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New approaches in biomonitoring of extraneous substances in aquatic environment

Nové postupy biomonitoringu cizorodých látek ve vodním prostředí

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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

1.1. INTRODUCTION

Many recent human activities lead to pollution of the environment. Thousands of production or fabrication processes produce huge amounts of materials and substances, which are potentially hazardous to biota, including humans. According to the data from CAS Registry, an average of four thousand new chemicals are registered every day and the increase has an exponential trend (Binetti et al. 2008). There are many recent examples of poor decisions of using new chemical substances or disregarding risks of using and disposing of dangerous materials, e.g. DDT and some other pesticides, freons, phosphates in washing powders, polychlorinated biphenyls, toxic metals, etc. Nearly three thousand chemicals are high-production-volume (HPV) chemicals with production of more than 1 million pounds per year; HPV chemicals are extensively used in homes, schools and communities (EPA, 1998). Some of these chemicals are classified as immediate danger, and specific restrictions are set for their use and disposal, but many of them have an unknown environmental impact.

Most of these substances find their way into surface or underground water bodies. Some of them into industrial or domestic wastewaters, some enter in precipitation after exposure to air pollution, some seep from waste dumps after disposal and some of them are flushed from agricultural sites (Abel 1996, Giesy et al. 2001, Lepom et al. 2012).

Unsuitable farm cultivation leads to runoff, which can carry fertilizers and pesticides. Different types of industry produce a huge amount of by-products. Present technologies in sewage treatment plants (STPs) are often unable to remove contaminants from wastewater and these substances enter the surface water and food chains. One group emerging pollutants is pharmaceuticals, which can affect aquatic organisms (Prado et al. 2014, Pyle et al. 2005) or alter fish behaviour (Brodin et al. 2013). Some pollutants e.g. toxic metals, organochlorine pesticides, perfluoroalkyl substances (PFASs) or polychlorinated biphenyls (PCBs) persist for a very long time in the environment, because of their low degradability (Visnjevec et al. 2014, Yamaguchi et al. 2003, Zhao et al. 2012).

1.2. BIOMONITORING OF EXTRANEOUS SUBSTANCES IN AN AQUATIC ENVIRONMENT

Considerable amounts of different synthetic chemicals are continuously entering aquatic environments. Because of their potential negative effects on water biota, there is a great need to monitor their occurrence and fate in the environment. Thus, biomonitoring of aquatic environments is conducted by authorities all over the world. The term “biomonitoring” as used here means *chemical monitoring of biota*.

Both abiotic (water, sediment) and biotic (biofilm, algae, benthos, fish tissues) materials are sampled and analysed to evaluate the behaviour of certain chemicals in the environment. Two decades ago, biomonitoring focused on benthic macroinvertebrates (recommended in 27% of studies), algae (25%), protozoa (17%), bacteria (10%), and fish (6%). More recently, attention has shifted to three groups: fish, benthic macroinvertebrates, and algae (Resh 2008). Nowadays, another important indicator should be mentioned – the passive sampler.

Some pollutants increase or biomagnify in the bodies of animal´s at higher trophic position (Berger et al. 2009, Campbell et al. 2008, Dusek et al. 2005, Goeritz et al. 2013), fish, as top consumers are commonly used as bioindicators of aquatic environmental contamination. Age is another important factor that affects the level of contamination (Burger and Gochfeld 2007, Pandelova et al. 2008, Verdouw et al. 2011) as some chemicals can bioaccumulation as fish live longer, thus sampling of adult fish is important.

1.2.1. Biota samples in biomonitoring

1.2.1.1. Fish

Fish are common indicators of contamination in aquatic environments. Ironically, the use of this group of aquatic organisms (especially adults) has the greatest technical, personnel and time requirements and it carries a big load of uncertainty.

Adults of many fish species are migratory. This behaviour can be for spawning, feeding, or to find new territory. Some fishes have very long spawning movements (up to several thousand kilometres), for example diadromous species such as eel or salmon, but others confine migration to riverine systems (Donnelly et al. 1998, Kotusz et al. 2006, Prchalova et al. 2011). Migration affect the accuracy adult sampling. In general, adults sampled in riverine ecosystems might not be resident to the sampled locality. Uncertainty of the fish origin could lead to biased evaluation of given localities or misrepresent the source of pollution identification.

Another crucial point in biomonitoring programmes is the choice of reference fish species. Concentrations of many pollutants differ between fish groups because of their different trophic position (Dusek et al. 2005, Goeritz et al. 2013, Miege et al. 2012). It is necessary to choose a reference species which can be caught at all investigated sites. From a practical point of view, this can cause complications as sampling in different habitat types, a particular species might be absent in some localities. Regardless, catching sufficient numbers of individuals of the same species at each locality requires a considerable effort, which will require more personnel, technical and financial resources.

Concentrations of certain pollutants differ not only between species (Dusek et al. 2005, Goeritz et al. 2013), but individuals of same species too (Pandelova et al. 2008, Verdouw et al. 2011). This is caused mostly by the different age of sampled animals, but migration can play a role in these differences as well. For this reason, a sufficient number of individuals must be sampled to obtain relevant results about the contamination at a given locality. On the other hand, sampling of high numbers of adult fish can cause a conflict. As these fish are in reproductive age, their sampling could significantly affect the broodstock at the locality in some fish species.

Pollutant may differ greatly between the tissues of the body within one individual. Where a wide range of different contaminants are to be analysed, different organs and tissues must be analysed to obtain a relevant data. Different chemicals accumulate in specific parts in the fish body (Boalt et al. 2014, Goeritz et al. 2013, Yamaguchi et al. 2003), thus, sampling of only muscle or liver is not sufficient, some contaminants are found in fat or in the brain in higher concentrations. Sampling and analysing of several tissues or organs is time consuming and expensive, thus whole body homogenates might be an optimal matrix. From a practical point of view, this approach is complicated in the case of adult fish with a large body size and weight, but it might be easier in the case of juvenile fish.

1.2.1.2. Benthic fauna

Benthos represents a frequently used group of organisms for the evaluation of aquatic environment contamination. In general, the evaluation of environment quality using benthic organisms can be done in two ways. One used for decades is I based on the occurrence of specific species at the monitored sites (Horsák et al. 2009, Medeiros et al. 2011, Moreno et al. 2009). The second is based on instrumental analysis of target pollutants in benthos biomass (Kolaříková et al. 2012, Milani et al. 2013); with advancing methods of instrumental analysis, there is an increasing interest in this approach and will be the focus of this study.

Benthic organisms have limited mobility, thus, they are more suitable for inferring local conditions than adult fish (Resh 2008). Nevertheless, the mobility rate of benthic organisms is species specific and this should be taken into consideration.

As the group of benthic organisms is relatively large and consists of organisms with different trophic positions, there is a need to choose one reference species (closely related taxa can be combined for analyses). If the same species does not occur at all sampling sites, a species at the same trophic position should be selected. Benthic organisms are usually abundant in streams, rivers or lakes which facilitates effective sampling. Unfortunately, the high diversity of this group is coupled with an increased level of expertise needed for successful species identification.

Benthic organisms are usually not long-lived so that age of the organism is less of a difficulty than for fishes. In addition, more individuals can be sampled without negative ecologic/ethic consequences, which help to suppress the negative effect of age divergency. This affirmation is not valid in groups of mussels, where some species may live to 100 years.

1.2.1.3. Algae and biofilm

Different groups of algae are frequently used in biomonitoring, but the applicability may for only specific purposes. The main advantage (and disadvantage as well) is the short generation time of algal organisms. This characteristic allows algae to be used in the evaluation of the current situation in the environment, but excludes them from studies focused on long-term occurrence and fate of monitored compounds.

The adsorption, phytosorption and affinity of algae for heavy metal cations gives algae an advantage as a heavy metal accumulator in view of biomonitoring of such elements in both marine and freshwater ecosystems affected by urban activities (Conti and Cecchetti 2003, Metian et al. 2008, Sekabira et al. 2011).

Biofilm (periphyton) is a complex mixture of algae (diatoms), cyanobacteria and other heterotrophic organisms that is attached to submerged surfaces in most aquatic ecosystems. The advantages of periphyton communities for monitoring purposes are several: fixed habitats; a short generation time and existing information about pollution tolerances of common taxa occurred in biofilm communities (Biggs 1989). The response of biofilm communities to aquatic contamination has been intensively studied (Biggs 1989, Lewis et al. 2002). As these methods are based on the biomass or the occurrence of specific species, it can be successfully used only for the evaluation of environmental quality in a general, without the knowledge of specific contaminants and their concentrations in the environment.

Only the bioaccumulation of mercury was more intensively investigated in periphyton communities (Desrosiers et al. 2006, Hintelmann et al. 1993). However, recent research found periphyton is a better indicator of some other metals such Pb, As and Cr compared, to fish, benthos or macrophytes (Soto et al. 2011). Only limited information exists about the ability of biofilm to indicate occurrence of emerging contaminants as pharmaceuticals, personal care products or PFAS.

1.2.2. Passive sampling

Passive samplers represent an innovative monitoring tool for the time-integrated measurement of bioavailable contaminants in water, air or sediment. Passive sampling technology is proving to be a reliable, robust and cost-effective tool that can be used in monitoring programmes across the world. These devices mimic biological systems to provide a measure of bioavailable pollutants in the air and both fresh and salt water. Its passive transport mechanism is similar to that of chemical transport through fish gills or human lungs.

The first passive sampling devices were developed in the 1970s to determine concentrations of contaminants in the air. In 1980, this technology was first adapted for the monitoring of organic contaminants in water (Alvarez 2013). The initial type of passive sampler developed for aquatic monitoring purposes was the semipermeable membrane device (SMPD). This lipid-filled device immediately proved to be an extremely useful tool for measuring trace concentrations of POPs in water.

In the 1990s a new type of passive sampler was developed to complement the SPMD and to allow sampling of a wider range of organic contaminants with higher water solubility. The polar organic chemical integrative sampler (POCIS) aroused a big scientific interest after its introduction (Alvarez et al. 2004) and from that time, more than 300 chemicals have been proven to accumulate in the POCIS.

After being installed, the device samples a certain volume of water per day. This volume varies from chemical to chemical and it is dependent on the physical and chemical properties of the compound and on the length of sampling. The sampling rate can vary with changes in the water flow, temperature, turbulence and the build-up of a biofilm on the membrane surface (Booij et al. 1998, Harman et al. 2012). The accumulation of contaminants into the sampler is the result of three simultaneous processes. First, the contaminants diffuse across the water boundary layer. The thickness of boundary layer is dependent mainly on turbulence around the sampler and can significantly affect sampling rates. Second, the contaminant must transport across the membrane. Finally, contaminants transfer from the membrane into the sorbent material, mainly through adsorption.

Accumulation of chemicals by passive sampler devices generally follows first order kinetics. The uptake is characterized by an initial integrative phase, followed by an equilibrium-partitioning phase. During the integrative phase, a passive sampling device accumulates residues linearly, if exposure concentrations are constant. In order to estimate the water concentration of contaminants adsorbed in a sampler, calibration data applicable for in situ conditions, regarding the target compound, must be available (Alvarez 2013, Harman et al. 2012).

Unfortunately, the current use of the SPMD and POCIS is mostly as a research tool. The SPMD is a better-characterized sampling device than the POCIS (e.g. performance reference compounds for site-specific uptake rates), leading to a greater confidence in the results and acceptance in regulatory and legal cases. A significant amount of ongoing research should increase understanding of sampling kinetics of the POCIS, which will improve the accuracy in the time-weighted average concentration estimates and may result in an expanded role in regulatory issues (Alvarez 2013).

SPMD

The Semipermeable Membrane Device (SPMD) is composed of lay flat, low-density polyethylene tubing containing a thin film of a pure, high-molecular weight lipid (triolein). The polymer, often thought to be non-permeable, actually consists of micropores of less than 1nm in diameter. These pores allow selective diffusion of hydrophobic organic chemicals, which are then isolated in the lipid phase (Booij et al. 1998).

SPMD samplers are usually used for sampling of lipophilic compounds as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), dioxins or brominated flame retardants (BFRs).

POCIS

The POCIS sampler consists of a solid sorbent enclosed between two sheets of polyethersulfone (PES) microporous membrane. The ends of the membranes are compressed between two stainless steel rings, which prevent sorbent loss, as the PES membranes cannot be heat-sealed. The POCIS is usually inserted and deployed within a protective container to avoid ripping of POCIS membrane during its deployment.

POCIS was developed to sample a wide range of hydrophilic compounds. The original triphasic admixture of solid-phase adsorbents included in first POCIS was replaced with a more universal sorbent, Oasis® HLB (Waters Corp). This modification expanded the utility of POCIS for monitoring of pharmaceuticals and illicit drugs (Fedorova et al. 2014), personal care products, pesticides and some hormones in an aquatic environment (Alvarez 2013).

DGT

The diffusive gradients in thin films (DGT) is another option of a passive sampler usually used for in situ detection of bioavailable toxic trace metal contaminants. The DGT passive sampler consists of a polypropylene piston, binding gel, diffusive gel and membrane filter. The gel layers and membrane filter are fixed to the piston by using a polypropylene cap. The elements (compounds) pass through the membrane filter and diffusive gel. The binding gel is the site of adsorption, where the target compounds accumulate.

DGT can be used for the sampling of water and is also suitable for the monitoring of sediments and soils (Banks et al. 2012, Jacobs 2003, Liu et al. 2011). Potential target compounds cover a wide range, because there are different binding agents (gels) which can be used. Frequently used are DGTs for the sampling of trace metals (Fe, Cu, Cd, Hg, Zn), gold, radionuclides, sulphide, phosphate or some polar organic compounds (Fernández-Gómez et al. 2011, Larner et al. 2006, Menzies et al. 2005).

1.3. METHODS OF NON-LETHAL SAMPLING OF FISH

On occasions when health risks for consumers relate to fish consumption, fish from that locality is necessary. Muscle tissue of adult fish is usually sampled and analysed as it represents the most commonly consumed part of a fish. The number of individuals needed to overcome the problem with migration, is relatively high and frequent sampling (national programmes) could affect the local fish population.

Several non-lethal approaches to evaluate contamination using fish as bioindicators have been reported. Some of them evaluated different biopsy techniques (Ackerson et al. 2014, Schmitt and Brumbaugh 2007) and some use scales or fin-clips for the analysis as a surrogate for muscle tissue concentrations (Cervenka et al. 2011, Gremillion et al. 2005, Ryba et al. 2008). In most of these studies, the content of mercury was investigated because it is very common in an aquatic environment and a highly toxic contaminant. Mercury is persistent in the environment, accumulates mainly in muscle tissue and its levels in foodstuff is limited by European Union legislation, high demand on its biomonitoring is still needed.

Concerning biopsy techniques, these methods are accurate and can yield almost identical results compared to the sampling of fish fillets (Schmitt and Brumbaugh 2007). Measured concentrations can be directly used for health risk analysis. Nevertheless, the use of biopsy punches or needles is limited by the fish size as well as the fact that the risk of secondary infection is relatively high.

The use of fish fin-clips or scales is reported in a few publications. In some works, a strong relationship was found between Hg concentrations in muscle and in scales and/or fins of

fish. However, the prediction of muscle concentrations was not as precise as needed for the evaluation of health risks for consumers and it was suggested to use it only for initial screening (Gremillion et al. 2005, Ryba et al. 2008).

1.4. CHEMICAL ANALYSES

Nowadays, an increasing number of emerging contaminants demand effective detection methods, and quantification in different parts of the environment. Most of the currently used detection methods comprise two steps. The first is the extraction of target compounds from the sample and the second their identification and quantification. In some analyses, the clean-up step is included after the extraction process (LeDoux 2011), especially if samples contain higher portion of fat.

Extraction methods depend on the type of analysed compound and the type of the sample, but usually are based on liquid-liquid (LLE) or solid-phase (SPE) extraction (Brandsma et al. 2013, Ramos et al. 2015). During this process, the target compounds are transferred from biological matrices, into various primarily organic solvents as acetone, methanol, toluene, dichloromethane or their specific mixtures. Extraction procedures are usually missing in the analyses of contaminants as mercury, cadmium, lead and other metals. These methods are frequently based on thermal decomposition of the samples and atomic absorption spectrometry (AAS) is a commonly used detection method (Kenšová et al. 2010, Sardans et al. 2010).

Following the extraction or clean-up procedures, target compounds are usually separated either by gas chromatography (GC) or liquid chromatography (LC), and then identified and quantified using various kinds of detection methods depending on the substances, which should be analysed (LeDoux 2011, Ramos et al. 2015). The expanding role of GC and LC coupled with mass spectrometry (MS) and tandem mass spectrometry (MS/MS) is evident in the last two decades. This type of detection is currently the most used in the analysis of water, soil, sediments, biota samples or passive samplers (Fedorova et al. 2013, Grabicova et al. 2015, Kodesova et al. 2016, Miege et al. 2012).

1.5. HEALTH RISK ASSESSMENT

The benefits of fish consumption are well known, as it contains a higher level of poly-unsaturated fatty acids (PUFA), that have a positive effect on brain development in children (Clayton et al. 2007) or can reduce incidences of cardiac arrhythmias and sudden cardiac death (Singer and Wirth 2004). This assertion is valid mainly for marine fish species, but it is disputable in the case of species living in inland water bodies because the total lipid content in meat of freshwater fish is usually much lower compared to marine species. Beside the lipid content, also the fatty acids composition is different, as the freshwater fish have a much higher n-6 PUFA than n-3 PUFA, compared to marine species (Li et al. 2011). A more important factor determining the health aspects of freshwater fish consumption is their contamination by various extraneous substances. This contamination is related to industry, human population and agriculture, and is usually higher in inland rivers or lakes, than in oceans.

Thus, the evaluation of health risks connected with fish consumption is of interest to scientists and authorities (Asefi and Zamani-Ahmadmahmoodi 2015, Olmedo et al. 2013, Urban et al. 2009, Zhuang et al. 2013), but it was not realised in Czech open waters until 2014. Health risk assessment is usually conducted in areas, where a higher level of contamination is

expected or known and where fish from these areas are consumed (Urban et al. 2009, Zhuang et al. 2013) or to validate a good quality of caught or farmed fish introduced to the market (Cheng et al. 2013, Muñoz et al. 2010, Yu et al. 2013).

The evaluation of health risks consists of two procedures. First, determination and quantification of target contaminants must be done in edible parts of the fish (or other foodstuff). Second, analysed concentrations of pollutants must be evaluated in view of the potential health risk for consumers. The usual way is to compare analysed concentrations of compounds with their maximum limits (ML) or maximum residue limits (MRL, in pesticides) for foodstuff, that are set by authorities such as the European Commission (EC) or the US Environmental Protection Agency (EPA). Moreover, for some eminent pollutants the toxicological recommendations are also given by EPA, Food and Agriculture Organization of the United Nations (FAO), World Health Organisation (WHO) or European Food Safety Authority (EFSA). These recommendations indicate the amount of certain contaminants that can be consumed lifelong without negative health effects and are expressed as tolerable daily/weekly/monthly intakes.

1.6. DIRECTION OF THE PRESENT STUDY

The main goal of this study was to develop and evaluate alternative approaches to monitor extraneous substances in aquatic environments that would have the potential to replace adult fish as bioindicators of contamination. Approaches were studied with the focus on emerging contaminants as pharmaceuticals or personal care products together with well known, but highly toxic and persistent pollutants such as toxic metals and organochlorine pesticides. The criterions for the evaluation of studied approaches were mainly demands on sampling and analysis, reproducibility of results and the environmental impact on the sampling localities.

The specific objectives of the thesis

- To evaluate the aquatic environmental contamination using fish as bioindicators and human health risk assessment related to the consumption of fish from open waters in the Czech Republic.
- To assess the bioavailable concentrations of selected PFASs in an aquatic environment using passive samplers (POCIS) and the comparison with real fish contamination.
- To evaluate the possibility of using YOY fish for the biomonitoring of a wide range of pollutants in an aquatic environment.
- To verify the method of nonlethal sampling of fish for a mercury biomonitoring purposes.

In chapter 2, the health risk assessment related to the consumption of different fish species from twenty-seven major fishing grounds was evaluated. Chapter 3 reports the possibility of a passive sampling approach to monitor PFAS in an aquatic environment and compare the data from POCIS with the data from fish tissues analysis at several sampling sites. The approach using young-of-the-year (YOY) fish as bioindicators of a wide range of contaminants is presented and discussed in chapter 4. An optimised method for the accurate prediction of muscle mercury concentrations from analysis of fish fin-clips is presented in chapter 5, as a representative of a nonlethal method of sampling adult fish. In chapter 6, the effect of sewage treatment plant (STP) effluent on a small stream is evaluated using benthic macroinvertebrates as bioindicators.

REFERENCES

Abel, P.D., 1996. Water pollution biology, Taylor & Francis, London ; Bristol, PA.

Ackerson, J.R., Mckee, M.J., Schmitt, C.J., Brumbaugh, W.G., 2014. Implementation of a non-lethal biopsy punch monitoring program for mercury in smallmouth bass, micropterus dolomieu lacepsde, from the eleven point river, Missouri. Bull. Environ. Contam. Toxicol. 92(2), 125–131.

Alvarez, D.A., 2013. Development of semipermeable membrane devices (SPMDs) and polar organic chemical integrative samplers (POCIS) for environmental monitoring. Environ. Toxicol. Chem. 32(10), 2179–2181.

Alvarez, D.A., Petty, J.D., Huckins, J.N., Jones-Lepp, T.L., Getting, D.T., Goddard, J.P., Manahan, S.E., 2004. Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. Environ. Toxicol. Chem. 23(7), 1640–1648.

Asefi, M., Zamani-Ahmadmahmoodi, R., 2015. Mercury concentrations and health risk assessment for two fish species, Barbus grypus and Barbus luteus, from the Maroon River, Khuzestan Province, Iran. Environ. Monit. Assess. 187(10).

Banks, J., Ross, D.J., Keough, M.J., 2012. Short-term (24 h) effects of mild and severe hypoxia (20% and 5% dissolved oxygen) on metal partitioning in highly contaminated estuarine sediments. Estuar. Coast. Shelf. Sci. 99, 121–131.

Berger, U., Glynn, A., Holmstrom, K.E., Berglund, M., Ankarberg, E.H., Tornkvist, A., 2009. Fish consumption as a source of human exposure to perfluorinated alkyl substances in Sweden – Analysis of edible fish from Lake Vattern and the Baltic Sea. Chemosphere 76(6), 799–804.

Biggs, B.J.F., 1989. Biomonitoring of organic pollution using periphyton, south branch, canterbury, new zealand. New Zealand Journal of Marine and Freshwater Research 23(2), 263–274.

Binetti, R., Costamagna, F.M., Marcello, I., 2008. Exponential growth of new chemicals and evolution of information relevant to risk control. Annali Dell Istituto Superiore Di Sanita 44(1), 13–15.

Boalt, E., Miller, A., Dahlgren, H., 2014. Distribution of cadmium, mercury, and lead in different body parts of Baltic herring (*Clupea harengus*) and perch (*Perca fluviatills*): Implications for environmental status assessments. Marine Pollution Bulletin 78(1–2), 130–136.

Booij, K., Sleiderink, H.M., Smedes, F., 1998. Calibrating the uptake kinetics of semipermeable membrane devices using exposure standards. Environ. Toxicol. Chem. 17(7), 1236–1245.

Brandsma, S.H., De Boer, J., Leonards, P.E.G., Cofino, W.P., Covaci, A., 2013. Organophosphorus flame-retardant and plasticizer analysis, including recommendations from the first worldwide interlaboratory study. TrAC – Trends. Anal. Chem. 43, 217–228.

Brodin, T., Fick, J., Jonsson, M., Klaminder, J., 2013. Dilute concentrations of a psychiatric drug alter behavior of fish from natural populations. Science 339(6121), 814–815.

Burger, J., Gochfeld, M., 2007. Risk to consumers from mercury in Pacific cod (*Gadus macrocephalus*) from the Aleutians: Fish age and size effects. Environ. Res. Forum 105(2), 276–284.

Campbell, L., Verburg, P., Dixon, D.G., Hecky, R.E., 2008. Mercury biomagnification in the food web of Lake Tanganyika (Tanzania, East Africa). Sci. Total Environ. 402(2–3), 184–191.

Cervenka, R., Bednarik, A., Komarek, J., Ondrackova, M., Jurajda, P., Vitek, T., Spurny, P., 2011. The relationship between the mercury concentration in fish muscles and scales/fins and its significance. Cent. Eur. J. Chem. 9(6), 1109–1116.

Clayton, E.H., Hanstock, T.L., Garg, M.L., Hazell, P.L., 2007. Long chain omega-3 polyunsaturated fatty acids in the treatment of psychiatric illnesses in children and adolescents. Acta Neuropsychiatrica 19(2), 92–103.

Conti, M.E., Cecchetti, G., 2003. A biomonitoring study: Trace metals in algae and molluscs from Tyrrhenian coastal areas. Environ. Res. 93(1), 99–112.

Desrosiers, M., Planas, D., Mucci, A., 2006. Total mercury and methylmercury accumulation in periphyton of Boreal Shield Lakes: Influence of watershed physiographic characteristics. Sci. Total Environ. 355(1–3), 247–258.

Donnelly, R.E., Caffrey, J.M., Tierney, D.M., 1998. Movements of a bream (*Abramis brama* (L.)), rudd x bream hybrid, tench (*Tinca tinca* (L.)) and pike (*Esox lucius* (L.)) in an Irish canal habitat. Hydrobiologia 372, 305–308.

Dusek, L., Svobodova, Z., Janouskova, D., Vykusova, B., Jarkovsky, J., Smid, R., Pavlis, P., 2005. Bioaccumulation of mercury in muscle tissue of fish in the Elbe River (Czech Republic): multispecies monitoring study 1991–1996. Ecotox. Environ. Safe. 61(2), 256–267.

Fedorova, G., Randak, T., Golovko, O., Kodes, V., Grabicova, K. and Grabic, R., 2014. A passive sampling method for detecting analgesics, psycholeptics, antidepressants and illicit drugs in aquatic environments in the Czech Republic. Sci. Total Environ. 487, 681–687.

Fedorova, G., Randak, T., Lindberg, R.H., Grabic, R., 2013. Comparison of the quantitative performance of a Q-Exactive high-resolution mass spectrometer with that of a triple quadrupole tandem mass spectrometer for the analysis of illicit drugs in wastewater. Rapid Commun. Mass Spectrom. 27(15), 1751–1762.

Fernández-Gómez, C., Dimock, B., Hintelmann, H., Díez, S., 2011. Development of the DGT technique for Hg measurement in water: Comparison of three different types of samplers in laboratory assays. Chemosphere 85(9), 1452–1457.

Giesy, J.P., Kannan, K., Jones, P.D., 2001. Global biomonitoring of perfluorinated organics. Sci. World J. 1, 627–629.

Goeritz, I., Falk, S., Stahl, T., Schafers, C., Schlechtriem, C., 2013. Biomagnification and tissue distribution of perfluoroalkyl substances (PFASs) in market-size rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 32(9), 2078–2088.

Grabicova, K., Grabic, R., Blaha, M., Kumar, V., Cerveny, D., Fedorova, G., Randak, T., 2015. Presence of pharmaceuticals in benthic fauna living in a small stream affected by effluent from a municipal sewage treatment plant. Water Res. 72, 145–153.

Gremillion, P.T., Cizdziel, J.V., Cody, N.R., 2005. Caudal fin mercury as a non-lethal predictor of fish-muscle mercury. Environ. Chem. 2(2), 96–99.

Harman, C., Allan, I.J. and Vermeirssen, E.L.M., 2012. Calibration and use of the polar organic chemical integrative sampler-a critical review. Environ. Toxicol. Chem. 31(12), 2724–2738.

Hintelmann, H., Ebinghaus, R. and Wilken, R.D., 1993. Accumulation of mercury(II) and methylmercury by microbial biofilms. Water Res. 27(2), 237–242.

Horsák, M., Bojková, J., Zahrádková, S., Omesová, M., Helešic, J., 2009. Impact of reservoirs and channelization on lowland river macroinvertebrates: A case study from Central Europe. Limnologica 39(2), 140–151.

Cheng, Z., Nie, X.P., Wang, H.S., Wong, M.H., 2013. Risk assessments of human exposure to bioaccessible phthalate esters through market fish consumption. Environ. Int. 57–58, 75–80.

Jacobs, P.H., 2003. Monitoring of subaqueous depots with active barrier systems for contaminated dredged material using dialysis samplers and DGT probes. Journal of Soils and Sediments 3(2), 100–107.

Kenšová, R., Čelechovská, O., Doubravová, J., Svobodová, Z., 2010. Concentrations of metals in tissues of fish from the Věstonice reservoir. Acta Vet. Brno 79(2), 335–345.

Kodesova, R., Kocarek, M., Klement, A., Golovko, O., Koba, O., Fer, M., Nikodem, A., Vondrackova, L., Jaksik, O., Grabic, R., 2016. An analysis of the dissipation of pharmaceuticals under thirteen different soil conditions. Sci. Total Environ. 544, 369–381.

Kolaříková, K., Stuchlík, E., Liška, M., Horecký, J., Tátosová, J., Hardekopf, D., Lapšanská, N., Hořická, Z., Hovorka, J., Mihaljevič, M., Fuksa, J.K., Von Tümpling, W., 2012. Long-term changes in the bioaccumulation of As, Cd, Pb, and Hg in macroinvertebrates from the Elbe River (Czech Republic). Water Air Soil Pollut. 223(6), 3511–3526.

Kotusz, J., Witkowski, A., Baran, M., Blachuta, J., 2006. Fish migrations in a large lowland river (Odra R., Poland) – based on fish pass observations. Folia Zool. 55(4), 386–398.

Larner, B.L., Seen, A.J., Snape, I., 2006. Evaluation of diffusive gradients in thin film (DGT) samplers for measuring contaminants in the Antarctic marine environment. Chemosphere 65(5), 811–820.

LeDoux, M., 2011. Analytical methods applied to the determination of pesticide residues in foods of animal origin. A review of the past two decades. J. Chromatogr. A 1218(8), 1021–1036.

Lepom, P., Irmer, U., Wellmitz, J., 2012. Mercury levels and trends (1993–2009) in bream (*Abramis brama* L.) and zebra mussels (*Dreissena polymorpha*) from German surface waters. Chemosphere 86(2), 202–211.

Lewis, M.A., Weber, D.L., Moore, J.C., 2002. An evaluation of the use of colonized periphyton as an indicator of wastewater impact in near-coastal areas of the Gulf of Mexico. Arch. Environ. Contam. Toxicol. 43(1), 11–18.

Li, G., Sinclair, A.J, Li, D., 2011. Comparison of lipid content and fatty acid composition in the edible meat of wild and cultured freshwater and marine fish and shrimps from China. J. Agric. Food Chem. 59(5), 1871–1881.

Liu, J., Feng, X., Qiu, G., Yao, H., Shang, L., Yan, H., 2011. Intercomparison and applicability of some dynamic and equilibrium approaches to determine methylated mercury species in pore water. Environ. Toxicol. Chem. 30(8), 1739–1744.

Medeiros, A.S., Luszczek, C.E., Shirley, J., Quinlan, R., 2011. Benthic biomonitoring in arctic tundra streams: A community-based approach in iqaluit, nunavut, Canada. Arctic 64(1), 59–72.

Menzies, N.W., Kusumo, B., Moody, P.W., 2005. Assessment of P availability in heavily fertilized soils using the diffusive gradient in thin films (DGT) technique. Plant Soil 269(1–2), 1–9.

Metian, M., Giron, E., Borne, V., Hédouin, L., Teyssié, J.L. and Warnau, M., 2008. The brown alga Lobophora variegata, a bioindicator species for surveying metal contamination in tropical marine environments. J. Exp. Mar. Biol. Ecol. 362(1), 49–54.

Miege, C., Peretti, A., Labadie, P., Budzinski, H., Le Bizec, B., Vorkamp, K., Tronczynski, J., Persat, H., Coquery, M., Babut, M., 2012. Occurrence of priority and emerging organic compounds in fishes from the Rhone River (France). Anal. Bioanal. Chem. 404(9), 2721–2735.

Milani, D., Grapentine, L.C., Fletcher, R., 2013. Sediment Contamination in Lyons Creek East, a Tributary of the Niagara River: Part I. Assessment of Benthic Macroinvertebrates. Arch. Environ. Contam. Toxicol. 64(1), 65–86.

Moreno, P., França, J.S., Ferreira, W.R., Paz, A.D., Monteiro, I.M., Callisto, M., 2009. Use of the BEAST model for biomonitoring water quality in a neotropical basin. Hydrobiologia 630(1), 231–242.

Muñoz, I., Martínez Bueno, M.J., Agüera, A. and Fernández-Alba, A.R., 2010. Environmental and human health risk assessment of organic micro-pollutants occurring in a Spanish marine fish farm. Environ. Pollut. 158(5), 1809–1816.

Olmedo, P., Pla, A., Hernández, A.F., Barbier, F., Ayouni, L., Gil, F., 2013. Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. Environ. Int. 59, 63–72.

Pandelova, M., Henkelmann, B., Roots, O., Simm, M., Jaerv, L., Benfenati, E., Schramm, K.W., 2008. Levels of PCDD/F and dioxin-like PCB in Baltic fish of different age and gender. Chemosphere 71(2), 369–378.

Prado, P.S., Pinheiro, A.P.B., Bazzoli, N., Rizzo, E., 2014. Reproductive biomarkers responses induced by xenoestrogens in the characid fish Astyanax fasciatus inhabiting a South American reservoir: An integrated field and laboratory approach. Environ. Res. 131, 165–173.

Prchalova, M., Horky, P., Slavik, O., Vetesnik, L., Halacka, K., 2011. Fish occurrence in the fishpass on the lowland section of the River Elbe, Czech Republic, with respect to water temperature, water flow and fish size. Folia Zool. 60(2), 104–114.

Pyle, G.G., Rajotte, J.W., Couture, P., 2005. Effects of industrial metals on wild fish populations along a metal contamination gradient. Ecotox. Environ. Safe. 61(3), 287–312.

Ramos, S., Homem, V., Alves, A., Santos, L., 2015. Advances in analytical methods and occurrence of organic UV-filters in the environment – A review. Sci. Total Environ. 526, 278–311.

Resh, V.H., 2008. Which group is best? Attributes of different biological assemblages used in freshwater biomonitoring programs. Environ. Monit. Assess. 138(1–3), 131–138.

Ryba, S.A., Lake, J.L., Serbst, J.R., Libby, A.D. and Ayvazian, S., 2008. Assessment of caudal fin-clip as a non-lethal technique for predicting muscle tissue mercury concentrations in largemouth bass. Environ. Chem. 5(3), 200–203.

Sardans, J., Montes, F., Peñuelas, J., 2010. Determination of As, Cd, Cu, Hg and Pb in biological samples by modern electrothermal atomic absorption spectrometry. Spectroc. Acta Pt. B-Atom. Spectr. 65(2), 97–112.

Sekabira, K., Origa, H.O., Basamba, T.A., Mutumba, G., Kakudidi, E., 2011. Application of algae in biomonitoring and phytoextraction of heavy metals contamination in urban stream water. Int. J. Environ. Sci. Technol. 8(1), 115–128.

Schmitt, C.J., Brumbaugh, W.G., 2007. Evaluation of potentially nonlethal sampling methods for monitoring mercury concentrations in smallmouth bass (*Micropterus dolomieu*). Arch. Environ. Contam. Toxicol. 53(1), 84–95.

Singer, P., Wirth, M., 2004. Can n-3 PUFA reduce cardiac arrhythmias? Results of a clinical trial. PProstaglandins Leukot. Essent. Fatty Acids 71(3), 153–159.

Soto, D.X., Roig, R., Gacia, E., Catalan, J., 2011. Differential accumulation of mercury and other trace metals in the food web components of a reservoir impacted by a chlor-alkali plant (Flix, Ebro River, Spain): Implications for biomonitoring. Environ. Pollut. 159(6), 1481–1489.

United States Environmental Protection Agency, 1998. Chemical hazard data availability study: what do we really know about the safety of high production volume chemicals? Washington, DC: Office of Pollution Prevention and Toxics.

Urban, J.D., Tachovsky, J.A., Haws, L.C., Wikoff Staskal, D., Harris, M.A., 2009. Assessment of human health risks posed by consumption of fish from the Lower Passaic River, New Jersey. Sci. Total Environ. 408(2), 209–224.

Verdouw, J.J., Macleod, C.K., Nowak, B.F., Lyle, J.M., 2011. Implications of age, size and region on mercury contamination in estuarine fish species. Water Air Soil Pollut. 214(1–4), 297–306.

Visnjevec, A.M., Kocman, D., Horvat, M., 2014. Human Mercury Exposure and Effects in Europe. Environ. Toxicol. Chem. 33(6), 1259–1270.

Yamaguchi, N., Gazzard, D., Scholey, G., Macdonald, D.W., 2003. Concentrations and hazard assessment of PCBs, organochlorine pesticides and mercury in fish species from the upper Thames: River pollution and its potential effects on top predators. Chemosphere 50(3), 265–273.

Yu, Z.Y., Jin, F., Sun, J.F., Yuan, S.G., Zheng, B., Zhang, W.J., An, W., Yang, M., 2013. Residual levels of pesticides in freshwater fish from Beijing aquatic product markets and health risk assessment. Huanjing Kexue/Environmental Science 34(1), 251–256.

Zhao, Y.G., Wong, C.K.C., Wong, M.H., 2012. Environmental contamination, human exposure and body loadings of perfluorooctane sulfonate (PFOS), focusing on Asian countries. Chemosphere 89(4), 355–368.

Zhuang, P., Li, Z.A., McBride, M.B., Zou, B., Wang, G., 2013. Health risk assessment for consumption of fish originating from ponds near Dabaoshan mine, South China. Environ. Sci. Pollut. Res. 20(8), 5844–5854.

CHAPTER 2

CONTAMINATION OF FISH IN IMPORTANT FISHING GROUNDS OF THE CZECH REPUBLIC

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Contamination of fish in important fishing grounds of the Czech Republic

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ABSTRACT

The aim of this study was to compare the contamination levels of certain important fishing grounds in the Czech Republic and to assess the health risk of consuming the fish from these localities. The assessment was performed from 2006 to 2010 in 27 fishing grounds. Within this project, 707 fish from 14 different species were sampled. The concentration of selected toxic metals (Hg, Pb, Cd) and persistent organic pollutants (POPs), such as non-dioxin-like polychlorinated biphenyls (NDL-PCBs), hexachlorocyclohexane (HCH) isomers, dichlorodiphenyltrichloroethane (DDT) and its metabolites (o,p´-DDE; p,p´-DDE; o,p´-DDD; p,p´-DDD; o,p´-DDT; p,p´-DDT) and hexachlorobenzene (HCB), were analysed in the muscle tissue of the sampled fish. Atomic absorption spectrometry (AAS) was used for the analysis of toxic metals. All of the POPs were analysed using gas chromatography with an electron capture detector (GC/ECD). Common bream (*Abramis brama*) was chosen as a reference fish species for the comparison of fishing grounds. Mercury was found as a major pollutant in fish flesh at all of the sampling sites. Concentrations in excess of the maximum level (ML) of mercury in the muscle tissue of fish (0.5 mg kg^{-1}) were registered in 32 samples. Concentrations of other monitored toxic metals in fish muscle were low, typically below the limit of quantification (LOQ). From the tested POPs, DDTs and NDL-PCBs were found as major pollutants. ML for NDL-PCBs (ICES-6) in muscle tissue of fish (0.125 mg kg^{-1}) was exceeded in 7 samples. In case of tested pesticides, concentrations in excess of the MRL were not registered.

1. Introduction

Many human activities cause some types of pollution that results in surface or underground water contamination. Certain xenobiotics, such as toxic metals or persistent organic pollutants (POPs), remain in the environment for a very long time because of their poor degradability. These xenobiotics, such as toxic metals, are commonly deposited in the water sediments of aquatic environments, which results in their presence in food chains at localities where there have been no sources of pollution for many years (Abel, 1996; van Hattum et al., 1993).

After being released into the air, mercury returns to the ground through precipitation and enters the aquatic environment; thus, atmospheric deposition is a dominant source of mercury (Lepom et al., 2012). Mercury is neurotoxic in both its organic and inorganic forms (Atchison and Hare, 1994). The commonly encountered form of mercury, methylmercury (MeHg), is the most toxic form affecting aquatic biota (Lasorsa and Allen-Gil, 1995; Maceda-Veiga et al., 2012). MeHg is primarily responsible for bioaccumulation in the muscle tissue of fish with a methylmercury-to-total mercury ratio of 83–90% (Kannan et al., 1998; Kruzikova et al., 2008; Lasorsa and Allen-Gil, 1995; Marsalek et al., 2005).

Cadmium has similar neurotoxic effects as some other heavy metals including mercury, but it has a specific impact on reproductive organs in long-term exposure to even trace concentrations. Several studies (De Conto et al., 1999; Svobodova et al., 2002) show that target organs for accumulation of cadmium are chiefly kidney and liver.

Important source of lead in environment are exhaust gases of vehicles. Lead petrol was restricted to use recently in developed countries, but lead from this source remains in environment and some countries are still using lead petrol. Also lead has neurotoxic effects in living organisms similar to cadmium and mercury (Gupta et al., 2009; Svobodova et al., 2002).

POPs are characterised by a high affinity for adipose tissue and slow biodegradability, which results in their bioaccumulation in

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various animal species, along the trophic chain, including fish species that are at the top of the food pyramid in an aquatic ecosystem (Bosnir et al., 2007; Marsalek et al., 2004; Siroka et al., 2005). The occurrence of POPs in the environment arouses scientific interest primarily because of their endocrine disrupting effects on animals and humans (Godduhn and Duffy, 2003; Petro et al., 2010; Yang and Xu, 2005).

In the Czech Republic, sport fishing is a traditional recreational activity. Currently, the majority of anglers are organised into two Anglers Unions, which host nearly 320,000 members organised into 585 local units. Nearly 4000 t of various fish species are caught annually by anglers in the Czech Republic. The most stocked fish is common carp (*Cyprinus carpio*), with 3200 t of stocking per year (Adamek et al., 2012).

