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Dredging Associated Effects: Maternally Transferred Pollutants and DNA Adducts in Feral Fish

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This study reports on the bioavailability, maternal transfer, and genotoxicity in feral fish of organic sediment pollutants. Northern pike (*Esox lucius*) and perch (*Perca fluviatilis*) were caught in a polluted bay before and during dredging activities and from reference areas. Polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) were analyzed in ovulating eggs to investigate if the bay sediment posed a threat to early life-stages of fish. On the basis of previous investigations in this area, the level of exposure via maternal transfer and diffusive uptake of water-borne pollutants after hatch is likely sufficient to cause abnormalities in early life-stages of fish. During dredging, hepatic DNA adducts were elevated in adult fish, demonstrating an increased release of genotoxic compounds, which may contribute to adverse effects in aquatic organisms for several years. Although no substantial increase of maternally transferred pollutants were observed during dredging, this is the first time a correlation between hepatic DNA adducts in fish and pollutant burden in their eggs is demonstrated. Our findings underline the importance of combining chemical and toxicological methods as well as a need for greater emphasis on other polycyclic aromatic compounds in environmental risk evaluations.

Introduction

Some of the ecological effects of greatest concern for organic sediment pollutants are chronic effects on adult fish (reduced reproductive output) and acute (abnormalities and survival) effects on early life-stages. Current risk predictions of sediment pollutants could be improved by identifying those biomarkers of exposure most closely associated with these adverse effects. While feral fish caught in areas polluted with polycyclic aromatic compounds (PACs) suffer from hepatic neoplasms (*1*) and abnormalities during early life-stages (*2*), their exposure history is difficult to estimate. PACs are generally metabolized rapidly and are thus below the limit of detection using chemical analysis of muscle (*3*). Although Carls et al. (*4*) concluded from a laboratory experiment that maternal PAC exposure is less important than direct exposure in causing detrimental effects in larvae of pacific herring (*Clupea pallasi*), maternal exposure may still pose a threat to early life-stages of feral fish. The polycyclic aromatic hydrocarbon (PAH) benzo[*a*]pyrene is readily transferred into the gonads of fish (*5*), and feral fish are demonstrated to have higher PAC concentrations in gonads than in muscle (*6*, *7*). PAC exposure is shown to be closely associated with reduced gonadal development (*8*).

Hepatic DNA adducts are another indicator of PAC exposure in feral fish. Reactive intermediates formed during PAC metabolism can covalently bind to subcellular structures, including nucleic acids, thus inducing genotoxicity (*3*). These DNA adducts are found in feral fish from PAC polluted areas (*9*) and are associated with degenerative/necrotic and preneoplastic hepatic lesions (*10*). Since hepatic DNA adducts are relatively persistent, enabling an integrated measurement over time of exposure, metabolism, and DNA repair, they are considered a good biomarker for genotoxic compounds (*9*).

By isolating different compound classes from extracts of environmental samples prior to toxicity evaluations, a number of studies has been able to demonstrate that PACs are potentially more toxic in aquatic environments than more stable compounds such as polychlorinated biphenyls (PCBs) reviewed in ref *11*. The bay Örserumsviken on Sweden's Baltic coast was recently remediated due to considerable PCB pollution (*12*), and the dredged bottom sediment was placed in a covered landfill. Despite high concentrations of PCBs, we demonstrated that a fraction from the bottom sediment extract containing PACs caused more abnormalities to early life-stages of rainbow trout (*Oncorhynchus mykiss*) than the fraction containing dicyclic aromatic compounds (DACs), including PCBs (*13*). The DAC fraction was a potent cytochrome P4501A (CYP1A) inducer and contributed to more than half of the total ethoxyresorufin *O*-deethylase (EROD) induction potential (*13*, *14*). Against this background, the polluted bay serves as an interesting area for investigating pollutant responses in feral fish.

