Available online at www.sciencedirect.com
ScienceDirect
Chemosphere 67 (2007) 527–536
CHEMOSPHERE
www.elsevier.com/locate/chemosphere

Chemical contamination of the Rybinsk Reservoir, northwest Russia: Relationship between liver polychlorinated biphenyls (PCB) content and health indicators in bream (*Abramis brama*)

Grigorii M. Chuiko [a,*], Donald E. Tillitt [b], James L. Zajicek [b], Boris A. Flerov [a,✠], Vera M. Stepanova [a], Yuri Y. Zhelnin [a], Vera A. Podgornaya [a]

[a] *Institute of the Biology of Inland Waters (IBIW), Russian Academy of Sciences, Main St. No. 1, 152742 Borok, Nekouz, Yaroslavl, Russia*
[b] *Columbia Environmental Research Center, US Geological Survey, Department of Interior, 4200 New Haven Road, Columbia, MO 65201, United States*

Received 9 December 2005; received in revised form 20 July 2006; accepted 24 September 2006
Available online 13 November 2006

Abstract

The Rybinsk Reservoir (Russia) is the largest artificial waterbody in Europe (4550 km^2) and provides drinking water for population of the cities located along the coast line. Industrialization in Cherepovets at the northeastern portion of the reservoir, including one of the largest metallurgical facilities in Europe, has resulted in chemical contamination of the reservoir. The extent of polychlorinated biphenyls (PCB) contamination in bream liver, a common fish species, taken from six locations in the Rybinsk Reservoir and Volga River, and biochemical and morphometric biomarkers of fish health were investigated. Liver PCB concentrations ranged from non-detected to 3.4 µg/g wet wt of liver, with the greatest concentrations found in fish taken near the industrialized area in Sheksna Reach of Rybinsk Reservoir. The source of the bream contamination is the PCB pollution of bottom organisms and sediments conditioned with industrialization facilities of Cherepovets. The patterns of the PCB congeners in the livers of bream taken near Cherepovets were similar at all of the stations that were sampled around the reservoir and Volga River. Among the common fish health biomarkers used only liver total ChE activity and liver-somatic index in bream near Cherepovets can reflect environmental pollution. Other morphometric (FCF, Clark's condition factors, and spleen-somatic index) and biochemical (protein content and acetylcholinesterase activity in the brain) biomarkers related with fish health varied among locations, but were not correlated to the concentrations of PCBs in the bream livers.

Keywords: PCBs; Brain and liver cholinesterases; Bream; Fish health; Morphometric indices; Rybinsk Reservoir; Russia

1. Introduction

The Rybinsk Reservoir (Fig. 1) is located in northeastern European Russia (58°30′N), (38°30′E) and was formed during 1941–1947 at the confluence of the Volga, the Sheksna and the Mologa Rivers. These rivers form the four parts of the reservoir: Volga Reach, Sheksna Reach, Mologa Reach, and Main Reach. The Rybinsk Reservoir is the largest reservoir in Europe. It is approximately 56 km wide by 80–90 km long and has a surface area of 4550 km^2 and total volume of 25.4 km^3. The reservoir is relatively shallow with an average depth of 5.6 m, while the area deeper than 20 m amounts to less than 1% of the total surface, and the deepest point is only 30.4 m. The period of complete water exchange in the reservoir is about 0.83 year (Fortunatov, 1979).

The Rybinsk Reservoir serves as the main source of fresh water and reliable transportation for cities located in the area. Fish from the Rybinsk Reservoir provide a large part of the dietary protein for the surrounding population. Two large cities, Rybinsk and Cherepovets (with

* Corresponding author. Tel.: +7 4854724116/4042; fax: +7 48547244042.
E-mail address: gko@ibiw.yaroslavl.ru (G.M. Chuiko).
✠ Deceased.

0045-6535/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2006.09.046

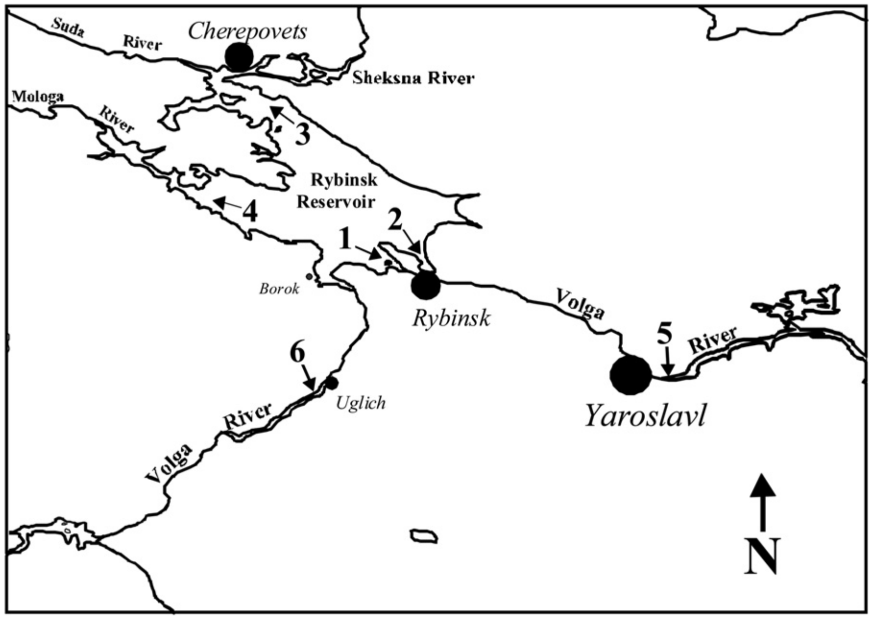

Fig. 1. Collection sites on the Upper Volga River and the Rybinsk Reservoir in the Yaroslavl Region of northwestern European Russia. Bream samples were collected at: (1) Lavrovo, on Volga Reach upstream of Rybinsk City near the settlement of Lavrovo; (2) Volkovo, on Sheksna Reach upstream of Rybinsk City near the settlement of Volkovo; (3) Miaksa, on Sheksna Reach downstream of Cherpovets near the village of Miaksa; (4) Pervomika, on Mologa Reach near the settlement of Pervomika; (5) Yaroslavl, on Volga River downstream of Yaroslavl City; (6) Uglich, on Volga River upstream of the city Uglich.

populations totaling 300–400 thousand people), and numerous smaller cities and settlements are directly located along the shores of the reservoir. Industrialization in large cities, especially in Cherepovets located upstream on the Sheksna River (Fig. 1) at the northern part of the reservoir, is a potential source of pollution. Industrial complex in Cherepovets consists of more 65 plants including two large chemical producing plants, steel rolling plants, and metallurgical facilities with full processing cycle which are among the largest in Europe. Wastewaters from these facilities are discharged directly or through a wastewater treatment plant into the Sheksna River and then go to the reservoir. Moreover, emissions from this large industrial complex also can contaminate the surrounding environment through atmospheric transport and deposition of chemicals.

