Ecotoxicology and Environmental Safety 164 (2018) 92–99

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Complex effects of pollution on fish in major rivers in the Czech Republic

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ARTICLE INFO

Keywords:
Biomarker
EROD
Vitellogenin
Passive sampling
Biomonitoring
Chub

ABSTRACT

Monitoring the contamination level in aquatic environments and assessing the impact on aquatic life occurs throughout the world. In the present study, an approach based on a combination of biomarkers and the distribution of various industrial and municipal pollutants was used to investigate the effect of aquatic environmental contamination on fish. Monitoring was performed in ten rivers in the Czech Republic (Berounka, Dyje, Elbe, Lužnice, Odra, Ohře, Otava, Sázava, Svratka, and Vltava rivers, with one or two locations in each river) at the same sites that were regularly monitored within the Czech National Monitoring Program in 2007–2011. Health status, hepatic ethoxyresorufin-O-deethylase (EROD) activity, total cytochrome P450 content, and the plasma vitellogenin concentration were assessed in wild chub (*Squalius cephalus*) males caught at the monitored sites. The contamination level was the highest in the Svratka River downstream of Brno. Among all measured persistent organic pollutants (POPs), polychlorinated biphenyls and dichlorodiphenyltrichloroethane and its metabolites were the major contributors of POPs in fish muscle. Elbe, Odra, and Svratka rivers were identified as the most polluted. Fish from these locations showed reduced gonad size, increased vitellogenin concentration in male plasma, EROD, and total cytochrome P450 content. These biomarkers can be used for future environmental monitoring assessments. Overall, this study improves our understanding of the relationship between human activities and pollutant loads and further contributes to the decision to support local watershed managers to protect water quality in this region.

1. Introduction

A high pollution level in the aquatic environment creates a warning situation throughout world. River systems in the Czech Republic are of concern because they are contaminated with many organic and inorganic substances. These substances includes a several of persistent organic pollutants such as organochlorinated (Prokeš et al., 2012), brominated flame retardants and perfluoroalkyl substances (Cerveny et al., 2016a; Hajslova et al., 2007; Hlouskova et al., 2013), pharmaceutical active compounds (Fick et al., 2017; Golovko et al., 2014; Koba et al., 2018), personal care products (Grabicova et al., 2013), pesticides (Cerveny et al., 2014) and metals such as mercury/methylmercury (Cerveny et al., 2016b; Sedlackova et al., 2014), lead and cadmium (Cerveny et al., 2016c, 2014; Rambouskova et al., 2014). Some of these compounds can bioaccumulate via the food chain (Grabicova et al., 2015; Kidd et al., 2014) posing a risk to human health (Cerveny et al., 2014). Reported levels of perfluoroalkyl substances and total mercury in fish muscle have ranged between 0.15–877 μg/kg (Hlouskova et al., 2013) and 71–236 μg/kg (Sedlackova et al., 2014), respectively. Increasing levels of environmental pollutants are recorded when rivers flood (Madsen et al., 2014). Prague had extreme floods nine times over the past 500 years (Elleder, 2010). The Lužnice river basin, which is a tributary of the Vltava River, was highly affected by a flood in 2006, causing increased concentrations of metals, organic compounds, fecal coliform bacteria, and nitrates (Hrdinka et al., 2012). At four other rivers in the Czech Republic (Odra, Otava, Elbe, and Lužnice) increased

Abbreviations: EROD, ethoxyresorufin O-deethylase; CYP, cytochrome P450; VTG, vitellogenin; HSI, hepatosomatic index; GSI, gonadosomatic index; POCIS, polar organic compounds integrative sampler; NADPH, nicotinamide adenine dinucleotide phosphate; SPMD, semipermeable membrane device

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https://doi.org/10.1016/j.ecoenv.2018.07.109
Received 12 April 2018; Received in revised form 23 July 2018; Accepted 26 July 2018

mercury levels were recorded resulting in its bioaccumulation in fish muscle (Cerveny et al., 2014; Randak et al., 2009; Sedlackova et al., 2014).

An important issue in aquatic toxicology is the adverse effects on fish of the organic compounds detected in water. The complex mixture of pollutants can affect the structure and function of biological systems, causing responses at the molecular, biochemical, histological, and behavioral levels before the community level is affected (Giang et al., 2017). However, the presence of environmental pollutants in water bodies does not necessarily indicate harmful effects. A link between external levels of exposure and internal levels of biological alterations must be identified using of multi-biomarker approach. Several categories of biomarkers of exposure and biological effects were suggested to assess the health of an organism in environmental monitoring experiments (Connon et al., 2012; Hook et al., 2014). The cytochrome P450 (CYP) enzymes involved in the first phase of xenobiotic metabolism are recognized as biomarkers of exposure because of their inducibility by exogenous compounds. These biomarkers often indicate exposure of an organism to toxicants. Several recent reviews discussed the potential use of CYP as a biomarkers in the aquatic toxicology (Reynaud and Deschaux, 2006). Changes in morphological indexes were considered to be early warning biomarkers to detect the response of an organism to exposure to various chemicals (Araújo et al., 2018; Li et al., 2013). The hepatosomatic index (HSI) has been linked to contaminants from several sources, such as pulp and paper mill effluents (Sepulveda et al., 2003), landfills (Noaksson et al., 2001), or to exposure to treatments in laboratory studies (Li et al., 2011; Steinbach et al., 2014), while the gonadosomatic index (GSI) has been widely used as a biomarker of reproductive allocation and the reproductive condition in fish physiology (Zeyl et al., 2014). Additionally, changes in the fish hormonal background can confirm the presence of endocrine-disrupting chemicals in water. The production of vitellogenin (VTG) is initiated by natural estrogen hormones. Females, therefore, have different levels of VTG depending on their hormonal cycles, while males and juveniles fish have very low levels throughout the year. When male and juveniles fish are exposed to xenoestrogens, they begin to produce VTG at similar levels as females. For example, in *in situ* and *in vivo* experiments, male fish exposed to water discharged from a wastewater effluent showed increased VTG levels (Rivas-Rivera et al., 2014; Sumpter, 2005), and this biomarker response promoted the inhibition of the spermatogenesis process.

