ARTICLE IN PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Ecotoxicology and Environmental Safety ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Ecotoxicology and Environmental Safety

www.elsevier.com/locate/ecoenv

Bioaccumulation of mercury in muscle tissue of fish in the Elbe River (Czech Republic): multispecies monitoring study 1991–1996

L. Dušek[a,b,*], Z. Svobodová[c], D. Janoušková[c], B. Vykusová[c], J. Jarkovský[b], R. Šmíd[a,b], P. Pavliš[a,b]

[a]RECETOX, Faculty of Science, Masaryk University, Kamenice 126/3, 625 00 Brno, Czech Republic
[b]Center of Biostatistics and Analyses, Masaryk University, Kamenice 126/3, 625 00 Brno, Czech Republic
[c]Research Institute of Fish Culture and Hydrobiology, University of South Bohemia, Zátiší 728/11, 389 25 Vodňany, Czech Republic

Received 22 September 2003; accepted 29 November 2004

Abstract

The study monitored mercury (Hg) contamination of fish muscle tissue at 13 geographical sites that can be regarded as crucial points for an ecotoxicological assessment of the Czech Republic section of the Elbe River. The descriptive part of the study was primarily aimed at comparative evaluation of the Hg load during the period 1991–1996. The conclusions were supported by multivariate statistical analyses of the content of Hg in the muscle tissue of 1251 fish belonging to 23 species with four dominant indicator species: *Perca fluviatilis* ($n = 163$), *Abramis brama* ($n = 173$), *Rutilus rutilus* ($n = 148$), and *Leuciscus cephalus* ($n = 166$). Considering data from 3- to 5-year-old fish, significantly increased contamination was detected in typical predators compared to the other fish species in all sites ($P < 0.001$). On the other hand, omnivorous and planctivorous species were ranked as the least sensitive for Hg pollution. Perch appeared to be the most contaminated species in the sample with muscle Hg concentration in the range of 0.840–1.398 mg Hg kg^{-1}. Although less contaminated than perch, muscle contamination of bream sensitively separated differently contaminated sites; the highest load ranged from 0.368 to 0.543 mg Hg kg^{-1}. Time-related comparison of sampling campaigns revealed no significant trend changes, in either sediment samples or fish tissue. Thus, the analyses documented an evidently rather stabilized total Hg pollution in the Elbe River environment. Multivariate multispecies analyses found the age of analyzed individuals and the feeding strategy of a given species as the most important, however mutually interactive, covariates for Hg accumulation in muscle tissue. The analyses revealed decreasing sensitivity of older predator individuals to differentiate highly and moderately contaminated sites. Benthophagous species mostly kept their discrimination capacity toward contaminated sites in all age categories, with the exception of bream that was rather linked to the pattern typical for predator species. The unclear position of omnivorous species, represented namely by roach, corresponded with their weak bioindicator power, mainly in the young age categories.

Keywords: Elbe River; Heavy metals; Mercury; Bioaccumulation; Fish bioindicator; Multivariate analysis

1. Introduction

In recent years a number of wide-ranging monitoring studies have been performed in order to estimate the degree of mercury (Hg) contamination in freshwater ecosystems (Sliggers and Jager, 1993; Yamaguchi et al., 2003). Knowledge regarding contamination of different levels of the food chain is necessary for estimation of total pollutant input fluxes and subsequent partitioning among different phases in the aquatic system. The growing international concern about this environmental data is closely related to the strongly developing ecological risk assessment activities. In addition, freshwater monitoring outputs hold a key position in the estimation of the Hg dose consumed by the human

*Corresponding author. Center of Biostatistics and Analyses, Masaryk University, Kamenice 126/3, 625 00 Brno, Czech Republic. Fax: +420 547 121 413.
E-mail address: dusek@cba.muni.cz (L. Dušek).

0147-6513/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2004.11.007

population as it is highly dependent on fish consumption.

The Elbe River represents one of the largest freshwater ecosystems in central Europe with a length of 1091 km and a total watershed area of 148,268 km^2. Monitoring of its environmental status reflects the potential influence of 25 million people (6 million in the Czech Republic). There had been several monitoring studies concerned with heavy metal and polychlorinated biphenyl (PCB) pollution of the Elbe River up to the 1990s (Tent, 1983; Forstner et al., 1990). The anthropogenic load has been reported as steadily increasing to its maximum in the 1980s and early 1990s when the Elbe River was considered as one of the most contaminated river in the Europe. Accordingly, an intensive working group under the auspices of the International Committee for Elbe Protection (Internationale Kommission zum Schutz der Elbe—www.ikse.de) was established in 1990 with programs dealing with complex monitoring studies, emission inventories, and ecological risk assessment surveys. The committee has initiated several complex monitoring programs. Long-term examination of 13 model sites of the Czech Republic section of the river, performed under projects Elbe I (1991–1994) and Elbe II (1995–1998), substantially contributed to the state of environment knowledge; data on Hg load reported herein also resulted from these projects. It should be emphasized that results are not only of simply descriptive importance—heavy contamination still present in many sites of the Elbe River should be regarded as an ideal model system for scientific monitoring programs due to a wide variety of geographically and anthropogenically distinguishable sites.

Both ecological and human risk assessments assume that evaluation is based on exposure data representative of important components of the investigated ecosystem. Within both approaches, a distinction is made between simple indicator systems (using the most vulnerable, interesting, or suspect part of an ecosystem) and more complex (multicomponent or multispecies) assessments including space and time variability. Contamination of selected indicator fish species appears to be a valuable early warning model (Marcovecchio and Moreno, 1993; van Dam et al., 1998) with rather limited interpretation toward the other parts of an ecosystem. Although a variety of biological species or environmental compartments are used in the monitoring of freshwater ecosystems, the analyses of bottom sediments, zoobenthos, and tissues of selected fish species are most frequently combined in the case of pollution by heavy metals (Kristensen and Hansen, 1980; Lodenius, 1991; Prego and Cobelo-Garcia, 2003). In Europe, various fish species were effectively used for bioindication of contamination, with dependence on the region, e.g., *Abramis brama* in monitoring of the Elbe River (Kammann, 1995) and the Weser River (Busch et al., 1995), *Leuciscus cephalus* in the Morava River (Gelnar et al., 1994), *L. cephalus* in the Rhone River (Devaux et al., 1998), *Esox lucius* in the Oder River (Meinelt et al., 1997), *Anguilla anguilla* in the Dutch Rhine delta (Pieters and Geuke, 1994), and the Thames Estuary (Langston et al., 2002).

On the other hand, more than one fish species should be analyzed in comparative environmental studies due to substantial variations in pollution processes (Burger et al., 2002). In addition to regional variability the results of monitoring programs might be biased due to different time profiles of exposure in examined sites. Multispecies comparisons covering different feeding habits of fish and a wide range of age categories have the potential to distinguish recent exposure from long-term load. However, although multispecies sampling strategy provides outputs close to real-life situations in the ecosystem, it also strongly increases requirements on the input data and subsequent statistical analyses. A reasonable application of statistical methods is then very much needed because it could greatly assist reliable comparison of pollution even in very different sites (Jensen and Cheng, 1987). A variety of quite different statistical techniques ranging from narrative descriptions to complex multivariate analyses could be applied for that purpose. Among others, age (or weight) of monitored fish populations appears to be the factor most frequently standardized by statistically oriented techniques. Some authors standardize the actual content of Hg to a selected young age category (e.g., Lange et al., 1993) prior to any statistical treatment. Bioconcentrations of Hg positively correlated with age or size of fish could be evaluated using regression models fitted to the experimental data (Jackson, 1991; Mueller and Serdar, 2002). Hg accumulated in tissues was also effectively related to the fish age as an independent variable in a Bertalanffy-type function (Morrison and Thérien, 1995).