In the winter of 1986–1987, several accidental spills of concentrated sulfuric acid (more than 1000 m^3) from metallurgical facilities and a chemical plant "Ammophos" destroyed the microbial processes of the wastewater treatment plant, and the untreated wastewaters flowed out to the reservoir for at least four months (Flerov, 1990). As a result, significant chemical contamination and ecological damages occurred to essentially all components of the Rybinsk Reservoir. The most striking changes were reported in Sheksna Reach near Cherepovets. For example, two months after the accidents the total concentrations of polycyclic aromatic hydrocarbons (PAH), and oil products in water were 54 and 10 μg/l, respectively. Metal concentrations in water and bottom sediments were also markedly increased (Flerov, 1990). Nine months later the total PAH concentrations in bottom sediments which included methylnaphthalenes, diphenyl, dimethylphthalenes, acenaphthene, dibenzofuran, and fluorene ranged from 0.12 to 22 μg/g wet weight (wet wt) in inverse proportion to their distance from Cherepovets (Kozlovskaya et al., 1990). As a result, the abundance and species diversity of zooplankton organisms decreased 10-fold (Riv'er, 1990), and six months afterward the reservoir water was still acutely toxic to ceriodaphnia (*Ceriodaphnia affinis*) (Chalova, 1990).

Environmental contamination of the Rybinsk Reservoir by PCBs has been examined in two studies; four to seven years after the accidental spills (Kozlovskaya and German, 1997; German and Kozlovskaya, 1999). Elevated amounts of PCB were reported in various components of the reservoir ecosystem near Cherepovets reaching maximal values of 7.16 μg/g dry wt in bottom sediments and 15.8 μg/g wet wt in fish liver. PCBs were also found in industrial wastewaters and bottom sediments near Cherepovets, suggesting that industry in this city as a source of PCB contamination of the reservoir ecosystem. The presence of PCBs in aquatic ecosystems has been reported throughout the world (Van der Oost et al., 1988; Marthinsen et al., 1991; Tillitt et al., 1992; De Boer et al., 1993; Lake et al., 1995). The persistent nature of PCBs along with their volatility has also lead to the global distribution of PCBs through atmospheric transport. Owing to their lipophilic character and stability, PCBs can accumulate in fish-eating birds, wildlife, and humans, and cause adverse effects (Tillitt et al., 1992).

The first objective of this study was to determine the extent of PCB contamination in fish from different locations around the Rybinsk Reservoir. Bream (*Abramis brama*), a common fish species in this reservoir, was used as the indicator species in our studies. Firstly, bream are widespread throughout the entire reservoir and are also the dominant species with the highest yield for the commercial fishery (Malinin and Strel'nikov, 1990). Secondly, bream are an important vector for the transfer of persistent contaminants from bottom sediments, through food chains to humans. Bream are a benthivorous fish that are the main dietary source of fish for consumption by the local populations. Therefore, it was also our objective to assess the general health status of bream in the Rybinsk Reservoir and to use the concentrations of PCBs as an indicator of chemical contamination at strategic locations in the reservoir. Brain AChE activity is more known as a biomarker of fish exposure to neurotoxins such as organophosphorus compound, carbamates, etc. However, earlier it has been demonstrated that the brain AChE activity can serve as a bioindicator of fish stress (Pavlov et al., 1994). Moreover, recently it was found that chub (*Leuciscus cephalus*) living in Lambro River (Italy) had a decreased brain AChE activity when the total PCB content in fish was increased (Barra et al., 2001). Also, liver enzyme activities including cholinesterases can be indicative of general health and well being or physiological condition of organism. Therefore, cholinesterase activity (in both the brain and liver) was used as a general biochemical indicator of organism health in our study. To further evaluate the general health of these fishes, we made morphometric measures, including condition factor, and organ-somatic indices on bream collected from six locations on the Rybinsk Reservoir.

2. Materials and methods

2.1. Sampling

Sites were selected for sampling in June–August 1994 (Fig. 1). Four sites were located on the Rybinsk Reservoir: two sites in Sheksna Reach; one in the northern part near Cherepovets City (Miaksa); and another in the southern part, downstream, near Rybinsk City (Volkovo). The third and fourth sampling sites were located in Mologa Reach (Pervomaika) and the southern part of the Volga Reach (Lavrovo), respectively. Two more sampling sites were on the Volga River, upstream (Uglich) and downstream (Yaroslavl) of the reservoir.

Six to ten bream of both sexes, ranging in standard lengths from 221 to 324 mm (263 ± 25; mean $\pm$ SD) and weights from 225 to 737 g (375 ± 114; mean $\pm$ SD) were sampled using a trawl net. The age of fish was not determined, but as it was reported previously that bream of this size correspond with five to eight-year old fish (Zhiteneva, 2000). Total weight, eviscerated weight (± 1 g), and standard length were measured. Liver and spleen were removed and weighed. Liver samples were immediately frozen at -18 °C and stored under these conditions until biochemical and PCB analyses were initiated.

2.2. Morphometric data

Fulton's and Clark's condition factors (CF) (Anderson and Gutreuter, 1983; Siddall et al., 1994), spleen-somatic (SSI) and liver-somatic (LSI) indices (Heath, 1995) were calculated for each fish based on total weight (TW) or eviscerated weight (EW), standard length (L), and total spleen (SW) or liver (LW) weight according to the following equations:

(1) $\text{Fulton's CF} = 100\,\text{TW}/\text{L}^3$ and

(2) $\text{Clark's CF} = 100\,\text{EW}/\text{L}^3$;

(3) $\text{SSI} = 1000\,\text{SW}/\text{EW}$ and

(4) $\text{LSI} = 100\,\text{LW}/\text{EW}$,

where all weights are in grams (g) and lengths are in centimeters (cm).

2.3. Sample preparation for PCB analysis

Three to six fish from each site were analyzed for PCB content. A 5-g liver portion from each fish was dried with 20 g of anhydrous sodium sulfate (99%; Merck, St. Louis, MO, USA) and ground. Samples were then shipped to the Columbia Environmental Research Center (Columbia, MO, USA) for further processing and analysis. The samples were homogenized and column extracted with CH_2Cl_2. Using methods similar to those described by Schmitt et al. (1985), a portion of each sample was used to gravimetrically determine the lipid content. The remainder of each extract was treated by two stages of reactive sulfuric acid-treated silica gel column clean-up, followed by a high performance gel permeation chromatography (Steingraeber et al., 1994). The purified sample extracts were brought to 1 ml in isooctane for immunoassay or gas chromatographic analyses (Zajicek et al., 1996).

