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ENZYMATIC AND ESTROGENIC RESPONSES IN FISH EXPOSED TO ORGANIC POLLUTANTS IN THE NEW YORK–NEW JERSEY (USA) HARBOR COMPLEX

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Abstract—This study examines biochemical and hormonal responses in resident and migratory fish from the New York–New Jersey (USA) Harbor Complex (NY–NJHC) and those treated with sediment-associated organic contaminants. Following laboratory exposures to organic extracts of NY–NJHC sediments (injection), livers from adult male mummichogs, *Fundulus heteroclitus*, were analyzed for vitellogenin (VTG), cytochrome P450 1A (CYP1A), CYP3A, and estradiol 2-hydroxylase (E2OHase) and ethoxyresorufin-*O*-deethylase (EROD) activities. Levels of CYP1A (311–391% of control) and EROD (1267–3611% of control) were elevated in mummichogs exposed to high doses of sediment extracts, while VTG, CYP3A, and E2OHase were unaffected. In field studies, reproductively mature male mummichogs collected from a highly contaminated area, Newark Bay (NJ, USA), did not have detectable levels of VTG but did exhibit elevated levels of CYP1A and EROD. Vitellogenin was also not detected in juvenile striped bass (*Morone saxatilis*) collected from the main stem of the lower Hudson River (NY, USA). Similar to results in the sediment extract–treated fish, CYP3A and E2OHase were unaltered in Newark Bay *F. heteroclitus*. The lack of response of CYP3A and E2OHase activities to contaminant mixtures, either environment or sediment derived, suggests that compounds in these mixtures either do not alter these enzymes, produce antagonistic effects in mixtures, are present at ineffective concentrations, or are regulated in a species-specific manner.

Keywords—Cytochrome P450 enzymes Fish Mixtures Vitellogenin

INTRODUCTION

A highly publicized and controversial issue in environmental science today is the potential risk posed by endocrine-disrupting compounds (EDCs) to humans and wildlife. Several studies document endocrine disruption in feral fish in urban waterways in England [1–3] and in the United States [4]. Mounting evidence indicates that municipal sewage treatment plant (MSTP) effluent and sludge is a substantial source of EDCs, such as natural and man-made estrogens, and alkylphenol ethoxylates to urban rivers worldwide [5–7]. A form of endocrine disruption manifested by male and juvenile fish exposed to EDCs is the inappropriate production of vitellogenin (VTG), an egg yolk precursor protein normally elevated only in spawning female fish. For example, male rainbow trout exposed to estradiol (E2) produced VTG levels up to 10,000-fold higher than those in control fish [6]. Fish may also respond to EDCs by altering levels of enzymes involved in hormone metabolism. Following exposure to a known xenoestrogen, 4-nonylphenol, juvenile Atlantic salmon had decreased levels of cytochrome P4503A (CYP3A)- and CYP2K-like proteins and 6β-, 16α-, and 17α-hydroxylase activities [8]. Although endocrine disruption has been observed in numerous laboratory and field settings, little is known of the consequences of such effects on fish populations.

Several measures of biochemical and hormonal response in fish were chosen for the present study. Inappropriate vitellogenesis in male and juvenile fish was used to assess the estrogenic response of exposed fish as it has been widely used in assessing estrogenicity of chemicals in laboratory and field studies [1–4,7]. The enzyme CYP1A is induced by organic contaminants and is widely used as a biomarker of exposure to these pollutants [9]. Hepatic CYP3A was chosen because this enzyme metabolizes steroid hormones and is inducible by organic pollutants [10]; thus, disruption of this enzyme could result in reproductive abnormalities. The activity of estradiol 2-hydroxylase (E2OHase), which is the primary metabolizing enzyme of E2 in fish [11], was used as a possible indicator of hormonal or reproductive disruption.

The test fish used in the present study were mummichogs (*Fundulus heteroclitus*) and striped bass (*Morone saxatilis*). These two species of fish are exposed to pollutants present in urban harbors differently as a result of their contrasting life histories (e.g., foraging behavior, range, and diet). Mummichogs are a nonmigratory omnivorous species with benthic eggs and limited home range [12], and, as such, represent species that are chronically exposed to pollutants in their environment. Striped bass are piscivores that forage throughout estuaries and bays and spend their critical early life stages in these areas. Therefore, they represent species that are potentially exposed to pollutants during sensitive developmental periods. Striped bass was also chosen because it is a commercially important species.

