CB28, which displayed the strongest TMSs in the same direction for both Jūrmalciems and Salacgrīva (Figure 6). Based on the correlation results of pooled data from both sites, consumption of Gobiidae and Polychaeta negatively correlated with BDE99 concentration in perch muscle tissues ($p = 0.016$ and 0.004, respectively). Although it is worth noting that the frequency of occurrence of Gobiidae positively correlated with frequency of Mysida ($r = 0.64$, $p = 0.048$) and negatively with that of Decapoda ($r = -0.70$, $p = 0.025$). Conversely, CB189, exhibited a strong positive correlation ($p = 0.019$) with the proportion of Gobiidae in perch diets. And concentration CB28 had only significant correlation with increase of Decapoda in perch diets ($p = 0.048$).

## 4 Discussion

### 4.1 Condition factor and feeding ecology

Body condition factor reflects growth patterns affected by numerous biotic and abiotic circumstances such as water quality, parasitic infections and metabolic rate (Cren, 1951; Datta et al., 2013; Famoofo and Abdul, 2020). Differences in fish body condition between locations could be attributed to a variety of factors, including feeding ecology, differences between littoral and pelagic feeding grounds, trade-offs in foraging efficiency, individual body morphology and variations in migratory strategy (Svanback and Eklov, 2004; Chapman et al., 2015; Hansson et al., 2019). Additionally, higher growth rates have been observed in lower salinity environments (Christensen et al., 2021). The European perch, a dominant piscivorous fish in coastal areas of the Baltic Sea (Hansson et al., 2018), is a visual predator (Jacobson et al., 2019). Accordingly, predation success and thus feeding ecology of perch is dependent on a relatively narrow range of water depths and temperatures (Persson, 1986; Bergman, 1987), vegetation cover (refugia) and turbidity levels (Andersson et al., 2009; Lunt and Smee, 2015). As both study areas are coastal with similar hydromorphological characteristics and relatively minor differences in salinity levels, the observed higher body condition factor values (Fulton's factor) in Jūrmalciems compared to those in Salacgrīva cannot be attributed to physical or hydromorphological condition, so differences in nutrition and feeding ecology should be explored.

Perch are highly opportunistic predators that will often switch to feeding on macroinvertebrates if fish are not available (Järv et al., 2011) or they will skip the shift to a piscivorous diet altogether (Jacobson et al., 2019). According to GIT content analysis, perch mostly followed a benthic diet with a few exceptions at both study sites. In Jūrmalciems the diet was more homogeneously distributed among various prey at lower trophic levels of specimens with mostly Decapoda and some Gobiidae at higher trophic levels while in Salacgrīva the perch diet primarily consisted of Amphipoda, followed by Gobiidae and Mysida, followed by lower proportions of other prey groups. It is highly likely that a predominantly Decapoda diet versus a predominantly Amphipoda diet is the main cause for the differences in condition factor among study sites. Although limited, the available data suggest that observed differences in diet is due to higher availability of decapods in Jūrmalciems compared to Salacgrīva, creating more favorable feeding conditions as described by (Ray, 2005) rather than lower competition for food with other species as described by (Martino and Able, 2003).

Previously, it was reported (Almqvist et al., 2010; Liversage et al., 2017) that perch are important predators of the invasive species round goby (*M. melanostomus*), and that when round goby densities are high predation on native fish species is mitigated (Liversage et al., 2017). The results of this study confirm that suggestion, e.g., perch at both sites exhibited almost complete indifference to any kind of fish prey, except round goby. However, the observed relative proportion among food items precludes concluding that perch are an important predator of the round goby.

Simultaneously, insects (Odonata, Epemeroptera, Trichoptera) appeared more frequently in the stomachs of perch with higher trophic levels, which indicates a tight relationship to the freshwater feeding grounds up the Salaca river. It was previously suggested that seasonal and natal migratory behavior (Siddika and Lehtonen, 2004; Christensen et al., 2021) as well as large migration distances (Järv, 2000) promote a diversity of feeding opportunities, including freshwater feeding grounds (Järv et al., 2011; Christensen et al., 2021; Suhareva et al., 2021).