The current study was designed to investigate if sediment pollutants posed a threat to feral fish inhabiting the polluted bay by studying (i) the maternal transfer of pollutants into fish eggs, (ii) the effects of dredging activities on the pollutant burden in eggs and DNA adducts in adult fish, and (iii) the relationships between DNA adducts in female fish and pollutant burden in their eggs. For these purposes, we caught northern pike (*Esox lucius*) and perch (*Perca fluviatilis*) both before and during dredging activities in the polluted bay and from reference areas. We analyzed PCBs and PAHs in fish eggs and hepatic DNA adducts in adult fish.

Materials and Methods

Chemicals. All solvents and chemicals for DNA purification and adduct analysis as well as PCB and PAH analyses were purchased from common commercial sources and were of analytical grade or higher.

Study Areas. The bottom sediment in the polluted bay Örserumsviken (57°43.60'N, 16°40.00'E; WGS-84) contains up to 21 mg of ΣPCB/kg of dry sediment (*14*) and up to 11 mg of ΣPAH/kg of dry sediment (*13*). From October 2001 to September 2003, the bottom sediment (0−1 m) of the inner bay was dredged. Reference material was collected from two areas, both located in the Baltic Sea: (i) pike from the Trosa archipelago (58°45.25'N, 17°30.00'E; WGS-84) and (ii) perch from the middle part of the Stockholm archipelago (59°23.75'N, 18°38.78'E; WGS-84).

Biological Material and Tissue Sampling. Three sampling campaigns were conducted in the polluted bay: (i) before dredging and during spawning (April 2001), (ii) during dredging and after spawning (October 2002), and (iii) during dredging and during spawning (April 2003). Feral fish were caught from the pike reference area during spawning (May 2001), and female perch were caught from the perch reference area after spawning (October 2000). Pike and perch were

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caught with gill nets and were caged until tissue sampling, which was performed within 24 h. Although their exposure history is unknown, both species can be considered rather territorial (*15*, *16*) and thus exposed to xenobiotics in the immediate area of their capture.

Fish were killed with a blow to the head, and approximately 1 g of the central part of the liver was immediately excised and plunged into liquid nitrogen for DNA adduct analysis. Eggs for PCB and PAH analysis were collected by dissection (perch) or, from ovulating female pike, by stripping. Since pike eggs can be collected without sacrificing the fish it is a more convenient biological matrix than muscle for analyzing pollutants. Gonads of sexually immature females were excluded from this investigation. The total number of egg samples was analyzed: four pike samples from the pike reference area, four pike samples before dredging, three pike samples during dredging, three perch samples before dredging, and 10 perch samples during dredging (Supporting Information Table S1). Age was determined in pike from the cleithrum and pterygoid bones (*17*); in perch, the operculum was used (*18*). Weight and length were measured on all fish; on pike, skin tumors (sarcomas) (*19*) were recorded.

PCB and PAH Analysis in Fish Eggs. Extractions were performed according to a method modified from Koslowski et al. (*20*). In short, 3 g of eggs was ground with anhydrous Na_2SO_4 at a ratio of 1:4. An internal standard solution of ^{13}C-labeled PCBs (IUPAC numbers 28, 52, 101, 105, 118, 138, and 180), d_{10}-pyrene, and d_{12}-perylene was added before extracting the sample 3 times in dichlormethane/*n*-hexane (1:4 v/v). Aliquots were taken out for determination of extractable lipids (10%) and for PCB analysis (1%). The aliquot for PCB analysis was cleaned up on an open SiO_2 (10% water w/w) column, followed by a triple layer column, containing from the top: 1 cm SiO_2/H_2SO_4, 1 cm SiO_2/KOH, and 1 cm SiO_2 (*12*). The remaining extract, used for PAH analysis, was cleaned up on an open SiO_2 column followed by liquid−liquid partitioning with cyclohexane/dimethylformamide/water (*21*). Blanks (without eggs) and external standards were extracted and treated identically as the samples. Extractable lipids were determined gravimetrically by evaporating the aliquot to dryness in a preweighed vial.

