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ESTROGENIC POTENTIAL OF ORGANIC CONTAMINANTS IN NEW YORK HARBOR

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

A laboratory and field study was conducted to assess the potential estrogenicity of contaminants in the Hudson-Raritan Estuary. The production of vitellogenin (VtG) in the livers of male and juvenile fish was used as a biomarker for estrogenicity in both parts of the study. VtG is a lipo-protein precursor to egg yolk synthesized by the liver under high levels of circulating 17β-estradiol (E2). Because VtG synthesis is induced by E2, and male and juvenile fish normally have very low levels of E2 and little to no VtG in their plasma, the production of VtG in males and juveniles is an indication of the presence of E2 or E2-acting compounds. VtG synthesis was measured by Western blot analysis. Injection with organic extracts of sediment from the Kill van Kull and the lower portion of the East River failed to induce VtG production in adult male *Fundulus heteroclitus*. In field studies of adult *F. heteroclitus* and juvenile striped bass, *Morone saxatilis* and bluefish, *Pomatomus saltatrix*, only gravid female *F. heteroclitus* had detectable levels of VtG. These results indicate that although areas of the Hudson-Raritan Estuary contain high levels of organic contaminants, concentrations of these compounds do not elicit an estrogenic response in fish.

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INTRODUCTION

The main objective of this research was to assess the estrogenic potential of organic contaminants in New York Harbor. Sediments of the Hudson-Raritan Estuary, like those of many other urban estuaries, contain high concentrations of hydrophobic organic contaminants (HOCs) introduced from multiple anthropogenic sources. These HOCs include a mixture of polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, and polychlorinated dibenzodioxins (dioxins) among other compounds. Due to their hydrophobic nature, a large fraction of HOCs adsorb onto particles and then settle to the sediment (Farrington 1989). In NOAA's National Status and Trends Program Report, five sites within the Hudson River Harbor Estuary were ranked among the most toxic in the nation (Long and Morgan 1990). Finding the sources and determining the toxicity of such contaminated sediments has been identified as a high priority research area by the U.S. EPA's New York/New Jersey Harbor Estuary's Proposed Comprehensive Conservation and Management Plan (EPA 1995). In addition to traditionally recognized organic contaminants, the alkylphenol polyethoxylates (APEs) and their more hydrophobic metabolites may also be of concern in urban estuaries due to their high release rates and potential to act as endocrine disruptors (Renner 1997).

Contaminants in Hudson-Raritan Estuary sediments are thought to have a high potential to cause toxicity in marine organisms (Squibb et al. 1991). In laboratory studies using environmentally realistic concentrations, many of these contaminants exert adverse biological effects. In addition, some of these compounds, such as PAHs, PCBs, pesticides, and dioxins, have the potential to be endocrine disruptors. APEs and their

metabolites, commonly found in sewage treatment plant effluent and sludge, have been recently recognized as weakly estrogenic (Jobling et al. 1996; White et al. 1994; reviewed by Nimrod and Benson 1996). Because they are not routinely measured, ambient levels of APEs have been poorly characterized and their potential effects on marine biota are yet to be resolved. Although mixtures of these lipophilic organic compounds are typical of contaminated urban harbors, very little work has addressed the endocrine disrupting potency of such mixtures.

Organic xenobiotics are known to have a battery of effects on reproduction. Some of these include decreased gonad size, decreased steroid hormone levels, delayed spawning, and developmental deformities (reviewed by Monosson 1997). The mechanisms underlying these actions cannot yet be explained. It has been proposed by many researchers that these effects and others could be the result of endocrine disruption. Xenoestrogens exert effects similar to those of the endogenous female hormone, 17β-estradiol (E2). In high concentrations (1000 x and higher than those of E2), metabolites of APEs compete with E2 for binding to estrogen receptors in fish (Jobling et al. 1996; White et al. 1994). Other compounds can have antiestrogenic activity as demonstrated for β-naphthoflavone (BNF) in rainbow trout (Anderson et al. 1996) and for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in mammals (Gierthy et al. 1987). Kelce et al. (1995) have shown that feminizing effects are not just a result of xenoestrogen exposure, but that antiandrogens can also cause feminization. Several studies have documented endocrine disruption in the field. Folmar et al. (1996) found estrogenic activity in 7 of 10 male carp caught near a U. S. sewage effluent discharge point. After a large spill of dichlorodiphenyltrichloroethane (DDT) and other pesticides in the 1980s, juvenile male

American alligators in Lake Apopka, FL had under-developed testes and reduced levels of testosterone (Vonier et al. 1996). A recent landmark study documents widespread reproductive disruption in feral fish of the UK (Jobling et al. 1998). Collectively these studies indicate that chemicals commonly found in coastal waters may be acting as endocrine disruptors in some cases.