2.4. Enzyme-linked immunosorbent assay analysis (ELISA)

The concentrations of total PCBs in all of the samples of bream livers were determined by ELISA analysis using the Ohmicron PCB RaPID Assay R after a solvent exchange to 50% methanol/water (Zajicek et al., 1996). A horseradish peroxidase-labeled PCB analog and H_2O_2/3,3′,5,5′-tetramethylbenzidine was used as PCB enzyme conjugate and substrate/chromogen, respectively.

Individual samples were analyzed in duplicate at room temperature (21–27 °C). The conjugation reaction was run for 15 min. The color reaction was stopped after 20 min, and the absorbance of each sample solution was measured at 450 nm with a model RPA-1™ spectrophotometer (Ohmicron). The concentrations of PCBs in the livers of bream were calibrated against four calibration standards of Aroclor 1254 (0, 0.25, 1.0 and 5 ng/ml) based upon the similarity of the congener profiles with this technical standard. The concentrations were calculated automatically, corrected for sample dilutions, and reported as μg/g liver wet wt.

2.5. Gas chromatographic analysis

Four of the samples of bream livers representing Volga Reach (Lavrovo), Sheksna Reach (Volkovo, Miaksa near Cherepovets), and Mologa Reach (Pervomaika) were selected for a more detailed analysis of PCBs by high resolution gas chromatography. The purpose of this analysis was to confirm the accuracy of the results from the ELISA assays and allow a comparison of the patterns of the congeners found in bream livers from the various locations on the Rybinsk Reservoir. One-microliter aliquots of purified extracts were analyzed by capillary gas chromatography with electron capture detection to determine total PCB concentration as the sum of 108 PCB congeners contained in 84 peaks and calibrated against a mixed Aroclor standard composed of equal parts of Aroclors 1242, 1248, 1254, and 1260 as percent weight (Steingraeber et al., 1994). The recovery of procedural internal standards, PCB 030 and PCB 204 (Ballschmiter and Zell, 1980) added to samples prior to extraction, were greater than 95% for all samples. Total PCB concentrations were reported as μg/g liver wet wt, uncorrected for recoveries.

2.6. Biochemical analysis

A 0.5 g portion of each liver or whole brain was homogenized (stainless steel homogenizer; East Germany) and diluted (1:20) with 0.1 M phosphate buffer (pH 7.5). The homogenates were centrifuged at 5000 rpm at 4 °C for 10 min, and the supernatant analyzed for acetylcholinesterase (AChE) in the brain or total (acetyl- and butyrylcholinesterase) cholinesterase (ChE) activity in the liver samples and soluble protein (SP) content in both. Supernatants were diluted 500-fold (liver) and 1500- or 3000-fold (brain) prior to analysis. AChE and ChE activities were determined following (Ellman et al., 1961) at a wavelength of 412 nm, temperature 30 °C, and run time 10 min. Acetylthiocholine iodide (Sigma, St. Louis, MO, USA; 4.3×10^{-4} M final concentration) was used as the substrate for both AChE and ChE determinations. Enzyme activity was expressed as μmol substrate hydrolyzed per g tissue per hour. SP content was determined by the methods described in Bradford (1976). Measurements were conducted using an UV–VIS SP26 spectrophotometer (LOMO, St. Petersburg, Russia). Each sample was run in duplicate and measured twice. Means and standard errors (SE) were calculated and results analyzed using ANOVA, followed by Tukey test procedure at 95% level of significance. Pearson correlation analysis was conducted to estimate relationships between pairs of measures (Zar, 1984).

3. Results

3.1. PCB content in bream liver

The concentrations of PCBs in the livers of bream collected from nearly all areas of the Rybinsk Reservoir were relatively low, with the exception of those collected near Cherepovets (Miaksa) (Table 1). The concentrations of PCBs in the livers of bream collected from Lavrovo, Volkovo, Pervomaika, Yaroslavl, and Uglich were all below 0.09 μg/g, except for one fish from Pervomaika which had a liver PCB content equal 0.64 μg/g (Table 1). In contrast, the livers of bream collected near Miaksa had significantly ($p < 0.05$) greater average concentrations of PCBs (0.22–3.4 μg/g wet wt) than the livers of fish collected in other parts of the reservoir.

3.2. PCB congener patterns

The gas chromatographic patterns of PCB congeners of the selected samples were compared with one another and that of technical Aroclor standards as a point of reference (Fig. 2). Penta- and hexa-chlorinated congeners were predominant in all of the samples analyzed, followed by the tetrachlorinated biphenyl congeners. The patterns of PCBs in the livers of bream were similar at all of the locations and were not remarkably different from the congener profile of Aroclor 1254 (Fig. 2). The similarity in PCB patterns among the samples and the Aroclor 1254 standard verified it as the appropriate standard for ELISA quantification

Table 1
Concentrations of polychlorinated biphenyls (PCBs) and lipid contents in liver of bream collected from locations on the Rybinsk Reservoir[1]

Sampling site[2]	N[3]	Lipid contents, %	μg PCB/g wet wt
(1) Lavrovo	5	1.3 ± 0.5	0.019 ± 0.006^{ab} (0.010–0.041)
(2) Volkovo	5	2.6 ± 1.3	0.019 ± 0.010^{b} (0–0.057)
(3) Miaksa	5	1.3 ± 0.4	1.092 ± 0.583^{c} (0.220–3.400)
(4) Pervomaika	5	2.8 ± 1.3	0.159 ± 0.121^{b} (0.015–0.640)
(5) Yaroslavl	3	1.0 ± 0.5	0.033 ± 0.007^{b} (0.020–0.045)
(6) Uglich	5	4.0 ± 1.3	0.031 ± 0.009^{b} (0.010–0.059)

[1] Mean ± standard error and extreme values in parentheses; means with same letter superscript are not significantly different (Tukey's HSD test, $p < 0.05$).
[2] The number before sampling site name corresponds to site location number given in Fig. 1.
[3] The number of fish analyzed.