PAH and PCB analyses were performed on a HP6890 high-resolution gas chromatograph (Hewlett-Packard, Avondale, PA) with a PTE-5 capillary column (30 m × 0.25 mm, 0.25 μm film thickness, Supelco, Bellefonte, PA) coupled to an Autospec Ultima high-resolution mass spectrometer (Micromass, Ultricham, UK) operating in selected ion mode. For calculation of internal standard recovery, ^{13}C-labeled PCB 153 or d_{12}-chrysene were added before PCB or PAH analysis, respectively. Concentrations were calculated using peak areas for the labeled internal standards and the response factors obtained from external standard solutions. Two alkylation degrees (C_1 to C_2) of phenanthrene/anthracene were qualitatively analyzed by the molecular ion and retention index (*22*). Peak detection was set at 3 times noise. Detection limits were set at 4 times blanks since the lowest recovery for PAH analyses in blanks was 24% and the highest in samples was 78%. PAH recoveries (mean% ± 95% confidence limit) were 31 ± 3.8% in blanks and 43 ± 3.7% in egg samples, and PCB recoveries were 92 ± 5.5% in blanks and 105 ± 3.7% in egg samples. Concentrations were compared on an extractable lipid basis since the lipoprotein vitellogenin, a significant circulating lipid pool in fish, is an important vector for the maternal transfer of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and benzo[*a*]pyrene (*23*), and it is reasonable to assume that other PAHs and PCBs would be transferred similarly.

DNA Extraction and ^{32}P-Postlabeling Analysis of Adducts. The entire procedure has recently been summarized (*24*). In short, liver tissue samples were semi-thawed, and the DNA was extracted, purified, quantified, and then radiolabeled. DNA adducts were then analyzed on thin layer chromatography (TLC) sheets. In parallel to the analysis of the perch and pike liver samples, quality assurance controls were run to facilitate correct measurements. As positive controls, a DNA adducted liver sample from perch (*24*) and the chromatography adduct standard 7*R*,8*S*,9*S*-trihydroxy-10*R*-(N^2-deoxyguanosyl-3′-phosphate)-7,8,9,10-tetrahydrobenzo[*a*]pyrene were used. Salmon sperm DNA was used as a negative control. Artificial adducts, which are related to enzyme batch (*25*), were excluded from analyses. The hepatic DNA adduct pattern displayed on the autoradiograms and the levels of DNA adducts (nmol of adducts/mol of normal nucleotides) did not differ between sexes of the same species from the same sampling campaign (not shown), and they were therefore pooled for statistical comparisons.

Statistics. Parametric tests (ANOVA, Tukey HSD) were used to investigate statistical differences in hepatic DNA adduct levels in pike and perch and in PCB and PAH concentrations in eggs from pike. Homogeneous variance was obtained by logarithmic transformations. To compare differences in concentrations of PCBs and PAHs in perch eggs collected before and during dredging, and to investigate differences in the relative contributions of PCB congeners between pike and perch eggs, the *t*-test was employed. To investigate statistical correlations between hepatic DNA adducts and ΣPCB or ΣPAH concentrations, and between morphometric variables, we used Spearman rank correlation test. As an α-level for statistically significant differences between groups, a *p*-value of less than 0.05 was used.

Results

Body Parameters. Age was positively correlated to length and weight in both species (Supporting Information Figures S1 and S2). We did not find a higher prevalence of pike suffering from skin tumors in the polluted bay as compared with the reference pike (Supporting Information Table S1). During dredging, 20% of pike and 23% of perch were in a non-reproducing state (few visible oocytes). These frequencies are lower than in Lake Molnbyggen, Sweden, where 75% of the female perch were immature (*26*), but still well above the 0−3% found in minimally polluted waters (*27*). Although reduced gonadal development in feral fish is demonstrated to correlate with PAC exposure (*8*), further studies with larger sample sizes are needed to investigate whether feral fish in the polluted bay have underdeveloped gonads.

PCB and PAH Concentrations in Feral Fish Eggs. Concentrations of individual PCB congeners in fish eggs are given in Supporting Information Table S2. Pike and perch eggs from the polluted bay contained on average 124 000 and 20 000 μg of ΣPCB/kg of extractable lipid, respectively. Pike eggs from the polluted bay had 24 times higher concentrations than reference pike eggs. No significant differences in ΣPCB concentration were observed in pike eggs ($p = 0.06$) or in perch eggs ($p = 0.37$) before and during dredging. While no significant differences in congener concentrations were found in perch eggs before and during dredging, pike eggs had significantly higher concentrations during dredging of the following congeners: IUPAC numbers 90 + 101, 110, 105, 149, 132 + 153, and 138. The lack of correlation between fish age and ΣPCB burden in their eggs (Supporting Information Figure S3) is most likely due to the annual release of all eggs during spawning.