### 4.2 Trophic magnification of Hg

The results of this study indicated that feeding ecology and Hg contamination of perch populations are interconnected. Despite similar $TMF_{prey}$ and $THg_{TL2}$ at both study sites (Salacgrīva and Jūrmalciems), TMF and $TMF_{perch}$ exhibited expected (Suhareva et al., 2020) THg magnification with trophic level increase, resulting in higher $THg_{TL3.5}$ in perch individuals caught in the site with higher magnification rate. Similar magnification rates among prey probably can be explained by the fact that at lower trophic levels feeding ecology is more straightforward and consists of a narrowerfood sources compared to perch, so Hg transfer is expected to be more or less equable. Simultaneously, any difference of exposure of benthic organisms, such as crustaceans, polychaetes and bivalves to methyl-Hg species via respiratory surfaces (Gray, 2002), which can potentially cause additional variation in the measured concentrations in these organisms, was not statistically supported by given the prey organisms analyzed in this study. Similarly, (Hilgendag et al., 2022) revealed that a lack of a significant relationship between methyl-Hg concentration (well correlated with THg) and trophic level in fish exhibited benthic diet trends could be due to exposure to methyl-Hg via non-dietary pathways. We argue that this was relevant for THg concentration in perch, especially due to diversity of feeding ecology. Opportunistic dietary ecology of perch can be highly affected by prey availability and habitat features (see above), therefore trophic transfer of Hg can follow numerous scenarios and dietary paths. More rapid trophic magnification of THg in perch from Jūrmalciems is very likely due to strongly pronounced dietary shift from the least polluted Amphipoda and Mysida to prey with higher THg concentrations, specifically Decapoda and *N. melanostomus*, which was not observed in Salacgrīva. Elevated levels of mercury concentration available in the coastal habitats and its biomagnification may result due to a

range of factors, including low rates of primary and secondary production, hydrological connection to methylation sites (Ward et al., 2010), larger amount of nutrients (Kidd et al., 2012) which probably correlates with inflow of terrigenous organic matter (Jędruch et al., 2019) via coastal erosion and river runoff (Jędruch et al., 2019; Hilgendag et al., 2022) and finally bioavailability of Hg species (Kidd et al., 2012; Jędruch et al., 2019).

This study reveals conflicting patterns of trophic magnification of Hg, where the concentration at lower trophic levels of the benthic food web do not always correspond to the same relative pollution level at higher levels of the trophic chain. Moreover, this uncertainty does not seem to have an easy solution as Hg concentrations in sediments do not always reflect Hg bioaccumulation potential (Xu et al., 2019), despite serving as a major reservoir of Hg (Chen et al., 2012; Gworek et al., 2016; Jędruch et al., 2019). A strong positive correlation in Jūrmalciems between the frequency of decapods found in perch GIT and the THg concentration must be critically considered, as it could be caused by the positive covariation of both variables with trophic level. However, a moderately positive correlation between decapod occurrence and THg concentration in the pooled data from both study sites did not exhibit any correlation with trophic level, thus indicating that decapods are a potential driver for more rapid Hg uptake. This suggests that higher proportion of more polluted decapods in the diet of perch from Jūrmalciems compared to less polluted amphipods that are more frequent in the diet of perch from Salacgrīva, may act as a significant Hg transfer channel in perch food chain at the studied feeding grounds.

Based on the results, we suggest that trophic magnification of THg is site specific, where key factors of variation line up in a complex scheme of interactions between biotic and abiotic drivers that form a nutritional prey base with unique pollution levels and availability. This founding is also supported also by previous studies on trophic magnification and biomagnification of Hg (Ward et al., 2010; Jędruch et al., 2019; Fioramonti et al., 2022; Hilgendag et al., 2022).

### 4.3 Trophic magnification of PCBs and PBDEs

The specific requirements for THg analysis allow for the measurement of individual fish, and almost all of the sampled prey, regardless of size. Thus the procedure provides high resolution of data including possible variations, easily detected errors and ability to trace uptake changes along the trophic scale. Detection of widely monitored persistent organic substances such as polybrominated diphenyl ethers (PBDE) and polychlorinated biphenyls (PCB) requires a certain sample size, which often compels researchers to aggregate numerous individuals into one pooled sample, thus the obtained concentration represents a pool average, canceling the opportunity of detailed trophic transfer analysis. PBDE and PCB are non-polar organic substances that tend to bioaccumulate in lipid-rich organisms (Kidd et al., 2019). Elevated concentrations of PBDE and PCB frequently found above the quantification level in the studied sites allowed us to examine trophic transfer features applying the approach approbated on Hg magnification and described above. A previous study which focused on PBDEs concentrations in sewage sludge, freshwater sediments and perch tissues at a variety of study sites in the region (Aigars et al., 2017) revealed a serious pollution problem. This study supports the previous findings by high PBDEs concentrations found in the majority of organisms caught in the coastal waters of gulf and sea.