In the present study, we focused on the detection of toxic metals (mercury, lead, cadmium) and certain POPs (α-, β-, γ-HCH, HCB, DDT and NDL-PCBs; evaluated as a sum of six indicator congeners) in reference fish species (common bream) and fish species that are preferred by anglers in the monitored fishing grounds. Common bream (*Abramis brama*) was chosen as a reference species due to its high abundance in all of the evaluated fishing grounds and because it is not artificially stocked in Czech Republic, so it means that concentrations of pollutants found in its body better reflect the pollution of given locality than in artificially stocked fish species (e.g. carp).

Contrary to fish from the markets of EU countries where hygienic quality is continuously controlled, there is no detailed evidence about contamination of fish from rivers and other water bodies. These data could provide important information about the contamination of fish to anglers, who commonly consume the fish from rivers and water reservoirs. By comparing with provisional tolerable weekly intake (PTWI) and provisional tolerable monthly intake (PTMI) for MeHg and Cd respectively and provisional tolerable daily intake (PTDI) for DDT, suggested by FAO/WHO (WHO, 2004, 2011; FAO, 2001), the potential health risk for consumers was evaluated.

2. Materials and methods

2.1. Monitored sites

From 2006 to 2010, samples were obtained from 27 of the most popular fishing grounds in the Czech Republic, as shown in Fig. 1. Fishing grounds with the highest attendance were chosen in cooperation with the Czech Angler´s Union as indicated by the preferences of anglers. Several industrial areas or sites with a higher level of another type of pollution were also included. A list of the sampling sites with specific characteristics is shown in the Supporting Information (Table S1).

2.2. Fish sampling

Fish were caught by electrofishing, gillnets and angling. Common bream (*Abramis brama*) was chosen as a reference species due to its high abundance in all of the evaluated fishing grounds. Bream is a typical benthic feeder and is not artificially stocked in the Czech Republic. Characteristics of reference species caught at monitored sites are given in Table 1. In addition to bream, fish species typical for each locality, such as asp (*Aspius aspius*) or perch (*Perca fluviatilis*), were sampled as well as those that are preferred by anglers, such as carp (*Cyprinus carpio*), zander (*Sander lucioperca*) pike (*Esox Lucius*) eel (*Anguilla anguilla*) and catfish (*Silurus glanis*).

All of the sampled fish were measured and weighed, and scales were removed for age determination. Muscle tissue from the dorsal side of the body was obtained for all analyses, because it is a common consumable part of fish body by anglers. Samples of reference fish species (bream) for toxic metal analyses were obtained individually at all of the monitored sites. Samples of bream for POPs analyses and samples of other fish species were obtained as single-species pooled samples at each sampling site. The same amount of muscle tissue was taken from each individual to make these pooled samples. Samples were packed into plastic bags, labelled and transported to the laboratory in thermo-boxes filled with ice. Samples were then stored at −18 °C until analyses.

Experimental animals were handled in accordance with the national and institutional guidelines for the protection of human subjects and animal welfare.

2.3. Chemical analyses

Analyses of the target pollutants were performed in the laboratory of the State Veterinary Institute in Prague, which is accredited for the range of analytes of interest in accordance with EN ISO/IEC 17025: 2005. Concentrations of all target

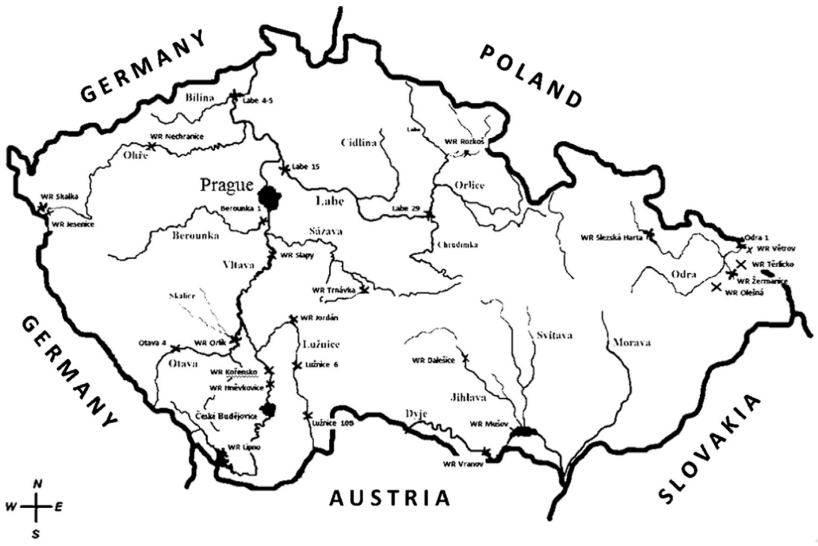

Fig. 1. Map of the Czech Republic with sampling sites.

Table 1
Characteristics of reference species (*Abramis brama*) caught at monitored sites.

Site name	n	Age (years) mean ± SD	Body weight (g) mean ± SD	Total length (mm) mean ± SD	Lipid content (%) pooled sample
Berounka River – Prague	5	7.4 ± 1.0	1144 ± 272	467 ± 37	1.55
Elbe River – Obristvi	5	4.8 ± 1.2	522 ± 92	364 ± 15	0.38
Elbe River – Pardubice	4	5.8 ± 1.6	656 ± 251	393 ± 66	0.32
Elbe River – Svadov	5	6.6 ± 1.4	850 ± 206	423 ± 43	4.06
Luznice River – Majdalena	5	4.0 ± 0.6	257 ± 79	292 ± 29	0.39
Luznice River – Sobeslav	5	7.4 ± 1.5	982 ± 387	432 ± 50	1.88
Odra River – Ostrava	5	8.6 ± 0.5	1150 ± 158	462 ± 24	3.08
Otava River – Strakonice	5	5.6 ± 2.0	741 ± 497	373 ± 79	1.77
WR Dalesice	5	7.0 ± 0.6	878 ± 122	417 ± 15	4.70
WR Hnevkovice	5	4.8 ± 1.2	497 ± 185	359 ± 38	6.19
WR Jesenice	5	6.2 ± 2.0	776 ± 210	400 ± 89	2.21
WR Jordan	5	5.0 ± 0.9	483 ± 193	349 ± 51	2.80
WR Korensko	5	5.0 ± 0.6	508 ± 69	375 ± 26	0.76
WR Lipno	5	4.6 ± 0.8	351 ± 87	315 ± 25	1.76
WR Musov	5	9.0 ± 0.9	1459 ± 335	476 ± 41	7.49
WR Nechranice	5	5.6 ± 0.5	642 ± 56	406 ± 14	3.66
WR Olesna	5	7.0 ± 0.6	282 ± 31	309 ± 15	0.90
WR Orlik	5	6.2 ± 0.8	801 ± 75	408 ± 10	3.50
WR Rozkos	5	5.0 ± 0.6	734 ± 69	430 ± 9	3.10
WR Skalka	5	5.4 ± 1.2	562 ± 124	371 ± 23	2.50
WR Slapy	5	5.2 ± 0.8	531 ± 100	363 ± 21	3.95
WR Slezska Harta	5	9.4 ± 1.0	637 ± 195	398 ± 44	0.40
WR Terlicko	5	5.6 ± 0.5	336 ± 84	314 ± 15	1.05
WR Trnavka	5	5.4 ± 0.8	558 ± 103	357 ± 20	0.65
WR Vetrov	5	5.0 ± 0.0	335 ± 32	314 ± 10	0.72
WR Vranov	5	6.6 ± 0.8	958 ± 99	416 ± 21	6.26
WR Zermanice	5	10.6 ± 0.5	1086 ± 108	437 ± 18	3.83

WR, water reservoir

analytes in the muscle tissue of analysed fish were determined and expressed in wet weight (w.w.).

The total mercury (THg) content was determined directly in the sample units by a selective mercury analyser (Advanced mercury analyser, AMA-254, Altec) based on atomic absorption spectroscopy (AAS). Other toxic metals were measured by electrothermal (flameless) atomic absorption spectrometry with Zeeman background correction (graphite furnace atomic absorption spectrometry (GF-AAS, SpectrAA 220Z, Varian)) after microwave mineralisation of the samples (EN 13 804, 13 805 and 14084). For calibration of the instruments, MERCK calibration solutions were used (THg – CertiPUR standard solution, lead and cadmium – Titrisol standard solutions).

The PCBs concentration was quantified as a sum of six indicator congeners (28, 52, 101, 138, 153 and 180). Organochlorine pesticides were quantified as a sum of DDT and its metabolites (p,p′-DDE; p,p′-DDD; o,p′-DDT; p,p′-DDT), a sum of α+β HCH isomers, γ-HCH (lindane) and HCB. The POPs were determined by two dimensional gas chromatography with electron capture detector – GC-ECD Agilent 6890 Series (Hajslova et al., 1995) after application GPC clean up. For calibration of the instrument, Dr. Ehrenstorfer standard solutions were used (PCB MIX3 and Pesticide MIX71). For the calculation of the pesticides and PCBs on w.w., content of fat in the samples was determined by extraction on Soxtec after hydrolysis by hydrochloric acid. Diethyl ether was used as a solvent for extraction of fat.

2.4. Analyses results

The determined concentrations of the contaminants were compared with the maximum levels (MLs) in foodstuff set by the European Regulations no. 629 and 1259 for toxic metals and NDL-PCBs (European Commission (EC), 2006; European Commission (EC), 2011) and the Czech nationwide regulation no. 305/2004 (Czech Republic, 2004) setting maximum residue levels (MRLs) in foodstuff for certain pesticides.

Statistical evaluation was performed using STATISTICA 10 software (StatSoft Inc., USA). If concerns toxic metals, the arithmetic mean and the standard deviation were calculated for reference fish species (bream) at each site. In case of toxic metals in samples of non-reference species and POPs in all samples, mean and standard deviations have not been calculated, because these samples were obtained as a pooled sample units of single species at each locality. The Pearson ´s correlation was used to express DDT and NDL-PCBs concentrations dependence on amount of lipid tissue.

2.5. Risk assessment

Toxicological recommendations given by FAO/WHO and a modified method which was described by Kannan et al. (1998) were used to calculate reference doses (RfDs) for certain analysed pollutants. This modification is explained in "Risk assessment" section. In agreement with studies focused on MeHg/THg ratio in muscle tissue of fish (Kannan et al., 1998; Kruzikova et al., 2008; Lasorsa and Allen-Gil, 1995; Marsalek et al., 2005), we used MeHg/THg ratio 0.85 for calculations. Evaluation of health risk caused by consumption of fish was done for most common fish species, which were caught at each monitored locality. All RfDs were transformed into number of portions (á 170 g of meat), which could be eaten monthly during a lifetime without negative health effects.

Toxicological recommendations for evaluated contaminants given by WHO/FAO are presented in Supporting information (Table S2).

3. Results and discussion

3.1. Toxic metals

Concentrations of toxic metals in bream muscle at the monitored sites are presented in Table 2.

From spectrum of analysed pollutants, we found THg as a major pollutant in monitored rivers and water reservoirs of the Czech Republic. Detectable levels of THg were found in all of the sample units at each of the monitored sites for all of the analysed fish species. The ML for THg concentration in fish muscle tissue (0.5 mg kg^{-1}), set by European Regulation no. 629/2008/EC, was exceeded in 32 individual or pooled samples of various fish species at 17 sampling sites (Supporting Information Table S3).

The highest concentrations of THg in bream (reference species) muscle were found at the Skalka water reservoir (0.593 ± 0.128 mg kg^{-1}). A majority (80%) of detected values exceeded the ML there. This site is affected by historical industrial pollution originating from the industrial zone of Germany situated along the Reslava River (Marsalek et al., 2005). The highest concentration of THg was observed in pooled sample of asp (*Aspius aspius*) at the level of 3.570 mg kg^{-1}, which was the highest concentration observed during whole study. Regular consumption of fish from this locality could not be recommended. In spite of high concentration of THg in almost all caught fish species in this locality, we found relatively low mean concentration (0.161 mg kg^{-1}) of THg in muscle tissue of carps, which are artificially stocked.

Other sites that were highly polluted with mercury contamination were Odra–Ostrava (0.459 ± 0.038 mg kg^{-1}), Korensko (0.445 ± 0.091 mg kg^{-1}) and Elbe–Obristvi (0.410 ± 0.068 mg kg^{-1}). These sites are affected by coal mining and pig iron processing, graphite processing and the chemical production industry, respectively.

Many studies have been focused on THg and methylmercury contamination in the Elbe River (Dusek et al., 2005; Lepom et al., 2012; Siroka et al., 2005; Zlabek et al., 2005). Lepom et al. (2012) reported concentrations of THg (arithmetic mean) in muscle of bream from 0.111 to 0.324 mg kg^{-1} w.w. at several sites from the Elbe River in Germany in 2009. We found similar mean concentrations (0.220 and 0.270 m kg^{-1}) at sampling sites Elbe–Pardubice and Elbe–Svadov respectively. Higher THg concentrations observed at locality Elbe–Obristvi might be caused by large chemical plant, which is located approximately 5 km upstream.

However, low concentrations of THg in bream muscle were found at the Vetrov (0.037 ± 0.014 mg kg^{-1}), Jesenice (0.061 ± 0.029 mg kg^{-1}) and Terlicko (0.069 ± 0.021 mg kg^{-1}) sampling sites. THg contamination of bream muscle from mentioned

Table 2
Concentrations of toxic metals in the muscle tissue of reference species (*Abramis brama*) from the monitored fishing grounds.

Site name	**THg** (mg kg^{-1} **w.w.**) **LOQ=0.001** mg kg^{-1}		**Pb** (mg kg^{-1} **w.w.**) **LOQ=0.02** mg kg^{-1}		**Cd** (mg kg^{-1} **w.w.**) **LOQ=0.002** mg kg^{-1}	
	min–max	**mean ± SD**	**min–max**	**mean ± SD**	**min–max**	**mean ± SD**
Berounka River – Prague	0.226–0.327	0.263 ± 0.035	0.02–0.05	0.028 ± 0.012	< LOQ–0.003	0.002 ± 0.001
Elbe River – Obristvi	0.286–0.477	0.410 ± 0.068	< LOQ–0.05	0.03 ± 0.013	< LOQ–0.002	0.001 ± 0.0005
Elbe River – Pardubice	0.189–0.249	0.220 ± 0.023	< LOQ–0.04	0.02 ± 0.012	< LOQ	nd
Elbe River – Svadov	0.133–0.401	0.270 ± 0.088	< LOQ–0.02	0.014 ± 0.005	< LOQ	nd
Luznice River – Majdalena	0.090–0.150	0.127 ± 0.022	< LOQ–0.02	0.012 ± 0.004	< LOQ–0.004	0.002 ± 0.001
Luznice River – Sobeslav	0.139–0.370	0.261 ± 0.087	0.02–0.1	0.05 ± 0.037	< LOQ–0.006	0.002 ± 0.002
Odra River – Ostrava	0.425–0.527	0.459 ± 0.038	< LOQ–0.067	0.053 ± 0.030	0.003–0.012	0.009 ± 0.003
Otava River – Strakonice	0.066–0.571	0.284 ± 0.186	0.01–0.02	0.016 ± 0.005	< LOQ–0.002	0.001 ± 0.0005
WR Dalesice	0.296–0.462	0.363 ± 0.059	< LOQ–0.015	0.007 ± 0.005	< LOQ–0.013	0.003 ± 0.005
WR Hnevkovice	0.169–0.267	0.200 ± 0.035	< LOQ–0.02	0.012 ± 0.004	< LOQ	nd
WR Jesenice	0.040–0.118	0.061 ± 0.029	< LOQ–0.28	0.112 ± 0.093	< LOQ–0.002	0.001 ± 0.0005
WR Jordan	0.161–0.388	0.248 ± 0.077	0.01–0.02	0.012 ± 0.004	< LOQ	nd
WR Korensko	0.296–0.536	0.445 ± 0.091	< LOQ	nd	< LOQ–0.004	0.002 ± 0.001
WR Lipno	0.109–0.214	0.160 ± 0.039	< LOQ–0.02	0.012 ± 0.004	< LOQ	nd
WR Musov	0.084–0.180	0.149 ± 0.034	< LOQ–0.061	0.038 ± 0.018	< LOQ–0.003	0.002 ± 0.001
WR Nechranice	0.108–0.180	0.142 ± 0.028	0.02–0.06	0.038 ± 0.015	< LOQ	nd
WR Olesna	0.091–0.289	0.225 ± 0.070	< LOQ–0.027	0.015 ± 0.006	< LOQ–0.031	0.015 ± 0.013
WR Orlik	0.298–0.466	0.365 ± 0.061	< LOQ–0.02	0.012 ± 0.004	< LOQ–0.002	0.001 ± 0.0004
WR Rozkos	0.088–0.235	0.141 ± 0.053	0.01–0.05	0.032 ± 0.015	< LOQ	nd
WR Skalka	0.359–0.742	0.593 ± 0.128	0.02–0.08	0.056 ± 0.021	< LOQ–0.005	0.003 ± 0.001
WR Slapy	0.065–0.083	0.071 ± 0.007	< LOQ	nd	< LOQ	nd
WR Slezska Harta	0.251–0.477	0.331 ± 0.078	0.045–0.087	0.058 ± 0.016	0.004–0.008	0.007 ± 0.001
WR Terlicko	0.044–0.106	0.069 ± 0.021	< LOQ–0.033	0.026 ± 0.008	< LOQ -0.009	0.004 ± 0.003
WR Trnavka	0.097–0.134	0.114 ± 0.014	0.01–0.02	0.014 ± 0.005	< LOQ	nd
WR Vetrov	0.013–0.054	0.037 ± 0.014	< LOQ–0.013	0.005 ± 0.004	< LOQ–0.006	0.002 ± 0.002
WR Vranov	0.268–0.331	0.305 ± 0.024	0.003–0.080	0.02 ± 0.03	< LOQ–0.021	0.006 ± 0.008
WR Zermanice	0.167–0.255	0.204 ± 0.029	< LOQ–0.031	0.014 ± 0.008	0.003–0.039	0.016 ± 0.013

WR, water reservoir; w.w., wet weight; LOQ, limit of quantification; nd, have not been done.

localities is comparable with this reported by Lepom et al. (2012) from reference site (Lake Belau), where concentration of 0.034 mg kg^{-1} w.w. was found in 2009. Relatively low Hg contamination is also reported by Zarski et al. (1997) from sampling sites along Vistula River in Poland. Concentrations of THg found in bream muscle tissue there ranged from 0.068 to 0.183 mg kg^{-1}. Kensova et al. (2010) found a THg mean concentration of 0.090 mg kg^{-1} in bream muscle from the Vestonice reservoir. We found a similar mean concentration (0.149 mg kg^{-1}) at the Musov locality, which is a dam on the upper part of same river system.

Concentrations of mercury in the muscle tissue of selected fish species are shown in Table 3. Higher concentrations were found in the muscle of predatory fish, which corresponds with previous studies (Kim et al., 2012; Mirlean et al., 2005; Noel et al., 2013; Yamaguchi et al., 2003). High concentrations of THg, including the highest observed concentration during this study (3.570 mg kg^{-1} w.w.), were found in the muscle of the cyprinid predatory fish Asp (*Aspius aspius*) at all of the sites where this species was caught.

However, low THg contamination was found in carp muscle (*Cyprinus carpio*) at a majority of the localities because it is typically stocked in open waters in a catchable size and thus comes from generally uncontaminated pond breeding facilities. Contamination of pond-farmed fish is typically very low (Svobodova et al., 2002). This finding corresponds with results of certain other authors (Kim et al., 2012; Celechovska et al., 2007; Kensova et al., 2010; Marsalek et al., 2007). In addition, Noel et al. (2013) found significantly lower concentrations of THg in carp muscle (the average concentration was 0.061 mg kg^{-1} w.w.) than in other fish species in French rivers.

Concentrations of cadmium found in bream (reference species) muscle tissue were low at all of the sampling sites and were below the LOQ in the majority of the samples. Higher concentrations were found only at the Zermanice (0.016 ± 0.013 mg kg^{-1}), Olesna (0.015 ± 0.013 mg kg^{-1}), and Terlicko (0.004 ± 0.003 mg kg$^{\pm 1}$) localities. Concentrations at the Zermanice and Olesna sites were characterised by a high variability. Significantly, the most cadmium-polluted site was Terlicko where one pooled sample (n5) of muscle tissue of zander (*Sander lucioperca*) exceeded the ML for cadmium in fish muscle (0.05 mg kg^{-1}) with a concentration of 0.058 mg kg^{-1}.

Contrary to mercury, concentrations of cadmium in fish muscle do not show an increase along the food chain (Kensova et al., 2010; Noel et al., 2013). Kensova et al. (2010) showed that lower concentrations of cadmium are found in the muscle of predatory fish species, such as pikeperch and pike, compared with non-predatory fish, such as bream or carp. This assertion was confirmed in a study by Noel et al. (2013) that found levels of cadmium in the muscle samples of pike, bream and roach in concentrations of 0.001, 0.004 and 0.005 mg kg^{-1} respectively. These findings correspond with our muscle tissue results, in which no accumulation of cadmium due to the trophic position of the fish species was recorded.

Similar to cadmium, we found low concentrations of lead in bream muscle tissue at all of the localities. The majority of the lead levels were under the LOQ, as shown in Table 2, and none of the levels exceeded the ML in fish muscle (0.3 mg kg^{-1}). The highest mean concentration of lead in bream muscle was found at the Jesenice locality (0.112 ± 0.093 mg kg^{-1}), where also the highest individual sample concentration of 0.280 mg kg^{-1} was recorded. Several studies have confirmed that the concentration of lead in fish muscle does not increase due to the trophic level of the fish (Kensova et al., 2010; Noel et al., 2013). These assertions correspond with our findings.

Maceda-Veiga et al. (2012) found concentrations of lead in barbell muscle (*Barbus meridionalis*) from the Ripoll River in the

Table 3
Concentration of THg in muscle tissue of selected fish species from monitored fishing grounds.

Sampling site	Carp (*Cyprinus carpio*)		Roach (*Rutilus rutilus*)		Chub (*Squalius cephalus*)		Asp (*Aspius aspius*)		Perch (*Perca fluviatilis*)	
	n	**THg (mg kg^{-1})**	***n***	**THg (mg kg^{-1})**	***n***	**THg (mg kg^{-1})**	***n***	**THg (mg kg^{-1})**	***n***	**THg (mg kg^{-1})**
Berounka River – Prague	4	0.066	5	0.103	–	–	4	**0.672**	–	–
Elbe River – Obristvi	3	**0.503**	–	–	5	0.456	4	**2.180**	5	0.451
Elbe River – Pardubice	2	0.069	–	–	5	0.252	2	**0.719**	5	0.127
Elbe River – Svadov	–	–	5	0.266	5	0.291	5	**0.896**	–	–
Luznice River – Majdalena	4	0.030	6	0.198	–	–	–	–	5	**0.580**
Luznice River – Sobeslav	3	0.119	5	0.189	5	0.190	4	**0.768**	–	–
Odra River – Ostrava	–	–	–	–	5	**0.533**	3	0.630	–	–
Otava River – Strakonice	–	–	5	0.202	5	0.169	–	–	5	**0.557**
WR Dalesice	3	**0.542**	4	0.209	–	–	1	**0.674**	3	0.426
WR Hnevkovice	4	0.227	5	0.297	5	0.313	4	0.360	–	–
WR Jesenice	5	0.043	5	0.075	–	–	–	–	5	0.082
WR Jordan	5	0.057	5	0.122	–	–	5	0.323	4	0.337
WR Korensko	4	0.096	5	0.227	–	–	–	–	5	0.274
WR Lipno	4	0.016	5	0.180	–	–	–	–	4	0.234
WR Musov	–	–	5	0.067	–	–	4	0.136	–	–
WR Nechranice	5	0.058	5	0.085	–	–	5	**0.545**	6	0.190
WR Olesna	5	0.035	–	–	–	–	–	–	4	0.271
WR Orlik	5	0.110	–	–	5	**0.608**	–	–	3	0.128
WR Rozkos	5	0.021	5	0.058	–	–	5	0.360	4	0.278
WR Skalka	2	0.161	–	–	–	–	2	**3.570**	5	**1.210**
WR Slapy	5	0.044	5	0.125	–	–	3	1.420	5	0.167
WR Slezska Harta	–	–	–	–	5	**0.620**	–	–	5	0.499
WR Terlicko	5	0.022	–	–	–	–	–	–	–	–
WR Trnavka	4	0.036	5	0.243	–	–	–	–	1	0.203
WR Vetrov	4	0.014	–	–	–	–	1	0.159	–	–
WR Vranov	5	0.086	–	–	1	0.241	4	**0.652**	–	–
WR Zermanice	–	–	–	–	5	0.451	–	–	–	–

Sampling site	Zander (*Sander lucioperca*)		Pike (*Esox lucius*)		Eel (*Anguilla anguilla*)		Catfish (*Silurus glanis*)	
	n	**THg (mg kg^{-1})**	***n***	**THg (mg kg^{-1})**	***n***	**THg (mg kg^{-1})**	***n***	**THg (mg kg^{-1})**
Berounka River – Prague	–	–	5	0.239	3	0.404	–	–
Elbe River – Obristvi	–	–	3	0.922	–	–	–	–
Elbe River – Pardubice	–	–	–	–	–	–	–	–
Elbe River – Svadov	1	0.362	–	–	–	–	–	–
Luznice River – Majdalena	3	0.320	3	0.539	–	–	–	–
Luznice River – Sobeslav	–	–	5	0.301	–	–	–	–
Odra River – Ostrava	–	–	2	0.484	–	–	2	**0.586**
Otava River – Strakonice	–	–	4	0.335	–	–	–	–
WR Dalesice	4	0.485	3	0.401	–	–	–	–
WR Hnevkovice	–	–	5	0.175	–	–	–	–
WR Jesenice	4	0.100	3	0.161	–	–	–	–
WR Jordan	–	–	–	–	5	0.361	–	–
WR Korensko	2	0.290	3	0.479	–	–	–	–
WR Lipno	4	0.368	2	0.232	–	–	–	–
WR Musov	1	0.198	–	–	–	–	–	–
WR Nechranice	3	0.449	–	–	–	–	–	–
WR Olesna	5	0.143	–	–	5	0.110	–	–
WR Orlik	–	–	-	–	5	0.325	2	0.357
WR Rozkos	5	0.245	–	–	–	–	–	–
WR Skalka	–	–	1	**1.600**	–	–	–	–
WR Slapy	5	0.382	–	–	–	–	–	–
WR Slezska Harta	5	**0.557**	5	0.455	4	0.537	–	–
WR Terlicko	5	0.118	4	0.157	3	0.116	–	–
WR Trnavka	1	0.321	–	–	1	0.456	–	–
WR Vetrov	4	0.136	–	–	1	0.171	–	–
WR Vranov	–	–	–	–	–	–	2	**0.541**
WR Zermanice	3	0.318	4	0.307	5	0.389	–	–

Figures in bold express values in excess of Maximum Limit for mercury in fish muscle tissue (629/2008/EC); WR, water reservoir; THg, total mercury; *n*, number of individuals in pooled sample.

range from 0.036 to 0.099 mg kg^{-1}, which are results that are comparable with our study. In addition, Noel et al. (2013) found similar concentrations of lead (0.017 ± 0.019 mg kg^{-1}) in bream muscle at several sampling sites in France. Djedjibegovic et al. (2012) reported higher concentrations of lead in fish muscle from the Neretva River (Bosnia and Herzegovina) in a range from 0.055 to 0.703 mg kg^{-1}. High concentrations of lead were found by Gupta et al. (2009) in the Allahabad region in India in which contamination of the muscle tissue of fish (*Chana punctatus*) reached mean concentrations in the range from 1.86 to 2.89 mg kg^{-1}.

3.2. POPs

NDL-PCBs and DDTs were found to be the major contaminants among the POPs analysed at all of the localities. The ML in fish

muscle in the Czech Republic for $\sum_6$ PCB indicator congeners (28, 52, 101, 138, 153 and 180) is 0.125 mg kg^{-1}. The MRLs for ∑DDT, $\sum\alpha+\beta$ HCH and HCB are 0.5, 0.02 and 0.05 mg kg^{-1}, respectively. All POPs were analysed in single species pooled samples of muscle tissue at each locality.

3.2.1. NDL-PCBs

The highest concentration of $\sum_6$ NDL-PCBs in pooled sample of bream muscle tissue was found at the Odra–Ostrava (0.159 mg kg^{-1}), where excess of ML (0.125 mg kg^{-1}; Regulation 1259/2011/EC) was registered. Some higher concentrations were found also at Elbe–Svadov (0.089 mg kg^{-1}) and Musov (0.078 mg kg^{-1}) sampling sites. The lowest concentrations in pooled samples of bream muscle tissue were found in samples from the Luznice–Majdalena (0.0006 mg kg^{-1}), Hnevkovice (0.0024 mg kg^{-1}), and Vetrov (0.0029 mg kg^{-1}). Concentrations of POPs in the pooled samples of muscle tissue of bream (reference species) from monitored sites are shown in Table 4.

Measured concentrations of $\sum_6$ NDL-PCBs were usually well below the ML at most of the localities, but we discovered 7 pooled samples of different fish species in excess of ML at 4 sampling sites (Supporting Information Table S4). The highest concentration (1.670 mg kg^{-1}) was observed in pooled sample of muscle tissue of eel (*Anquilla anguilla*) from WR Zermanice.

Siroka et al. (2005) found concentrations of NDL-PCBs in the muscle of chub (*Squalius cephalus*) at several sampling sites along the Elbe River in the range of 0.11–0.16 mg kg^{-1}. This result corresponds with our results from this locality. Boscher et al. (2010) reported concentrations of $\sum_{13}$NDL-PCBs from rivers in Luxembourg in whole body homogenates of barbel and chub in the range from 0.008 to 0.105 mg kg^{-1} and from 0.005 to 0.153 mg kg^{-1}, respectively. Orban et al. (2007) found mean concentrations of $\sum_7$NDL-PCBs in perch muscle (*Perca fluviatilis*) in the range from 0.0012 to 0.0023 mg kg^{-1} in Italian lakes. In our study, the mean values of $\sum_6$NDL-PCBs content found in the muscle of perch varied from < 0.00005 mg kg^{-1} (Korensko) to 0.0084 mg kg^{-1} (Olesna). A study by Waszak and Dabrowsak (2009) from Poland showed similar contamination levels compared with the Czech Rivers. The mean concentrations of $\sum_7$NDL-PCBs in the muscle of pikeperch and perch were 0.0016 and 0.0044 mg kg^{-1}, respectively.

Higher concentrations of NDL-PCBs were found in fish with higher lipid content in its body tissues. We found correlation between concentration of NDL-PCBs in muscle tissue of different fish species and its lipid content. Pearson´s correlation was used to express this dependence and $r > 0.5$ was observed at 18 sampling sites (64%). In European fresh water courses, eel (*Anquilla Anguilla*) is usually fish with the highest lipid content, so higher concentrations of NDL-PCBs are found in its body compared with other fish species. Szlinder-Richert et al. (2010) reported concentrations of $\sum_7$NDL-PCBs in the muscle of eel from the Vistula and Szczecin lagoons (Poland) from 0.003 to 0.534 mg kg^{-1}. In addition, Yamaguchi et al. (2003) found higher concentrations of NDL-PCBs in eels compared with other fish species. Concentrations of $\sum_6$NDL-PCBs analysed in muscle of eels in our study were in the range from 0.013 (Jordan) to 1.670 (Zermanice) mg kg^{-1} (pooled samples; n5).

The concentrations of NDL-PCBs in the muscle tissue of some of the none-reference fish species are shown in the Supporting Information (Table S5).

3.2.2. Pesticides

Although DDT was banned for agricultural use in 1974 in the Czech Republic, its occurrence in the environment is still evident. The highest concentrations of DDTs in bream muscle were found at the Musov, Elbe–Svadov and Dalesice sampling sites (0.263, 0.173 and 0.097 mg kg^{-1}, respectively). These sampling sites represent lowland areas with intensive agriculture, where

Table 4
Concentrations of POPs in the pooled samples of muscle tissue of reference species (*Abramis brama*) from the monitored fishing grounds.

LOQs (mg kg^{-1}) **Site name**	**$\sum_6$ PCB 0.00024** (mg kg^{-1} **w.w.**)	**∑ DDT 0.00005**	**α-HCH 0.00002**	**β-HCH 0.00004**	**γ-HCH 0.00003**	**∑ HCH**	**HCB 0.00003**
Berounka River – Prague	0.059	0.036	< LOQ	< LOQ	< LOQ	–	< LOQ
Elbe River – Obristvi	0.016	0.005	< LOQ	< LOQ	< LOQ	–	0.0001
Elbe River – Pardubice	0.009	0.004	< LOQ	< LOQ	< LOQ	–	< LOQ
Elbe River – Svadov	0.089	0.173	< LOQ	< LOQ	< LOQ	–	0.0035
Luznice River – Majdalena	0.001	0.001	< LOQ	< LOQ	< LOQ	–	< LOQ
Luznice River – Sobeslav	0.018	0.014	< LOQ	< LOQ	< LOQ	–	< LOQ
Odra River – Ostrava	0.159	0.065	0.0001	0.0002	0.0004	0.0007	0.0017
Otava River – Strakonice	0.017	0.022	< LOQ	< LOQ	< LOQ	–	< LOQ
WR Dalesice	0.041	0.097	0.0002	0.0003	0.0007	0.0012	0.0024
WR Hnevkovice	0.002	0.017	< LOQ	< LOQ	< LOQ	–	0.0003
WR Jesenice	0.005	0.006	< LOQ	< LOQ	< LOQ	–	< LOQ
WR Jordan	0.004	0.006	< LOQ	< LOQ	< LOQ	–	< LOQ
WR Korensko	0.005	0.004	< LOQ	< LOQ	< LOQ	–	< LOQ
WR Lipno	0.004	0.002	< LOQ	< LOQ	< LOQ	–	< LOQ
WR Musov	0.078	0.263	< LOQ	< LOQ	0.0002	0.0002	0.0034
WR Nechranice	0.009	0.009	< LOQ	< LOQ	< LOQ	–	< LOQ
WR Olesna	0.018	0.017	< LOQ	< LOQ	0.0002	0.0002	0.0005
WR Orlik	0.009	0.022	< LOQ	< LOQ	< LOQ	–	< LOQ
WR Rozkos	0.005	0.020	< LOQ	< LOQ	< LOQ	–	< LOQ
WR Skalka	0.010	0.003	< LOQ	< LOQ	< LOQ	–	0.0001
WR Slapy	0.014	0.018	< LOQ	< LOQ	< LOQ	–	0.0001
WR Slezska Harta	0.027	0.045	< LOQ	< LOQ	0.0001	0.0001	0.0003
WR Terlicko	0.004	0.010	< LOQ	< LOQ	0.0003	0.0003	0.0005
WR Trnavka	0.009	0.003	< LOQ	< LOQ	< LOQ	–	< LOQ
WR Vetrov	0.003	0.013	< LOQ	< LOQ	0.0001	0.0001	0.0001
WR Vranov	0.030	0.052	< LOQ	0.0001	0.0010	0.0011	0.0026
WR Zermanice	0.014	0.021	0.0001	< LOQ	0.0001	0.0002	0.0013

WR, water reservoir; LOQ, limit of quantification; w.w., wet weight.

historical use of target pesticides was high. The lowest concentrations were observed at the Luznice–Majdalena, Lipno and Trnavka sites (0.0011, 0.0018 and 0.0027 mg kg^{-1}, respectively). The wide range of detected concentrations indicates the variation in the pollution levels at different localities.

Marsalek et al. (2004) conducted a long-term study from 1995 to 1999 at the Musov site in which the concentrations of DDT in bream muscle tissues were in the range from 0.012 to 0.305 mg kg^{-1}. This study identified a significant decrease in the DDT contamination level of bream during the sampling period, which was calculated from regression equations. Bosnir et al. (2007) reported a DDT concentration of 0.002 mg kg^{-1} in cyprinid fish muscle from the Sava River (Croatia). Relatively low concentrations of DDT were found in fish from the Nestos River in Greece. Mean concentrations in the muscle of chub and barbel were 0.00040 and 0.00047 mg kg^{-1}, respectively (Christoforidis et al., 2008).

Similar to other POPs, the DDT concentration in fish muscle increase with its lipid content. We found high correlation between concentration of DDT in muscle tissue of different fish species and it´s lipid content at most evaluated localities. Pearson´s correlation was used to express this dependence and $r > 0.5$ was observed at 20 sampling sites (74%). Concentrations of DDTs in muscle tissue of some of non-reference fish species are shown in the Supporting Information (Table S6).

Concentrations of other monitored pesticides – α-, β-, γ-HCH and HCB were low, usually below their LOQs at most sampling sites as it could be seen in Table 4.

3.3. Risk assessment

Maximum tolerable/acceptable intakes of fish meat were calculated according to Eq. (1). Counted amount of fish meat was expressed in kg $person^{-1}$ of 70 kg body weight and it was transferred into number of portions according to Eq. (2). Calculations were made for most common fish species which were caught at each sampling locality (fishing ground). Finally, the amount of fish meat which could be eaten lifelong without negative health effects was expressed in number of portions per month for all evaluated contaminants. These recommendations come from the known contamination of caught fish, but there is definitely more sources of contamination in angler´s diet which should be considered. On the other hand, fish from open waters represent usually main source of mercury, PCBs and OCPs in human diet compare to other types of common food (Cuadrado et al., 1995; Martí-Cid et al., 2008; Törnkvist et al., 2011).

$$w_m = \frac{EL\, b_w}{c} \quad (1)$$

$$N_p = \frac{w_m}{w_P} \quad (2)$$

EL – exposition limit (PTWI, PTMI, PTDI, ADI);
w_m – tolerable intake of fish meat (kg);
b_w – body weight (70 kg);
c – measured concentration of contaminant (mg kg^{-1});
N_p – tolerable number of portions per day/week/month (according to EL);
w_P – weight of one portion (0.17 kg of fish meat).

Consumption rate of fish (all) in general population of the Czech Republic is 5.7 kg $person^{-1}$ $year^{-1}$, but freshwater fish participate with only 1.4 kg $person^{-1}$ $year^{-1}$. However, higher consumption rates are found in group of anglers and their families. Mean consumption rate of fish meat in this group of population in the Czech Republic is 12.1 kg $person^{-1}$ $year^{-1}$, which corresponds to 5.9 portions (meals) of fish meat per month. Most of the fish consumed by anglers represent their own catches and thus this fish originated from open waters of the Czech Republic.

Table 5
Maximum tolerable/allowable amount of meat of reference fish species (*Abramis brama*) in portions per month (limiting values for consumption are in bold).

Site name	MeHg	Cd	Σ DDT
	Portions (meals) per month*		
Berounka River – Prague	**13**	nd	6946
Elbe River – Obristvi	**8.5**	nd	46,880
Elbe River – Pardubice	**15.5**	nd	65,882
Elbe River – Svadov	**19,0**	nd	1424
Luznice River – Majdalena	**27**	nd	222,576
Luznice River – Sobeslav	**14.5**	nd	17,362
Odra River – Ostrava	**7.5**	1281	3778
Otava River – Strakonice	**20.5**	nd	11,225
WR Dalesice	**9.5**	nd	2555
WR Hnevkovice	**16.5**	nd	14,490
WR Jesenice	**62.5**	nd	40,172
WR Jordan	**14.5**	nd	38,663
WR Korensko	**8**	nd	64,506
WR Lipno	**21**	nd	138,022
WR Musov	**22.5**	3843	939
WR Nechranice	**23.5**	nd	26,283
WR Olesna	**15**	1081	14,448
WR Orlik	**12.5**	5065	11,240
WR Rozkos	**27**	nd	12,182
WR Skalka	**6**	3843	89,840
WR Slapy	**46.5**	nd	13,642
WR Slezska Harta	**10.5**	1747	5552
WR Terlicko	**48.5**	2477	24,955
WR Trnavka	**29.5**	nd	92,186
WR Vetrov	**115.5**	5764	18,576
WR Vranov	**11**	1921	4797
WR Zermanice	**16.5**	721	11,709

* Portion = 170 g of fish meat; WR, water reservoir; nd, have not been done (concentrations below LOQ).

As it was mentioned above, THg was found as a major pollutant in muscle tissue of fish at all sampling sites. In accordance with these findings, MeHg was found as a limiting contaminant (from the range of evaluated) for consumption of fish at all monitored localities. The tolerable monthly intake of reference fish species (bream) which could be eaten lifelong without negative health effects is given in Table 5. These amounts vary from 6 to 115 portions (mean = 24) of bream meat per month, depending on given locality.

From our results, it is obvious, that health risk caused by consuming fish from open waters in the Czech Republic is locality and species dependent. The most preferred fish species by Czech anglers is common carp, which made approximately 80% of total weight of catches in years 2009–2013. Common carp is bred in ponds and then artificially stocked into open waters, thus contamination of its meat by monitored pollutants is very low compared to native fish originated from natural spawning in the rivers and water reservoirs. The tolerable monthly intakes of carp meat varied from 6 to 244 portions (mean = 77) per month, depending on given locality.

On the other hand, there is a quite big group of anglers who are specialized in angling of predator fish species like pike, zander or catfish. These fish species are usually more contaminated because they are at the top of the food web in aquatic environment. For example, tolerable monthly intakes of two predatory fishes, pike and asp, were in range from 2 to 21 (mean = 10) and from 1 to 24 (mean = 7) portions per month respectively, depending on given locality.

4. Conclusions

From the range of the analysed pollutants within the monitored period, it is evident that THg was the major pollutant at all of the sampled fishing grounds in the Czech Republic. The results proved a consistently high THg contamination of the Skalka Reservoir where regular consumption of fish could not be recommended. Increased THg concentrations were also recorded at sampling sites located in the middle stretch of the Elbe River (locality near Neratovice), in the Odra River (near Ostrava) and in the Dalesice, Korensko and Vranov Reservoirs. Despite this finding, the hygienic quality of fish meat from a majority of the Czech fishing grounds is acceptable, and it can be consumed without any health risks. However, a high consumption of predatory fish from mentioned sampling sites could result in an increased risk of negative health effects, because of higher THg content due to higher trophy level of these fish species. Common carp (*Cyprinus carpio*), which is the most frequently caught species in the fishing grounds of the Czech Republic, typically showed the lowest contamination of its meat by the monitored pollutants.