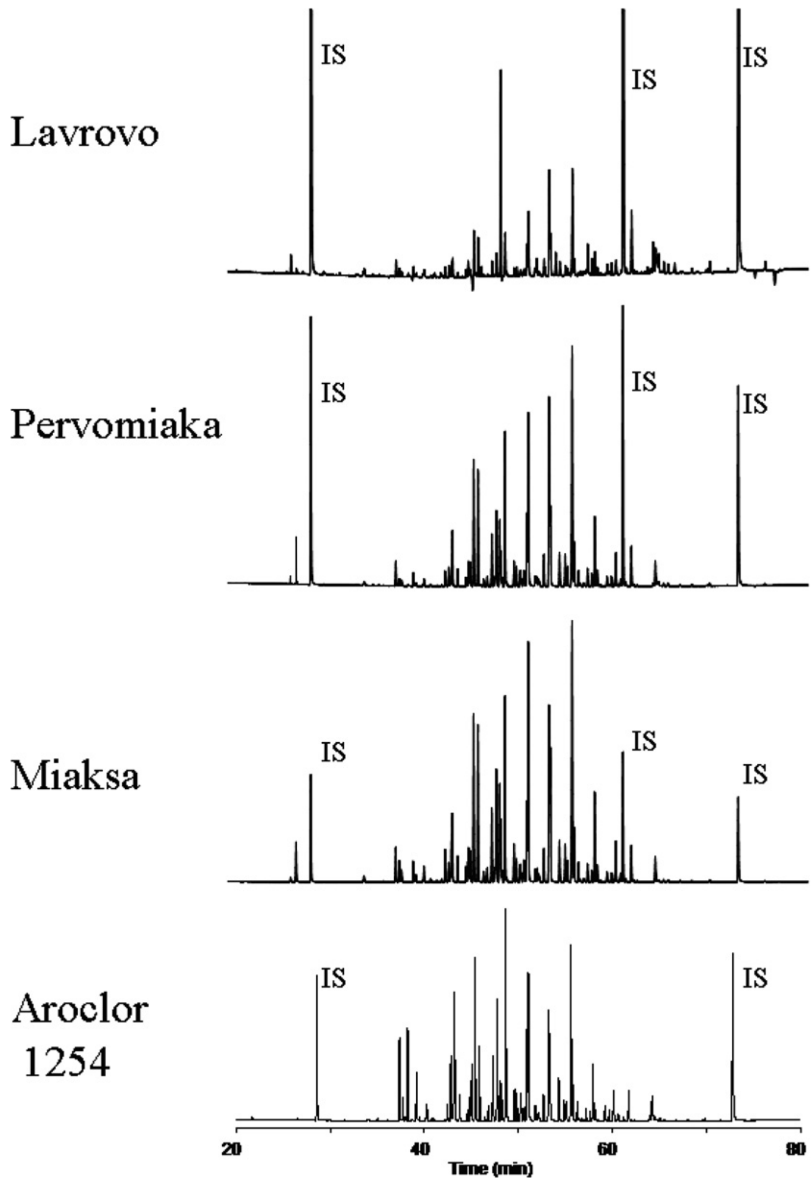

Fig. 2. Gas chromatographic patterns of polychlorinated biphenyls observed in the livers of bream collected from the Lavrovo, Miaksa, and Pervomika on the Rybinsk Reservoir, referenced against the Aroclor 1254 technical standard.

procedures. The source and type of technical PCB mixture in this reservoir is unknown, but is thought to be Sovol, the technical mixture most commonly used in Russia. Additionally, the consistent patterns of PCB congeners in the livers of bream suggest a single source of contamination was predominant in this reservoir system.

3.3. Morphometric analysis

No significant differences were found in Fulton's and Clark's CFs among bream from different sites (Table 2). In contrast, spleen-somatic indices and LSI of bream varied significantly ($p < 0.05$) among different sites (Table 2). The highest values of spleen-somatic indices were found in fish from Yaroslavl (4.57), Volkovo (4.12) and Uglich (3.94), while the smallest value was observed from Pervomaika (2.20). The largest LSI values were in bream collected near Miaksa (3.00) and Lavrovo (2.93), while the lowest were in bream collected from Pervomaika (1.87) and Uglich (1.76). No significant correlations were found between liver PCB content and any morphometric measures of these fish (Table 4).

3.4. Biochemical analysis

Brain AChE activity and SP content was found to be significantly different ($p < 0.05$) among fish from different sites (Table 3). The greatest brain AChE activity values were found in bream from Volkovo (1197 μmol/g/h), while the lowest were in the fish from Pervomaika (630 μmol/g/h).

Table 2
Morphometric indicators of health in bream from the Rybinsk Reservoir[1]

Sampling site[2]	*N*[3]	Condition factor		Index	
		Fulton's	Clark's	Spleen-somatic	Liver-somatic
(1) Lavrovo	9	2.01 ± 0.09^{a}	1.71 ± 0.07^{a}	3.67 ± 0.31^{ab}	2.93 ± 0.20^{a}
(2) Volkovo	10	2.04 ± 0.03^{a}	1.83 ± 0.03^{a}	4.12 ± 0.42^{a}	2.61 ± 0.16^{ab}
(3) Miaksa	10	2.07 ± 0.03^{a}	1.79 ± 0.03^{a}	2.91 ± 0.42^{ab}	3.00 ± 0.15^{a}
(4) Pervomaika	6	1.98 ± 0.03^{a}	1.78 ± 0.03^{a}	2.20 ± 0.33^{b}	1.85 ± 0.10^{c}
(5) Yaroslavl	6	1.98 ± 0.06^{a}	1.80 ± 0.06^{a}	4.57 ± 0.65^{a}	1.96 ± 0.33^{bc}
(6) Uglich	10	1.96 ± 0.04^{a}	1.75 ± 0.03^{a}	3.94 ± 0.03^{a}	1.76 ± 0.08^{c}

[1] Mean ± standard error are presented; means with same letter superscript are not significantly different within the same column (Tukey's HSD test, $p < 0.05$).
[2] The number before sampling site name corresponds to site locations number given in Fig. 1.
[3] The number of fish analyzed.

Table 3
Acetylcholinesterase (AChE) and total cholinesterase (ChE) activities and soluble protein content (SP) in the brain and liver of bream collected from the locations on the Rybinsk Reservoir[1]

Sampling site[2]	*N*[3]	Brain		Liver	
		SP[4]	AChE[5]	SP	ChE
(1) Lavrovo	9	49.3 ± 2.1^{a}	1149 ± 83^{ab}	53.0 ± 3.6^{a}	83.2 ± 8.6^{ab}
(2) Volkovo	10	63.7 ± 3.7^{b}	1197 ± 123^{b}	67.8 ± 6.6^{ab}	73.0 ± 9.1^{a}
(3) Miaksa	10	59.1 ± 2.0^{ab}	900 ± 56^{abc}	51.8 ± 4.1^{a}	48.6 ± 5.5^{a}
(4) Pervomaika	6	53.0 ± 3.9^{ab}	630 ± 81^{c}	58.2 ± 4.9^{ab}	73.6 ± 8.3^{a}
(5) Yaroslavl	6	58.7 ± 3.8^{ab}	1031 ± 144^{abc}	72.9 ± 4.9^{ab}	148.9 ± 23.9^{c}
(6) Uglich	10	58.7 ± 1.4^{ab}	840 ± 52^{ac}	75.5 ± 3.0^{b}	131.0 ± 15.8^{bc}

[1] Mean ± standard error is presented; means with same letter superscript are not significantly different in the same column (Tukey's HSD test, $p < 0.05$).
[2] The number before sampling site name corresponds to site locations number given in Fig. 1.
[3] The number of fish analyzed.
[4] SP content is presented as mg/g (wet wt).
[5] AChE and ChE activities are presented as μmol/g/h (wet wt).