Since Hg has the potential to bioaccumulate in food chains, the representative selection of an indicator species for a specified ecosystem appears to be a crucial step for reliable interpretation, particularly in long-term studies. The descriptive part of the study was primarily aimed at comparative evaluation of the Hg load in 13 model sites along the Czech section of the Elbe River sampled during the period 1991–1996. The conclusions were supported by multivariate statistical analyses of the content of Hg in the muscle tissue of 1251 fish belonging to 23 species with three dominant indicator species: *Perca fluviatilis*, *A. brama*, and *Rutilus rutilus*. The indicator potential of these species, which represent three different feeding strategies, was related to the whole spectrum of analyzed species in order to identify and classify their information value. Uncertainty associated with sediment-related input data and data reflecting contamination of fish species differing in feeding habits was previously discussed. We endeavoured to document the utility of the multivariate comparative approach as an effective tool for the treatment of a heterogeneous environmental data set including the variability related to multispecies differences, age of fish, space, and time attributes.

2. Materials and methods

2.1. Geographical site description

Pollution of the Elbe River originated mainly from indirect loads reaching the river via water from the catchment area contaminated by municipal wastes and industrial discharges (chemical industries, paper mills, waste water works, shipbuilding yards, and docks). That is why sites located downstream from the large cities on the river are the most representative model points for long-term monitoring surveys (Beuge and Kramer, 1982; Truckenbrodt and Einax, 1995). However, industrial and municipal wastes were not the only sources responsible for the total metal burden in this region. The Elbe River also flows through agricultural regions and nonpoint sources could have accounted for the pollution by Hg, namely due to uncontrolled erosion, soil leaching, and surface runoff (Adams et al., 2001). For example, for decades, cereals grown in the area of interest were treated by the fungicide Agronal containing phenylmercury. The geographical locations of the study sites are displayed in Fig. 1 and major environmental characteristics are described in Table 1.

2.2. Sampling design and analyses

Four sampling campaigns have been performed at all 13 sites of the Elbe I and Elbe II projects. At all the sites, sediment samples were taken and analyzed for total Hg content in both 1994 and 1995 according to Blomqvist (1985) and Fukuhara and Sakamoto (1987). Standard fishing by gill net was performed throughout the experimental period. Each collected fish was immediately measured, weighed, and aged (according to its scales). Samples of muscle, liver, kidney, and gonads were stored at −18 °C. Total Hg content was determined by flameless atomic absorption spectrometry after previous mineralization of analyzed tissues (Studnicka et al., 1974).

A total of 1251 fish individuals (915 herbivorous and 336 piscivorous) belonging to 23 species was analyzed for content of Hg in the muscle tissue. Most representative samples were obtained from the four indicator fish species (*P. fluviatilis*: $n = 163$; *A. brama*: $n = 173$; *R. rutilus*: $n = 148$; and *L. cephalus*: $n = 166$) due to their relatively high abundance in the studied ecosystems. These four species formed nearly 52% of the whole data set. The list of examined fish species with their feeding guilds is displayed in Table 2.

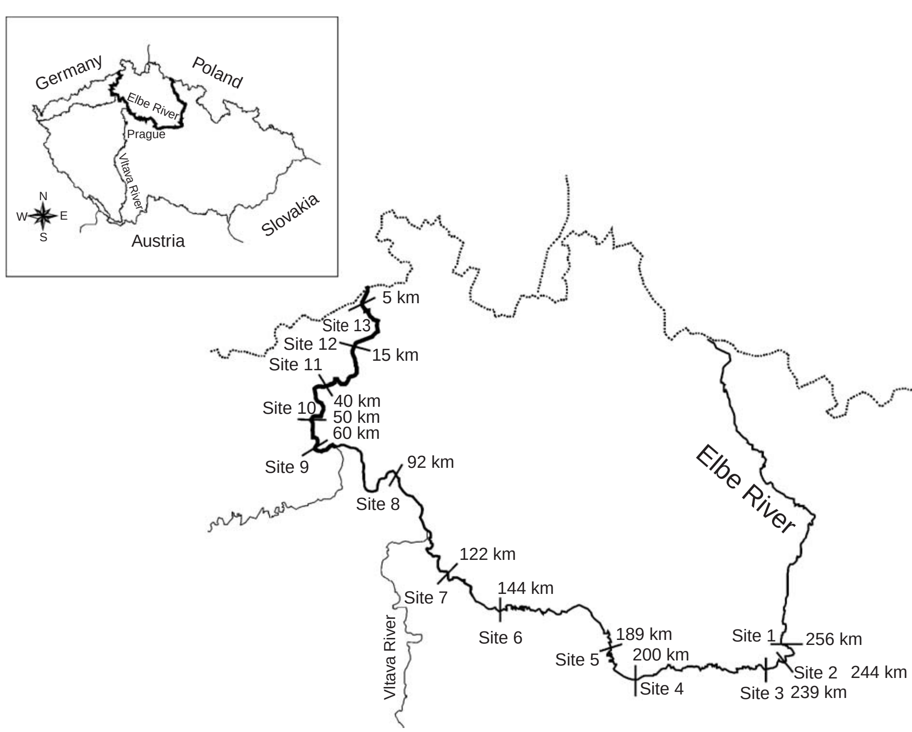

Fig. 1. Site geographic location.

Table 1
Basic environmental characteristics of the sites examined during the period 1991–1996[a]

Site (influencing town)	Location (km)	Sample size (n) [a,b]	Mean annual flow ($m^3 s^{-1}$)	Mean annual temperature (°C)	Major anthropogenic influence
I. Opatovice	Downstream (256)	144	30	10.6	Typical background sites, without significant anthropogenic influence
II. Pardubice	Upstream (244)	100	32	10.8	
III. Pardubice	Downstream (239)	67	32	11.0	Municipal wastes from Pardubice town
IV. Kolín	Upstream (200)	50	45	11.1	Industrial production—chemical plant
V. Kolín	Downstream (189)	74	45	11.5	Industrial + municipal wastes from Kolín town
VI. Čelákovice	Downstream (144)	71	56	11.5	
VII. Neratovice	Downstream (122)	127	68	11.6	Chemical production
VIII. Štětí	Downstream (90)	32	165	11.7	Paper mills located upstream (Vltava River)
IX. Lovosice	Downstream (60)	77	190	11.9	Chemical industry
X. Vaňov	Downstream (50)	49	215	12.0	Ship-building yard, docks
XI. Ústí n. Labem	Downstream (40)	67	265	12.1	Municipal wastes from towns located upstream, chemical industry, organic waste discharges
XII. Děčín	Downstream (15)	316	295	12.1	
XIII. Hřensko	Downstream (5)	77	308	12.2	

[a]Data collected in four sampling campaigns: 1991, 1992, 1993, 1996.
[b]Total number of examined fish.