Concentrations of individual PAHs in fish eggs are given in Supporting Information Table S3. The average ΣPAH burden in pike and perch eggs from the polluted bay were 130 and 23 μg of ΣPAH/kg of extractable lipid, respectively. Pike eggs from the polluted bay contained 4.2 times higher ΣPAH concentrations than reference pike eggs. No significant differences in the ΣPAH concentration were observed in pike

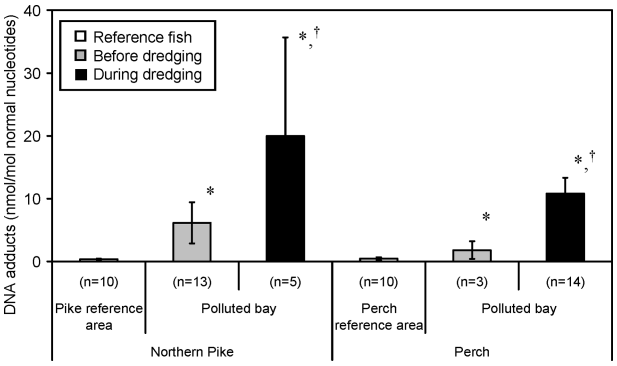

FIGURE 1. Hepatic DNA adducts in pike and perch (males and females pooled since no statistical difference was observed between sexes) collected in the polluted bay before and during dredging. Values are presented as mean ± 95% confidence limit for the values derived from *n* fish. Fish were also collected from the pike reference area and from the perch reference area. *Significantly ($p < 0.05$, Tukey HSD) different as compared with their reference species. †Significantly ($p < 0.05$, Tukey HSD) higher than before dredging.

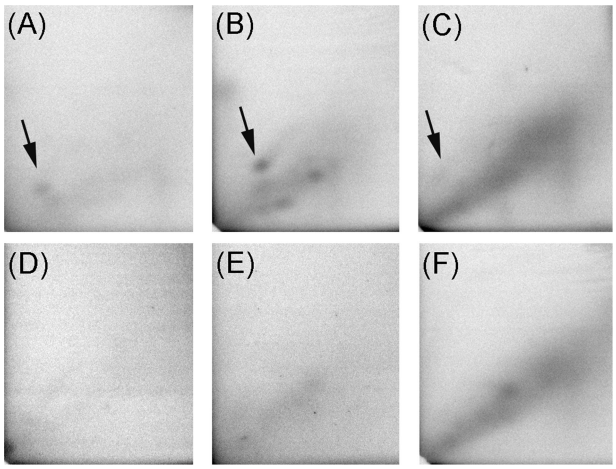

FIGURE 2. Representative autoradiograms of ^{32}P-postlabeled DNA adducts from livers of (A) pike from the pike reference area, (B) pike collected before dredging from the polluted bay, (C) pike collected during dredging from the polluted bay, (D) perch from perch reference area, (E) perch collected before dredging from the polluted bay, and (F) perch collected during dredging from the polluted bay. Arrows indicate artificial adducts, which were excluded from analyses.

eggs ($p = 0.96$) or in perch eggs ($p = 0.73$) before and during dredging. A significant positive correlation was observed between age and ΣPAH burden in pike eggs during dredging (Supporting Information Figure S4). The increase of ΣPAH in eggs as compared with age was, however, not substantial, and in all other fish, this correlation was not observed. Note that the differences in the amounts of extractable lipids between PCB and PAH analyses arise from the re-extraction of some samples for PAH analyses. Detectable concentrations of 1-methylphenanthrene, the qualitatively analyzed C_1- and C_2-phenanthrene/anthracene (43 and 25 μg/kg of extractable lipid, respectively), pyrene, 1-methylpyrene, and 2-methylpyrene, totaling 300 μg of ΣPAH/kg of extractable lipid (not shown) were only found in eggs from the female pike with skin tumors collected from the polluted bay before dredging.