This study aimed to evaluate the biomarker responses in the wild chub (*Squalius cephalus* L.) at 11 locations situated on ten major rivers and their tributaries in the Czech Republic, regularly monitored within framework of the Czech Republic National Monitoring Program from 2007 to 2011, and to link the observed effects with the contamination level at the monitored sites. The presence of pollutants was confirmed using passive samplers.

2. Materials and methods

2.1. Area of study

A 5-year study was conducted from 2007 to 2011 at 11 locations in ten rivers in the Czech Republic (Fig. 1). Fish were collected in the localities regularly monitored within the Czech National Monitoring Program. Also, the most contaminated localities on selected rivers were revealed and reviewed earlier (Havelková et al., 2008). Each selected site was located in the downstream part of the river, close to its confluence with a higher-order flow or downstream of an important industrial agglomeration. A brief description of each site is given in Supplementary material 1. Wild adult male chub (approximately 121–129 fish at all sites per year) were captured by electrofishing during the first half of May (Supplementary material 2). The age of the captured fish ranged from 2 to 8 years but most (76%) of the fish were 3–5 years old.

2.2. Fish sampling and sample processing

Fish were transported to the lab and held in aerated tanks until they were processed (less than 1 h). A total of 615 male chub, with a body weight 260 ± 186 g (mean ± standard deviation) and length 252 ± 62 mm, were sampled and analyzed. Blood was taken via caudal vein puncture using a heparinized syringe and inserted into heparinized Eppendorf tubes (heparin sodium salt; 5000 IU/mL) to avoid blood coagulation. Blood plasma for biochemical analysis was obtained from cooled centrifuged blood (10 min, 837 × *g*, 4 °C) and stored at − 80 °C for determination of VTG concentration. Liver tissues (approx. 0.5 g) were stored at −80 °C until microsomes were prepared. Gonads and livers were weighed to calculate the gonadosomatic and hepatosomatic indices as follows: GSI = gonad weight / body weight × 100; HSI = liver weight / body weight × 100.

Additionally, muscle tissue from three random fish from each location was used for chemical analyses (n = 165 for 5-year period).

2.3. Biochemical analyses

Eight fish from each location were used for determination of EROD activity and total CYP content, while VTG concentration was measured in all fish. Microsomal proteins were extracted from liver tissue using differential centrifugation, as described by Burkina et al. (2013). The colorimetric method based on bicinchoninic acid was used to determine total protein concentration (Smith et al., 1985).

Total CYP content was determined using the method described by Omura and Sato (1964a,b). The catalytic activity of ethoxyresorufin O-deethylase (EROD) was determined as the rate of transformation of ethoxyresorufin to resorufin. The incubation procedures were previously described by Steinbach et al. (2014). Briefly, incubation mixtures contained 0.2 mg microsomal protein in an incubation medium of 50 mM potassium phosphate buffer (pH 7.4) with 1.0 mM nicotinamide adenine dinucleotide phosphate and 2 μM of 7-ethoxyresorufin. The fluorescence detector (Infinite 200 – Photometer TECAN, Männedorf, Switzerland) was used to detect resorufin with excitation and emission at 544 and 590 nm, respectively. The enzymatic activities were expressed as pmol of resorufin produced per mg of microsomal proteins per minute (the limit of detection (LOD) for resorufin was 2 pmol). The variation in absorbance in each reaction well was linear over time ($R^2 > 0.96$).

The total concentration of plasma VTG in the male chub was assessed using a carp vitellogenin ELISA kit (Biosense Laboratories, Norway). Specific binding between antibodies and VTG was performed in pre-coated 96-well microtiter plates, according to the manufacturer's instructions (V01003402-096) using a calibration curve. All assays were performed spectrophotometrically at 405 nm in duplicate. The LOD for VTG was 4.8 ng/mL.

2.4. Water quality and chemical analysis

All analysis were performed in the laboratory of the State Veterinary Institute in Prague and in National Reference Laboratory for POP analysis at Institute of Public Health in Ostrava, which are accredited for the range of analyses of interest in accordance with EN ISO/IEC 17025: 2005. They use wide range of reference materials and annually participate in international inter-laboratory tests.

Three semipermeable membrane devices (SPMDs) and one polar organic compounds integrative sampler (POCIS) were used for assessment of water quality with specific organic pollutants. SPMDs with performance reference compounds (PRC) and polar organic chemical integrative samplers (POCISs) in triphasic sorbent configuration (Pest) were purchased from Exposmeter (Trehorningen, Tavelsjo, Sweden). The passive samplers were deployed for a period of 4 weeks in end of May and beginning of June in 2010.

Polar pesticides, pharmaceuticals and perfluorinated compounds