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Effects of pollution on chub in the River Elbe, Czech Republic

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ABSTRACT

The Elbe River is one of the most polluted aquatic ecosystems in the Czech Republic. The effect of three major chemical plants located on the Elbe River (at Pardubice, Neratovice, and Usti nad Labem) on fish was studied in 2004. Health status, chemical concentrations (Hg, PCB, DDT, HCH, HCB, OCS, 4-*tert*-nonylphenols, 4-*tert*-octylphenol) in muscle, and biomarkers (hepatic ethoxyresorufin-*O*-deethylase (EROD), plasma vitellogenin, and plasma 11-ketotestosterone) were assessed in male chub (*Leuciscus cephalus* L.). Differences between localities upstream (US) and downstream (DS) from the monitored source of pollution were identified. Fish from DS sampling sites showed significantly higher levels of contaminants than fish from US sampling sites. Generally, the concentrations of pollutants in fish from the Elbe sites were significantly higher compared to the reference site. Reduced gonad size, decreased plasma levels of 11-ketotestosterone, EROD and vitellogenin induction, and histopathologies of male gonads indicated harmful effects of aquatic pollution in fish from the Czech portion of the Elbe River.

1. Introduction

The Elbe River, part of an important European aquatic ecosystem, is one of the most polluted aquatic ecosystems in the Czech Republic. It is 1103.5 km long with a catchment area of 148 268 km^2, and flows through the Czech Republic (51 336 km^2) and Germany (96 932 km^2). Heavy metals and persistent organic compounds are the major contaminants monitored in the aquatic environment, both in biotic and abiotic compartments, in recent years. Mercury (Hg), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane and its metabolites (DDTs), α-, β-, and γ-isomers of hexachlorocyclohexane (HCHs), hexachlorobenzene (HCB), octachlorostyrene (OCS) were pointed out as the most important pollutants of the Elbe River (Rudis, 2000; Hilscherova et al., 2001; Celechovska et al., 2005; Marsalek et al., 2006; Randak et al., 2006; Hajslova et al., 2007).

Various categories of contaminants may cause adverse effects on endocrine systems of aquatic organisms. Endocrine disrupting chemicals (EDCs) are man-made and naturally occurring chemicals which can affect the balance of hormone function of organisms, e.g. pharmaceuticals, pesticides, industrial chemicals (PCBs, PAHs, phthalates, styrenes), degradation products of tensides (alkylphenols), personal care products, Hg, and others (Keith, 1998; Tyler et al., 1998; Mills et al., 2001). The majority of the above-mentioned pollutants are persistent and/or lipophilic chemicals, which can accumulate in various environmental compartments and also enter the food chain, thus negatively affecting non-aquatic as well as aquatic organisms. Exposure to a number of planar organic contaminants (e.g. PAHs, PCBs) activates induction of the mixed function oxygenase (MFO) system of organisms (Palace et al., 1996; Kirby et al., 1999).

The assessment of surface water contamination by various xenobiotics may be performed, not only by ‘classic’ chemical monitoring of selected pollutants, but also through examination for indicators of adverse effects of pollution on organisms. Selected biochemical parameters, so-called biomarkers in an indicator fish, can be used for this purpose (Van der Oost et al., 2003).

The use of biomarkers has become widespread and is integral to many monitoring programs. The presence of plasma vitellogenin (VTG) and 11-ketotestosterone (KT) in male fish, gonadosomatic index (GSI), and hepatic ethoxyresorufin-*O*-deethylase (EROD) activity are used as biomarkers in the Czech national monitoring program.

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Synthesis of VTG, a lipophosphoprotein, is induced by estradiol in the liver of female fish (Schwaiger and Negele, 1998). In the presence of substances with estrogenic effects, synthesis of VTG is carried out in the liver of male fish, which may lead to degenerative alterations of male gonads, reproductive breakdown, and, in extreme cases, sex reversal (Sumpter and Jobling, 1995). In recent years, plasma VTG levels have been viewed as an important biomarker for the determination of exposure to estrogenic endocrine disruptors (Jobling et al., 1998; Kolarova et al., 2005; Kirby et al., 2007).

Supplemental biomarkers, which can identify effects related to endocrine disruptors, are variations in steroid hormone concentrations in fish blood plasma. Steroid hormones affect sex differentiation, maturation, spawning, sexual behavior, and secondary sexual characteristics. Some studies reported that exposure of fish to substances having endocrine disruptor effects lead to decreased levels of sex steroid hormones, e.g. KT (Hecker et al., 2002). GSI has been widely used as a biomarker in fish both in field studies and laboratory experiments (Kime, 1995).

CYP1A1 is the terminal component of the MFO system and is of pivotal importance in the detoxification of certain organic contaminants (e.g. PAH, PCBs). EROD activity is CYP1A dependent and is measured as a marker of induction of the MFO system. EROD is widely used as biomarker of aquatic environment contamination (Kirby et al., 1999, 2007; Siroka and Drastichova, 2005; Havelkova et al., 2008).

The aim of the study was to examine the effects of effluents from three major chemical plants along the Elbe River in the Czech Republic on fish. The concentrations of pollutants (Hg, PCBs, DDTs, HCHs, HCB, OCS, 4-*tert*-nonylphenols, and 4-*tert*-octylphenol) were measured in fish muscle as proof of their exposure and accumulation. Health status evaluation and analyses of biomarkers (EROD, VTG, KT) were used to evaluate the effects of contaminants present in the aquatic environment on fish. Chub (*Leuciscus cephalus* L.) was chosen as the sentinel fish, due to its reported sensitivity to particular biomarkers (Flammarion et al., 2000; Machala et al., 2001) and its relative abundance at the sampling sites. Wild chub have previously been used for some field studies (Devaux et al., 1998; Larno et al., 2001; Flammarion et al., 2002; Krca et al., 2007; Christoforidis et al., 2008).

2. Material and methods

2.1. Fish sampling

Wild male chub were captured by electrofishing in the first half of May 2004, from six sites located both upstream (US) and downstream (DS) from three potential sources of pollution of aquatic ecosystem on the Elbe River. Adult fish of similar size were targeted at each site to reduce variation due to age; however, the age of fish varied. At each location, 4–10 chubs were collected. A total of 51 specimens were analyzed. Length and body weights were measured and scales collected for age determination. Sex was determined macroscopically and later confirmed by light microscopic evaluation of hematoxylin–eosin-stained sections. The main characteristics of fish samples are summarized in Table 1.