In the other sites the differences were not significant. The greatest difference in brain SP content was between fish from Volkovo and Lavrovo (63.7 and 49.3 mg/g, respectively). Fish from other sites had values for brain SP content that did not differ from bream collected near Volkovo and Lavrovo. There was a strong positive correlation between these two biochemical measures ($r = 0.56$, $p < 0.0001$) among fish from different locations. No significant correlation was observed between liver PCB content and these biochemical measures from the brains of the bream collected in the Rybinsk Reservoir (Table 4).

Biochemical measures of the liver were found to be significantly different ($p < 0.05$) among fish from different sites (Table 3). Both, ChE activity and liver SP content values were lowest in fish from Miaksa while the highest values were found in fish from Yaroslavl and Uglich. There was a strong positive correlation between these two biochemical measures ($r = 0.56$, $p < 0.0001$) among fish from different

Table 4
Pearson correlation matrix (*r*, *p*-value) for chemical, biochemical, and morphometric measures in bream collected from the locations in the Rybinsk Reservoir, Russia

	$[PCB]_l$	$[SP]_l$	$[ChE]_l$	$[AChE]_b$	$[SP]_b$	FCF	CCF	SSI	LSI
$[PCB]_l$[a]	–	−0.34; 0.077	**−0.43**[b]; **0.023**	−0.25; 0.192	−0.22; 0.253	−0.16; 0.405	−0.32; 0.099	0.03; 0.864	0.27; 0.165
$[SP]_l$	–	–	**0.56; 0.0000**	−0.03; 0.812	0.08; 0.553	0.05; 0.742	0.08; 0.577	0.06; 0.677	−0.35; 0.011
ChE_l	–	–	–	0.02; 0.911	0.07; 0.620	−0.23; 0.098	−0.11; 0.461	0.26; 0.068	**−0.6; 0.0000**
$AChE_b$	–	–	–	–	**0.56; 0.0000**	−0.18; 0.205	−0.19; 0.179	0.33; 0.012	0.01; 0.942
$[SP]_b$	–	–	–	–	–	0.002; 0.985	0.11; 0.435	0.26; 0.061	−0.19; 0.195
FCF	–	–	–	–	–	–	**0.91; 0.0000**	−0.04; 0.78	**0.43; 0.002**
CCF	–	–	–	–	–	–	–	−0.05; 0.71	0.15; 0.293
SSI	–	–	–	–	–	–	–	–	−0.08; 0.594
LSI	–	–	–	–	–	–	–	–	–

[a] Subscripts "l" and "b" indicate measures for liver and brain, respectively.
[b] Bold numbers indicate significant ($p < 0.05$) correlation between measures.

locations (Table 4). Moreover, liver ChE activity was negatively correlated with the concentrations of liver PCBs ($r = -0.43$; $p < 0.05$). Both liver ChE and liver SP content were also negatively correlated with LSI and positively correlated with FCF values ($r = -0.6$, -0.35, and 0.43; $p < 0.01$, respectively).

4. Discussion

In our study total PCB concentrations in bream livers varied among two locations in Volga River (Uglich, Yaroslavl) and three locations in Rybinsk Reservoir (Lavrovo, Volkovo, and Pervomaika) from non-detectable quantities (<0.02 μg/g) to 0.09 μg/g wet wt. Only a single fish from Pervomaika had a PCB concentration of 0.64 μg/g wet wt in liver. This concentration is within the range that was found in livers of fish caught in the Sheksna Reach (Miaksa) where the average liver PCB content is 1.092 μg/g wet wt. That is, 30–60-fold greater than in the livers of fish collected from other five locations. The reason for the elevated amount of PCBs in this specimen was not known exactly. However, it might be the result of a causal migration of this individual from the Sheksna Reach toward Mologa Reach. As a rule, bream in Rybinsk Reservoir form schools in accordance with reaches. The migratory patterns of bream are such that they usually travel only short distances related with seasonal periods of spawning, summer feeding, and over-wintering. However, sometimes long distance migrations by individuals or small groups of bream among reservoir reaches can occur (Poddubny, 1971).

In general, our results are in a good agreement with the results of another study on PCB distribution in the Rybinsk Reservoir, carried out in 1989–1993. In that study bream liver PCB content, measured as Clophen A-40 and A-50, varied among locations from non-detectable quantities in Mologa Reach to 15.8 μg/g wet wt, observed in bream taken from Sheksna Reach in locations closest to Cherepovets (Kozlovskaya and German, 1997; German and Kozlovskaya, 1999). PCB concentrations found in the bream livers from Rybinsk Reservoir in both studies spanned the range of PCB concentrations observed in fish biomonitoring conducted in the United States (Schmitt et al., 1990).

Discriminate PCB contents in bream liver from different parts of Rybinsk Reservoir are corresponded very well with PCB contents in bottom sediments. Earlier it has been shown that the PCB concentrations in grey mud sediments of the Sheksna Reach, determined as Clophen A-40 and A-50, reached 7.16 μg/g dry wt at Cherepovets and decreased to 0.06 μg/g dry wt at a distance of 30 km downstream from the city. PCB traces were only found in the bottom sediments that were collected upstream of Cherepovets and in Mologa Reach of the Rybinsk Reservoir. A similar tendency was reported for PCB content in benthic organisms. The highest PCB concentrations were found in oligocheate worms (1.8 μg/g), mosquito's larvae (0.67 μg/g), and zebra mussels (1.4 μg/g) collected just downstream of Cherepovets (Kozlovskaya and German, 1997). As known, the basic diet of bream in Rybinsk Reservoir after two-year age consists of mosquito larvae, oligocheate worms, mollusks, as well as bottom mud (Zhiteneva, 1981). Feeding bottom organisms and bottom mud bream accumulate large amount of persistent, hydrophobic chemicals, such as PCB. These chemicals are not water-soluble. After discharging into the water they adsorb to suspend organic and inorganic particles, settle together with them to the bottom, and accumulate in bottom sediments enriched with organic carbon not so far from the sources of discharge (Suffet et al., 1994).