In the present study, fish were exposed to potential EDCs using two different regimes: laboratory exposure to organic extracts of New York–New Jersey Harbor Complex (NY–NJHC) sediments and field exposure in the NY–NJHC (Fig. 1). Because many organic aquatic pollutants, such as polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and EDCs, tend to bind to particles, sediments act as pollutant reservoirs for these chemicals. For this reason, we exposed fish to organic extracts of NY–NJHC sediments. In

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the extraction process, even tightly associated (nonbioavailable) fractions of organic compounds are extracted from the sediments; thus, exposure to sediment extracts represents a worst-case scenario of the potential effect of sediment-associated contaminants. To assess a more representative exposure of fish to EDCs and other pollutants in the NY-NJHC, we collected resident (mummichogs) and migratory (striped bass) fish from two sites in this system. Fish inhabiting the harbor should exhibit the integrated effect of all chemical exposure pathways (e.g., industrial effluent, sewage effluent, and so on) at their locations.

We have previously demonstrated that the effluent of the MSTP located near the collection site of striped bass (Fig. 1) is estrogenic to juvenile sunshine bass and induces CYP1A in this species and in adult male mummichogs [13]. Ferguson et al. [14] confirmed the presence of xenoestrogens in the MSTP effluent used in that study. The concentrations of alkylphenol ethoxylates (14.8–15.8 μg nonylphenol/L, 0.18–0.67 μg octylphenol/L, and 55.2–74.0 μg total alkylphenol ethoxylates/L) in the effluent are comparable to those associated with estrogenic effects in fish [3]. Here we report results of hormonal and biochemical responses in field fish, some of which (striped bass) were collected near the MSTP, as well as those responses in fish exposed to NY-NJHC sediments.

MATERIALS AND METHODS

Chemicals

Bicinchoninic acid protein kits and 17β-estradiol (E2) were purchased from Sigma Chemical (St. Louis, MO, USA). The [2,4,6,7-^{3}H(N)]17β-estradiol (3.7×10^4 Bq/μmol; 97% pure) was supplied by New England Nuclear (Boston, MA, USA). The 7-ethoxyresorufin was obtained from Molecular Probes (Eugene, OR, USA). Enhanced chemiluminescence (ECL) Western blotting detection systems and Hyperfilm were purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). Reagents for protein assays and 0.45-μm nitrocellulose membrane were obtained from Bio-Rad (Hercules, CA, USA). Precast 4 to 12% Tris-glycine 1.5 mm, 15-well minigels were supplied by Invitrogen (San Diego, CA, USA). All other reagents were obtained from Sigma Chemical, Fisher Scientific (Fair Lawn, NJ, USA), J.T. Baker (Phillipsburg, NJ, USA), and EM Science (Gibbstown, NJ, USA).

Animals

Reproductively mature male *F. heteroclitus* were collected from Flax Pond in Long Island (NY, USA) by minnow trap. We have previously determined that the organic contaminant body burden of this population is low [15]. Therefore, fish from this site were used as a reference population in the present work. Male *F. heteroclitus* were collected in May during their spawning season and acclimated to appropriate test conditions for one week. Fish were maintained on an ambient photoperiod and fed daily with Tetramin® fish flakes (Tetra Sales, Blacksburg, VA, USA). Spawning adult male and female *F. heteroclitus* were collected by minnow trap from the Roanoke Yacht Club in Newark Bay (NJ, USA). Our laboratory has previously shown that Newark Bay *F. heteroclitus* ovaries contain 1.6 ± 0.4 μg PCB/g wet weight, which is eightfold higher than the concentrations in ovaries from the Flax Pond population (0.2 ± 0.03 μg PCB/g wet wt) [16]. Newark Bay *F. heteroclitus* were held in the laboratory for 2 d before sacrifice. Young of the year (YOY) striped bass, *M. saxatilis*, were collected from the lower Hudson River in July 1998 as part of a New York State fish survey. Body burdens of PCBs in Hudson River YOY striped bass collected in 1993 ranged from 0.24 to 13.40 μg/g [17]. Striped bass were collected by seine and sacrificed on site.