Concerning POPs, all of the measured concentrations of DDTs, HCH and HCB were below their MRLs at all sampling sites. We detected 7 samples in excess of ML for NDL-PCBs at 4 sampling sites, most of them from locality Odra-Ostrava, where ML was exceeded in pooled samples of muscle tissue of bream, barbell (*Barbus barbus*), Asp (*Aspius aspius*) and catfish (*Silurus glanis*). Strong correlation between concentration of PCB, DDT and lipid content of fish body was verified at most of sampling sites.

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Appendix A. Supporting information

Supporting information associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2014.07.034.

References

Abel, P.D., 1996. Water Pollution Biology. Taylor & Francis, London; Bristol, PA.

Adamek, Z., Linhart, O., Kratochvil, M., Flajshans, M., Randak, T., Policar, T., Masojidek, J., Kozak, P., 2012. Aquaculture in the Czech Republic in 2012: modern European prosperous sector based on thousand-year history of pond culture. Aquac. Eur. 37, 5–14.

Atchison, W.D., Hare, M.F., 1994. Mechanisms of methylmercury-induced neurotoxicity. Faseb J. 8, 622–629.

Boscher, A., Gobert, S., Guignard, C., Ziebel, J., L'Hoste, L., Gutleb, A.C., Cauchie, H.M., Hoffmann, L., Schmidt, G., 2010. Chemical contaminants in fish species from rivers in the North of Luxembourg: potential impact on the Eurasian otter (*Lutra lutra*). Chemosphere 78, 785–792.

Bosnir, J., Puntaric, D., Smit, Z., Klaric, M., Grgic, M., Kosanovic, L.M., 2007. Organochlorine pesticides in freshwater fish from the Zagreb area. Arh. Hig. Rada Toksikol. 58, 187–193.

Celechovska, O., Svobodova, Z., Zlabek, V., Macharackova, B., 2007. Distribution of metals in tissues of the common carp (*Cyprinus carpio* L.). Acta Vet. Brno 76, 93–100.

Cuadrado, C., Kumpulainen, J., Moreiras, O., 1995. Lead, cadmium and mercury contents in average Spanish market basket diets from Galicia, Valencia, Andalucia and Madrid. Food Addit. Contam. 12, 107–118.

Czech Republic, 2004. The decree No. 305/2004 of 6 May 2004 Defines the Types of Contaminants and Toxicological Substances and Setting their Maximum Levels in Foodstuff. Collection of Laws of the Czech Republic.

Christoforidis, A., Stamatis, N., Schmieder, K., Tsachalidis, E., 2008. Organochlorine and mercury contamination in fish tissues from the River Nestos, Greece. Chemosphere 70, 694–702.

De Conto, Cinier C., Petit-Ramela, M., Faurea, R., Garin, D., Bouvet, Y., 1999. Kinetics of cadmium accumulation and elimination in carp Cyprinus carpio tissues. Comp. Biochem. Physiol. Part C: Pharmacol. Toxicol. Endocrinol. 122, 345–352.

Djedjibegovic, J., Larssen, T., Skrbo, A., Marjanovic, A., Sober, M., 2012. Contents of cadmium, copper, mercury and lead in fish from the Neretva river (Bosnia and Herzegovina) determined by inductively coupled plasma mass spectrometry (ICP-MS). Food Chem. 131, 469–476.

Dusek, L., Svobodova, Z., Janouskova, D., Vykusova, B., Jarkovsky, J., Smid, R., Pavlis, P., 2005. Bioaccumulation of mercury in muscle tissue of fish in the Elbe River (Czech Republic): multispecies monitoring study 1991–1996. Ecotoxicol. Environ. Saf. 61, 256–267.

European Commission (EC), 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, L 364.

European Commission (EC), 2011. Commission Regulation (EC) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs. Official Journal of the European Union, L 320.

FAO, Food and Agriculture Organization of the United Nations, 2001. Pesticide Residues in Food – 2000 (Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group). FAO Plant Production and Protection Paper, 163.

Godduhn, A., Duffy, L.K., 2003. Multi-generation health risks of persistent organic pollution in the far north: use of the precautionary approach in the Stockholm Convention. Environ. Sci. Policy 6, 341–353.

Gupta, A., Rai, D.K., Pandey, R.S., Sharma, B., 2009. Analysis of some heavy metals in the riverine water, sediments and fish from river Ganges at Allahabad. Environ. Monit. Assess. 157, 449–458.

Hajslova, J., Schoula, R., Holadova, K., Poustka, J., 1995. Analysis of PCB in biotic matrices by two-dimensional GC-ECD. Int. J. Environ. Anal. Chem. 60, 163–173.

Kannan, K., Smith Jr., R.G., Lee, R.F., Windom, H.L., Heitmuller, P.T., Macauley, J.M., Summers, J.K., 1998. Distribution of total mercury and methyl mercury in water, sediment, and fish from South Florida estuaries. Arch. Environ. Contam. Toxicol. 34, 109–118.

Kensova, R., Celechovska, O., Doubravova, J., Svobodova, Z., 2010. Concentrations of metals in tissues of fish from the Vestonice reservoir. Acta Vet. Brno 79, 335–345.

Kim, C.K., Lee, T.W., Lee, K.T., Lee, J.H., Lee, C.B., 2012. Nationwide monitoring of mercury in wild and farmed fish from fresh and coastal waters of Korea. Chemosphere 89, 1360–1368.

Kruzikova, K., Randak, T., Kensova, R., Kroupova, H., Leontovycova, D., Svobodova, Z., 2008. Mercury and methylmercury concentrations in muscle tissue of fish caught in major rivers of the Czech Republic. Acta Vet. Brno 77, 637–643.

Lasorsa, B., Allen-Gil, S., 1995. The methylmercury to total mercury ratio in selected marine, freshwater, and terrestrial organisms. Water Air Soil Pollut. 80, 905–913.

Lepom, P., Irmer, U., Wellmitz, J., 2012. Mercury levels and trends (1993-2009) in bream (*Abramis brama* L.) and zebra mussels (*Dreissena polymorpha*) from German surface waters. Chemosphere 86, 202–211.

Maceda-Veiga, A., Monroy, M., de Sostoa, A., 2012. Metal bioaccumulation in the Mediterranean barbel (*Barbus meridionalis*) in a Mediterranean River receiving effluents from urban and industrial wastewater treatment plants. Ecotoxicol. Environ. Saf. 76, 93–101.

Marsalek, P., Svobodova, Z., Randak, T., 2007. The content of total mercury and methylmercury in common carp from selected Czech ponds. Aquac. Int. 15, 299–304.

Marsalek, P., Svobodova, Z., Randak, T., Svehla, J., 2005. Mercury and methylmercury contamination of fish from the Skalka reservoir: a case study. Acta Vet. Brno 74, 427–434.

Marsalek, P., Weiss, V., Kolejka, J., 2004. Chlorinated hydrocarbon residues in fish from Nove Mlyny reservoirs along Dyje river, Czech Republic. Acta Vet. Brno 73, 275–281.

Martí-Cid, R., Llobet, J.M., Castell, V., Domingo, J.L., 2008. Dietary intake of arsenic, cadmium, mercury, and lead by the population of catalonia, Spain. Biol. Trace Elem. Res. 125, 120–132.

Mirlean, N., Larned, S.T., Nikora, V., Kutter, V.T., 2005. Mercury in lakes and lake fishes on a conservation-industry gradient in Brazil. Chemosphere 60, 226–236.

Noel, L., Chekri, R., Millour, S., Merlo, M., Leblanc, J.C., Guerin, T., 2013. Distribution and relationships of As, Cd, Pb and Hg in freshwater fish from five French fishing areas. Chemosphere 90, 1900–1910.

Orban, E., Nevigato, T., Masci, M., Di Lena, G., Casini, I., Caproni, R., Gambelli, L., De Angelis, P., Rampacci, M., 2007. Nutritional quality and safety of European perch (*Perca fluviatilis*) from three lakes of Central Italy. Food Chemistry 100, 482–490.

Petro, E.M.L., Covaci, A., Leroy, J.L.M.R., Dirtu, A.C., De Coen, W., Bols, P.E.J., 2010. Occurrence of endocrine disrupting compounds in tissues and body fluids of Belgian dairy cows and its implications for the use of the cow as a model to study endocrine disruption. Sci. Total Environ. 408, 5423–5428.

Siroka, Z., Krijt, J., Randak, T., Svobodova, Z., Peskova, G., Fuksa, J., Hajslova, J., Jarkovsky, J., Janska, M., 2005. Organic pollutant contamination of the River Elbe as assessed by biochemical markers. Acta Vet. Brno 74, 293–303.

Svobodova, Z., Zlabek, V., Celechovska, O., Randak, T., Machova, J., Kolarova, J., Janouskova, D., 2002. Content of metals in tissues of marketable common carp and in bottom sediments of selected ponds of South and West Bohemia. Czech J. Anim. Sci. 47, 339–350.

Szlinder-Richert, J., Usydus, Z., Pelczarski, W., 2010. Organochlorine pollutants in European eel (*Anguilla anguilla* L.) from Poland. Chemosphere 80, 93–99.

Törnkvist, A., Glynn, A., Aune, M., Darnerud, P.O., Ankarberg, E.H., 2011. PCDD/F, PCB, PBDE, HBCD and chlorinated pesticides in a Swedish market basket from 2005 – levels and dietary intake estimations. Chemosphere 83, 193–199.

van Hattum, B., Korthals, G., Van Straalen, N.M., Govers, H.A.J., Joosse, E.N.G., 1993. Accumulation patterns of trace metals in freshwater isopods in sediment bioassays – influence of substrate characteristics, temperature and pH. Water Res. 27, 669–684.

Waszak, I., Dabrowsak, H., 2009. Persistent organic pollutants in two fish species of Percidae and sediment from the Sulejowski Reservoir in central Poland. Chemosphere 75, 1135–1143.

WHO, World Health Organisation, 2004. Evaluation of Certain Food Additives and Contaminants (61st report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 922.

WHO, World Health Organisation, 2011. Evaluation of Certain Food Additives and Contaminants (73st Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 960.

Yamaguchi, N., Gazzard, D., Scholey, G., Macdonald, D.W., 2003. Concentrations and hazard assessment of PCB, organochlorine pesticides and mercury in fish species from the upper thames: river pollution and its potential effects on top predators. Chemosphere 50, 265–273.

Yang, F.X., Xu, Y., 2005. Hydroxylated metabolites of polychlorinated biphenyls and their endocrine disrupting mechanism. Prog. Chem. 17, 740–748.

Zarski, T.P., Zarska, H., Sokol, J., Beseda, I., Valka, J., 1997. The influence of season on mercury metabolism in bream (*Abramis brama* L.). Ekologia Bratisl. 16, 331–336.

Zlabek, V., Svobodova, Z., Randak, T., Valentova, O., 2005. Mercury content in the muscle of fish from the Elbe River and its tributaries. Czech J. Anim. Sci. 50, 528–534.

SUPPLEMENT MATERIALS

Table S1. *List of the monitored fishing grounds.*

Site name	Fishing ground ID	Area	GPS
Berounka River - Prague	401 001 Berounka 1	55 ha	N 49° 57.299´; E 14° 19.312´
Elbe River - Obristvi	411 047 Labe 15	75 ha	N 50° 17.762´; E 14° 28.934´
Elbe River - Pardubice	451 032 Labe 29	51 ha	N 50° 8.339´; E 15° 48.204´
Elbe River - Svadov	441 022 Labe 4-5	165 ha	N 50° 39.908´; E 14° 06.139´
Luznice River - Majdalena	421 042, Luznice 10B	180 ha	N 48° 57.584´; E 14° 51.430´
Luznice River - Sobeslav	421 037 Luznice 6	101 ha	N 49° 15.328´; E 14° 42.216´
Odra River - Ostrava	471 063 Odra 1	52 ha	N 49° 55.252´; E 18° 19.483´
Otava River - Strakonice	421 056 Otava 4	64 ha	N 49° 15.277´; E 13° 53.170´
WR Dalesice	461 056 Jihlava 7-8	397 ha	N 49° 9.285´; E 16° 4.525´
WR Hnevkovice	421 073 Vltava 21 – 22	262 ha	N 49° 17.404´; E 14° 20.600´
WR Jesenice	431 200 Odrava 1	700 ha	N 50° 4.369´; E 12° 27.392´
WR Jordan	421 068 Tismenice 1	55 ha	N 49° 25.377´; E 14° 39.500´
WR Korensko	421 090 Vltava 20	130 ha	N 49° 14.277´; E 14° 23.566´
WR Lipno	421 200 Vltava 30 – 32	4870 ha	N 48° 44.221´; E 14° 6.181´
WR Musov	461 026 Dyje 7	530 ha	N 48° 53.550´; E 16° 32.250´
WR Nechranice	441 043 Ohre 9	1300 ha	N 50° 20.478´; E 13° 21.477´
WR Olesna	471 077 Olesna 2A	74 ha	N 49° 39.490´; E 18° 18.590´
WR Orlik	481 501 Vltava 16-19	2300 ha	N 49° 24.717´; E 14° 9.418´
WR Rozkos	451 200, Rozkos	1080 ha	N 50° 22.926´; E 16° 03.057´
WR Skalka	431 040 Ohre 19	340 ha	N 50° 5.264´; E 12° 18.268´
WR Slapy	401 022 Vltava 10 – 14	1000 ha	N 49° 45.222´; E 14° 25.272´
WR Slezska Harta	471 200 Harta 1A	900 ha	N 49° 53.271´; E 17° 34.561´
WR Terlicko	471 131 Stonavka 2A	267 ha	N 49° 45.520´; E 18° 30.900´
WR Trnavka	421 503 Trnava	80 ha	N 49° 30.467´; E 15° 11.529´
WR Vetrov	471 084 Olse 3A	52 ha	N 49° 53.210´; E 18° 30.109´
WR Vranov	461 032 Dyje 15	537 ha	N 48° 56.122´; E 15° 46.337´
WR Zermanice	471 043 Lucina 2A	248 ha	N 49° 43.104´; E 18° 27.364´

WR, water reservoir

Table S2. *Toxicological recommendations for certain contaminants given by WHO/FAO.*

Contaminant	Limit	Criteria		Source
MeHg	1,6	PTWI	µg x kg bw.$^{-1}$ x week^{-1}	WHO 2004
Cd	25	PTMI	µg x kg bw.$^{-1}$ x moth^{-1}	WHO 2011
Σ DDT	10	PTDI	µg x kg bw.$^{-1}$ x day^{-1}	FAO 2001

PTWI, Provisional tolerable weekly intake; PTMI, Provisional tolerable monthly intake; PTDI, Provisional tolerable daily intake; bw., body weight

Table S3. *List of samples that were in excess of the ML for mercury in fish muscle (629/2008/EC).*

Site name	Indicator	n (pcs)	THg concentration
		individual/pooled sample *	(mg kg^{-1} w.w.)
Berounka River - Prague	*Aspius aspius*	4*	0.672
Elbe River – Svadov	*Aspius aspius*	5*	0.896
Elbe River - Obristvi	*Cyprinus carpio*	2*	0.503
	Aspius aspius	4*	2.180
Elbe River - Pardubice	*Aspius aspius*	2*	0.719
Lužnice River - Majdalena	*Perca fluviatilis*	5*	0.580
Lužnice River - Sobeslav	*Aspius aspius*	4*	0.768
Odra River - Ostrava	*Abramis brama*	1	0.500
	Squalius cephalus	5*	0.533
	Barbus barbus	4*	0.523
	Aspius aspius	3*	0.630
	Silurus glanis	2*	0.586
Otava River - Strakonice	*Abramis brama*	1	0.571
	Perca fluviatilis	5*	0.557
WR Dalesice	*Cyprinus carpio*	3*	0.542
	Aspius aspius	1	0.674
WR Korensko	*Abramis brama*	1	0.527
	Abramis brama	1	0.536
WR Nechranice	*Aspius aspius*	5*	0.545
WR Orlik	*Squalius cephalus*	5*	0.608
WR Skalka	*Abramis brama*	1	0.584
	Abramis brama	1	0.650
	Abramis brama	1	0.631
	Abramis brama	1	0.742
	Abramis bjoerkna	5*	0.654
	Perca fluviatilis	5*	1.210
	Esox lucius	1	1.600
	Aspius aspius	2*	3.570
WR Slapy	*Aspius aspius*	3*	1.420
WR Slezska Harta	*Sander lucioperca*	3*	0.557
	Squalius cephalus	5*	0.620
	Anguilla anguilla	4*	0.537
WR Vranov	*Silurus glanis*	1	0.640
	Aspius aspius	4*	0.652
WR Zermanice	*Scardinius erythrophtalmus*	5*	0.776

WR, water reservoir; w.w., wet weight; ML, maximum limit (0.5 mg kg^{-1} w.w.); THg, total mercury

Table S4. *List of samples that were in excess of the ML for NDL-PCBs in fish muscle (1259/2011/EC).*

Site name	Indicator	n (pcs)	$\sum_6$PCBs concentration
		individual/pooled sample *	(mg kg^{-1} w.w.)
Elbe River - Obristvi	*Aspius aspius*	4*	0.200
Odra River - Ostrava	*Abramis brama*	5*	0.164
	Barbus barbus	4*	0.196
	Aspius aspius	3*	0.191
	Silurus glanis	1*	1.170
Dalesice	*Aspius aspius*	1*	0.131
WR Zermanice	*Anguilla anguilla*	5*	1.670

WR, water reservoir; w.w., wet weight; ML, maximum limit (0.125 mg kg^{-1} w.w.)

Table S5. *Concentration of $\sum_6$ PCB in muscle tissue of selected fish species from monitored fishing grounds.*

Sampling site	Cyprinus carpio		Rutilus rutilus		Squalius cephalus		Aspius aspius		Perca fluviatilis		Sander lucioperca		Esox lucius		Anguilla anguilla		Silurus glanis	
	n	Σ_6 PCB (mg.kg^{-1})	n	Σ_6 PCB (mg.kg^{-1})	n	Σ_6 PCB (mg.kg^{-1})	n	Σ_6 PCB (mg.kg^{-1})	n	Σ_6 PCB (mg.kg^{-1})	n	Σ_6 PCB (mg.kg^{-1})	n	Σ_6 PCB (mg.kg^{-1})	n	Σ_6 PCB (mg.kg^{-1})	n	Σ_6 PCB (mg.kg^{-1})
Berounka River - Prague	4	0.01038	5	0.00335	-	-	4	0.02825	-	-	-	-	5	0.00070	3	0.07035	-	-
Elbe River - Obristvi	3	0.01348	-	-	5	0.03337	4	0.20027	5	0.00333	-	-	3	0.00215	-	-	-	-
Elbe River - Pardubice	2	0.01606	-	-	5	0.06557	2	0.36680	5	0.00268	-	-	-	-	-	-	-	-
Elbe River - Svadov	-	-	5	0.02840	5	0.03750	5	0.06340	-	-	1	0.00740	-	-	-	-	-	-
Luznice River - Majdalena	4	0.01460	6	0.00125	-	-	-	-	5	0.00482	3	0.00070	3	nd	-	-	-	-
Luznice River - Sobeslav	3	0.00301	5	0.00433	5	0.00753	4	0.00665	-	-	-	-	5	0.00092	-	-	-	-
Odra River - Ostrava	-	-	-	-	5	0.10200	3	0.19100	-	-	-	-	2	0.03260	-	-	2	0.60440
Otava River - Strakonice	-	-	5	0.00555	5	0.01334	-	-	5	0.00537	-	-	4	0.00071	-	-	-	-
WR Dalesice	3	0.01370	4	0.01010	-	-	1	0.13100	3	0.00710	4	0.02490	3	0.02910	-	-	-	-
WR Hnevkovice	4	0.00462	5	0.00062	5	0.02983	4	0.00257	-	-	-	-	5	0.00162	-	-	-	-
WR Jesenice	5	0.00159	5	0.00088	-	-	-	-	5	0.00074	4	0.00084	3	0.00017	-	-	-	-
WR Jordan	5	0.01360	5	0.00860	-	-	5	0.00042	4	0.00149	-	-	-	-	5	0.01314	-	-
WR Korensko	4	0.00072	5	0.00542	-	-	-	-	5	nd	2	0.00030	3	nd	-	-	-	-
WR Lipno	4	0.00285	5	0.00306	-	-	-	-	4	0.00152	4	0.00203	2	0.00142	-	-	-	-
WR Musov	-	-	5	0.00890	-	-	4	0.06900	-	-	1	0.07040	-	-	-	-	-	-
WR Nechranice	5	0.00171	5	0.00492	-	-	5	0.00173	6	0.00064	3	0.00034	-	-	-	-	-	-
WR Olesna	5	0.00690	-	-	-	-	-	-	4	0.00840	5	0.00930	-	-	5	0.38700	-	-
WR Orlik	5	0.00500	-	-	5	0.00770	-	-	3	0.00030	-	-	-	-	5	0.29489	2	0.00210
WR Rozkos	5	0.00042	5	0.00099	-	-	5	0.00701	4	0.00355	5	nd	-	-	-	-	-	-
WR Skalka	2	0.00185	-	-	-	-	2	0.00360	5	0.00095	-	-	1	0.00139	-	-	-	-
WR Slapy	5	0.00970	5	0.00450	-	-	3	0.01616	5	0.00147	5	0.00021	-	-	-	-	-	-
WR Slezska Harta	-	-	-	-	5	0.03780	-	-	5	0.00410	5	0.00200	5	0.00450	4	0.07615	-	-
WR Terlicko	5	0.00250	-	-	-	-	-	-	-	-	5	0.00270	4	0.00460	3	0.01460	-	-
WR Trnavka	4	0.00107	5	0.00266	-	-	-	-	1	0.00044	1	0.00023	-	-	1	0.20092	-	-
WR Vetrov	4	0.00380	-	-	-	-	1	0.07880	-	-	4	0.00340	-	-	1	0.17400	-	-
WR Vranov	5	0.00920	-	-	1	0.03860	4	0.05470	-	-	-	-	-	-	-	-	2	0.00300
WR Zermanice	-	-	-	-	5	0.10800	-	-	-	-	3	0.00850	4	0.06920	5	1.67000	-	-

WR, water reservoir

Table S6. *Concentration of DDT in muscle tissue of selected fish species from monitored fishing grounds.*

Sampling site	Cyprinus carpio		Rutilus rutilus		Squalius cephalus		Aspius aspius		Perca fluviatilis		Sander lucioperca		Esox lucius		Anguilla anguilla		Silurus glanis	
	n	Σ DDT (mg kg^{-1})	n	Σ DDT (mg kg^{-1})	n	Σ DDT (mg kg^{-1})	n	Σ DDT (mg kg^{-1})	n	Σ DDT (mg kg^{-1})	n	Σ DDT (mg kg^{-1})	n	Σ DDT (mg kg^{-1})	n	Σ DDT (mg kg^{-1})	n	Σ DDT (mg kg^{-1})
Berounka River - Prague	4	0.01296	5	0.00245	-	-	4	0.01170	-	-	-	-	5	0.00090	3	0.06979	-	-
Elbe River - Obristvi	3	0.01760	-	-	5	0.03180	4	0.13600	5	0.00110	-	-	3	0.00066	-	-	-	-
Elbe River - Pardubice	2	0.01088	-	-	5	0.05146	2	0.01920	5	0.00036	-	-	-	-	-	-	-	-
Elbe River - Svadov	-	-	5	0.04690	5	0.02880	5	0.03710	-	-	1	0.00570	-	-	-	-	-	-
Luznice River - Majdalena	4	0.01395	6	0.00317	-	-	-	-	5	0.00142	3	0.00008	3	0.00009	-	-	-	-
Luznice River - Sobeslav	3	0.00229	5	0.00246	5	0.00364	4	0.00273	-	-	-	-	5	0.00064	-	-	-	-
Odra River - Ostrava	-	-	-	-	5	0.03050	3	0.08280	-	-	-	-	2	0.01170	-	-	2	0.24580
Otava River - Strakonice	-	-	5	0.00589	5	0.01251	-	-	5	0.00350	-	-	4	0.00086	-	-	-	-
WR Dalesice	3	0.02700	4	0.01740	-	-	1	0.16190	3	0.01230	4	0.04030	3	0.05080	-	-	-	-
WR Hnevkovice	4	0.00384	5	0.00009	5	0.02159	4	0.00084	-	-	-	-	5	0.00060	-	-	-	-
WR Jesenice	5	0.00274	5	0.00036	-	-	-	-	5	0.00018	4	0.00018	3	0.00009	-	-	-	-
WR Jordan	5	0.00846	5	0.00990	-	-	5	0.00014	4	0.00046	-	-	-	-	5	0.00696	-	-
WR Korensko	4	0.00069	5	0.00316	-	-	-	-	5	0.00009	2	0.00021	3	nd	-	-	-	-
WR Lipno	4	0.00328	5	0.00084	-	-	-	-	4	0.00006	4	0.00010	2	0.00005	-	-	-	-
WR Musov	-	-	5	0.02140	-	-	4	0.10040	-	-	1	0.21800	-	-	-	-	-	-
WR Nechranice	5	0.00119	5	0.00273	-	-	5	0.00040	6	0.00090	3	nd	-	-	-	-	-	-
WR Olesna	5	0.01190	-	-	-	-	-	-	4	0.00750	5	0.00870	-	-	5	0.40300	-	-
WR Orlik	5	0.00840	-	-	5	0.00980	-	-	3	0.00060	-	-	-	-	5	0.51500	2	0.00130
WR Rozkos	5	0.00050	5	0.00455	-	-	5	0.03691	4	0.01237	5	nd	-	-	-	-	-	-
WR Skalka	2	0.00034	-	-	-	-	2	0.00047	5	0.00010	-	-	1	0.00025	-	-	-	-
WR Slapy	5	0.01161	5	0.00327	-	-	3	0.01271	5	0.00057	5	nd	-	-	-	-	-	-
WR Slezska Harta	-	-	-	-	5	0.04090	-	-	5	0.00530	5	0.00220	5	0.00470	4	0.08645	-	-
WR Terlicko	5	0.00390	-	-	-	-	-	-	-	-	5	0.00500	4	0.00910	3	0.04720	-	-
WR Trnavka	4	0.00536	5	0.00186	-	-	-	-	1	0.00170	1	0.00265	-	-	1	1.06140	-	-
WR Vetrov	4	0.01060	-	-	-	-	1	0.22900	-	-	4	0.01820	-	-	1	0.64100	-	-
WR Vranov	5	0.02070	-	-	1	0.31900	4	0.05950	-	-	-	-	-	-	-	-	2	0.00330
WR Zermanice	-	-	-	-	5	0.06370	-	-	-	-	3	0.00300	4	0.00830	5	0.26900	-	-

WR....water reservoir

CHAPTER 3

PERFLUOROALKYL SUBSTANCES IN AQUATIC ENVIRONMENT – COMPARISON OF FISH AND PASSIVE SAMPLING APPROACHES

Cerveny, D., Grabic, R., Fedorova, G., Grabicova, K., Turek, J., Kodes, V., Golovko, O., Zlabek, V., Randak, T., 2016. Perfluoroalkyl substances in aquatic environment-comparison of fish and passive sampling approaches. Environmental Research 144: 92–98.

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Perfluoroalkyl substances in aquatic environment-comparison of fish and passive sampling approaches

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ABSTRACT

The concentrations of seven perfluoroalkyl substances (PFASs) were investigated in 36 European chub (*Squalius cephalus*) individuals from six localities in the Czech Republic. Chub muscle and liver tissue were analysed at all sampling sites. In addition, analyses of 16 target PFASs were performed in Polar Organic Chemical Integrative Samplers (POCISs) deployed in the water at the same sampling sites. We evaluated the possibility of using passive samplers as a standardized method for monitoring PFAS contamination in aquatic environments and the mutual relationships between determined concentrations.

Only perfluorooctane sulphonate was above the LOQ in fish muscle samples and 52% of the analysed fish individuals exceeded the Environmental Quality Standard for water biota. Fish muscle concentration is also particularly important for risk assessment of fish consumers. The comparison of fish tissue results with published data showed the similarity of the Czech results with those found in Germany and France.

However, fish liver analysis and the passive sampling approach resulted in different fish exposure scenarios. The total concentration of PFASs in fish liver tissue was strongly correlated with POCIS data, but pollutant patterns differed between these two matrices. The differences could be attributed to the metabolic activity of the living organism. In addition to providing a different view regarding the real PFAS cocktail to which the fish are exposed, POCISs fulfil the Three Rs strategy (replacement, reduction, and refinement) in animal testing.

1. Introduction

Perfluoroalkyl substances (PFASs) are artificially fluorinated hydrocarbons and their derivatives, which have a wide range of industrial, agricultural, and household uses. Due to their surface-active properties, PFASs are used as lubricants, components of fire-fighting foams, and leather and paper surface protectors (Lindstrom et al., 2011; Zhao et al., 2012; Zareitalabad et al., 2013). They are also used in many herbicide and insecticide formulations or in cosmetics (Zhang and Lerner, 2012). PFASs have been produced and used for over sixty years, but due to the lack of suitable analytical methods, they did not arouse scientific interest until early 2000 (Zhao et al., 2012; Valsecchi et al., 2013). Nowadays, PFASs are detected in various environmental matrices all over the world, even in the regions where they have never been used. PFASs are persistent in the environment due to the strong carbon–fluorine bond, which makes them resistant to thermal, chemical, and biological degradation. PFASs are released into the environment from direct (manufacture and application) and indirect sources (release from consumer goods during their use). Aquatic environments are usually the final recipient of these pollutants originating from the industrial areas and waste water treatment plants (WWTPs) of urban centres (Giesy et al., 2001; Prevedouros et al., 2006; Zhao et al., 2012; Zareitalabad et al., 2013).

The most studied PFAS is perfluorooctane sulphonate (PFOS). This compound was reported to be the most prevalent PFAS found in biota samples from various regions of the world (Giesy et al., 2001; Berger et al., 2009; Yeung et al., 2009; Zhao et al., 2012; Naile et al., 2013). Higher PFOS concentrations were found in aquatic mammals and birds that ate fish (Giesy et al., 2001). Significant differences in PFOS concentrations were also found between piscivorous and non-piscivorous fish (Ye et al., 2008b). Besides PFOS, there are some other PFASs, like PFOA, perfluorononanoic acid (PFNA), perfluorohexane sulphonate (PFHxS), or perfluoropentanoic acid (PFPeA), that are usually found in aquatic biota (Giesy et al., 2001; Miege et al., 2012). In contrast to

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other persistent organic pollutants (POPs), which accumulate mainly in the lipid tissues of the body, greater PFAS concentrations are found in the liver, blood plasma, or gall bladder of fish (Giesy and Kannan, 2001; Murakami et al., 2011; Naile et al., 2013). This can be assigned to the indirect exposure pathway, including transformation of precursors such as perfluorooctane sulphonamido ethanols (FOSEs), FOSAs, fluorotelomer alcohols (FTOHs), or polyfluoroalkyl phosphate esters (PAPs) in living organisms (Gebbink et al., 2015).

The toxic effects of some PFASs, including perfluorinated octanesulphonamides (FOSAs), on fish and zooplankton were confirmed by several studies (Sanderson et al., 2004; Zheng et al., 2012). Relationships were found between the vitellogenin gene expression in fish liver, the vitellogenin plasma activity and the PFAS concentrations in fish plasma (Houde et al., 2013). Johansson et al. (2009) found that neonatal exposure to PFOS and perfluorooctanoic acid (PFOA) in mice has negative effects on brain development. For such reasons, PFOS was added to Annex B of the Stockholm Convention on Persistent Organic Pollutants in 2009. Additionally, PFOS was identified as a priority hazardous substance and an Environmental Quality Standard (EQS) of 9.1 $\mu g\ kg^{-1}$ of wet weight (w.w.) for water biota (fish) was set by the Directive of the European Parliament and Council (2013). The European Food Safety Authority (EFSA), (2008) also published a Scientific Opinion on PFOS and PFOA, where the tolerable daily intakes (TDI) of 150 ng kg^{-1} bw d^{-1} and 1.5 $\mu g\ kg^{-1}$ bw d^{-1}, respectively, were set.

Increased knowledge of PFASs characteristics leads to higher demand of its biomonitoring in the environment. Several authors focused on PFASs occurrence in aquatic environment during last decade e.g. (Giesy et al., 2001; Ye et al., 2008a; Berger et al., 2009; Yeung et al., 2009).However, there is no uniform approach for its evaluation in fish; different authors performed analysis in different fish tissues (muscle/muscle with skin/liver/plasma/whole body homogenates) and the units of measurement varied (dry weight/wet weight), which makes it difficult to compare the obtained data and evaluate the real contamination of study areas.

The objectives of this study were to investigate the concentrations of PFAS in water and fish from the Czech Republic and to evaluate differences between monitoring approaches. Polar Organic Chemical Integrative Samplers (POCIS) data were compared with the data from analysis of fish muscle and liver tissue. It seems that POCIS and other passive sampling methods may have the potential to become a standardized approach for biomonitoring of aquatic environments, which fulfils the internationally-established principles of Replacement, Reduction, and Refinement-the Three Rs. These principles are also included in the EU Directive on the protection of animals used for scientific purposes (European Parliament and Council, 2010).

2. Material and methods

2.1. Monitored sites

Six localities belonging to different water courses of the Labe (Elbe) and Morava (Danube) catchments were selected as sampling sites (Fig. 1). These sampling sites were chosen due to higher probability of occurrence of the target pollutants, as they are situated downstream of large cities or industrial areas. There is no evidence about PFAS producers in the Czech Republic, but various kinds of industry could use some of these compounds as ingredients or operating substances in production processes. Communal waste waters can also be an important source of PFASs in rivers downstream urban centres. Detailed information on selected localities, including flow rate or catchment area, is attached in the Supplementary material (Table S1).

Dluhonice lies on the Becva River, downstream of Prerov city

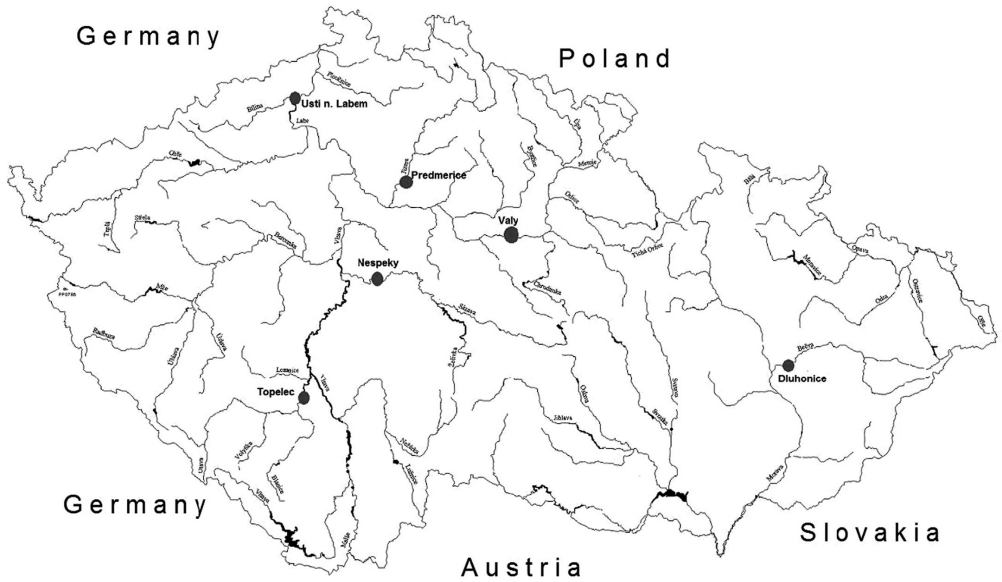

Fig. 1. Map of the Czech Republic with sampling sites.

(47,000 inhabitants), where metal processing, chemical, and photographic industries are located. Valy lies on the Elbe River. This sampling site is situated downstream of Pardubice city (90,000 inh.), where chemical plants, an oil refinery, a heavy machinery factory, and an electronic equipment plant are located. Usti nad Labem lies on the Bilina River, just before its confluence with the Elbe River. The Bilina River is classified as a highly polluted river in the Czech Republic. It flows through the northwestern part of the Czech Republic, which is greatly affected by coal mining activities and has several urban centres, chemical and pharmaceutical industry plants, oil refineries, and metal processing industry plants on its shores. Predmerice is situated on the Jizera River. The Jizera River begins in Poland and flows through mountain areas with several ski resorts (located about 100 km upstream of the sampling site). Two industrial areas are located about 20 and 26 km upstream of Benatky nad Jizerou and Mlada Boleslav, respectively, where the abrasives producers and big automobile producers operate. Nespeky is situated on the Sazava River, where no sources of pollution by PFASs are assumed. Topelec is situated on the Otava River, downstream of Pisek city (30,000 inh.), where several electronic and textile industry producers are located.

2.2. Fish sampling and sample preparation

Six individuals of the European chub (*Squalius cephalus*) were caught at each sampling site in 2012. Chub was chosen as a reference species due to its abundance at all sampling sites and due to its usage as a bio-indicator species in the Czech Hydrometeorological Institute's national programme of surface water quality monitoring.

Experimental animals were handled in accordance with the national and institutional guidelines for the protection of human subjects and animal welfare (European Parliament and Council, 2010). Electrofishing devices were used to obtain a sufficient number of reference fish species at monitored sites. All caught fish were sacrificed then measured, weighed, and scales were taken to determine their age. The characteristics of the sampled fish are given in Table 1. Samples of muscle tissue without skin from the mid-dorsal part of the body were taken, packed into plastic bags, labelled, and stored in insulated boxes on ice during transport to the laboratory; liver samples were taken, packed, labelled, and stored in the same way. For the localities of Predmerice and Valy, only 5 and 3 samples of liver tissue were taken due to the small size of some experimental fish at these sampling sites. All samples were taken individually and then transported to the laboratory, where they were kept frozen at -20 °C until analysis.

2.3. Chemicals and standards

Mixtures of seventeen native (PFAC-MXB) and nine mass-labelled (MPFAC-MXA) perfluorinated acids and perfluoroalkylsulphonates were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). Working mixtures of native compounds and surrogate standards were prepared in methanol at 1 μg mL^{-1} and stored at 4 °C. Methanol (LiChrosolv Hypergrade), acetonitrile (LiChrosolv, Hypergrade), toluene (Suprasolv), and dichloromethane (Suprasolv) were purchased from Merck (Darmstadt, Germany). Formic acid, used for acidification of mobile phase, was purchased from Labicom (Olomouc, Czech Republic). Ultrapure water was obtained from an aqua-MAX-Ultra system (Younglin, Kyounggi-do, Korea).

Table 1
Characteristics of caught fish (chub).

Locality	*n*	Age (years)	Body weight (g)	Total body length (mm)
		mean ± SD	mean ± SD	mean ± SD
Nespeky	6	3.2 ± 0.4	140.8 ± 86.8	228.3 ± 35.7
Valy	6	2.3 ± 0.5	67.5 ± 60.7	168.8 ± 46.8
Dluhonice	6	3.7 ± 0.5	255.0 ± 71.6	279.3 ± 26.1
Predmerice	6	3.7 ± 1.1	279.2 ± 352.0	259.8 ± 90.1
Usti nad Labem	6	3.0 ± 0.0	88.3 ± 17.2	207.3 ± 11.4
Topelec	6	3.8 ± 0.9	304.2 ± 150.9	282.2 ± 42.2

2.4. Muscle and liver sample extraction

A modification of the method of (Fedorova et al., 2014) was used for extraction of seven target compounds from fish tissues. Briefly, samples of fish tissue (0.5 g) with added internal standard (20 ng per sample) and 1 mL of extraction solvent (acetonitrile with 1% formic acid) were homogenised at 1800 revolutions per min for 10 min (TissueLyser II, Qiagen, Germany) and centrifuged at 10,000*g* for 10 min (Micro 200R, Hettich Zentrifugen, Germany). The supernatant was filtered through a syringe filter (0.45 μm pores, regenerated cellulose) and allowed to evaporate to about 0.5 mL at room temperature overnight. An aliquot of the extract was diluted with water (1:1) and this sample was analysed by liquid chromatography with high resolution mass spectrometry (LC-HRMS).

2.5. Passive sampler deployment and extraction

Based on passive sampler calibration results published by (Fedorova et al., 2013), POCIS in the pesticide configuration (triphasic admixture of a hydroxylated polystyrene-divinylbenzene resin, Isolute ENV+) and a carbonaceous adsorbent (Ambersorb 1500) dispersed on a styrene divinylbenzene copolymer (S-X3 Bio Beads) were used (Nya Exposmeter AB, Tavelsjö, Sweden). The samplers were deployed in the riverine water at sampling sites for 21 days during the spring of 2012. After the exposure period, the samplers were retrieved, cleaned with ultrapure water, and transported on ice to the laboratory, where they were stored at −18 °C until analysis. The sampling period of twenty-one days was set to achieve high accumulation of target compounds and representative overview in longer time span.

Sixteen PFASs were analysed in passive samplers after standardized extraction procedures (Alvarez et al., 2005). Briefly, sorbent was transferred into glass gravity-flow chromatography columns filled with glass wool (2 cm layer). Target analytes were recovered from the sorbent by elution with 50 mL of a dichloromethane/methanol/toluene mixture (8:1:1, v/v/v). Extracts were reduced to 2 mL by rotary evaporation. The internal standards (2 ng) were added to the 100 μl sample aliquots in autosampler vials and analysed with LC-HRMS. The complete data set of PFASs in POCIS is reported in the Supplementary materials (Table S2).

2.6. LC-HRMS analysis

A hybrid quadrupole/orbital trap mass spectrometer QExactive (Thermo Fisher Scientific, San Jose, CA, USA), coupled to an Accela 1250 LC pump (Thermo Fisher Scientific, San Jose, CA, USA) and a HTS XT-CTC autosampler (CTC Analytics AG, Zwingen, Switzerland), was used for the analysis. A Cogent Bidentate C18 column (50 mm × 2.1 mm ID × 4 μm particles; Microsolv Technology Corporation, Eatontown, NJ, USA) was used for the separation of target analytes. The gradient and flow of the mobile phase and the basic set-up of the HESI-II ionisation interface are described in the Supplementary materials (Tables S3 and S4, respectively). The mass spectrometer was operated in high resolution product scan

(HRPS) mode to selectively detect the target compounds. The mass width at the isolation quadrupole was set as 0.7 mu. The orbital trap was operated at a resolution of 17,500 FWHM.