2.3. Statistical data analyses

A log-normal distribution, typically with an extreme position of the upper tail, was clearly proved for the content of Hg in analyzed tissues. Prior to statistical analyses, these data were logarithmically transformed in order to reach normal distribution (χ^2 test, Shapiro–Wilks' test) and homogeneity of variance within selected categories (Bartlett's test). All numeric and graphic outputs were based on the geometric mean and its confidence limits (Parkin and Robinson, 1992). If necessary, the final estimates of the geometric mean were corrected to reduce bias due to small n (Parkin et al., 1990). One-way ANOVA followed by Tukey's multiple-range test was applied for comparison of more than two fish species, sites, or time periods. Both parametric Pearson's correlation coefficient and Spearman's rank correlation coefficient were applied to find significant relationships between Hg concentration in tissues and fish age (Zar, 1984). Comparison of the sites was summarized by multivariate clustering based on median content of Hg in muscle tissues. Matrix of Euclidean distances and furthest neighbor algorithms were found to provide the most reliable discrimination among the sites.

The principal component analysis (PCA) was used to characterize the multispecies relations through linear transformation of median Hg content in muscle tissues arranged according to individual samples (rows) and species with distinguished age category (columns). The new variables, called principal components (PCs), were calculated as eigenvectors of a multispecies correlation matrix. In order to prevent certain species from having more influence than others due to the scaling effect, the median Hg content data were standardized prior to PCA (i.e., original variables were centered to obtain a zero average and normalized to obtain a variance of 1). Data pretreated in this way provided the most meaningful bilinear projections showing the relation between species and groups of species (Arnold and Collins, 1993). PCA was carried out in order to provide (1) component loading vectors sufficient to explain the relationships among species and their mutual position with respect to feeding habits, and (2) component score vectors as pairwise noncorrelated variables that were used for additional multispecies comparisons (Jolliffe, 1986; Legendre and Legendre, 1998). Component weight vectors were scaled to the length of 1.

Table 2
List of examined fish species

Fish species		Feeding guilds
Abramis brama	Bream	Benthophagous
Alburnoides bipunctatus	Spirlin, riffle minnow	Planctivorous
Alburnus alburnus	Bleak	Planctivorous
Anguilla anguilla	European eel	Predator
Aspius aspius	Asp	Predator
Barbus barbus	Barbel	Benthophagous
Blicca bjoerca	White bream, silver bream	Benthophagous
Carassius auratus	Gibel carp	Planctivorous
Esox lucius	Pike	Predator
Gobio gobio	Gudgeon	Benthophagous
Gymnocephalus cernua	Ruffe, pope	Benthophagous
Ictalurus nebulosus	Catfish, brown bullhead	Benthophagous
Leuciscus cephalus	Chub	Omnivorous
Leuciscus idus	Ide, orfe	Omnivorous
Leuciscus leuciscus	Dace	Omnivorous
Perca fluviatilis	Perch	Predator
Rutilus rutilus	Roach	Benthophagous
Salmo trutta	Trout	Predator
Scardinius erythrophthalmus	Rudd	Phytophagous
Silurus glanis	Wels, sheatfish	Predator
Stizostedion lucioperca	Pikeperch, zander	Predator
Thymallus thymallus	Grayling	Benthophagous
Vimba vimba	Vimba bream	Benthophagous

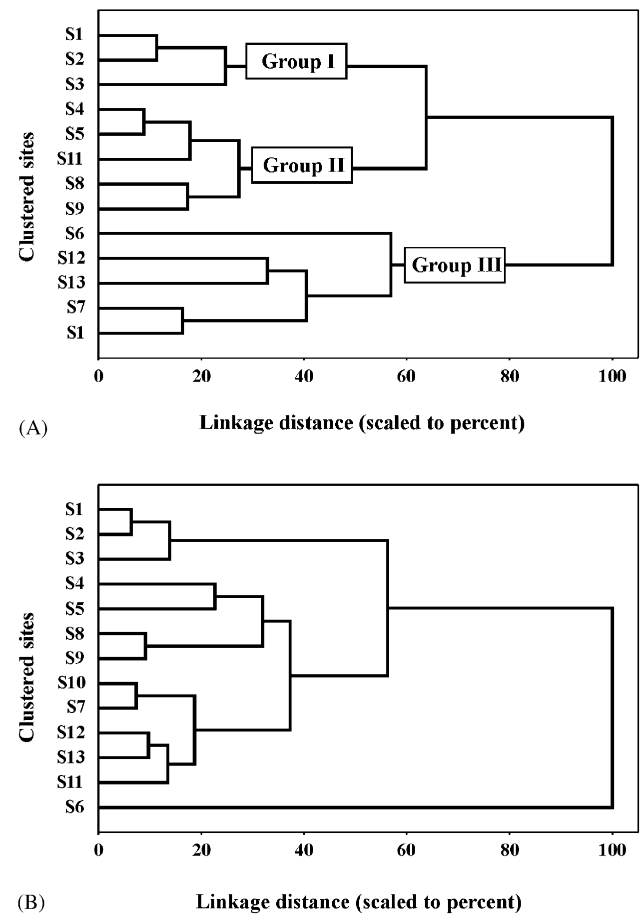

Fig. 2. Multivariate clustering of examined sites based on Euclidean distance. (A) Individuals 3–5 years old; (B) individuals 1–2 years old.

3. Results

3.1. General comparison and multivariate grouping of model sites

Keeping species identity as variables, the spatial similarity pattern of the sites was identified by clustering of the median Hg levels in muscle tissues of fish 3–5 years old (Fig. 2A) and younger individuals (1–2 years, Fig. 2B). Contamination of older fish clearly defined three distinct groups of sites with the slightly outstanding position of site 6 (Fig. 2A): background sites (Group I: Opatovice and Pardubice); sites with moderate Hg load (Group II: Kolín, Štětí, Lovosice, Ústí n. Labem); and heavily contaminated sites (Group III: Čelákovice, Neratovice, Vaňov, Děčín, Hřensko). Contamination of younger fish significantly separated background sites (Group I) from most of the heavily polluted sites (Group III); the middle polluted group of sites revealed a rather unclear position and was partially superimposed with heavily polluted ones (Fig. 2B). The outstanding position of site 6 was significantly more extreme than in the case of older individuals (Figs. 2A and B).

The results of multispecies clustering suggested ranking of the sites qualitatively similar to Hg levels in sediments (Table 3). This fact indicates rather long-term environmental load in most of the sites belonging to groups II and III. Because of data homogeneity within these groups (clustered data reached a very reasonable coefficient of variance up to 24%), the trinomial categorization of the sites was retained in any further species-specific and time-related analyses. Using data from fish 3–5 years old, Table 3 numerically defines long-term bioaccumulated levels of Hg in analyzed fish communities. Although contamination of muscle of all the evaluated species was significantly lower in background sites S1–3, only data from bream and clustered benthophagous species contributed to statistically significant separation of heavily polluted sites (Group III) from moderate ones (Group II).