The US and DS locations were separated by weirs. The weirs make US migrations of fish hardly possible. The DS locations were situated as near as possible to the discharges from suspected sources of pollution. The distances between US and DS sampling sites were usually 3–10 km. A site on the Vltava River in South Bohemia was selected as an unpolluted reference area. The locations of the sampling sites are illustrated in Fig. 1. The locations were:

- Pardubice (population 90 000) is the capital city of the Pardubice Region, situated on the River Elbe, 104 km east of Prague. It is a major industrial city with a chemical factory, an oil refinery, a heavy machinery manufacturer, and an electronic equipment plant. The chemical factory Alliachem Synthesia is the most important producer of paints, pigments, and explosives in the Czech Republic. Its production of organic specialities includes primarily active components for making medicines and pesticides. The factory grounds are contaminated with a broad range of substances including simple aromatics, chlorinated aromatics, toxic metals (Hg, As), PCBs, organochlorine pesticides (OCPs), etc. (Heinisch et al., 2006, 2007).

Table 1
The main characteristics of male chub (*Leuciscus cephalus* L.)

Locality	n	Body weight (g) M±SD	Standard length (mm) M±SD	Age (years) M±SD
Reference site	7	764±120	333±19	7.0±0.5
US Pardubice	8	298±70	254±15	3.6±0.9
DS Pardubice	10	374±173	272±35	5.1±1.2
US Neratovice	8	421±135	278±38	4.3±1.0
DS Neratovice	8	378±240	254±44	4.5±1.2
US Ústí nad Labem	6	213±8	190±10	3.5±1.0
DS Ústí nad Labem	4	606±371	289±60	5.5±1.5

n = number of fish examined, M±SD = mean±standard deviation.

Fig. 1. Sampling sites.

- Neratovice (population 16 400) is located in the Central Bohemian Region of the Czech Republic. The chemical plant Spolana is located there. It currently produces caprolactam as a raw material for polyamide fiber and the making of plastics, polyvinylchloride (PVC) and several inorganic compounds (e.g. ammonium sulphate, hydrochloric acid, sulphuric acid). In the past, it manufactured a broader range of substances, e.g. linear olefins, viscose staple and chlorinated pesticides. The chemical plant grounds are contaminated with dioxins, mercury, chlorinated aliphatic hydrocarbons, and OCPs (Heinisch et al., 2006, 2007; Stachel et al., 2004).
- Usti nad Labem, the regional capital (population 100 000), at the lower reaches of the Elbe River, is in an area affected by agricultural and industrial activity. The most important source of pollution of the Elbe River is the Spolchemie chemical plant. The production at the plant is divided into three basic segments, namely the manufacturing of inorganic chemicals, synthetic resins, and organic paints. Grounds of the chemical plant are contaminated mainly with chlorinated aliphatic hydrocarbons, simple aromatics, polyaromatic hydrocarbons, PCBs, metals, organochlorinated pesticides, and related substances (DDTs, HCB) (Heinisch et al., 2006, 2007).
- The reference site was a location on the upper reaches of the Vltava River (the tributary of the Elbe River) US from the Lipno reservoir, with no known sources of significant anthropogenic pollution. This part of the Vltava River is situated in Sumava National Park, it has a more natural character and it passes mainly through grazing grounds and woodland. It was not possible to obtain an unpolluted site on the Elbe River with the presence of chub.

2.2. Tissue sample processing

Fish were held in aerated tanks until processed (usually less than 1 h) and blood samples were taken by caudal venipuncture into heparinized tubes to keep capture and handling stress to a minimum. Samples were centrifuged on site, and plasma samples were immediately frozen in liquid nitrogen.

The health status of the fish was assessed, and external features were recorded. Gonads and livers were dissected and weighed. Liver samples were placed in tubes and frozen in liquid nitrogen for subsequent analysis of EROD activity. Muscle samples were put into polyethylene bags, labeled, and stored at −18 °C. Gonads and grossly visible tissue anomalies were preserved in 10% formalin for histopathological examination. The GSI was calculated as follows:

$$GSI = \text{gonad weight}/\text{body weight} \times 100(\%).$$

2.3. Biochemical analyses

Measurement of VTG and KT in the blood plasma was performed by using pre-coated ELISA kits (Biosense laboratories® Norway) according to the manufacturer's instructions. The use of carp vitellogenin ELISA for determination of vitellogenin in chub was validated by Flammarion et al. (2000). Absorbance was measured using a SLT Spectra (A5082) instrument set at 492 nm for VTG and 420 nm for KT detection. The limit of detection (LOD) of the procedure for VTG and KT was 10 ng ml^{-1} and 13 pg ml^{-1}, respectively.

The EROD activity was determined spectrofluorometrically (LOD 2 pmol mg^{-1} min^{-1}). In the presence of NADPH, EROD activity converts the substrate ethoxyresorufin, which is a fluorescent product. Standard phosphate buffer, NADPH and suspension adequate for 0.2 mg ml^{-1} protein were put into a cell. Ethoxyresorufin was added, and the increase in fluorescence was monitored for 5 min (excitation/emission wavelengths were 535/585 nm). The EROD activity was subsequently calculated based on a comparison with fluorescence of the standard (resorufin) of known concentration (Rutten et al., 1992; Siroka et al., 2005).

2.4. Chemical analysis

Persistent organochlorine pollutants, including PCB indicator congeners (IUPAC no. 28, 52, 101, 118, 138, 153, and 180); HCB; α-, β-, γ-isomers of HCH; OCS, *o,p′*- and *p,p′*-isomers of DDT and its metabolites DDE and DDD) were measured in individual muscle samples using methods described in detail by Hajslova et al. (1995). Isolation of target analytes from fish muscle was carried out by Soxhlet extraction using the solvent mixture, hexane:dichloromethane (1:1, v/v). Crude extracts were purified employing gel permeation chromatography (GPC) on a Bio Beads S-X3 column using cyclohexane:ethylacetate (1:1, v/v) as a mobile phase. An HP 5890 gas chromatograph equipped with two electron capture detectors (Agilent Technologies, USA) and two parallel columns possessing different selectivity (DB-5 and DB-17; 60 m × 0.25 mm × 0.25 μm), both J&W Scientific, USA) were employed for analyses of PCBs and OCPs. Performance characteristics obtained within a validation study were as follows: limits of quantification (LOQ) for individual PCBs and OCPs ranged between 0.2 and 1.0 μg kg^{-1} lipid weight. Recoveries for target analytes varied between 85% and 103% (RSD < 11%).

Mercury content of individual muscle samples was measured by cold vapor atomic absorption spectrometry on an AMA-254 (Altec Ltd., Czech Republic) single-purpose mercury analyzer (detection limit 0.001 mg kg^{-1}; recovery 82 ± 6%). Accuracy of the results was validated using standard reference material BCR-CRM 463 (Tuna fish).