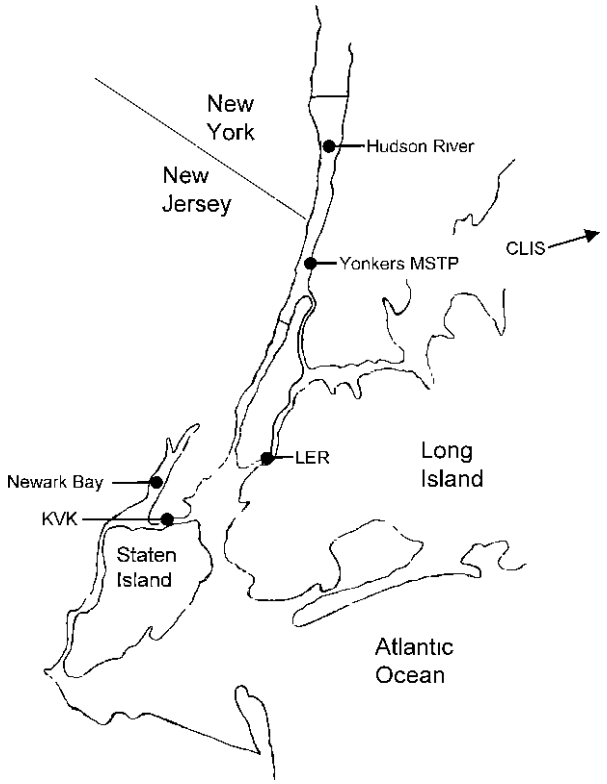

Fig. 1. Map of the study area, the New York–New Jersey Harbor (USA) Complex, and the sites from which sediment samples were taken: Kill van Kull (KVK) 40°38.970′N; 74°06.368′W; lower East River (LER) 40°42.569′N; 73°58.255′W. Central Long Island Sound (CLIS) 41°07.999′N; 72°52.741′W is off the map, midway between Mt. Sinai (NY, USA) and New Haven (CT, USA), and was used as reference sediment. Newark Bay, where *Fundulus heteroclitus* (mummichogs) were collected, and the location of the Yonkers municipal sewage treatment plant (MSTP) are also on this map.

Study 1: Exposure to organic extracts of NY-NJHC sediments

The goal of this first study was to evaluate whether in situ organic contaminants in NY-NJHC sediments have the potential to affect pollutant- and steroid-metabolizing enzymes or provoke an estrogenic response in fish.

Sediments from a reference site, Central Long Island Sound (CLIS) (41°07.999′N; 72°52.741′W), and from two sites within NY-NJHC, the Kill van Kull waterway (KVK) (40°38.970′N; 74°06.368′W) and the lower portion of the East River (LER) (40°42.569′N; 73°58.255′W), were used in this study because of their range of measured toxicities and variable chemical composition [18]. Chemical characterization revealed that CLIS sediments contain 1,690 ng/g total PAHs and 93.6 ng/g total PCBs, LER sediments contain 41,900 ng/g total PAHs and 4,540 ng/g total PCBs, and KVK sediments contain 89,200 ng/g total PAHs and 111 ng/g total PCBs, all on a dry-weight basis [18]. The dose of organic sediment extract injected was based on PCB levels in extruded eggs of resident female mummichogs in Newark Bay (2 μg PCB/g wet eggs),

and the PCB dose needed to achieve those levels (10 μg PCB/g body wt) determined in previous studies [15]. Based on the PCB content of sediment extracts for LER, we determined that 640 g of wet LER sediment would yield an extract containing sufficient PCBs for making up our dosing solutions: 10 μg PCB/g body weight (high dose) and 1 μg/g body weight (low dose). To expose fish to equivalent amounts of nonpollutant sediment–associated organic compounds, extract doses were made from the same amount of sediment for all three sites: LER, KVK, and CLIS.

Dosing solutions were prepared from sediments as follows. Sediment from each site was Soxhlet-extracted overnight with a 1:1 hexane:acetone solution, and the extract was back-extracted against water, volume reduced, and exchanged to dimethylsulfoxide (DMSO). Dosing solutions were prepared from these extracts using a modification of the method developed by Elskus [19] in which corn oil was added to the appropriate amount of extract in DMSO and vortexed to achieve an emulsion with a final DMSO concentration of 33%. Estradiol doses (8 μg of 17β-E2/g body wt) were prepared similarly using corn oil and DMSO and used as a positive control for estrogenic effects. Dosing solutions made using CLIS extract served as a negative sediment control. All dosing solutions contained 33% DMSO.

