Muscle chemical content and hepatic biotransformation in bream (*Abramis brama*) and asp (*Aspius aspius*) in a PCB-contaminated lake

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During the period of 1956–1983, an approximated amount of 900 liters of polychlorinated biphenyls (PCBs) was discharged into a small freshwater lake in southern Finland. Biomonitoring study of the lake in 1998 revealed clearly detectable muscle PCB concentrations in the two feral fish species studied, bream (*Abramis brama*) and asp (*Aspius aspius*), suggesting persistent PCB contamination in the lake. The muscle PCB contents in bream and asp were 18 and 59 times higher than the corresponding values measured in reference locations. The hepatic monooxygenase EROD activities were significantly induced in fish caught from the lake, underlining the biomarker value of EROD measurements in monitoring long-term chemical exposure in field studies. The conjugation GST activities showed mostly seasonal differences and did not indicate the chemical stress of fish.

Introduction

Polychlorinated biphenyls (PCBs) are highly persistent, lipophilic environmental pollutants that accumulate in the fatty tissues of humans and other animals and have caused toxic effects particularly if prolonged exposure occurs (WHO 1993). PCBs are bioavailable from the sediments to benthic aquatic species, can be transported and the effects biomagnified through the food
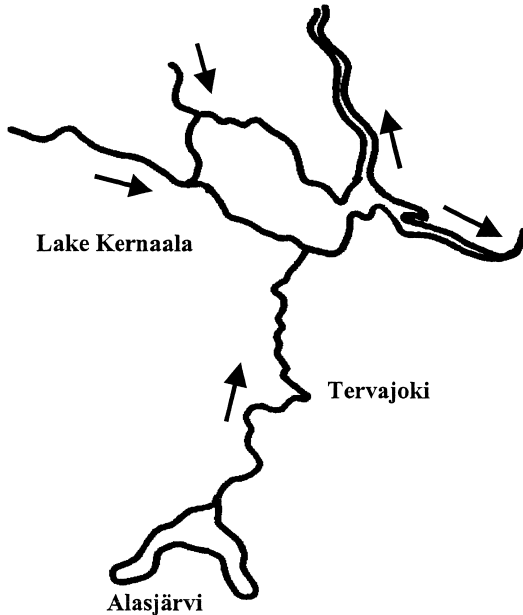
Fig. 1. Lakes Kernaala and Alasjärvi, the reference site (another reference location, Kokemäenjoki, is not shown).

chain at the higher trophic levels (Stein *et al.* 1987, Smith *et al.* 1990, De Wit *et al.* 1992).

The enzyme-catalysed metabolism of chemicals is called biotransformation. Biotransformation through the phase I (cytochrome P450 monooxygenase enzymes, or CYPs) and phase II (conjugating enzymes) is a requisite for detoxication and excretion of lipophilic chemicals (Goksøyr and Förlin 1992). Measurements of two particular enzyme activities, 7-ethoxyresorufin *O*-deethylase (EROD) and glutathione transferase (GST) have been commonly used to detect phase I and phase II reactions (Safe 1994, Stegeman *et al.* 1992).

Coplanar PCBs are potent inducers of biotransformation enzyme systems in teleosts (Lindström-Seppä *et al.* 1994, Janz and Metcalfe 1991). Subsequently, good correlation has been found between tissue levels of total PCBs and rates of monooxygenase enzyme activities in flatfish *Platichthys flesus* (Stegeman *et al.* 1988). Both monooxygenase and conjugation enzyme activities have been shown to respond to halogenated hydrocarbon exposure in juvenile rainbow trout (*Oncorhynchus mykiss*) (Huuskonen *et al.* 1996, Fisk *et al.* 1997). PCB-mediated EROD induction has been detected already at the

embryonic stage of fish, while embryonic GST was not affected by PCB exposure (Koponen *et al.* 2000). Furthermore, Huuskonen *et al.* (1998) demonstrated EROD induction in fish hepatoma cells (PLHC-1) exposed to sediment extracts of PCB-polluted lake.