For determination of alkylphenols, pooled fish samples were desiccated with anhydrous sodium sulphate and transferred to an Erlenmeyer flask. After addition of surrogate standards (4-*n*-nonylphenol and 4-*n*-octylphenol), samples were extracted by sonication using an extraction mixture, *n*-hexane/dichlormethane (1:1, v/v). Crude extracts were purified employing GPC on a Bio Beads S-X3 column using cyclohexane:ethylacetate (1:1, v/v) as a mobile phase. Final identification and quantification of target analytes were performed by gas chromatography (HP 6890 gas chromatograph) employing a mass selective detector HP 5973 (Agilent Technologies, USA) operated in selected ion monitoring (SIM) mode. Isotopically labeled d_8-4-*n*-nonylphenol was used as an internal standard for correction of matrix effects. Separation of sample components was carried out on a DB-5ms column (60 m × 0.25 mm × 0.25 μm). An aliquot of 1 μl of the purified extract was injected (pulsed splitless mode) at 250 °C. The oven temperature was: 60 °C (2 min), 45 °C min^{-1} to 180 °C, 2 °C min^{-1} to 230 °C, 60 °C min^{-1} to 300 °C (9 min). Helium was used as a carrier gas (1 ml min^{-1} flow). The recovery of analytical method obtained by repeated analyses ($n = 6$) of fortified fish samples was 85 ± 12% for 4-*tert*-octylphenol (spiking level 1 ng g^{-1} wet weight) and 77 ± 16% for 4-*tert*-nonylphenols (spiking levels 10 ng g^{-1} muscle). The LOQs were 0.2 ng g^{-1} wet weight and 2 ng g^{-1} wet weight for 4-*tert*-octylphenol and 4-*tert*-nonylphenols, respectively.

2.5. Statistical analysis

As the data obtained did not meet the requirements for parametric statistical tests (normality), they were analyzed non-parametrically. The descriptive statistics were expressed as the median and percentiles in graphs, and mean ± SD (standard deviation) and median in tables. The results were subjected to Kruskal–Wallis test to analyze regional differences in the parameters. The differences between sites US and DS of potential sources of pollution were assessed using Mann–Whitney *U*-test. Significance was accepted when $P < 0.05$. The Spearman rank correlation coefficient was used for analysis of relationships between analyzed parameters. The analyses were performed in Statistica for Windows 8.0 (Statsoft Inc., 2007).

2.6. Ethical statement

The study was conducted in accordance with national and institutional guidelines for the protection of animal welfare (Act no. 246/1992 Coll., Animal Welfare, Czech Republic). During the whole study, the principles of the Ethical Committee for the Protection of Animals in Research of the University of South Bohemia, Research Institute of Fish Culture and Hydrobiology Vodnany were strictly followed.

3. Results

3.1. Health status and histology

Fish collected at the reference site showed no pathological changes. Males exhibited fully matured gonads and were ready to spawn, which corresponded to the spring season. The lumen of the cyst was filled with spermatozoa (Fig. 2).

Fish DS from Neratovice showed the worst health status. Fins of most specimens exhibited uneven edges with mechanically damaged surfaces. All specimens exhibited large open lesions and abscesses on the skin and exophthalmia and large keratoleukomas of the eyes. However, no gonad histopathologies were observed in fish from the Neratovice site, with the exception of a single male showing macroscopically visible parasite infection of the gonad. Gonad sections were filled with cysts containing spores of Pleistophora spp.

Two males (50%) DS from Usti nad Labem exhibited gonad anomalies. Light micrographs showed intersex characteristics (Fig. 3). Perinucleolar oocytes were dispersed in testicular tissue. Fig. 3D shows neoplastic cell masses adjacent to normal lobules.

No pathologies of gonads were found at the remaining locations.

3.2. Biomarkers

The highest levels of VTG in plasma of male chub were measured in fish from the DS Usti nad Labem location (Table 3),

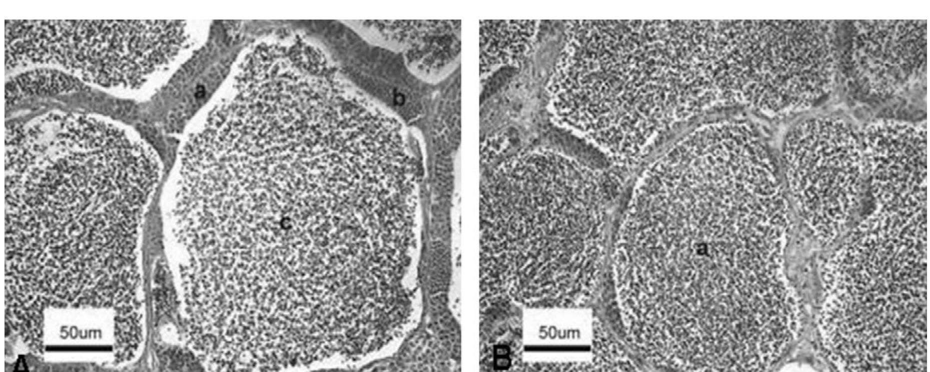

Fig. 2. Testis of chub (*Leuciscus cephalus* L.) from reference locality. (A) Late spermatogenic testes containing spermatocytes (a), spermatids (b), and spermatozoa (c). (B) Late spermatogenic testes containing mostly spermatozoa (a).

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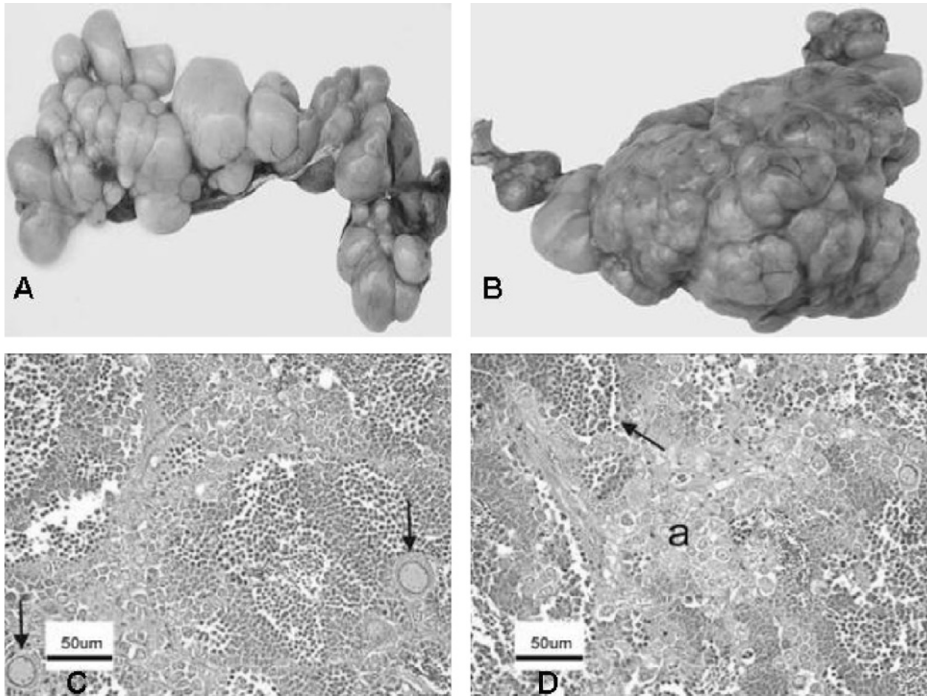

Fig. 3. Testis of chub (*Leuciscus cephalus* L.) from locality downstream Usti nad Labem. (A and B) Nodular mass in the testis of chub. (C) Light micrograph showing intersex. Perinucleolar oocytes (arrow) are dispersed in testicular tissue. (D) Neoplastic cell masses (a) adjacent to normal lobules (arrow).

