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Is hepatic cytochrome P4501A1 expression predictive of hepatic burdens of dioxins, furans, and PCBs in Atlantic tomcod from the Hudson River estuary?

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Abstract

Hepatic cytochrome P4501A1 (CYP1A1) expression in fishes is frequently used to evaluate bioavailable aromatic hydrocarbon contamination of aquatic ecosystems. In controlled laboratory experiments, CYP1A1 expression in naïve fishes is usually dose-responsive to aromatic hydrocarbons and in field studies levels of gene expression in natural populations often correspond with known levels of sediment-borne contaminants. We quantified CYP1A1 mRNA levels in juvenile Atlantic tomcod *Microgadus tomcod* from 42 sites in the Hudson River estuary to evaluate the correspondence between hepatic CYP1A1 expression and hepatic concentrations of persistent halogenated aromatic hydrocarbons and to determine the utility of CYP1A1 expression as a biomarker in evaluating the microgeographic distribution of bioavailable contaminants within a large aquatic ecosystem. We found significant spatial heterogeneity in CYP1A1 mRNA levels among collection sites with levels of gene expression differing in some cases by 23–34 folds. CYP1A1 mRNA expression was highest in tomcod from the Newark Bay complex and lowest in tomcod from the most upriver collection sites in the main stem of the Hudson River. Although levels of PCDDs, PCDFs, and PCBs expressed as TCDD TEQs and CYP1A1 mRNA were highest in tomcod from the Newark Bay complex, there was no relationship between hepatic halogenated aromatic hydrocarbon levels and hepatic CYP1A1 mRNA in tomcod from sites in the main stem of the Hudson River. These results suggest that levels of CYP1A1 expression in fish from sites highly polluted with mixtures of halogenated aromatic hydrocarbons and other xenobiotics may not always be reflective of levels of bioavailable aromatic hydrocarbon contaminants. Based on these results and earlier controlled laboratory experiments, we hypothesize that elevated levels of CYP1A1 expression in tomcod from the Hudson River may be due primarily to PAHs or other contaminants not measured in this study.
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Keywords: Biomarkers; Gene expression: Dioxins; Furans: PCBs; Hudson River

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1. Introduction

A paradigm in aquatic toxicology is that hepatic cytochrome P4501A1 (CYP1A1) induction in fishes is sensitive to environmentally relevant concentrations of aromatic hydrocarbons, that the window of gene expression is broad resulting in potentially high levels of induction, and that the levels of expression are responsive to graded doses of these contaminants. As a result, CYP1A1 expression in fishes is frequently used as a biomarker of exposure to aromatic hydrocarbon contaminants and early biological effect. CYP1A1 transcription is mediated by activation of the aryl hydrocarbon receptor (AhR) pathway and it is believed that AhR function is necessary for most toxic consequences from exposure to aromatic hydrocarbons (Hahn, 1998). Thus, induced hepatic CYP1A1 in natural populations from polluted sites is often associated with teratogenicity, early life stage mortality (Wright and Tillitt, 1999), elevated levels of overall hepatic DNA damage (Stein et al., 1994) and increased prevalence of liver tumors (Wirgin et al., 1994).

In naïve fish, CYP1A1 transcription is dose responsively induced at the transcriptional (Courtenay et al., 1999) and translational (Stegeman and Hahn, 1994) levels by controlled laboratory exposures to certain polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), coplanar polychlorinated biphenyls (PCB) congeners and polycyclic aromatic hydrocarbons (PAHs). PAHs are very rapidly metabolized by CYP1A1-encoded enzymes while metabolism of halogenated aromatic hydrocarbons such as PCBs is far slower and the prototypical CYP1A1 inducer 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) may be refractory to metabolism by cytochrome P450-encoded enzymes. As a result, the halogenated aromatic hydrocarbons are highly persistent in lipophilic tissues (Landi et al., 1998; Burton et al., 1998) and their concentrations often biomagnify at the apex of aquatic food chains. In contrast, PAHs are not directly quantifiable in fish livers because of their rapid metabolism (Varanasi and Stein, 1991) and thus surrogate indices, fluorescent metabolites of PAHs in bile (Krahn et al., 1986, 1987) and ^{32}P postlabeling of hepatic DNA adducts, are frequently used to quantify exposures to PAHs (Stein et al., 1994).