The patterns of the PCB congeners observed in bream liver collected from different locations in the Rybinsk Reservoir and Volga River were all quite similar (Fig. 2). Congener-specific analysis was not studied before and performed simply to provide an initial screen of the congener profiles in the bream and an indication of the appropriateness of our calibration standard for the ELISA analysis. We did not analyze enough samples by congener-specific analysis to allow more comprehensive investigation of the patterns, such as by principle components analysis. However, the rank-order of the concentrations of the major PCB congener peaks is similar among all locations (data not presented). Congeners PCB 138 (2,2′,3′,4,4′,5-hexachlorobiphenyl) or PCB 101 (2,2′,4,5,5′-pentachlorobiphenyl) were found at the highest concentrations in all of the samples of bream liver analyzed. The congeners found in the next three greatest concentrations within a given sample were PCB 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl), PCB 118 (2,3′,4,4′,5-pentachlorobiphenyl), or (2,2′,4,4′,5-pentachlorobiphenyl). The rest of the predominant congeners were also similar in the five individual samples of bream collected from Volga Reach (Lavrovo), Sheksna Reach 80 km downstream of Cherepovets (Volkovo), near Cherepovets (Miaksa), and Pervomaika (data not presented).

The elevated concentrations of PCBs in samples from the Cherepovets region of the reservoir, in conjunction with the similarity in congener PCB profiles suggest a single source of PCB contamination in the reservoir. In other words, the contamination of bream liver with PCB in Sheksna Reach has a local origination and relates to their high concentrations in bottom sediments and benthic organisms near Cherepovets. Thus, evidence from our study, as well as previous reports, suggest that the PCB contamination of bream, bottom organisms, and sediments in Seksna Reach of Rybinsk Reservoir has the single source of industrial activities in Cherepovets. However, definitive conclusions regarding this require further more detailed investigations.

The general health of bream collected from different locations was evaluated with several measures in our study. Fulton's and Clark's CF of bream were similar from all sampling sites, including Cherepovets (Miaksa). These measures reflect the nutritional state or "well-being" of fish and are sometimes interpreted as an index of growth rate

(Busacker et al., 1990). Therefore, our data suggest that there are no differences in nutritional states and growth rates among bream from the different locations. We also observed no correlation between the liver PCB content and either CFs. In general, total metabolic expenditures and growth in these fish appeared to be balanced and were not affected by PCB contamination. However, four years prior to our study (summer of 1990) bream of similar size collected from Sheksna Reach near Cherepovets had markedly lower Clark's CF and higher PAH content in liver than the fish from other parts of reservoir (Siddall et al., 1994; Kozlovskaya and German, 1997). A decrease in Clark's CF has been associated with fish weight loss (loss of muscle), which may take place as a result of decreases in feeding efficiency or fish food conversion capability. It is possible that the lower Clark's CF observed in the summer of 1990 was due to the chronic effects of contaminant exposure on fish and benthic organisms in Sheksna Reach after an accidental wastewater spill from Cherepovets in the winter 1987 (Flerov, 1990). Such poisonings can result in altered behavior including feeding efficiency, etc. (Little et al., 1993). Also, contaminants can alter the numbers and diversity of food organisms, which may affect foraging efficiency (Munkittrick and Dixon, 1989). The contaminant content (for instance, total PAHs) in fish immediately after the spill in 1987 approached 1000-fold greater than was observed prior to the spill (Kozlovskaya et al., 1990). Mortality and pathological alterations of the internal organs of the fish were reported during and even one year after the accident (Kozlovskaya et al., 1990; Mikryakov et al., 1990; Volodin, 1990). PCB analysis has not been undertaken for that time. Six years later in 1993, when the contamination decreased, the condition of the fish improved in Sheksna Reach, as indicated by an increase of Clark's CF from 1.33 to 1.79 in bream obtained in our study.

Conversely, the LSI values of bream indicated that health differences still existed among fish from different locations. Measurement of the LSI as well as of the CFs is a common tool to assess fish health or general condition. LSI generally reflects the changes in the liver size and is related to the nutritional status of fish at the time of capture. These changes in the liver size are associated with energy accumulation and are largely caused by fluctuations in glycogen and fat content (Busacker et al., 1990; Heath, 1995). Chronic stress and exposure to toxicants can affects LSI values also. Usually chronic stress is followed by energy expenditures, which are supplied by glycogen and fat stores from the liver. These expenditures can result in decreases of LSI values. Thus, a simultaneous decrease in CFs and LSI may indicate the exhaustion of energy stores and the nutritional status of both individuals and populations. Long-term exposure of fish to organic contaminants leads to an increase in LSI (Slooff et al., 1983; Everaarts et al., 1993; Heath, 1995). The enlargement of fish liver can be due to hyperplasia and/or hypertrophy or fat degeneration of hepatocytes. It may be associated with an increased capacity to metabolize xenobiotics and indicates adaptation to contaminated conditions (Hinton et al., 1992; Heath, 1995). In our study, we did not do histopathology analysis. However, liver hypertrophy followed with proliferation of endoplasmic reticulum was reported earlier in channel catfish after subacute exposure to Aroclor 1254 (Klaunig et al., 1979). Although other study have not shown significant increases of the LSI values in rainbow trout chronically exposed *per os* to Aroclor 1254 (Nestel and Budd, 1975). These contradictory findings are more likely due to the time of toxic exposure and PCB doses accumulated with fish. We observed that bream near Cherepovets (Miaksa) had the greatest LSI values ($p < 0.05$) in the Rybinsk Reservoir and Volga River. Since bream in all locations had similar CFs it may be supposed that the high LSI values are a result of a chronic stress, possibly exposure to some organic chemical, rather than alterations in the nutritional state. Even though we did not observe a strong correlation between liver PCB content and LSI in bream, the health status of bream collected from Cherepovets appeared to have been compromised by some factor(s), of which may include PCBs. Also, the enlargement of the liver caused by hypertrophy and accompanied with activation of xenobiotic-metabolizing enzymes has been reported in bream that inhabited more contaminated locations in the River Rhine and its tributaries in Netherlands. The LSI values of those bream were similar to the ones reported in present study (Slooff et al., 1983). However, in order to confirm hepatotoxic reason of liver enlargement in bream from Miaksa further studies with histopathology analysis should be undertaken.

The distribution pattern of SSI values in bream among locations is other than those of LSI. Highest bream SSI values are in southern sites (Yaroslavl, Uglich, Volkovo) while lowest ones are in northern sites (Pervomaika, Miaksa). The distance between southern and northern sites is more than 80 km. Since Pervomaika is reference and more low polluted sites, but Miaksa is more contaminated therefore the reasons of these differences may be rather a geographic-spatial factor than contamination.