Prior to injection, male *F. heteroclitus* (8.1 ± 1.8 g) were acclimated to laboratory conditions for one week in flow-through seawater tanks. Fish were intraperitoneally injected on days 1 and 3 with one of the following treatment solutions: KVK 1 μg/g or 10 μg/g, LER 1 μg/g or 10 μg/g, CLIS 10 μg/g, and E2 8 μg/g. Positive (E2), negative (CLIS), and vehicle (corn oil/DMSO) control fish were injected at one concentration only. Either E2 or organic extracts of sediment were injected twice (days 1 and 3) to provoke the well-known primary–secondary response of VTG to E2 [20]. Fish were maintained in flow-through seawater (~28 parts per thousand salinity). On day 5, fish were killed by cervical section. This dosing regime reliably elevates VTG levels in male mummichogs treated with E2 [19]. Fish livers were removed, frozen immediately on dry ice, and stored in liquid nitrogen until analyzed. Analyses for CYP1A, CYP3A, ethoxyresorufin-*O*-deethylase (EROD), E2OHase, and VTG were performed on these samples.

Study 2: Field collections from Newark Bay, New Jersey, and the lower Hudson River

The aim of the field study was to determine whether resident, nonmigratory male mummichogs from Newark Bay and juveniles of migratory striped bass from the lower Hudson River manifest signs of exposure to estrogenic compounds. In addition, the effects of chronic field exposure on pollutant- and steroid-metabolizing enzymes were evaluated in Newark Bay mummichogs.

Reproductively mature male *F. heteroclitus* (8.8 ± 1.6 g) were collected from Newark Bay, a contaminated site in northern New Jersey, in June 1998. Reproductively mature female *F. heteroclitus* (7.1 ± 1.6 g) were also collected from this site as positive controls for estrogenic effects. Twenty-three juvenile *M. saxatilis* (64 ± 14 mm in total length) were collected near Dobbs Ferry in the lower Hudson River, which is approximately 5 mi north of the Yonkers MSTP. Hepatic CYP1A, CYP3A, EROD, E2OHase, and VTG analyses were evaluated in *F. heteroclitus*. Young of the year *M. saxatilis* were analyzed only for hepatic VTG.

Microsomal preparation and protein assays

Hepatic microsomes of *F. heteroclitus* and *M. saxatilis* from tissues were isolated using a modification of the technique of Stegeman et al. [21]. Liver cell fractions were stored in liquid nitrogen (−192°C) until analyzed. Microsomal proteins were measured spectrophotometrically using a bicinchoninic acid protein assay kit with bovine serum albumin as the standard.

Cytochrome P450 proteins

Microsomal proteins of sediment extract-treated *F. heteroclitus*, resident Newark Bay *F. heteroclitus*, and Hudson River YOY *M. saxatilis* were electrophoretically separated by sodium dodecylsulfate–polyacrylamide gel electrophoresis as described by Laemmli [22] using 1.5-mm × 15-well, 4 to 12% Tris-Glycine precast minigels and transferred via electroblotter to 0.45-μm nitrocellulose membranes (Bio-Rad). The transfer efficiency of proteins was evaluated by Ponceau staining the nitrocellulose membranes and Coomassie-blue staining the residual gels. Membranes were immunoblotted for CYP1A protein content using the MAb 1-12-3, a monoclonal antibody (MAb) (gift of J.J. Stegeman, Woods Hole Oceanographic Institution, Woods Hole, MA, USA) raised against scup that recognizes CYP1A in several teleost species [9] and ECL-linked horseradish peroxidase–labeled anti-mouse antibody. Membranes were stripped and reprobed for CYP3A protein content using a polyclonal antibody (PAb) (gift of M. Celander, Göteborg University, Göteborg, Sweden) raised against cytochrome $P450_{con}$ in rainbow trout [23] and ECL-linked horseradish peroxidase–labeled anti-rabbit antibody. In the absence of purified CYP1A and CYP3A proteins from *F. heteroclitus*, microsomal protein from 50 μg/g β-naphthoflavone (BNF), a CYP1A inducer, and vehicle-treated male *F. heteroclitus* were loaded at 10 μg into the wells of each gel to normalize gel to gel variability. The PAb to trout CYP3A used in this study recognized a 56-kDa molecular weight primary band and a secondary, slightly smaller molecular weight band in *F. heteroclitus* (blots not shown).

Protein band intensities were quantified using Sigma Gel (Statistical Product and Service Solutions, Chicago, IL, USA). The intensity (in optical density units) of the CYP1A or CYP3A band of each sample was normalized to the CYP1A or CYP3A band of either the BNF- or the vehicle-treated male on the same membrane.

Catalytic assays

Microsomal CYP1A activity was measured using the CYP1A-specific substrate 7-ethoxyresorufin. The CYP1A catalytic activity was measured by EROD and was evaluated fluorometrically on a Cytofluor 2300 (Millipore, Bedford, MA, USA) multiwell plate reader using the method of Hahn et al. [24].