Hudson River biota have been chronically challenged with exposure to unusually high levels of mixtures of aromatic hydrocarbon contaminants including PCBs, PCDDs, PCDFs, PAHs and a variety of metals (Squibb et al., 1991). PCBs were discharged at Hudson River, River Miles (RM) 195 and RM 197 from two facilities that manufactured electrical capacitors. It has been estimated that between 209,000 and 1,330,000 pounds of PCBs were released in total from these two sources between 1947 and 1977 (TAMS Consultants, 1999). With the removal of the Fort Edwards Dam at RM 195 in 1973, PCBs were transported down river and their distribution in sediments and biota formed a gradient from upriver to the mouth of the estuary in New York City (Feng et al., 1998). Between the early 1970s and 1989, it was estimated that approximately two-thirds of the PCBs deposited in the lower main stem of the Hudson River were derived from upper Hudson River sources and their downstream transport (Bopp et al., 1998). PCB loading from New York City wastewater treatment plants and combined sewer outflows probably also contributed significantly, particularly in recent years, to PCB contamination in the lower river (Farley and Thomann, 1998). Elevated levels of sediment-borne PCBs were also reported in the Newark Bay complex probably attributable to several electrical manufacturing facilities, paint manufacturers, and other industries (Wenning et al., 1994).

The largest known source of PCDDs and PCDFs to the Hudson River estuary was a chemical manufacturing facility on the Passaic River, NJ (a tributary of Newark Bay) (Fig. 1), which between 1948 and 1969 synthesized the herbicide 2,4,5-T, a major component of Agent Orange. Bopp et al. (1991) estimated that approximately 4–8 kg of TCDD were released from this site into Newark Bay resulting in sediment concentrations of up to 21,000 ppt TCDD near the Passaic River source. PCDDs and PCDFs in the main stem of the Hudson River probably originate from municipal solid waste incineration and biomass combustion (Skinner et al., 1997) and very high levels of

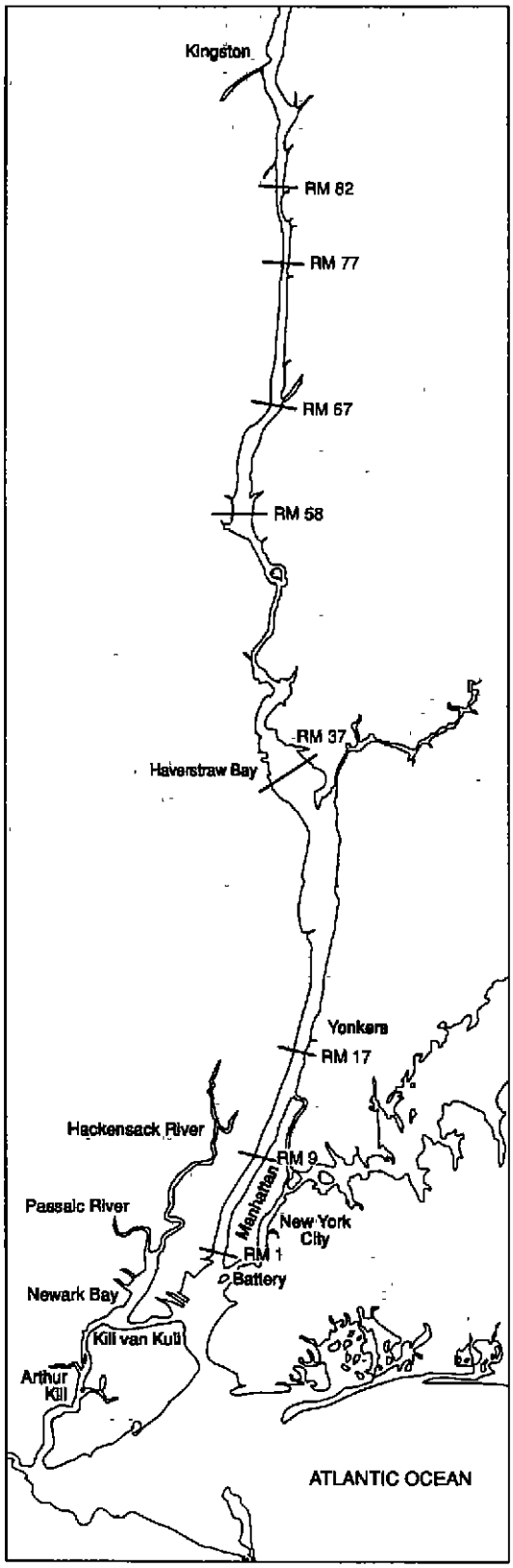

Fig. 1.