Considering data from 3- to 5-year-old fish, significantly increased contamination was detected in typical predators compared to herbivorous or omnivorous fish species in all groups of sites (Table 3, $P < 0.001$). Perch, as partially benthophagous species with a substantial portion of fish in food, appeared to be the most contaminated species in the sample (muscle Hg concentration in the range of 0.840–1.398 mg Hg kg^{-1}). Although less contaminated than perch, muscle contamination of bream sensitively separated all three groups of sites; the highest metal load ranged from

Table 3
Grouping of the sites (S1–S13) into three categories reflecting gradient in mercury load (I < II < III): characteristics summarized for the whole sampling period 1991–1996[a]

Characteristics	Group I (S1, S2, S3)	Group II (S4, S5, S8, S9, S11)	Group III (S6, S7, S10, S12, S13)
Total no. of fish individuals	331	290	630
Hg in sediments ($mg\,kg^{-1}_{d.w.}$)[c]	0.46 (0.29; 1.16)[A]	3.08 (2.24; 5.16)[B]	6.12 (4.72; 9.30)[C]
Hg in muscle tissue (mg kg^{-1})[b]: major indicator species and groups of species joined according to their feeding strategy			
Perca fluviatilis (predator)	$n = 22$	$n = 19$	$n = 33$
	0.152 (0.089; 0.219)[A]	0.729 (0.559; 0.973)[B]	1.06 (0.840; 1.398)[B]
Rutilus rutilus (omnivorous)	$n = 16$	$n = 22$	$n = 35$
	0.106 (0.089; 0.124)[A]	0.353 (0.265; 0.445)[B]	0.375 (0.335; 0.415)[B]
Abramis brama (benthophagous)	$n = 15$	$n = 16$	$n = 21$
	0.171 (0.108; 0.237)[A]	0.309 (0.243; 0.359)[B]	0.415 (0.391; 0.597)[C]
Other predator species[d,e]	$n = 26$	$n = 25$	$n = 45$
	0.237 (0.196; 0.282)[A]	0.781 (0.614; 0.868)[B]	0.814 (0.712; 0.953)[B]
Other omnivorous species[d,f]	$n = 48$	$n = 34$	$n = 81$
	0.091 (0.072; 0.129)[A]	0.221 (0.182; 0.294)[B]	0.306 (0.244; 0.417)[B]
Other benthophagous species[d,g]	$n = 28$	$n = 57$	$n = 47$
	0.133 (0.109; 0.157)[A]	0.336 (0.297; 0.369)[B]	0.428 (0.361; 0.564)[C]

[a]Statistical comparison of grouped sites: numbers within one row followed by the same superscript capital letter are not significantly different.
[b]Only individuals 3–5 years old were selected for the comparisons.
[c]Numbers represent geometric mean supplied with corresponding 95% confidence limits (in parentheses).
[d]Data from these species were joined on the basis of preliminary ANOVA test indicating statistically negligible differences between single species.
[e]*Esox lucius*, *Silurus glanis*, *Stizostedion lucioperca*, *Anguilla anguilla*, *Aspius aspius*.
[f]*Alburnus alburnus*, *Alburnoides bipunctatus*, *Scardinius erythrophthalmus*, *Leuciscus cephalus*.
[g]*Blicca bjoerkna*, *Barbus barbus*, *Gobio gobio*, *Carassius auratus*, *Ictalurus nebulosus*, *Vimba vimba*.

0.368 to 0.543 $mg\,Hg\,kg^{-1}$. Data from omnivorous and benthophagous species could not be statistically distinguished in sites with low and moderate metal burden; benthophagous species as a category, however, revealed significantly increased contamination of muscle tissues in heavily polluted sites ($P < 0.05$; Group III).

3.2. Time-related comparisons of mercury load

Pairwise comparison of two major sampling campaigns, 1991–1992 and 1993–1996, objectively divided periods of increasing international interest focused on the Elbe River environment. Based on strict statistical analyses of data arranged in a temporal sequence, no significant trend changes were detected in any examined fish species, although some fish species revealed a shift of values associated with time, namely in young individuals (Figs. 3A and B). There were no significant time-related changes, after 1990, in either sediment samples or fish tissue of older individuals (3–5 years). Thus, we have observed an evidently rather stabilized total Hg pollution (Fig. 3, Table 3). Indeed, Hg levels in muscle tissues of young perch and other predator species (1–2 years old, Figs. 3A-I and B-III) caught in sites with moderate contamination even indicated a decreasing trend in bioaccumulation of Hg over time. On the other hand, heavily contaminated sites clustered in Group III seemed to be steadily polluted up to 1996 as reflected by the increased Hg levels in young individuals of all indicator fish species and predator species (Figs. 3A-I and B-III).

3.3. Bioindicator potential of different species—multivariate comparison

Apart from time trends, data shown in Fig. 3 also revealed substantial differences between fish species in their potential to discriminate sites according to gradient of Hg pollution. Reasonably expressed confidence limits justified the clustering of less abundant predator, omnivorous, and benthophagous species (coefficient of variance did not exceed 28% within these groups). Predator species, and namely the most contaminated perch, revealed important indicator potential even at a young age for the maximum content of Hg accumulated in muscle tissue. However, predator individuals older than 3 years living in moderately and heavily polluted sites (groups II and III) were all strongly contaminated with negligible statistically significant differences. A similar pattern, i.e., decreasing sensitivity of older individuals to differentiate highly contaminated sites, was obtained analyzing muscle tissue of bream that appeared to be more similar to the predator species than to the other benthophagous species. Clustered data of other benthophagous species (bream not included) reflected a Hg gradient in sites, including the stage between moderately and heavily polluted sites (Fig. 3).

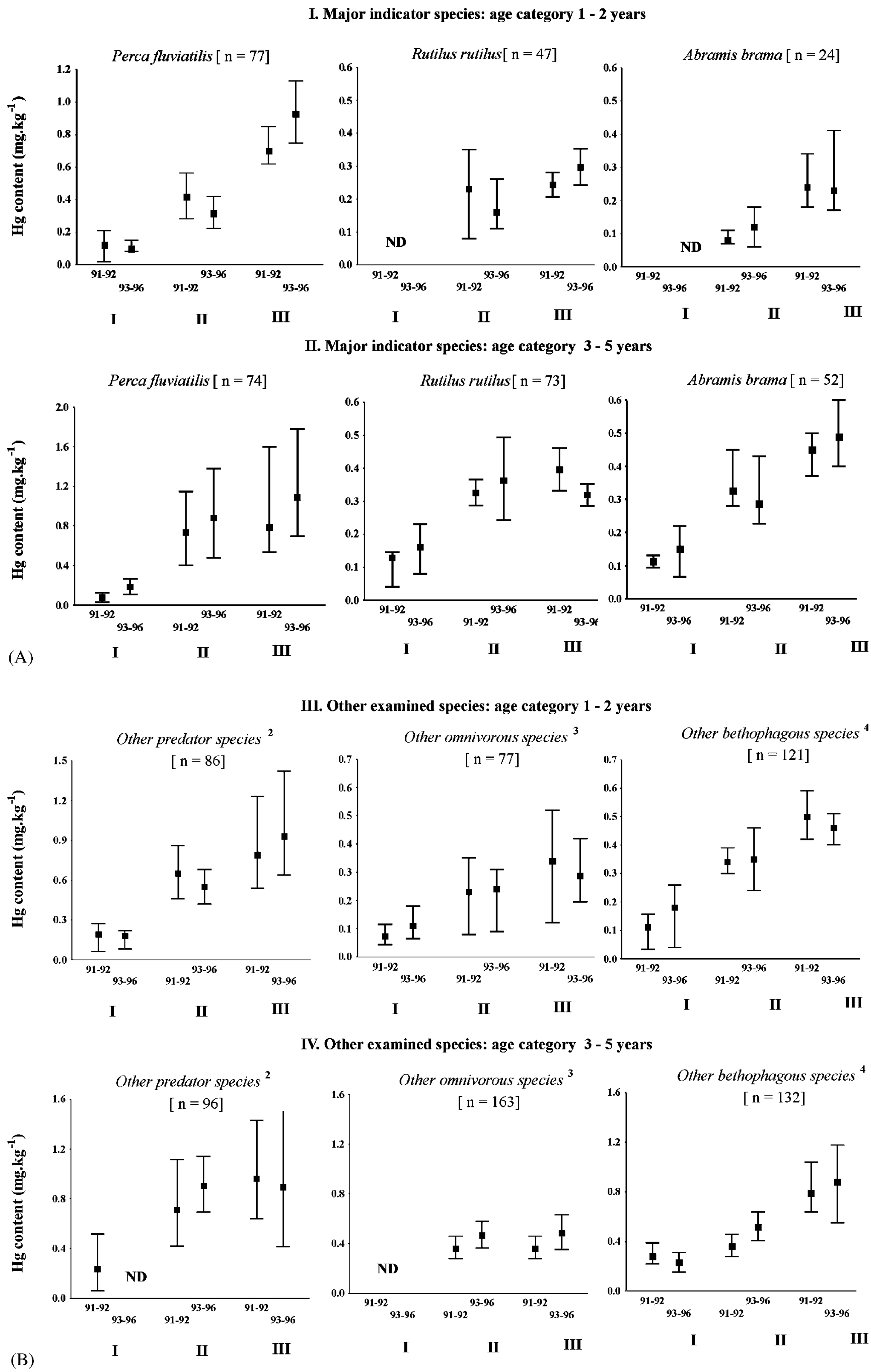

Fig. 3. (A) Time profile of Hg content in tissue of indicator species. (B) Time profile of Hg content in tissue of other examined species.