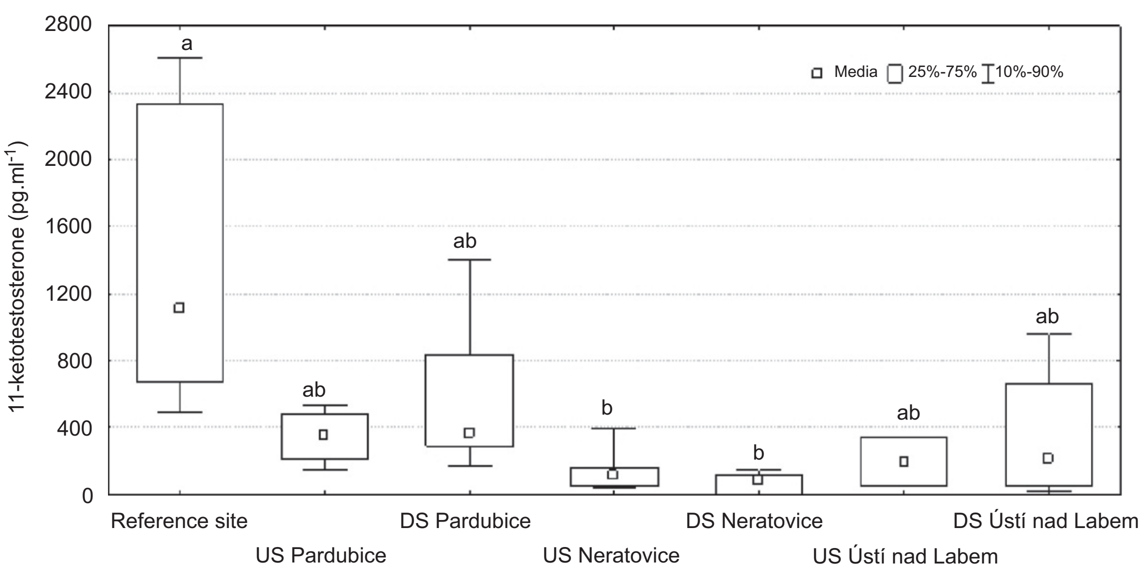

Fig. 4. Comparison of 11-ketotestosterone concentrations in blood plasma (pg ml^{-1}) of male chub (*Leuciscus cephalus* L.) from the monitored sites. Different superscript letters indicate significant differences ($P<0.05$).

while the lowest value of VTG content was in those from the US Neratovice site. The VTG level in fish from the reference site was not significantly different from those in other localities.

The highest levels of KT in plasma were observed in male chub from the reference site (Fig. 4). The lowest KT values were found in chub collected from DS Neratovice. KT levels in fish from the reference site were significantly higher ($P<0.01$) than in fish from both US and DS Neratovice. Two males from DS Usti nad Labem with low KT (80.8 and 16.2 pg ml^{-1}) were intersex, while two other males from the same site showed much higher KT concentrations (349.2 and 962.2 pg ml^{-1}) and exhibited no pathological anomalies of gonads.

The highest GSI was in fish from the reference location (Fig. 5). The GSI in fish from the reference site was significantly higher than in those from sites DS of Pardubice ($P<0.01$) and DS of Neratovice ($P<0.05$).

The highest EROD values were found in samples collected from DS Neratovice (Fig. 6). The EROD values detected in fish from the reference site were significantly lower in comparison to those from US Neratovice ($P<0.01$), DS Pardubice ($P<0.05$), and DS Neratovice ($P<0.05$).

When comparing sites located US to those DS of potential sources of pollution, no significant differences were found in VTG, KT, and EROD activity values. Significant differences

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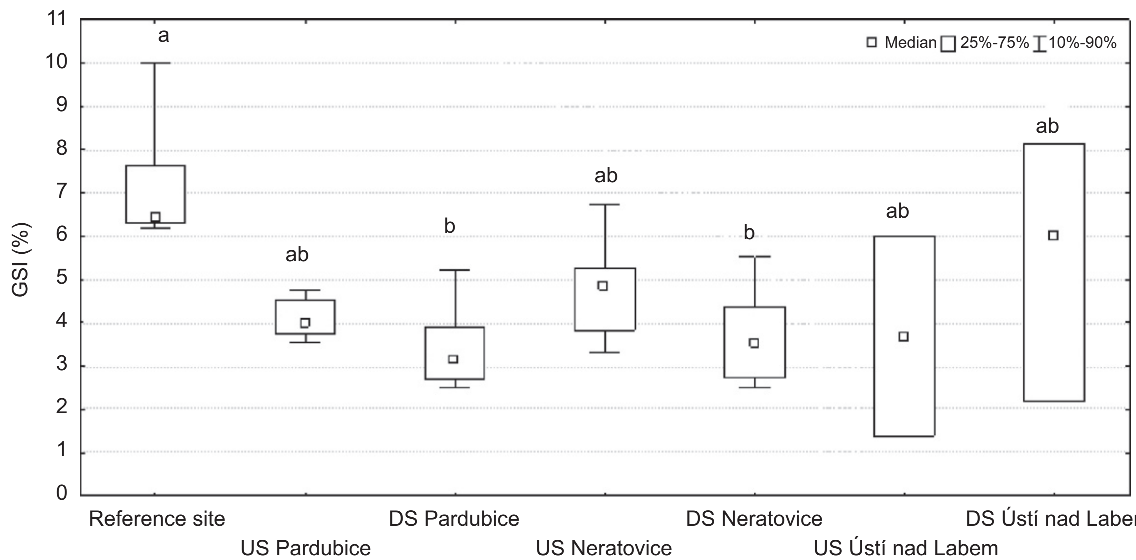

Fig. 5. Gonado somatic index (GSI) of male chub from the monitored sites. Different superscript letters indicate significant differences ($P<0.05$).