sediment-borne octachlorodibenzo-*p*-dioxin with TCDD TEQs up to 2100 ppt have recently been reported at an upper Hudson River site near Troy, NY, (RM 155) (Bopp et al., 1998).

Sediments in the Upper Harbor of the Hudson River-Raritan Bay estuary exhibited the second highest levels of total PAHs among all U.S. estuaries monitored from 1984 to 1987 in the NOAA National Status and Trends Program (NOAA, 1988). Numerous industrial and municipal sources of PAHs to the Hudson River estuary include creosote wood preserving facilities, petroleum storage and refining facilities, chemical and paint manufacturing, combined sewer and stormwater outfalls, and municipal sewage treatment plants. The Newark Bay complex has been contaminated by numerous oil spill events in the Arthur Kill and a multitude of chemical manufacturing facilities and combined sewer and stormwater outfalls on the lower Passaic River (Huntley et al., 1993).

Atlantic tomcod (*Microgadus tomcod*) is an abundant, anadromous species in Atlantic coast estuaries from Labrador to the Hudson River. Since it is bottom-dwelling, dependent on benthic prey, has a lipid-rich liver, and a tendency to reside year-round in its natal estuaries, tomcod from highly polluted sites often bioaccumulate unusually high concentrations of halogenated aromatic hydrocarbon contaminants (Courtenay et al., 1999; Roy et al., 2001). In the early 1980s, tomcod from the Hudson River population exhibited one of the highest prevalences of liver cancer ever observed in a natural population and at the same time, the age structure of the Hudson River population was truncated compared with tomcod from elsewhere (Dey et al., 1993).

Earlier, we reported that levels of hepatic CYP1A1 mRNA expression were significantly higher in environmentally exposed adult tomcod from the Hudson River compared with the tomcod from four other less contaminated Atlantic coast estuaries (Kreamer et al., 1991; Wirgin et al., 1994). In these studies, elevated levels of

Fig. 1. A map of the mid and lower Hudson River estuary including the Newark Bay complex showing those sites from which young-of-the-year Atlantic tomcod were sampled in this study for both hepatic halogenated aromatic hydrocarbon contaminants and hepatic CYP1A1 mRNA expression.

CYP1A1 expression directly correlated with levels of bile metabolites of PAHs and hepatic DNA adducts detected by ^{32}P postlabeling, indicating that Hudson River adults were exposed to elevated levels of PAHs and that these had incurred DNA damage. Subsequent studies revealed that hepatic levels of coplanar PCBs that induce CYP1A1, TCDD, total PCDDs and PCDFs were substantially higher in adult tomcod from the Hudson River compared with the adult tomcod from cleaner rivers (Courtenay et al., 1999; Roy et al., 2001). Yet, in controlled laboratory studies, CYP1A1 mRNA was not inducible in tomcod from the Hudson River that were i.p. injected with PCBs or TCDD, but was inducible in tomcod i.p. injected with benzo[a]pyrene (B[a]P) or beta-naphthoflavone (β-NF) (Wirgin et al., 1992; Courtenay et al., 1999).

While these results show that CYP1A1 mRNA expression is higher in tomcod from polluted compared with cleaner estuaries, they also suggest that levels of gene expression may be lower than predicted, based on sediment concentrations or hepatic burdens of halogenated aromatic hydrocarbons. In this study, we tested this hypothesis by comparing levels of CYP1A1 mRNA expression and hepatic loads of PCDDs, PCDFs, and PCB congeners in juvenile tomcod samples from multiple sites in the lower Hudson River estuary including the Newark Bay complex. We also evaluated the use of CYP1A1 expression as a biomarker in the fine scale mapping of sediment loads of bioavailable aromatic hydrocarbons within a large ecosystem such as the tidal Hudson River estuary.