Our study found that although concentrations of ΣPBDE and ΣPCB in prey did not vary significantly between the two locations and showed no significant difference in trophic magnification, the concentrations in perch reflected opposite tendencies (differed both, concentration and $TMF_{consumer}$). As stated above, starting with TL=3.6, the diet of Jūrmalciems perch mostly consists of Decapoda,

which had comparably low PBDE concentration compared to juvenile and small fish species in the respective feeding ground. This, however, fails to explain the PCB decrease, because PCB concentrations in Gobiidae and Decapoda were in similar ranges. Therefore, relatively high ΣPCB concentration in smaller perch (TL≤3.6), can be related to the early life stages, which perch likely spend in a nursing ground with higher levels of available PCBs. For example, ΣPCB concentration in a pooled perch sample from one of the freshwater bodies adjacent to the Jūrmalciems sampling site (Lake Liepājas) was 11.5 μg $kg^{-1}$ LW, which is substantially higher than the concentration range measured in perch from both Jūrmalciems and Salacgrīva. As a proxy, juvenile smelt (*O. eperlanus*) caught in Jūrmalciems can be used for the comparison of concentrations. Anadromous smelt generally inhabit marine coastal zones, spawn in freshwater bodies, where larvae spend the summer season, and then migrate to the sea in autumn (Plikšs and Aleksejevs, 1998). ΣPCB concentrations measured in the pooled sample of smelt juveniles were higher than in any other analyzed prey. High concentrations of PCBs have also previously been reported for European eel (*Anquilla anquilla*) caught in Lake Liepājas (Zacs et al., 2016). Although, there is insufficient evidence to avoid speculation and indicate exactly where perch spend early life stages.

In the analyzed GIT of perch from Salacgrīva, at TL=3.7, the occurrence of Amphipoda switches from being the predominant prey item to being comparable with that of Gobiidae, as TL increases. Another perceptible shift in the diet composition was the increasing frequency of insects. As previously discussed, the insects found in the diets of perch from Salacgrīva are more likely to inhabit freshwater ecosystems. Therefore, it could be possible thatin Salacgrīva there is a stronger influence of freshwater prey on the uptake of PBDE and PBC during the later life-stages, when compared to perch from Jūrmalciems.

Based on our results, we agree with other studies stating that although trophic position correlates with concentration of at least some congeners, the trophic transfer mechanism of these hazardous substances is more complex (Olsson et al., 2000; Burreau et al., 2004; Kobayashi et al., 2019). Hanson et al., (2020) stated that, after a previous downward trend in Hg, PCB118 and PCB153 concentrations in perch (also Szlinder-Richert et al., 2009 for PCBs in different species), there was an increase in pollutant levels. More specifically, they claim that the concentration rise is likely related to diet changes and the ecology of macroinvertebrates, rather than a change in pollution pressure. Moreover, they speculate that the concentration increase coincides with the distribution of *Marenzelleria virdis*, an invasive species, which can dig deep into sediments and remobilize buried PCBs. Other studies suggest that, besides feeding ecology (Hanson et al., 2020; Madgett et al., 2022) multiple biotic factors such as sex and body size (Burreau et al., 2004), tissue type, reproductive status and metabolic efficiency can affect concentrations of PCB and PBDE accumulated in marine organisms (Buckman et al., 2006; Szlinder-Richert et al., 2009; Tian et al., 2010; Zhang et al., 2016). Additionally, the fate and potential of persistent organic pollutants depends on physical-chemical properties of substances, i.e., hydrophobicity and molecular size (Russell et al., 1999; Olsson et al., 2000; Nfon et al., 2008). Theoretically, higher biomagnification and thus trophic magnification potential is related to more pronounced hydrophobic properties (Burreau et al., 2004). Therefore, penta-BDE show maximum biomagnification, whereas hexa- to hepta-BDE, same hexa- to nona-PCB, exhibit lower biomagnification properties, and large octa- to deca-BDE were suggested to be unable to biomagnify (Burreau et al., 2004; Mizukawa et al., 2013). The highest concentrations among PBDEs in all analyzed organisms during this study were detected for (tetra-) BDE47 and (deca-) BDE209 at both sampling sites, which agrees with PBDE concentrations detected in perch from inland freshwater bodies (Aigars et al., 2017). Opposite to the expected trends, BDE47 exhibited moderate or no trophic magnification, while BDE209 showed very high TMS among both perch and their prey at Salacgrīva. Like Kierkegaard et al. (1999) and (Burreau et al., 2004) this finding confirms the its bioavailability of

BDE209 despite its large molecular size. Moreover, PCB congeners with the highest detected concentration at both sites were (penta-) CB105, (penta-) CB118 and (hexa-) CB156, also failed to show signs of trophic magnification, as evidenced by negligible TMSs for the gulf and the sea coast.