The E2OHase activity was measured in liver microsomes using a slight modification of the tritiated water release method described by Kupfer et al. [25]. All reactions were run in duplicate. Samples were counted in a LKB Wallac 1217 RackBeta (Turku, Finland) liquid scintillation counter.

Vitellogenin

Because of methodological difficulties in obtaining blood in these fish species, we measured VTG in microsomal liver fractions of *F. heteroclitus* and *M. saxatilis*. We expected VTG to be present in this tissue [19]. Others [26] used VTG in

Table 1. Sediment extract[a]-treated *Fundulus heteroclitus* males: gonadosomatic index (GSI), hepatic vitelogenin (VTG), cyctochrome P4501A (CYP1A), ethoxyresorufin-*O*-deethylase (EROD), CYP3A, and estradiol 2-hydroxylase (E2OHase)

			CYP1A		CYP3A	
Treatment	GSI	VTG[b]	Normalized OD units[c]	EROD (pmol/min/mg)	Normalized OD units[d]	E2OHase (pmol/min/mg)
Vehicle control	4.00 ± 1.78 (8)[e]	− (8)	0.55 ± 0.60 (5)	116 ± 95 (5)	1.58 ± 1.15 (5)	69.2 ± 14.5 (5)
17β-Estradiol	5.55 ± 0.78 (8)	+ (8)	0.47 ± 0.24 (5)	123 ± 57 (5)	1.26 ± 0.12 (5)	81.0 ± 37.2 (5)
CLIS 10 μg/g	5.29 ± 0.98 (8)	− (8)	1.25 ± 0.73 (5)	208 ± 107 (5)	1.00 ± 0.08 (5)	92.0 ± 26.4 (5)
LER 1 μg/g	5.76 ± 1.14 (8)	− (8)	1.05 ± 0.51 (5)	144 ± 127 (6)	1.09 ± 0.28 (5)	5.13 ± 28.0 (5)
LER 10 μg/g	4.39 ± 0.58 (8)	− (8)	2.15 ± 0.71 (5)[f,g]	420 ± 122 (5)[f,g,h]	1.18 ± 0.20 (5)	86.9 ± 15.6 (5)
KVK 1 μg/g	5.61 ± 1.44 (7)	− (8)	1.28 ± 0.85 (5)	310 ± 159 (5)	1.05 ± 0.14 (5)	80.2 ± 39.4 (5)
KVK 10 μg/g	5.44 ± 0.86 (8)	− (8)	1.71 ± 0.91 (5)	310 ± 159 (5)	1.04 ± 0.10 (5)	61.9 ± 9.1 (5)

[a] Sediments are from the New York-New Jersey Harbor Complex (USA): CLIS = Central Long Island Sound; LER = Lower East River; KVK = Kill van Kull.
[b] The same microsomal protein level was analyzed for VTG in all treatment samples. + = VTG detected; − = VTG not detected.
[c] OD = optical density. Band intensity normalized to BNF-treated male on same blot; the same microsomal protein level was analyzed in all treatment samples.
[d] Band intensity normalized to vehicle-treated male on same blot; the same microsomal protein level was analyzed in all treatment samples.
[e] Mean ± standard deviation (number of individuals). Significantly different from [f] vehicle-treated controls ($p < 0.05$), [g] low-dose ($p < 0.05$), [h] CLIS-treated fish ($p < 0.05$).

cytosol liver fractions to assess exposure to estrogenic chemicals in fish. As described by McArdle et al. [27], VTG concentrations in hepatic microsomal samples of treated and resident Newark Bay *F. heteroclitus* were determined by sodium dodecylsulfate–polyacrylamide gel electrophoresis and Western blotting using *F. heteroclitus* VTG antiserum (gift of K. Selman, University of Florida, Gainesville, FL, USA). Liver microsomes of YOY *M. saxatilis* were immunoblotted for VTG as described previously for *F. heteroclitus*, using a PAb that recognizes *M. saxatilis* VTG and purified *M. saxatilis* VTG as the standard (gifts of C. Sullivan, North Carolina State University, Raleigh, NC, USA).