2. Methods

2.1. Sample collections

Juvenile tomcod were used for analysis, for two reasons. First, young-of-the-year tomcod probably move less than adults, making the biomarker response more reflective of the fine scale distribution of bioavailable contaminants. Second, modulation of CYP1A1 mRNA expression associated with sexual development and maturation in both male and female tomcod is avoided (Williams et al., 1998).

Young-of-the-year tomcod ($n = 444$) were collected from late June to early August of 1994–1998 at 39 sites in the main stem of the Hudson River between the Battery in New York City (RM 0) and Kingston, NY, (RM 85) (Fig. 1). Additionally, three sites were sampled in the Newark Bay complex including two sites in the lower Hackensack River (RM 2 and RM 4) and one site at the northern apex of Newark Bay just below where the Passaic River enters the Bay. All fish were collected with bottom trawls; fish from the main stem of the Hudson River were collected at night; those from the Newark Bay complex were collected during the day. Fish were immediately sacrificed, measured, their livers excised, snap frozen in liquid nitrogen in 1.5 ml microcentrifuge tubes, and stored at −70°C until processing. A portion of each liver was used for RNA analysis and the remaining tissue from a subset of collections ($n = 11$ sites) was used to quantify hepatic burdens of halogenated aromatic hydrocarbons.

2.2. RNA isolations

Frozen individual livers were homogenized in 1 ml of Ultraspec reagent (Biotecx, Houston, TX) directly in the 1.5 ml microcentrifuge tubes. The homogenates were incubated on ice for 10 min, 200 μl of chloroform was added, vortexed, and incubated on ice for an additional 10 min. Homogenates were centrifuged at $15\,000 \times g$ for 15 min at 4°C, the aqueous phase was saved and mixed with an equal volume of cold isopropanol and incubated on ice for 45 min. Homogenates were centrifuged at $15\,000 \times g$ for 15 min at 4°C to pellet total RNA. RNA pellets were washed twice with cold 70% EtOH, centrifuged, air-dried, resuspended in 50 μl of DEPC-treated water and stored at −70°C until use.

2.3. RNA analyses

Spectrophotometric analysis at 260 and 280 nm was used to initially evaluate the purity of RNA isolations and to quantify total RNA concentra-

tions. All RNA samples were further analyzed for their integrity using northern gels. RNAs were denatured and electrophoresed in 1.0% MOPS agarose gels (Fourney et al., 1988), stained in ethidium bromide solution and photographed under UV illumination to evaluate the integrity of the 28S and 18S ribosomal RNA bands. RNAs were reisolated from degraded samples.

CYP1A1 mRNA levels were quantified using slot blot analyses. About 2 μg of total RNAs were applied directly to Nytran Plus membranes (Scheicher and Schuell (S&S)) using an S&S slot blot manifold as described in Courtenay et al. (1999). Membranes were baked for 2 h under vacuum and stored under vacuum until use. Prehybridizations for 3 h at 65°C and hybridizations overnight at 65°C were performed as described in Kreamer et al. (1991) using ^{32}P radiolabeled, β-NF induced, full length, tomcod CYP1A1 cDNA probes (Roy et al., 1995). After hybridizations, membranes were washed three times for 5 min each in 6 × SSPE/0.1% SDS at room temperature. Final wash stringencies were 1 × SSPE/0.1% SDS at 65°C for 1 h and autoradiography or phosphorimaging were used to visualize mRNA bands. CYP1A1 mRNA levels were quantified from X-ray films using the Whole Band Analysis package in the Millipore Bio Image Analysis System or from phosphor imaging screens using a Storm 860 Scanner and Molecular Dynamics ImageQuant™ for Macintosh software version 1.0.

To ensure equal loading of RNAs, membranes were stripped of the tomcod CYP1A1 probes by their immersion 2–3 times in boiling water and rehybridized exactly as described above to a ^{32}P radiolabeled rat 18S rRNA probe, pHRR118 (Chan et al., 1984). rRNA concentrations in individual specimens were quantified as described above. Our earlier studies have shown little variability in 18S rRNA expression in tomcod exposed to heterogeneous environments, whereas variability in β-actin expression was often high (Wirgin et al., 1996).