Table 4
Correlation structure of data expressing mercury level in muscle tissue of indicator and the other fish species[a]

	Perca fluviatilis	*Rutilus rutilus*	*Abramis brama*	Other predator species[b]	Other omnivorous species[b]
(A) *Individuals with age<3 years*					
Other benthophagous species[b]			+	+ +	
Other omnivorous species[b]		+ +			
Other predator species[b]	+		+ +		
Abramis brama	+				
Rutilus rutilus					
(B) *Individuals with age 3–5 years*					
Other benthophagous species[b]			+	+	
Other omnivorous species[b]	+	+ +			
Other predator species[b]	+		+		
Abramis brama	+				
Rutilus rutilus					

+ Correlation significant at the level $P< 0.05$; + + Correlation significant at the level $P<0.01$.
[a]Pearson's correlation coefficients evaluated on geometric means of mercury level in each sample.
[b]Data from these species were combined because of statistically negligible heterogeneity within the subgroups (see Table 3).

Omnivorous and planctivorous species were ranked as the least sensitive indicators for Hg pollution. Contamination of young omnivorous individuals did not permit a statistically significant separation of background sites from the others; individuals older than 3 years revealed increased Hg content with dependence on environmental conditions, however, without any difference between moderately and heavily polluted sites. Roach as an abundant indicator species appeared to be the most representative species of this group (Fig. 3).

Hg levels in muscle tissues of species grouped according to feeding strategy were mutually related in correlation matrices displayed in Table 4. As expected, benthophagous species strongly correlated with predator species only in the case of young individuals; data from individuals 3–5 years old led to a weaker, but still significant, correlation. Omnivorous species were not correlated significantly either with predator or with benthophagous groups. Roach as a representative species of this group correlated highly significantly with data from omnivorous species. Similarly, perch and bream correlated with groups of species they belong to but their representative position was less significant, namely in the case of bream.

Multivariate comparison of different species was summarized on the basis of median levels of Hg in muscle tissues. A PCA was successful within both included age categories; two-dimensional projections of extracted PCs exhausted more than 80% of total variability. Biplots displayed in Fig. 4 summarized two types of analyses: (1) interrelationship of primary data from single species (Figs. 4A and C) and (2) analysis relating separated indicator species to groups of species clustered according to different feeding strategies (Figs. 4B and D). The analysis again concluded a high indicator potential of young (1–2 years) predator species, namely perch, toward differently contaminated sites (Figs. 4A and B), while data from older predator individuals could not distinguish moderately and heavily polluted sites (Figs. 4C and D). Benthophagous species kept their discrimination capacity toward highly contaminated sites in both age categories, with the exception of bream that was rather linked to the pattern typical for predator species. The unclear position of omnivorous species, represented by roach, corresponded with their weak bioindicator power, mainly in the young age categories (Fig. 4).

4. Discussion

Clearly proved increasing accumulation of Hg in tissues over the lifetime of examined fish can be regarded as an indication of contamination (Rincon et al., 1993; Niimi and Kissoon, 1994; Mueller and Serdar, 2002) and age-related changes then represent a crucial variability component in biomonitoring studies. The significantly varying contamination of tissues in relation to the fish age or weight has been reported for numerous fish species both for Hg and for methylmercuric salts (Bache et al., 1971; Svobodová and Hejtmánek, 1985; Barak and Mason, 1990; Jackson, 1991; Friedmann et al., 1996; Neumann and Ward, 1999; de Pinho et al., 2002). Another widely known factor is the tendency of fish species to accumulate Hg relative to their position in the food web and feeding habits of indicator fish species are therefore emphasized in many ecotoxicological studies (Rincon et al., 1993; Burger et al., 2002; Sager, 2002).

Similarly in our study, quantitatively more significant differentiation of background and polluted sites was reached in the case of older fish as a consequence of higher Hg levels accumulated in their muscle tissues (Figs. 3 and 4). However, data obtained from older fish

