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Environmental Effects of Dredging Technical Notes

RELATIONSHIP BETWEEN PCB TISSUE RESIDUES AND REPRODUCTIVE SUCCESS OF FATHEAD MINNOWS

PURPOSE: This technical note provides initial guidance for interpreting the biological consequences of bioaccumulation in aquatic organisms. Specifically, the relationship between polychlorinated biphenyl (PCB) tissue residues and reproductive success in the fathead minnow, *Pimephales promelas*, is examined.

BACKGROUND: The US Army Corps of Engineers often conducts, or requires to be conducted, an assessment of potential bioaccumulation of environmental contaminants from sediments scheduled for dredging and open-water disposal. At present, however, there is no generally accepted guidance to assist in the interpretation of the biological consequences of specific levels of bioaccumulation. To provide an initial basis for such guidance, the Environmental Laboratory of the US Army Engineer Waterways Experiment Station is conducting both literature data base analyses and experimental laboratory studies as part of its Long-Term Effects of Dredging Operations (LEDO) Program. This technical note discusses a portion of the laboratory effort.

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Materials and Methods

Experimental design

Adult fathead minnows, *Pimephales promelas*, were obtained from Northeastern Biologists, Rhinebeck, N.Y. The response of *P. promelas* in a variety of toxicity tests has been shown to be representative of most freshwater fish (Suter et al. 1987). Following a 30-day acclimation period to all conditions except sediment, fish were placed in 40-ℓ glass aquaria containing a 2- to 4-cm layer of sediment. Sediments containing 0.82, 14.0, and 27.0 μg/g dry weight PCB, expressed as Aroclor 1254 equivalents, were identified as low, medium, and high treatments, respectively. Sediment was collected from

Sheboygan Harbor, Wis., an inland waterway known to contain substantial amounts of PCBs. Aquaria containing only clear water served as the control treatment. Moderate aeration and a continuous flow (80 mℓ/min) of aged, charcoal-filtered tap water were provided to all aquaria.

Fathead minnows were exposed for 16 weeks total to the four treatments. For the first 5 weeks fish were maintained at 20° C with a photoperiod of 12 hr light:12 hr dark. The water temperature was then increased 1 degree per day to 26° C and the photoperiod lengthened 1 hr every other day to 16 hr light:8 hr dark. Spawning substrates were also introduced into each aquarium. These steps were taken to induce gametogenesis, sexual dimorphism, and reproductive activities in fathead minnows (Denny 1987). Fecundity and frequency of egg production were monitored daily for the remainder of the 16-week exposure.

Chemical analysis

Fish were collected after 7 and 16 weeks of exposure, frozen, and saved for determinations of tissue PCB residues. All chemical analyses were conducted by the Tennessee Valley Authority, Chattanooga, Tenn., using a Hewlett-Packard gas chromatograph equipped with a 30-meter DB-5 fused capillary column and electron capture detector. The detection limit for Aroclor 1254 was 0.10 μg/g and for individual PCB congeners, 0.01 μg/g. Nomenclature for PCB congeners follows Ballschmiter and Zell (1980) as adopted by the International Union of Pure and Applied Chemists (IUPAC).

Statistical analysis

Treatment effects on all parameters were analyzed via one-way analysis of variance. Differences were considered statistically significant at $p < 0.05$. Square root or log 10 transformations were used if data sets were not homogeneous. Arc sine transformations were used for nonhomogeneous data expressed as percentages. Mean separation for homogeneous data was achieved via the Waller-Duncan k-ratio t test. When transformations were unsuccessful in achieving homogeneity a Proc Rank nonparametric procedure was used for mean separation (SAS Institute, Inc. 1985). PCB concentrations reported to be less than the detection limit were considered equal to the detection limit in all calculations. To facilitate residue-effects observations, the reproduction data were normalized to control values and expressed as a percentage.