In the present study, we found considerable variability in liver ChE and brain AChE activities in bream among locations. Inhibition of these enzymatic activities by anticholinesterase inhibitors such as organophosphorus or carbamate pesticides is hardly probable since the use of these chemicals is extremely rare around Rybinsk Reservoir at that time. Although it is known PCBs are not ChE inhibitors, negative correlations between PCB content and liver total ChE activity, on the one hand, and liver total ChE activity and LSI, on the other hand, may indicate an indirect manifestation of hepatotoxic effect. More than likely, the observed decrease of ChE activity, as well as liver SP content, was due to an increase in liver weight caused by hypertrophy. In this case the total amount of ChE protein remains constant, but total liver weight is increased. Such conditions occur when liver hypertrophy is associated with fat degeneration. Increases in lipids are known to occur in hepatocytes of rainbow trout after chronic administration of Aroclor 1254 (Hacking et al., 1977). The variability of brain AChE activity in bream from different locations may also be due to geographic-spatial factors, since the lowest values were observed in fish inhabiting the northern portion of Rybinsk Reservoir, Miaksa and Pervomaika.

Thus, our study showed bream from Sheksna Reach of Rybinsk Reservoir is more contaminated with PCB. The source of the bream contamination is PCB pollution of bottom organisms and sediments conditioned with industrialization facilities of Cherepovets. Among the common fish health biomarkers used only liver total ChE activity and liver-somatic index in bream near Cherepovets can reflect environmental pollution. Further investigations are required which include a broader range of specific fish health indicators and the determination of more chemical contaminants (i.e. PAHs, organochlorines, heavy metals) in order to identify the true extent of chemical effects on fish population in the Rybinsk Reservoir.

Acknowledgements

The authors would like to thank John Meadows and Kathy Echols for their technical assistance in preparation and gas chromatographic analysis of these samples, Greg Potts for assistance with ELISA determinations, and Dennis Schroeder for preparation of the samples for analysis. We also thank Denny Buckler and David Fabacher for thoughtful comments on earlier drafts and Robert Little for the preparation of maps.

References

Anderson, R.O., Gutreuter, S.J., 1983. Length, weight, and associated structural indices. In: Nielsen, L.A., Johnson, D.L. (Eds.), Fisheries Techniques. American Fisheries Society, Southern Printing Company, Blacksburg, VA, USA, pp. 283–300 (Chapter 15).

Ballschmiter, K., Zell, M., 1980. Analysis of polychlorinated biphenyls (PCB) by glass capillary gas chromatography: composition of technical Aroclor– and Clopen–PCB mixtures. Fresenius Z. Anal. Chem. 302, 20–31.

Barra, R., Notarianni, V., Gentili, G., 2001. Biochemical biomarker responses and chlorinated compounds in the fish *Leusciscus cephalus* along a contaminant gradient in a polluted river. Bull. Environ. Contam. Toxicol. 66, 582–590.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principal of protein-dye binding. Anal. Biochem. 72, 248–254.

Busacker, G.P., Adelman, I.R., Goolish, E.M., 1990. Growth. In: Schreck, C.B., Moyle, P.B. (Eds.), Methods for Fish Biology. American Fisheries Society, Bethesda, MD, USA, pp. 363–387 (Chapter 11).

Chalova, IV., 1990. Study of water toxicity in Rybinsk Reservoir using seven days biotest on *Ceriodaphnia affinis*. In: Flerov, B.A. (Ed.), Impact of Waste Waters of Cherepovets Industrial Complex on Ecological Situation in Rybinsk Reservoir. Goskomizdat, Rybinsk, Russia, pp. 20–23 (in Russian).

De Boer, J., Stronck, C.J.N., Traag, W.A., Van der Meer, J., 1993. Non-ortho and mono-ortho substituted chlorobiphenyls and chlorinated dibenzo-*p*-dioxins and dibenzofurans in marine and freshwater fish and shellfish from the Netherlands. Chemosphere 26, 1823–1842.

Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, I., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 70, 88–95.

Everaarts, J.M., Shugart, L.R., Gustin, M.K., Hawkins, W.E., Walker, W.W., 1993. Biological markers in fish: DNA integrity, hematological parameters and liver somatic index. Mar. Environ. Res. 35, 101–107.

Flerov, B.A., 1990. Ecological situation in Rybinsk Reservoir after accidents on Cherepovets waste water treatment plant in 1987. In: Flerov, B.A. (Ed.), Impact of Waste Waters of Cherepovets Industrial Complex on Ecological Situation in Rybinsk Reservoir. Goskomizdat, Rybinsk, Russia, pp. 3–11 (in Russian).

Fortunatov, M.A., 1979. Physical geography of the Volga basin. In: Mordukhai-Boltovskoi (Ed.), The Volga River and its Life. Dr. W. Junk by Publishers, The Hague, Boston, London, Netherlands, pp. 1–29 (Chapter 1).

German, A.V., Kozlovskaya, V.I., 1999. Polychlorinated biphenyls content in bream *Abramis brama* from Rybinsk Reservoir. Quest. Ichthyol. 39 (1), 139–142 (in Russian).

Hacking, M.A., Budd, J., Hodson, K., 1977. The ultrastructure of the liver of the rainbow trout: normal structure and modifications after chronic administration of a polychlorinated biphenyl Aroclor 1254. Can. J. Zool. 56, 477–491.

Heath, A.G., 1995. Water Pollution and Fish Physiology, second ed. Lewis, Boca Raton, FL, USA.

Hinton, D.E., Baumann, P.C., Gardner, G.R., Hawkins, W.E., Hendricks, J.D., Murchelano, R.A., Okihiro, M.S., 1992. Histopathologic biomarkers. In: Huggett, R.J., Kimerle, R.A., Mehrle, P.M. (Eds.), Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress. Lewis Publishers, Boca Raton, FL, USA, pp. 155–209 (Chapter 4).

Klaunig, J.E., Lipsky, M.M., Trump, B.F., Hinton, D.E., 1979. Biochemical and ultrastructural changes in teleost liver following subacute exposure to PCB. J. Environ. Pathol. Toxicol. 2, 953–963.

Kozlovskaya, V.I., German, A.V., 1997. Polychlorinated biphenyls and polycyclic aromatic hydrocarbons in ecosystem of Rybinsk Reservoir. Water Resour. 24 (5), 563–569 (in Russian).

Kozlovskaya, V.I., Pavlov, D.F., Chuiko, G.M., Khalko, V.V., Vinnikov, Y.Y., Anokhin, S.V., 1990. Effect of contaminants upon fish condition in Sheksna Reach of Rybinsk Reservoir. In: Flerov, B.A. (Ed.), Impact of Waste Waters of Cherepovets Industrial Complex on Ecological Situation in Rybinsk Reservoir. Goskomizdat, Rybinsk, Russia, pp. 123–143 (in Russian).

Lake, J.L., McKinney, R., Lake, C.A., Osterman, F.A., Heltshe, J., 1995. Comparison of patterns of polychlorinated biphenyl congeners in water, sediment, and indigenous organisms from New Bedford Harbor, Massachusetts. Arch. Environ. Contam. Toxicol. 29, 207–220.