Statistical analysis

The Shapiro–Wilk test in STASTICA (StatSoft, Tulsa, OK, USA) was used to determine whether the data for treatments and groups follow normal distributions. If the groups being compared were normally distributed ($p > 0.05$), significant differences among the treatments (sediment extract study) or groups (field study) were determined by analysis of variance followed by Tukey's post hoc test in STASTICA. If the groups being compared were not normally distributed ($p < 0.05$), significant differences among the treatments (sediment extract study) or groups (field study) were determined by Kruskal–Wallis multiple comparison test (nonparametric test) in STASTICA. Differences were considered significant at $p < 0.05$.

RESULTS

Differential response of CYP3A, CYP1A, EROD, and E2OHase

Both CYP1A protein and EROD activity were elevated in adult male *F. heteroclitus* injected with NY-NJHC sediment extracts. The high dose of organic extract from LER sediment significantly induced CYP1A protein (391%) and EROD activity (267%) in male *F. heteroclitus* over vehicle controls in a dose-dependent-response manner (Table 1). Although organic extracts of KVK sediment induced CYP1A and elevated EROD activities relative to the controls, the results were not statistically significant. No significant effects of the sediment extract treatment on either CYP3A content or E2OHase activity were observed (Table 1).

Resident Newark Bay male *F. heteroclitus* had elevated hepatic levels of CYP1A protein (218%) and EROD activity (241%) compared to vehicle-treated male fish from a reference site, Flax Pond (NY, USA) (Table 2). Significant sex differences were observed in EROD activity, with female *F. heteroclitus* from Newark Bay having significantly lower EROD activity than Newark Bay males. The levels of CYP1A protein were also lower in Newark Bay female *F. heteroclitus* than Newark Bay males, though not significantly. In contrast, CYP3A protein content and E2OHase activity did not differ between the sexes, nor did those levels differ between Flax and Newark mummichogs (Table 2).

Estrogenic responses

Sediment extract treatment failed to induce detectable levels of hepatic microsomal VTG in male *F. heteroclitus* (Table 1). However, male *F. heteroclitus* treated with E2 did produce VTG (Table 1) as expected, demonstrating that our assay can detect VTG in this species. None of the 22 Newark Bay male *F. heteroclitus* exhibited detectable levels of hepatic microsomal VTG. All five female *F. heteroclitus* from Newark Bay showed expected levels of VTG (Table 2). Both the Flax Pond and the Newark Bay *F. heteroclitus* were gonadally mature (Tables 1 and 2). None of the pooled hepatic microsomal samples from Hudson River YOY *M. saxatilis* had detectable levels of VTG. The detection limit of the striped bass assays was 0.1 μg VTG as determined by the lowest concentration of purified *M. saxatilis* VTG detectable by Western blot analysis (data not shown).

Table 2. Field-caught *Fundulus heteroclitus* and *Morone saxatilis* gonadosomatic index (GSI), hepatic levels of vitellogenin (VTG), cytochrome P4501A (CYP1A), ethoxyresorufin-*O*-deethylase (EROD), CYP3A, and estradiol 2-hydroxylase (E2OHase)

					CYP1A		CYP3A	
Species	Collection site[a] or treatment	Sex	GSI or repoductive status	VTG[b]	Normalized OD units[c]	EROD (pmol/min/mg)	Normalized OD units[d]	E20Hase (pmol/min/mg)
F. heteroclitus	Vehicle treated	M	4.00 ± 1.78 (8)[e]	− (8)	0.55 ± 0.60 (5)	116 ± 95 (5)	1.58 ± 1.1 (5)	69.2 ± 14.5 (5)
F. heteroclitus	Newark	F	7.87 ± 3.86 (5)	+ (5)	0.45 ± 0.24 (4)	127 ± 81 (4)	0.78 ± 0.21 (4)	95.1 ± 30.3 (4)
F. heteroclitus	Newark	M	4.74 ± 0.97 (25)	− (22)	1.19 ± 0.18 (5)[f]	279 ± 50 (5)[f]	0.83 ± 0.25 (5)	95.1 ± 22.3 (4)
M. saxatilis	Hudson River	—	Juvenile	− (5)	NA	NA	NA	NA

[a] Study locations are in the New York-New Jersey Harbor Complex (USA).
[b] The microsomal protein level analyzed for VTG in female *F. heteroclitus* was half the level analyzed in male *F. heteroclitus* and juvenile *M. saxatilis*. + = VTG detected; − = VTG not detected.
[c] OD = optical density. Band intensity normalized to vehicle-treated male on same blot; the same microsomal protein level was analyzed in all treatment samples.
[d] Band intensity normalized to vehicle-treated male on same blot; the same microsomal protein level was analyzed in all treatment samples.
[e] Mean ± standard deviation (*n* = individual *F. heteroclitus* or pools of four *M. saxatilis*). NA = not analyzed.
[f] Significantly different from vehicle-treated controls ($p < 0.05$) and resident Newark bay female fish ($p < 0.05$).