Hepatic DNA Adducts in Adult Fish. Adduct levels in pike from the polluted bay were 17 times higher before dredging and 55 times higher during dredging as compared with the pike reference area (Figure 1). In perch, adduct levels were 4.1 times higher before dredging and 25 higher during dredging as compared with the perch reference area (Figure 1). Adduct spots were found in some reference fish (Figure 2A,D), although mainly below the limit of detection (<1.5 times background). Before dredging, several adduct spots were detected in pike (Figure 2B), while fewer spots were evident in perch (Figure 2E). During dredging, a composite of multiple overlapping DNA adduct spots (i.e., diagonal radioactive zone) were observed in pike (Figure 2C) and perch (Figure 2F), indicating exposure to complex mixtures of genotoxic compounds (*28*). In addition, an apparently oily film was observed during dredging, suggesting an increased release of organic compounds to the water column.

Discussion

Contribution of Maternally Transferred Pollutants to Toxicity. Maternally transferred pollutants in feral fish eggs likely contribute to adverse effects in developing embryo and larvae. Our previous studies (*13*, *14*) investigated the potential toxicity of different compound classes from the bay sediment by injecting fractions separated by degrees of aromaticity from the sediment extract into newly fertilized rainbow trout eggs. The DAC fraction, where PCBs were isolated, caused fewer abnormalities in rainbow trout larvae than the PAC fraction but was a more potent EROD inducer. Rainbow trout eggs injected with the highest dose of the DAC fraction contained equal ΣPCB concentrations (*14*) to the pike eggs from the polluted bay. Although the PCBs' contribution to the EROD induction potential was deemed minor, feral fish may experience co-exposure of PCBs with other currently unidentified potent CYP1A inducing DACs.

Although rainbow trout eggs injected with the highest dose of the PAC fraction (*13*) contained 530 times higher ΣPAH concentrations than pike eggs, developing feral fish larvae may be exposed to equal or even higher concentrations of PACs after hatch. Uptake rates of water-borne PAHs occur at a faster rate in fish larvae than in juveniles and adults due to the relatively larger surface of membranes (*29*). When using the analyzed concentrations in the polluted sediment before dredging and assuming that physicochemical characteristics of benzo[*a*]pyrene could be extrapolated to environmental conditions, the estimated dose of benzo[*a*]pyrene received by feral fish larvae in the bay is 32 μg/kg of wet weight (Supporting Information Figure S5). The highest doses we injected into rainbow trout eggs contained 40 μg of benzo[*a*]pyrene/kg of wet egg (*13*). There is, however, a growing body of studies demonstrating the minor contribution of commonly analyzed PAHs to potential toxicity. Alkylated PAHs, for instance, are found in higher concentrations in polluted bottom sediment than their commonly analyzed non-alkylated homologues (*30*), and they are shown to be more toxic to early life-stages of aquatic animals (*31*, *32*). In the polluted bay sediment, 17 of the most commonly analyzed PAHs were unable to account for the abnormalities in rainbow trout larvae (*33*). A substantial part of the toxic potential was isolated in a subfraction containing three- and four-ring compounds, including alkylated and sulfur-heterocyclic compounds (*13*). The increasing alkylation degree of naphtalene and phenanthrene is demonstrated to increase uptake rates and decrease elimination rates in sheepshead minnows (*Cyprinodon variegatus*) (*34*). The toxicity of benzo[*a*]pyrene in channel catfish (*Ictalurus punctatus*) is enhanced by co-exposure to PCB metabolites (*35*). Taken together, these lines of reasoning provide a basis for increased concern for the combined effect of complex mixtures of pollutants that will certainly adversely affect feral fish. Investigating artificially fertilized feral fish eggs may provide further knowledge of the effects on fish early life-stages by maternally transferred pollutants. The development of analytical methods and toxicological evaluations for a larger number of PACs is necessary for more reliable environmental risk evaluations.