A well accepted assay of estrogenic response is the measurement of vitellogenin production (Sumpter and Jobling 1995; Heppell et al. 1995; Palmer et al. 1998). Vitellogenin (VtG) is a precursor to egg yolk protein synthesized by hepatocytes in response to the binding of E2 to estrogen receptors in the liver. VtG is released into the blood and absorbed by developing oocytes in the ovaries. It is a female-specific protein, but male and juvenile individuals have liver receptors for E2 and are capable of producing VtG if challenged with E2 (Lim et al. 1991; Elskus 1992). Measuring VtG levels in male or juvenile blood plasma or hepatic tissues eliminates the need to consider the cyclical nature of female reproductive hormones and directly determines if xenobiotics are acting as estrogens.

Using VtG production in male and juvenile fish as a measure of estrogenicity, we assessed the estrogenic potential of contaminants in New York Harbor. In the laboratory study, the production of VtG was measured in male killifish, *Fundulus heteroclitus*, from a reference site (Flax Pond at the State University of NY at Stony Brook's Marine Laboratory) injected with whole sediment organic extracts from contaminated sites in New York Harbor. A field study was also conducted in order to measure VtG synthesis in resident male killifish, as well as juveniles of migratory species, striped bass, *Morone saxatilis* and bluefish, *Pomatomus saltatrix* in the Hudson-Raritan Estuary.

METHODS

Experiment 1: Laboratory analysis of VtG in male *Fundulus heteroclitus* from Flax Pond injected with whole organic extracts of sediment

Treatment Solutions

Sediments from a reference site, Central Long Island Sound (CLIS), and from two sites within New York Harbor, the Kill van Kull waterway (KVK) and the lower portion of the East River (LER) (Figure 1), were used in this part of the study due to their range of measured toxicities and variable compositions of HOCs. The top 5 cm layer of the sediments from these sites was collected by a Smith-MacIntyre grab in late April 1998 for other ongoing Hudson River Foundation-funded work in this laboratory. As part of this study and other HRF-supported work (A. McElroy and A. Elskus; B. Brownawell), concentrations of organic contaminants (total PAHs and total PCBs) in sediments from these sites are being characterized.

Dosing Solution Preparation

We dosed fish with organic extracts of New York Harbor sediments to evaluate the estrogenicity of the *in situ* contaminant mixtures to which benthic fish in the Harbor may be exposed. We considered this a worse case scenario since such extracts contain a vast mixture of organic contaminants present in the sediment, not all of which are necessarily bioavailable to fish.

The dose of sediment extract injected was based on PCB levels in resident female killifish in Newark Bay (2 μg PCB/g wet eggs) and the PCB dose needed to achieve those levels (10 mg PCB/kg body weight), determined by studies previously done in this

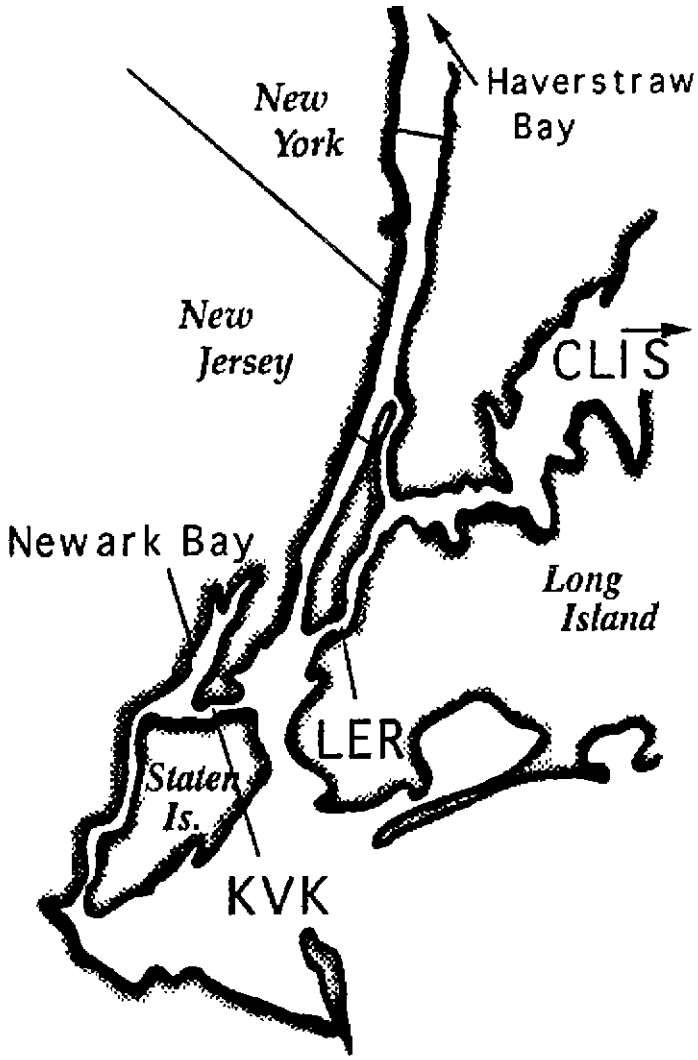

Figure 1: This map of the Hudson-Raritan Estuary indicates the sites from which sediment samples were taken: Kill van Kull (KVK), Lower East River (LER). Central Long Island Sound (CLIS) is off the map, midway between Mt. Sinai and Manhattan and was used as a reference sediment.

laboratory (Elskus et al. in press). Based on the PCB content of sediment extracts for LER (unpublished data), we determined the amount of LER sediment needed to yield an extract containing sufficient PCBs for making up our dosing solutions: 10 mg PCB/kg body weight (high dose) and 1 mg/kg body weight (low dose). This amount was 640 g wet LER sediment.