## 5 Conclusion

This study demonstrates the importance of accounting for feeding ecology in the analysis of trophic transfer and magnification of hazardous substances such as THg, PCBs, and PBDEs. Considering these results, we conclude that HS uptake is influenced not only by dietary composition, but also by the proportion of consumed prey in the diet specific to the feeding ground. Thus, higher concentrations found in predatory fish may not necessarily indicate higher pollution loads in the site, but rather more successful trophic transfer as a result of dietary characteristics and food availability. Furthermore, the potential ability of accumulated pollution to be transported from other adjacent waterbodies to the assessment area will require further studies to examine consumer migration behavior.

## Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Author contributions

NS, RP, JT, LB, NAH contributed to sample collection and pre-treatment. JT and LB conducted GIT content analysis. RP and NS were responsible for management of laboratory analyses and making the operational data available for further proceeding. NS conducted the data processing, produced the figures, analyzed the results, and wrote the manuscript based on discussion with JA, RP and N.A.H. JA is a project leader, contributed to conceptualization and design of the manuscript, together with NS and RP. All authors provided comments on the manuscript. All authors contributed to the article and approved the submitted version.

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## List of abbreviations

DW – dry weight

GIT - gastrointestinal tract

Hg - mercury

HS – hazardous substances

JM – Jūrmalciems

LW – lipid weight

THg – total mercury

TL – trophic level

TMF – trophic magnification factor

TMS – trophic magnification slope

PBDE (BDE) – Polybrominated diphenyl ethers

PCB (CB) - Polychlorinated biphenyls

SG - Salacgrīva

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**Data Availability Statement**

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

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**Table 1.** Concentrations of THg, PCB and PBDE measured in perch and its potential prey collected at sampling site Salacgrīva SG (THg concentrations per perch individual and concentrations for PCB and PBDE congeners are given in Table S3a and S3b in Supplementary materials).

| Study site | | Organism | $\delta^{15}$N±SE (‰) | TL±SE | THg±SE (μg·kg$^{-1}$DW) | ΣPCBs (μg·kg$^{-1}$LW) | ΣPBDEs (μg·kg$^{-1}$LW) |
|---|---|---|---|---|---|---|---|
| SG | *Consumer* | *P. fluviatilis* | 16.5±0.0 (12.9-18.1) | 3.6±0.0 (2.5-4.0) | 236.4±5.5 (82.6-1455.5) | 2.15±0.19 (1.53-2.69) | 0.64±0.13 (0.42-1.12) |
| | Prey invertebrates | Polychaeta | 14.7±1.0 | 3.0±0.3 | 81.7±9.5 | | |
| | | Oligochaeta | 10.9±0.0 | 1.9±0.0 | 83.5±0 | | |
| | | Cardiida *(M. balthica)* | 11.2±0.1 | 2.0±0.0 | 112.7±19.7 | | |
| | | Isopoda *(Idotea sp.)* | 11.7±0.0 | 2.2±0.0 | 91.5±0.0 | | |
| | | Amphipoda | 11.2±0.2 | 2.0±0.1 | 52.9±11.5 | | |
| | | Mysida | 13.0±0.2 | 2.5±0.1 | 80.0±9.2 | 0.70 | 0.22 |
| | | Decapoda *(C. crangon)* | 14.3±0.5 | 2.9±0.1 | 79.2±2.8 | | |
| | | Decapoda *(P. elegans)* | 13.2±0.4 | 2.6±0.1 | 101.5±2.0 | | |
| | Prey vertebrates | *G. aculeatus* | 13.9±0.0 | 2.8±0.0 | 157±0 | 1.73 | 0.24 |
| | | *N. melanostomus* (≤50 mm) | 12.1±0.0 | 2.3±0.0 | NA | 1.95 | 0.77 |
| | | *N. melanostomus* (100-120 mm) | 14.0±0.7 | 2.8±0.2 | 117.4±24.2 | 2.03 | 0.97 |
| | | *A. tobianus* | 13.5±0.4 | 2.7±0.1 | 128.6±54.8 | | |
| | | *O. eperlanus* (juv) | 16.2±0.0 | 3.5±0.0 | 54.9±0.0 | | |
| | | *C. harengus membras* (juv) | 15.2±0.0 | 3.2±0.0 | 97.7±0.0 | | |
| | | *P. flesus (juv)* | 15.6±0.0 | 3.3±0.0 | 109.3±0.0 | | |
| | | *A. alburnus* | 12.9±0.0 | 2.5±0.0 | NA | 2.06 | 0.52 |