Lake Kernaala in southern Finland has a history of PCB pollution. A local paper mill used PCBs in quality control processes during 1956–1983. During that time, approximately 900 liters of PCBs were discharged into the Tervajoki, which runs into the southern end of the lake. The total PCB effluent consisted of about 100 liters of Clophen A50 (1956–1963) and approximately 800 liters of Aroclor 1242, Aroclor 1342, as well as Pyralene 1499, 3000-3010, and 3001-3011 (1961–1983) (M. Korpio pers. comm.). In 1987, fish in lake Kernaala were found to be contaminated by PCBs (Koistinen *et al.* 1989). Although the chemical burden of the lake has decreased since those days, considerable amounts of PCBs have still been measured from muscle samples of lake Kernaala feral fish. For instance, a total PCB concentration in the muscle of eel (*Anguilla anguilla*) in 1993, bream (*Abramis brama*) in 1995 and asp (*Aspius aspius*) in 1996 have all been higher than the Finnish National Board of Health's tolerance limit of 2.0 mg kg^{-1} (Piiroinen 1993, 1995, 1996).

The main objective of our work was to study the effects of PCB exposure on feral fish in lake Kernaala, using biotransformation enzyme measurements as an endpoint. Another goal was to study the biomarker value of new potential fish species. First, the total PCB concentrations in the muscle tissue of two feral fish species, bream and asp, were determined. In addition, both hepatic monooxygenase (EROD) and conjugation (GST) enzyme activities were measured in order to evaluate the value of the biotransformation enzyme system as a biomarker in monitoring long-term environmental pollution.

Materials and methods

Study area

Lake Kernaala is a freshwater lake (length 3.5 km, width 1.5 km, area 405 ha) located in southern

Finland (Fig. 1). In a paper mill upstream from the lake, an approximated amount of 900 l of PCBs were used for laboratory testing of condensate paper during 1956–1983. The resulting effluent was discharged through Tervajoki into the southern part of lake Kernaala. A reference lake of this study, Alasjärvi, is situated upstream from the mill, about 6 km upstream from lake Kernaala.

Field sampling

Two behaviorally and physiologically different *Cyprinidae*, bream and asp, were studied. In early May 1997 (spring sampling), a total of 17 bream (8 males/9 females) were caught by gill nets from lake Kernaala (water temperature +14 °C). Another 16 bream (3/13) were caught in August 1997 (summer sampling), when the spawning season of bream was over (water temperature +24 °C). Simultaneously with the Kernaala summer sampling, an additional 14 bream (4/10) were caught from Alasjärvi (water temperature +24 °C), a small unpolluted lake adjacent to Kernaala, which served as a reference lake for bream in this study. Only adult bream were selected from each sampling location.

During the Kernaala spring sampling, 13 specimens of asp (9 males/4 females) were also caught from the lake. Asp were introduced into lake Kernaala as one-summer-old juveniles in 1991 (O. Piiroinen pers. comm.). While asp reach their sexual maturity at the age of 8 (male) to 9 (female), it was assumed that all the fish we caught from lake Kernaala were immature juveniles. Since asp could not be found from any other lake in the study area, reference asp (3 males/3 females) were caught by gill nets from the Kokemäenjoki, southwest Finland, in the fall of 1998 (water temperature +9 °C). Asp caught from both locations were histologically verified to be immature.

In each sampling, the gill nets were frequently checked, and undamaged, live fish were placed in containers filled with fresh water and immediately transported to the nearby shore. The fish were weighed, measured, and killed by severing the spinal cord. The abdominal cavity of the fish was opened, and a portion of the liver was excised and stored in liquid nitrogen for enzyme and protein analyses. In addition, a lateral muscle sample was taken and placed in liquid nitrogen for chemical analyses. Triplicate scale sample was taken from each bream for age determination. Asp from lake Kernaala were aged by comparing their weight/length data with existing stocking data. Asp from Kokemäenjoki were aged by scale analyses.