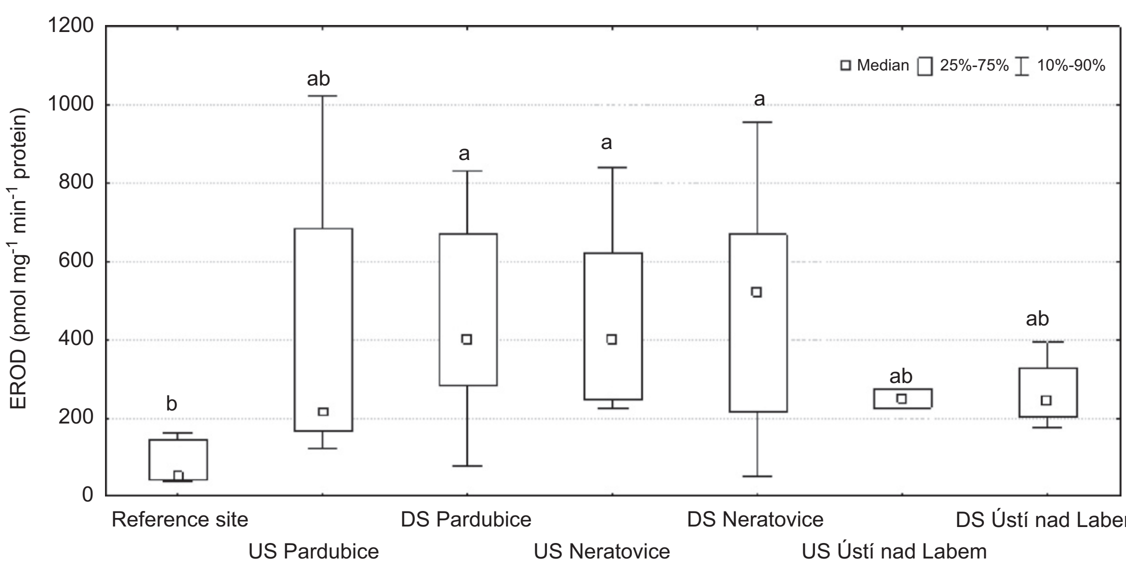

Fig. 6. Comparison of ethoxyresorufin-*O*-deethylase (EROD) activity in liver samples (pmol mg^{-1} min^{-1}) of male chub (*Leuciscus cephalus* L.) from the monitored sites. Different superscript letters indicate significant differences ($P<0.05$).

($P<0.05$) in GSI values was observed between sites DS and US Pardubice.

3.3. Concentrations of pollutants

The highest concentration of PCBs in chub muscle was found in fish from the Neratovice sites (Fig. 7). Significantly lower levels ($P<0.01$) of PCBs were found at the reference location as compared to sites at Pardubice and Neratovice. Higher ($P<0.05$) concentrations of PCBs were measured in all sites located DS of potential sources of pollution compared to those located US.

When we compared the relative contribution of individual PCB congeners, the most abundant were the higher chlorinated congeners nos. 138, 153, and 180, which contributed 40–80% of the total amount of measured PCBs (Fig. 8). This pattern was observed at all sampling sites.

Other measured organochlorine pollutants were residues of OCPs, represented by isomers of DDT and its metabolites DDD and DDE, HCB and HCH isomers. Similar to the previously discussed indicator PCBs, the lowest concentration of DDTs (sum of *o,p′*- and *p,p′*-isomers DDE, DDD, and DDT) in fish muscle was measured at the reference site (Table 2), mean 0.92 mg kg^{-1} lipid weight. Highest levels of DDTs were found at DS Usti nad Labem (mean 6.48 mg kg^{-1} lipid weight) and DS Neratovice (mean 4.83 mg kg^{-1} lipid weight). Significant differences ($P<0.05$) in DDTs levels were observed between DS and US Neratovice as well as DS and US Pardubice. Contributions of individual DDT isomers and its metabolites are shown in Fig. 9. With the exception of at DS Pardubice, *p,p′*-DDE were the most abundant chemical, contributing up to 85% of the total DDTs in fish muscle.

Concentrations of HCB were detected in all samples, but levels were one order of magnitude lower than those of PCBs and DDTs. The sampling site with the highest levels of HCB in fish muscle (0.521 ± 0.229 mg kg^{-1} lipid) was DS Usti nad Labem, while the lowest concentration (0.033 ± 0.005 mg kg^{-1} lipid) of HCB in chub muscle was found in samples collected at the

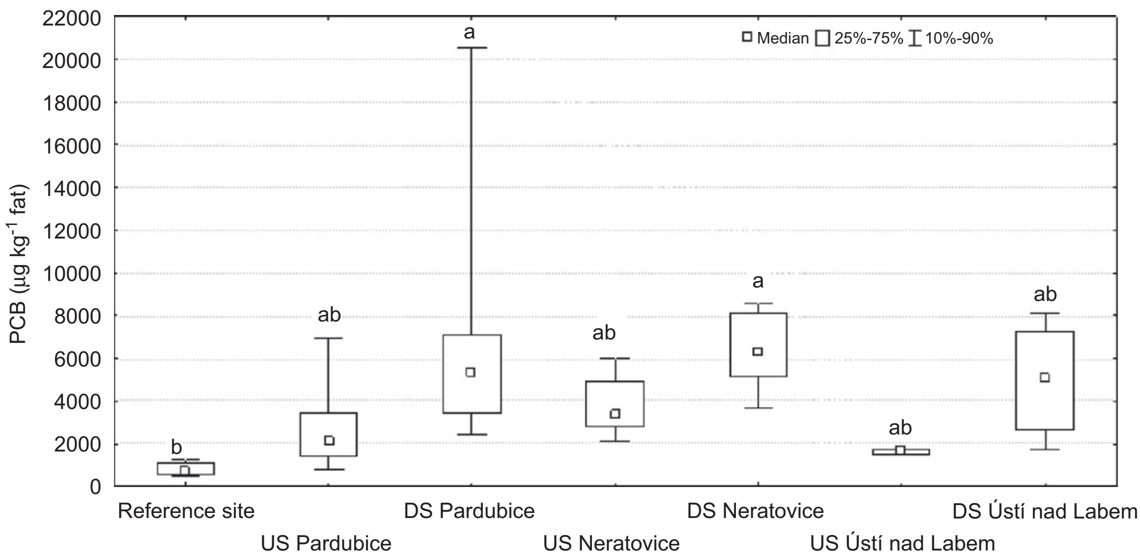

Fig. 7. Levels of indicator PCBs in chub in all sampling sits ($\mu g\,kg^{-1}$ lipid weight). Different superscript letters indicate significant differences ($P<0.05$).

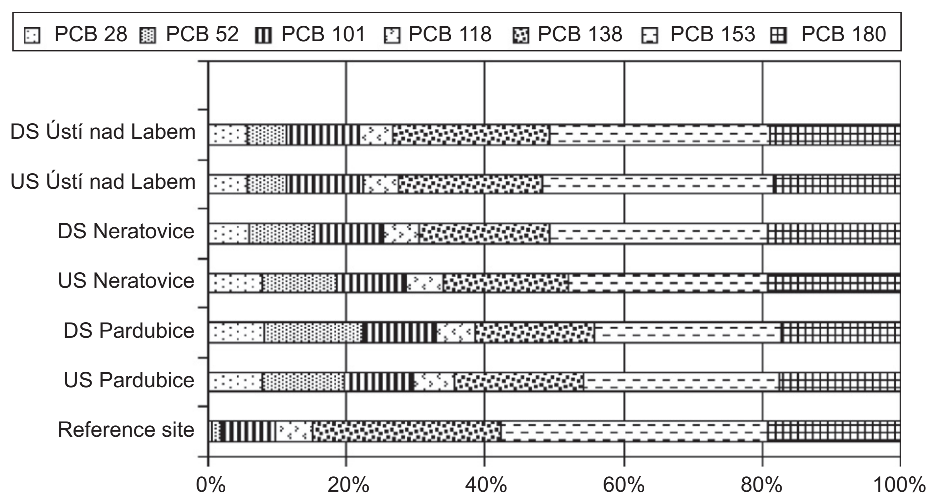

Fig. 8. Relative contributions of individual indicator PCB congener in chub muscle.