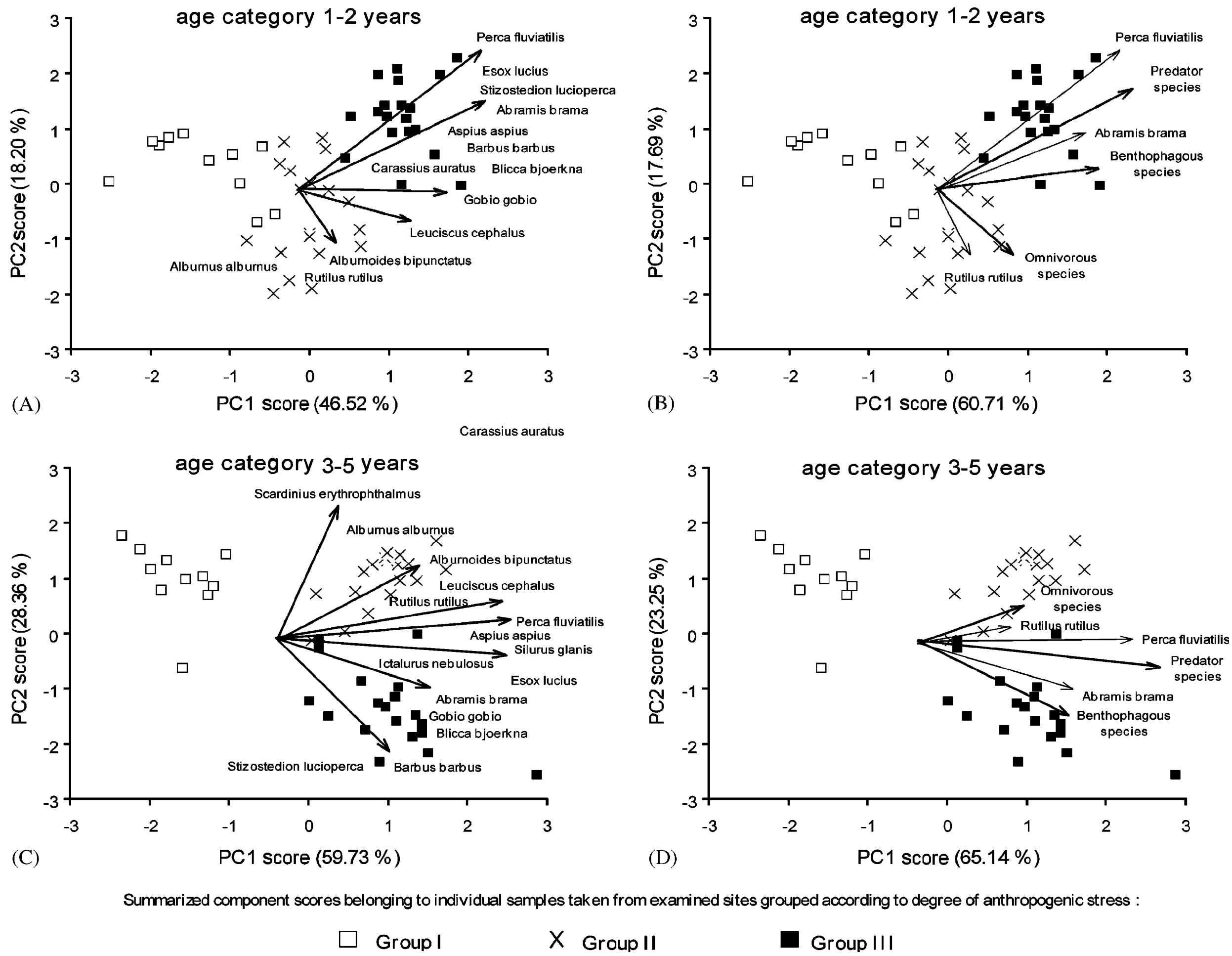

Fig. 4. Biplot presentation of bioindicator potential of (A, C) individual examined species and (B, D) indicator groups of species.

individuals reflected long-term exposure with many influencing factors and furthermore, not all included species equally contributed to the final pattern. Although sensitive to increased contamination, resulting biological data could not necessarily indicate a real environmental gradient of pollution. Uncertainty associated with retrospective studies should be sharply reduced by an optimum combination of qualitative and quantitative points of view, which of course implies analysis of more than one indicator species represented by individuals of at least two distinct age categories. Therefore, we found the age of analyzed individuals and the feeding strategy of a given species as the most important, however mutually interactive, covariates for Hg accumulation in muscle tissue.

Young predator species (1–2 years) very sensitively differentiated contaminated sites while older predator individuals (3–5 years) living in moderately and heavily polluted sites were all strongly contaminated with negligible statistical differences. It seems that their enormous sensitivity to Hg bioaccumulation in the trophic structure reduced the potential to react on continually increasing metal load. On the other hand, benthophagous species of any age category very effectively indicated underlying Hg gradient in sites, with the exception of bream, which was rather similar to predator species. Omnivorous and planctivorous species were ranked as the least sensitive indicators for Hg pollution. Contamination of young omnivorous individuals did not permit a statistically significant separation of background sites from the others, indicating that increased Hg level in these species is strongly dependent on long-term exposure.

Based on these results, one can propose a dual concept in the interpretation of biomonitoring of Hg load in freshwater ecosystems. The first component of the concept reflects the absolute amount of Hg accumulated in the analyzed tissues that depends to a great degree on the position of a given species in the trophic chain (Van der Zanden and Rasmussen, 1996; Bowles et al., 2001). The second component implements the discriminative power of a given species toward environmental pollution gradient and time profile of exposure, and deals with an interactive interpretation of the age structure and spectrum of feeding habits that occurred in the actual analyzed sample.

The long-term evaluation of 13 heterogeneous model sites presented here enabled identification of three environmentally important parts of the whole fish community:

1. *Early warning indicators*. Young individuals (1–2 years) of predator species, and of bream, appeared to be a sensitive part of the community, indicating recent increases in metal load in even highly polluted sites. Even at a young age, predator species could accumulate relatively high absolute amounts of Hg, which further potentiated their informative value. Young perch individuals appeared to be the most representative for this group.
2. *Sensitive indicators of long-term exposure to Hg*. Individuals of predator species and of bream 3–5 years old were unambiguously assorted to this indicator group. These species are characterized by high sensitivity to Hg accumulation due to their feeding strategy and therefore, under long-term exposure, they could reach relatively high Hg load in even moderately polluted sites. On the other hand, however, these characteristics appeared to reduce their potential to discriminate moderately and heavily polluted freshwater ecosystems.
3. *Safe indicators of underlying environmental gradient*. Benthophagous species with the exception of bream appeared to accumulate Hg in positive correlation with its sediment content. Although the accumulated Hg content was not as high as in the case of predator species, it has significant potential to discriminate between differently polluted sites in even higher age categories of fish.

As previously specified in the Introduction, the study was scientifically aimed mainly on multivariate comparison of different fish species with respect to their ages and feeding strategies. We can conclude that selection of indicator species should not be restricted only to certain levels of the food chain. The early warning monitoring programs should be primarily directed at top predators in low age categories, but without neglecting other, mainly benthophagous, species. Input data from larger predator fish species are frequently limited in sample size in common river ecosystems and thus the other fish species can be regarded as important receptors with potential to transfer pollution load to higher levels of the food chain. In long-term or retrospective studies, special emphasis should be placed on examination of benthophagous species that are subjected to a more or less direct impact of the pollution substance. Multi-species sampling could be recommended particularly for comparative retrospectively oriented studies because it reflects the biological integrity of a given ecosystem.

Apart from the suggested ecotoxicological classification, the levels of Hg content in muscle tissue of different species analyzed in the presented study corresponded to a commonly known pattern. Contamination of predators typically more than twice exceeded Hg levels found in herbivorous or omnivorous fish species (Table 3, Fig. 3). Although the included species have very heterogeneous habitat preferences, they could be grouped according to Hg levels in muscle tissues in the following decreasing order: benthophagous and predator species with a substantial portion of fish in their food (e.g., *P. fluviatilis*) $>$ typical predators with fish prominent in food (*Silurus glanis*, *E. lucius*) $\gg$ typical benthophagous species (*A. brama*, *Blicca bjoerkna*, *Carassius auratus*) $>$ omnivorous and clearly planctivorous fish species (*R. rutilus*, *Scardinius erythrophthalmus*). The study apparently emphasized the degree of sediment ingestion and associated direct exposure to contaminated particles as the most important factors influencing indicator values of a given fish species. Benthophagous species preying close to the bottom were recognized as susceptible indicators of environmental gradient of Hg as has already been reported, for example, by Žarski et al. (1995) and Svobodova et al. (1999).

The species composition of the samples reflected the actual situation in the biodiversity of fish communities inhabiting the investigated part of the Elbe River with abundant perch, roach, chub, and particularly bream as the most dominant species (Vostradovský et al., 1994). Bream, particularly, has very frequently occurred in catches from highly polluted sites, which could indicate eutrophic changes and subsequent oxygen disturbances. *A. brama* is a fish of relatively low requirements to water purity and oxygen content and it then becomes a predominant and ecotoxicologically important species in common stretches of European rivers (Žarski et al., 1995).

Temporal oxygen deficiency is widely known to enhance methylation of Hg and its mobility in freshwater ecosystems (Friedmann et al., 1996; Boening, 2000; Boszke et al., 2002). In addition to aeration, temporal fluctuations in water pH values or in content of dissolved organic carbon could also partially contribute to the increased bioaccumulation of Hg. The contamination of fish tissues in freshwater reservoirs or lakes was frequently documented to be inversely correlated with the pH of water (Lange et al., 1993; Greenfield et al., 2001; Boszke et al., 2002). Highly probable oxygen disturbances (<7 mg of dissolved $O_2\,kg^{-1}$) in polluted parts of the Elbe River (Guhr et al., 1999) could intensify bacterial methylation processes and thus potentiate uptake of Hg especially by benthophagous species including bream. Levels of methylmercury in the Elbe River sediments are estimated to range from 1% to 2% (Robertson et al., 1987) to 10% of total Hg content (Wilken and Hitelman, 1991). The increase in methylmercury concentration can be strongly site specific, not necessarily related to the absolute anthropogenic enrichment of Hg. Even temporal changes in physical and chemical conditions can substantially enhance the bioavailability of Hg to fish, even in the absence of high Hg concentrations in sediments and water (Phillips et al., 1987; Benoit et al., 2003).