To expose fish to equivalent amounts of sediment, extract doses were made from the same amount of sediment for all three sites: LER, KVK, and CLIS. It is important to note that these extracts do not contain equivalent amounts of either PCBs or other contaminants. Previous work in our laboratory indicates that KVK sediment extract contains lower concentrations of PCBs and higher concentrations of PAHs than LER, while CLIS contains low levels of chemical contaminants overall (unpublished data).

Sediment extracts were prepared by solvent extraction. Briefly, sediment from each site was soxhlet-extracted overnight using a 1:1 hexane:acetone solution. The resulting extract was back-extracted against water, volume reduced, and exchanged to dimethylsulfoxide (DMSO). Dosing solutions were prepared from the extracts using a modification of the method of Elskus (1992) in which corn oil was added to the appropriate amount of extract (dissolved in DMSO) and vortexed to achieve an emulsion with a final DMSO concentration of 33%.

Estradiol (E2) treatment served as a positive control. Estradiol dosing solutions (8 mg of 17β-estradiol/kg body weight) were prepared similarly using corn oil and DMSO. The vehicle control contained only 33% DMSO in corn oil.

Fish and Treatment Injections

F. heteroclitus were collected from Flax Pond in Long Island, NY by minnow trap in early May 1998. McElroy et al. (1997) have previously determined that the organic contaminant body burden of this population is low. Therefore, fish from this site were used as a reference population in this study. Six- to ten-gram males were transported to Flax Pond Marine Laboratory, held in flow-through seawater and maintained on an ambient photoperiod. Fish were fed daily with Tetramin fish flakes (Royal Pet Supply, Brentwood, NY).

Fish were divided into 7 groups of 8 fish each. Positive (E2), negative (CLIS), and vehicle (corn oil/DMSO) control fish were injected at one concentration only. Sequential injections of E2 or sediment extracts were performed to mimic the well-known primary-secondary response of VtG to E2 (Tata and Smith 1979). Based on their body weight, 7 groups of 8 male *F. heteroclitus* were intraperitoneally injected twice on Day 1 and 3 with one of the following treatment solutions: KVK 1 mg/kg, KVK 10 mg/kg, LER 1 mg/kg, LER 10 mg/kg, CLIS 10 mg/kg, vehicle 33% DMSO, E2 8 mg/kg. Fish were killed by cervical section on Day 5. This dosing regime reliably elevates VtG levels in male killifish treated with E2 (Elskus 1992). Fish livers were removed and frozen immediately on dry ice and stored in liquid nitrogen. Whole sediment extracts, if estrogenic, would induce a primary-secondary response in VtG similar to that of E2.

Tissue Preparation and Protein Assay

Individual frozen livers were homogenized in Tris Buffer (50 mM Tris at pH 7.4) using a Glas-Col 099C K44 variable speed homogenizer stirrer (Terre Haute, IN). Liver

homogenates were differentially centrifuged to isolate the microsomal from the cytosolic portion of the cells as described by Stegeman et al. (1979). The liver cell fractions were stored in liquid nitrogen (-192°C) until analysis.

Microsomal and cytosolic protein was measured using a Bicinchoninic Acid Protein Assay Kit (Sigma, St. Louis, MO) with bovine serum albumin (BSA) (Sigma, St. Louis, MO) as the standard.

Gel Electrophoresis and Western Blotting

Proteins were electrophoretically resolved using a modified method of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) described by Laemmli (1970). Total cytosolic or microsomal protein containing VtG from a vitellogenic female *F. heteroclitus* collected from Flax Pond, served as a relative standard for vitellogenin in the absence of purified *F. heteroclitus* VtG. Forty µg of cytosolic or microsomal protein were loaded along with 3 concentrations (1, 5, 10 µg) of cytosolic or microsomal protein from the vitellogenic female and a molecular weight marker (Novex, San Diego, CA) into 1.5 mm x 15 well, 4-12% Tris-Glycine pre-cast mini-gels.

The separated microsomal proteins were transferred from the gel onto a solid membrane (0.45 µm nitrocellulose, BioRad Laboratories, Hercules, CA), using a semi-dry electroblotter (Owl Separation Systems, Portsmouth, NH), and membranes were stained with Ponceau stain to evaluate the efficiency of protein transfer (Rosenberg 1996).