Isotope dilution or internal standard method was used in combination with matrix matching standard approach i.e. standard at enough high concentration level was prepared in sample extract.

2.7. Analytical method validation

The method performance for POCIS was already published (Fedorova et al., 2013). Our analytical method for fish tissues was validated regarding its linearity, repeatability, limit of quantification (LOQ), and recovery. The method was linear over the range of 1 to 500 ng g^{-1} (R^2 = 0.999). Method repeatability was tested for ten replicates; the relative standard deviation (RSD) of replicates was 9%. The recovery of target compounds from fish tissue was studied by spiking "clean" fish samples with a mixture of the target compounds before the extraction procedure. The average recovery of target compounds ranged from 94 to 121% (recovery was tested for 4 concentration levels, 5 replicates for each level). Instrumental LOQ was derived from the calibration curve. Peak area corresponding to this LOQ was used for calculation of LOQs in individual samples with using S/N ratio > 10 as auxiliary parameter in some (mainly liver extract) samples. Corresponding values reflect differences among IS recovery, weight of the samples and final volumes of the extract. LOQs for target compounds in individual samples ranged from 0.27 to 5.4 ng g^{-1} in muscle and from 1.2 to 15.0 ng g^{-1} in liver tissues. All method performance parameters are presented in the Supplementary Materials (Table S5). As internal QA/QC we processed fortified samples with each series of samples. Recoveries of target analytes based on fortified samples were in range from 97% to 123% with exception of PFHxS (152%). Blank samples were prepared during extraction process for both fish tissues and POCIS analyses according to its extraction method. Concentrations of target analytes above the LOQ were not found in blank samples.

2.8. Statistical analysis

A correlation between concentrations of PFAS found in muscle/liver tissues and those found in POCIS was evaluated using Statistica 10 software (StatSoft Inc., USA). As the data did not show a normal distribution, Spearman's correlation was used to quantify the strength of this relation.

3. Results and discussion

The concentrations of target pollutants measured in a set of 72 muscle/liver samples collected in 2012 at 6 sampling sites are presented in Table 2. Similar to other results from the Czech Republic and other geographic regions (Ye et al., 2008b; Rudel et al., 2011; Hradkova et al., 2012; Wang et al., 2012), PFOS was the

Table 2
Concentration of target compounds in samples of fish tissue.

ng g^{-1} (w.w.)	PFPeA		PFHxA		PFHpA		PFHxS		PFOA		PFNA		PFOS		Total analysed PFASs	
	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver
Sampling site Nespeky																
No. of samples	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Mean	nd	56.0	nd	53.3	nd	14.0	nd	nd	nd	nd	nd	2.3	2.9	10.8	2.9	131.8
SD	nd	27.1	nd	22.3	nd	5.5	nd	nd	nd	nd	nd	1.0	1.5	4.0	1.5	51.6
Minimum	< LOQ	18.0	< LOQ	25.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1.0	1.0	7.1	1.0	61.1
Maximum	< LOQ	96.0	< LOQ	91.0	< LOQ	23.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	3.8	5.8	18.0	5.8	206.4
Sampling site Valy																
No. of samples	6	3	6	3	6	3	6	3	6	3	6	3	6	3	6	3
Mean	nd	60.3	nd	54.0	nd	nd	nd	nd	nd	nd	nd	2.8	8.8	64.0	8.8	185.5
SD	nd	30.5	nd	26.5	nd	nd	nd	nd	nd	nd	nd	1.4	1.7	22.6	1.7	86.8
Minimum	< LOQ	30.0	< LOQ	30.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1.5	6.3	47.0	6.3	108.5
Maximum	< LOQ	102.0	< LOQ	91.0	< LOQ	13.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	4.8	11.0	96.0	11.0	306.8
Sampling site Dluhonice																
No. of samples	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Mean	nd	65.3	nd	39.7	nd	nd	nd	nd	nd	nd	nd	1.7	27.3	221.5	27.3	334.0
SD	nd	46.9	nd	19.4	nd	nd	nd	nd	nd	nd	nd	0.6	3.7	86.0	3.7	58.7
Minimum	< LOQ	27.0	< LOQ	21.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1.0	22.0	122.0	22.0	230.5
Maximum	< LOQ	161.0	< LOQ	73.0	< LOQ	24.0	< LOQ	< LOQ	< LOQ	11.0	< LOQ	3.0	33.0	381.0	33.0	430.0
Sampling site Predmerice																
No. of samples	6	5	6	5	6	5	6	5	6	5	6	5	6	5	6	5
Mean	nd	81.0	nd	66.8	nd	27.2	nd	nd	nd	nd	nd	2.3	17.1	185.6	17.1	346.5
SD	nd	34.5	nd	38.7	nd	20.9	nd	nd	nd	nd	nd	0.8	6.1	78.4	6.1	130.7
Minimum	< LOQ	26.0	< LOQ	25.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1.6	7.5	86.0	7.5	191.6
Maximum	< LOQ	123.0	< LOQ	138.0	< LOQ	48.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	3.9	26.0	294.0	26.0	531.9
Sampling site Usti nad Labem																
No. of samples	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Mean	nd	23.0	nd	22.2	nd	5.6	nd	nd	nd	nd	nd	2.0	38.3	466.8	38.3	518.7
SD	nd	5.7	nd	3.9	nd	2.0	nd	nd	nd	nd	nd	1.3	6.1	178.1	6.1	172.2
Minimum	< LOQ	16.0	< LOQ	17.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.7	31.0	319.0	31.0	374.7
Maximum	< LOQ	33.0	< LOQ	29.0	< LOQ	8.4	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	4.6	49.0	804.0	49.0	849.5
Sampling site Topelec																
No. of samples	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Mean	nd	55.0	nd	21.5	nd	nd	nd	nd	nd	nd	nd	1.2	3.2	18.4	3.2	96.7
SD	nd	34.0	nd	5.6	nd	nd	nd	nd	nd	nd	nd	0.5	1.0	7.9	1.0	35.4
Minimum	< LOQ	13.0	< LOQ	13.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.8	2.2	9.2	2.2	64.4
Maximum	< LOQ	123.0	< LOQ	29.0	< LOQ	3.6	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	2.4	5.3	33.0	5.3	169.8

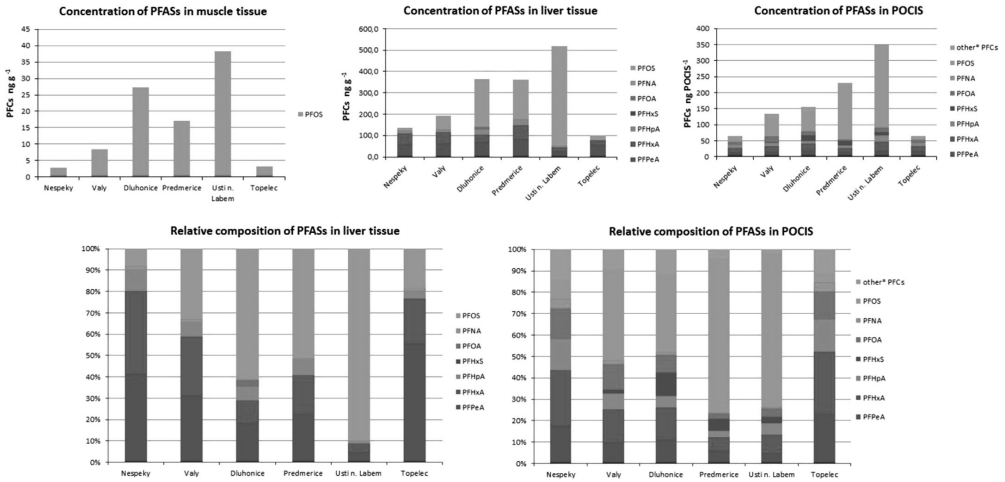

Fig. 2. Concentrations and relative composition of target PFASs in muscle and liver tissue of fish and in POCIS.* 16 PFASs were analysed in POCIS (Supplementary material, Table S2).

prevailing pollutant from a group of monitored PFASs in the aquatic environment.

3.1. Fish muscle tissue

In muscle tissue samples, only PFOS was found above the LOQ. On the other hand, this compound was found in all samples. The arithmetic mean ($n=6$) of PFOS concentrations varied from 2.9 to 38.3 ng g^{-1} w.w., depending on the sampling site. Concentrations exceeding the EQS for aquatic biota were found in 52% of the individual samples. The highest mean and individual (49 ng g^{-1} w.w.) concentrations were found at Usti nad Labem (Bilina River), where the mean concentration of PFOS was more than nine-fold higher than the EQS. The Bílina River is relatively small and, due to low dilution of contaminated inputs, a highly polluted water course, which flows through an area of intensive surface coal mining, heavy chemical industry, and oil refineries.

Few studies focused on monitoring of PFASs in fish from Czech rivers. Unfortunately, some of these works were focused on fish species with different feeding strategies caught at different sampling sites, and the number of samples taken was insufficient to obtain reliable results. Hradkova et al. (2012) found mean concentrations of PFOS that ranged from < LOD to 193 ng g^{-1} w.w. in the muscle tissue of different fish species. The highest mean concentrations in their work were found in the Elbe River, at localities Obristvi (62 ng g^{-1} w.w., $n=3$) and Usti nad Labem (193 ng g^{-1} w.w., $n=1$), in the muscle tissue of bream (*Abramis brama*) and roach (*Rutilus rutilus*), respectively. High concentrations of PFOS were reported by (Hlouskova et al., 2013) from the Bilina River, where the mean concentration of 752 ng g^{-1} w.w. ($n=3$) was detected in the muscle tissue of fish (undefined species). Agreement with our data was found in recent publication, where concentration of PFOS analysed in chub muscle from nine different sampling sites along Elbe and Vltava Rivers ranged between 0.722 and 36.1 ng g^{-1} w.w. (Svihlikova et al., 2015). In any case, PFOS seems to be a relevant environmental pollutant in the Czech aquatic environment.

Similar investigations were made in France at several sampling sites along the Rhone River. Samples of muscle tissue with the skin of several fish species were analysed and the concentrations of PFOS ranged from 15.7 to 308.9 ng g^{-1} d.w. (Miege et al., 2012). The occurrence of PFASs in fish from Lake Vättern (Sweden) was reported by (Berger et al., 2009), who observed median concentrations of PFOS in the muscle tissue of different fish species from this locality that varied between 2.86 ng g^{-1} w.w. (*Coregonus lavaretus*) and 12 ng g^{-1} w.w. (*Lota lota*). (Ye et al., 2008b) examined the Mississippi River and analysed the muscle tissue of carp (*Cyprinus carpio, n 30*) at three different sampling sites; concentrations of PFOS ranged from 4.3 to 90 ng g^{-1} w.w. As occurred in our study, PFOS was the dominant compound at each of the three monitored localities of the Mississippi River, comprising 77% to 89% of the total amount of analysed PFASs in muscle tissue. Lower concentrations of Perfluoroalkyl acids (PFAAs) than in freshwater ecosystems are reported in fish from Baltic Sea (Koponen et al., 2015). Concentrations of PFOS in muscle samples of various fish species caught in the open sea sampling sites varied from 0.31 to 7.5 ng g^{-1} w.w. Similar concentrations (from < LOD to 5.66 ng g^{-1} w.w.) were found in fish from 9 sampling sites within Aegean Sea (Greece, Turkey). Ten sites were investigated in total during this study and different fish species was caught at each site. Different from all others was Picarel (*Spicara smaris*) as 20.4 ng g^{-1} w.w. of PFOS was analysed in its muscle (Vassiliadou et al., 2015). Because of the design of experiment it is impossible to say, if this difference is species or site specific.

3.2. Fish liver tissue

In contrast to samples of muscle tissue, concentrations of four analytes, PFOS, PFNA, PFPeA, and PFHxA, were above the LOQ in 100% of individual liver samples (Table 2). PFHpA was above the LOQ in 44% of individual liver samples. Concentrations of PFHxS and PFOA were below their LOQs in all individual liver samples with only one exception in case of PFOA at locality Dluhonice. PFASs pattern and higher concentrations of target analytes correspond with other reports that analysed PFASs in the liver samples of fish, and this tissue is recognised as a main organ of

bioaccumulation of these extraneous substances in the fish body (Giesy and Kannan, 2001; Prevedouros et al., 2006; Rudel et al., 2011).

PFOS was dominant in liver samples from sampling sites Valy, Dluhonice, Predmerice, and Usti nad Labem, where it reached 35%, 66%, 55%, and 90%, respectively, of total PFAS concentrations (Fig. 2).

In sampling sites Nespeky and Topelec, the contribution of PFOS to the total PFAS concentration was only 8% and 19%, respectively. PFPeA was the dominant compound from a group of analysed PFASs in these two localities, with contributions of 43% and 57%, respectively. Locality Topelec lies on the Otava River downstream the city of Pisek (population of 30,000), where several electronic and textile industry producers are located, while locality Nespeky lies on the Sazava River in a rural area, where no sources of pollution by PFASs are known.

The Bilina River (sampling site Usti nad Labem) was the most contaminated among the monitored localities. As it was mentioned before, the Bilina River is one of the most polluted water courses in the Czech Republic, in general. A total PFAS concentration of 519 ng g^{-1} w.w. was registered there (arithmetic mean, $n=6$), 90% of which was PFOS. Several authors, focusing on the occurrence and fate of PFASs, mentioned that PFOS and PFOA can be formed as breakdown products of other PFASs (Takagi et al., 2008; Kaserzon et al., 2012; Rahman et al., 2014). Based on these findings, we assume that there are many local sources of pollution in the catchment area of the Bilina River. As the sampling site is located near the confluence with the Elbe River, it is possible that some of these parental PFASs are transformed into PFOS, leading to its great contribution on total PFAS concentration there. On the other hand, PFOA, which also can be formed by breakdown of certain precursor compounds, was not found in fish tissues.

Rudel et al. (2011) analysed the liver samples of bream that originated from several water courses (Elbe, Rhine, and Saar Rivers) and one reference background site (Lake Belau) in Germany. These samples were retrieved from the German ESB archive and correspond to the years 2007 and 2008. The concentrations of PFOS in liver samples of bream from the rivers ranged from 130 to 257 ng g^{-1} w.w.; it was only 6.4 ng g^{-1} w.w at the reference site. In our study, concentrations of PFOS in samples of chub liver ranged from 10.8 to 466 ng g^{-1} w.w. (arithmetic mean, $n=6$). This observation fits surprisingly well to Rudel's finding, as the lowest PFOS level Czech site is considered a “clean” area and high ones correspond to similarly industrialised areas in Germany.

3.3. POCIS

Unlike fish tissues, seven target analytes were found in POCIS at levels above the LOQ at all monitored sites, with two exceptions concerning PFHxS at sampling sites Topelec and Nespeky (Table 3). At most of the monitored sites, PFOS was the dominant compound among the analysed PFASs, with concentrations from 2.6 to 250 ng^{-1} per POCIS. Two exceptions were found at Topelec and Nespeky again, where PFOS reached only 5% and 10%, respectively, of the total PFAS concentrations. PFPeA and PFHxA were the predominant compounds in POCIS extracts from these sampling sites. The results from POCIS analyses correspond to those from fish liver, where the Topelec and Nespeky sites also significantly differed from others. Besides the seven target analytes, another nine were analysed in POCIS. The concentrations of these nine compounds varied from $<$ LOQ to 8.3 ng^{-1} per POCIS and they were only a minor component of PFAS pollution; see Supplemetary material (Table S2).

3.4. Comparison of studied approaches

The total PFAS concentrations found in the POCIS strongly correlate with those obtained from the liver tissue of fish ($r=0.94$, $p<0.05$). From target analytes, only PFOS was found in muscle tissue of fish and its concentrations also positively correlated between fish and POCIS ($r=0.84$, $p<0.05$).

The comparison of data obtained from analysis of different fish tissues and from POCIS at all monitored sites are presented in Fig. 2. From the relative composition of target compounds in liver tissue and POCIS, it is obvious that we can obtain quite different pictures about PFAS contamination patterns. This fact is supported by the lack of significant correlation between concentrations of individual PFASs found in liver tissue and in POCIS at sampling sites. These differences could be caused by fish metabolic activity, which can transform some of the PFASs into others (Takagi et al., 2008; Rahman et al., 2014) or to fast excretion of some compounds.

Concerning adult fish data, its reliability could be affected by fish size and age too. Different view on real PFASs cocktail could be also made because of metabolic activity of live fish. Besides its relevance and ecological/ethical consequences, the economy and time costs of these two approaches differ significantly.

As the fish liver was recognised as a relevant tissue for PFAS monitoring (Giesy et al., 2001; Naile et al., 2013), passive sampling seems to be an even better alternative to live animals for monitoring these compounds in aquatic environments. This approach avoids the sacrifice of aquatic biota (mostly fish), so it fulfils the Three Rs (Replacement, Reduction, and Refinement). These principles are also included in the EU Directive on the protection of animals used for scientific purposes (European Parliament and Council, 2010).

Table 3
Concentration of target compounds (ng $POCIS^{-1}$) in passive samplers at monitored sampling sites.

Sampling site	t (°C)[a]	PFPeA ng $POCIS^{-1}$	PFHxA ng $POCIS^{-1}$	PFHpA ng $POCIS^{-1}$	PFHxS ng $POCIS^{-1}$	PFOA ng $POCIS^{-1}$	PFNA ng $POCIS^{-1}$	PFOS ng $POCIS^{-1}$	other PFASs[b] ng $POCIS^{-1}$	Total PFASs ng $POCIS^{-1}$
Nespeky	17	11	17	9.1	0	9.1	3	5.3	9.5	64
Valy	19	13	21	9.9	2.1	16	2.9	56	13.1	134
Dluhonice	19	17	24	8.3	17	13	2	56	18.7	156
Predmerice	16	13	15	7.3	13	5.4	1.3	165	10	230
Usti n. Labem	17	16	31	19	10	14	2.7	250	7.3	350
Topelec	[c]	15	19	9.7	0	8.4	2.9	2.6	7.4	65

[a] Mean water temperature counted from daily measured values.
[b] Sixteen different PFASs were analysed in POCIS, for more information see Supplementary material (Table S2).
[c] No data because of technical problem.

4. Conclusion

As other authors have indicated, there is a high variability in the levels of PFAS contamination of aquatic environments. The level of contamination is affected by the occurrence of large urban centres and various types of industrial production. Fish livers were better indicators of PFAS pollution than muscle because five of the seven target compounds were found above the LOQ in liver, while only PFOS was found above the LOQ in muscle.

Passive sampling (POCIS) seems to be a more effective approach for monitoring of PFASs in aquatic environments than analyses of fish. Although the total concentrations of PFASs found in POCIS strongly correlated to those found in the liver tissue of fish, the composition of the individually measured substances and their ratios in POCIS and fish liver were different. This difference is probably caused by metabolic transformation of some PFASs in fish or their excretion. There are also other reasons to eliminate the use of fish for this type of monitoring, such as expenses and ecological and ethical aspects. On the other hand, the muscle tissue of fish remains an important indicator for risk assessment of consumers.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2015.11.010.

References

Alvarez, D.A., Stackelberg, P.E., Petty, J.D., Huckins, J.N., Furlong, E.T., Zaugg, S.D., Meyer, M.T., 2005. Comparison of a novel passive sampler to standard water-column sampling for organic contaminants associated with wastewater effluents entering a New Jersey stream. Chemosphere 61, 610–622.

Berger, U., Glynn, A., Holmstrom, K.E., Berglund, M., Ankarberg, E.H., Tornkvist, A., 2009. Fish consumption as a source of human exposure to perfluorinated alkyl substances in Sweden-Analysis of edible fish from Lake Vattern and the Baltic Sea. Chemosphere 76, 799–804.

European Food Safety Authority, 2008. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. Scientific Opinion of the Panel on Contaminants in the Food chain. EFSA J. 653, 1–131.

European Parliament and Council, 2010. Directive 2010/63/EU of 22 September 2010 on the protection of animals used for scientific purposes. Off. J. Eur. Union 276, 33–79.

European Parliament and Council, 2013. Directive 2013/39/EU of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Off. J. Eur. Union 226, 1–17.

Fedorova, G., Golovko, O., Randak, T., Grabic, R., 2013. Passive sampling of perfluorinated acids and sulfonates using polar organic chemical integrative samplers. Environ. Sci. Pollut. Res. 20, 1344–1351.

Fedorova, G., Nebesky, V., Randak, T., Grabic, R., 2014. Simultaneous determination of 32 antibiotics in aquaculture products using LC-MS/MS. Chem. Pap. 68, 29–36.

Gebbink, W.A., Berger, U., Cousins, I.T., 2015. Estimating human exposure to PFOS isomers and PFCA homologues: The relative importance of direct and indirect (precursor) exposure. Env. Int. 74, 160–169.

Giesy, J.P., Kannan, K., 2001. Global distribution of perfluorooctane sulfonate in wildlife. Environ. Sci. Technol. 35, 1339–1342.

Giesy, J.P., Kannan, K., Jones, P.D., 2001. Global biomonitoring of perfluorinated organics. Sci. World J. 1, 627–629.

Hlouskova, V., Lankova, D., Kalachova, K., Hradkova, P., Poustka, J., Hajslova, J., Pulkrabova, J., 2013. Occurrence of brominated flame retardants and perfluoroalkyl substances in fish from the Czech aquatic ecosystem. Sci. Total Environ. 461, 88–98.

Houde, M., Douville, M., Despatie, S.P., De Silva, A.O., Spencer, C., 2013. Induction of gene responses in St. Lawrence River northern pike (Esox lucius) environmentally exposed to perfluorinated compounds. Chemosphere 92, 1195–1200.

Hradkova, P., Pulkrabova, J., Kalachova, K., Hlouskova, V., Tomaniova, M., Poustka, J., Hajslova, J., 2012. Occurrence of halogenated contaminants in fish from selected river localities and ponds in the Czech Republic. Arch. Environ. Contam. Toxicol. 62, 85–96.

Johansson, N., Eriksson, P., Viberg, H., 2009. Neonatal exposure to PFOS and PFOA in mice results in changes in proteins which are important for neuronal growth and synaptogenesis in the developing brain. Toxicol. Sci. 108, 412–418.

Kaserzon, S.L., Kennedy, K., Hawker, D.W., Thompson, J., Carter, S., Roach, A.C., Booij, K., Mueller, J.F., 2012. Development and calibration of a passive sampler for perfluorinated alkyl carboxylates and sulfonates in water. Environ. Sci. Technol. 46, 4985–4993.

Koponen, J., Airaksinen, R., Hallikainen, A., Vuorinen, P.J., Mannio, J., Kiviranta, H., 2015. Perfluoroalkyl acids in various edible Baltic, freshwater, and farmed fish in Finland. Chemosphere 129, 186–191.

Lindstrom, A.B., Strynar, M.J., Libelo, E.L., 2011. Polyfluorinated compounds: past, present, and future. Environ. Sci. Technol. 45, 7954–7961.

Miege, C., Peretti, A., Labadie, P., Budzinski, H., Le Bizec, B., Vorkamp, K., Tronczynski, J., Persat, H., Coquery, M., Babut, M., 2012. Occurrence of priority and emerging organic compounds in fishes from the Rhone River (France). Anal. BioAnal. Chem. 404, 2721–2735.

Murakami, M., Adachi, N., Saha, M., Morita, C., Takada, H., 2011. Levels, temporal trends, and tissue distribution of perfluorinated surfactants in freshwater fish from Asian countries. Arch. Env. Contam. Toxicol. 61, 631–641.

Naile, J.E., Khim, J.S., Hong, S., Park, J., Kwon, B.O., Ryu, J.S., Hwang, J.H., Jones, P.D., Giesy, J.P., 2013. Distributions and bioconcentration characteristics of perfluorinated compounds in environmental samples collected from the west coast of Korea. Chemosphere 90, 387–394.

Prevedouros, K., Cousins, I.T., Buck, R.C., Korzeniowski, S.H., 2006. Sources, fate and transport of perfluorocarboxylates. Environ. Sci. Technol. 40, 32–44.

Rahman, M.F., Peldszus, S., Anderson, W.B., 2014. Behaviour and fate of perfluoroalkyl and polyfluoroalkyl substances (PFASs) in drinking water treatment: a review. Water Res. 50, 318–340.

Rudel, H., Muller, J., Jurling, H., Bartel-Steinbach, M., Koschorreck, J., 2011. Survey of patterns, levels, and trends of perfluorinated compounds in aquatic organisms and bird eggs from representative German ecosystems. Environ. Sci. Pollut. Res. 18, 1457–1470.

Sanderson, H., Boudreau, T.M., Mabury, S.A., Solomon, K.R., 2004. Effects of perfluorooctane sulfonate and perfluorooctanoic acid on the zooplanktonic community. Ecotoxicol. Environ. Saf. 58, 68–76.

Svihlikova, V., Lankova, D., Poustka, J., Tomaniova, M., Hajslova, J., Pulkrabova, J., 2015. Perfluoroalkyl substances (PFASs) and other halogenated compounds in fish from the upper Labe River basin. Chemosphere 129, 170–178.

Takagi, S., Adachi, F., Miyano, K., Koizumi, Y., Tanaka, H., Mimura, M., Watanabe, I., Tanabe, S., Kannan, K., 2008. Perfluorooctanesulfonate and perfluorooctanoate in raw and treated tap water from Osaka, Japan. Chemosphere 72, 1409–1412.

Valsecchi, S., Rusconi, M., Polesello, S., 2013. Determination of perfluorinated compounds in aquatic organisms: a review. Anal. BioAnal. Chem. 405, 143–157.

Vassiliadou, I., Costopoulou, D., Kalogeropoulos, N., Karavoltsos, S., Sakellari, A., Zafeiraki, E., Dassenakis, M., Leondiadis, L., 2015. Levels of perfluorinated compounds in raw and cooked Mediterranean finfish and shellfish. Chemosphere 127, 117–126.

Wang, T.Y., Lu, Y.L., Chen, C.L., Naile, J.E., Khim, J.S., Giesy, J.P., 2012. Perfluorinated compounds in a coastal industrial area of Tianjin, China. Environ. Geochem. Health 34, 301–311.

Ye, X.B., Schoenfuss, H.L., Jahns, N.D., Delinsky, A.D., Strynar, M.J., Varns, J., Nakayama, S.F., Helfant, L., Lindstrom, A.B., 2008a. Perfluorinated compounds in common carp (Cyprinus carpio) fillets from the Upper Mississippi River. Environ. Int. 34, 932–938.

Ye, X.B., Strynar, M.J., Nakayama, S.F., Varns, J., Helfant, L., Lazorchak, J., Lindstrom, A.B., 2008b. Perfluorinated compounds in whole fish homogenates from the Ohio, Missouri, and Upper Mississippi Rivers, USA. Environ. Pollut. 156, 1227–1232.

Yeung, L.W.Y., Yamashita, N., Taniyasu, S., Lam, P.K.S., Sinha, R.K., Borole, D.V., Kannan, K., 2009. A survey of perfluorinated compounds in surface water and biota including dolphins from the Ganges River and in other waterbodies in India. Chemosphere 76, 55–62.

Zareitalabad, P., Siemens, J., Hamer, M., Amelung, W., 2013. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in surface waters, sediments, soils and wastewater-A review on concentrations and distribution coefficients. Chemosphere 91, 725–732.

Zhang, S., Lerner, D.N., 2012. Review of physical and chemical properties of perfluorooctanyl sulphonate (PFOS) with respect to its potential contamination on the environment. Adv. Mater. Res. Switz. 518–523, 2183–2191.

Zhao, Y.G., Wong, C.K.C., Wong, M.H., 2012. Environmental contamination, human exposure and body loadings of perfluorooctane sulfonate (PFOS), focusing on Asian countries. Chemosphere 89, 355–368.

Zheng, X.M., Liu, H.L., Shi, W., Wei, S., Giesy, J.P., Yu, H.X., 2012. Effects of perfluorinated compounds on development of zebrafish embryos. Environ. Sci. Pollut. Res. 19, 2498–2505.

SUPPLEMENT MATERIALS

Table S1. *Characteristics of sampling sites.*

Sampling site	GPS	Water course	Mean flow rate (m^3/sec.)	Catchment area (km^2)
Nespeky	N 49° 51.37´; E 14° 39.07´	Sazava River	23,4	4038,25
Valy	N 50° 03.31´; E 15° 61.64´	Labe River	56,4	6397,79
Dluhonice	N 49° 26.59´; E 17° 24.36´	Becva River	17,3	1592,69
Predmerice	N 50° 14.57´; E 14° 46.38´	Jizera River	24,3	2158.71
Usti nad Labem	N 50° 65.73´; E 14° 04.02´	Bilina River	6,5	1070
Topelec	N 49° 34.30´; E 14° 15.18´	Otava River	23,4	2948,85

Table S2. *Concentrations of all analysed PFCs in POCIS from monitored sites (ng $POCIS^{-1}$).*

	Dluhonice	Valy	Predmerice	Usti n. Labem	Topelec	Nespeky
PFBA	7.6	8.3	5.3	5.4	7.2	5.8
PFBS	6.1	<0.95	<0.90	<0.87	<0.93	1.8
PFDA	1.9	4.1	0.4	1.6	<0.48	1.4
PFDoA	<0.42	<0.41	0.7	<0.46	<0.48	<0.48
PFDS	<2.1	<2.1	<1.9	<2.3	<2.4	<2.5
PFHpA	8.3	9.9	7.3	19	9.7	9.1
PFHpS	2.7	<1.3	4.1	<1.3	<1.4	<1.2
PFHxA	24	21	15	31	19	17
PFHxS	17	2.1	13	10	<1.9	<1.6
PFNA	2	2.9	1.3	2.7	2.9	3
PFOA	13	16	5.4	14	8.4	9.1
PFOS	56	56	165	250	2.6	5.3
PFPeA	17	13	13	16	15	11
PFTeDA	<0.39	<0.38	<0.35	<0.43	<0.45	<0.45
PFTrDA	<0.35	<0.34	<0.31	<0.38	0.5	<0.4
PFUdA	<0.5	0.6	<0.45	0.6	<0.56	0.7

Table S3. *LC gradient for the separation of target compounds.*

Time, min	A, %	B, %	Flow, µL min^{-1}
0.00	100	0	300
1.00	100	0	300
7.00	60	40	350
10.00	0	100	400
12.00	0	100	400
12.01	100	0	300
15.00	100	0	300

A water + 0.1% FA. B Acetonitrile + 0.1% FA.

Table S4. *Parameters for HESI ion source.*

Parameter	Value
Capilary temperature (°C)	325
Vaporiser temperature (°C)	300
Auxillary gas pressure (a.u.)	15
Sheath gas (nitrogen) pressure (a.u.)	30
Spray voltage (V)	2700

Table S5. *Method performance parameters for fish tissue analysis.*

Compound	Linearity[a]	Average LOQ[b] [ng g^{-1}]		Average recovery[c] [%]							
		(muscle)	(liver)	1 ng.g^{-1}	RSD [%]	5 ng.g^{-1}	RSD [%]	25 ng.g^{-1}	RSD [%]	100 ng.g^{-1}	RSD [%]
PFPeA	1	2.8	4.4	109	4	107	1	96	1	119	5
PFHxA	0.9999	0.43	9.8	115	1	121	1	101	2	117	7
PFHpA	1	3.2	1.7	92	8	98	3	103	1	123	5
PFHxS	0.9997	0.7	1.1	130	11	94	7	95	3	114	13
PFOA	0.9998	3.2	2.8	86	8	104	4	105	7	119	4
PFNA	0.9997	0.22	0.50	130	10	102	9	89	9	104	3
PFOS	0.9998	0.24	0.33	100	5	110	5	102	3	113	5

[a] R-squared value for the calibration curve in the range 1–100 ng g^{-1}
[b] Average of all measured samples
[c] 5 replicates were tested for each concentration level

CHAPTER 4

YOUNG-OF-THE-YEAR FISH AS A PROSPECTIVE BIOINDICATOR FOR AQUATIC ENVIRONMENTAL CONTAMINATION MONITORING

Cerveny, D., Turek, J., Grabic, R., Golovko, O., Koba, O., Fedorova, G., Grabicova, K., Zlabek, V., Randak T., 2016. Young-of-the-year fish as a prospective bioindicator for aquatic environmental contamination monitoring. Water Research 103: 334–342.

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Young-of-the-year fish as a prospective bioindicator for aquatic environmental contamination monitoring

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ABSTRACT

Toxic metals (Hg, Cd, Pb) and fifteen perfluoroalkyl substances (PFASs) were determined in different fish samples at two locations on the Elbe River in the Czech Republic. The muscle tissue of the two adult fish species most commonly used as bioindicators in central Europe and whole body homogenates of various species of young-of-the-year (YOY) fish were used. The purpose of this study was to evaluate the potential to replace adult fish muscle tissue with YOY fish for contamination monitoring. All of the toxic metals and five of the fifteen PFASs were found in the YOY fish samples while only mercury and PFOS were detected in the muscle tissue of adults. The concentration of total mercury (THg) in the YOY fish homogenates ranged between 0.014 and 0.062 μg g^{-1}. Of the spectrum of analysed pollutants, only the THg concentrations were lower in YOY fish homogenates than in adult muscle tissue. The cadmium concentration varied from 0.004 to 0.024 μg g^{-1} and the lead concentration varied from 0.032 to 0.396 μg g^{-1} in YOY fish homogenates, while in most of the adult samples, Cd and Pb were below the detection limit of the analytical methods employed. The PFOS concentrations in YOY fish homogenates were comparable to the concentrations frequently found in adult liver tissue. These results show that mixed shoals of YOY fish can be successfully used for aquatic bio-monitoring. Interspecific variability in the concentrations of the target pollutants in YOY fish whole body homogenates is usually lower than the intraspecific variability of the concentrations of the pollutants in adult fish muscle. YOY fish were found to be a suitable bioindicator and have several advantages compared to adult fish.

1. Introduction

Human activity impacts quality of life both positively and negatively. Environmental pollution is one of the most negative side effects of the development of civilization. Worldwide, authorities monitor the level of environmental contamination to evaluate its negative effects on wild biota and to protect humans from health risks related to damaged living space.

The aquatic environment is probably the most endangered portion of the Earth's biosphere because it is the final destination of most of the pollution produced by humans (Abel, 1996). This is why the contamination level of various pollutants is scrutinized all over the world in marine (Fujii et al., 2015; Net et al., 2015; Pan and Wang, 2012; Wu et al., 2012) and freshwater ecosystems (Djedjibegovic et al., 2012; Murakami et al., 2011; Noel et al., 2013; Stahl et al., 2014; Svihlikova et al., 2015). A commonly used tool for describing the quality of the aquatic environment is biomonitoring, which makes use of various aquatic biota, such as plants, planktonic/benthic communities of invertebrates and fish. There are several reasons for the use of organisms for evaluating of environmental contamination. The sensitivity of the detection (or quantification) of those pollutants that have the potential to bioaccumulate (Grabicova et al., 2015; Maceda-Veiga et al., 2012; Net et al., 2015) or to become biomagnified (Campbell et al., 2008; Kelly et al., 2009; Misztal-Szkudlinska et al., 2011; Ouedraogo et al., 2015) in the trophic chain is much higher than the sensitivity of their detection in water. In addition, it reflects a much longer time frame than conventional sampling or 24-h composite samples. Another reason for using organisms for monitoring is the potential to perform a human risk assessment. The biomagnification of certain contaminants in the higher levels of the aquatic food web, such as

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fish, can affect the health of consumers of popular and commonly used foodstuffs (Koponen et al., 2015; Marti-Cid et al., 2008; Orban et al., 2007).

Most scientific research and monitoring programs are based on the sampling of adult fish. This approach is not substitutable for evaluating health risks for consumers because adult fish constitute a typical source of fish-based foodstuffs, but it has several limitations when used to evaluate the level and trends of contamination at specific sites. Different pollutants accumulate in different tissues in the body of a fish (Boalt et al., 2014; Cinier et al., 1999; Goeritz et al., 2013; Grabicova et al., 2014), and it is very expensive and practically impossible to sample all fish organs or tissues to obtain reliable data from the chemical analysis of such samples. Because the level of contamination in the body of fish is species and age-specific (Cerveny et al., 2014; Miege et al., 2012; Noel et al., 2013), it is difficult to compare results from different localities within a single study or among different studies. Additionally, the migration of adult fish plays a role in the accuracy of the results because some species have long migration routes during certain periods of their lives (Kuliskova et al., 2009; Prchalova et al., 2011; Slavik et al., 2009), so bodily contamination does not necessarily correspond to the location where a fish was caught. Ecological and ethical consequences exist as well. Annual sampling of adults (broodstocks) of an indicator fish species in sufficient numbers for analysis could affect its natural reproduction at a regularly monitored locations, especially in small streams.

The purpose of this study was to evaluate the potential for the use of young-of-the-year (YOY) fish (especially *Cyprinids* that spawn in the spring) for the biomonitoring of extraneous substances in the aquatic environment. To the best of our knowledge, no evidence concerning this approach exists. Whole body homogenates of YOY fish composed of several species were used for the chemical analyses of certain toxic metals and perfluoroalkyl substances (PFAS). Together with whole body homogenates of YOY fish, the muscle tissue of adult bream (*Abramis brama*) and chub (*Squalius cephalus*) (the principal piscine bioindicators in the Czech Republic) caught at the same sampling sites were investigated. The specific aims of the study were: (i) to investigate the variability in the concentrations of target pollutants among different species of shoals of YOY fish at the localities and (ii) to compare the concentrations of these pollutants and their variability in whole body homogenates of YOY fish to the muscle tissue of adults caught at the same locality. Our hypotheses were that (i) the variability of the concentration of the target pollutants among different species of YOY fish (interspecific variability) is lower than that among individuals within a single bioindicator species of adult fish (intraspecific variability) caught at the same locality and (ii) the concentrations of most pollutants are higher in the whole body homogenates of YOY than in adult fish muscle.

Because of the high mortality of YOY fish during the first winter season – up to 80% (Johnson and Evans, 1990; Kirjasniemi and Valtonen, 1997; Miranda and Hubbard, 1994), sampling of this age group of fish in late summer would not significantly affect their populations at a given locality.

2. Materials and methods

2.1. Fish sampling

All fish sampled were caught during September 2014 at two sampling sites along Elbe River in the Czech Republic. The Kozly sampling site (N 50°15.17730′, E 14°33.23882′) lies in the upper stretch of the Elbe River close to Prague, and the Decin sampling site (N 50°46.27710′, E 14°12.66213′) is situated in the middle part of the Elbe River close to the border with Germany. Both sites are affected by upstream chemical industrial activity (former old fashion chloralkali plants that have an ongoing production of chlorine for PVC, oil refineries, etc.). These localities were selected because of the expected high biodiversity and abundance of YOY fish as well as already documented POP and toxic metal contamination (Hajšlová et al., 2007; Havelková et al., 2008; Randak et al., 2009). A wide spectrum of fish species combined with a sufficient number of individuals was the key factor for evaluating the interspecific variability in the concentrations of the target contaminants.

YOY fish were caught by electrofishing (back-pack equipment EFKO FEG 1500) close to the river bank. A considerable amount of juveniles is usually found in the shallow parts of the river in late summer or in autumn. Mixed shoals of YOY fish were present, and cyprinid fish species predominated. Older fish were determined by size and immediately removed from the samples. After capture, the YOY fish were immediately sacrificed using carbon dioxide in the water, chilled to 4 °C and transported to the laboratory, where species determination and sample preparation were performed. Together with the YOY fish, ten adult bream and ten adult chub were caught using an electrofishing boat (equipment AGK Kronawitter GmbH EL65II) at the same sampling sites. The adults were measured and weighed, and scales were taken for age determination. Samples of muscle tissue without skin from the mid-dorsal part of the body were taken from the adult fish, chilled to 4 °C and transported to the laboratory. The characteristics of the adult and YOY fish caught at the sampling sites are shown in Tables 1 and 2, respectively.

All experimental animals were handled in accordance with national and institutional guidelines for the protection of human subjects and animal welfare (European Parliament and Council, 2010).

2.2. Preparation of the YOY fish samples

After transport to the laboratory, the YOY fish were divided into two portions. The first was designated as the real pooled sample (RS) and was immediately homogenized in a knife mill GRINDMIX GM 200 (RETSCH, Germany). This homogenate was then divided into subsamples for the analyses of different target pollutants according to the methods described below. Five repetitions of each subsample were analysed.

In the second portion, species were determined according to morphology and meristic traits. The characteristics of individual species were calculated as well as their percentage in the catch

Table 1
Characteristics of adult fish caught at the sampling sites.

Locality	Species	n	Age (years)	Body weight (g)	Total length (mm)
			Mean ± SD	Mean ± SD	Mean ± SD
Kozly	*Squalius cephalus*	10	5.1 ± 2.0	435.6 ± 341.9	308.2 ± 73.1
	Abramis brama	10	6.2 ± 3.0	758.0 ± 593.8	369.5 ± 98.8
Decin	*Squalius cephalus*	10	6.7 ± 1.8	840.4 ± 394.9	412.0 ± 81.7
	Abramis brama	10	7.0 ± 1.2	738.5 ± 445.7	403.0 ± 79.9

Table 2
Characteristics of YOY fish caught at the sampling sites.

Locality	Species	n	Total weight (g)	Mean weight (g)	% of total
Decin	barbel (*Barbus barbus*)	231	371	1.6	23.2
	gudgeon (*Gobio gobio*)	18	49	2.7	3.1
	roach (*Rutilus rutilus*)	69	152	2.2	9.5
	bleak (*Alburnus alburnus*)	54	36	0.7	2.3
	vimba (*Vimba vimba*)	9	49	5.4	3.1
	dace (*Leuciscus leuciscus*)	40	124	3.1	7.8
	stone moroko (*Pseudorasbora parva*)	16	53	3.3	3.3
	chub (*Squalius cephalus*)	461	766	1.7	47.9
Kozly	bitterling (*Rhodeus sericeus*)	64	70	1.1	7.4
	bream (*Abramis brama*)	26	25	1.0	2.6
	gudgeon (*Gobio gobio*)	27	45	1.7	4.8
	roach (*Rutilus rutilus*)	72	185	2.6	19.6
	ide (*Leuciscus idus*)	8	30	3.8	3.2
	dace (*Leuciscus leuciscus*)	17	40	2.4	4.2
	chub (*Squalius cephalus*)	487	550	1.1	58.2

(Fig. 1). After the separation of species, a defined pooled sample (DS) was then prepared using an equivalent weight of each species present in the catch to achieve a species ratio 1:1 for all species. The DS was then homogenized and divided into subsamples for analyses performed in the same fashion as in RS. Five repetitions of the DS were also analysed.