Chemical analyses

The data for muscle chemistry is obtained from three separate samplings during 1996–1998. To estimate the lowest chemical load in adult fish during the annual cycle, only postspawning female bream were selected for analysis. In lake Kernaala, adult bream were collected from two different locations: from the supposedly less polluted end of the lake (north) and from the more polluted end of the lake (south). The reference asp caught from Kokemäki were sexually immature. The analysis of total PCBs in the muscle tissue of bream from both lakes (1997), as well as the reference asp (1998) was performed as follows: homogenized (in liquid NO_2) muscle samples (3 g) mixed with 12 g Na_2SO_4 were extracted in a Soxhlet apparatus (Soxhlet system HT 6) for 1.5 hours at 200 °C. Samples were rinsed for one hour in 60 ml of toluene, which was then evaporated and samples were transferred into kimax glass vials with 1 ml of hexane, 4 ml of sulfuric acid (H_2SO_4) and 1 ml of internal standard 2,4,6-TCB (128.2 ng ml^{-1} of toluene). The samples were shaken for few minutes, centrifuged, and let to settle until the hexane layer was separated. The upper hexane layer was then transferred into aluminum oxide column and eluted with 10 ml of 2% dichloromethane-hexane. Finally, the samples were evaporated down to 1 ml and transferred to autosampler vials. PCB contents were analyzed with high resolution gas chromatograph coupled to mass spectrometer.

The total PCB content in the muscle tissue of sexually immature asp (1996) from lake Kernaala was analyzed by the Water Protection Association of Kokemäenjoki (Tampere, Finland) as a part of a local paper mill Tervakoski

Inc's statutory environmental quality control survey of lake Kernaala and surrounding lakes. The muscle samples were weighed, homogenized and dried. Before the extraction, known volume of 2,4,6-trichlorobiphenyl was injected into each sample as internal standard. Samples were then extracted with the solvent mixture of acetone-hexane-petrolhexane-ether (5.5:2.5:9:1) in a Soxhlet apparatus for six hours. The extracts were weighted to obtain the fat content, redissolved in hexane, and washed with H_2SO_4. Finally, the total PCB content of the organic phase was analyzed with the dual column gas chromatograph with electron-capture detection. PCB contents were expressed both as wet weight (ww) and as lipid weight values (lw).

Enzyme and protein assays

All the fish for biotransformation enzyme activity studies were caught during 1997–1998. To assess the sexual and seasonal variation in biotransformation enzyme activities, bream of both gender were caught before and after their spawning season in 1997. Also lake Kernaala asp were caught in 1997, but the reference asp were collected from Kokemäenjoki in 1998. In sample

preparation, microsomes and cytosolic fractions were prepared as described by Koponen *et al.* (1997). The hepatic monooxygenase activities of the fish were measured from the microsomal fraction using 7-ethoxyresorufin (final concentration 2 μM) as a substrate. The deethylation of 7-ethoxyresorufin (EROD) was measured with a Shimadzu fluorescence spectrophotometer (RF-5001PC) in a kinetic reaction with resorufin as a reference (Burke and Mayer 1974). Glutathione S-transferase (GST) activity in the cytosolic fraction was analyzed with a Perkin-Elmer Lambda 2 UV/VIS spectrophotometer with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate (Habig *et al.* 1974).

Statistics

The data were analyzed statistically using the SPSS/PC+ (release 7.5) program, employing routine statistical methods (Zar 1984). Normality test of the variables was made with the Kolmogorov-Smirnov (Lilliefors) test, followed by Levene's test for homogeneity of variance. Since the data did not meet the requirements for normal distribution, further analyses were run with nonparametric methods. Differences between groups

Table 1. Total polychlorinated biphenyls (tPCB) in the muscle tissue of bream and asp in lake Kernaala and at reference locations. Numerical data are presented as mean ± S.D.

Species	Sampling location	Year	Gender and reprod. stage	n	Body weight (g)	Lipid (mean %)	Muscle tPCB content in	
							whole tissue (ng g^{-1} ww)	muscle lipid (μg g^{-1} lw)
Bream	Alasjärvi (reference)	1997	female, post-spawning	6	255 + 53	0.3 ± 0.2	9 ± 4[ab]	3.0 ± 1.4[ab]
Bream	Kernaala (North)	1997	female, post-spawning	8	372 + 80	0.3 ± 0.1	59 ± 27[ac]	20.6 ± 7.8[a]
Bream	Kernaala (South)	1997	female, post-spawning	6	306 + 59	0.4 ± 0.2	159 ± 80[bc]	38.0 ± 29.4[b]
Asp	Kokemäenjoki (reference)	1998	both sexes immature	5	1219 + 114	1.5 ± 0.8[d]	47 ± 27[d]	3.1 ± 0.8[d]
Asp*	Kernaala (South)	1996	both sexes immature	5	1092 + 156	3.6 ± 0.6[d]	2764 ± 946[d]	78.7 ± 27.4[d]

* Muscle chemistry data was analyzed by Water Protection Association of Kokemäenjoki and obtained from Tervakoski Inc's environmental quality control survey of lake Kernaala. [a] = significant difference ($p < 0.05$) between Alasjärvi and Kernaala North (bream); [b] = sig. difference between Alasjärvi and Kernaala South (bream); [c] = sig. difference between Kernaala South and Kernaala North (bream); [d] = sig. difference between Kernaala South and Kokemäenjoki (asp). ww = wet weight; lw = lipid weight.

were detected with the nonparametric Kruskal-Wallis one-way analysis of variance and Mann-Whitney's test with Bonferroni's correction.