**Table 2.** Concentrations of THg, PCB and PBDE measured in perch and its potential prey collected at sampling site Jūrmalciems JM (THg concentrations per perch individual and concentrations for PCB and PBDE congeners are given in Table S3a and S3b in Supplementary materials).

| Study site | | Organism | $\delta^{15}$N±SE (‰) | TL±SE | THg±SE (μg·kg$^{-1}$DW) | ΣPCBs (μg·kg$^{-1}$LW) | ΣPBDEs (μg·kg$^{-1}$LW) |
|---|---|---|---|---|---|---|---|
| JM | *Consumer* | *P. fluviatilis* | 13.6±0.0 (9.4-15.4) | 3.6±0.0 (2.4-4.1) | 342.9±8.2 (46.9-1110.3) | 4.19±0.48 (3.19-5.89) | 0.53±0.10 (0.4-0.83) |
| | Prey invertebrates | Polychaeta | 13.9±0.0 | 3.7±0.0 | 92.3±0.0 | | |
| | | Oligochaeta | 8.5±0.2 | 2.1±0.1 | 43.5±0.0 | | |
| | | Cardiida *(M. balthica)* | 8.1±0.0 | 2.0±0.0 | 106.0±0.0 | | |
| | | Isopoda | 8.1±0.5 | 2.0±0.1 | 23.1±2.9 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *(Idotea sp.)* | | | | | |
| | Amphipoda | 8.2±0.2 | 2.0±0.1 | 36.6±1.0 | | |
| | Mysida | 10.3±0.0 | 2.6±0.0 | 61.6±2.4 | | |
| | Decapoda *(C. crangon)* | 11.3±0.4 | 2.9±0.1 | 80.7±21.4 | 3.48 | 0.20 |
| | Decapoda *(P. elegans)* | 10.4±0.1 | 2.7±0.0 | 71.9±17.6 | 2.92 | 0.27 |
| Prey vertebrates | *G. aculeatus* | 9.8±0.3 | 2.5±0.1 | 88.0±2.0 | | |
| | *N. melanostomus* (100-120 mm) | 10.2±0.0 | 2.6±0.0 | 126.2±0.0 | 3.98 | 0.67 |
| | *A. tobianus* | 12.4±0.0 | 3.2±0.0 | 37.7±0.0 | 1.70 | 0.54 |
| | *O. eperlanus* (juv) | 13.3±0.0 | 3.5±0.0 | 23.0±0.0 | 7.68 | 1.28 |
| | *C. harengus membras* (juv) | 10.5±0.0 | 2.7±0.0 | 32.5±0.0 | 1.17 | 0.36 |
| | *P. flesus, S. maximus* (juv) | 11.7±0.2 | 3.0±9.1 | 68.0±28 | 6.44 | 0.67 |

**Table 3**. Descriptors of trophic magnification calculated from Hg, PCB and PBDE concentrations in prey and consumer organisms: $TL_2$ – concentration normalized to trophic level 2, $TL_{3.5}$ – concentration normalized to trophic level 3.5, TMF – trophic magnification factor, $TMF_{consum}$ – trophic magnification factor calculated on perch data only, $TMF_{prey}$ – trophic magnification factor calculated on prey data only.