DISCUSSION

This study examined biochemical and hormonal endpoints in fish exposed to organic extracts of NY-NJHC sediments and those responses in feral fish from the NY-NJHC. We did not observe an estrogenic response in either adult resident (*F. heteroclitus*) or YOY migratory (*M. saxatilis*) fish naturally exposed to contaminants in the NY-NJHC. Field-caught YOY striped bass did not exhibit an estrogenic response despite their proximity to the MSTP from which effluent used in a previous study [13] was estrogenic to juvenile sunshine bass. Our results suggest either that the levels of estrogenic compounds at these sites are too low to induce an estrogenic effect in resident or migratory fish or that the chemicals present in the mixture are acting antagonistically. In England, researchers find evidence of estrogenic effects in field fish living in MSTP effluent–contaminated waterways [1,6,7]. However, those observations may be due to higher levels of effluent-derived contaminants in those rivers compared to those in the NY-NJHC.

Organic extracts of LER or KVK sediments did not induce VTG but did induce CYP1A in mummichogs. These results indicate that estrogenic chemicals are not found at levels capable of inducing estrogenic effects in sediments from these sites or that antagonism from other contaminants present is diminishing these effects. In addition, Newark Bay mummichogs exhibited elevated levels of CYP1A protein and EROD activity. These results demonstrate that fish in the NY-NJHC can or are responding to organic contaminants (e.g., PAHs, PCBs, and dioxins) present in sediments that may be potentially harmful.

While organic extracts of LER sediment elicited a significant dose-related response in CYP1A protein content and EROD activity in *F. heretoclitus*, organic extracts of KVK sediment did not. Gooch et al. [28] simultaneously observed elevated levels of CYP1A messenger RNA and decreased EROD activities at higher coplanar PCB doses, and this phenomenon correlated with residual PCB concentrations in the hepatic tissue from which the microsomes were isolated. Those authors suggested that coplanar PCBs may be competing with the substrate used in the EROD assays since some congeners of PCBs are substrates for CYP1A enzymes themselves. Kill van Kull Waterway sediments have twice the concentration of total PAHs than LER sediments. Because PAHs are known substrates for CYP1A enzymes and relatively high concentrations of PAHs exist in KVK sediments, we suggest that the lack of a dose-related increase in EROD activity in fish treated with organic extracts of KVK sediment may be a result of kinetic suppression at the higher dose examined.

In our studies, CYP3A in laboratory- and field-exposed fish did not respond to contaminant mixtures in organic extracts of NY-NJHC sediments or in Newark Bay, suggesting that these compounds either do not alter this enzyme or are present at ineffective concentrations. Compared to CYP1A, relatively few studies have been conducted on the effects of environmental contaminants on CYP3A. Our findings agree with those of Arukwe and Goksøyr [29], who demonstrated that BNF, a model PAH, had no affect on CYP3A levels in spawning turbot. In contrast, Nile tilapia in a highly contaminated reservoir in São Paulo, Brazil, exhibited fivefold-higher levels of CYP3A protein compared to reference site fish [30]. Laboratory and field studies have shown that while CYP1A and its associated activity can be induced in contaminant-exposed freshwater and marine fish by several- to 100-fold over controls or reference fish [31,32], CYP3A appears to be much less responsive to pollutants, has higher constitutive expression than CYP1A, and is slightly inducible by steroids (e.g., pregnenolone-16α-carbonitrile) [23]. The CYP3A may have a larger role in the metabolism of endogenous rather than exogenous compounds in fish [33].

In the present study, CYP3A in laboratory-exposed fish did not respond to E2 injection, suggesting that estrogenic compounds either do not alter this enzyme or are present at ineffective concentrations. Similar to our findings, common carp treated with 17α-ethynlestradiol, a potent synthetic estrogen, did not alter CYP3A levels [34]. In contrast, CYP3A levels in trout species were lower after E2 treatment [35,36]. These results suggest that species differences in CYP3A regulation may exist.