Two antibodies were used to visualize VtG. The primary antibody recognizes VtG bound to the membrane. The secondary antibody recognizes the primary antibody

and is labeled with a luminescent tracer. The primary antibody in this study was a polyclonal antibody (antiserum) raised against VtG in *F. heteroclitus* (a generous gift of Dr. Kelly Selman, Univ. of Florida). The secondary antibody was a horseradish peroxidase-labeled anti-rabbit antibody, which is part of the ECL Western blotting analysis system (Amersham, Buckinghamshire, England). The primary and secondary antibodies were diluted 1:33 and 1:1,000, respectively, in 5% milk block. VtG was identified by its characteristic molecular weight band at 200 kDa in *F. heteroclitus* (Selman and Wallace 1983).

In the absence of a purified solution of VtG from *F. heteroclitus*, microsomal and cytosolic protein containing VtG from a vitellogenic female was loaded at 3 concentrations, 10, 5, and 1 μg into the wells of the gel. The intensity of the VtG band of each sample was compared to those of the 3 concentrations of a vitellogenic female sample. A consistent scale of band intensity was used to visually rate the VtG signal of the vitellogenic female sample, in which the highest total protein concentration (10 μg) was scored as +++++, the median total protein concentration (5 μg) was scored as +++, and the lowest total protein concentration (1 μg) of vitellogenic female sample was scored as +.

Experiment 2: New York Harbor field analysis of VtG in resident fish, *Fundulus heteroclitus* and migratory fish, *Morone saxatilis* and *Pomatomus saltatrix*

Fish Collections

A total of 25 adult male and 5 female killifish, *F. heteroclitus*, were collected by minnow trap from the Roanoke Yacht Club in Newark Bay, NJ where we have sampled.

Young-of-the-year (YOY) striped bass, *Morone saxatilis* and bluefish, *Pomatomus saltatrix* were collected from the lower Hudson River July 27, 1998 as part of the NYS DEC beach seine surveys (Figure 2). Twenty-three *M. saxatilis* were collected from a site in Dobbs Ferry along the Hudson River. Nine *P. saltatrix* were collected from four sites along the lower Hudson River. Total lengths (mm) were recorded and livers excised on site. The livers were immediately frozen on dry ice, transported back to SUNY Stony Brook and stored in liquid nitrogen until processed.

Vitellogenin Analysis

Because the livers obtained from individual *M. saxatilis* were too small to process as individuals, livers from 5 to 6 *M. saxatilis* were pooled to make a total of 4 groups. For *P. saltatrix*, 2 livers were large enough to process as individuals. Livers from the remaining 7 *P. saltatrix* were combined to produce 3 pools of 2 to 3 fish per pool. All of the *F. heteroclitus* livers were processed individually.

VtG protein analysis of resident *F. heteroclitus* was performed as described above, except 20 µg instead of 40 µg of total microsomal protein female *F. heteroclitus* were subjected to SDS-PAGE and VtG Western blotting. Liver microsomes of *M. saxatilis* and *P. saltatrix* were immunoblotted for VtG as described above for *F. heteroclitus*, except the primary antibody used was a 1:10,000 dilution of a polyclonal antibody that recognizes *M. saxatilis* VtG (generously provided by Dr. Craig Sullivan, North Carolina State Univ.). Purified *M. saxatilis* was used as the protein standard. It was hoped that if the bluefish samples did contain VtG, the antibody raised against VtG of striped bass would also recognize VtG of bluefish.