| | | Jūrmalciems | Salacgrīva | Statistically significant difference between sampling sites |
|---|---|---|---|---|
| **Hg** | **$TL_2$** | 25.4 ± 2.2 μg·kg$^{-1}$ DW | 33.9 ± 3.0 μg·kg$^{-1}$ DW | p = 0.069 |
| | **$TL_{3.5}$** | 338.3 ± 37.6 μg/kg DW | 225.7 ± 6.2 μg/kg DW | p < 0.001 |
| | **TMF** | 5.5 | 2.93 | Slope: p < 0.001<br>Intercept: p < 0.001 |
| | **$TMF_{consum}$** | 9.8 | 4.7 | Slope: p < 0.001<br>Intercept: p < 0.001 |
| | **$TMF_{prey}$** | 1.9 | 1.7 | Slope: p = 0.718<br>Intercept: p = 0.018 |
| **PCB** | **$TL_2$** | 0.31 ± 0.066 μg·kg$^{-1}$LW | 0.35 ± 0.11 μg·kg$^{-1}$ LW | p = 1.000 |
| | **$TL_{3.5}$** | 3.67 ± 1.13 μg·kg$^{-1}$LW | 1.46 ± 0.20 μg·kg$^{-1}$ LW | p = 0.005 |
| | **TMF** | 1.4 | 1.3 | Slope: p = 0.888<br>Intercept: p=0.005 |
| | **$TMF_{consum}$** | 0.3 | 1.9 | Slope: p = 0.029<br>Intercept: p = 0.003 |
| | **$TMF_{prey}$** | 1.4 | 1.4 | Slope: p = 0.979<br>Intercept: p = 0.225 |
| **PBDE** | **$TL_2$** | 0.063 ± 0.016 μg·kg$^{-1}$ LW | 0.12 ± 0.06 μg·kg$^{-1}$ LW | p = 0.715 |
| | **$TL_{3.5}$** | 0.63 ± 0.058 μg·kg$^{-1}$ LW | 1.40 ± 0.38 μg·kg$^{-1}$ LW | p = 0.029 |
| | **TMF** | 1.2 | 1.3 | Slope: p = 0.846<br>Intercept: p = 0.454 |
| | **$TMF_{consum}$** | 0.1 | 8.1 | Slope: p = 0.040<br>Intercept: p = 0.560 |
| | **$TMF_{prey}$** | 1.4 | 1.2 | Slope: p = 0.928<br>Intercept: p = 0.684 |

Figure captions:

**Figure 1.** Map of sampling sites: coastal zone of the Gulf of Riga – Salacgrīva, coastal zone of the Baltic Sea – Jūrmalciems.

**Figure 2. A:** Fulton factor as a fish condition metric for perch caught in coastal waters of the Gulf of Riga (Salacgrīva) and the Baltic Sea (Jūrmalciems). Letters A-D and a-d indicate statistically significant differences in Fulton's factor among Gabelhouse length categories. **B:** Relation between trophic level of caught perch and the total body length (mm), JM - Jūrmalciems, SG - Salacgrīva.

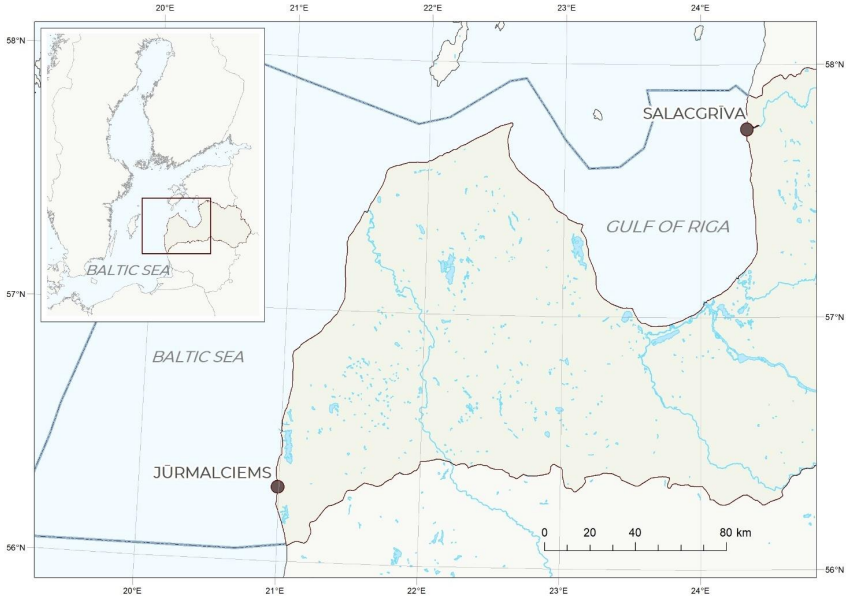
**Figure 3.** Occurrence of the most common prey found in stomachs of perch caught at coastal waters of the Gulf of Riga (Salacgrīva) and the Baltic Sea (Jūrmalciems); values are available in supplementary material (Table S4).