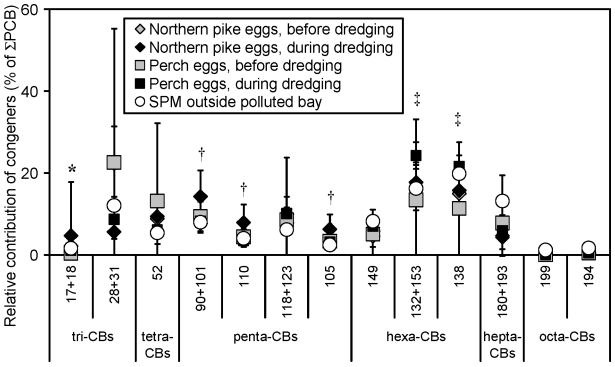

FIGURE 3. Relative contribution of individual PCB congeners (% of ΣPCB, w/w) in feral fish eggs and settling particulate matter (SPM). Eggs were collected from mature female pike and perch caught using a gillnet during their spawning period from the polluted bay. SPM was collected outside the polluted bay before dredging, data obtained from ref *14*. Values are presented as mean; error bars depict ± 95% confidence limit. Total number of egg samples analyzed: pike eggs before dredging $n = 4$; pike eggs during dredging $n = 3$; perch eggs before dredging $n = 3$; and perch eggs during dredging $n = 10$. *Significantly ($p < 0.05$, *t*-test) higher contribution in pike eggs than in perch eggs before dredging. †Significantly ($p < 0.05$, *t*-test) higher contribution in pike eggs than in perch eggs during dredging. ‡Significantly ($p < 0.05$, *t*-test) higher contribution in perch eggs than in pike eggs during dredging. The SPM data presented in this figure are reprinted from ref *14* with permission from Elsevier.

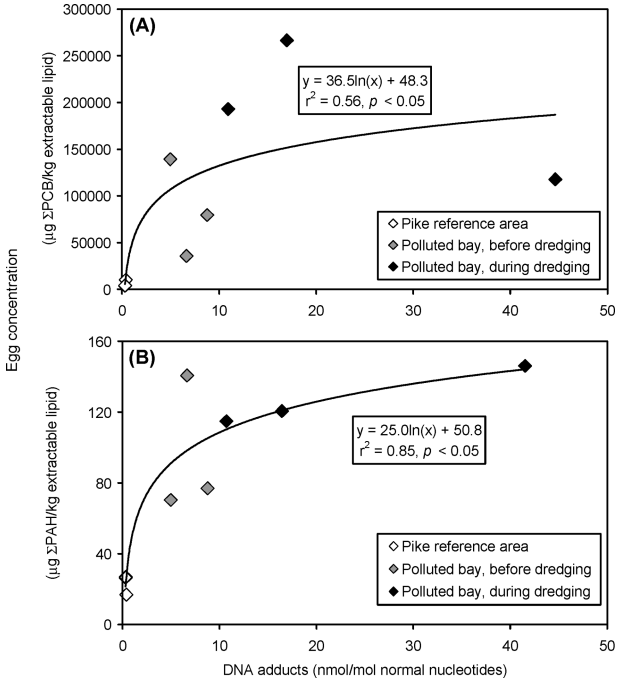

FIGURE 4. Relationships between levels of hepatic DNA adducts in female pike and their egg concentrations (µg/kg of extractable lipid) of ΣPCB (A) and of ΣPAH (B). The correlations were statistically significant between hepatic DNA adducts and their egg concentration of ΣPCB ($p < 0.05$, Spearman's $\rho = 0.81$) and of ΣPAH ($p < 0.05$, Spearman's $\rho = 0.85$). The best curve-fit was obtained by the logarithmic equation.