Results

Scale analysis revealed bream to be over 9 years-old with no statistical differences in age distribution between samplings (data not shown). Furthermore, there were no notable differences in their body weights or muscle lipid contents (Table 1). The asp collected from lake Kernaala were estimated to be 4-year-old by comparing their weight and length data with existing stocking statistics. Scale analysis of the reference asp from Kokemäenjoki revealed them to be 6 years-old. The muscle lipid content in reference asp was over 2-times lower than in asp caught from lake Kernaala (Table 1).

PCB in muscle tissue

The amount of total PCBs in the muscle (ww) and also in the lipid proportion of the muscle tissue (lw) of bream and asp in lake Kernaala and in fish from the reference waters, Alasjärvi and Kokemäenjoki, are presented in Table 1.

The PCB content in fish caught from the reference waters was extremely low in comparison to that in lake Kernaala fish. Both bream and asp from lake Kernaala had significantly elevated PCB muscle contents, which indicates apparent prolonged chemical exposure. Significant intra-lake variation in PCB uptake was also apparent (Table 1). Bream caught from the northern end of lake Kernaala revealed three times lower PCB content in muscle and two times lower amount of PCB in lipid proportion than bream from the southern sampling point. When compared with the chemical data of Alasjärvi bream, the muscle/lipid PCB content was 18/13 times higher in Kernaala South and 7/7 times higher in Kernaala North, respectively. Differences were even more pronounced in asp: the muscle/lipid PCB content in lake Kernaala asp were 59/26 times higher than in reference samples (Table 1).

Biotransformation enzymes

The results of hepatic monooxygenase (EROD) and conjugation (GST) enzyme activity measurements of bream and asp are presented in Table 2. Since there was no statistically significant difference in the enzyme activity data of bream between northern and southern sampling points

Table 2. Hepatic monooxygenase and conjugation activities in feral bream and asp in lake Kernaala and at reference locations. Numerical data are presented as mean ± S.D.

Species	Sampling location	Year	Water T (°C)	Sex	*n*	Reprod. stage	Biotransformation enzyme activities	
							EROD	GST
Bream	Kernaala	1997 (spring)	14	male	8	pre-spawning	333 ± 69[ab]	973 ± 127[ab]
				female	9		188 ± 64[ab]	579 ± 104[ab]
Bream	Kernaala	1997 (summer)	24	male	3	post-spawning	53 ± 30[abc]	1337 ± 78[b]
				female	13		25 ± 13[abc]	1250 ± 207[b]
Bream	Alasjärvi (reference)	1997 (summer)	24	male	4	post-spawning	6 ± 4[c]	1290 ± 70
				female	10		6 ± 2[c]	1332 ± 202
Asp	Kernaala	1997 (spring)	14	male	9	immature	177 ± 37[ad]	502 ± 8[d]
				female	4		260 ± 44[ad]	407 ± 40
Asp	Kokemäenjoki (reference)	1998 (autumn)	9	male	3	immature	1.5 ± 0.3[d]	347 ± 44[d]
				female	3		0.8 ± 0[d]	313 ± 28

[a] = significant difference ($p < 0.05$) between male and female fish inside each sampling; [b] = significant difference between Kernaala spring and Kernaala summer (bream); [c] = significant difference between Alasjärvi summer and Kernaala summer (bream); [d] = significant difference between Kernaala spring and Kokemäki fall (asp). EROD = 7-ethoxyresorufin *O*-deethylase [pmol/(min × mg prot.)], GST = glutathione S-transferase [nmol/(min × mg prot.)].