**Figure 4.** ΣPCBs and ΣPBDEs trophic magnification slope for combined prey data collected at Jūrmalciems and Salacgrīva sampling sites.

**Figure 5.** Trophic magnification slopes calculated for log-concentration of PCB and PBDE, normalized to 5% lipid content.

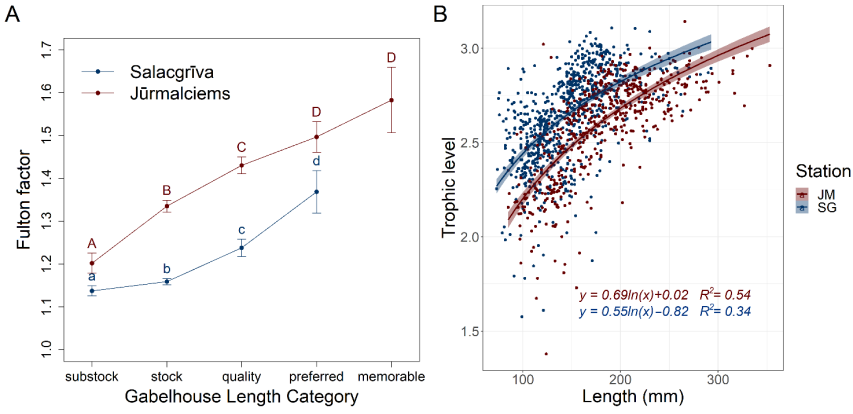
**Figure 6.** Spearman's rank correlation analysis of the log concentrations of POPs (BDE99, CB189, and CB28) in perch from Jūrmalciems and Salacgrīva and the frequency of occurrence of different prey types in their stomachs. The POPs were selected for analysis based on two criteria: 1) large trophic magnification slopes, and 2) similar slope directions in both locations. Empty cells indicate insignificant correlation relationships ($p<0.05$). Full correlation matrix see in the supplementary materials (Table S5).

843 Figure 1.




844

845 Figure 2.

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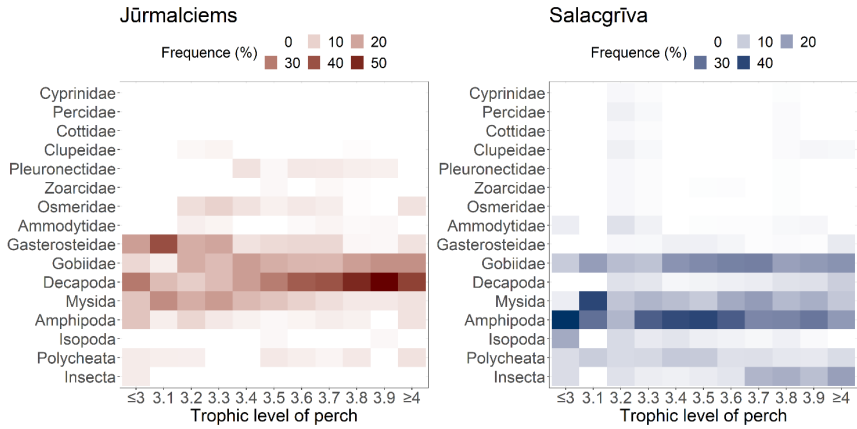
847 Figure 3.


Jūrmalciems
Salacgrīva
Frequence (%)
0
10
20
30
40
50
Cyprinidae
Percidae
Cottidae
Clupeidae
Pleuronectidae
Zoarcidae
Osmeridae
Ammodytidae
Gasterosteidae
Gobiidae
Decapoda
Mysida
Amphipoda
Isopoda
Polycheata
Insecta
≤3 3.1 3.2 3.3 3.4 3.5 3.6 3.7 3.8 3.9 ≥4
Trophic level of perch