Results and Discussion

A clear and inverse relationship existed between the reproductive success of sediment-exposed minnows and their internal PCB tissue concentrations (Figure 1). Reproduction was significantly impaired in fish exposed to the

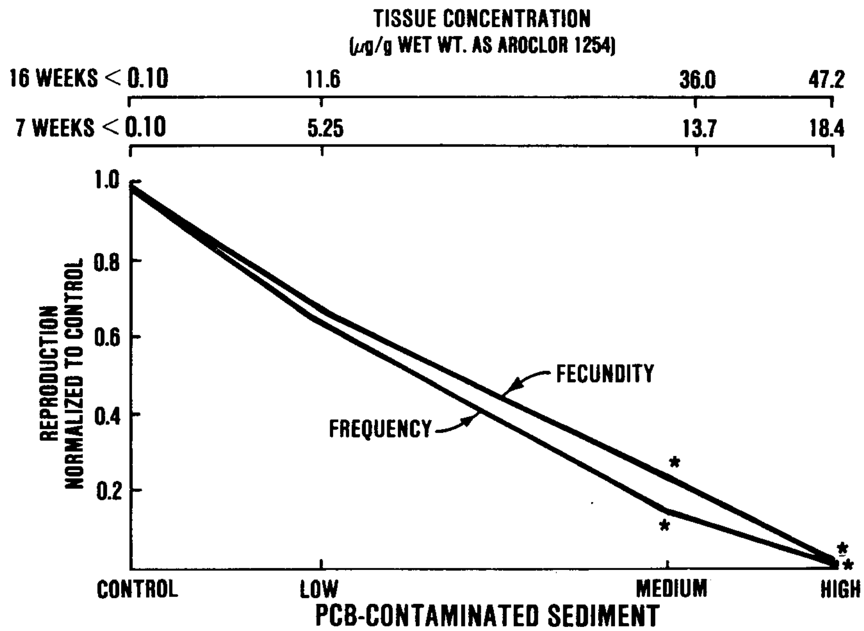

Figure 1. The relationship between reproduction and PCB tissue concentrations in fish exposed to PCB-contaminated sediment; asterisk indicates significantly different from controls

medium and high PCB-contaminated sediment treatments compared to fish in the controls and low PCB-contaminated sediment. The magnitude of this inhibition was large; approximately 80 to 100 percent of control values. Mean PCB concentrations in these affected fish ranged from 13.7 to 47.2 µg/g (ppm) wet weight, expressed as Aroclor 1254 equivalents. Bengtsson (1980) reported similar results for the saltwater minnow, *Phoxinus phoxinus*, chronically exposed to PCBs. In that study, mean tissue concentrations of 170 mg/kg (ppm) fresh weight were associated with a significant decrease in reproduction while fish with lower tissue concentrations, 0.2 to 15 mg/kg (ppm) fresh weight, were apparently unaffected.

Numerous reviews have demonstrated that background PCB residues in feral fish throughout the United States generally range from a few tenths of a ppm up to low single-digit ppm's (Peakall 1975, Butler and Schutzmann 1978, Wassermann et al. 1979, and Veith et al. 1981). Freshwater fish collected from some of the more highly industrialized waterways in Lake Michigan contained PCB concentrations (as Aroclor 1254) ranging from a few ppm wet weight to a high of 15 ppm (Veith 1975). Saltwater fish collected from one of the most highly PCB-contaminated estuaries in the United States, New Bedford Bay, Mass., generally had single-digit ppm concentrations of PCB with two fish species, cunner and American eel, being exceptionally high--30 to 130 ppm (Weaver 1984). In this experiment fathead minnows with significantly impaired reproduction had double-digit ppm PCB concentrations in their tissue. This level of PCB in the tissue is associated with fish inhabiting some of the more highly contaminated waterways in the United States.