in lake Kernaala (not shown), the data from those two points were pooled to represent the whole lake. In Kernaala spring sampling, clearly elevated EROD activities were measured in bream. Male bream showed significantly higher hepatic monooxygenase activities (1.8-fold) than female bream. In postspawning sampling, EROD activities were remarkably diminished both in male (6.3-fold) and in female (7.5-fold) bream. Gender difference in EROD activities was still apparent (male 2.1-fold higher than female). However, due to a low number of male bream in the latter sampling event, the difference was not statistically significant. Despite the decline in EROD activity levels between the two samplings in lake Kernaala, bream from post-spawning sampling showed significantly higher EROD activities than bream caught at the same time from adjacent Alasjärvi, the reference lake. In the Alasjärvi sampling, both male and female bream showed notably lower (8.8-fold/4.2-fold) monooxygenase activities when compared to the Kernaala sampling. Interestingly, EROD activities measured from Alasjärvi bream were at the same low level in both male and female fish (Table 2).

Lake Kernaala asp showed distinctly induced hepatic EROD activities when compared to control fish caught from Kokemäenjoki (Table 2). Female asp displayed significantly higher (1.5-fold) EROD activities than male asp in lake Kernaala. Hepatic monooxygenase enzyme activities in reference asp were at the same basal level in both genders.

Differences between sexes, as well as seasonal differences were apparent in conjugation (GST) enzyme activities in lake Kernaala bream (Table 2). Prespawning male fish showed significantly higher (1.7-fold) GST activities than female fish. There was also a notable increase in conjugation enzyme activities from spring to fall both in male (1.4-fold) and female (2.2-fold) bream. However, GST values of both gender from Kernaala fall sampling were essentially at the same level than their reference values caught from Alasjärvi. In general, male asp showed somewhat higher hepatic GST values than female fish. However, no significant gender-specific differences in hepatic GST activities were found inside either sampling. Conjugation

enzyme activities were higher in both male (1.5-fold) and female (1.3-fold) asp in lake Kernaala than in asp caught from reference location (Table 2). Due to a low number of female fish in both samplings, the difference in female fish was not statistically significant.

Discussion

Our results on chemical residues showed that PCBs are still present at high concentrations in lake Kernaala fish. Together with elevated PCB muscle content, the sublethal chemical exposure of aquatic biota was demonstrated via induced xenobiotic metabolism of feral fish: when biotransformation enzyme activities of both bream and asp of the lake were compared to corresponding values measured at reference locations, significantly higher hepatic monooxygenase EROD activities were evident in lake Kernaala fish. At the same time, GST conjugation enzyme activities showed mainly seasonal, and to some extent also sexual variation, addressing the limited bioindicator value of GST measurements in such field studies.

PCB accumulation

The cyprinid species sampled in our study, bream and asp, both share the same habitat where the biomagnification of effects of PCBs into fish most likely results from eating contaminated prey at lower trophic levels. Adult bream is known to feed close to the bottom, its food consisting mainly of bottom-living invertebrates, chiefly insect larvae, worms, and mollusks. Bream is an ideal monitoring species because their feeding behavior is characterized by direct contact with the sediment and their small migration radius (Marth *et al.* 1997). Asp, in contrast, is a predatory fish. Its long life-span, late sexual maturation, predatory behavior, high muscle lipid content, and apparent success in avoiding parasitic infections (Koponen *et al.* 2001) give it a high value as a potential new species for environmental biomonitoring studies. The young asp eat invertebrates, but at a comparatively early age they begin to eat fish. For that reason, asp,

being one of the top predator species in aquatic food web on that lake, most probably bioconcentrates organochlorine compounds already at an early age. These differences between the two species could explain the higher muscle PCB contents of asp than of bream in our study.

During the period of chemical discharge, the theoretical daily volume of PCBs released into lake Kernaala was less than 0.2 litres per day (O. Piiroinen pers. comm.). Regardless of seemingly low discharge rate over the time, PCBs are very much present in the sediment of the lake. In the study of Huuskonen *et al.* (2000), total PCB contents in lake Kernaala sediment were 798 ng g^{-1} dry weight (dw) at the southern sampling point and 521 ng g^{-1} dw at the northern end of the lake. In the same study the average amount of total polycyclic aromatic hydrocarbons (PAHs) in the sediment of the lake (510 μg kg^{-1} dw) was measured to be about five times higher than in Alasjärvi sediment. The authors also analyzed total PCBs in Alasjärvi sediment to be less than 20 ng g^{-1} dw. In comparison, the sediment PCB concentrations in two subarctic lakes in northern Finland were less than 4 ng g^{-1} dw (Vartiainen *et al.* 1997). In Germany, the mean levels of sediment PCBs at sites rated as highly polluted were over 200 ng g^{-1} dw and less than 50 ng g^{-1} dw at sites rated as slightly polluted (Fromme *et al.* 1999). We were unable to get any chemical data on Kokemäenjoki sediment. However, the muscle PCB content in reference asp caught from that location were at the background exposure level.