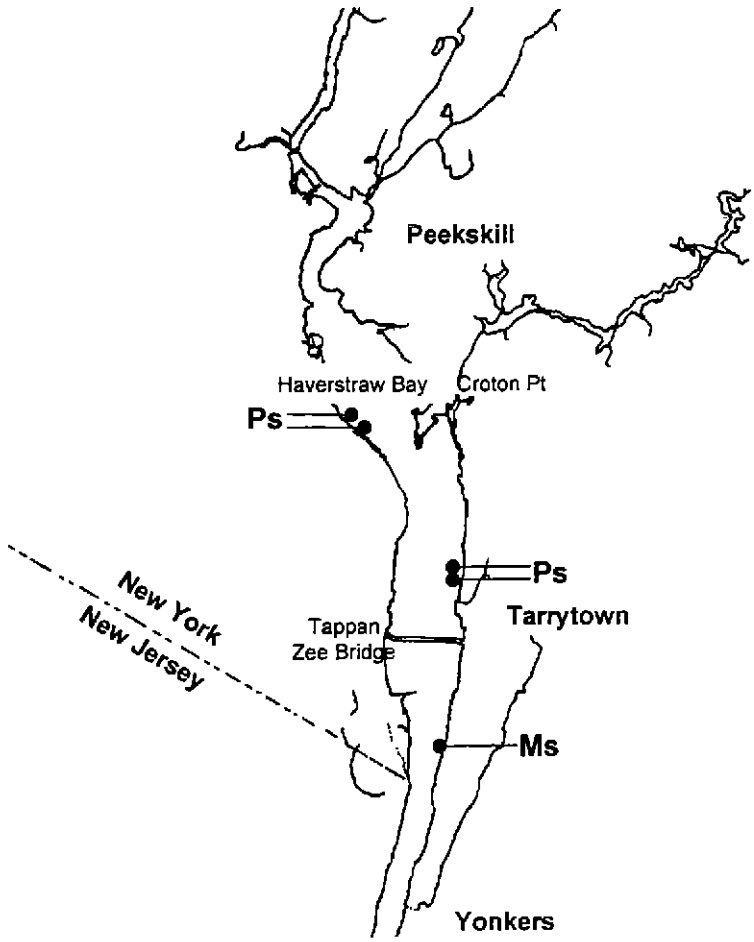

Figure 2: *Morone saxatilis* and *Pomatomus saltatrix* were caught during a New York State Dept. of Environmental Conservation YOY beach seining cruise. Twenty three *Morone saxatilis* (Ms) were collected from a station near Dobbs Ferry. A total of 9 *Pomatomus saltatrix* (Ps) were collected from stations near Haverstraw Bay and Tarrytown.

Statistical Analysis

Data from the laboratory and field studies are reported as mean values +/- standard deviations from the mean. In Microsoft Excel®, a two-tailed t-test of equal variance was applied to the laboratory data sets when comparing the treatment groups to the vehicle control. Treatment groups (17β-estradiol, CLIS 10 mg/kg, LER 1 mg/kg, LER 10 mg/kg, KVK 1 mg/kg, and KVK 10 mg/kg) were considered significantly different from the vehicle control (vehicle treatment), if $P<0.05$.

RESULTS

Experiment 1

No mortalities resulted from the injections of *F. heteroclitus* and all fish lived until sacrificed on Day 5. The average body weight, hepatosomatic index (HSI), and gonadosomatic index (GSI) per treatment group are listed in Table 1. The mean GSI of the LER 10 mg/kg and the 17β-estradiol-treated fish was significantly ($p=0.05$) higher than the of the vehicle controls.

Total protein concentration was higher in liver cytosols than liver microsomes (Table 2). VtG concentrations were also higher in cytosols than microsomes. However, we were unable to use cytosols for VtG analysis because our cytosolic VtG was unstable, having not been prepared with glycerol, a reagent which protects proteins from degradation during freezing. The repeated freeze/thaw regime necessitated by aliquot removal quickly degraded our cytosolic VtG. For this reason, we used the microsomal fraction of the liver, to which glycerol had been added, for all further VtG analyses.

Table 1: Body weight, HSI, and GSI of sediment extract-dosed *Fundulus heteroclitus*[a]

Treatment	N	Body Wt (g)	HSI[b]	GSI[c]
17β-Estradiol	8	8.53 +/-1.15	1.90 +/-0.25	5.55 +/-0.78*
Vehicle	8	7.55 +/-1.82	1.59 +/-0.47	4.00 +/-1.78
CLIS 10 mg/kg	8	7.93 +/-1.45	1.52 +/-0.37	5.29 +/-0.98
LER 1 mg/kg	8	8.43 +/-1.47	1.63 +/-0.75	5.76 +/-1.14*
LER 10 mg/kg	8	8.80 +/-0.96	1.68 +/-0.35	4.39 +/-0.58
KVK 1 mg/kg	7[d]	7.91 +/-1.24	1.52 +/-0.41	5.61 +/-1.44
KVK 10 mg/kg	8	7.93 +/-1.09	1.67 +/-0.35	5.44 +/-0.86

[a]Data are given as mean values +/- standard deviations. [b]HSI, hepatosomatic index = liver wt (g) / total body wt (g) x 100. [c]GSI, gonadosomatic index = gonad wt (g) / total body wt (g) x 100. [d]One individual from this treatment group was not analyzed because it was female. *Significantly different from the vehicle-treated fish ($P<0.05$).

Table 2: Hepatic microsomal and cytosolic protein concentrations of treated male *F. heteroclitus*[a]

Treatment	N	Hepatic Microsomal Protein	N	Hepatic Cytosolic Protein
17β-Estradiol	7	21.92 +/-12.63	7	87.54 +/- 9.88
Vehicle	6	34.64 +/-14.95	7	100.21 +/-70.03
CLIS 10 mg/kg	5	27.35 +/- 7.15	8	89.07 +/-21.87
LER 1 mg/kg	6	35.12 +/-12.00	2	74.44 +/-29.96
LER 10 mg/kg	6	37.69 +/-13.48	2	111.37 +/-20.65
KVK 1 mg/kg	6	35.57 +/- 7.55	7	105.99 +/-48.86
KVK 10 mg/kg	6	45.36 +/-11.21	5	101.07 +/-14.76

[a]Data are given as mean values +/- standard deviations. None of the treatment groups are significantly different from the vehicle control at $P<0.05$.