All remaining individuals of each fish species were then divided into five portions, and each portion was homogenized separately to evaluate the variability in the concentration of each target pollutant within each species of YOY fish. The number of individuals in these pooled samples varied according to the abundance of a specific species in the catch. An insufficient number of individuals was caught at the Kozly sampling site for four of seven fish species. Only four samples of YOY bream, gudgeon (*Gobio gobio*), and ide (*Leuciscus idus*) and three samples of YOY dace (*Leuciscus leuciscus*) were prepared for all analyses. All samples of YOY fish together with the adult samples were kept frozen (−18 °C) until analysis.

2.3. Chemical analyses

2.3.1. Toxic metals

The total mercury (THg) content was directly determined in the sample by a selective mercury analyser (Advanced mercury analyser, AMA-254, Altec) based on atomic absorption spectroscopy (AAS). The sample was thermally decomposed in an oxygen flow. The mercury was then captured by a gold amalgamator, and the absorbance of the mercury vapour was measured after its thermal release from the amalgamator. An aliquot of 100–200 mg of YOY fish whole body homogenate or adult muscle tissue was used for analysis. The final concentration of the sample was calculated as the mean of two independent measurements. The detection limit for the THg content was 0.1 ng g^{-1}. Other toxic metals were measured by electrothermal (flameless) atomic absorption spectrometry with Zeeman background correction (graphite furnace atomic absorption spectrometer GF-AAS, SpectrAA 220Z, Varian) after the microwave mineralization of the samples. The detection limits for cadmium and lead were 0.002 μg g^{-1} and 0.02 μg g^{-1}, respectively. To calibrate the instruments, MERCK calibration solutions were used (THg – CertiPUR standard solution, lead and cadmium - Titrisol standard solutions). The certified reference material (BCR-422) consisted of cod muscle powder was used to verify the measurement accuracy in all toxic metal analyses.

2.3.2. Perfluoroalkyl substances

Fifteen PFASs, including perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulfonate (PFHxS) or perfluoropentanoic acid (PFPeA), were determined in YOY fish whole body homogenates and adult muscle tissue.

A modified method (Fedorova et al., 2014) was used to extract the target compounds from fish tissue. Briefly, a fish tissue sample (0.5 g) with internal standards (10 ng $sample^{-1}$) and 1 ml of extraction solvent (acetonitrile acidified with 1% of formic acid) added was homogenized at 1800 oscillations per min for 5 min (TissueLyser II homogenizer, Quiagen, Germany). The extract was then centrifuged at 10 000 × g for 10 min (centrifuge Micro 200R, Hettich Zentrifugen, Germany). The supernatant was filtered through a syringe filter (0.45-μm pore size, regenerated cellulose filter) and frozen overnight to ensure protein precipitation. The final extract was then centrifuged again at 10 000 × g for 10 min and an aliquot was taken and analysed by liquid chromatography with high resolution mass spectrometry (LC-HRMS).

2.3.3. Chemicals and standards

Mixtures of seventeen native (PFAC-MXB) and nine mass-labelled (MPFAC-MXA) perfluorinated acids and perfluoroalkyl sulfonates were purchased from Wellington Laboratories, Inc. (Guelph, ON, Canada). Working mixtures of native compounds and surrogate standards were prepared in methanol at a concentration of 1 μg mL^{-1} and stored at 4 °C. Methanol (LiChrosolv Hypergrade), acetonitrile (LiChrosolv, Hypergradewere), a CertiPUR standard solution and Titrisol standard solutions were purchased from Merck (Darmstadt, Germany). The certified reference material (BCR-422) was obtained from Institute for Reference Materials and Measurements under the European Commission (IRMM, Geel, Belgium). The formic acid used for acidification of mobile phases was purchased from Labicom (Olomouc, Czech Republic). Ultrapure water was obtained by the aqua-MAX-Ultra system (Younglin, Kyounggi-do, Korea).

2.3.4. LC- HRMS analysis

A hybrid quadrupole/orbital trap mass spectrometer QExactive (Thermo Fisher Scientific, San Jose, CA, USA) coupled to an Accela 1250 LC pump (Thermo Fisher Scientific, San Jose, CA, USA) and a HTS XT-CTC autosampler (CTC Analytics AG, Zwingen, Switzerland) was used for the analysis. A Cogent Bidentate C18 column (50 mm × 2.1 mm ID × 4-μm particles; Microsolv Technology Corporation, Eatontown, NJ, USA) was used for the separation of the target analytes. The gradient and flow of the mobile phase and the basic set-up of the HESI-II ionization interface are described in the Supplementary material (Tables S1 and S2, respectively). The mass spectrometer was operated in the high resolution product scan

(HRPS) mode for the selective detection of the target compounds. The mass width at the isolation quadrupole was set to 0.7 μ. The orbital trap was operated at a resolution of 17 500 FWHM.

Isotope dilution or an internal standard method was used in combination with a matrix matching standard approach, i.e., a standard at a sufficiently high concentration was prepared in the sample extract.

2.3.5. Quality control of LC – HRMS analysis

Our analytical method for the fish tissue PFAS analysis was validated for linearity, repeatability, quantification limit (LOQ), and recovery (Cerveny et al., 2016). In this study, an instrumental LOQ was derived from the calibration curve. The peak area corresponding to this LOQ was used to calculate the LOQs in individual samples. The corresponding values reflect the differences among IS recovery, sample weight and final volume of the extract. The mean LOQ for the target compounds ranged from 0.5 to 6.5 ng g^{-1} for YOY fish homogenates and from 1.9 to 7.5 ng g^{-1} in adult muscle tissue. Fortified and blank samples were processed with each sample series as an internal QA/QC.

2.4. Statistical analysis

Statistical evaluation of the data was performed using the Statistica 12 software (StatSoft Inc., USA). The interspecific variability of the concentrations of the target analytes among different YOY fish species was evaluated using one-way analysis of variance (ANOVA) or a Kruskal-Wallis nonparametric test after testing for the normality of the data with a Shapiro–Wilk test. The concentrations of target compounds in five subsamples of each YOY fish species were used to evaluate the interspecific variability at both sampling sites.

The intraspecific variability among individual adult chub and bream was evaluated in the same way. The concentrations of target compounds in ten individuals of both adult bream and chub were used to evaluate the intraspecific variability at both sampling sites.

Comparison of studied approaches was performed using the coefficient of variation. Differences in contamination between two sampling sites were evaluated using one-way ANOVA or nonparametric Kruskal-Wallis test after testing for the normality of the data with a Shapiro–Wilk test.

3. Results and discussion

3.1. Toxic metals

3.1.1. Mercury

Low interspecific variability in THg concentrations was found between different species of YOY fish. The concentration (species specific mean value, n = 5) of THg measured in YOY fish whole body homogenates ranged between 0.031 and 0.049 μg g^{-1} at Kozly and between 0.014 and 0.062 μg g^{-1} at Decin (Supplementary material, Table S3). At Kozly, a significant difference ($p < 0.05$) in the THg concentration between dace and bitterling (*Rhodeus amarus*) was found. At Decin, the THg concentration in vimba (*Vimba vimba*) was significantly different ($p < 0.01$) from all other fish, except for barbell (*Barbus barbus*). These differences could have originated from the slightly different spawning seasons of the sampled YOY fish species. Because the YOY fish were several months old, a few weeks could have played a role in the bioaccumulation of mercury. Small differences in the THg concentration were also found between individuals of the same species. These could be partly due to the multiple spawning strategy of most cyprinid species, in which several cohorts are present in YOY fish shoals. Regardless of this variability in concentration, no significant difference was found between the RS and DS pooled samples at both localities, thus multispecies samples of YOY fish could be successfully used for biomonitoring of mercury in aquatic environment.

High intraspecific variability of THg concentrations occurs in samples of adult fish at both sampling sites. The THg concentrations found in adult fish muscle tissue varied between 0.076 and 0.465 μg g^{-1} depending on the site and the species (Supplementary material, Table S4). Differently from all other contaminants investigated, higher concentrations of THg were found in the adult muscle tissue than in the YOY fish homogenates because of the potential for mercury to primarily bioaccumulate in fish muscle

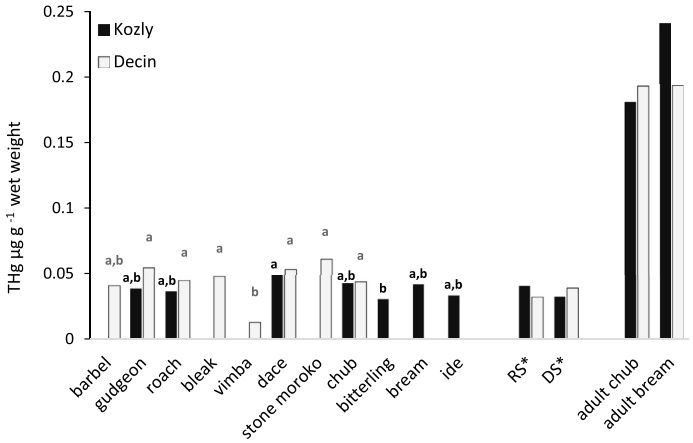

Fig. 1. Mercury concentrations analysed in whole body homogenates of different YOY fish species, multispecies pooled samples and muscle of adult fish. Data sharing the same superscript are not significantly different from one another: "grey" for Decin; "black" for Kozly. *RS, real pooled sample (YOY fish species contribution according to the abundance of each species in mixed shoals at the sampling site); *DS, defined pooled sample (an equal contribution of all YOY fish species).

tissue (Boalt et al., 2014; Kannan et al., 1998; Lasorsa and Allengil, 1995). Concerning THg contamination, evaluation of investigated localities was dependent on fish species which was used as an indicator. Average concentration of THg in chub muscle did not differ between sites, but that found in bream at Kozly was higher. This difference was not significant (ANOVA, $p = 0.26$) due to high intraspecific variability, which was much higher than in YOY fish. High variability in the THg concentrations between individual adults of the same species can primarily be explained by the age of the fish (Burger and Gochfeld, 2007; Verdouw et al., 2011), but also by the migration and feeding behaviour. Some studies focused on sex–related differences in mercury accumulation in fish tissues were published recently. Significant differences was found if analysis was conducted directly in perch (*Perca fluviatilis*) gonadal tissue (Jankovská et al., 2014), but gender was not found as a key factor when muscle tissue of forty six another fish species was analysed (Bastos et al., 2016; Gewurtz et al., 2011). Both age divergence and migration, but not sex of adults, suggest that adult fish may not be an optimal biological matrix to compare contamination among different localities.

In agreement with our hypothesis, lower interspecific variability (counted from species specific mean value) in the THg concentrations was found in YOY fish (Coef. Var. = 15% Kozly and 16% Decin) compared to the intraspecific variability in adults (Coef. Var. from 35% to 56% depending on fish species and sampling site). An analysis of mercury in tissues of adult fish provides an information about contamination of given locality by target compounds during specific time period determined by the age of sampled fish. If fish caught at different localities differ in their age as it is usual in biomonitoring programmes, the comparison of investigated localities may be affected by the different time of bioaccumulation in indicator fish. The use of YOY fish as bioindicator eliminates fouling related to age divergence and allows an evaluation of current contamination at monitored sites. Precisely, by analysis of YOY fish homogenates we can obtain data about mercury contamination during closer defined period of several months. That seems to be advantageous compare to adult fish that usually vary in their age (Cerveny et al., 2014; Noel et al., 2013) and are characterised with migration behaviour (Prchalova et al., 2011; Slavik et al., 2009) and grab samples of water, which is not a relevant matrix for evaluating of mercury contamination in aquatic environment (Ramalhosa et al., 2006).

3.1.2. Lead and cadmium

Compared to mercury, both the interspecific and intraspecific variation in the lead and cadmium concentration in YOY fish were higher at both localities. The results of the lead analyses are summarized in Fig. 2, and complete information about the cadmium and lead concentrations in the YOY fish homogenates is given in the Supplementary material (Table S5) (see Fig. 3).

The lead concentration (the species specific mean value) in YOY fish whole body homogenates varied between 0.032 and 0.304 $\mu g\ g^{-1}$ at Decin and between 0.060 and 0.396 $\mu g\ g^{-1}$ at Kozly. A significant difference ($p < 0.01$) in the lead concentration was found only at Kozly, between bitterling (*Rhodeus amarus*) and ide. High concentration of lead analysed in bitterling might be partly explained by the feeding behaviour of this species. As bitterling feeds mainly on plants, where a significant loads of metals can be expected (Basile et al., 2012; Ebrahimpour and Mushrifah, 2008; Fayed and Abd-El-Shafy, 1985), it can results in higher concentrations of these bioaccumulative pollutants in fish tissues.

The cadmium concentration (the species specific mean value) in YOY fish whole body homogenates ranged from 0.005 to 0.024 $\mu g\ g^{-1}$ at Decin and from 0.004 to 0.016 $\mu g\ g^{-1}$ at Kozly. In case of Decin locality, the highest concentrations was found in samples of gudgeon and it results in significant difference in mean Cd concentration ($p < 0.05$) between this species and stone moroko. This difference could be caused by different spawning seasons of these species or by the possibility that gudgeon from this locality was not YOY fish but one year old juvenile. As gudgeon represents a small fish species which usually grow up to 12 cm and it is characteristic with multiple spawning strategy during the season and fast grow rate, it can be difficult to distinguish between YOY and juvenile fish. Big difference in mean weight occurs between gudgeon from Decin and from Kozly (Table 1), which indicate that gudgeon from Decin was probably juvenile and not YOY fish. In geographical region of the Czech Republic, gudgeon has minor contribution in mixed shoals of YOY fish (3% and 5% at Decin and Kozly sites in this study). From this point of view, uncertainty caused by age determination in case of gudgeon should not affect the evaluation based on real pooled sample of YOY fish whole body homogenate. But it must be taken into consideration in localities where fish with similar reproduction and grow rate characteristics dominate in YOY fish shoals. At Kozly, the cadmium concentration in chub was significantly different ($p < 0.05$) from that in gudgeon and bream, which was most likely caused by the different spawning seasons of these fish species within the year of sampling. As mean weight of gudgeon on this locality was very small, we assumed that this cohort origin from late summer spawning, differently from chub that spawn usually in spring.

The lead and cadmium concentrations in YOY fish whole body homogenates were significantly different among some species. However, the interspecific variability did not result in a significant difference in the cadmium and lead concentrations between the RS and DS pooled samples at both sites, thus multispecies samples of YOY fish could be used for biomonitoring of these toxic metals in aquatic environment.

Very low lead and cadmium concentrations were found in adult fish muscle tissue. A cadmium concentration above the LOQ was found only in one individual chub in Decin. For lead, 20% and 25% of the samples were above the LOQ at Decin and Kozly, respectively (Supplementary material, Table S4). The concentrations measured in all of the positive samples were either equal to or very close to the LOQ value. Obviously, adult fish muscle tissue is not the most suitable matrix for the evaluation of lead and cadmium contamination. Despite reports that the main site of lead or cadmium deposits in fish is the liver and the kidney (Boalt et al., 2014; Cinier et al., 1999), we only sampled adult fish muscle (as it is given by sampling protocol of Czech Hydrometeorological Institue – authority for monitoring of surface water quality in Czech Republic) to compare conventional biomonitoring protocol with a novel approach using YOY fish whole body homogenates.

Because the lead and cadmium concentrations were below LOQ in the most of the adult fish samples, statistical evaluation between studied approaches could not be performed. However, an analysis of YOY fish whole body homogenates could yield an information about the lead and cadmium contamination at a given locality. The mean lead and cadmium concentrations in RS were 0.134 and 0.012 $\mu g\ g^{-1}$ at Decin and 0.094 and 0.017 $\mu g\ g^{-1}$ at Kozly. No significant difference was found in the RS lead or cadmium concentration between investigated sites.

3.2. Perfluoroalkyl substances

Five of the fifteen target PFASs were found in YOY fish whole body homogenates at concentrations above the LOQ. The total PFAS concentrations measured in different YOY fish species ranged from 13 $ng\ g^{-1}$ to 94 $ng\ g^{-1}$ at Decin and from 111 $ng\ g^{-1}$ to 819 $ng\ g^{-1}$ at Kozly (Supplementary material, Tables S6 and S7). The most prevalent compound was perfluorooctane sulphonate (PFOS), which

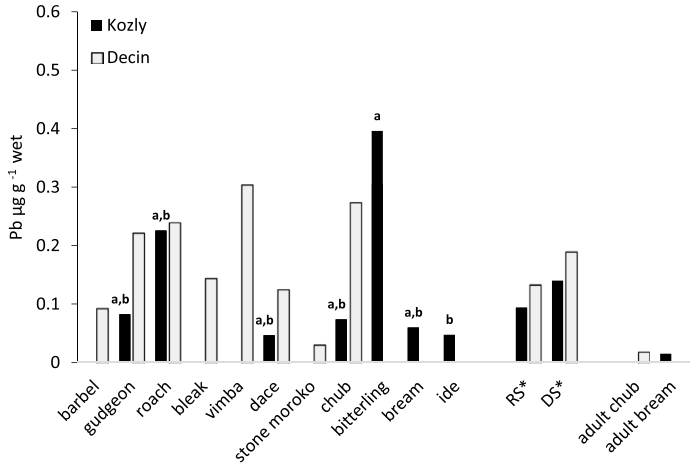

Fig. 2. Lead concentrations analysed in whole body homogenates of different YOY fish species, multispecies pooled samples and muscle of adult fish. Data sharing the same superscript are not significantly different from one another. *RS, real pooled sample (YOY fish species contribution according to the abundance of each species in mixed shoals at the sampling site); *DS, defined pooled sample (an equal contribution of all YOY fish species).

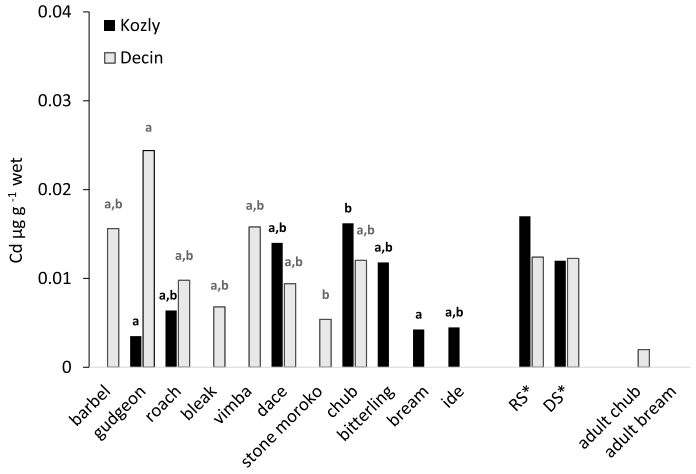

Fig. 3. Cadmium concentrations analysed in whole body homogenates of different YOY fish species, multispecies pooled samples and muscle of adult fish. Data sharing the same superscript are not significantly different from one another: "grey" for Decin; "black" for Kozly. *RS, real pooled sample (YOY fish species contribution according to the abundance of each species in mixed shoals at the sampling site); *DS, defined pooled sample (an equal contribution of all YOY fish species).

comprised 83%–100% of the total PFAS concentration (species specific mean value) at Decin and from 86% to 100% at Kozly. The PFOS concentrations in YOY fish homogenates correspond to the concentrations that are frequently found in the liver of adult fish (Cerveny et al., 2016; Rudel et al., 2011). The other four PFASs (perfluorodecanoic acid (PFDA), Perfluoro-undecanoic acid (PFUdA), Perfluorododecanoic acid (PFDoA), Perfluorotridecanoic acid (PFTrDA)) detected were present at low concentrations, and their contribution to the contamination pattern was not greater than 7% of the total PFASs concentration.

The mean PFOS concentration (803 ng g^{-1}) and the total PFASs in YOY bream at Kozly significantly differed from all other (PFOS 111–287 ng g^{-1}) YOY fish species ($p < 0.05$) at that locality. Because YOY fish live in mixed shoals and occupy the same territory with a restricted food supply, we did not expect such a great effect of different feeding strategies to occur. On the other hand, bream is a typical benthic feeder, and this could lead to higher contamination, even at this age, because sediment is an important sink for PFASs as

well as for other pollutants (Gao et al., 2015; Prevedouros et al., 2006). Admittedly, this assumption was not affirmed by increased level of other analysed contaminants in YOY bream samples, especially mercury for that sediment is an important sink in aquatic environment (Kannan et al., 1998). Also adult bream muscle samples did not show higher levels of PFOS compare to samples of adult chub at the locality, thus extremely high content of PFOS in YOY bream samples remains unexplained. Unfortunately, bream did not occur in the mixed YOY fish shoals at Decin, thus we were not able to confirm this phenomena at both sites. Although the variability in the PFAS concentration between different YOY fish species was relatively high, a significant difference between the RS and DS pooled samples was not found at any of locality.

Only PFOS was found in adult bream and chub muscle tissue. The PFOS concentration in bream varied from 7 ng g^{-1} to 23 ng g^{-1} and from 7 ng g^{-1} to 57 ng g^{-1} at Kozly and Decin, respectively. The PFOS concentration in chub fell in a range from 4 ng g^{-1} to 24 ng g^{-1} and from 2 ng g^{-1} to 19 ng g^{-1} at Kozly and Decin, respectively (Supplementary material, Table S8). Concentrations of PFOS analysed in fish muscle within this study lie within the range usually reported by other authors (Kovarova et al., 2012; Pan et al., 2014; Squadrone et al., 2015). All PFASs analysis data are summarized in Fig. 4. PFOS is usually the most prevalent PFAS found in fish tissues (Koponen et al., 2015; Kovarova et al., 2012; Svihlikova et al., 2015) and is frequently the only PFAS found in fish muscle (Cerveny et al., 2016; Squadrone et al., 2015).

Divergent pictures of the contamination at given localities were obtained based on the approach used. No significant difference in PFAS concentration between sites was found in adult muscle tissue. Actually, a higher mean PFOS concentration was found in bream from Decin than from Kozly, whereas the reverse outcome was found for chub, but these differences were not significant.

Contrary to the adult samples, the PFOS and total PFAS concentrations in the RS YOY fish samples were significantly higher at Kozly. The mean RS concentration of PFOS was 142 ng g^{-1} and 39 ng g^{-1} at Kozly and Decin, respectively. The different results for the two approaches could be explained by different amounts of time needed for bioaccumulation to occur in the indicator organisms. The adult fish reflect the long-term contamination at a given locality, but the YOY fish reflect the actual situation more accurately. However, this assumption is correct only if the adults at both sampling site have the same behavioural pattern.

The PFOS concentration was used to compare variability between studied approaches because it was the only PFAS present in both the adult muscle tissue and the YOY fish homogenates. The results did not agree with our hypothesis because the interspecific variability in the PFOS concentration in YOY fish (Coef. Var. = 103% Kozly and 46% Decin) was higher than the intraspecific variability in adults (Coef. Var. from 37% to 76% depending on species and site). This discrepancy can be assigned to the large difference (three times higher than in other YOY fish species) in the PFOS level in juvenile bream at Kozly, which was the main contributor to high variability between the YOY fish species.

4. Conclusion

Within this study, a novel approach for biomonitoring of extraneous substances in aquatic environment using a whole body homogenates of multispecies samples of YOY fish was evaluated and compared with a conventional approach using adult fish as bioindicators. The results indicate that YOY fish can be a useful bioindicator of aquatic environmental contamination for a wide range of pollutants as the most of target contaminants were found at higher concentrations in YOY fish homogenate than in adult's muscle tissue. However more research is needed to ensure suitability of this approach for monitoring of other groups of important contaminants as POPs, pesticides, pharmaceuticals, etc. Although some variability in the concentration of the target pollutants occurred between different YOY fish species, the differences in mean concentrations between the real and defined pooled samples were relatively low (20% and 15% for THg, 29% and 1% for cadmium, 33% and 29% for lead, 6% and 32% for total PFASs at Kozly and Decin, respectively) and not statistically significant. The data show that mixed YOY fish shoals of cyprinid fishes can be sampled without

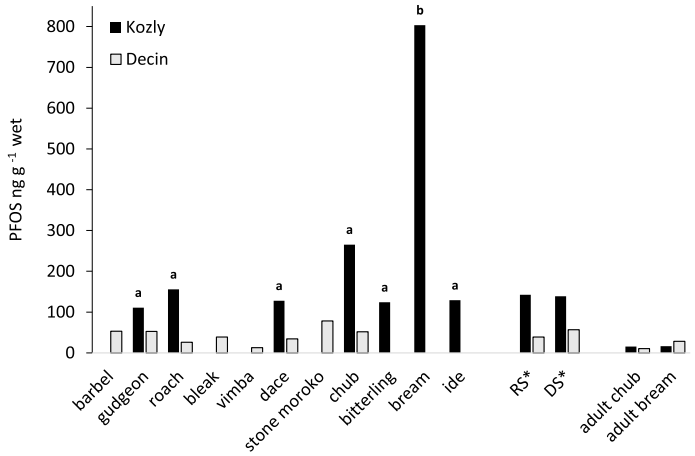

Fig. 4. PFOS concentrations analysed in whole body homogenates of different YOY fish species, multispecies pooled samples and muscle of adult fish. Data sharing the same superscript are not significantly different from one another. *RS, real pooled sample (YOY fish species contribution according to the abundance of each species in mixed shoals at the sampling site); *DS, defined pooled sample (an equal contribution of all YOY fish species).

species determination and selection. Concentrations of target pollutants analysed in YOY fish samples at investigated sites were comparable regardless of the species structure and ratio. Consequently, they might be suitable for the comparison of contamination levels at different sites within biomonitoring programmes carried out by authorities in European countries. As use of YOY fish approach can simplify procedures at both, field and laboratory level, benefits from decreased time and cost spends could be of concern. Our results affirm the limitations of using adult fish for biomonitoring studies due to their migration patterns, as well as species and age effects. Conversely, the YOY fish approach has a high potential to generate more relevant data concerning the contamination of aquatic ecosystems. Finally, use of YOY fish as biological matrix has a minimal impact on the fish population, so this approach conforms to the internationally established principles of Replacement, Reduction, and Refinement (the Three R's strategy) that are also embodied in European Union legislation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2016.07.046.

References

Abel, P.D., 1996. Water Pollution Biology. Taylor & Francis, London; Bristol, PA.
Basile, A., Sorbo, S., Conte, B., Castaldo Cobianchi, R., Trinchella, F., Capasso, C., Carginale, V., 2012. Toxicity, accumulation, and removal of heavy metals by three aquatic macrophytes. Int. J. Phytoremed. 14 (4), 374–387.
Bastos, W.R., Dórea, J.G., Bernardi, J.V.E., Manzatto, A.G., Mussy, M.H., Lauthartte, L.C., Lacerda, L.D., Malm, O., 2016. Sex-related mercury bioaccumulation in fish from the Madeira river, Amazon. Environ. Res. 144, 73–80.
Boalt, E., Miller, A., Dahlgren, H., 2014. Distribution of cadmium, mercury, and lead in different body parts of Baltic herring (Clupea harengus) and perch (Perca fluviatills): implications for environmental status assessments. Mar. Pollut. Bull. 78 (1–2), 130–136.
Burger, J., Gochfeld, M., 2007. Risk to consumers from mercury in Pacific cod (Gadus macrocephalus) from the Aleutians: fish age and size effects. Environ. Res. 105 (2), 276–284.
Campbell, L., Verburg, P., Dixon, D.G., Hecky, R.E., 2008. Mercury biomagnification in the food web of lake Tanganyika (Tanzania, East Africa). Sci. Total Environ. 402 (2–3), 184–191.
Cerveny, D., Grabic, R., Fedorova, G., Grabicova, K., Turek, J., Kodes, V., Golovko, O., Zlabek, V., Randak, T., 2016. Perfluoroalkyl substances in aquatic environment-comparison of fish and passive sampling approaches. Environ. Res. 144, 92–98.
Cerveny, D., Zlabek, V., Velisek, J., Turek, J., Grabic, R., Grabicova, K., Fedorova, G., Rosmus, J., Lepic, P., Randak, T., 2014. Contamination of fish in important fishing grounds of the Czech Republic. Ecotoxicol. Environ. Saf. 109, 101–109.
Cinier, C.D., Petit-Ramel, M., Faure, R., Garin, D., Bouvet, Y., 1999. Kinetics of cadmium accumulation and elimination in carp Cyprinus carpio tissues. Comp. Biochem. Physiol. C-Pharmacol. Toxicol. Endocrinol. 122 (3), 345–352.
Djedjibegovic, J., Larssen, T., Skrbo, A., Marjanovic, A., Sober, M., 2012. Contents of cadmium, copper, mercury and lead in fish from the Neretva river (Bosnia and Herzegovina) determined by inductively coupled plasma mass spectrometry (ICP-MS). Food Chem. 131 (2), 469–476.
Ebrahimpour, M., Mushrifah, I., 2008. Heavy metal concentrations (Cd, Cu and Pb) in five aquatic plant species in Tasik Chini, Malaysia. Environ. Geol. 54 (4), 689–698.
European Parliament and Council, 2010. Directive 2010/63/EU of 22 September 2010 on the protection of animals used for scientific purposes. Off. J. Eur. Union L 276.
Fayed, S.E., Abd-El-Shafy, H.I., 1985. Accumulation of Cu, Zn, Cd, and Pb by aquatic macrophytes. Environ. Int. 11 (1), 77–87.
Fedorova, G., Nebesky, V., Randak, T., Grabic, R., 2014. Simultaneous determination of 32 antibiotics in aquaculture products using LC-MS/MS. Chem. Pap. 68 (1), 29–36.
Fujii, Y., Sakurada, T., Harada, K.H., Koizumi, A., Kimura, O., Endo, T., Haraguchi, K., 2015. Long-chain perfluoroalkyl carboxylic acids in Pacific cods from coastal areas in northern Japan: a major source of human dietary exposure. Environ. Pollut. 199, 35–41.
Gao, Y., Fu, J.J., Meng, M., Wang, Y.W., Chen, B.W., Jiang, G.B., 2015. Spatial distribution and fate of perfluoroalkyl substances in sediments from the Pearl River Estuary, South China. Mar. Pollut. Bull. 96 (1–2), 226–234.
Gewurtz, S.B., Bhavsar, S.P., Fletcher, R., 2011. Influence of fish size and sex on mercury/PCB concentration: importance for fish consumption advisories. Environ. Int. 37 (2), 425–434.
Goeritz, I., Falk, S., Stahl, T., Schafers, C., Schlechtriem, C., 2013. Biomagnification and tissue distribution of perfluoroalkyl substances (PFASs) in market-size rainbow trout (Oncorhynchus mykiss). Environ. Toxicol. Chem. 32 (9), 2078–2088.
Grabicova, K., Grabic, R., Blaha, M., Kumar, V., Cerveny, D., Fedorova, G., Randak, T., 2015. Presence of pharmaceuticals in benthic fauna living in a small stream affected by effluent from a municipal sewage treatment plant. Water Res. 72, 145–153.
Grabicova, K., Lindberg, R.H., Ostman, M., Grabic, R., Randak, T., Larsson, D.G.J., Fick, J., 2014. Tissue-specific bioconcentration of antidepressants in fish exposed to effluent from a municipal sewage treatment plant. Sci. Total Environ. 488, 46–50.
Hajšlová, J., Pulkrabová, J., Poustka, J., Čajka, T., Randák, T., 2007. Brominated flame retardants and related chlorinated persistent organic pollutants in fish from river Elbe and its main tributary Vltava. Chemosphere 69 (8), 1195–1203.
Havelková, M., Dušek, L., Némethová, D., Poleszczuk, G., Svobodová, Z., 2008. Comparison of mercury distribution between liver and muscle – a biomonitoring of fish from lightly and heavily contaminated localities. Sensors 8 (7), 4095–4109.
Jankovská, I., Miholová, D., Romočuský, Š., Petrtýl, M., Langrová, I., Kalous, L., Sloup, V., Válek, P., Vadlejch, J., Lukešová, D., 2014. Importance of fish gender as a factor in environmental monitoring of mercury. Environ. Sci. Pollut. Res. 21 (9), 6239–6242.
Johnson, T.B., Evans, D.O., 1990. Size-dependent winter mortality of young-of-the-year white perch – climate warming and invasion of the Laurentian great-lakes. Trans. Am. Fish. Soc. 119 (2), 301–313.
Kannan, K., Smith, R.G., Lee, R.F., Windom, H.L., Heitmuller, P.T., Macauley, J.M., Summers, J.K., 1998. Distribution of total mercury and methyl mercury in water, sediment, and fish from south Florida estuaries. Arch. Environ. Contam. Toxicol. 34 (2), 109–118.
Kelly, B.C., Ikonomou, M.G., Blair, J.D., Surridge, B., Hoover, D., Grace, R., Gobas, F.A.P.C., 2009. Perfluoroalkyl contaminants in an arctic marine food web: trophic magnification and wildlife exposure. Environ. Sci. Technol. 43 (11), 4037–4043.
Kirjasniemi, M., Valtonen, T., 1997. Size-dependent over-winter mortality of young-of-the-year roach, Rutilus rutilus. Environ. Biol. Fishes 50 (4), 451–456.
Koponen, J., Airaksinen, R., Hallikainen, A., Vuorinen, P.J., Mannio, J., Kiviranta, H., 2015. Perfluoroalkyl acids in various edible Baltic, freshwater, and farmed fish in Finland. Chemosphere 129, 186–191.
Kovarova, J., Marsalek, P., Blahova, J., Jurcikova, J., Kasikova, B., Svobodova, Z., 2012. Occurrence of perfluoroalkyl substances in fish and water from the Svitava and Svratka Rivers, Czech Republic. Bull. Environ. Contam. Toxicol. 88 (3), 456–460.
Kuliskova, P., Horky, P., Slavik, O., Jones, J.I., 2009. Factors influencing movement behaviour and home range size in ide Leuciscus idus. J. Fish Biol. 74 (6), 1269–1279.
Lasorsa, B., Allengil, S., 1995. The methyl mercury to total mercury ratio in selected marine, fresh-water, and terrestrial organisms. Water Air Soil Pollut. 80 (1–4), 905–913.
Maceda-Veiga, A., Monroy, M., de Sostoa, A., 2012. Metal bioaccumulation in the Mediterranean barbel (Barbus meridionalis) in a Mediterranean river receiving effluents from urban and industrial wastewater treatment plants. Ecotoxicol. Environ. Saf. 76, 93–101.
Marti-Cid, R., Llobet, J.M., Castell, V., Domingo, J.L., 2008. Dietary intake of arsenic, cadmium, mercury, and lead by the population of Catalonia, Spain. Biol. Trace Elem. Res. 125 (2), 120–132.
Miege, C., Peretti, A., Labadie, P., Budzinski, H., Le Bizec, B., Vorkamp, K., Tronczynski, J., Persat, H., Coquery, M., Babut, M., 2012. Occurrence of priority and emerging organic compounds in fishes from the Rhone River (France). Anal. Bioanal. Chem. 404 (9), 2721–2735.
Miranda, L.E., Hubbard, W.D., 1994. Length-dependent winter survival and lipid-composition of age-0 Largemouth bass in bay springs reservoir, Mississippi. Trans. Am. Fish. Soc. 123 (1), 80–87.
Misztal-Szkudlinska, M., Szefer, P., Konieczka, P., Namiesnik, J., 2011. Biomagnification of mercury in trophic relation of great cormorant (Phalacrocorax carbo) and fish in the vistula Lagoon, Poland. Environ. Monit. Assess. 176 (1–4), 439–449.
Murakami, M., Adachi, N., Saha, M., Morita, C., Takada, H., 2011. Levels, temporal trends, and tissue distribution of perfluorinated surfactants in freshwater fish from Asian countries. Arch. Environ. Contam. Toxicol. 61 (4), 631–641.
Net, S., Henry, F., Rabodonirina, S., Diop, M., Merhaby, D., Mahfouz, C., Amara, R., Ouddane, B., 2015. Accumulation of PAHs, Me-PAHs, PCBs and total mercury in sediments and marine species in coastal areas of Dakar, Senegal: contamination level and impact. Int. J. Environ. Res. 9 (2), 419–432.

Noel, L., Chekri, R., Millour, S., Merlo, M., Leblanc, J.C., Guerin, T., 2013. Distribution and relationships of As, Cd, Pb and Hg in freshwater fish from five French fishing areas. Chemosphere 90 (6), 1900–1910.

Orban, E., Nevigato, T., Masci, M., Di Lena, G., Casini, I., Caproni, R., Gambelli, L., De Angelis, P., Rampacci, M., 2007. Nutritional quality and safety of European perch (Perca fluviatilis) from three lakes of Central Italy. Food Chem. 100 (2), 482–490.

Ouedraogo, O., Chetelat, J., Amyot, M., 2015. Bioaccumulation and trophic transfer of mercury and selenium in African sub-tropical fluvial reservoirs food webs (Burkina Faso). Plos One 10 (4).

Pan, C.G., Zhao, J.L., Liu, Y.S., Zhang, Q.Q., Chen, Z.F., Lai, H.J., Peng, F.J., Liu, S.S., Ying, G.G., 2014. Bioaccumulation and risk assessment of per- and polyfluoroalkyl substances in wild freshwater fish from rivers in the Pearl River Delta region, South China. Ecotoxicol. Environ. Saf. 107, 192–199.

Pan, K., Wang, W.X., 2012. Trace metal contamination in estuarine and coastal environments in China. Sci. Total Environ. 421, 3–16.

Prevedouros, K., Cousins, I.T., Buck, R.C., Korzeniowski, S.H., 2006. Sources, fate and transport of perfluorocarboxylates. Environ. Sci. Technol. 40 (1), 32–44.

Prchalova, M., Horky, P., Slavik, O., Vetesnik, L., Halacka, K., 2011. Fish occurrence in the fishpass on the lowland section of the River Elbe, Czech Republic, with respect to water temperature, water flow and fish size. Folia Zool. 60 (2), 104–114.

Ramalhosa, E., Segade, S.R., Pereira, E., Vale, C., Duarte, A., 2006. Mercury cycling between the water column and surface sediments in a contaminated area. Water Res. 40 (15), 2893–2900.

Randak, T., Zlabek, V., Pulkrabova, J., Kolarova, J., Kroupova, H., Siroka, Z., Velisek, J., Svobodova, Z., Hajslova, J., 2009. Effects of pollution on chub in the river Elbe, Czech Republic. Ecotoxicol. Environ. Saf. 72 (3), 737–746.

Rudel, H., Muller, J., Jurling, H., Bartel-Steinbach, M., Koschorreck, J., 2011. Survey of patterns, levels, and trends of perfluorinated compounds in aquatic organisms and bird eggs from representative German ecosystems. Environ. Sci. Pollut. Res. 18 (9), 1457–1470.

Slavik, O., Horky, P., Bartos, L., 2009. Occurrence of cyprinids in fish ladders in relation to flow. Biologia 64 (5), 999–1004.

Squadrone, S., Ciccotelli, V., Prearo, M., Favaro, L., Scanzio, T., Foglini, C., Abete, M.C., 2015. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA): emerging contaminants of increasing concern in fish from Lake Varese, Italy. Environ. Monit. Assess. 187 (7).

Stahl, L.L., Snyder, B.D., Olsen, A.R., Kincaid, T.M., Wathen, J.B., McCarty, H.B., 2014. Perfluorinated compounds in fish from US urban rivers and the Great Lakes. Sci. Total Environ. 499, 185–195.

Svihlikova, V., Lankova, D., Poustka, J., Tomaniova, M., Hajslova, J., Pulkrabova, J., 2015. Perfluoroalkyl substances (PFASs) and other halogenated compounds in fish from the upper Labe River basin. Chemosphere 129, 170–178.

Verdouw, J.J., MacLeod, C.K., Nowak, B.F., Lyle, J.M., 2011. Implications of age, size and region on mercury contamination in estuarine fish species. Water, Air, Soil Pollut. 214 (1–4), 297–306.

Wu, Y.N., Wang, Y.X., Li, J.G., Zhao, Y.F., Guo, F.F., Liu, J.Y., Cai, Z.W., 2012. Perfluorinated compounds in seafood from coastal areas in China. Environ. Int. 42, 67–71.

SUPPLEMENT MATERIALS

Table S1. *LC gradient for the separation of target compounds.*

Time, min	A, %	B, %	Flow, µL min^{-1}
0.00	100	0	300
1.00	100	0	300
7.00	60	40	350
10.00	0	100	400
12.00	0	100	400
12.01	100	0	300
15.00	100	0	300

A water + 0.1% FA. B Acetonitrile + 0.1% FA.