Although the mechanism responsible for the observed decrease in fathead minnow reproduction is unknown, several possibilities, based on supporting data, can be considered. For example, one might suspect that the large decrease in reproduction in fish was due to high mortalities. However, this was not the case. At the end of the 16-week exposure, percent survival in all treatments was quite high (80 to 100 percent) and there was no significant effect among treatments for either male or female fish (Figure 2). Neither

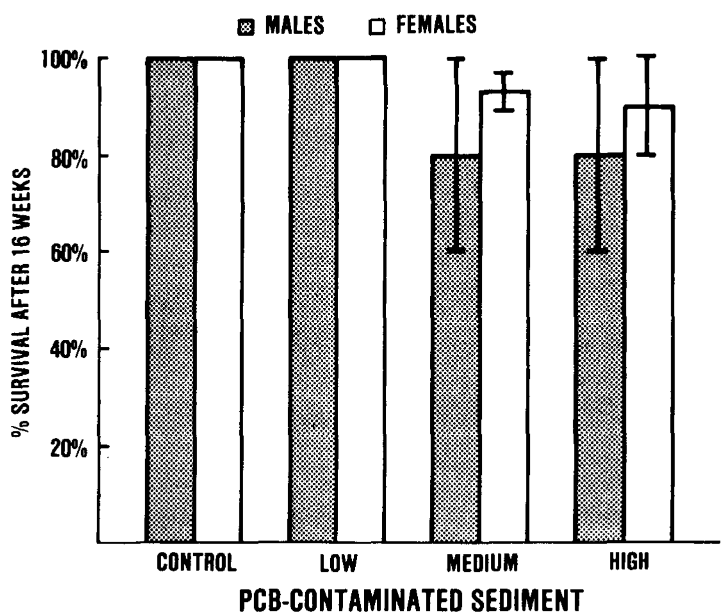

Figure 2. Survival of fish exposed to PCB-contaminated sediment for 16 weeks

does the impaired reproduction appear to be due to a lack of feeding or a general wasting of the exposed fish. There was no significant treatment effect on the mass of male or female fish at the end of the experiment (Figure 3).

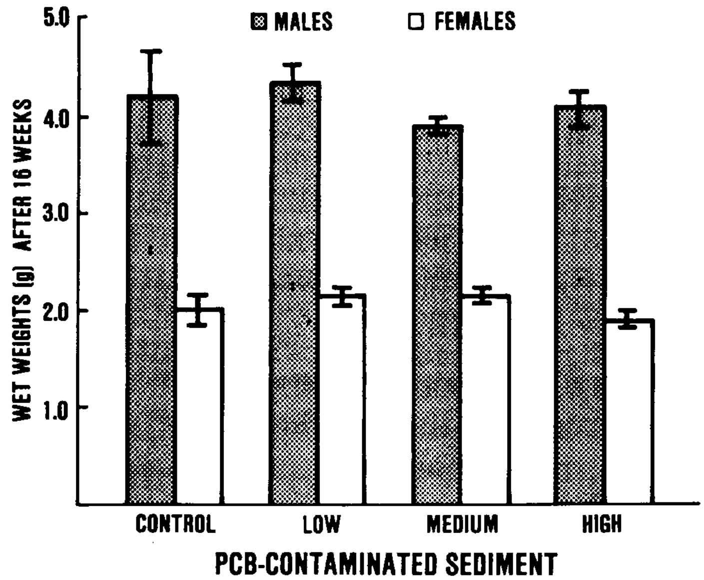

Figure 3. Wet weights of fish exposed to PCB-contaminated sediment for 16 weeks

There was, however, a significant increase in percent lipid at the end of the experiment in affected fish exposed to the medium and high sediment treatments (Figure 4). This indicates that lipid metabolism was somehow disrupted.

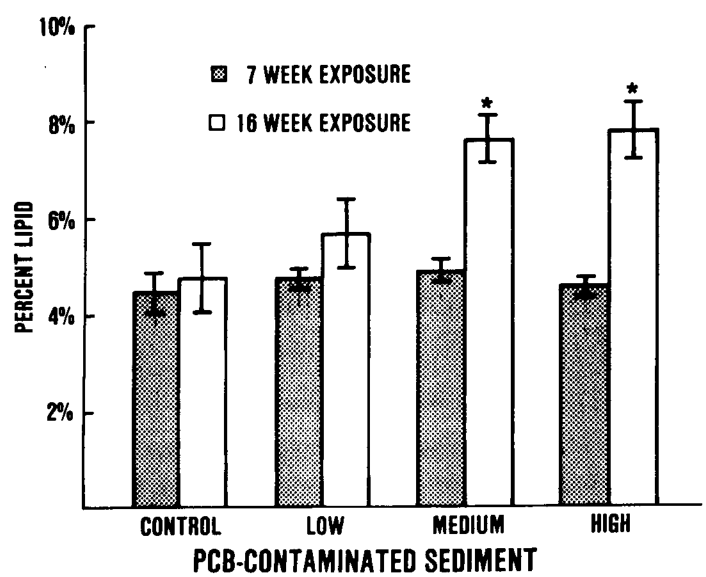

Figure 4. Percent lipid in fish exposed to PCB-contaminated sediment; asterisk indicates significantly different from controls