Little, E.E., Fairchild, J.F., DeLonay, A.J., 1993. Behavioral methods for assessing impacts of contaminants on early life stage fishes. Am. Fish. Soc. Symp. 14, 67–76.

Malinin, L.K., Strel'nikov, A.S., 1990. Condition of ichtyophauna as a result of Sheksna Reach contamination in Rybinsk Reservoir for 1987–1988. In: Flerov, B.A. (Ed.), Impact of Waste Waters of Cherepovets Industrial Complex on Ecological Situation in Rybinsk Reservoir. Goskomizdat, Rybinsk, Russia, pp. 89–100 (in Russian).

Marthinsen, I., Staveland, G., Skaare, J.U., Ugland, K.I., Haugen, A., 1991. Levels of environmental pollutants in male and female flounder (*Platichthys flesus* L.) and Cod (*Gadus morphua* L.) caught during the year 1988 near or in the Waterway of Glomma, the largest river of Norway. I. Polychlorinated biphenyls. Arch. Environ. Contam. Toxicol. 20, 353–360.

Mikryakov, V.R., Andreeva, A.M., Lapirova, T.B., Silkina, N.I., 1990. Immune system responses in fish from Sheksna Reach after accident on Cherepovets industrial complex. In: Flerov, B.A. (Ed.), Impact of Waste Waters of Cherepovets Industrial Complex on Ecological Situation in Rybinsk Reservoir. Goskomizdat, Rybinsk, Russia, pp. 144–155 (in Russian).

Munkittrick, K.R., Dixon, D.G., 1989. Use of white sucker (*Catostomas commersoni*) populations to assess the health of aquatic ecosystems exposed to low-level contaminant stress. Can. J. Fish. Aquat. Sci. 46, 1455–1462.

Nestel, H., Budd, J., 1975. Chronic oral exposure of rainbow trout (*Salmo gairdneri*) to a polychlorinated biphenyl (Aroclor 1254): pathological effects. Can. J. Comp. Med. 39, 208–215.

Pavlov, D.F., Chuiko, G.M., Shabrova, A.G., 1994. Adrenaline induced changes of acetylcholinesterase activity in the brain of perch (*Perca fluviatilis* L.). Comp. Biochem. Physiol. 108C (1), 113–115.

Poddubny, A.G., 1971. Ecological Topography of Populations of Fishes in Reservoirs. Nauka, Leningrad, Russia (in Russian).

Riv'er, I.K., 1990. Impact of Cherepovets waste water effluents on zooplankton in Sheksna Reach. In: Flerov, B.A. (Ed.), Impact of Waste Waters of Cherepovets Industrial Complex on Ecological Situation in Rybinsk Reservoir. Goskomizdat, Rybinsk, Russia, pp. 42–58 (in Russian).

Schmitt, C.J., Zajicek, J.L., Ribick, M.A., 1985. National pesticide monitoring program: residues of organochlorine chemicals in freshwater fish, 1980–1981. Arch. Environ. Contam. Toxicol. 14, 225–260.

Schmitt, C.J., Zajicek, J.L., Peterman, P.H., 1990. National contaminant biomonitoring program: residues of organochlorine chemicals in U.S. freshwater fish, 1976–1984. Arch. Environ. Contam. Toxicol. 19, 748–781.

Siddall, R., Robotham, P.W.J., Gill, R.A., Pavlov, D.F., Chuiko, G.M., 1994. Relationship between polycyclic aromatic hydrocarbon (PAH) concentrations in bottom sediments and liver tissue of bream (*Abramis brama*) in Rybinsk Reservoir, Russia. Chemosphere 29, 1467–1476.

Slooff, W., Van Kreijl, C.F., Baars, A.J., 1983. Relative liver weights and xenobiotic-metabolizing enzymes of fish from polluted surface waters in the Netherlands. Aquat. Toxicol. 4, 1–14.

Steingraeber, M.T., Schwartz, T.R., Wiener, J.G., Lebo, J.A., 1994. Polychlorinated biphenyl congeners in emergent mayflies from the upper Mississippi River. Environ. Sci. Technol. 28, 707–714.

Suffet (Mel), I.H., Jafvert, C.T., Kukkonen, J., Servos, M.R., Spacie, A., Williams, L.L., Noblet, J.A., 1994. Synopsis of discussion session: influence of particulate and dissolved material on the bioavailability of organic compounds. In: Hamelink, J.L., Landrum, P.F., Bergman, H.L., Benson, W.H. (Eds.), Bioavailability: Physical, Chemical, and Biological Interactions. Lewis Publishers, Boca Raton, FL, USA, pp. 93–108.

Tillitt, D.E., Ankley, G.T., Giesy, J.P., Ludwig, J.P., Kurita-Matsuba, H., Weseloh, D.V., Ross, P.S., Bishop, C.A., et al., 1992. Polychlorinated biphenyl residues and egg mortality in double-crested cormorants from the Great Lakes. Environ. Toxicol. Chem. 11, 1281–1288.

Van der Oost, R., Heida, H., Opperhuizen, A., 1988. Polychlorinated biphenyl congeners in sediments, plankton, mollusks, crustaceans, and eel in a freshwater lake: implications of using reference chemicals and indicator organisms in bioaccumulation studies. Arch. Environ. Contam. Toxicol. 17, 721–729.

Volodin, V.M., 1990. Fish reproduction system condition and productivity in North Sheksna Reach of Rybinsk Reservoir. In: Flerov, B.A. (Ed.), Impact of Waste Waters of Cherepovets Industrial Complex on Ecological Situation in Rybinsk Reservoir. Goskomizdat, Rybinsk, Russia, pp. 101–122 (in Russian).

Zajicek, J.L., Tillitt, D.E., Huckins, J.N., Petty, J.D., Potts, M.E., Nardone, D.A., 1996. Application of enzyme-linked immunosorbent assay for measurement of polychlorinated biphenyls from hydrophobic solutions: extracts of fish and dialysates of semipermeable membrane devices. In: Van Emon, J.M., Gerlach, C.L., Johnson, J.C. (Eds.), Environmental Immunochemical Methods. ACS Symposium Series 647. American Chemical Society, Washington, DC, pp. 307–325.

Zar, J.H., 1984. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, NJ, USA.

Zhiteneva, T.C., 1981. Feeding peculiarities of bream (*Abramis brama* L.) on a grey mud biotope in the different reaches of the Rybinsk Reservoir. In: Monakov, A.V. (Ed.), Intrapopulation Variability in Fish Feeding and Growth. Proc. IBIW RAS, vol. 47 (50). Russian Acad. Sci., Yaroslavl, Russia, pp 53–63 (in Russian).

Zhiteneva, T.S., 2000. Changes of body proportions of bream *Abramis brama* (Cyprinidae) from different population in relationship with periods of late ontogenesis. Biol. Inland Waters 4, 103–115 (in Russian).