Table 2
Content of hexachlorocyclohexane (HCHs), dichlorodiphenyltrichloroethane and its metabolites (DDTs), hexachlorobenzene (HCB) in muscle of male chub (*Leuciscus cephalus* L.)

Locality	Fat (%) M±SD	HCHs* ($mg\,kg^{-1}$ lipid) M±SD (median)	DDTs** ($mg\,kg^{-1}$ lipid) M±SD (median)	HCB ($mg\,kg^{-1}$ lipid) M±SD (median)
Reference site	2.3±0.6	0.018±0.004 (0.018)^{d}	0.92±0.17 (0.98)^{c}	0.033±0.005 (0.035)^{b}
US Pardubice	2.4±0.6	0.021±0.006 (0.020)^{cd}	1.29±0.79 (0.95)^{bc}	0.144±0.047 (0.122)^{ab}
DS Pardubice	1.6±0.6	0.024±0.005 (0.025)^{bcd}	2.85±1.62 (2.61)^{abc}	0.195±0.603 (0.184)^{a}
US Neratovice	2.1±0.4	0.040±0.006 (0.040)^{abc}	1.39±0.46 (1.30)^{abc}	0.138±0.023 (0.137)^{ab}
DS Neratovice	1.4±0.4	0.486±0.970 (0.054)^{a}	4.83±1.94 (4.01)^{a}	0.288±0.104 (0.245)^{a}
US Usti nad Labem	1.7±0.3	0.056±0.003 (0.056)^{abc}	1.14±0.27 (1.14)^{abc}	0.109±0.012 (0.109)^{ab}
DS Usti nad Labem	2.4±0.7	0.053±0.009 (0.055)^{ab}	6.48±4.52 (5.81)^{ab}	0.521±0.229 (0.601)^{a}

M±SD = mean±standard deviation.
US—upstream from.
DS—downstream from.
Values with the same alphabetical superscript within each column do not differ significantly ($P<0.05$).
* Sum of HCH isomers (α, β, γ).
** Sum of DDT (*o,p′*-DDE; *p,p′*-DDE; *o,p′*-DDD; *p,p′*-DDD; *o,p′*-DDT; *p,p′*-DDT).

reference site (Table 2). Significantly more contaminated fish were situated DS from the potential source of pollution in all localities ($P<0.05$).

Isomers of HCHs were also found in all analyzed samples of chub muscle. Fish from the reference site showed significantly lower concentrations of HCHs compared to US Neratovice

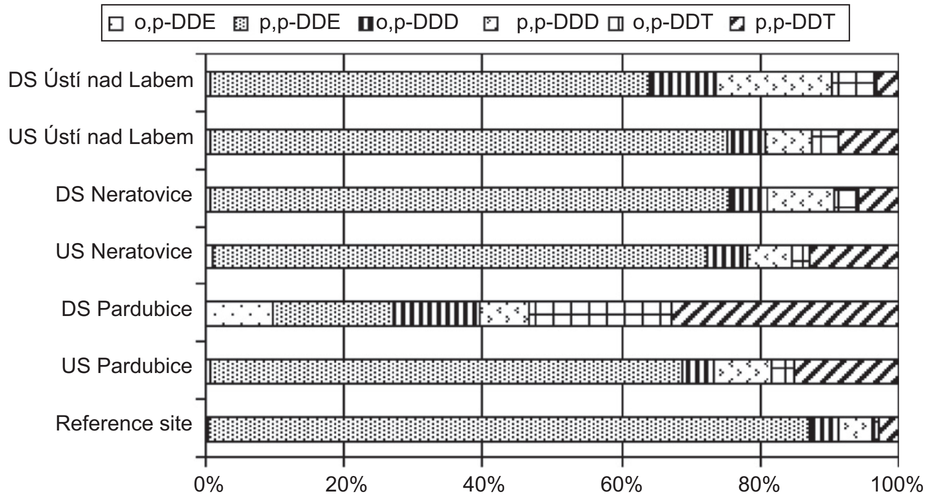

Fig. 9. Relative contributions of individual DDT isomers and its metabolites DDD and DDE in chub muscle.

Table 3
Content of octachlorostyrene (OCS), mercury (Hg), and alkylphenols (AF) in muscle and levels of vitellogenin (VTG) in blood plasma of the analysed male chub (*Leuciscus cephalus* L.)

Locality	Fat (%) M ± SD	OCS (mg kg^{-1} lipid) M ± SD (median)	Hg (mg kg^{-1} muscle) M ± SD (median)	AF* (μg kg^{-1} muscle)	VTG (μg ml^{-1}) M ± SD (median)
Reference site	2.3 ± 0.6	0.004 ± 0.001 (0.004)^{c}	0.789 ± 0.180 (0.803)^{a}	1.1	0.97 ± 0.19 (0.96)^{a}
US Pardubice	2.4 ± 0.6	0.153 ± 0.212 (0.065)^{abc}	0.157 ± 0.080 (0.140)^{c}	3.0	62 ± 165 (0.70)^{a}
DS Pardubice	1.6 ± 0.6	1.013 ± 0.764 (0.928)^{a}	0.351 ± 0.110 (0.380)^{abc}	2.7	12431 ± 26471 (377)^{a}
US Neratovice	2.1 ± 0.4	0.045 ± 0.014 (0.042)^{bc}	0.664 ± 0.387 (0.498)^{ab}	1.1	1.9 ± 3.5 (0.13)^{a}
DS Neratovice	1.4 ± 0.4	0.100 ± 0.022 (0.097)^{ab}	0.543 ± 0.096 (0.528)^{ab}	1.1	98 ± 210 (15)^{a}
US Usti nad Labem	1.7 ± 0.3	0.024 ± 0.018 (0.024)^{abc}	0.110 ± 0.037 (0.110)^{bc}	2.3	321 ± 437 (321)^{a}
DS Usti nad Labem	2.4 ± 0.7	0.221 ± 0.124 (0.269)^{ab}	0.260 ± 0.046 (0.239)^{bc}	2.7	3226 ± 5896 (426)^{a}

M ± SD = mean ± standard deviation.
US—upstream from.
DS—downstream from.
Values with the same alphabetical superscript within each column do not differ significantly ($P<0.05$).
* Sum of AF (4-*tert*-nonylphenol and *tert*-octylphenol) pooled samples.