Elevated lipids, as well as changes in lipid-synthesizing enzymes, have been observed in PCB-exposed mammals (Hansell and Ecobichon 1974, Holub et al. 1975, and Ishidate et al. 1978). Some evidence suggests that these elevations are due to an inhibition of catabolism rather than an active synthesis of lipids (Ishidate and Nakazawa 1976). An increase in the proportion of saturated fatty acids has also been observed in saltwater fish exposed to PCBs (Caldwell et al. 1979). This response was similar to that observed in fish acclimating to elevated temperatures. Since the lipid data from this experiment is not qualitative, effects on specific lipid pools cannot be determined at this time.

The bioaccumulation of specific PCB congeners may have also adversely affected fish reproduction (Figure 5). Some of these congeners, especially IUPAC numbers 52, 128, 138, 153, and 180, are known or suspected inducers of the mixed-function oxidase (MFO) enzyme system in fish (Chambers and Yarbrough 1976, Lech et al. 1982, Clarke 1986, and Kleinow et al. 1987). As the name implies, the MFO system is responsible for the insertion of molecular oxygen into numerous organic substrates which are required for a variety of biochemical reactions. The MFO system is embedded in the lipid matrix of biological membranes and any disruption in that matrix will affect normal MFO activity (Stier 1976). Certain PCB congeners, because of their MFO-inducing properties, could be exerting a direct toxic effect by disrupting normal MFO activity. They may also be acting indirectly by generating excessive numbers of electrophilic metabolites. These highly reactive compounds, typified by the diol epoxide of benzo(a)pyrene, are known to interact detrimentally with biological membranes and genetic material (Ahokas 1979).

One of the major functions of the MFO system is the metabolism of lipids. Two specific types of lipid metabolism which are essential to fish reproduction are the synthesis of sex hormones (steroidogenesis) and the production of egg yolk. The latter process is dependent on the production of the phospholipoprotein, vitellogenin, in the liver. Both processes are initiated and controlled by the hypothalmic-pituitary-gonadal axis in fish (Peter and Crim 1979). Although many investigators have shown that exposure of fish to PCBs can have a significant effect on both steroidogenesis and vitellogenesis (Sivarajah et al. 1978, Hansson et al. 1980, Chen and Sonstegard 1984, Forlin et al. 1984, and Wester et al. 1985), no clear pattern of effect has emerged. This is probably due, at least in part, to the complexity of the