None of the fish treated with vehicle solution or with sediment extracts from Kill van Kull, Lower East River, or Central Long Island Sound, had detectable levels of VtG as determined by Western blot analysis (Figure 3). The detection limit for VtG was 1 μg of total microsomal protein, which was the lowest concentration of microsomal protein derived from a vitellogenic female in which VtG was detectable. Microsomal samples from male fish injected with 2 x 8 mg E2/kg body weight appeared to contain average levels of VtG intermediate between the 1 and 5 μg of microsomal protein from the vitellogenic female standard (Figure 3).

Experiment 2

Newark Bay *Fundulus heteroclitus*:

Average body weight, HSI, GSI, and total protein concentration for 25 male and 5 female *F. heteroclitus* caught in Newark Bay are provided in Table 3. Female *F. heteroclitus* had significantly higher mean HSI, GSI, and hepatic microsomal protein concentrations than males. None of the 22 Newark Bay male *F. heteroclitus* contained detectable levels of VtG. All five female *F. heteroclitus* appeared to contain levels of VtG intermediate between the 1 and 5 μg of microsomal protein from the vitellogenic female from Flax Pond (Figure 4).

Hudson River YOY *Morone saxatilis* and *Pomatomus saltatrix*:

Twenty three *M. saxatilis* and nine *P. saltatrix* larger than 50 mm in total length were analyzed in this part of the study. Average fish length and liver microsomal protein concentration are listed in Table 4. None of pooled samples of microsomal protein from

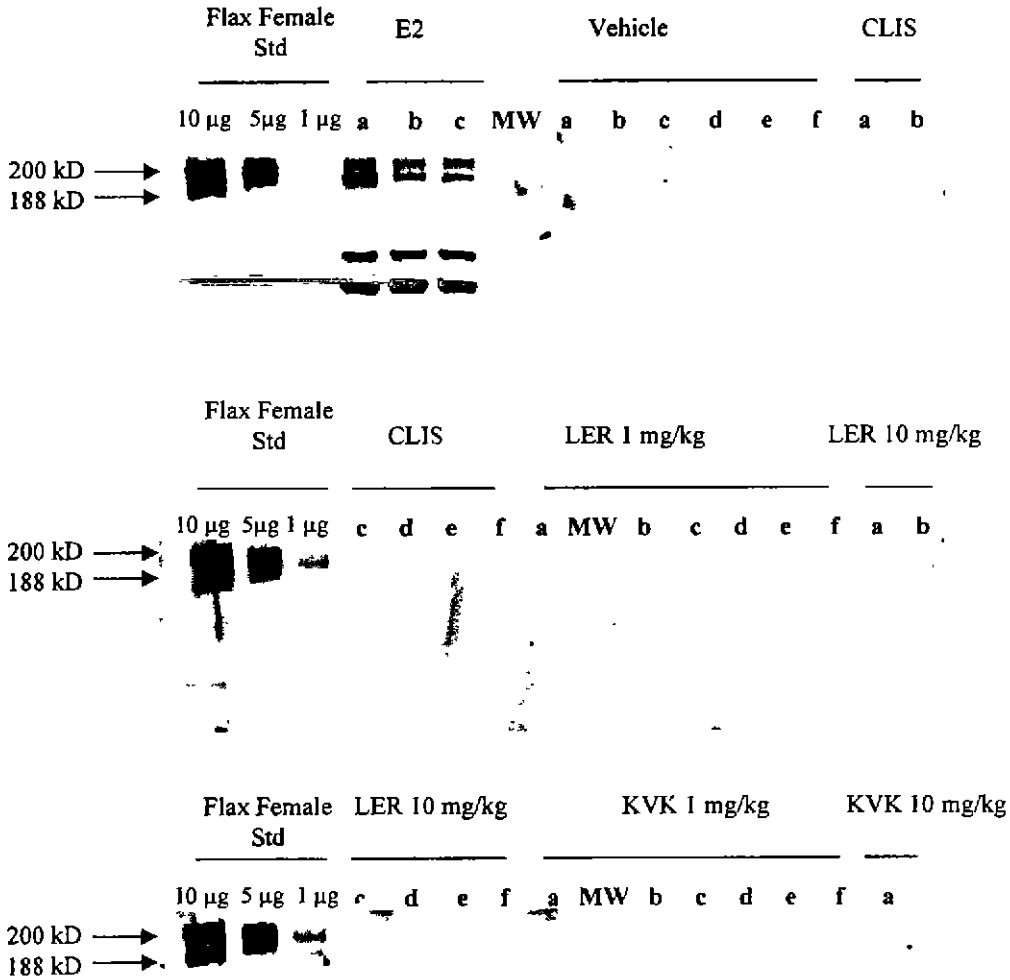

Figure 3: Microsomal protein (40 µg/lane) from treated male *Fundulus heteroclitus* analyzed for VtG by Western blot. Letters refer to individual fish. This species' vitellogenin is 200 kDa in size. Note that E2-treated males (positive control) have elevated VtG levels, but none of the sediment extract-treated male fish show detectable bands of vitellogenin.