Although 13 selected sites cover a wide spectrum of environmental conditions in the Elbe River ecosystem, the results of biological monitoring suggested that a substantial part of the pollution is received from closely located towns, the chemical industry being the most important contributor. More or less intensive local influences cause progressively increasing heavy metal burden with increasing distance downstream of the town of Pardubice as was reflected by the resulting grouping of the sites (Table 1, Fig. 2). In agreement with previously performed investigations (Svobodová et al., 1993), the outstanding site 6 (Čelákovice) was correctly linked to the cluster of heavily polluted sites.

In conclusion, the results should be compared with other surveys of the Elbe River performed during the period 1985–1994 (Svobodová and Hejtmánek, 1985; Svobodová et al., 1996; Guhr et al., 1993; Reincke, 1993). Considering the overall situation in the Czech Republic, the obtained data documented a still high exposure of fish populations to Hg in all identified heavily polluted sites. Already published decreasing trends in Hg levels in sediments and fresh water could be biased due to frequent flood situations and flow variability (Guhr et al., 1994); biological monitoring concluded only generally stabilized environmental conditions. However, no negative and statistically significant trends in the contamination of the fish population since 1991 were detected in this 6-year study as a consequence of new water treatment plants constructed in the main upstream sources of pollution (Adams et al., 2001).

Acknowledgments

This research was supported by the Ministry of Environment of the Czech Republic (Project Elbe I., II., III.) and by the Ministry of Education of the Czech Republic (MSM 126100003, MSM 122200003/3).

References

Adams, M.S., Ballin, U., Gaunery, T., Hale, B.W., Kausch, H., Kruse, R., 2001. Monitoring selected indicators of ecological change in the Elbe River since the fall of the Iron Curtain. Environ. Conserv. 28, 333–344.

Arnold, G.M., Collins, A.J., 1993. Interpretation of transformed axes in multivariate analysis. Appl. Stat. 42, 381–400.

Bache, C.A., Gutenmann, W.H., Lish, D.J., 1971. Residues of total mercury and methylmercuric salts in lake trout as a function of age. Science 172, 951–953.

Barak, N.A.E., Mason, C.F., 1990. Mercury, cadmium and lead in eels and roach: the effects of size, season and locality on metal concentrations in flesh and liver. Sci. Total Environ. 92, 249–256.

Benoit, J.M., Gilmour, C.C., Heyes, A., Mason, R.P., Miller, C.L., 2003. Geochemical and biological controls over methylmercury production and degradation in aquatic ecosystems. ACS Symp. Ser. 835, 262–297.

Beuge, P., Kramer, W., 1982. Detection and characterization of fault zones by means of mercury survey. Z. Geol. Wiss. 10, 607–616.

Blomqvist, S., 1985. Reliability of core sampling of soft bottom sediment—an in situ study. Sedimentology 32, 602–612.

Boening, D.W., 2000. Ecological effects, transport, and fate of mercury: a general review. Chemosphere 40, 1335–1351.

Boszke, L., Glosinska, G., Siepak, J., 2002. Some aspects of speciation of mercury in a water environment. Pol. J. Environ. Stud. 11, 285–298.

Bowles, K.C., Apte, S.C., Maher, W.A., Kawei, M., Smith, R., 2001. Bioaccumulation and biomagnification of mercury in Lake Murray, Papua New Guinea. Can. J. Fish. Aquat. Sci. 58, 888–897.

Burger, J., Gaines, K.F., Boring, C.S., Stephens, W.L., Snodgrass, J., Dixon, C., McMahon, M., Shukla, S., Shukla, T., Gochfeld, M., 2002. Metal levels in fish from the Savannah River: potential hazards to fish and other receptors. Environ. Res. 89, 85–97.

Busch, D., Cetinkaya, M., Vosniok, W., 1995. Contamination of bream (*Abramis brama,* Linne 1785) with heavy metals and organochlorines in the Bremen part of the river Weser, 1985 and 1987. Part I. Heavy metals. In: Gerken, B., Schirmer, M. (Eds.), The River Weser. Fischer-Verlag, Stuttgard, pp. 123–137.

de Pinho, A.P., Guimaraes, J.R.D., Martins, A.S., Costa, P.A.S., Olavo, G., Valentin, J., 2002. Total mercury in muscle tissue of five shark species from Brazilian offshore waters: effects of feeding habit, sex, and length. Environ. Res. 89, 250–258.

Devaux, A., Flammarion, P., Bernardon, V., Garric, J., Monod, G., 1998. Monitoring of the chemical pollution of the river Rhone through measurement of DNA damage and cytochrome P4501A induction in chub (*Leuciscus cephalus*). Mar. Environ. Res. 46, 257–262.

Forstner, U., Schode, J., Knauth, H.D., 1990. Metal pollution in the tidal Elbe River. Sci. Total Environ. 97–8, 347–368.

Friedmann, A.S., Watzin, M.C., Brinck-Johnsen, T., Leiter, J.C., 1996. Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). Aquat. Toxicol. 35, 265–278.

Fukuhara, H., Sakamoto, M., 1987. An improved Ekman-Birge grab for sampling an undisturbed bottom sediment core sample. Jpn. J. Limnol. 48, 127–132.

Gelnar, M., Koubková, B., Pláňová, H., Jurajda, P., 1994. Report on metazoan parasites of fishes of the river Morava with remarks on the effects of water pollution. Helminthologia 31, 47–56.

Greenfield, B.K., Hrabik, T.R., Harvey, C.J., Carpenter, S.R., 2001. Predicting mercury levels in yellow perch: use of water chemistry, trophic ecology, and spatial traits. Can. J. Fish. Aquat. Sci. 58, 1419–1429.

Guhr, H., Büttner, O., Dreyer, U., Krebs, D., Spott, D., Suhr, U., Weber, E., 1993. Zusammenstellung, Auswertung und Bewertung des vorhandenen Datenmaterials über die stoffliche Belastung der Gewässergüte der Mittelelbe nach einheitlichen gemeinsamen Kriterien (Vorstudie)—Band I. GKSS, Forschungszentrum Geesthacht GmbH, GKSS 93/E/18.

Guhr, H., Prange, A., Punčochář, P., Wilken, R.D., Büttner, B. (Eds.), 1994. Die Elbe im Spannungsfeld zwischen Okologie und

Okonomie. 6. Magdeburger Gewässerschutzseminar. B.G. Teubner Verlagsgesellschaft Stuttgart, Leipzig, pp. 499–502.

Guhr, H., Karrasch, B., Spott, D., 1999. Shifts in the processes of oxygen and nutrient balances in the river Elbe since the transformation of the economic structure. Acta Hydrochim. Hydrobiol. 28, 155–161.

Jackson, T.A., 1991. Biological and environmental control of mercury accumulation by fish in lakes and reservoirs of northern Manitoba, Canada. Can. J. Fish. Aquat. Sci. 48, 2449–2470.

Jensen, A., Cheng, Z., 1987. Statistical analysis of trend monitoring data of heavy metals in flounder (*Platichthys flesus*). Mar. Pollut. Bull. 18, 230–238.