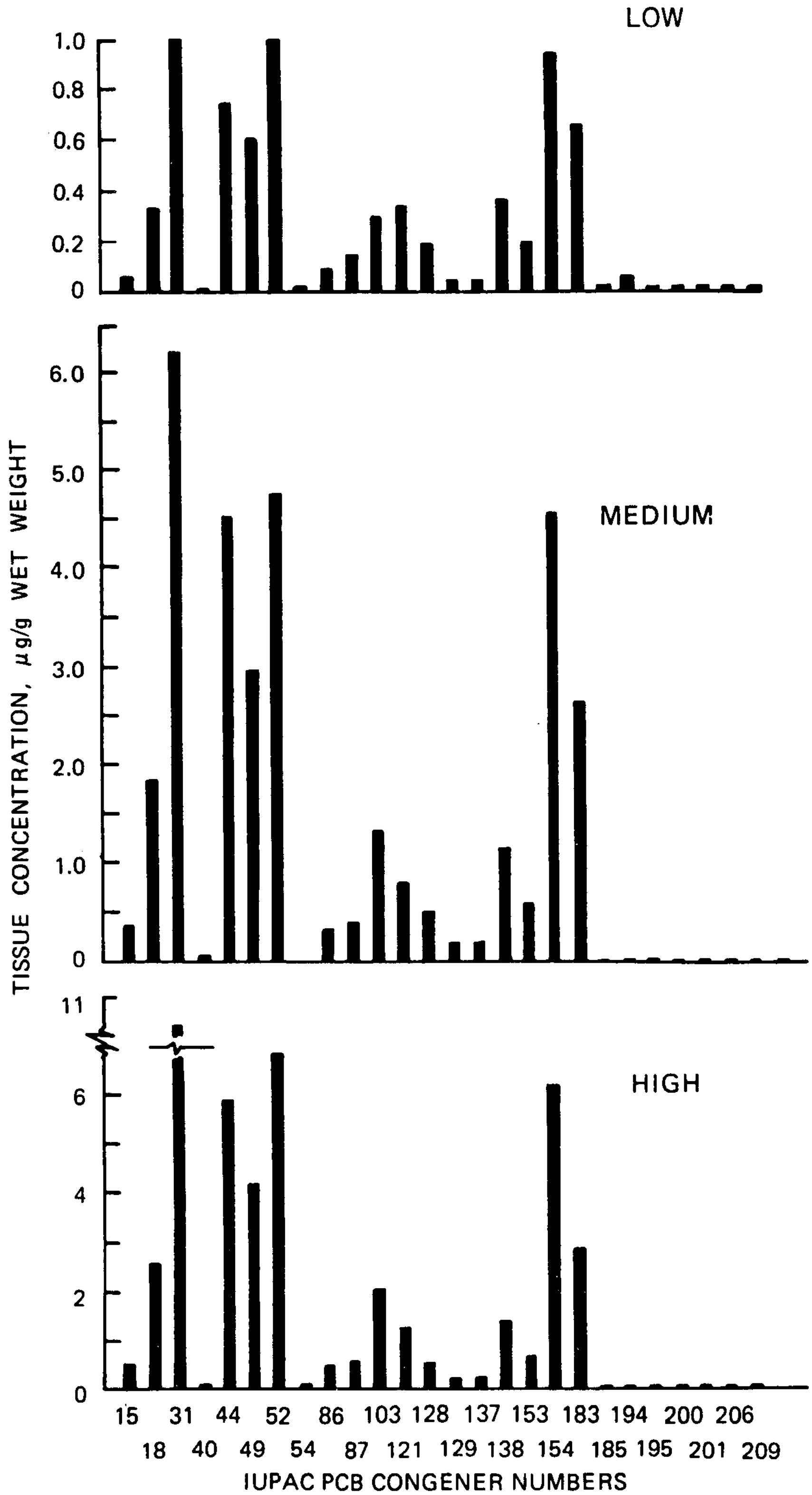

Figure 5. Concentrations of individual PCB congeners in fish exposed to PCB-contaminated sediment for 16 weeks

biochemistry of fish reproduction (Stegeman and Woodin 1984). Nevertheless, the accumulation of significant amounts of MFO-inducing PCB congeners, coupled with elevated lipid levels in the affected fish, strongly suggests that a disruption in lipid metabolism may be responsible for the impaired reproduction observed in fish exposed to PCBs and PCB-contaminated sediment.

Conclusions

Results from the testing of adult fathead minnows, *Pimephales promelas*, indicated that PCB-contaminated sediments had a significant deleterious effect of the species' fecundity and frequency of reproduction. Affected fish had double-digit ppm tissue concentrations of PCBs, expressed as Aroclor 1254. These tissue concentrations correspond to PCB residues of fish inhabiting highly contaminated waterways in the United States. The manner in which PCBs exert their detrimental effect on fish reproduction is unknown, but may involve some aspect of lipid metabolism. Pathways responsible for steroidogenesis and vitellogenesis may be especially vulnerable. Affected fish accumulated substantial amounts of specific PCB congeners which are known or suspected MFO inducers. Further investigation as to their potential biological effect will assist interpretation of the biological consequences of PCB bioaccumulation in aquatic animals.

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