2.4. Measurement of hepatic levels of halogenated aromatic hydrocarbons

Concentrations of PCDDs, PCDFs, and PCBs were measured in the pooled livers ($n = 5–10$ livers per site) of tomcod collected from 11 sites in the Hudson River estuary (Fig. 1). Analyses were carried out at Fisheries and Oceans Canada's Institute of Ocean Sciences in Sidney, BC. Dioxin/furan analyses were performed in accordance with the Environment Canada Reference Methods 1/RM/19 and 1/RM/23 (Environment Canada, 1992a,b). During carbon fiber cleanup of the dioxin samples, all 209 PCB congeners were also collected from an automated high-performance liquid chromatography system. From this system four fractions were collected:fraction I contained the di-*ortho*-PCBs, fraction II contained the mono-*ortho*-PCBs, fraction III the non-*ortho*-PCBs, and fraction IV the PCDDs and PCDFs. Fractions were concentrated to less than 10 μl, spiked with corresponding ^{13}C-labeled method performance standards and analyzed by high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). Details on methods, surrogate internal standard solution composition, and on the solvents and conditions used in all the clean-up and fractionation steps are in MacDonald et al. (1997). Tissue samples were analyzed in batches of 12, which included one procedural blank, one certified reference material, and nine real samples of which, one was analyzed in duplicate. The criteria for PCB identification and quantification, and the quality assurance and quality control measures undertaken for the sample work-up and the HRGC/HRMS analysis were similar to those outlined in the Environment Canada protocols for PCDDs/PCDFs analyses (Environment Canada, 1992a,b). Hepatic wet weight concentrations of PCDD, PCDF, and PCB congeners are presented as well as original (untransformed) TCDD TEQs (2,3,7,8 tetrachlorodibenzo-*p*-dioxin Toxic Equivalent Quotients) using fish-specific toxic equivalent factors (TEFs) proposed by Van den Berg et al. (1998).

All statistical analyses were carried out on wet-weight data. Due to the small quantities of tissues available for chemical analyses (generally less than 1 g), percent moisture was not quantified and percent lipid while quantified was considered

likely to be over inflated (39–79%) and so was not reported or used for lipid-normalization of data for comparisons.

2.5. Analyses of CYP1A1 expression data

Both CYP1A1 mRNA and chemical contaminant data were log-transformed for analysis following examination in probability plots. Sites at which at least 5 fish were sampled were compared by ANOVA followed by Tukey's multiple range test. Associations of CYP1A1 mRNA expression with River Mile within the main stem of the Hudson River and with hepatic contaminant burdens measured in the same fish from the same sites were analyzed by linear regression. Regressions against contaminant burdens were calculated as recommended by Zar (1999) for multiple dependent data (CYP1A1 mRNA levels) for each independent datum (contaminant burdens in tissue pools from 11 sites). Where relationships were found to be significantly non-linear despite transformations, significance of slope deviation from zero was tested against within-group mean sum of squares rather than residual mean sum of squares and this is reflected in the reported degrees of freedom of those tests. For CYP1A1 mRNA levels, sample means and 95% confidence intervals were back-transformed to original units for presentation.

3. Results

3.1. Hepatic concentrations of halogenated aromatic hydrocarbons

Wet-weight concentrations of halogenated aromatic hydrocarbons expressed in units of TCDD-TEQs were dominated at most sites in the Hudson River estuary by PCDDs and PCDFs (Table 1). Hepatic TEQs were much higher in tomcod from the Newark Bay complex than in fish from elsewhere in the lower Hudson River estuary (Fig. 2). Tomcod from the Hackensack River and the apex of Newark Bay exhibited hepatic TEQs of 1052 and 743, respectively, compared with 85 in tomcod from the most contaminated sample (RM 17) from the main stem of the Hudson River. Within the main stem of the Hudson River, TEQs declined both downstream of RM 17 to 52 TEQ's at RM 1 and upstream of RM 17 to 7 TEQ's at RM 82. PCDDs and PCDFs contributed 97% of the TEQs at the most contaminated sites (Hackensack River and Newark Bay), but only 14% at the least contaminated site (RM 82), where coplanar PCBs contributed 52% of the TEQs and mono-ortho PCBs contributed the remaining 34% of the TEQs. At the most contaminated Hudson River site (RM 17), PCDDs and PCDFs contributed 78% of TEQs.