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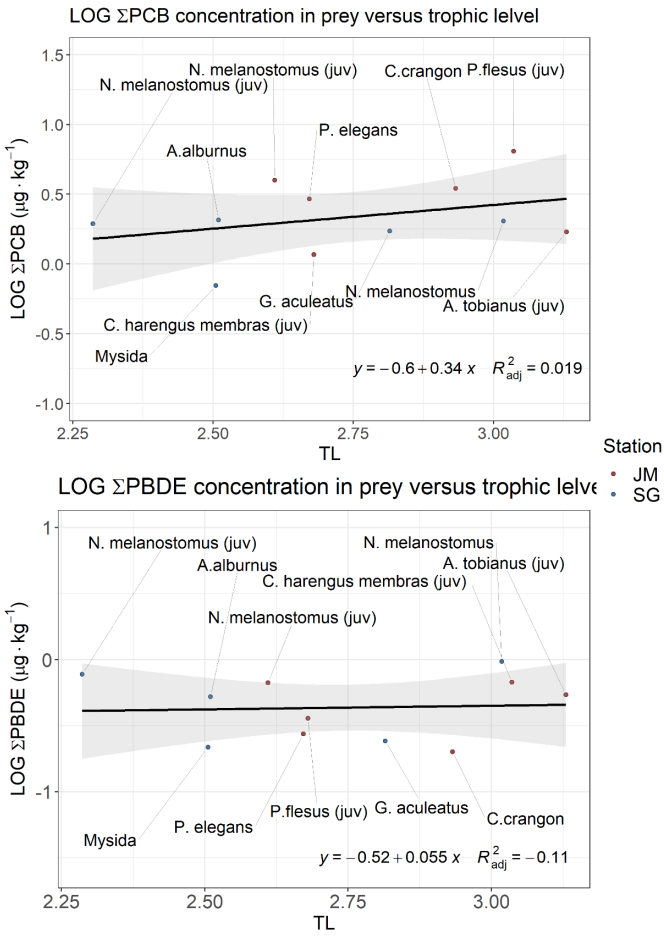
850 Figure 4.

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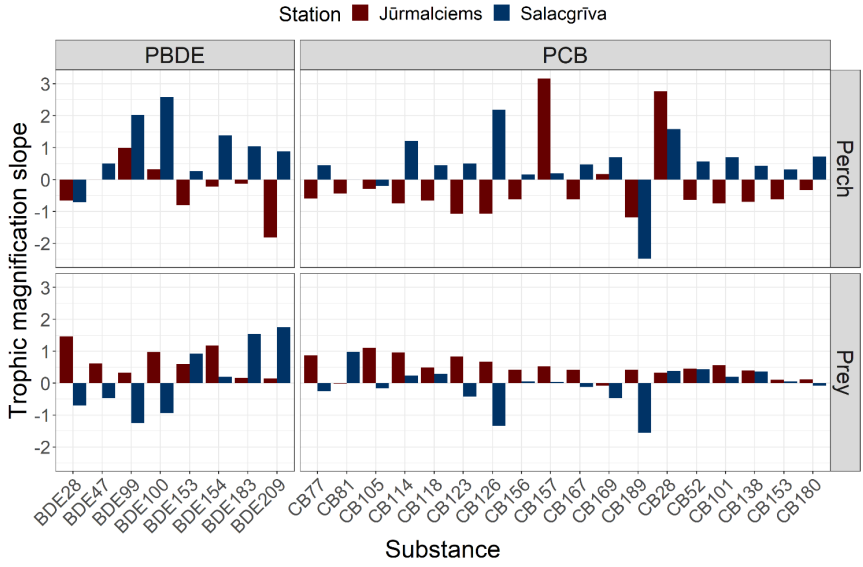
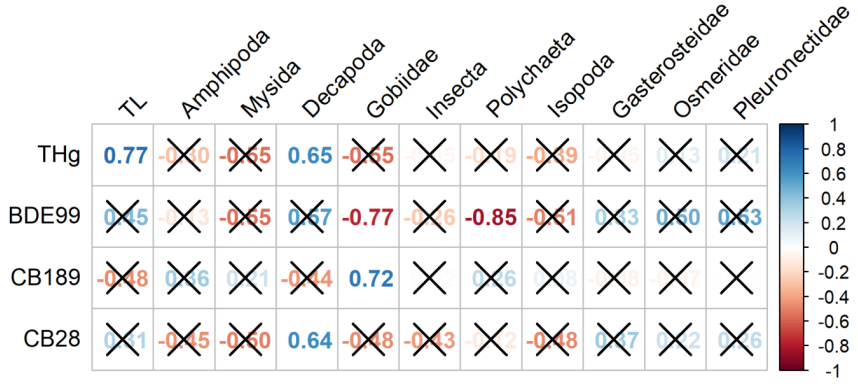
852 Figure 5.




853

854 Figure 6.

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