Table S2. *Parameters for HESI ion source.*

Parameter	Value
Capilary temperature (°C)	325
Vaporiser temperature (°C)	300
Auxillary gas pressure (a.u.)	15
Sheath gas (nitrogen) pressure (a.u.)	30
Spray voltage (V)	2700

Table S3. *THg concentrations in samples of YOY fish homogenates.*

Species	n	Kozly			n	Decin		
		mean	±	SD		mean	±	SD
bitterling *Rhodeus amarus*	5	0.031	±	0.005				
bream *Abramis brama*	4	0.042	±	0.004				
gudgeon *Gobio gobio*	4	0.039	±	0.007	5	0.056	±	0.011
roach *Rutilus rutilus*	5	0.037	±	0.002	5	0.045	±	0.013
ide *Leuciscus idus*	4	0.034	±	0.009				
dace *Leuciscus leuciscus*	3	0.049	±	0.007	5	0.054	±	0.011
chub *Squalius cephalus*	5	0.043	±	0.008	5	0.044	±	0.005
barbel *Barbus barbus*					5	0.041	±	0.013
bleak *Alburnus alburnus*					5	0.048	±	0.016
vimba *Vimba vimba*					5	0.014	±	0.000
rasbora *Pseudorasbora parva*					5	0.062	±	0.020

Species	n	Kozly			n	Decin		
		mean	±	SD		mean	±	SD
Real pooled sample *(RS)*[a]	5	0.041	±	0.002	5	0.033	±	0.001
Defined pooled sample *(DS)*[b]	3	0.033	±	0.004	5	0.039	±	0.001

[a] YOY fish species ratio according to their abundance in mixed shoals at sampling site
[b] YOY fish species in equal ratio

Table S4. *Toxic metals concentrations in samples of adult´s muscle tissue.*

Sampling site		Kozly			Decin		
Species	fish no.	concentration (μg g^{-1})			concentration (μg g^{-1})		
		THg	Cd	Pb	THg	Cd	Pb
bream *Abramis brama*	1	0.384	<0.002	0.02	0.154	<0.002	0.02
	2	0.368	<0.002	<0.02	0.114	<0.002	<0.02
	3	0.370	<0.002	0.02	0.113	<0.002	<0.02
	4	0.263	<0.002	<0.02	0.168	<0.002	<0.02
	5	0.294	<0.002	<0.02	0.179	<0.002	<0.02
	6	0.076	<0.002	<0.02	0.157	<0.002	<0.02
	7	0.165	<0.002	0.02	0.157	<0.002	<0.02
	8	0.177	<0.002	0.02	0.326	<0.002	<0.02
	9	0.198	<0.002	0.02	0.128	<0.002	<0.02
	10	0.117	<0.002	<0.02	0.320	<0.002	<0.02
chub *Squalius cephalus*	1	0.222	<0.002	<0.02	0.215	0.002	<0.02
	2	0.180	<0.002	<0.02	0.253	<0.002	<0.02
	3	0.163	<0.002	<0.02	0.127	<0.002	<0.02
	4	0.196	<0.002	<0.02	0.229	<0.002	<0.02
	5	0.298	<0.002	<0.02	0.106	<0.002	0.02
	6	0.254	<0.002	<0.02	0.139	<0.002	<0.02
	7	0.275	<0.002	<0.02	0.156	<0.002	0.03
	8	0.124	<0.002	<0.02	0.465	<0.002	0.02
	9	0.123	<0.002	<0.02	0.145	<0.002	<0.02
	10	0.104	<0.002	<0.02	0.103	<0.002	<0.02

Table S5. *Cadmium and lead concentrations in samples of YOY fish homogenates.*

Species	Kozly							Decin						
	n	Concentration µg g^{-1}						n	concentration µg g^{-1}					
		Cadmium			Lead				Cadmium			Lead		
		mean	±	SD	mean	±	SD		mean	±	SD	mean	±	SD
bitterling *Rhodeus amarus*	5	0.012	±	0.002	0.396	±	0.064							
bream *Abramis brama*	4	0.004	±	0.001	0.060	±	0.049							
gudgeon *Gobio gobio*	4	0.004	±	0.001	0.083	±	0.019	5	0.024	±	0.013	0.222	±	0.157
roach *Rutilus rutilus*	5	0.006	±	0.002	0.226	±	0.127	5	0.010	±	0.004	0.240	±	0.121
ide *Leuciscus idus*	4	0.005	±	0.002	0.060	±	0.029							
dace *Leuciscus leuciscus*	3	0.014	±	0.006	0.047	±	0.025	5	0.009	±	0.004	0.126	±	0.099
chub *Squalius cephalus*	5	0.016	±	0.007	0.074	±	0.063	5	0.012	±	0.007	0.274	±	0.305
barbel *Barbus barbus*								5	0.016	±	0.008	0.094	±	0.044
bleak *Alburnus alburnus*								5	0.007	±	0.004	0.145	±	0.142
vimba *Vimba vimba*								5	0.016	±	0.006	0.304	±	0.259
stone moroko *Pseudorasbora parva*								5	0.005	±	0.002	0.032	±	0.022
Real pooled sample *(RS)*[b]	5	0.017	±	0.001	0.094	±	0.015	5	0.012	±	0.001	0.134	±	0.008
Defined pooled sample *(DS)*[c]	3	0.012	±	0.002	0.140	±	0.050	5	0.012	±	0.002	0.190	±	0.056

[a] species was absent in mixed shoals of YOY fish at the sampling site
[b] YOY fish species ratio according to their abundance in mixed shoals at sampling site
[c] YOY fish species in equal ratio

Table S6. *PFASs concentrations in samples of YOY fish homogenates from sampling site Kozly.*

Species	n	Concentration (ng g^{-1})					
		PFOS	PFDA	PFUdA	PFDoA	PFTrDA	total PFASs
bitterling *Rhodeus amarus*	5	124.1	<LOQ	<LOQ	<LOQ	<LOQ	124.1
bream *Abramis brama*	4	803.3	20.6	1.0	4.6	<LOQ	819.1
gudgeon *Gobio gobio*	4	111.0	<LOQ	<LOQ	<LOQ	<LOQ	111.0
roach *Rutilus rutilus*	5	155.9	<LOQ	<LOQ	1.4	<LOQ	157.2
ide *Leuciscus idus*	4	129.0	<LOQ	<LOQ	2.0	<LOQ	131.0
dace *Leuciscus leuciscus*	3	127.8	<LOQ	1.0	14.9	4.2	147.9
chub *Squalius cephalus*	5	265.4	2.5	1.1	15.7	2.0	286.7
Real pooled sample *(RS)*[a]	5	142.6	1.3	<LOQ	8.9	1.1	154.0
Defined pooled sample *(DS)*[b]	3	138.9	<LOQ	<LOQ	5.1	<LOQ	144.1

[a] YOY fish species ratio according to their abundance in mixed shoals at sampling site
[b] YOY fish species in equal ratio

Table S7. *PFASs concentrations in samples of YOY fish homogenates from sampling site Decin.*

Species	n	Concentration (ng g^{-1})					
		PFOS	PFDA	PFUdA	PFDoA	PFTrDA	total PFASs
barbel *Barbus barbus*	5	53.3	<LOQ	<LOQ	2.3	<LOQ	55.6
gudgeon *Gobio gobio*	5	52.4	0.7	<LOQ	3.2	<LOQ	56.3
roach *Rutilus rutilus*	5	25.9	<LOQ	<LOQ	<LOQ	<LOQ	25.9
bleak *Alburnus alburnus*	5	38.7	<LOQ	<LOQ	2.4	<LOQ	41.1
vimba *Vimba vimba*	5	12.8	<LOQ	<LOQ	<LOQ	<LOQ	12.8
dace *Leuciscus leuciscus*	5	34.3	<LOQ	<LOQ	2.0	<LOQ	36.4
stone moroko *Pseudorasbora parva*	5	78.3	2.7	2.5	9.8	0.5	93.9
chub *Squalius cephalus*	5	51.7	<LOQ	<LOQ	0.8	<LOQ	52.4
Real pooled sample *(RS)*[a]	5	39.0	<LOQ	<LOQ	1.9	<LOQ	40.9
Defined pooled sample *(DS)*[b]	5	56.8	0.2	<LOQ	3.4	<LOQ	60.5

[a] YOY fish species ratio according to their abundance in mixed shoals at sampling site
[b] YOY fish species in equal ratio

Table S8. *PFOS concentration analysed in muscle tissue of adults.*

Sampling site	Fish no.	Bream	Chub
		Abramis brama	*Squalius cephalus*
		PFOS concentration (ng g^{-1})	
Kozly	1	8.2	14.6
	2	22.0	23.9
	3	22.3	20.0
	4	6.7	9.5
	5	18.4	15.4
	6	22.5	22.6
	7	17.0	11.3
	8	13.2	12.3
	9	22.8	22.4
	10	11.6	4.2
Decin	1	7.2	11.2
	2	12.5	8.7
	3	56.7	19.1
	4	54.7	10.5
	5	7.5	12.5
	6	11.6	1.6
	7	8.0	7.9
	8	39.6	12.8
	9	55.3	1.7
	10	[a]	19.3

[a] sample was lost

CHAPTER 5

FISH FIN-CLIPS AS A NON-LETHAL METHOD FOR BIOMONITORING OF MERCURY CONTAMINATION IN AQUATIC ENVIRONMENT AND HUMAN HEALTH RISK ASSESSMENT

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Fish fin-clips as a non-lethal approach for biomonitoring of mercury contamination in aquatic environments and human health risk assessment

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HIGHLIGHTS

- Use of fish fin-clips is a suitable non-lethal approach for mercury monitoring.
- THg muscle concentration and muscle/fin-clip THg ratio are negatively correlated.
- Precise prediction of THg muscle concentrations in bream and chub is possible.

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ABSTRACT

Muscle tissue and pectoral fins of two important indicator fish species, frequently used in biomonitoring programs, were sampled and analysed for total mercury content (THg) at six localities within the Czech Republic. The relationship between mercury concentration in muscle and in fin-clips was described. Mean values of THg fin-clip concentration correlate significantly ($p < 0.01$) with those measured in muscle of indicator fish. Concerning comparison among localities, a coefficient of determination (r^2) of 0.85 and 0.91 was found between studied approaches in the case of chub (*Squalius cephalus*) and bream (*Abramis brama*), respectively. THg muscle concentrations (mean, $n = 10$) varied from 0.181 to 0.491 μg g^{-1} wet, depending on indicator species and locality. A concentration-dependent relationship between muscle and fin-clip THg content was found in both species. Based on this finding, a novel method for the prediction of muscle THg concentration from fin-clips analysis was developed. The difference between measured and predicted muscle concentration was below 10% in both indicator species at most sampling sites. Use of fish fin-clips was found as an appropriate nonlethal approach for the evaluation of mercury contamination in aquatic environments as well as for human health risk assessment.

1. Introduction

Many human activities cause some type of pollution that results in surface or underground water contamination. Certain xenobiotics, such as toxic metals remain in the environment for a very long time, as these are not degradable under natural conditions. Mercury is considered as the most dangerous toxic metal, due to its neurotoxicity and levels found in the aquatic environment (Noel et al., 2013; Cerveny et al., 2014; Asefi and Zamani-Ahmadmahmoodi, 2015). Water sediments, soils and vegetation present an important sink for mercury, which results in its presence in food chains at localities where there have been no sources of pollution for many years (Vanhattum et al., 1993; Abel, 1996; Nguetseng et al., 2015).

After being released into the air, mercury returns to the ground through precipitation and enters the aquatic environment; thus, atmospheric deposition is a dominant source of mercury (Lepom et al., 2012). Mercury is neurotoxic in both its organic and inorganic forms (Atchison and Hare, 1994; Fretham et al., 2012), and the commonly encountered form of mercury, methylmercury (MeHg), is the most toxic form affecting aquatic biota (Lasorsa and Allengil,

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1995; Maceda-Veiga et al., 2012). MeHg is primarily responsible for bioaccumulation in the muscle tissue of fish with a methylmercury fraction of 83–90% of the-total mercury concentration (Lasorsa and Allengil, 1995; Kannan et al., 1998; Marsalek et al., 2005; Kruzikova et al., 2008).

Because of these mentioned characteristics, mercury has become one of the most monitored xenobiotics in aquatic environments. Use of aquatic organisms, especially fish, for monitoring of mercury is necessary as concentrations of mercury in water are usually very low. Furthermore, water is not a relevant matrix for assessing human health risks (Orban et al., 2007; Cerveny et al., 2014). No biodegradation of Hg and ability to enter the food web results in its bioaccumulation in various animal species along the trophic chain, especially in fish that are at the top of the food pyramid in an aquatic ecosystem (Dusek et al., 2005; Li et al., 2015).

As Hg is listed as a priority substance in the field of water policy and it is regulated under the Water Framework Directive, an Environmental Quality Standard (EQS) of 0.02 $\mu g\ g^{-1}$ (wet weight) for water biota was set by the Directive of the European parliament and Council (2013). Furthermore, Maximum Level (ML) of 0.5 $\mu g\ g^{-1}$ for fish muscle and fishery products intended for human consumption was set by European Commission (2008).

Fish consumption represents the main exposure pathway for mercury contamination in humans (Cuadrado et al., 1995; Cerveny et al., 2014), thus muscle tissue of adult fish is of interest to researchers and regulatory bodies all over the world. This approach is appropriate in the case of marketable fish, but in the case of wild fish, it can induce a conflict of interests with the internationally accepted guidelines for the protection of human subjects and animal welfare. These principles are also included in the EU Directive on the protection of animals used for scientific purposes (European parliament and Council, 2010). Moreover, due to the valid legislation, fish that are sampled for scientific or biomonitoring purposes cannot be used as a human food or for feeding of livestock, thus it ends as a veterinary waste.

Several studies have been published that deal with non-lethal approaches for the evaluation of mercury contamination in aquatic environment using fish as bioindicators. Some of them evaluated different biopsy techniques (Schmitt and Brumbaugh, 2007; Ackerson et al., 2014), and some were using scales or fin-clips for analysis and prediction of muscle tissue mercury concentrations (Gremillion et al., 2005; Ryba et al., 2008; Cervenka et al., 2011; Piraino and Taylor, 2013). The aim of the present study is to contribute to the discussion and extend the knowledge of the relationship between muscle and fin-clips mercury concentration in freshwater fish. Muscle and fin-clip mercury concentrations analysed in the two most used indicator fish species from six localities within Czech Republic are presented.

2. Material and methods

2.1. Monitored sites

Six sampling sites were chosen within Czech Republic. These sites were chosen according to occurrence of indicator fish species and they were supposed to cover both areas with low and higher mercury contamination. Sampling site Neratovice lies on the Elbe River in an area, where important source of pollution – the chemical plant Spolana Neratovice is located. Another three sampling sites were chosen in the vicinity. Kostelec and Obristvi are located upstream and downstream, respectively, from the Neratovice, and Mlekojedy is a sandpit without connection to the Labe River watercourse close to the Neratovice. The remaining two sampling sites are located in the southern part of the Czech Republic. Hnevkovice and Rimov are the dams located on Vltava and Malse River, respectively.

2.2. Fish sampling and sample preparation

European bream (*Abramis brama*) and European chub (*Squalius cephalus*) were chosen as the indicator fish species to be caught at all experimental sampling sites. Both bream and chub are native species of Czech Republic and they are not artificially stocked. These species are most often used as fish bioindicators in the central Europe monitoring programs. Fish were caught by an electrofishing device, gillnets and angling. Both indicator species were caught at all sampling sites except for Mlekojedy, where only bream was caught.

Ten specimens of both fish species were caught at each sampling site. All of the sampled fish were measured and weighed. Muscle tissue from the mid-dorsal part of the body and the fin-clip of pectoral fin were obtained from all individuals. The samples were placed into 2-ml Eppendorf tubes, cooled down and stored at 4 °C during transport to the laboratory, where they were kept frozen (−20 °C) until analysis was performed. Fish sampling was realized from April to June 2015 and analyses were completed in July of the same year. All experimental animals were handled in accordance with the national and institutional guidelines for the protection of human subjects and animal welfare (European parliament and Council, 2010).

2.3. Mercury analysis

The total mercury (THg) content was determined directly in the sample units by a selective mercury analyser (Advanced mercury analyser, AMA-254, Altec) based on atomic absorption spectroscopy (AAS). This method is based on thermal decomposition of a sample in a flow of oxygen, the capture of mercury by a gold amalgamator, and measurements of the mercury vapour absorbance after thermal release from the amalgamator. Briefly, 100–200 mg of thawed muscle/fin-clip was loaded in nickel boat and analysed. No pre-treatment of muscle samples was made, and fin-clips were only washed with deionized water prior to analysis. Final concentration of the sample was calculated as a mean of two independent measurements and it is expressed as wet weight (ww) concentration. For calibration of the instrument, MERCK calibration solution–CertiPUR was used. To demonstrate quality assurance/quality control performance of the analytical method, blank samples and certified reference material BCR-422 (lyophilized cod muscle) were used. THg concentrations in blank samples were below the limit of detection. Based on BCR–422, the method uncertainty of 3.54% was found and expressed as a relative standard deviation (RSD) of seven measurements (at $0.559 \pm 0.016\ \mu g\ g^{-1}$ Hg). Recovery for the same reference material was from 100% to 105% ($n = 7$).

2.4. Prediction of THg muscle concentration from fin-clips analysis

Individuals of each species from all sampling sites were divided into three groups according to the THg concentration measured in fin-clips. A median of muscle/fin-clips THg ratio (quotient) was then calculated for each of these three groups and it was indicated as a "prediction factor" (PF). The prediction factors and ranges of fin-clip THg concentrations that were used for the calculation are given in Table 1. Concentrations of THg measured in fin-clips were transferred into predicted muscle concentration using appropriate PF in all individuals at all sampling sites. The mean predicted muscle concentration was then calculated for each locality from individually acquired values in both indicator species.

Table 1
Factors for prediction of muscle mercury concentration from fin-clips analysis.

Range[a]	Chub (*Squalius cephalus*)		Bream (*Abramis brama*)	
	Muscle/fin-clip THg ratio (quotient)			
μg g^{-1}	Median (PF[b])	(min–max)	Median (PF[b])	(min–max)
<0.03	14	(8.9–20.2)	18	(9.6–33.0)
0.03–0.06	9	(6.7–12.2)	13	(6.8–17.1)
>0.06	7	(6.1–9.1)	7	(6.8–8.9)

[a] Ranges of fin-clip THg concentration for counting of prediction factors.
[b] Prediction factor.

2.5. Statistical analyses

Statistical evaluation of the data was completed using Statistica 12 software (StatSoft Inc., USA). Pearson's correlation coefficient and a coefficient of determination (r^2) defining the relationship between muscle and fin THg concentrations were calculated using regression analysis at each sampling site. Differences between sampling sites were evaluated using analysis of variance (one-way ANOVA) after testing of normal distribution of data (Shapiro–Wilk test).

3. Results and discussion

Measurable concentrations of THg were found in all samples of muscle tissue and fin-clips of indicator fish species. The limit of detection for THg content was 0.07 ng g^{-1}. The relative standard deviation of measurements (n = 2) varied between 0.1% and 0.9% in fin-clips and 0.1%–2.5% in muscle tissue, respectively. The data from all of the sampling sites are summarized in Table 2, together with characteristics of sampled fish. The obtained data indicate that high variability occurs in muscle THg concentration between individuals of same species at each locality. This can be mainly caused by the differing ages of sampled fish and partly by their migration behaviour, as different individuals spent different amounts of time in the locality where they were caught. Interdependence between the age and the body burden of individual was described in case of contaminants with bioaccumulation potential (Dusek et al., 2005; Cerveny et al., 2014). The age together with the species trophic level are the main factors determining the level of mercury contamination (Misztal-Szkudlinska et al., 2011; Ouedraogo et al., 2015).

ML of 0.5 μg g^{-1} for fish muscle was exceeded in 25% of individual bream samples and 10% of individual samples of chub. No mean concentration (n = 10) of both species at all sampling sites exceed this ML, but in case of bream, mean concentration over 0.4 μg g^{-1} in muscle was detected at four of six localities. Concerning EQS, the level of 0.02 μg g^{-1} was exceeded in all individual samples of fish muscle analysed within this study. Mean Hg concentrations (n = 10) exceed the EQS by 9- to 24-fold, depends on fish species and locality.

Table 2
Characteristics of sampled fish and concentrations of total mercury (THg) in muscle and fin-clips samples.

Locality	Species	n	Total length (mm)		Weight (g)		THg muscle concentration (μg g^{-1})		THg fin concentration (μg g^{-1})	
			Mean	(min–max)	Mean	(min–max)	Mean	(min–max)	Mean	(min–max)
Neratovice	*Abramis brama*	10	401	(340–470)	673	(420–1050)	0.454	(0.258–0.643)	0.043	(0.024–0.094)
	Squalius cephalus	10	355	(290–440)	569	(280–1125)	0.303	(0.113–0.646)	0.03	(0.012–0.056)
Kostelec	*Abramis brama*	10	430	(380–460)	822	(530–1010)	0.415	(0.205–0.525)	0.037	(0.023–0.069)
	Squalius cephalus	10	305	(190–490)	452	(65–1535)	0.248	(0.107–0.644)	0.029	(0.009–0.105)
Obristvi	*Abramis brama*	10	386	(310–445)	596	(299–866)	0.491	(0.365–0.661)	0.043	(0.024–0.074)
	Squalius cephalus	10	385	(270–575)	596	(230–1030)	0.305	(0.163–0.601)	0.032	(0.012–0.083)
Mlekojedy	*Abramis brama*	10	364	(330–445)	444	(345–620)	0.222	(0.059–0.327)	0.012	(0.005–0.026)
	Squalius cephalus	0[a]								
Hnevkovice	*Abramis brama*	10	395	(275–470)	691	(265–1115)	0.272	(0.188–0.385)	0.014	(0.011–0.019)
	Squalius cephalus	10	348	(220–500)	614	(115–1580)	0.181	(0.04–0.356)	0.012	(0.003–0.033)
Rimov	*Abramis brama*	10	418	(340–470)	824	(480–965)	0.433	(0.255–0.586)	0.028	(0.012–0.041)
	Squalius cephalus	10	335	(230–440)	424	(120–835)	0.223	(0.094–0.504)	0.02	(0.005–0.072)

[a] Species was not present at the locality.

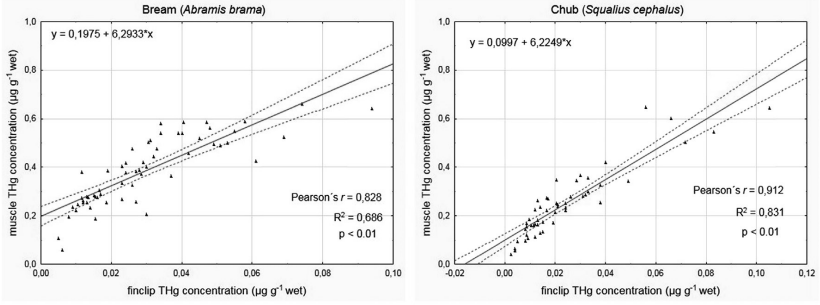

Fig. 1. Regression analysis of muscle/fin-clip total mercury (THg) concentration relationship in indicator fish species.

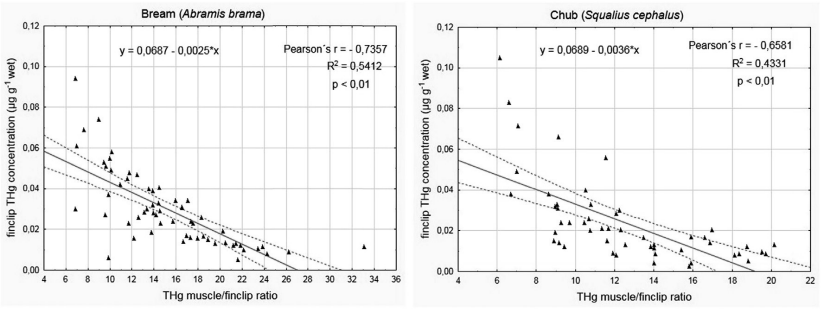

Fig. 2. Regression analysis of relationship between fin-clip total mercury (THg) concentration and muscle/fin-clip THg ratio.

3.1. Muscle to fin-clips THg relationship

In chub samples, the significant correlation ($p < 0.01$) between muscle and fin-clips THg content was found to be present at all localities. The coefficient of determination (r^2) varied from 0.83 to 0.95 depending on the sampling site. However, significant correlations ($p < 0.05$) between bream muscle and fin-clips THg content were found in three of six localities only. The coefficient of determination varied from 0.29 to 0.77 (all six localities). Based on a statistical analysis of the data, a poor relationship between THg content in muscle and in fin-clips exists in case of bream, but in reality, the relationship has a similar correlation as in case of chub. As the higher variability in THg content occurs between individuals of chub at all sampling sites (Table 2), higher coefficient of determination and stronger significance is achieved. Compared to chub, individuals of bream did not differ in size (assumed age as well) at most of sampling sites, which lead to smaller variability in THg content (Table 2). As the differences in muscle THg concentration were relatively low, concentrations found in fin-clips cannot follow these differences as exact as it is needed to gain significance or high values of the coefficient of determination. The lowest coefficient of determination (0.29) was achieved at locality Hnevkovice, where also the lowest variability in muscle THg concentration was found. Similar results were observed by (Cervenka et al., 2011), who found higher coefficient of determination between muscle and fin-clips THg content in bream from Hamry reservoir when all age groups were evaluated together ($r^2 = 0.86$). If individuals were divided into groups according to their age, the value of r^2 decreased to 0.71 and 0.51. Another study evaluating the same relationships was done in Rhode Island, where black bass (*Micropterus salmoides*) was used as an indicator fish species (Ryba et al., 2008). An r^2 of 0.85 between fish muscle and caudal fin THg concentrations (dry weight) was found in set of 169 fish collected from 26 freshwater sites. The content of THg measured in muscle samples varied greatly (from 0.450 to 16.95 µg g^{-1}) between individuals of black bass. Comparable correlations between muscle and fin-clip THg content was found in pike (*Esox lucius*) and walleye (*Sander vitreus*) from three northern-Carolina reservoirs, where an r^2 of 0.78 and 0.83, respectively (Gremillion et al., 2005). In the present study, a global coefficient of determination (all sites together) of 0.69 and 0.83 was achieved in case of bream and chub, respectively (Fig. 1).

The relationship between muscle and fin-clip THg concentration was found to be not linear. This finding was mentioned in a previous study (Ryba et al., 2008), but in our study, this concentration-dependent relationship was significantly negatively correlated ($p < 0.01$). As a higher level of mercury contamination in fish body was found, the smaller difference in muscle/fin-clip THg concentration was observed in both indicator species. An r^2 of 0.54 and 0.43 between THg fin-clips concentration and muscle/fin-clips THg ratio was found in case of bream and chub, respectively (Fig. 2).

3.2. Comparison of sites

All sampling sites were compared using the mean values ($n = 10$) of the muscle and fin-clip THg concentrations measured in both fish species (Fig. 3). The same picture of contamination in various localities was obtained using both approaches; however, concentrations found in fin-clips were approximately one order of magnitude lower than those found in muscle tissue. This level of difference between muscle and fin-clips THg concentration

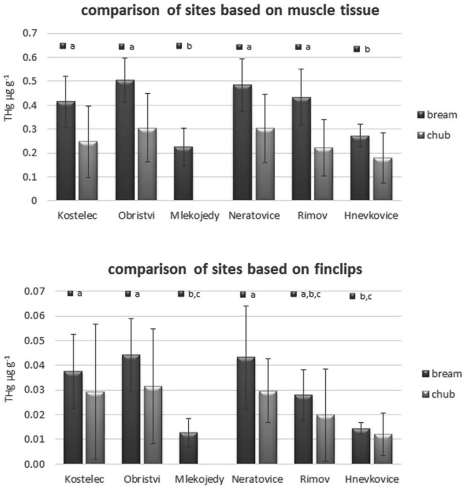

Fig. 3. Comparison of total mercury (THg) contamination of sampling sites based on both muscle and fin-clip approaches ^{a,b,c} superscripts indicate significant ($p < 0.01$) differences in THg concentrations between localities.

Table 3
Results of muscle total mercury (THg) prediction from fin-clips analysis.

Locality	Bream (*Abramis brama*)			Chub (*Squalius cephalus*)		
	Measured	Predicted	Difference	Measured	Predicted	Difference
	THg (μg g^{-1} wet)			THg (μg g^{-1} wet)		
Kostelec	0.415	0.481	0.066	0.247	0.312	0.065
Obristvi	0.472	0.509	0.037	0.305	0.289	−0.016
Mlekojedy	0.211	0.218	0.007	[a]		
Neratovice	0.453	0.560	0.107	0.303	0.313	0.010
Rimov	0.433	0.409	−0.024	0.223	0.228	0.005
Hnevkovice	0.272	0.259	−0.013	0.181	0.153	−0.028

[a] Species was not present at the locality.

corresponds with previously published works of other authors (Gremillion et al., 2005; Ryba et al., 2008; Cervenka et al., 2011). Concerning comparison of sampling site's contamination, strong correlation between mean THg concentrations in muscle and in fin-clips of both indicator species was found. Coefficient of determination was found significant at $p < 0.05$ and $p < 0.01$ with an r^2 of 0.85 and 0.91 in chub and bream, respectively.

Significant differences ($p < 0.01$) in contamination of sampling sites were found based on bream samples. Same differences were found using both muscle and fin-clips approaches, with only one exception in locality Rimov. Contrary to muscle approach, Rimov did not differ from any other site based on the bream fin-clip analysis (Fig. 3). Levels of THg measured in both muscle and fin-clips of chub did not significantly differ between the localities. This absence of significant differences could partly be caused by high variability in THg concentrations among chub individuals within sampling sites.

Information about contamination of given localities brought by both muscle and fin-clip approaches were almost identical, thus using of fish fin-clips for evaluation of THg contamination in aquatic environment can be recommended. The authors believe that the main advantage is in sampling more individuals per locality without ecologic/ethic consequences, which can help to reduce negative effects of migration and different age of sampled fish.

3.3. Prediction of THg muscle concentrations

Some authors have evaluated the possibility to predict muscle mercury concentrations from fin-clips analysis (Ryba et al., 2008; Piraino and Taylor, 2013). In both studies, high uncertainty of prediction was found, thus this method could not be recommended for developing human consumption advisories. A different method for predicting was applied in this study according to our findings on concentration-dependent relationship between muscle and fin-clip THg concentrations.

As the relationship between fin-clip and muscle THg concentration was found concentration-dependent, use of a linear function for estimation results in a relatively high error (Ryba et al., 2008). According to this finding, fish of both species were divided into three groups based on THg concentrations measured in fin-clips and three different prediction factors (PFs) were used to calculate a predicted muscle concentration.

Concentrations of THg measured in all individual samples of fin-clips were transferred using PFs into "predicted individual muscle concentration" as described in the Material and Methods section. The "mean predicted muscle concentrations" were then calculated for both indicator species at all sampling sites from individually predicted values. The difference between real (measured) and predicted THg muscle concentrations ranged between 3% and 24% in bream samples and between 2% and 26% in chub samples, depending on locality. In both species, a difference higher than 16% was found in only one (but not the same) locality and most of the sites have this difference below 10% (Table 3). These differences could be only partly explained by the uncertainty of analytical method, as that was quite low (3.5%).

Prediction of THg muscle concentration from fin-clip analysis based on using a prediction factor was found as a relatively precise method in the case of chub and bream. We suppose that a similar concentration-dependent relationship between muscle and fin-clip THg content should exist in other economically more important fish species. After a description of PFs for specific fish species, it could be used for health risk assessments. In the case of chub and bream, described PFs were found appropriate within the range of THg muscle concentrations from 0.039 to 0.661 μg g^{-1} (wet weight). This range represents common values of THg muscle concentration found in these fish species within Europe (Dusek et al., 2005; Lepom et al., 2012; Cerveny et al., 2014).

4. Conclusion

From the results of the present study, the use of fish fin-clips can be considered as an appropriate biomonitoring approach for evaluation of THg contamination in aquatic environments. Concerning the level of contamination among sampling sites, such results were brought by fin-clip analysis comparison to muscle tissue of indicator fish species. The only difference was observed in THg concentrations, which was found approximately one order of magnitude lower in fin-clips compared to muscle tissue.

The concentration-dependent relationship between muscle and fin-clip THg content was found in the case of bream and chub. On the basis of this finding, a suitable method for prediction of muscle THg concentration was developed. The use of three different prediction factors for three different ranges of fin-clip concentrations was found as an effective and a relatively precise prediction method. The difference between measured and predicted muscle concentrations was below 10% at most of localities in both indicator fish species.

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References

Abel, P.D., 1996. Water Pollution Biology. Taylor & Francis, London; Bristol, PA.
Ackerson, J.R., Mckee, M.J., Schmitt, C.J., Brumbaugh, W.G., 2014. Implementation of

a non-lethal biopsy punch monitoring program for mercury in smallmouth bass, *Micropterus dolomieu* LacepSde, from the eleven point river, Missouri. B. Environ. Contam. Toxicol. 92, 125–131.
Asefi, M., Zamani-Ahmadmahmoodi, R., 2015. Mercury concentrations and health risk assessment for two fish species, Barbus grypus and Barbus luteus, from the Maroon River, Khuzestan Province, Iran. Environ. Monit. Assess. 187.
Atchison, W.D., Hare, M.F., 1994. Mechanisms of methylmercury-induced neurotoxicity. Faseb J. 8, 622–629.
Cervenka, R., Bednarik, A., Komarek, J., Ondrackova, M., Jurajda, P., Vitek, T., Spurny, P., 2011. The relationship between the mercury concentration in fish muscles and scales/fins and its significance. Cent. Eur. J. Chem. 9, 1109–1116.
Cerveny, D., Zlabek, V., Velisek, J., Turek, J., Grabic, R., Grabicova, K., Fedorova, G., Rosmus, J., Lepic, P., Randak, T., 2014. Contamination of fish in important fishing grounds of the Czech Republic. Ecotoxicol. Environ. Saf. 109, 101–109.
Cuadrado, C., Kumpulainen, J., Moreiras, O., 1995. Lead, cadmium and mercury contents in average spanish market basket diets from galicia, valencia, andaluíca and madrid. Food Addit. Contam. 12, 107–118.
Dusek, L., Svobodova, Z., Janouskova, D., Vykusova, B., Jarkovsky, J., Smid, R., Pavlis, P., 2005. Bioaccumulation of mercury in muscle tissue of fish in the Elbe River (Czech Republic): multispecies monitoring study 1991-1996. Ecotoxicol. Environ. Saf. 61, 256–267.
European Commission (EC), 2008. Commission regulation (EC) No 629/2008 of 2 July 2008 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Union L 173–176.
European parliament and Council, 2010. Directive 2010/63/EU of 22 September 2010 on the protection of animals used for scientific purposes. Off. J. Eur. Union L 276.
European parliament and Council, 2013. Directive 2013/39/EU of 12 August 2013 amending directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Off. J. Eur. Union L 226.
Fretham, S.J.B., Caito, S., Martinez-Finley, E.J., Aschner, M., 2012. Mechanisms and modifiers of methylmercury-induced neurotoxicity. Toxicol. Res. UK (1), 32–38.
Gremillion, P.T., Cizdziel, J.V., Cody, N.R., 2005. Caudal fin mercury as a non-lethal predictor of fish-muscle mercury. Environ. Chem. 2, 96–99.
Kannan, K., Smith, R.G., Lee, R.F., Windom, H.L., Heitmuller, P.T., Macauley, J.M., Summers, J.K., 1998. Distribution of total mercury and methyl mercury in water, sediment, and fish from south Florida estuaries. Arch. Environ. Contam. Toxicol. 34, 109–118.
Kruzikova, K., Randak, T., Kensova, R., Kroupova, H., Leontovycova, D., Svobodova, Z., 2008. Mercury and methylmercury concentrations in muscle tissue of fish caught in major rivers of the Czech Republic. Acta Vet. Brno 77, 637–643.
Lasorsa, B., Allengil, S., 1995. The methylmercury to total mercury ratio in selected marine, fresh-water, and terrestrial organisms. Water Air Soil Pollut. 80, 905–913.
Lepom, P., Irmer, U., Wellmitz, J., 2012. Mercury levels and trends (1993-2009) in bream (Abramis brama L.) and zebra mussels (Dreissena polymorpha) from German surface waters. Chemosphere 86, 202–211.
Li, J., Zhou, Q., Yuan, G.L., He, X.G., Xie, P., 2015. Mercury bioaccumulation in the food web of Three Gorges Reservoir (China): tempo-spatial patterns and effect of reservoir management. Sci. Total. Environ. 527, 203–210.
Maceda-Veiga, A., Monroy, M., de Sostoa, A., 2012. Metal bioaccumulation in the Mediterranean barbel (Barbus meridionalis) in a Mediterranean River receiving effluents from urban and industrial wastewater treatment plants. Ecotoxicol. Environ. Saf. 76, 93–101.
Marsalek, P., Svobodova, Z., Randak, T., Svehla, J., 2005. Mercury and methylmercury contamination of fish from the Skalka reservoir: a case study. Acta Vet. Brno 74, 427–434.
Misztal-Szkudlinska, M., Szefer, P., Konieczka, P., Namiesnik, J., 2011. Biomagnification of mercury in trophic relation of great cormorant (Phalacrocorax carbo) and fish in the Vistula Lagoon, Poland. Environ. Monit. Assess. 176, 439–449.
Nguetseng, R., Fliedner, A., Knopf, B., Lebreton, B., Quack, M., Rudel, H., 2015. Retrospective monitoring of mercury in fish from selected European freshwater and estuary sites. Chemosphere 134, 427–434.
Noel, L., Chekri, R., Millour, S., Merlo, M., Leblanc, J.C., Guerin, T., 2013. Distribution and relationships of As, Cd, Pb and Hg in freshwater fish from five French fishing areas. Chemosphere 90, 1900–1910.
Orban, E., Nevigato, T., Masci, M., Di Lena, G., Casini, I., Caproni, R., Gambelli, L., De Angelis, P., Rampacci, M., 2007. Nutritional quality and safety of European perch (Perca fluviatilis) from three lakes of Central Italy. Food Chem. 100, 482–490.
Ouedraogo, O., Chetelat, J., Amyot, M., 2015. Bioaccumulation and trophic transfer of mercury and selenium in African sub-tropical fluvial reservoirs food webs (Burkina Faso). Plos One 10.
Piraino, M.N., Taylor, D.L., 2013. Assessment of nonlethal methods for predicting muscle tissue mercury concentrations in coastal marine fishes. Arch. Environ. Contam. Toxicol. 65, 715–723.
Ryba, S.A., Lake, J.L., Serbst, J.R., Libby, A.D., Ayvazian, S., 2008. Assessment of caudal fin clip as a non-lethal technique for predicting muscle tissue mercury concentrations in largemouth bass. Environ. Chem. 5, 200–203.
Schmitt, C.J., Brumbaugh, W.G., 2007. Evaluation of potentially nonlethal sampling methods for monitoring mercury concentrations in smallmouth bass (Micropterus dolomieu). Arch. Environ. Contam. Toxicol. 53, 84–95.
Vanhattum, B., Korthals, G., Vanstraalen, N.M., Govers, H.A.J., Joosse, E.N.G., 1993. Accumulation patterns of trace-metals in fresh-water isopods in sediment bioassays – influence of substrate characteristics, temperature and ph. Water Res. 27, 669–684.

SUPPLEMENTARY MATERIAL

Table S1. *Characteristics of sampled fish and concentrations of total mercury (THg) in muscle and fin-clips samples.*

Locality	Species	n	Total length (mm)			Weight (g)			THg muscle concentration (μg g^{-1})			THg fin concentration (μg g^{-1})		
			mean	(min – max)		mean	(min – max)		mean	(min – max)		mean	(min – max)	
Neratovice	*Abramis brama*	10	401	(340 – 470)		673	(420 – 1050)		0.454	(0.258 – 0.643)		0.043	(0.024 – 0.094)	
	Squalius cephalus	10	355	(290 – 440)		569	(280 – 1125)		0.303	(0.113 – 0.646)		0.03	(0.012 – 0.056)	
Kostelec	*Abramis brama*	10	430	(380 – 460)		822	(530 – 1010)		0.415	(0.205 – 0.525)		0.037	(0.023 – 0.069)	
	Squalius cephalus	10	305	(190 – 490)		452	(65 – 1535)		0.248	(0.107 – 0.644)		0.029	(0.009 – 0.105)	
Obristvi	*Abramis brama*	10	386	(310 – 445)		596	(299 – 866)		0.491	(0.365 – 0.661)		0.043	(0.024 – 0.074)	
	Squalius cephalus	10	385	(270 – 575)		596	(230 – 1030)		0.305	(0.163 – 0.601)		0.032	(0.012 – 0.083)	
Mlekojedy	*Abramis brama*	10	364	(330 – 445)		444	(345 – 620)		0.222	(0.059 – 0.327)		0.012	(0.005 – 0.026)	
	Squalius cephalus	0[a]												
Hnevkovice	*Abramis brama*	10	395	(275 – 470)		691	(265 – 1115)		0.272	(0.188 – 0.385)		0.014	(0.011 – 0.019)	
	Squalius cephalus	10	348	(220 – 500)		614	(115 – 1580)		0.181	(0.04 – 0.356)		0.012	(0.003 – 0.033)	
Rimov	*Abramis brama*	10	418	(340 – 470)		824	(480 – 965)		0.433	(0.255 – 0.586)		0.028	(0.012 – 0.041)	
	Squalius cephalus	10	335	(230 – 440)		424	(120 – 835)		0.223	(0.094 – 0.504)		0.02	(0.005 – 0.072)	

CHAPTER 6

PRESENCE OF PHARMACEUTICALS IN BENTHIC FAUNA LIVING IN A SMALL STREAM AFFECTED BY EFFLUENT FROM A MUNICIPAL SEWAGE TREATMENT PLANT

Grabicova, K., Grabic, R., Blaha, M., Kumar, V., Cervený, D., Fedorova, G., Randak, T., 2015. Presence of pharmaceuticals in benthic fauna living in a small stream affected by effluent from a municipal sewage treatment plant. Water Research 72: 145–153.

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Presence of pharmaceuticals in benthic fauna living in a small stream affected by effluent from a municipal sewage treatment plant

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ABSTRACT

Aquatic organisms can be affected not only via polluted water but also via their food. In the present study, we examined bioaccumulation of seventy pharmaceuticals in two benthic organisms, *Hydropsyche* sp. and *Erpobdella octoculata* in a small stream affected by the effluent from a sewage treatment plant (STP) in Prachatice (South Bohemia region, Czech Republic).

Furthermore, water samples from similar locations were analyzed for all seventy pharmaceuticals. In water samples from a control locality situated upstream of the STP, ten of the seventy pharmaceuticals were found with average total concentrations of 200 ng L^{-1}. In water samples collected at STP-affected sites (downstream the STP's effluent), twenty-nine, twenty-seven and twenty-nine pharmaceuticals were determined at average total concentrations of 2000, 2100 and 1700 ng L^{-1}, respectively.

Six of the seventy pharmaceuticals (azithromycin, citalopram, clarithromycin, clotrimazole, sertraline, and verapamil) were found in *Hydropsyche*. Four pharmaceuticals (clotrimazole, diclofenac, sertraline, and valsartan) were detected in *Erpobdella*. Using evaluation criterion bioconcentration factor (BCF) is higher than 2000 we can assign azithromycin and sertraline as bioaccumulative pharmaceuticals. Even pharmaceuticals present at low levels in water were found in benthic organisms at relatively high concentrations (up to 85 ng g^{-1} w.w. for azithromycin). Consequently, the uptake of pharmaceuticals via the food web could be an important exposure pathway for the wild fish population.