Bioavailability of Sediment Compounds. Increased release of genotoxic compounds during dredging was demonstrated by elevated DNA adducts in both fish species. Egg analyses, however, gave no substantial evidence of increased release of sediment pollutants during dredging. This indicates that analyses of other sediment compounds may better reflect the increased release of genotoxic compounds and/or that the maternal transfer of PCBs and PAHs is influenced by several biological processes. Nevertheless, pike eggs from the polluted bay had higher concentrations of pollutants than reference pike eggs, demonstrating the maternal transfer of sediment pollutants into fish eggs. Interspecies differences in maternal transfer of sediment pollutants may be illustrated by comparing the relative contributions of congeners (% of ΣPCB) in eggs of perch and pike and in settling particulate matter (SPM) collected immediately outside the bay before dredging (SPM concentrations obtained from ref *14*). Although the number of chlorines in PCB congeners positively correlates with the log K_{ow} (*36*), and some congener contributions were significantly different between species, the PCB pattern in feral fish eggs of both species was similar to the pattern found in SPM (Figure 3), indicating that differences in lipophilic characteristics do not substantially alter the bioavailability and thus maternal transfer of sediment PCBs in the polluted bay.

Relationships of DNA Adducts to Pollutants in Eggs. Different metabolic persistence between PCBs and PAHs is probably the main reason that fish egg concentrations of ΣPAH were one-thousand of ΣPCB, while sediment concentrations of ΣPAH were half of ΣPCB (*13*, *14*). Higher levels of DNA adducts in fish from the polluted bay than in those from reference areas suggest increased metabolic activity since DNA adducts can provide an integrated measurement of exposure, relative activities of metabolic phase I enzymes, detoxification of environmental mutagens, and efficiency of DNA repair (*9*). The presence of potent CYP1A inducers in the polluted sediment (*13*, *14*) also suggest that metabolic phase I enzymes are induced in feral fish. Benzo[*a*]pyrene was more rapidly metabolized when channel catfish were pre-exposed to the CYP1A inducer β-naphthoflavone (*37*).

Significant positive correlations between hepatic DNA adducts in spawning pike and concentrations of ΣPCB (Figure 4A) and ΣPAH (Figure 4B) in their eggs may reflect the capacity of the fish to accommodate exposure and detoxify pollutants. Pollutant burden in eggs may constitute an integrated exposure over a comparatively long period of time since the previous spawning period ended about 10 months earlier. To the best of our knowledge, this is the first time a correlation between hepatic DNA adducts in feral fish and xenobiotics in their eggs is demonstrated. This correlation excludes pike with skin tumors, owing to the indications of decreased health status and altered metabolism. Detectable concentrations of alkylated PAHs were only found in eggs from a female pike with skin tumors. Flounder (*Platichthys flesus*) with liver tumors inhabiting the highly polluted Elbe estuary have decreased CYP1A activity (*38*). Although increased maternal transfer of PAHs might be due to a decreased metabolic activity in this specimen, further investigations are needed. The significant positive correlation between DNA adduct levels and ΣPCB (Figure 4A) is likely a result of co-exposure of fish to PCBs and PAHs since PCB is generally not considered to form bulky DNA adducts in vivo (*39*). Several studies have demonstrated the causal relationship between PAC exposure and hepatic DNA adducts (reviewed in ref *9*), and ^{32}P-postlabeling analysis is more suitable for large hydrophobic xenobiotics (e.g., PACs containing more than three rings (*40*)). The shape of Figure 4B suggests that increased metabolic phase I activity and thus elevated DNA damage in female fish, induced by xenobiotic exposure, reduces the maternal transfer of PAHs. How these genome damages affect pike and perch is unknown, but laboratory experiments with several fish species have demonstrated that DNA adductscorrelate with hepatic neoplasms (*10*, *41*, *42*). Although hepatic DNA adducts are generally recognized as potential initiators of carcinogenic processes, they may also lead to disruption of vital liver functions. Hepatic DNA adducts did

not correlate with age (Supporting Information Figure S6) but are demonstrated to be persistent in pike (*40*), suggesting that the elevated levels of hepatic DNA adducts decline slowly. While a significant part of the polluted bottom sediment is now placed in a dry deposit on land, dredging might have caused adverse effects in aquatic organisms for several years, indicating that more studies over a longer time scale are needed for investigating environmental improvement. The findings presented in this study underline the importance of combining chemical and toxicological methods for reliable environmental risk evaluations.

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Supporting Information Available

Three tables and six figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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