Studies of vertebrates suggest that CYP3A and its associated activity, testosterone-6β-hydroxylase (T-6β-Ohase), may be sexually differentiated in some species. In sexually mature rainbow trout and turbot, females had lower CYP3A levels than males [23,29]. The T-6β-OHase activity was significantly

lower in maturing females than in juvenile and mature rainbow and brook trout [36,37]. Sexually mature female winter flounder, on the other hand, exhibited higher T-6β-OHase activity [38] and P450A (putative CYP3A) levels than sexually mature males [39]. Sexually mature mummichogs ([33]; present study) and sexually mature scup [39] do not exhibit gender differences in this protein. Our results further suggest that levels of CYP3A protein are not altered by exposure to E2 in mummichogs. The gender specificity of CYP3A also appears to be species specific in mammals, with levels of CYP3A higher in adult male rats than in adult females, higher in adult female humans than in adult males, and not sexually differentiated in rabbits [40]. Further work is needed to elucidate the regulation of CYP3A in vertebrates.

The E2OHase activities in laboratory- and field-exposed fish in the present study did not respond to either steroids or contaminant mixtures. Studies of E2OHase in vertebrates indicate that this enzyme may be regulated differentially in vertebrate groups, with fish showing increases or no response to hormonal and/or chemical treatments. English sole treated with aromatic hydrocarbon– and chlorinated hydrocarbon–contaminated sediment extracts from the Duwamish Waterway (WA, USA) had 1.5-fold lower E2OHase activity levels than controls [41]. Similarly, hepatic E2OHase activity was reduced by BNF treatment in scup [11]. In contrast, hepatic E2OHase activity was unaltered in winter flounder treated with the PAH and CYP1A inducer BNF [11], similar to our findings in contaminant-exposed mummichog. Hormonal effects on E2OHase also appear to be species specific in fish. We found that treatment with the female hormone E2 did not affect E2OHase activity in mummichogs. However, sexually mature scup and winter flounder do exhibit sex differences in E2OHase [11,39], with E2 treatment lowering E2OHase activities in winter flounder [39]. The E2OHase in mammals also exhibits species specificity. The E2OHase activity is higher in adult male rats than in adult females and higher in adult female humans than in adult males [42]. Clearly, more work is needed to elucidate endogenous and xenobiotic regulation of this enzyme in fish.

Probing with anti-trout $P450_{con}$ PAb, we observed a single CYP3A band of approximately 56 kDa in most mummichog (*F. heteroclitus*) microsomal samples. Up to two secondary bands of slightly lower molecular weight were seen in some samples (data not shown), possibly indicating either degradation of the protein or multiple CYP3A forms. Celander et al. [33] have reported multiple CYP3A proteins in some teleost species, including mummichogs. The CYP3A proteins of scup, Atlantic cod, winter flounder [33], and common carp [34], ranging from 50 to 59 kDa, are recognized by this PAb. Our results are consistent with work by others reporting CYP3A in a variety of teleost species [7,8,23,29,30,33,35,36,39,43].

Unlike CYP3A, CYP1A is strongly and consistently regulated by sex hormones in all species of fish studied to date. Consistent with studies in other fish [29,39,44,45], we found that CYP1A levels failed to be induced in sexually mature female mummichogs from a contaminated site (Newark Bay) relative to male fish from the same site. It is well documented that the high levels of E2 in sexually mature female fish are responsible for this suppression [29,39,45].

Our results agree with previous reports suggesting that CYP1A is not an estradiol 2-hydroxylase in fish as it is in mammals. Both CYP1A protein and activity were uncoupled from E2OHase activity in pollutant-exposed fish, suggesting that CYP1A does not catalyze this reaction in fish. Similarly, Snowberger and Stegeman [11] found that E2OHase activity is not inhibited in hepatic microsomes from teleosts incubated with antibodies against P450E (putative CYP1A).

CONCLUSION

These results suggest that endocrine disruption is not widespread in this system. NY-NJHC resident fish from two sites are not experiencing estrogenic effects from either the sediments or the natural waters at those locations. These fish are responding to compounds associated with toxic effects in wildlife, as demonstrated by their elevated CYP1A levels. However, the long-term effects on these fish are not fully understood, although some populations have developed tolerance to chemicals in the NY-NJHC (Newark Bay mummichogs [15]). While the NY-NJHC experiences heavy contaminant inputs from commercial harbor traffic, urban runoff, and the effluent inputs of several MSTPs, the enormous volume of water and strong currents in this system may be sufficient to ameliorate the estrogenic, if not other, effects of these pollutants on resident and migratory fishes. Finally, E2OHase activity and CYP3A protein do not respond to organic extracts of contaminated sediments or E2, suggesting that these are not sensitive biomarkers of exposure.

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