1. Introduction

Effluent of municipal sewage treatment plants (STPs) contains numerous organic and inorganic pollutants due to insufficient removal efficiency during the treatment processes (Golovko et al., 2014a; Halling-Sorensen et al., 1998; Heberer, 2002; Petrovic et al., 2003). This incomplete removal broadly reflects the pharmaceuticals mixture to which fish and other aquatic organisms are typically exposed (Verlicchi et al., 2012).

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Most of the research examining the possible bioconcentration of environmental contaminants in fish has focused on either controlled laboratory conditions associated with known individual pharmaceuticals or mixture exposure (e.g. Brozinski et al., 2013; Cuklev et al., 2012; Lahti et al., 2011; Nallani et al., 2011; Steinbach et al., 2013) or on fish placed into cages and exposed directly to the effluent (e.g. Lajeunesse et al., 2011; Togunde et al., 2012). It means that bioconcentration is the prevailing or only exposure mechanism. However, intake of pollutants via contaminated natural food is completely ignored in these experimental setups. Depending on the exposure period, fish are either not fed or artificial feed is used. In the case of real conditions, fish in cages can be stressed and outcomes of these experiments do not truly mimic the natural conditions.

Aquatic organisms are exposed not only via the discharge of sewage waters but also via their food web. Benthic fauna is a very important part of the food web in aquatic environments (e.g. Hellmann et al., 2013; Hildrew, 1992). Benthic organisms are often used as bioindicators for assessing environmental pollution due to their limited movability and relatively easy sampling (Clews et al., 2014; Cortes et al., 2013; Pan et al., 2012; Smith et al., 1999). Larvae of caddisflies (*Hydropsyche* sp.) and leeches (*Erpobdella* sp.) are often used as indicators of water quality or pollution in streams (Azrina et al., 2006; Koperski, 2005, 2010; Sola and Prat, 2006; Stuijfzand et al., 1999; Tessier et al., 2000). These species have been used for monitoring of riverine pollution in the Czech Republic during the last decade (Kolarikova et al., 2012; Macova et al., 2009). These organisms significantly contribute to a fish diet as well (Elliott, 1967; Greenberg and Dahl, 1998; Laine, 2001; Reiriz et al., 1998).

The aim of this study was to investigate pharmaceutical levels in benthic organisms and consequently to reveal their importance in the exposure pathways of the fish.

2. Material and methods

2.1. *Sampling location*

Benthic organisms and water samples were collected in the Zivny stream (a tributary of Blanice River) situated in the south part of the Czech Republic, during May 2013. The stream is 13 km long with an average depth of 30 cm and an average width of 3 m. Flow varied from 0.150 to 0.600 $m^3\ s^{-1}$. The stream is highly impacted by effluent from the Prachatice STP. Prachatice (12,000 inhabitants) is a district town. There is only light industry (food, machinery and electronics) and a hospital. The STP's effluent can make up approximately 25% of the water flow in the Zivny stream. The details of treatment processes and permissible limits for final effluent from the STP are given in Supplementary material (S1). Except for stretches in Prachatice, the stream habitat is natural or seminatural. The fish community is dominated by brown trout (*Salmo trutta* m. *fario* L.) with occurrences of other riverine species, including stone loach (*Barbatula barbatula* L.) and bullhead (*Cottus gobio*). Benthic invertebrate organisms, specifically *Hydropsyche* sp. and *Erpobdella octoculata*, were chosen for this study. Both of organisms are present in all (or in a majority) of sampled sites situated along the stream and make up an important part of the fish diet. Larvae of *Hydropsyche* are net-spinning omnivores (Benke and Wallace, 1997; Hellmann

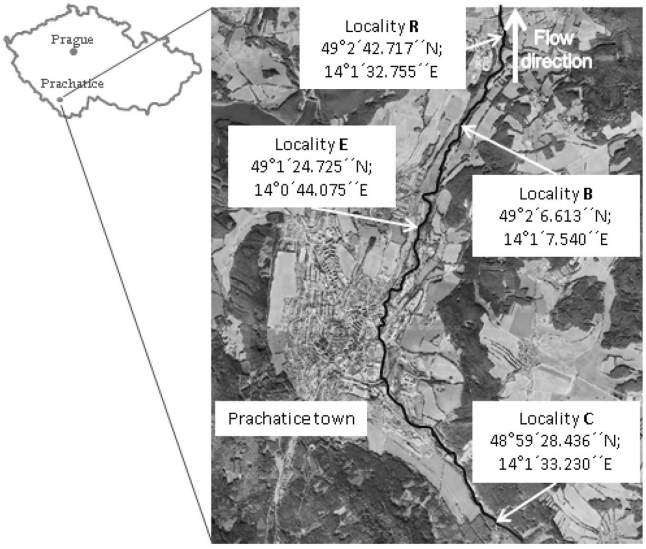

Fig. 1 – Sampling localities of the Zivny stream. Site E – area where the STP's effluent enters the Zivny stream; Site B – approx. 1.5 km downstream of the STP; Site R – approx. 3 km downstream of the STP; Site C – non-polluted area (approx. 4.5 km upstream of the STP).

et al., 2013), using resources from multiple trophic levels. Leeches, *E. octoculata* (*Hirudinea*) are predators feeding especially on oligochates and chironomid larvae (Schenkova et al., 2007).

2.2. Sample collection

Grab water samples were collected seven times during two weeks in April–May 2013 with the typical weather conditions for this season (total precipitation 38 mm during the sampling period – about 70% of long term average) in three locations: the area 100 m downstream the place where the STP's effluent enters the stream (site E), 1.5 km downstream (site B), and 3 km downstream (site R), every time on the same place. Water samples from a non-contaminated area – 4.5 km upstream from the source of pollution (site C) – were used as a control (Fig. 1). Samples were filtered (0.45 μm regenerated cellulose filters, Labicom, Olomouc, Czech Republic) and frozen at −20 °C until the analysis.

The benthic organisms were collected from the same localities as the water samples. Individuals were picked up with tweezers, immediately frozen and stored at −20 °C until the analysis. *Hydropsyche* sp. was found in all of the sampled sites in sufficient amount, *E. octoculata* was found only in the sites situated downstream of the STP Prachatice (i.e. sites E, B, R).

2.3. Chemicals

Liquid chromatography mass spectrometry- (LC/MS-) grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Formic acid (LC/MS grade) was obtained from Sigma–Aldrich (Steinheim, Germany).

Internal standard stocks were prepared from mass-labeled crystalline compounds of amitriptyline, atenolol, carbamazepine, clarithromycin, sulfamethoxazole, and trimethoprim. Sulfamethoxazole ($^{13}C_6$) and trimethoprim ($^{13}C_3$) were purchased from Cambridge Isotope Laboratories; carbamazepine (D_{10}) and amitriptyline (D_6) from CDN Isotopes; atenolol (D_6) from Alsa Chim; and clarithromycin (D_3) from Toronto Research Chemicals, Inc.

Stock solutions of native compounds were prepared from the crystalline compounds (Supplementary material S2) at the concentration of 1 mg mL^{-1} in methanol and stored at −20 °C. Working solutions were prepared mixing and diluting stock solutions to concentration 1 μg mL^{-1}.

2.4. Water analysis

A triple-stage quadrupole MS/MS TSQ Quantum Ultra EMR (Thermo Fisher Scientific), equipped with Accela 1250 and 600

Table 1 – Average concentration [ng L^{-1}] of positive pharmaceuticals in the water samples collected from the Zivny stream from 24th April to 10th May 2013. For average concentration of all analyzed pharmaceuticals see Supplementary material S7.

Therapeutic class	Pharmaceutical	Concentration in water [ng L^{-1}]				LOQ range [ng L^{-1}]
		Locality C	Locality E	Locality B	Locality R	
Analgesics	Diclofenac	<LOQ	94 ± 52	91 ± 24	65 ± 23	2–3
	Tramadol	7 ± 3	230 ± 150	270 ± 100	200 ± 80	1–2
Antihistaminic	Fexofenadine	<LOQ	15 ± 11	15 ± 8	9 ± 6	1
Antibiotics	Azithromycin	<LOQ	8 ± 7	6 ± 5	2 ± 1	1–2
	Clarithromycin	<LOQ	120 ± 70	130 ± 60	88 ± 45	1
	Clindamycin	<LOQ	7 ± 4	8 ± 4	5 ± 3	1
	Erythromycin	<LOQ	23 ± 19	22 ± 13	15 ± 9	1–2
	Sulfamethizole	<LOQ	<LOQ	<LOQ	3 ± 1	1–4
	Sulfamethoxazole	<LOQ	32 ± 20	28 ± 12	20 ± 11	3–4
	Trimethoprim	2 ± 1	75 ± 48	84 ± 28	55 ± 20	1–3
Antifungal	Clotrimazole	4 ± 1	5 ± 3	7 ± 4	6 ± 2	2–3
Cardiovascular drugs	Atenolol	<LOQ	95 ± 61	100 ± 40	69 ± 29	6–21
	Bisoprolol	<LOQ	40 ± 24	38 ± 13	29 ± 9	3–5
	Diltiazem	<LOQ	2 ± 1	<LOQ	<LOQ	1
	Dipyridamole	<LOQ	13 ± 10	11 ± 7	5 ± 1	1
	Eprosartan	6 ± 2	67 ± 32	37 ± 17	22 ± 15	1
	Irbesartan	5 ± 4	79 ± 43	88 ± 24	74 ± 25	1–2
	Metoprolol	7 ± 4	210 ± 140	190 ± 70	130 ± 60	1–4
	Sotalol	<LOQ	22 ± 3	24 ± 3	17 ± 2	7–23
	Valsartan	8 ± 3	200 ± 110	210 ± 80	150 ± 50	1
	Verapamil	<LOQ	9 ± 6	8 ± 3	4 ± 1	1
Lipid regulator	Atorvastatin	<LOQ	25 ± 31	7 ± 5	8 ± 7	2–4
Psychoactive drugs	Carbamazepine	<LOQ	110 ± 60	100 ± 40	76 ± 27	1–2
	Citalopram	<LOQ	29 ± 19	27 ± 10	19 ± 3	3–5
	Memantine	<LOQ	4 ± 2	< LOQ	4 ± 1	2–3
	Mirtazapine	3 ± 2	3 ± 1	3 ± 1	2 ± 1	1–2
	Risperidone	4 ± 2	2 ± 1	2 ± 1	4 ± 1	1
	Sertraline	2 ± 1	5 ± 3	3 ± 1	2 ± 1	1–2
	Venlafaxine	<LOQ	74 ± 42	76 ± 28	55 ± 21	4–5
Sedative	Oxazepam	<LOQ	12 ± 6	11 ± 4	10 ± 3	1

For legend, see Fig. 1. LOQ = limit of quantification; $n = 7$.

Table 2 – Concentration of positive findings of pharmaceuticals [ng g^{-1} w.w.] in *Hydropsyche* sp.

Pharmaceutical	Concentration [ng g^{-1}]								LOQ [ng g^{-1}]	Recovery [%]
	Locality C	F	Locality E	F	Locality B	F	Locality R	F		
Azithromycin	<LOQ[a]	0/6	40 ± 7[b]	6/6	85 ± 8[d]	5/5	68 ± 9[c]	5/5	3.0–4.2	139
Clarithromycin	<LOQ[a]	0/6	3.1 ± 1.6[a]	6/6	2.2 ± 0.8[a]	3/5	2.9 ± 0.1[a]	2/5	1.4–1.9	85
Verapamil	<LOQ[a]	0/6	3.5 ± 0.9[b]	6/6	3.1 ± 0.9[b]	5/5	2.7 ± 0.4[b]	5/5	1.0–1.4	85
Citalopram	<LOQ[a]	0/6	4.2 ± 1.1[b]	6/6	3.8 ± 0.8[b]	5/5	3.6 ± 0.9[b]	5/5	1.4–2.8	150
Sertraline	<LOQ[a]	0/6	4.6 ± 0.8[b]	6/6	5.5 ± 1.6[b]	5/5	4.3 ± 0.8[b]	5/5	1.1–2.0	143
Clotrimazole	3.6 ± 0.3[a]	3/6	2.3 ± 1.2[a]	5/6	1.4 ± 0.1[a]	4/5	1.4 ± 0.2[a]	4/5	1.1–4.4	91

For legend see Fig. 1; [a,b,c,d] – statistically significant differences ($p < 0.05$); F – frequency of detection, number of positive sample/number of all samples.

LC pumps (Thermo Fisher Scientific) and a PAL HTC autosampler (CTC Analytics AG) operated using Xcalibur software (Thermo Fisher Scientific), was connected with in-line solid phase extraction (SPE LC-MS/MS) and used for analysis of water.

Water samples were thawed and filtered through a regenerated cellulose syringe filter (0.45 μm pores). The samples were analyzed by the in-line SPE/LC-MS/MS method after internal standards addition. The description of the method can be found elsewhere (Fedorova et al., 2014a; Lindberg et al., 2014). Briefly, two analytical columns in two runs were used for separation of target analytes – Hypersil Gold column (50 mm × 2.1 mm ID × 3 μm particles; Thermo Fisher Scientific) and Cogent Bidentate column (50 mm × 2.1 mm ID × 4 μm particles; Microsolv Technology Corporation). In-line extraction was performed at Hypersil Gold column (20 mm × 2.1 mm ID × 12 μm particles; Thermo Fisher Scientific) in both LC runs, in accordance with the method described by Fedorova et al. (2014a). A detailed description of the analytical method is given in the Supplementary material (S3). The limits of quantification (LOQs) for the water samples are given in Table 1. The trueness of the analytical method has been reported previously (Fedorova et al., 2014a).

2.5. *Benthos analysis*

Extracts from benthos were prepared according to Fedorova et al. (2014b). Collected amounts of both *Hydropsyche* sp. and *E. octoculata* from each locality were sufficient for five to six parallel samples. Briefly, three individuals of one species from the same locality were cut into small pieces when thawed for each replicate sample. Around one half gram of this sample was weighted and internal standards were added to each sample. The mixture was then homogenized (TissueLyser II, Quiagen, Germany; 1800 min^{-1} for 10 min) with acetonitrile (acidified 0.1% formic acid) using a stainless steel ball. The samples were then centrifuged (Micro 200R centrifuge, Hettich Zentrifugen, Germany; 9500 × *g* for 10 min) and filtered (0.45 μm regenerated cellulose filters, Labicom, Olomouc, Czech Republic). The supernatant was frozen at −20 °C for 24 h then centrifuged again to remove precipitated proteins and other solid particles from the samples. 100 μL aliquot was taken to vials for analysis. A Q-Exactive mass spectrometer (Thermo Fisher Scientific) coupled with an Accela 1250 LC pump (Thermo Fisher Scientific) and HTS XT-CTC autosampler (CTC Analytics AG), was used for the analysis of the extracts from benthic organisms. Extracts were analyzed by liquid chromatography with tandem mass spectrometry in high resolution product scan mode using the same two chromatography columns mentioned above. The analytical method is described in Supplementary Material (S4, S5). The trueness of the method (as a recovery of fortified samples) together with the LOQs is given in Table 2 and Table 3 (LOQs for all compounds analyzed in extracts from benthos are given in Supplementary Material S6).

2.6. *Statistical analysis*

The data are presented as mean ± SD. Statistical analysis of the data was conducted with the STATISTICA v.12 software for Windows (StatSoft, Czech Republic). Normally distributed and homoscedastic data (as assessed by Cochran, Hartley and Barlett tests) were appraised by a one-way ANOVA (Tukey test). When data were not normally distributed and homoscedastic, the non-parametric method of data analysis was used (Kruskal–Wallis test). Differences were considered statistically significant at $p < 0.05$. One half of the LOQ value was used in the data sets when some of the results were found below the LOQ.

Table 3 – Concentration of positive findings of pharmaceuticals [ng g^{-1} w.w.] in *Erpodella octoculata*.

Pharmaceutical	Concentration [ng g^{-1}]						LOQ [ng g^{-1}]	Recovery [%]
	Locality E	F	Locality B	F	Locality R	F		
Valsartan	2.3 ± 1.0[a]	6/6	1.3 ± 0.5[b]	6/6	<LOQ[b]	0/5	0.49–0.73	101
Sertraline	5.6 ± 0.8[a]	6/6	4.9 ± 0.6[a]	6/6	4.2 ± 2.6[a]	5/5	0.82–1.1	125
Diclofenac	33 ± 13[a]	6/6	14 ± 3[b]	6/6	12 ± 4[b]	5/5	1.3–1.9	120
Clotrimazole	2.5 ± 0.3[a]	6/6	1.4 ± 0.2[b]	6/6	1.2 ± 0.1[b]	5/5	0.72–0.94	68

For legend see Fig. 1; [a,b] – statistically significant differences ($p < 0.05$); F – frequency of detection, number of positive sample/number of all samples.

3. Results and discussion

3.1. *Pharmaceuticals in water*

Mean water concentrations of positive pharmaceuticals measured in water samples are given in Table 1 (mean water concentrations of all seventy analyzed pharmaceuticals are given in Supplementary material S7). Fifteen of the seventy analyzed pharmaceuticals with the highest observed concentrations belong to a group of antibiotics (clarithromycin, trimethoprim and sulfamethoxazole), cardiovascular drugs (irbesartan, valsartan, eprosartan, sotalol, atenolol, metoprolol and bisoprolol), psychoactive drugs (venlafaxine, citalopram and carbamazepine), and analgesics (diclofenac and tramadol). The highest concentrations were 130 (clarithromycin), 210 (metoprolol and valsartan), 110 (carbamazepine), and 270 (tramadol) ng L^{-1} for antibiotics, cardiovascular drugs, psychoactive drugs, and analgesics, respectively. This pattern corresponds to consumption within the Czech population and the resistance of pharmaceuticals to the treatment processes (Golovko et al., 2014a, b).

Fig. 2 shows total concentration of pharmaceuticals in a longitudinal profile of the stream and the data normalized to carbamazepine concentration (daily results normalized and then summed) were added to graph. Total concentration in site C (upstream), as well as the number of pharmaceuticals above LOQ, is lower than those in sites downstream of the effluent released from the STP. Statistical testing of the data was performed for all pharmaceuticals to determine whether or not the differences among the localities are significant. This observation suggests that this STP potentially contributed to the pollution by pharmaceuticals in the downstream sites. However, a few exceptions to this pattern were identified. For example, antifungal and psychoactive drugs, namely clotrimazole (4–7 ng L^{-1}), mirtazapine (2–3 ng L^{-1}), sertraline (2–5 ng L^{-1}) and risperidone (2–4 ng L^{-1}), were found in almost the same (very low) levels in all localities. This finding could be attributed to relatively high K_{ow} and K_d of this compounds and their presence at particle surface rather than in water phase

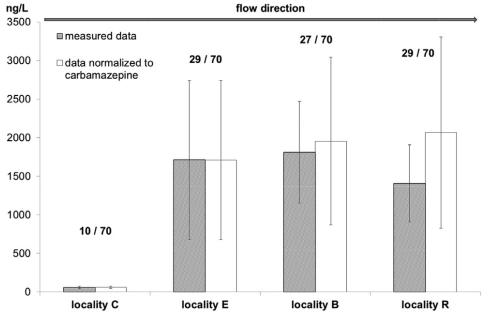

Fig. 2 – Total concentrations of pharmaceuticals in water samples in a longitudinal profile of the Zivny stream. Numbers given in the graph refer to positive/analyzed pharmaceuticals. For legend see Fig. 1.

(Horsing et al., 2011). Some other pharmaceuticals, such as memantine (4 ng L^{-1}) and diltiazem (2 ng L^{-1}), were determined at low levels but only at sites E and R. Statistically significant differences between upstream and all polluted downstream sites were found in the concentrations of all other pharmaceuticals. However, for most of pharmaceuticals, there were only small differences in concentration among the downstream sites (R, E and B), particularly between E and R. Considering the variability of water concentrations at each locality, the differences seem to be within this variability. These observations could be explained by the short distance between site B and site E (approx. 1.5 km), hence less time for degradation, sorption or transformation processes. However, we observed a decrease in atorvastatin, azithromycin, citalopram, clindamycin, diclofenac, dipyridamole, eprosartan, erythromycin, sotalol, sulfamethoxazole, trimethoprim, and verapamil concentrations at site R. To reveal potential dilution by tributaries, water concentration of each pharmaceutical was normalized to carbamazepine concentration in the site E. The same trend between sites E and B was confirmed, however, normalized sum of pharmaceuticals concentration in locality R was slightly higher. With respect to enhanced variability of normalized data we can attribute slightly lower concentration in the site R to dilution.

3.2. *Pharmaceuticals in benthic organisms*

The same range of pharmaceuticals as in the water samples was analyzed in benthic organisms living in the recipient stream.

Six of the pharmaceuticals analyzed were found above LOQ in *Hydropsyche* sp. – the antibiotics azithromycin and clarithromycin, the cardiovascular drug verapamil, the antifungal drug clotrimazole, and the antidepressants citalopram and sertraline (Table 2). In particular, clarithromycin, diclofenac, and valsartan represent the major water pollutants. All other compounds were found in water at very low concentrations (maximally low tens of ng L^{-1}). Four pharmaceuticals were detected in *E. octoculata* – the cardiovascular drug valsartan, the antidepressant sertraline, the anti-inflammatory drug diclofenac, and the antifungal drug clotrimazole (Table 3). All the compounds determined in benthic organisms have moderate to high partition coefficient (log *P*) ranging from 3.2 to 6.3, hence proportion of sorption to benthic organisms is expected. Longitudinal profile of particular compounds concentrations is shown in Fig. 3. A comparison of the pharmaceuticals concentrations in extracts from *Erpobdella* between polluted sites (E, B and R) and non-polluted site (locality C) is unfortunately not possible, because *Erpobdella* were not found in the non-polluted site. The localities where *Erpobdella* were collected in the Zivny stream (localities E, B, R, it means localities with sufficient amount of nutrients) correspond to areas in the Malaysian Langat river, where this subclass of benthic organisms was also found only in eutrophic areas (Azrina et al., 2006).

The concentration of pharmaceuticals in the benthic biota ranged from 1.2 to 85 ng g^{-1} wet weight (w.w.) in samples from polluted sites, whilst below LOQ in the upstream control site except for levels of clotrimazole (3.6 ng g^{-1}) in three of six samples. However, this finding corresponds to a trend observed in concentrations of this compound in water. There

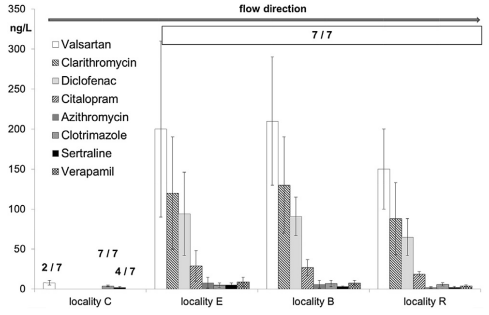

Fig. 3 – Concentrations of in benthos positively found pharmaceuticals in water samples in a longitudinal profile of the Zivny stream. Numbers given in the graph refer to positive/analyzed pharmaceuticals. For legend see Fig. 1.

was a significant decrease of concentration in sites downstream of the STP compared to the locality C; on the other hand, only one half of the clotrimazole samples from the control site were found above LOQ. Unfortunately, the other sampled species was not present in the control site (locality C), so we could not confirm the presence of clotrimazole in biota there. *Erpobdella* samples from sites E, B and R contained clotrimazole at about the same level as in *Hydropsyche*. Sertraline and clotrimazole were detected in both species. Sertraline was not present in samples from the control site; concentration levels were about the same in *Hydropsyche* (4.3–5.5 ng g^{-1}) and *Erpobdella* (4.2–5.6 ng g^{-1}), and concentrations did not shown significant differences among polluted localities.

Azithromycin (up to 85 ng g^{-1} w.w.) and diclofenac (up to 33 ng g^{-1} w.w.) were the highest measured pharmaceuticals in *Hydropsyche* and *Erpobdella*, respectively. While bioaccumulation in fish was previously reported for diclofenac (Lahti et al., 2011; Mehinto et al., 2010), azithromycin has not yet been detected in aquatic organisms.

There was no relationship between water and benthos azithromycin concentration. Based on previously reported strong sorption of azithromycin to bottom sediment (Gibs et al., 2013) and low concentration in sampled water, we can conclude that particles associated with this compound will be main source of this antibiotic in *Hydropsyche*. Diclofenac concentrations in *Erpobdella* followed a decrease of water concentration with longer distances from the STP. This trend was also obvious for the valsartan found in *Erpobdella*. Another three compounds found in *Hydropsyche* (citalopram, clarithromycin and verapamil) were not present in the control group, and there were no differences in these pharmaceuticals among all three polluted sites. However, clarithromycin was very close to LOQ and, contrary to all other compounds found in benthos, it was not present in all samples from sites B and R.

3.2.1. *Bioaccumulation*

As we cannot exclude exposure via contaminated food web, bioaccumulation factors (BAF) were calculated from the specific compound concentrations inside the benthos tissues to that of surrounding water (Table 4). Nevertheless, there is only limited information on the bioconcentration or bioaccumulation of pharmaceuticals in invertebrates. However, comparison with predicted BCF values is possible. Only two of six compounds exhibit lower BAF compare to predicted BCF. Valsartan in *Erpobdella* has five to ten times lower BAF then predicted BCF, while its water concentration is high. In this case the steady state between water and organism could be considered instead of the bioconcentration. Another compound with lower calculated BAF then predicted BCF is clotrimazole. The predicted BCF value is order of magnitude higher then calculated BAFs. However, it seems to be close to BCF which was estimated from measured K_{ow} (OSPAR, 2005) and in accordance with BCF previously published for fish (Corcoran et al., 2014). Anyhow, good agreement with BAF values for both species is a bit surprising taking into account the low clotrimazole levels in water and tissue.

Clarithromycin and azithromycin are structurally very similar antibiotics. Clarithromycin shows a good agreement of

Table 4 – Measured plasma bioaccumulation factors (BAF) in *Hydropsyche* sp. ($n = 6$) and *Erpobdella octoculata* ($n = 6$), and predicted plasma bioconcentration factors (BCF).

Pharmaceutical	BAF *Hydropsyche* sp.				BAF *Erpobdella octoculata*				Predicted BCF[c]
	Lokality C	E	B	R	Lokality C[a]	E	B	R	
Azithromycin		5000	14,000	34,000					29
Citalopram		145	140	190					71
Clarithromycin		26	17	33					29
Clotrimazole	900	460	200	230	–	500	200	200	610[d]
Diclofenac					–	350	150	180	93
Sertraline	<360[b]	920	1800	2100	–	1100	1600	2100	959
Valsartan					–	12	6.2	<3.3[b]	61
Verapamil		390	390	670					40

LOQ is shown in Tables 2 and 3.

[a] No *Erpobdella octoculata* were present at this site.

[b] Maximum estimated BAF. Analyte was not detected in biota so the concentration was set at the LOQ to estimate maximum BCF.

[c] Fick et al., 2010.

[d] Ospar 2005.

measured BAF with predicted BCF. Together with relatively high water concentration it can be steady state or bioconcentration assumed. Despite of the same predicted BCF, calculated BAF for azithromycin was several orders of magnitude higher. As mentioned above, high sorption to particles was observed for this compound. It can be concluded that different exposure pathways are involved.

For the rest of the pharmaceuticals, calculated BAFs were higher than the predicted BCFs. This could be simply connected with the uncertainties or variability of water concentration of those pharmaceuticals, however, exposure via food web or contact with contaminated sediment can contribute to resulting BAF value.

Presence of diclofenac at relatively high level in *Erpobdella* only is a bit surprising results as this pharmaceutical was previously reported as bioaccumulating in fish (Fick et al., 2010; Brown et al., 2007). On the other hand diclofenac was proved as cumulating in the worm body by C14 study but consequent LC/HRMS analysis revealed it presence as metabolite (Carter et al., 2014).

Evaluating bioaccumulation potential of pharmaceuticals with criterion of BCF (BAF in our case) > 2000, one can conclude that azithromycin and sertraline were found bioaccumulated. Citalopram, clotrimazole, diclofenac and verapamil have potential to be accumulated in the organisms but accumulation is in equilibrium with metabolic transformation and/or excretion from the body. Whilst, presence of valsartan and clarithromycin in the organisms could be attributed to steady state with water concentration.

Both sampled species belong to predator benthic fauna, but they have different body composition, metabolism, behavior, and different feeding strategy. While *Hydropsyche* has a soft body with a high content of fat [up to 8% of w.w. (Meier et al., 2000)], *Erpobdella* has hard muscle body created by protein fibers.

Hydropsyche species are opportunistic in their feeding functions, because of large differences in food availability or abiotic conditions (Scott, 1958; Fuller and Mackay, 1980). Thus, the proportion of animal prey in the diet varies during season (Hellmann et al., 2013), in some stream ecosystems *Hydropsychid* larvae feed almost exclusively on animal prey (Benke et al., 2001) whilst other authors found out less than 50% animal proportion in theirs gut (Basaguren et al., 2002). *Erpobdella octoculata* is a predator which mostly feed on chironomid larvae, which composed up to 80% of gut content and oligochates (Schönborn, 1985; Toman and Dall, 1997). However, laboratory experiments have shown that this species is also sucking the body fluids from dead, decaying bodies of vertebrates (Kutschera, 2003). Further experiments focused on the studied compound metabolites are needed to clarify if feeding strategy or specific metabolisms are responsible for differences in the pharmaceutical patterns observed in whole body homogenates between these organisms.

Benthic organisms are major components of the brown trout diet. *Trichoptera* (including *Hydropsychidae*) contributes usually more than 50% of consumed food (Fochetti et al., 2003, 2008). According to our experiences, *Erpobdella* was seasonally (especially in spring after rain) found in stomach of fish caught in this sampling stream but *Annelida* (Erpobdellidae) does not contribute significantly to brown trout diet (Fochetti et al., 2003, 2008). Daily food intake for brown trout is about 2% of body weight. In the worst case scenario (*Hydropsyche* is only the food), pharmaceuticals uptake via food can be estimated from about 3 ng per 100 g of body weight for clotrimazole at the site R to 170 ng per 100 g of body weight for azithromycin. Therefore pharmaceutical uptake via the food web can play an important role, especially for compounds which were found at relatively low concentration level in water, i.e. azithromycin, sertraline, citalopram, verapamil and clotrimazole.

There are only a limited number of articles reporting the fate and effect of STP-originated pharmaceutical mixtures on benthic organisms. The studies mainly focus on biochemical responses (Damasio et al., 2011), biodiversity (Azrina et al., 2006; Koperski, 2010; Pan et al., 2012), and survival (Stuijfzand et al., 1999). To our knowledge, this is the first report on pharmaceuticals in benthic fauna living in a stream affected by an STP's effluent.

4. Conclusions

The results clearly indicate that STP effluent was the major source of pharmaceuticals in the Zivny stream (a tributary of Blanice River). Extended measurement of these pharmaceuticals in benthic organisms further showed their bioaccumulation potential. Eight of the seventy analyzed pharmaceuticals were found in benthic organisms *Hydropsyche* sp. or *E. octoculata* (azithromycin, citalopram, clarithromycin, clotrimazole, diclofenac, sertraline, valsartan, and verapamil), supporting the environmental stability of these compounds. Using evaluation criterion BCF >2000 we can assign azithromycin and sertraline as bioaccumulative.

As benthic organisms are an important part of the fish diet (especially *Hydropsyche*) the uptake of pharmaceuticals via the food web could be an important exposure pathway in the wild fish population. This study illustrates how the pharmaceuticals can enter into the aquatic food web, which is not necessarily limited to water-phase concentrations.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2014.09.018.

REFERENCES

Azrina, M.Z., Yap, C.K., Ismail, A.R., Ismail, A., Tan, S.G., 2006. Anthropogenic impacts on the distribution and biodiversity of benthic macroinvertebrates and water quality of the Langat River, Peninsular Malaysia. Ecotoxicol. Environ. Saf. 64 (3), 337–347.

Basaguren, A., Riano, P., Pozo, J., 2002. Life history patterns and dietary changes of several caddisfly (Trichoptera) species in a northern Spain stream. Arch. für Hydrobiol. 155, 23–41.

Benke, A.C., Wallace, J.B., 1997. Trophic basis of production among riverine caddisflies: implications for food web analysis. Ecology 78 (4), 1132–1145.

Benke, A.C., Wallace, J.B., Harrison, J.W., Koebel, J.W., 2001. Food web quantification using secondary production analysis: predaceous invertebrates of the snag habitat in a subtropical river. Freshw. Biol. 46, 329–346.

Brown, J.N., Paxeus, N., Förlin, L., Larsson, D.G.J., 2007. Variations in bioconcentration of human pharmaceuticals from sewage effluents into fish blood plasma. Environ. Toxicol. Pharmacol. 24, 267–274.

Brozinski, J.M., Lahti, M., Meierjohann, A., Oikari, A., Kronberg, L., 2013. The anti-inflammatory drugs diclofenac, naproxen and ibuprofen are found in the Bile of wild fish caught downstream of a wastewater treatment plant. Environ. Sci. Technol. 47, 342–348.

Carter, L.J., Garman, C.D., Ryan, J., Dowle, A., Bergstrom, E., Thomas-Oates, J., Boxall, A.B.A., 2014. Fate and uptake of pharmaceuticals in soil-earthworm systems. Environ. Sci. Technol. 48, 5955–5963.

Clews, E., Low, E.W., Belle, C.C., Todd, P.A., Eikaas, H.S., Ng, P.K.L., 2014. A pilot macroinvertebrate index of the water quality of Singapore's reservoirs. Ecol. Indic. 38, 90–103.

Corcoran, J., Lange, A., Cumming, R.I., Owen, S.F., Ball, J.S., Tyler, C.R., Winter, M.J., 2014. Bioavailability of the imidazole antifungal agent clotrimazole and its effects on key biotransformation genes in the common carp (*Cyprinus carpio*). Aquat. Toxicol. 152, 57–65.

Cortes, R.M.V., Hughes, S.J., Pereira, V.R., Varandas, S.D.P., 2013. Tools for bioindicator assessment in rivers: the importance of spatial scale, land use patterns and biotic integration. Ecol. Indic. 34, 460–477.

Cuklev, F., Fick, J., Cvijovic, M., Kristiansson, E., Forlin, L., Larsson, D.G.J., 2012. Does ketoprofen or diclofenac pose the lowest risk to fish? J. Hazard. Mater. 229, 100–106.

Damasio, J., Fernandez-Sanjuan, M., Sanchez-Avila, J., Lacorte, S., Prat, N., Rieradevall, M., Soares, A., Barata, C., 2011. Multi-biochemical responses of benthic macroinvertebrate species as a complementary tool to diagnose the cause of community impairment in polluted rivers. Water Res. 45 (12), 3599–3613.

Elliott, J.M., 1967. The food of trout (*Salmo trutta*) in a Dartmoor stream. J. Appl. Ecol. 4, 59–71.

Fedorova, G., Golovko, O., Randak, T., Grabic, R., 2014a. Storage effect on the analysis of pharmaceuticals and personal care products in wastewater. Chemosphere 111, 55–60.

Fedorova, G., Nebesky, V., Randak, T., Grabic, R., 2014b. Simultaneous determination of 32 antibiotics in aquaculture products using LC-MS/MS. Chem. Pap. 68 (1), 29–36.

Fick, J., Lindberg, R.H., Parkkonen, J., Arvidsson, B., Tysklind, M., Larsson, D.G.J., 2010. Therapeutic levels of Levonorgestrel detected in blood plasma of fish: results from screening Rainbow Trout exposed to treated sewage effluents. Environ. Sci. Technol. 44, 2661–2666.

Fochetti, R., Amici, I., Argano, R., 2003. Seasonal changes and selectivity in the diet of brown trout in the River Nera (Central Italy). J. Freshw. Ecol. 18 (3), 437–444.

Fochetti, R., Argano, R., de Figueroa, J.M.T., 2008. Feeding ecology of various age-classes of brown trout in River Nera, Central Italy. Belg. J. Zool. 138, 128–131.

Fuller, R.L., Mackay, R.J., 1980. Feeding ecology of three species of Hydropsyche (Trichoptera: Hydropsychidae) in southern Ontario. Can. J. Zool. 58 (12), 2239–2251. http://dx.doi.org/10.1139/z80-306.

Gibs, J., Heckathorn, H.A., Meyer, M.T., Klapinski, F.R., Alebus, M., Lippincott, R.L., 2013. Occurrence and partitioning of antibiotic compounds found in the water column and bottom sediments from a stream receiving two wastewater treatment plant effluents in Northern New Jersey, 2008. Sci. Total Environ. 458, 107–116.

Golovko, O., Kumar, V., Fedorova, G., Randak, T., Grabic, R., 2014a. Removal and seasonal variability of selected analgesics/anti-inflammatory, anti-hypertensive/cardiovascular pharmaceuticals and UV filters in wasterwater treatment plant. Environ. Sci. Pollut. Res. http://dx.doi.org/10.1007/s11356-014-2654-9.

Golovko, O., Kumar, V., Fedorova, G., Randak, T., Grabic, R., 2014b. Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. Chemosphere (accepted 11th April 2014).

Greenberg, L.A., Dahl, J., 1998. Effect of habitat type on growth and diet of brown trout, *Salmo trutta* L., in stream enclosures. Fish. Manag. Ecol. 5, 331–348.

Halling-Sorensen, B., Nors Nielsen, S., Lanzky, P.F., Ingerslev, F., Holten Lutzheft, H.C., Jorgensen, S.E., 1998. Occurrence, fate and effects of pharmaceutical substances in the environment – a review. Chemosphere 36 (2), 357–393.

Heberer, T., 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol. Lett. 131 (1–2), 5–17.

Hellmann, C., Wissel, B., Winkelmann, C., 2013. Omnivores as seasonally important predators in a stream food web. Freshw. Sci. 32 (2), 548–562.

Hildrew, A., 1992. Food webs and species interactions. In: The Rivers Handbook: Hydrological and Ecological Principles. Blackwell Scientific, Oxford, England, pp. 309–330.

Horsing, M., Ledin, A., Grabic, R., Fick, J., Tysklind, M., Jansen, J.L., Andersen, H.R., 2011. Determination of sorption of seventy-five pharmaceuticals in sewage sludge. Water Res. 45, 4470–4482.

Kolarikova, K., Stuchlik, E., Liska, M., Horecky, J., Tatosova, J., Hardekopf, D., Lapsanska, N., Horicka, Z., Hovorka, J., Mihaljevic, M., Fuksa, J.K., von Tumpling, W., 2012. Long-term changes in the bioaccumulation of As, Cd, Pb, and Hg in macroinvertebrates from the Elbe river (Czech Republic). Water Air Soil Pollut. 223 (6), 3511–3526.

Koperski, P., 2005. Testing the suitability of leeches (Hirudinea, Clitellata) for biological assessment of lowland streams. Pol. J. Ecol. 53 (1), 65–80.

Koperski, P., 2010. Diversity of macrobenthos in lowland streams: ecological determinants and taxonomic specificity. J. Limnol. 69 (1), 88–101.

Kutschera, U., 2003. The feeding strategies of the leech *Erpobdella octoculata* (L.): a laboratory study. Int. Rev. Hydrobiol. 88 (1), 94–101.

Lahti, M., Brozinski, J.M., Jylha, A., Kronberg, L., Oikari, A., 2011. Uptake from water, biotransformation, and biliary excretion of pharmaceuticals by Rainbow Trout. Environ. Toxicol. Chem. 30 (6), 1403–1411.

Laine, A., 2001. Effects of peatland drainage on the size and diet of yearling salmon in a humic northern river. Arch. Fur Hydrobiol. 151 (1), 83–99.