($P<0.05$), DS Neratovice ($P<0.01$), and DS Usti nad Labem ($P<0.01$). Those from DS Neratovice showed the highest levels of HCH isomers (Table 2). A significant difference ($P<0.05$) was observed in HCHs levels between DS and US Neratovice. When comparing the relative contribution of individual isomers, the most abundant were β- (35–50%) and γ-isomer (i.e. lindane), both of which contributed 35–50% of the total HCHs, while α-HCH was a relatively minor isomer in chub muscle (approx. 15%).

Levels of OCS were comparable to those observed for HCB. Lowest levels were found at the reference site (mean 0.004 mg kg^{-1} lipid weight). Highest levels (mean 1.01 mg kg^{-1} lipid weight) were found in fish from DS Pardubice. In all cases, fish from sampling sites DS of the potential source of pollution showed higher OCS levels compared to those US.

No significant differences among sampling sites on the Elbe River, or between locations US and DS from a potential pollution source, were found in organochlorine pollutants, alkylphenols (4-*tert*-nonylphenol and 4-*tert*-octylphenol).

The lowest Hg concentrations were found in fish from US Neratovice, while the highest were in fish from the reference site. Mercury concentrations in fish muscle from the reference location were significantly higher than those measured in fish collected at US Pardubice ($P<0.01$) and US Usti nad Labem ($P<0.05$). Significant differences ($P<0.05$) of Hg levels were observed between DS and US sites at both Pardubice and Usti nad Labem.

3.4. *Correlations between biomarkers and pollutant concentrations*

Results of chemical monitoring and measures of biomarkers revealed significant negative correlations ($P<0.05$) between KT levels and the PCBs ($R=-0.389$), HCB ($R=-0.421$) and HCH ($R=-0.716$) concentrations in muscle in all groups of fish analyzed. Significant negative correlations ($P<0.05$) were also found between VTG levels and the concentrations of PCBs ($R=-0.426$) and OCS ($R=-0.444$) and between GSI and PCBs ($R=-0.471$), HCB ($R=-0.441$), DDTs ($R=-0.392$), and OCS ($R=-0.585$) concentrations in muscle. A significant correlation ($P<0.05$) was also observed between EROD activity and the concentration of PCBs ($R=0.557$), HCB ($R=0.568$), HCHs ($R=0.490$), and OCS ($R=0.524$).

4. Discussion

Male chub inhabiting the Elbe River were used as a sentinel species to examine the effects of effluents from three major chemical plants situated along this river. Males are generally more sensitive to, and more affected by, endocrine disruptors than females (Tejeda-Vera et al., 2007).

Gonads of male fish from DS Usti nad Labem locality were negatively affected in several cases. The study was not focused on the determination of sex ratios at sampling sites, but the sex of

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captured chubs was determined. Among 50 adult chub caught at the locality DS of Usti nad Labem, only four were males, two of which were identified as intersexes. Theoretically it is possible that males were present in other parts of the river due to spawning or food migration. However, this distribution of sexes was not observed in all remaining sampling sites which were sampled during the same season. For comparison, the sex ratio of males to females varied between 1:1 and 1:2.5 at the remaining Elbe River sites and 1.7: 1 at the reference site. Additionally, one intersex male chub from DS Usti nad Labem site contained tumor tissue throughout the gonads. Skewed sex ratio and pathological changes in gonads clearly showed severe disturbances in the male chub population from this location. It is likely that affected males would not be capable of successful reproduction.

Extensive pollution of the Czech environment has occurred in the last decades due to uncontrolled use of hazardous chemicals, resulting in continuous run-off of pollutants from industrial/municipal emission sources and/or dumping sites. As in various countries worldwide, the major sink for many organic contaminants, such as PCBs and OCPs, is the aquatic ecosystem, and their accumulation in both abiotic and biotic compartments has occurred (Svobodova et al., 1995; Kolarova et al., 2005). Fish sampling is a suitable tool for assessing aquatic ecosystem pollution through measurement of the bio-available fraction of large groups of organic contaminants, since fish are available everywhere in the aquatic environment (de Boer and Brinkman, 1994).

The differences of PCB concentrations in chub muscle between US and DS sites showed that the monitored chemical plants were important sources of PCB contamination of the Elbe River. The indicator congeners of PCBs (nos. 28, 52, 101, 118, 138, 153, and 180) that are commonly used for characterization of extent and type of technical mixture responsible for contamination were found in all sampling locations. The PCBs profiles in chub from all sites were similar in dominance of hexa- and heptachloro-congeners (PCB 138, 153, 180). At the reference site, a slightly lower contribution of lower chlorinated congeners PCB 28 and 52 was observed. This was probably due to an extremely low concentration of these chemicals, which was close to the LOQ. The predominance of higher chlorinated PCB congeners at all sampling sites may indicate the use of preparations containing Delor 106 (Taniyasu et al., 2003) in previous decades. Values of PCBs found in fish from the major tributaries of the Czech portion of the Elbe River were approximately two-fold lower than the fish from the main stream (Havelkova et al., 2007). This would indicate that the main sources of PCBs pollution of the Elbe River were situated directly on the river.

DDTs consists mainly of *p,p′*-DDE, with *p,p′*-DDD, and *p,p′*-DDT comprising only a minor portion. The relative contribution of *o,p′*-isomers is very low for all compounds. The low ratio between parent compound *p,p′*-DDT and its metabolite *p,p′*-DDE (about 0.1) at all test sites indicated that the application of DDT-based insecticides ceased several decades ago (DDT has been banned from Czech agriculture since the mid-1970s). Surprisingly, a significantly different DDTs profile was observed at DS Pardubice, where the major components were *p,p′*-DDT and *o,p′*-DDT, contributing more than 50% to the total DDTs amount (Fig. 9). This could indicate recent use or release of DDT in the area. Levels of DDTs found in fish from the most contaminated sites (DS Neratovice and DS Usti nad Labem) were approximately three- and six-fold, respectively, higher than those of fish from central Stockholm (Sweden; Linderoth et al., 2006) and many times higher than the values found in chub muscle from the Moselle River (France) (Flammarion et al., 2002).

HCH isomers and HCB represent another group of persistant organic pollutants (POPs) typically included in monitoring studies. Some release of these compounds into the environment was due to their use as pesticides (γ-HCH as insecticide, HCB as fungicide). HCB is a by-product of the industrial synthesis of chemicals, including carbon tetrachloride and perchloroethylene. However, due to the lower persistence of these compounds in the environment and/or due to the lower environmental concentrations, compared for example to DDT and PCBs, only low levels of HCH isomers and HCB were found at the test sites compared to levels of PCBs and DDTs (Table 2).

Although OCS was never used as a commercial product, it may be produced during incineration and combustion of chlorinated compounds. The highest levels of OCS were found in fish from DS Pardubice. Hence, this location was shown to contain a major source of OCS pollution, confirming the findings of a previous study (Randak et al., 2006). Levels similar to those in DS Pardubice have been found in fish from the lower reaches of the Elbe (Bester et al., 1998). Lower OCS levels have been detected in fish species from Belgium and Romania (Chu et al., 2003) compared to those in fish from our study.