In addition, there were no historical data of the bream or asp muscle chemistry from the reference locations, Alasjärvi and Kokemäenjoki. However, the average muscle PCB content in Alasjärvi pike (*Exos lucius*) in 1996 was 9.7 μg g^{-1} lw (Piiroinen 1997). The average PCB concentrations (lw) in the Simojoki salmon (4.97 μg g^{-1}) during 1988–1992 (Vuorinen *et al.* 1997) as well as those in char (0.1 and 0.5 μg g^{-1}) caught from two subalpine lakes in Finnish Lapland (Korhonen *et al.* 1997) are at the same level as the PCB muscle concentrations in Alasjärvi bream (3.0 μg g^{-1} lw) and in Kokemäenjoki asp (3.1 μg g^{-1}).

The amount of accumulated PCBs in bream muscle in the present study (59–159 ng g^{-1} ww)

was surprisingly low, when compared to muscle PCB contents of bream measured in southern Kernaala in 1992/1995 (800/2100 ng g^{-1} ww, with corresponding lipid normalized amounts of about 100/285 μg g^{-1} lw) (Piiroinen 1993, 1996). The average weight of the bream caught in 1992/1995 (979/960 g) reveals them to be significantly older and bigger than bream sampled in the present field study (just over 300 g). In addition, the muscle lipid content of the earlier sampled bream was clearly higher (0.8%/0.67%). This may be partly linked to seasonal differences, since those samplings were executed 2–4 months later than the current study (the bream caught later in the year had already picked up extra lipid stores for the winter). Significant individual variation in hepatic PCB levels and lipid content inside the same fish species have been reported earlier (Westernhagen *et al.* 1989, Marthinsen *et al.* 1991).

In lake Kernaala, where the muscle chemistry of pike has been monitored yearly since 1986, a decreasing trend in its muscle PCB concentrations was evident from 1986 to the early 1990s (Piiroinen 1995). Recently that decline has leveled off to a somewhat steady state between 500 and 1500 ng g^{-1} ww (Lintinen 1998). In the same way, the concentrations of organochlorines in fish from the Great Lakes in USA have initially decreased, but have recently begun to show signs of settling down to a steady level (Miller 1993). In 1982 the total PCBs in muscle tissue of Lake Michigan chinook salmon and lake trout were as high as 4300 ng g^{-1} and 13 000 ng g^{-1} ww. In the same year, however, the corresponding value in Lake Superior lake trout was considerably less, 800 ng g^{-1} ww (Miller 1993). When Lake Superior lake trout was investigated again in 1994, the total PCBs in muscle tissue had gone down significantly, close to 100 ng g^{-1} ww, and in the non-salmonid fish the average muscle PCBs ranged from 20 to 200 ng g^{-1} ww (Kucklick and Baker 1998). Those concentrations are well in accordance with the PCB load of feral fish recently collected from lake Kernaala and the reference locations.

Lake Kernaala asp, regardless of their younger age, appeared to be more potent accumulators of PCBs than were adult bream. Total PCBs in asp muscle (2700 ng g^{-1} ww) were at the

level where the use of fish for human consumption is not recommended (by the Finnish National Board of Health). Age/size, lipid content, and trophic position of an organism (Kucklick and Baker 1998), as well as the season (Marthinsen *et al.* 1991) may all influence the organochlorine burden of fish. In this study, the muscle lipid content in asp was nine times greater than in bream. Therefore, the finding that immature asp had taken up more lipophilic PCBs than adult bream could be dependent on the muscle lipid content rather than on the age and size of the fish. The lower PCB content in adult bream could also be caused by maternal transfer of the chemical into their eggs: earlier studies (Westin *et al.* 1983, Black *et al.* 1988) have shown the mobilization and loss of organochlorines from the body tissues of fish during the gamete production. Interestingly, the muscle lipid content of asp caught from reference location was significantly lower than in lake Kernaala asp. The reason for such a high variation is not known, but can be assumed to arise from nutritional and/or seasonal factors.