Jolliffe, I.T., 1986. Principal Component Analysis. Springer, New York.

Kammann, U., 1995. Metallothioneins and polychlorinated biphenyls in fish from the river Elbe and the North Sea. Schr. Bundesforschungsanst. Fisch. Hamb. 101, 103.

Kristensen, P., Hansen, J.C., 1980. On routine estimation of mercury pollution in running waters. Environ. Technol. Lett. 1, 81–88.

Lange, T.R., Royals, H.E., Connor, L.L., 1993. Influence of water chemistry on mercury concentration in largemouth bass from Florida lakes. Trans. Am. Fish. Soc. 122, 74–84.

Langston, W.J., Chesman, B.S., Burt, G.R., Pope, N.D., McEvoy, J., 2002. Metallothionein in liver of eels *Anguilla anguilla* from the Thames Estuary: an indicator of environmental quality? Mar. Environ. Res. 53, 263–293.

Legendre, P., Legendre, L., 1998. Numerical Ecology. Elseviere Science, Amsterdam.

Lodenius, M., 1991. Mercury concentrations in an aquatic ecosystem during twenty years following abatement of the pollution source. Water Air Soil Pollut. 56, 323–332.

Marcovecchio, J.E., Moreno, V.J., 1993. Cadmium, zinc and total mercury levels in the tissues of several fish species from La Plata River estuary, Argentina. Environ. Monit. Assess. 25, 119–130.

Meinelt, T., Kruger, R., Pietrock, M., Osten, R., Steinberg, C., 1997. Mercury pollution and macrophage centres in pike (*Esox lucius*) tissues. Environ. Sci. Pollut. Res. 4, 32–36.

Morrison, K.A., Thérien, N., 1995. Changes in mercury levels in lake whitefish (*Coregonus clupeaformis*) and northern pike (*Esox lucius*) in the LG-2 reservoir since flooding. Water Air Soil Pollut. 80, 819–828.

Mueller, K.W., Serdar, D.M., 2002. Total mercury concentrations among fish and crayfish inhabiting different trophic levels in Lake Whatcom, Washington. J. Freshwater Ecol. 17, 621–633.

Neumann, R.M., Ward, S.M., 1999. Bioaccumulation and biomagnification of mercury in two warmwater fish communities. J. Freshwater Ecol. 14, 487–497.

Niimi, A.J., Kissoon, G.P., 1994. Evaluation of the critical body burden concept based on inorganic and organic mercury toxicity in rainbow trout (*Oncorhynchus mykiss*) Arch. Environ. Contam. Toxicol. 26, 169–178.

Parkin, T.B., Robinson, J.A., 1992. Analysis of lognormal data. Adv. Soil Sci. 20, 193–235.

Parkin, T.B., Chester, S.T., Robinson, J.A., 1990. Evaluation of methods for calculating confidence intervals about the mean of a lognormally distributed variable. Soil Sci. Soc. Am. J. 54, 321–326.

Phillips, G.R., Medvick, P.A., Skaar, D.R., Knight, D.E., 1987. Factors affecting the mobilization, transport, and bioavailability of mercury in reservoirs of the upper Missouri River Basin. Tech. Rep. US Fish. Wildl. Serv. Montana Coop. Fish. Res. Unit, Montana State University.

Pieters, H., Geuke, V., 1994. Methyl mercury in the Dutch Rhine delta. Water Qual. Int. 30, 213–219.

Prego, R., Cobelo-Garcia, A., 2003. Twentieth century overview of heavy metals in the Galician Rias (NW Iberian Peninsula). Environ. Pollut. 121, 425–452.

Reincke, H., 1993. Die Elbe—Entwicklung der Wasserbeschaffenheitheit. Wasserwirtschaft-Wassertechnik 7, 24–29.

Rincon, F., Zurera, G., Moreno, R., Amaro-Lopez, M., 1993. Importance of eating habits and sample size in the estimation of environmental mercury contamination using biological indicators. Environ. Monit. Assess. 27, 193–200.

Robertson, D.E., Sklarew, D. S., Olsen, K.B., Bloom, N. S., Crecelius, E.A., Apts, C. W., 1987. Measurement of bioavailable mercury species in fresh water and sediments. Research Project 2020–2023, Final Report, Bastelle Pacific Northwest Laboratories.

Sager, D.R., 2002. Long-term variation in mercury concentrations in estuarine organisms with changes in releases into Lavaca Bay, Texas. Mar. Pollut. Bull. 44, 807–815.

Sliggers, C.J., Jager, H.H., 1993. Matrix International Working Groups/Task Forces on Heavy Metals and Persistent Organic Pollutants. Ministry of VROM, Directorate General for Environmetal Protection, The Hague, The Netherlands.

Studnicka, M., Hejtmánek, M., Svobodová, Z., 1974. Determination of mercury contents in muscles of fish from the river Vltava and its tributary streams. Acta Vet. Brno. 43, 145–151.

Svobodová, Z., Hejtmánek, M., 1985. Total mercury content in the components of running water, reservoir and pond ecosystems in Czechoslovakia. Symp. Biol. Hung. 29, 171–177.

Svobodova, Z., Dusek, L., Hejtmánek, M., Vykusova, B., Smid, R., 1999. Bioaccumulation of mercury in various fish species from Orlik and Kamyk water reservoirs in the Czech Republic. Ecotoxicol. Environ. Saf. 43, 231–240.

Svobodová, Z., Vykusová, B., Máchová, J., Bastl, J., Brbková, M., Svobodník, J., 1993. Monitoring of foreign substances in fishes from the Elbe river in the Čelákovice locality. Bull. VÚRH Vodňany 29, 47–61.

Svobodová, Z., Vykusová, B., Piačka, I., Dušek, L., Groch, L., 1996. Evaluation of contamination of fish sampled from Labe river in the period 1991–1994. (Auswertung der Kontamination der aus der Elbe und ihren Nebenflüssen gefangenen Fische im Zeitraum 1991–1994). In: Proceedings of Seventh Magdeburg Seminar on Water Preservation. České Budějovice, Czech Republic, pp. 496–499.

Tent, L., 1983. Heavy-metals in suspended matter and sediments of the Hamburg Elbe region. Vom Wasser 61, 99–109.

Truckenbrodt, D., Einax, J., 1995. Sampling representativity and homogeneity of river sediments. Fresen. J. Anal. Chem. 352, 437–443.

Van Dam, R.A., Camilleri, C., Finlayson, C.M., 1998. The potential of rapid assessment techniques as early warning indicators of wetland degradation: a review. Environ. Toxicol. Water 13, 297–312.

Van der Zanden, M.J., Rasmussen, J.B., 1996. A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout. Ecol. Monogr. 66, 451–477.

Vostradovský, J., Pivnička, K., Čihař, M., Poupě,, J., 1994. Species diversity, abundance, biomass and hunting of fish in the Elbe rive and its confluents. Fol. Environ. Sb. Bohemia Centralis 23, 121–127 (in Czech).

Wilken, R.D., Hitelman, H., 1991. Mercury and methylmercury in sediments and suspended particles from the river Elbe, North Germany. Water Air Soil Pollut. 56, 427–437.

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, 265–273.

Zar, J.H., 1984. Biostatistical Analysis, second ed. Prentice-Hall, London.

Žarski, T.P., Debski, B., Beseda, I., Vál'ka, J., 1995. Mercury pollution of bream (*Abramis brama* L.) caught in the middle course of the Vistula river in 1990 and 1993. Ekol. Bratislava 14 (3), 317–321.