Higher levels of the persistent organic pollutants in muscle were found in chub DS from the chemical plants and agglomerations that were selected as the potential sources. These findings confirmed that all of them are the sources of emission of monitored chemicals. Remarkably high amounts of POPs were also identified in sediments of middle (Pardubice, Neratovice) and lower (Usti nad Labem) reaches of the Elbe River (Heinisch et al., 2006, 2007).

The chemicals discharged by the monitored chemical plants involved could be responsible for activating EROD detoxification mechanisms in local fishes. The data on PCBs and OCP concentrations in muscle of indicator fish correlated with the induction of EROD activity. This is consistent with results of several studies reporting elevated levels of EROD activity in livers of fish exposed to organic contaminants such as PCBs, OCP, PAHs, dioxins, and agricultural and urban wastewater (Vigano et al., 1998; Machala et al., 2000; Behrens and Segner, 2005; Hansson et al., 2006). Induction of the EROD activity in hepatic tissue was demonstrated at all sampling sites except at the reference. The EROD, PCBs, and OCPs values seen in chub from the Elbe River were generally higher than those reported in wild chub from other rivers (Vigano et al., 1998; Machala et al., 2000, 2001; Flammarion et al., 2002; Winter et al., 2005). Similar values for these parameters were detected in chub from the Elbe River in a previous study (Randak et al., 2006).

The persistent organochlorine pollutants such as metabolites of DDT and some PCB congeners have been reported in fish as estrogens, anti-estrogens (Vaccaro et al., 2005; Zhang and Hu, 2008), and anti-androgens (Baatrup and Junge, 2001; Metcalfe et al., 2000; Thomas, 2000; Van den Berg et al., 2003). Therefore, the final effect of a complex pollutant mixture on fish is a function of many factors. The presence of chemicals with different modes of action has produced conflicting results in fish from sites on the Elbe River. Higher VTG levels in fish from contaminated sites on the Elbe River might be expected, but the presence of chemicals with antagonistic effects on VTG synthesis may be the key factor in the lower VTG induction. Simultaneous exposure of organisms to a variety of pollutants may result in additive, synergistic, or antagonistic effects on wild populations of fish (Tyler et al., 1998). Registered VTG concentrations in fish from DS Usti nad Labem were comparable with values found by Flammarion et al. (2000) in blood plasma of chub experimentally exposed to E_2. They found plasma VTG concentration higher than $1\,mg\,ml^{-1}$ in both females and males. Surprisingly, VTG concentrations in male fish from the reference site were 100 times higher of those reported by Flammarion et al. (2000) for untreated males. VTG values found in males from DS Pardubice and DS Usti nad

Labem were comparable with values reported for E_2-treated males in that study.

Data from laboratory studies have shown that exposure to a variety of EDCs suppresses the sex steroids, testosterone (T), and KT (Kime, 1995; Loomis and Thomas, 2000). Flammarion et al. (2002) found a decrease in GSI as a result of the exposure of chub to effluents containing organic pollutants and heavy metals. The present study has confirmed these findings. Only fish from the reference site showed KT levels consistent with concentrations of androgens typical for chub in the spawning season, as reported by Guerriero et al. (2005). Levels of KT in blood plasma of males from all other locations were comparable to levels of androgens typical for stasis and the post-spawning period. The role of sex steroids in controlling the developmental cycle in teleosts, especially during spawning, can be altered by environmental or hormonal manipulation (Guerriero et al., 2005). Persistent organochlorine pollutants, such as metabolites of DDT and some PCB congeners, have been reported as potent anti-androgens in fish (Baatrup and Junge, 2001; Metcalfe et al., 2000; Thomas, 2000; Van den Berg et al., 2003). In the present study, only minimal concentrations of DDTs and PCBs were reported in the reference fish, when compared to those from sites on the Elbe River. The highest GSI was also found in chub from the reference site. Gonads of males from the reference site were fully matured and fish were ready for spawning.

Surprisingly, the highest concentrations of Hg were found in muscle of fish from the reference locality but its origin at this site is unknown. One reason of this fact can be higher age of fish from the reference site in comparison with fish from the Elbe River sites. Mercury is accumulated in fish muscle during their life (Dusek et al., 2005) which means that concentrations of mercury in the muscle of older fish are usually higher than in the muscle of younger fish. Mercury is also listed as an EDC (Keith, 1998) so the link between the Hg concentrations and elevated levels of VTG in males from the reference locality is a concern. The most contaminated sites on the Elbe River were found to be US Neratovice and DS Neratovice as also reported in previous studies (Dusek et al., 2005; Zlabek et al., 2005; Marsalek et al., 2006). A decrease has been found in Hg concentrations in muscle of fish in comparison to historical data (Dusek et al., 2005) collected from the Neratovice section of the Elbe River. Nevertheless, stretches of the Elbe River adjacent to the Neratovice site remain among the most contaminated sites in the Czech Republic. Mercury concentrations in fish living near inflows into the Elbe River are generally many times lower. One order of magnitude lower Hg concentrations are reported for muscle of fish which were grown in Czech pond aquaculture and in the main tributaries of the Elbe River (Svobodova et al., 2002, 2004; Kruzikova et al., 2008).

Values of alkylphenols (sum of 4-*tert*-nonylphenols and 4-*tert*-octylphenol) found in fish from the Elbe River in this study were comparable to results of a previous study (Randak et al., 2006) and with levels in fish from the Kalamazoo River, Michigan (Kannan et al., 2003).

5. Conclusions

Fish captured upstream (US) and downstream (DS) of the monitored chemical plants showed varying degrees of contamination. In the majority of cases, fish from DS sampling sites showed significantly higher contamination than fish from US sites. Concentrations of pollutants in fish from the Elbe locations were generally significantly higher compared to the reference site. The data indicated important effects, at monitored sources, on contamination of the Elbe River. Reduced gonad size, decreased plasma levels of 11-ketotestosterone, EROD, and vitellogenin induction, histopathological changes in gonads and correlations between biomarkers and pollutant concentrations indicated that factors in the aquatic environment have affected important physiological functions in wild fish in the Elbe River. However other anthropogenic compounds (pharmaceuticals, personal care products, etc.), not analyzed, may also play a role in the observed levels of biomarkers. In fact, other studies have indicated that the major biological effects may be related to substances other than the classical environmental pollutants usually analyzed (Tyler and Routledge, 1998; Ying et al., 2002; Snyder et al., 2003; Douxfils et al., 2007). As mentioned, exposure of chemicals with different modes of action is also one of the reasons for unclear causal relationships between measured contaminants and biomarkers. Nevertheless, the data support the use of wild chub as a sentinel species in monitoring of aquatic ecosystems contamination.

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