Table 3: Physical characteristics of resident fish, *F. heteroclitus* collected from Newark Bay, NJ[a]

Sex	N	Body Wt (g)	HSI	GSI	Microsomal Protein (mg/g wet liver)
Male	25	8.76 +/-1.62	1.92 +/-0.41	4.74 +/-0.97	28.24 +/-6.66
Female	5	7.90 +/-1.64	3.55 +/-1.29*	7.87 +/-3.86*	35.89 +/-5.89*

[a]Data is given as mean values +/- standard deviations. *Significantly different from males ($P<0.05$).

Table 4: Physical characteristics of migratory fish, YOY *Morone saxatilis* and *Pomatomus saltatrix* collected from the Hudson River[a]

Species	N	Total Length (mm)	N	Microsomal Protein (mg/ g wet liver)
M. saxatilis	23	64 +/-14.10	4	20.79 +/-2.39
P. saltatrix	9	121 +/- 8.27	5	16.40 +/-3.27

[a]Data is given as mean values +/- standard deviations.

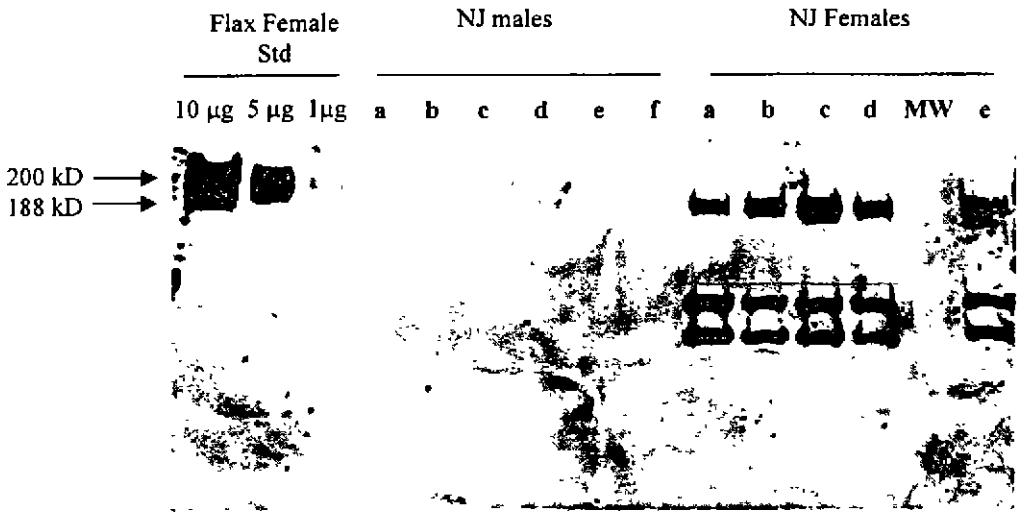

Figure 4: Hepatic microsomal protein from Newark Bay *Fundulus heteroclitus* analyzed by Western blot. Letters refer to individual fish. Microsomal samples from male and female fish were loaded at 40 and 20 µg of protein/lane, respectively. The vitellogenin band is indicated by the 200 kDa arrow. Note that only the female fish have detectable levels of vitellogenin.

either species contained detectable levels of VtG. The detection limit was 0.1 μg of VtG as determined by the lowest concentration of purified *M. saxatilis* VtG detected by Western blot analysis (data not shown).

Table 5 summarizes the results of the VtG analyses in both the laboratory and field experiments.

DISCUSSION

The Western blot analyses of E2-treated male *F. heteroclitus* clearly illustrates that the male individuals used in this study are capable of responding to estrogenic compounds. These findings are consistent with Lim et al. (1991) and Elskus (1992), who also demonstrated that estradiol-induced vitellogenin synthesis does occur in male fish. In this study, E2-treated male *F. heteroclitus* did not, however, produce nearly as much VtG as a reproductively mature female *F. heteroclitus*, even though the E2 dose regime we used (2 x 8 mg/kg body weight) produces plasma E2 levels orders of magnitude higher than those typically found in the plasma of gravid females (Elskus 1992). This may reflect the low concentration of estrogen receptors and short duration of E2 exposure in male livers relative to female livers.

Female *F. heteroclitus* collected from Newark Bay, NJ had lower levels of VtG than the female collected from Flax Pond, LI, NY. This may be due to fish from the two sites being collected at different phases of the lunar cycle. *F. heteroclitus* have semi-lunar reproductive cycles and spawn with the new and full moon during late spring to early fall, thus ovarian weight and GSI, peak during new and full moons (Taylor and DiMichele 1980). Because developing eggs sequester VtG, an elevation in VtG levels

Table 5: Summary of the vitellogenin results for the laboratory and field studies

Group*	N	Band of intensity
Treated Male *F. heteroclitus*:		
Stds (vitellogenic Flax Pond female) :		
10 µg microsomal protein	--	+++++
5 µg microsomal protein	--	++½
1 µg microsomal protein	--	+
17β-Estradiol	8	+½
Vehicle	8	ND
CLIS 10 mg/kg	7	ND
LER 1 mg/kg	8	ND
LER 10 mg/kg	8	ND
KVK 1 mg/kg	7	ND
KVK 10 mg/kg	8	ND
Field Collected Fish:		
Newark Bay Male *F. heteroclitus*	22	ND
Newark Bay Female *F. heteroclitus***	5	+½
Hudson River Y.O.Y. *M. saxatilis*	4	ND
Hudson River Y.O.Y. *P. saltatrix*	5	ND

ND = Not Detected

* All samples were loaded onto SDS-PAGE gels at a concentration of 40 µg of hepatic microsomal protein except where otherwise noted.