Lajeunesse, A., Gagnon, C., Gagne, F., Louis, S., Cejka, P., Sauve, S., 2011. Distribution of antidepressants and their metabolites in brook trout exposed to municipal wastewaters before and

after ozone treatment – evidence of biological effects. Chemosphere 83 (4), 564–571.
Lindberg, R.H., Östman, M., Olofsson, U., Grabic, R., Fick, J., 2014. Occurrence and behaviour of 105 active pharmaceutical ingredients in sewage waters of a municipal sewer collection system. Water Res. 58, 221–229.
Macova, S., Harustiakova, D., Kolarova, J., Machova, J., Zlabek, V., Vykusova, B., Randak, T., Velisek, J., Poleszczuk, G., Hajslova, J., Pulkrabova, J., Svobodova, Z., 2009. Leeches as sensor-bioindicators of river contamination by PCBs. Sensors 9 (3), 1807–1820.
Mehinto, A.C., Hill, E.M., Tyler, C.R., 2010. Uptake and biological effects of environmentally relevant concentrations of the nonsteroidal anti-inflammatory pharmaceutical diclofenac in Rainbow Trout (*Oncorhynchus mykiss*). Environ. Sci. Technol. 44 (6), 2176–2182.
Meier, G.M., Meyer, E.I., Meyns, S., 2000. Lipid content of stream macroinvertebrates. Arch. Fur Hydrobiol. 147 (4), 447–463.
Nallani, G.C., Paulos, P.M., Constantine, L.A., Venables, B.J., Huggett, D.B., 2011. Bioconcentration of ibuprofen in fathead minnow (Pimephales promelas) and channel catfish (Ictalurus punctatus). Chemosphere 84 (10), 1371–1377.
OSPAR Commission, 2005. OSPAR Background Document on Clotrimazole. http://www.ospar.org/documents/dbase/publications/p00199/p00199_bd%20on%20clotrimazole.pdf (accessed 02.09.14.).
Pan, B.Z., Wang, Z.Y., Xu, M.Z., 2012. Macroinvertebrates in abandoned channels: assemblage characteristics and their indications for channel management. River Res. Appl. 28 (8), 1149–1160.
Petrovic, M., Gonzalez, S., Barcelo, D., 2003. Analysis and removal of emerging contaminants in wastewater and drinking water. Trac-Trends Anal. Chem. 22 (10), 685–696.
Reiriz, L., Nicieza, A.G., Brana, F., 1998. Prey selection by experienced and naive juvenile Atlantic salmon. J. Fish Biol. 53 (1), 100–114.
Schenkova, J., Jarkovsky, J., Helesic, J., 2007. Strategies of coexistence of two species: *Erpobdella octoculata* and E-vilnensis (Hirudinea : Erpobdellidae). Int. Rev. Hydrobiol. 92 (4–5), 527–538.
Schönborn, W., 1985. Die ökologische Rolle von *Erpobdella octoculata* (L.) (Hirudinea: Erpobdellidae) in einem abwasserbelasteten Fluß. Zool. J. Syst. 112, 477–494.
Scott, D., 1958. Ecological studies on the Trichoptera of the river Dean, Cheshire. Arch. für Hydrobiol. 54, 340–392.
Smith, M.J., Kay, W.R., Edward, D.H.D., Papas, P.J., Richardson, K.S., Simpson, J.C., Pinder, A.M., Cale, D.J., Horwitz, P.H.J., Davis, J.A., Yung, F.H., Norris, R.H., Halse, S.A., 1999. AusRivAS: using macroinvertebrates to assess ecological condition of rivers in Western Australia. Freshw. Biol. 41 (2), 269–282.
Sola, C., Prat, N., 2006. Monitoring metal and metalloid bioaccumulation in Hydropsyche (Trichoptera, Hydropsychidae) to evaluate metal pollution in a mining river. Whole body versus tissue content. Sci. Total Environ. 359 (1–3), 221–231.
Steinbach, C., Fedorova, G., Prokes, M., Grabicova, K., Machova, J., Grabic, R., Valentova, O., Kocour Kroupova, H., 2013. Toxic effects, bioconcentration and depuration of verapamil in the early life stages of common carp (*Cyprinus carpio* L.). Sci. Total Environ. 461–462, 198–206.
Stuijfzand, S.C., Engels, S., van Ammelrooy, E., Jonker, M., 1999. Caddisflies (Trichoptera : Hydropsychidae) used for evaluating water quality of large European rivers. Archives Environ. Contam. Toxicol. 36 (2), 186–192.
Tessier, L., Boisvert, J.L., Vought, L.B.M., Lacoursiere, J.O., 2000. Effects of 2,4-dichlorophenol on the net-spinning behavior of *Hydropsyche slossonae* larvae (Trichoptera; Hydropsychidae), an early warning signal of chronic toxicity. Ecotoxicol. Environ. Saf. 46 (2), 207–217.
Togunde, O.P., Oakes, K.D., Servos, M.R., Pawliszyn, J., 2012. Optimization of solid phase microextraction for non-lethal in vivo determination of selected pharmaceuticals in fish muscle using liquid chromatography-mass spectrometry. J. Chromatogr. A 1261, 99–106.
Toman, M.J., Dall, P.C., 1997. The diet of *Erpobdella octoculata* (Hirudinea: Erpobdellidae) in two Danish lowland streams. Arch. für Hydrobiol. 140, 549–563.
Verlicchi, P., Al Aukidy, M., Zambello, E., 2012. Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment-A review. Sci. Total Environ. 429, 123–155.

CHAPTER 7

GENERAL DISCUSSION

As our progress in the field of analytical chemistry has been accelerated during last two decades, the insight on the fate of artificially produced chemicals is going to be changed in many ways. New possibilities in the identification and quantification of environmental pollutants have been brought by advanced techniques of instrumental analysis, especially in the case of detection methods. Current methods of liquid or gas chromatography coupled with different kinds of mass spectrometers have lowered limits of detection/quantification to levels <1 ng g^{-1}. What is probably more important, these methods allow us to detect, identify and describe the fate of unknown contaminants, which could help to assess their negative effects in the environment. The early identification of adverse effects of fabricated compounds is crucial to prevent examples well known from history like the extensive use of organochlorine pesticides, polychlorinated biphenyls or consequences related to releasing wastewaters containing toxic metals into the environment. Together with the improvement of analytical methods, the biomonitoring approaches should be optimised to obtain the most reliable data. Not only the need for reliable data, but also ethical and ecologic consequences, should guide how contemporary biomonitoring of environmental contamination is conducted. One of the main goals of scientists and authorities providing this kind of biomonitoring is to encourage companies to produce chemicals with less impact on the environment, thus biomonitoring should follow these claims and try to reduce its own environmental impact as well.

As objectives for biomonitoring studies are different and sometimes very specific, the choice of a suitable approach is crucial. In case of human health, risk assessments related to the consumption of fish from open waters, sampling of adult fish has been necessary. Health risk evaluation based on edible muscle tissue is rational in these cases, but in some species e.g. codfish, also the liver represents a valuable commodity and its contamination is frequently studied (Falandysz et al. 1994, Hrádková et al. 2010). However, sampling of fillets is appropriate for freshwater species from inland water bodies. Twenty-seven fishing grounds in the Czech Republic were monitored for fish contamination and human health risks assessment was conducted using fish fillets. Contamination of fish from different trophic levels was investigated, moreover one reference species (*Abramis brama*) was caught at all sites. Besides the scientific value of this work based on the number of samples and sampling sites, the important goal was to inform the community of anglers about the health aspects of eating fish from open waters in Czech Republic.

Most studies dealing with human health risk assessment focus on market products originating from oceans (Muñoz et al. 2010, Olmedo et al. 2013, Stankovic et al. 2012, Struciński et al. 2013). The monitoring of certain contaminants in inland waters is also done, but such studies on sport fishing species is less common (Noël et al. 2013), especially with respect to human health risks (Dong et al. 2015, Shatenstein et al. 1999). On the basis of our study (chapter two), health risks related to the consumption of fish from Czech fishing grounds was dependent on species and locality. Carp (*Cyprinus carpio*) was recognised as a species with the lowest contamination by target pollutants. This species is bred in a relatively clean environment (ponds) until it reaches marketable size and then after stocking into it can be exposed to contaminants. The number of carp portions (á 170 g) which can be eaten monthly without negative health effects, varied between 6 and 244, depending on the sampling locality. The opposite situation was found for predatory species. For example, the tolerable monthly intake of meat of pike (*Esox lucius*) was in the range from 2 to 21 portions, depending on the sampling locality. Many sport fishermen in the Czech Republic target

predatory fishes, therefore, health risks from these fisheries should not be underestimated, especially at localities with a higher level of contamination. Mercury was found to be a limiting contaminant for fish consumption in all fish species.

In the second study, the use of passive samplers (POCIS) for the monitoring of PFASs in an aquatic environment was evaluated and the data were compared with those obtained from the fish samples (chapter 3). Six localities within the Czech Republic were chosen according to the expected levels of contamination. All the sampling sites were from key profile localities which are regularly sampled by the Czech Hydrometeorological Institute (CHMI). POCIS were deployed and muscle and liver tissues from six individuals of chub (*Squalius cephalus*) were analysed. From sixteen target PFASs, only Perfluorooctane sulfonate (PFOS) was found in chub muscle at concentrations above LOQ and another three PFASs were found in liver samples. PFOS was found previously as the most prevalent PFAS found in biota samples from various regions of the world (Berger et al. 2009, Naile et al. 2013, Yeung et al. 2009, Zhao et al. 2012) with bioaccumulation and bomagnification potential (Giesy and Kannan 2001, Goeritz et al. 2013). Moreover, PFOS was identified as a priority hazardous substance. An Environmental Quality Standard (EQS) of 9.1 μg kg^{-1} of wet weight (w.w.) for water biota (fish) was set (European parliament and Council, 2013). Seven target compounds in concentrations above LOQ were found in POCIS, thereby establishing it as a suitable indicator for the monitoring of PFAS in aquatic environments. More target pollutants were found in POCIS extracts than in fish tissues, the total PFAS concentrations in POCIS strongly correlated with those measured in fish liver ($r=0.94$, $p<0.05$) at each sampling site. The total PFAS concentration was highly correlated with studied approaches, a different situation was in the contribution of individual compounds in the total amount of PFAS measured in liver and in POCIS. Compared to living organisms (fish), passive samplers do not provide information relative to metabolism (Vrana et al. 2005), thus, the measured tissue concentrations is in the environment. The use of passive samplers in monitoring a wide range of pollutants has become more extensive in the last two decades as the number of chemicals, for which calibration data are available is constantly increasing (Bailly et al. 2013, Belles et al. 2014, Ibrahim et al. 2013, Vrana et al. 2006). The calibration data are needed to estimate target pollutants in water concentrations (Alvarez et al. 2004, Fedorova et al. 2014), but passive samplers could be successfully used even for contaminants, where these data are not available or in cases, where water concentrations are not information of interest. Our data showed that there is a close relationship between levels of contaminants (PFASs) in POCIS and in biota (fish). In light of this knowledge, the use of passive samplers for the prediction of contaminant´s tissue concentrations in aquatic organisms (Fernandez and Gschwend 2015) present a challenge for future research in this field. There is a need to evaluate the ecological status of surface waters, which is embedded in the Water Framework Directive (Directive 2000/60/EC). As one part of this evaluation (EQS) demands data about levels of certain contaminants in aquatic biota (fish), the replacement of fish by other suitable bioindicators should be of interest as it fulfils the principles of Three R´s (Replacement, Reduction, Refinement).

In chapter 4, a promising method in the field of aquatic contamination biomonitoring was studied. The method is based on juvenile fish sampling. This method has not been extensively studied. Within this experiment, young-of-the–year (YOY) fish samples were compared with muscle tissue of adult bream and chub at two sampling sites in the Elbe River. Comparisons were made for a wide range of target pollutants (pharmaceuticals, PFASs, toxic metals).

Shoals of YOY fish in central Europe are mostly different species of cyprinids. Spawning is usually from April to June, thus these fish are a few months old in late summer or in autumn. Whole body homogenates can be analysed for a wide range of pollutants. From our point

of view, results from the analysis of whole body homogenates are more representative of environmental contamination. The young fish have low concentrations of chemicals with bioaccumulation potential, but with the current level of analytical chemistry, we were able to get over this issue. From a practical point of view, using YOY fish simplifies biomonitoring at all levels – grab of samples, sample preparation and analysis, results interpretation. The advantages/disadvantages will be discussed in more detail in the subsequent text.

As it was described in the introduction, sampling of adult fish demands a considerable amount of technical, personnel and financial resources. Adult fish sampling presents considerable uncertainty due to migration, age divergence, etc. In contrast, sampling YOY fish in the autumn is relatively easy due to their abundance in shallow parts of rivers and reservoirs. Analysis is simpler YOY are homogenised and then divided into subsamples for the analysis of target contaminants. However, some additional verification will be needed for this approach.

Age divergence in YOY from different cohorts is a variable. We did not expect variability in the concentrations of target pollutants between individuals of same species of YOY fish but some was evident. A few weeks difference in age can be a factor in total exposure time of aquatic contaminants. The concentrations of contaminants were much lower in YOY fish than those found in adults within the same experiment and the number of YOY in the final sample is great enough to suppress the fact that several cohorts are included. On the other hand, our work confirmed, that differences in the contamination between various species of YOY are usually not as significant as with adults (Miege et al. 2012, Noël et al. 2013, Struciński et al. 2013). These results suggest a practical solution because species structure of YOY fish shoals differ between localities (Copp 1992) and the comparison between different sampling sites must be possible without species determination.

The second question relates to YOY mobility. The YOY of some river species are displaced downstream after hatching (Harvey 1987, Reichard and Jurajda 2004, Reichard et al. 2002). The degree of downstream displacement depends on the morphological characteristics of the watercourse (Freeman et al. 2001, Grift et al. 2003, Jurajda 1999). Fish can take refuge in shallow water areas connected to the river. Fish can reside there throughout the summer, so these habitats should be selected as sampling sites for YOY.

YOY were suitable as a bioindicator for the range of selected contaminants. Analysis of YOY represent a short-term condition at that locality. In all of the target compounds, except mercury, higher concentrations with lower variability were found in the YOY homogenates, than in muscle tissue of adults. Concentrations of mercury were approximately one order of magnitude lower in YOY than those in adults, but still well above the detection limit. Mercury accumulates mainly in muscle tissue, so lower concentrations should be expected in YOY (Boalt et al. 2014, Djedjibegovic et al. 2012). Concentrations of other target toxic metals (cadmium, lead) were ten times higher in YOY fish compared to adults; only a few individuals of adults had concentrations above the LOQ.

Concerning PFASs, ive of the fifteen target compounds were found in YOY fish samples, while only PFOS was found in adults. PFOS was the most prevalent compound in YOY fish samples as well (comprise >80% of total PFASs), which corresponds with findings of our previous work and works of other authors (Squadrone et al. 2015, Zareitalabad et al. 2013, Zhao et al. 2012). The concentrations of PFASs in samples of YOY fish homogenates were comparable to those which are frequently found in the liver of adult fish from moderately contaminated water bodies.

From the selected target pharmaceuticals, tramadol and diltiazem were found in concentrations above LOQ in both YOY homogenates and muscles from adults. Higher concentrations were found in YOY than in adults, furthermore another three pharmaceuticals

were analysed in YOY fish homogenate. Unfortunately, data were highly variable and were relatively close to the LOQ; thus the analysis from pharmaceuticals were not included in the submitted manuscript.

If concerns comparison of contamination at given localities, YOY were a better indicator than adults which was species dependent. The mercury concentrations in chub samples were not different between sites, but were different in bream. In the case of PFOS, results were totally opposite between chub and bream and sampling sites. YOY fish did not show differences between localities in contamination by toxic metals and pharmaceuticals, but a significant difference was found in the case of PFASs contamination.

Another goal of the present thesis was to evaluate a nonlethal method of fish sampling for mercury contamination. Monitoring of mercury contamination remains important. Mercury is pertinent relative to consumption of fish from open waters in the Czech Republic (chapter 2). The study discussed in chapter 5 presents an optimised method for the estimating muscle mercury concentrations using fish fin–clips.

During this experiment, the muscle tissue and pectoral fins of two important indicator fish species were analysed for total mercury content (THg) at six localities within the Czech Republic. Our work agreed with previous studies; there is a strong correlation between mercury concentrations in fins and in muscle tissue (Cervenka et al. 2011, Gremillion et al. 2005, Rolfhus et al. 2008). Our data indicates that this relationship is concentration dependent. The levels found in fins were about one order of magnitude lower than those in muscle tissue, however, the relationship is not linear. Therefore, previously published methods that based muscle concentrations on fin–clips analysis assumed to be linear must be considered flawed (Gremillion et al. 2005, Ryba et al. 2008). Based on our findings about concentration dependent relationship, a novel method for the estimation of muscle tissue concentrations from fin–clips analysis was introduced.

The method estimates the muscle mercury concentrations using different prediction factors. These factors use a median of muscle/fin–clips Hg ratio (quotient) for three ranges of concentrations from fin-clip analysis (<0.03; 0.03–0.06; >0.06 $\mu g\ g^{-1}$). The relationship in Hg concentration between muscle tissue and fin–clips is also species specific and thus different estimation factors should be used for different fish species. We set these factors for bream and chub. The range of Hg concentrations, from which the calculation of estimation factors is grounded, cover the levels of mercury which could be mostly found in these species in Europe (Djedjibegovic et al. 2012, Kruzikova et al. 2008, Lepom et al. 2012).

Differences in estimated and actual mean (n=10) muscle mercury concentrations were found less than 10% at most of the investigated localities in both fish species. Thus, fin–clips were found to be suitable as a nonlethal method to estimate mercury in aquatic environments and as a surrogate for estimating muscle mercury concentrations.

The last experiment presented in chapter 6 used macroinvertebrates to evaluate levels of certain pharmaceuticals in small streams affected by STP. Benthic organisms are important in the diet of many fishes, consequently they are a significant source of fish contamination. The experiment took place in the Zivny stream, which is a watercourse affected by STP effluent from Prachatice (12000 inhabitants). Two benthic species, Hydropsyche sp. and Erpobdella octoculata were sampled at several points relative to STP. Most studies on pollution and the benthic community use biodiversity (Horsák et al. 2009, Medeiros et al. 2011, Moreno et al. 2009) and only a few focus on bioaccumulation of contaminants (Kolaříková et al. 2012, Milani et al. 2013). In our study, several pharmaceuticals bioaccumulated including the antibiotics azithromycin and clarithromycin, a cardiovascular drug valsartan, an anti-inflammatory drug

(diclofenac) or psychoactive drugs (sertraline and citalopram). The highest bioaccumulation potential was described in the case of diclofenac and azithromycin. In the case of diclofenac, the deterrent example of its fate in the environment is well known (Oaks et al. 2004, Shultz et al. 2004). Concerning psychoactive drugs, behavioural changes in fish were also reported (Brodin et al. 2013), but the negative effects of other pharmaceutical´s in the environment are mostly unknown.

CONCLUSIONS AND FUTURE PERSPECTIVES

Due to the amount of extraneous substances released into the aquatic environments, monitoring is important. Several approaches were evaluated as means of biomonitoring. In general, the use of specific approaches depend on the needs. Analysis of muscle tissue of adult fishes is usually required for health risk assessment, but in specific cases nonlethal fin–clip samples can be substituted. For the monitoring of a wide range of pollutants, YOY fish homogenates seem to be a suitable bioindicator as it measures the whole body burden of aquatic organisms, but further research is needed to confirm our results. The main task in the case of YOY fish approach will be to determine if these fish really represent the level of contamination at a given locality. Passive sampling represents a promising approach for contamination monitoring. With increasing knowledge about uptake rates of different chemicals, the passive samplers have the potential to become one of the most important indicators of aquatic contamination. Nevertheless, aquatic organism samples continue to be an important source of biomonitoring. As it was shown in the case of benthic fauna, contaminants can be detected in these aquatic organisms, but they are also the source affecting fish through the food web. From this perspective, it is important to examine even lower trophic levels by study of primary producers and decomposers so as to more thoroughly understand the pathways of the contaminants.

REFERENCES

Alvarez, D.A., Petty, J.D., Huckins, J.N., Jones-Lepp, T.L., Getting, D.T., Goddard, J.P., Manahan, S.E., 2004. Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. Environ. Toxicol. Chem. 23(7), 1640–1648.

Bailly, E., Levi, Y., Karolak, S., 2013. Calibration and field evaluation of polar organic chemical integrative sampler (POCIS) for monitoring pharmaceuticals in hospital wastewater. Environ. Pollut. 174, 100–105.

Belles, A., Tapie, N., Pardon, P., Budzinski, H., 2014. Development of the performance reference compound approach for the calibration of „polar organic chemical integrative sampler" (POCIS). Anal. Bioanal. Chem. 406(4), 1131–1140.

Berger, U., Glynn, A., Holmstrom, K.E., Berglund, M., Ankarberg, E.H., Tornkvist, A., 2009. Fish consumption as a source of human exposure to perfluorinated alkyl substances in Sweden – Analysis of edible fish from Lake Vattern and the Baltic Sea. Chemosphere 76(6), 799–804.

Boalt, E., Miller, A. and Dahlgren, H., 2014. Distribution of cadmium, mercury, and lead in different body parts of Baltic herring (*Clupea harengus*) and perch (*Perca fluviatills*): Implications for environmental status assessments. Marine Pollution Bulletin 78(1–2), 130–136.

Brodin, T., Fick, J., Jonsson, M., Klaminder, J., 2013. Dilute Concentrations of a Psychiatric Drug Alter Behavior of Fish from Natural Populations. Science 339(6121), 814–815.

Cervenka, R., Bednarik, A., Komarek, J., Ondrackova, M., Jurajda, P., Vitek, T., Spurny, P., 2011. The relationship between the mercury concentration in fish muscles and scales/fins and its significance. Cent. Eur. J. Chem 9(6), 1109–1116.

Copp, G.H., 1992. Comparative microhabitat use of cyprinid larvae and juveniles in a lotic floodplain channel. Environ. Biol. Fishes 33(1–2), 181–193.

Djedjibegovic, J., Larssen, T., Skrbo, A., Marjanovic, A., Sober, M., 2012. Contents of cadmium, copper, mercury and lead in fish from the Neretva river (Bosnia and Herzegovina) determined by inductively coupled plasma mass spectrometry (ICP-MS). Food Chem. 131(2), 469–476.

Dong, Z., Jim, R.C., Hatley, E.L., Backus, A.S.N., Shine, J.P., Spengler, J.D. and Schaider, L.A., 2015. A longitudinal study of mercury exposure associated with consumption of freshwater fish from a reservoir in rural south central USA. Environ. Res. 136, 155–162.

European parliament and Council, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal of the European Union, L 327.

European parliament and Council, 2013. Directive 2013/39/EU of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Official Journal of the European Union, L 226.

Falandysz, J., Tanabe, S. and Tatsukawa, R., 1994. Most toxic and highly bioaccumulative PCB congeners in cod-liver oil of Baltic origin processed in Poland during the 1970s and 1980s, their TEQ-values and possible intake. Sci. Total Environ. 145(3), 207–212.

Fedorova, G., Randak, T., Golovko, O., Kodes, V., Grabicova, K., Grabic, R., 2014. A passive sampling method for detecting analgesics, psycholeptics, antidepressants and illicit drugs in aquatic environments in the Czech Republic. Sci. Total Environ. 487, 681–687.

Fernandez, L.A., Gschwend, P.M., 2015. Predicting bioaccumulation of polycyclic aromatic hydrocarbons in soft-shelled clams (*Mya arenaria*) using field deployments of polyethylene passive samplers. Environ. Toxicol. Chem. 34(5), 993–1000.

Freeman, M.C., Bowen, Z.H., Bovee, K.D., Irwin, E.R., 2001. Flow and habitat effects on juvenile fish abundance in natural and altered flow regimes. Ecol. Appl. 11(1), 179–190.

Giesy, J.P., Kannan, K., 2001. Global distribution of perfluorooctane sulfonate in wildlife. Environmental Science & Technology 35(7), 1339–1342.

Goeritz, I., Falk, S., Stahl, T., Schafers, C., Schlechtriem, C., 2013. Biomagnification and tissue distribution of perfluoroalkyl substances (PFASs) in market-size rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 32(9), 2078–2088.

Gremillion, P.T., Cizdziel, J.V., Cody, N.R., 2005. Caudal fin mercury as a non-lethal predictor of fish-muscle mercury. Environ. Chem. 2(2), 96–99.

Grift, R.E., Buijse, A.D., Van Densen, W.L.T., Machiels, M.A.M., Kranenbarg, J., Klein Breteler, J.G.P., Backx, J.J.G.M., 2003. Suitable habitats for 0-group fish in rehabilitated floodplains along the lower River Rhine. River Research and Applications 19(4), 353–374.

Harvey, B.C., 1987. Susceptibility of young-of-the-year fishes to downstream displacement by flooding. Trans. Am. Fish. Soc. 116(6), 851–855.

Horsák, M., Bojková, J., Zahrádková, S., Omesová, M., Helešic, J., 2009. Impact of reservoirs and channelization on lowland river macroinvertebrates: A case study from Central Europe. Limnologica 39(2), 140–151.

Hrádková, P., Poustka, J., Hloušková, V., Pulkrabová, J., Tomaniová, M., Hajšlová, J., 2010. Perfluorinated compounds: Occurrence of emerging food contaminants in canned fish and seafood products. Czech. J. Food Sci. 28(4), 333–342.

Ibrahim, I., Togola, A., Gonzalez, C., 2013. In-situ calibration of POCIS for the sampling of polar pesticides and metabolites in surface water. Talanta 116, 495–500.

Jurajda, P., 1999. Comparative nursery habitat use by 0+ fish in a modified lowland river. River Res. Appl. 15(1–3), 113–124.

Kolaříková, K., Stuchlík, E., Liška, M., Horecký, J., Tátosová, J., Hardekopf, D., Lapšanská, N., Hořická, Z., Hovorka, J., Mihaljevič, M., Fuksa, J.K., Von Tümpling, W., 2012. Long-term changes in the bioaccumulation of As, Cd, Pb, and Hg in macroinvertebrates from the Elbe River (Czech Republic). Water Air Soil Pollut. 223(6), 3511–3526.

Kruzikova, K., Randak, T., Kensova, R., Kroupova, H., Leontovycova, D., Svobodova, Z., 2008. Mercury and Methylmercury concentrations in muscle tissue of fish caught in major rivers of the Czech Republic. Acta Vet. Brno 77(4), 637–643.

Lepom, P., Irmer, U., Wellmitz, J., 2012. Mercury levels and trends (1993–2009) in bream (*Abramis brama* L.) and zebra mussels (*Dreissena polymorpha*) from German surface waters. Chemosphere 86(2), 202–211.

Medeiros, A.S., Luszczek, C.E., Shirley, J., Quinlan, R., 2011. Benthic biomonitoring in arctic tundra streams: A community-based approach in iqaluit, nunavut, Canada. Arctic 64(1), 59–72.

Miege, C., Peretti, A., Labadie, P., Budzinski, H., Le Bizec, B., Vorkamp, K., Tronczynski, J., Persat, H., Coquery, M., Babut, M., 2012. Occurrence of priority and emerging organic compounds in fishes from the Rhone River (France). Anal. Bioanal. Chem. 404(9), 2721–2735.

Milani, D., Grapentine, L.C., Fletcher, R., 2013. Sediment contamination in Lyons creek east, a tributary of the Niagara River: Part I. assessment of benthic macroinvertebrates. Arch. Environ. Contam. Toxicol. 64(1), 65–86.

Moreno, P., França, J.S., Ferreira, W.R., Paz, A.D., Monteiro, I.M., Callisto, M., 2009. Use of the BEAST model for biomonitoring water quality in a neotropical basin. Hydrobiologia 630(1), 231–242.

Muñoz, I., Martínez Bueno, M.J., Agüera, A., Fernández-Alba, A.R., 2010. Environmental and human health risk assessment of organic micro-pollutants occurring in a Spanish marine fish farm. Environ. Pollut. 158(5), 1809–1816.

Naile, J.E., Khim, J.S., Hong, S., Park, J., Kwon, B.O., Ryu, J.S., Hwang, J.H., Jones, P.D., Giesy, J.P., 2013. Distributions and bioconcentration characteristics of perfluorinated compounds in environmental samples collected from the west coast of Korea. Chemosphere 90(2), 387–394.

Noël, L., Chekri, R., Millour, S., Merlo, M., Leblanc, J.C., Guérin, T., 2013. Distribution and relationships of As, Cd, Pb and Hg in freshwater fish from five French fishing areas. Chemosphere 90(6), 1900–1910.

Oaks, J.L., Gilbert, M., Virani, M.Z., Watson, R.T., Meteyer, C.U., Rideout, B.A., Shivaprasad, H.L., Ahmed, S., Chaudhry, M.J.I., Arshad, M., Mahmood, S., Ali, A., Khan, A.A., 2004. Diclofenac residues as the cause of vulture population decline in Pakistan. Nature 427(6975), 630–633.

Olmedo, P., Pla, A., Hernández, A.F., Barbier, F., Ayouni, L., Gil, F., 2013. Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. Environ. Int. 59, 63–72.

Reichard, M., Jurajda, P., 2004. The effects of elevated river discharge on the downstream drift of young-of-the-year cyprinid fishes. J. Freshw. Ecol. 19(3), 465–471.

Reichard, M., Jurajda, P., Ondračková, M., 2002. Interannual variability in seasonal dynamics and species composition of drifting young-of-the-year fishes in two European lowland rivers. J. Fish Biol. 60(1), 87–101.

Rolfhus, K.R., Sandheinrich, M.B., Wiener, J.G., Bailey, S.W., Thoreson, K.A., Hammerschmidt, C.R., 2008. Analysis of fin-clips as a nonlethal method for monitoring mercury in fish. Environ. Sci. Technol. 42(3), 871–877.

Ryba, S.A., Lake, J.L., Serbst, J.R., Libby, A.D., Ayvazian, S., 2008. Assessment of caudal fin-clip as a non-lethal technique for predicting muscle tissue mercury concentrations in largemouth bass. Environ. Chem. 5(3), 200–203.

Shatenstein, B., Kosatsky, T., Nadon, S., Lussier-Cacan, S., Weber, J.P., 1999. Reliability and relative validity of fish consumption data obtained in an exposure assessment study among Montreal-area sportfishers. Environ. Res. 80(2 II), S71–S86.

Shultz, S., Baral, H.S., Charman, S., Cunningham, A.A., Das, D., Ghalsasi, G.R., Goudar, M.S., Green, R.E., Jones, A., Nighot, P., Pain, D.J., Prakash, V., 2004. Diclofenac poisoning is widespread in declining vulture populations across the Indian subcontinent. Proceedings of the Royal Society B: Biological Sciences 271(SUPPL. 6), S458–S460.

Squadrone, S., Ciccotelli, V., Prearo, M., Favaro, L., Scanzio, T., Foglini, C., Abete, M.C., 2015. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA): emerging contaminants of increasing concern in fish from Lake Varese, Italy. Environ. Monit. Assess. 187(7).

Stankovic, S., Jovic, M., Stankovic, A.R. and Katsikas, L., 2012. Environmental Chemistry for a Sustainable World, pp. 311–373.

Struciński, P., Piskorska-Pliszczyńska, J., Maszewski, S., Góralczyk, K., Warenik-Bany, M., Mikołajczyk, S., Czaja, K., Hernik, A., Ludwicki, J.K., 2013. PCDD/Fs and DL-PCBs intake from fish caught in Polish fishing grounds in the Baltic Sea – Characterizing the risk for consumers. Environ. Int. 56, 32–41.

Vrana, B., Allan, I.J., Greenwood, R., Mills, G.A., Dominiak, E., Svensson, K., Knutsson, J., Morrison, G., 2005. Passive sampling techniques for monitoring pollutants in water. Trac-Trends Anal. Chem. 24(10), 845–868.

Vrana, B., Mills, G.A., Dominiak, E., Greenwood, R., 2006. Calibration of the Chemcatcher passive sampler for the monitoring of priority organic pollutants in water. Environ. Pollut. 142(2), 333–343.

Yeung, L.W.Y., Yamashita, N., Taniyasu, S., Lam, P.K.S., Sinha, R.K., Borole, D.V., Kannan, K., 2009. A survey of perfluorinated compounds in surface water and biota including dolphins from the Ganges River and in other waterbodies in India. Chemosphere 76(1), 55–62.

Zareitalabad, P., Siemens, J., Hamer, M., Amelung, W., 2013. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in surface waters, sediments, soils and wastewater – A review on concentrations and distribution coefficients. Chemosphere 91(6), 725–732.

Zhao, Y.G., Wong, C.K.C., Wong, M.H., 2012. Environmental contamination, human exposure and body loadings of perfluorooctane sulfonate (PFOS), focusing on Asian countries. Chemosphere 89(4), 355–368.

ENGLISH SUMMARY

Many chemicals with a wide range of uses are currently fabricated, and more of these products are being developed every day. Pesticides enter the aquatic environment as runoff from farming. Pharmaceuticals are excreted from humans and farm animals, and some enter sewage treatment plants, but not all are removed before entering the surface waters. Different types of contaminants including toxic metals are released into environment by various types of industry. These pollutants enter the aquatic systems both directly and with precipitation. Some of these extraneous substances, such as Persistent Organic Pollutants or toxic metals, are able to enter the food web and bioaccumulate. Even though the toxic effects of some common pollutants are well known, the fate of most new chemicals remain unknown. I It is impossible to describe the possible negative effects of all new chemicals. For this reason, compounds which are frequently found in aquatic biota should monitored so as to identify potentially hazardous substances.

We examined several approaches of biomonitoring in the present study. A well-established method for human health risk assessment was used to evaluate the quality of fish targeted by sport fishermen from open waters in the Czech Republic; a brochure was distributed to anglers via the Czech Fishing Union. Health risks to those who eat wild fish were species and locality dependent. Frequent consumption of predatory fish should be avoided at some fishing grounds, but no risks were found from eating carp (*Cyprinus carpio*) at all investigated sites.

The use of passive samplers is becoming increasingly attractive. As these devices are able to mimic the biological uptake of chemicals, their potential for replacing fish as bioindicators in routine biomonitoring programmes is evident. The comparison between analysis of fish tissues and POCIS extracts from selected sampling sites confirmed the potential of interchangeability of these indicators in the case of PFASs as target pollutants. Moreover, no metabolic transformation of contaminants take place in passive samplers, thus actual contamination is represented more precisely. The use of passive samplers completely fulfills the internationally accepted principles of animal use Replacement, Reduction, Refinement (the three R´s).

Another promising approach uses YOY fish as bioindicators. Multispecies samples of YOY fish homogenates were found to be better indicators for a wide range of pollutants than muscle tissue of adult fish. More target pollutants in higher concentrations are detected compared to the muscles of adults, partially because of the inclusion of organs in the homogenate. Beside the higher sensitivity in pollutants detection and quantification, practical and economic benefits accrue from using YOY fishes.

Mercury is a limiting contaminant relative to eating for the fish from open waters in the Czech Republic; a nonlethal method of fin-clip sampling can be used to assess this chemical in aquatic environments. Besides monitoring, our optimised method for the estimation of muscle tissue concentrations could be used for human health risk assessment as well. The difference between real muscle concentrations and concentrations estimated using our method differs less than 10% in most of the investigated localities.

Benthic organisms are an important part of the food web in aquatic environments, but insufficient information about contamination is available. First evidence about bioaccumulation of certain pharmaceuticals in benthic organisms was described in this and a companion study. Although, pharmaceuticals are generally considered to be not cumulative in organisms, our study demonstrated that certain pharmaceuticals do bioaccumulate. Thus, it is evident that dissolved pharmaceutical as well as contamination in food can be important exposure pathway of chemicals for organisms (fish) inhabiting aquatic environments.

CZECH SUMMARY

Množství chemických látek vyráběných za různými účely je v současné době ohromné a mnoho dalších je pro budoucí výrobu a použití každý den vyvíjeno. Pesticidy pronikají do vodního prostředí při nevhodném zemědělském obhospodařování, kdy dochází k plošnému splachování těchto látek z polí. Lidé i hospodářská zvířata vylučují ze svého těla množství léčiv, která jsou jim podávána, a tato posléze procházejí přes čistírny odpadních vod, neboť současné čisticí procesy je nedokáží z odpadních vod odstranit. Průmysl produkuje různé druhy kontaminantů, včetně toxických kovů. Tyto látky se do vodního prostředí dostávají buďto přímo, nebo ve formě srážek. Některé z těchto cizorodých látek jako například persistentní organické polutanty nebo těžké kovy vstupují do potravních řetězců a kumulují se v živých organizmech. Zatímco u některých dobře známých polutantů byly popsány jejich negativní účinky na organizmus, chování většiny tzv. „nových" kontaminantů je zcela neznámé. Protože množství cizorodých látek, které se dostávají do vodního prostředí, je obrovské, není možné v krátké době popsat případné negativní dopady u všech z nich. Z tohoto důvodu je nutné se věnovat primárně těm látkám, které jsou nejčastěji nacházeny ve vzorcích vodních organizmů. Biomonitoring představuje účinný nástroj, který pomáhá tyto potenciálně nebezpečné látky ve vodním prostředí identifikovat.

V rámci dizertační práce byly studovány a hodnoceny různé přístupy biomonitoringu. Metoda hodnocení zdravotních rizik byla v minulosti dobře popsána a používá se k hodnocení složek potravy v lidské výživě. V rámci této práce byla metoda aplikována na hodnocení rizik spojených s konzumací masa volně žijících ryb z významných rybářských revírů České republiky. Studie byla zaměřena na část populace věnující se sportovnímu rybolovu, která často ryby z volných vod konzumuje. Z tohoto důvodu nebyly výsledky práce publikovány pouze ve vědeckém časopise, ale také byly zájemcům z řad rybářů distribuovány ve formě brožury prostřednictvím Českého rybářského svazu. Zdravotní rizika spojená s konzumací ryb z volných vod závisí na druhu ryby a lokalitě. Zatímco zcela bez rizik je na všech sledovaných lokalitách konzumace masa kapra obecného (*Cyprinus carpio*), častou konzumaci většího množství masa dravých ryb nelze na některých lokalitách doporučit.

Použití pasivních vzorkovačů se stává v posledních dvou desetiletích stále atraktivnější. Vzhledem k tomu, že tato zařízení dokáží napodobit absorpci kontaminantů, která probíhá v živých organizmech, mají v rutinních programech monitorujících výskyt cizorodých látek značný potenciál, neboť jsou schopné nahradit ryby jako bioindikátory znečištění. V rámci naší práce byla tato teorie potvrzena v případě perfluoralkylových a polyfluoralkylových sloučenin (PFASs), kdy koncentrace těchto polutantů byly na vybraných lokalitách porovnány v tkáních ryb a v extraktech z pasivních vzorkovačů. V porovnání s živými organizmy neprobíhá v pasivních vzorkovačích metabolická přeměna kontaminantů vyskytujících se ve vodním prostředí, a proto výsledky analýz lépe odpovídají reálné situaci na dané lokalitě. Použití pasivních vzorkovačů navíc naplňuje mezinárodně uznávané principy Replacement, Reduction, Refinement (3R).

Dalším slibným přístupem se jeví použití juvenilních (tohoročních) ryb jako bioindikátorů znečištění. Směsné vícedruhové vzorky homogenátu ryb této věkové kategorie byly v rámci práce vyhodnoceny jako lepší indikátor širokého spektra polutantů než dospělé ryby jednoho druhu odlovené ve stejné lokalitě. Vzhledem k tomu, že homogenát tvořený celými těly vzorkovaných jedinců obsahuje veškeré vnitřní orgány, je možné v těchto vzorcích detekovat širší spektrum kontaminantů než ve svalovině dospělců. Kromě nižších hodnot limitů detekce a kvantifikace přináší tato metoda značné praktické a ekonomické výhody. Využití juvenilních ryb částečně naplňuje principy 3R, protože odlovem této věkové kategorie dochází k menšímu ovlivnění rybích populací než při využití ryb v reprodukčním věku.

V případě rtuti, která je limitujícím kontaminantem pro konzumaci ryb z volných vod v ČR, je možné pro posouzení kontaminace vodního prostředí použít metodu založenou na analýze ústřižků ploutví, která nevyžaduje usmrcení vzorkovaných jedinců. Kromě monitoringu výskytu rtuti na monitorovaných lokalitách je možné tento přístup využít také k odhadu koncentrace rtuti ve svalovině ryb. Rozdíl mezi skutečnou a odhadnutou průměrnou koncentrací rtuti ve svalovině ryb činil při použití námi optimalizované metody na většině posuzovaných lokalit méně než 10 %.

Přestože bentos představuje významný článek potravního řetězce ve vodním prostředí, informace ohledně kontaminace cizorodými látkami jsou u něj značně omezené. V další studii zařazené do této práce se podařilo poprvé prokázat kumulaci některých léčiv v bentických organizmech, které jsou významnou složkou potravních řetězců ve vodním prostředí. Ačkoliv léčiva jsou obecně považována za látky, které se nekumulují v organizmech, naše studie prokázala, že některá farmaka mají bioakumulační potenciál. Existuje tedy reálné riziko kontaminace vodních organizmů nejen prostřednictvím koncentrace těchto polutantů ve vodě, ale i prostřednictvím přijímané potravy.

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LIST OF PUBLICATIONS

PEER–REVIEWED JOURNALS WITH IF

Cerveny, D., Grabic, R., Fedorova, G., Grabicova, K., Turek, J., Kodes, V., Golovko, O., Zlabek, V., Randak, T., 2016. Perfluoroalkyl substances in aquatic environment-comparison of fish and passive sampling approaches. Environmental Research 144: 92–98. (IF 2015 = 3.088)

Cerveny, D., Roje, S., Turek, J., Randak, T. 2016. Fish fin-clips as a non-lethal method for biomonitoring of mercury contamination in aquatic environment and human health risk assessment. Chemosphere 163: 290–295. (IF 2015 = 3.698)

Cerveny, D., Turek, J., Grabic, R., Golovko, O., Koba, O., Fedorova, G., Grabicova, K., Zlabek, V., Randak T., 2016. Young-of-the-year fish as a prospective bioindicator for aquatic environmental contamination monitoring. Water Research 103: 334–342. (IF 2015 = 5.991)

Grabicova, K., Grabic, R., Blaha, M., Kumar, V., **Cerveny, D.**, Fedorova, G., Randak, T., 2015. Presence of pharmaceuticals in benthic fauna living in a small stream affected by effluent from a municipal sewage treatment plant. Water Research 72: 145–153. (IF 2015 = 5.991)

Cerveny, D., Zlabek, V., Velisek, J., Turek, J., Grabic, R., Grabicova, K., Fedorova, G., Rosmus, J., Lepic, P., Randak, T., 2014. Contamination of fish in important fishing grounds of the Czech Republic. Ecotoxicology and Environmental Safety 109: 101–109. (IF 2014 = 2.762)

APPLICATION OF METHODOLOGIES

Randák, T., Turek, J., **Červený, D.**, Kolarova, J., Lepic, P., Grabic, R., Zlabek, V., 2014. Produkce juvenilních kategorií pstruha obecného a lipana podhorního pro zarybňování volných vod. Edice Metodik, FROV JU, no. 155, 29 s. (in Czech)

Zlabek, V., **Cerveny, D.**, Grabic, R., Turek, J., Randak, T., 2014. Metodika hodnocení hygienické kvality masa ryb z hlediska obsahu cizorodých látek. Edice Metodik, FROV JU, č. 157, 25 s. (in Czech)

ABSTRACTS AND CONFERENCE PROCEEDINGS

Červený, D., Grabic, R., Fedorova, G., Grabicova, K., Turek, J., Kodes, V., Golovko, O., Zlabek, V., Randak, T., 2015. Perfluorované látky ve vodním prostředí – porovnání klasického přístupu využívajícího ryby jako bioindikátory a pasivního vzorkování. Toxicita a biodegradabilita odpadů a látek významných ve vodním prostředí, XVII. toxikologická konference. Vodňany, s. 12

Cerveny, D., Turek, J., Randak, T., 2014. Use of a young-of-the-year fish for assessment of mercury contamination in aquatic environment. EUROTOX 2014 meeting in Edinburgh, Scotland. Toxicology Letters 229S, S40–S252, S 111

Cerveny, D., Zlabek, V., Velisek, J., Turek, J., Grabic, R., Grabicova, K., Fedorova, G., Lepic, P., Randak, T., 2013. Kontaminace ryb ve významných rybářských revírech v České republice. Toxicita a biodegradabilita odpadů a látek významných ve vodním prostředí, XVI. toxikologická konference. Vodňany, s. 13.

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