Biotransformation

The results of our study showed significantly higher monooxygenase enzyme activities in lake Kernaala bream in comparison to those from the reference lake, Alasjärvi. Low EROD activities in Alasjärvi fish were characteristic for unstressed fish living in unpolluted environment. Similar EROD enzyme activities for bream caught from reference areas of lake Saimaa, Finland, have been reported (Lindström-Seppä and Oikari 1990, Kantoniemi *et al.* 1996). Both the gender and reproductive stage of adult bream also had an effect on EROD activities measured in lake Kernaala: enzyme activities were more pronounced in spring (prespawning) than in late summer (postspawning) sampling, with greater induction in male than in female bream in both events. These results are in agreement with the study of Hansson *et al.* (1980), in which the male rainbow trout expressed greater monooxygenase activities than females due to hormonal factors. Also, in several fish species the monooxygenase activities have been shown to decrease just before or during the spawning season (Koivusaari *et al.*

1984, Lindström-Seppä and Stegeman 1995).

Lake Kernaala asp showed also significantly higher EROD activities than those caught from the Kokemäenjoki. The extremely high muscle lipid content of lake Kernaala asp when compared to the reference fish must be a major factor on the high PCB accumulation into the muscle, and therefore also on the greatly induced hepatic EROD activities. The level of EROD induction in asp in Kernaala spring sampling was multifold to that of bream at the same time. Evidently, the interspecies variation observed here could have been partly due to a currently passed spawning season of bream. In contrast to adult bream, the immature female asp showed significantly higher EROD activities than male. When Koponen *et al.* (1997) studied the intra- and interstrain variability in biotransformation enzyme activities of rainbow trout, juvenile all-female populations were shown to express the greatest EROD activities when exposed to a model xenobiotic compound. In the present study the reference asp were older, they were acquired at a different time of the year and from a different geographical location. Therefore, a full collation of the data from reference fish with the data obtained from lake Kernaala might not be relevant. However, according to Collier *et al.* (1995), juvenile fish are supposedly free from sexual and seasonal variations of monooxygenase activities.

In field studies, where PAHs, PCBs and heavy metals often coexist (Schrank *et al.* 1997), it is very difficult to track down the particular chemical or chemical group causing the biotransformation enzyme induction in exposed fish. The impacts of PCBs on the environment are due to the individual components of these mixtures, their additive and/or nonadditive interactions with themselves and other chemical classes of pollutants (Safe 1994). Thus, it is noteworthy to mention that the presence of polycyclic aromatic compounds in lake Kernaala might have had some effect on biotransformation enzyme levels in studied fish. However, a total PAH load of lake Kernaala sediment can be considered to be low: in highly polluted Duwamish Waterway, WA, for instance, the total PAH content for corresponding aromatic hydrocarbons has been measured to be 28 mg kg^{-1} (Varanasi *et al.* 1992). That is about 55 times more than in lake

Kernaala sediment and over 260 times more than in Alasjärvi. Thus, significantly higher EROD activities in lake Kernaala fish in comparison to reference values were most probably due to exposure to PCBs.

Today, the history of PCB pollution of lake Kernaala can still be seen in chemical and biochemical analysis of affected fish population. PCBs have been shown to be able to mimic endogenous hormones, and it has been hypothesized that they could have a disrupting effect on the sex determination, sexual differentiation, and sexual development of fish (Tyler *et al.* 1998). However, the histopathological study of lake Kernaala bream and asp revealed their gonads to be structurally intact (Koponen *et al.* 2001). Because PCBs are apparently affecting the biotransformation enzyme system of fish, their potential to cause harmful effect on the overall fitness, fecundity or reproduction success of fish in the lake should be thoroughly studied. Meanwhile, solely the common knowledge of the bioexistence of the notorious chemical has effectively chased the local game fishermen away from the lake (O. Keijälä pers. comm.). This is quite unfortunate, since the most cost-effective way to lessen the PCB load of the lake would probably be the efficient fishing (but not for human consumption) of the potent PCB accumulating fish species out of the lake's ecosystem.

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