** 20 µg of microsomal protein

accompanies increased ovary size. The female from Flax Pond was collected at a time when the GSI, and hence VtG, was increasing and the Newark Bay females were collected during a time when the GSI was decreasing.

Previous studies on the Hudson-Raritan Estuary comparing concentrations of contaminants in sediment and organisms in laboratory and field-based toxicity tests provides strong evidence that many sediments from the region are toxic to at least some organisms and potentially toxic to others (Long and Morgan 1990; Squibb et al. 1991). We have demonstrated here that organic extracts of contaminated sediments from the Hudson-Raritan Estuary do not elicit an estrogenic response in killifish. Although we would never expect fish to be exposed to the full suite of contaminants found in an organic extract of sediment, this experiment was designed to be a worst case exposure scenario to these contaminants that are present in these sediments.

We also failed to observe an estrogenic response in either adult resident (*F. heteroclitus*) or YOY migratory (*M. saxatilis* and *P. saltatrix*) fish naturally exposed to contaminants in this system, either through the water column or sediment reservoir. Our results suggest that the levels of estrogenic compounds in the Hudson-Raritan Estuary may be too low to cause an estrogenic effect or that the mixture of chemicals present are acting in opposition to each other to limit the effect observed.

Most of the organic compounds found to be estrogenic are only weak mimics of this steroid and many of the estrogenic responses observed in the laboratory are at concentrations that may be environmentally unrealistic. A survey of 30 U.S. rivers found that water and sediment concentrations of APEs and their metabolites were relatively low (Naylor et al. 1992). A 1996 review on the effects of APEs in the environment concludes

that based on the environmental concentrations reported at the time, the estrogenic potential of these compounds is low in U.S. ecosystems (Nimrod and Benson 1996).

Studies in the United Kingdom have found inappropriate VtG production in male fish from rivers receiving sewage treatment plant effluent (Jobling et al. 1998; Harries et al. 1996; Harries et al. 1997). It has been suggested that sex steroids, natural (estradiol and estrone) and synthetic (from hormone therapy), may be a large contributor to the estrogenicity of British sewage treatment plant (STP) effluent (Sumpter and Jobling 1995). A recent UK study demonstrated that exposure to natural and synthetic estrogens in the laboratory at concentrations found in STP effluent were high enough to cause vitellogenin synthesis in male fish. Because undegraded natural and synthetic sex steroids and APE metabolites, as well as other estrogenic compounds, have been found in UK (Routledge et al. 1998) and U.S. (Folmar et al. 1996) STP effluent, the presence of these compounds in mixtures may pose a risk to the reproductive health of aquatic organisms.

Although widespread reproductive disruption has been documented in the UK, many U.S. river systems may contain lower concentrations of estrogenic compounds. Researchers have attributed contrasting findings to differences in sewage treatment practices, as well as human population densities, and size of receiving waters of the two countries. U.S. sewage treatment plants typically use aerobic degradation and activated sludge treatment systems, which could result in the higher APE degradation efficiency of U.S. plants over European plants (Renner 1997). In the UK, as a result of the high population density, 50% of the river flow may consist of sewage treatment plant effluent

(Routledge et al. 1998). Due to the relatively larger size of the receiving waters, many U.S. rivers have greater dilution factors than those in the UK (Renner 1997).

Urban harbors are home to many anthropogenic activities that introduce various contaminants into the water and sediments. Therefore, compounds are found in complex, heterogeneous mixtures. Because few studies have addressed the toxic potential of such mixtures, little is known about how co-occurring compounds interact. In the same sense, little is known about how the presence of different types of endocrine disrupting agents (i.e., estrogenic, antiestrogenic, androgenic) affect the toxicity of other chemicals in mixtures. It has been proposed by Safe and other researchers that these endocrine disruptors may be working in opposition and thus neutralizing toxic effects (Stone 1994).

In summary, a detectable estrogenic response was not found in any of the fish exposed to extracts from contaminated sediments from the New York Harbor. Nor does it appear that resident or migratory fish from the Hudson-Raritan Estuary are being exposed to significant levels of estrogenic compounds. Therefore, the estrogenic potential of the Hudson-Raritan Estuary appears to be low. Currently, we, along with investigators from the University of Mississippi (Drs. Benson and Schlenk) are assessing the potential estrogenicity of STP effluent from one of New York's largest plants by exposing adult male and juvenile fish directly to the effluent. The results from the current study along with the results present here, will help determine the overall risk for endocrine disruption in New York Harbor.

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