Across most sites sampled, there was a strong correlation between TEQs from PCDDs and PCDFs and contribution (i.e. % TEQ) by TCDD. TCDD contributed 85 and 88% of the TCDD TEQs at the Hackensack River and Newark Bay sites, respectively (Table 1). Hepatic levels of TCDD differed greatly among sites with the highest levels found in fish from the Hackensack River (897 pg/g ww) and Newark Bay (655 pg/g ww). For comparison, hepatic levels of TCDD in tomcod from the main stem of the Hudson River were much lower and ranged from 0 to 28 pg/g (ww) with highest levels observed in tomcod from

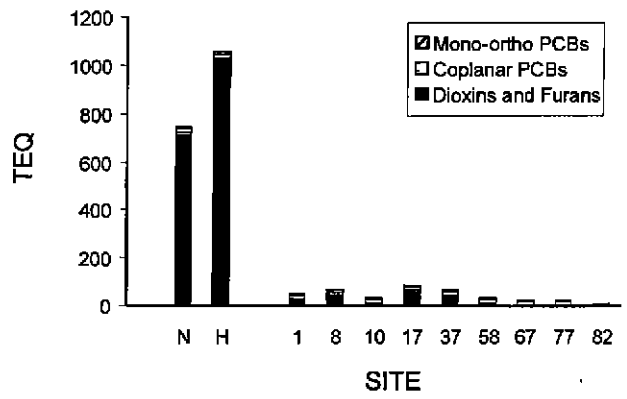

Fig. 2 Dioxins and furans, coplanar PCBs, and mono-ortho PCBs expressed as TCDD Toxic Equivalent Quotients (TEQs) in livers of young-of-the-year Atlantic tomcod from the Hudson River estuary collected between 1994 and 1998 N: Newark Bay, H: Hackensack River, Numbers 1–82: Hudson River Miles from the Battery at the southern tip of Manhattan Island.

Table 1
Wet-weight concentrations of halogenated aromatic hydrocarbons in livers of young-of-the-year tomcod from various River Miles (RM) of the Hudson River expressed in pg/g or ng/g and TCDD Toxic Equivalent Units (TEQs) based on fish-specific Toxic Equivalent Factors (TEF) (Van den Berg et al., 1998)

		Newark Bay	Hackensack River	Hudson River								
				RM1	RM8	RM10	RM17	RM37	RM58	RM67	RM77	RM82
Dioxins and Furans												
2,3,7,8-TCDD[a]	pg/g	655	897	26	28	14	27	9	4	0	1	0
	TEQ	655	897	26	28	14	27	9	4	0	1	0
Total PCDD[b]	pg/g	728	1039	100	84	75	98	143	101	52	38	88
	TEQ	671	921	31	36	14	35	16	8	3	4	0
2,3,7,8-TCDF[c]	pg/g	103	99	71	98	65	146	94	51	42	39	17
	TEQ	5	5	4	5	3	7	5	3	2	2	1
Total PCDF[d]	pg/g	380	697	129	200	93	287	282	155	149	133	49
	TEQ	50	103	13	20	12	31	27	13	9	7	1
Coplanar PCBs												
Total Coplanars[e]	pg/g	100 472	118 085	36 418	58 234	29 528	73 301	111 759	70 627	57 364	52 301	22 668
PCB77[f]	pg/g	32 224	37 886	12 747	17 970	10 067	25 256	31 674	23 167	16 549	14 855	6459
	TEQ	3	4	1	2	1	3	3	2	2	1	1
PCB81[g]	pg/g	1945	2628	514	810	355	1031	1957	1354	714	653	351
	TEQ	1	1	0	0	0	1	1	1	0	0	0
PCB126[h]	pg/g	2251	2945	824	1371	599	1790	2282	1271	817	765	593
	TEQ	11	15	4	7	3	9	11	6	4	4	3
PCB169[i]	pg/g	91	110	42	61	31	82	88	45	30	28	28
	TEQ	0	0	0	0	0	0	0	0	0	0	0
Mono-ortho PCBs												
Total Mono-orthos[j]	ng/g	4233	4663	1731	2726	1841	3286	6947	4117	3191	3029	1572
Subset[k]	ng/g	1246	1581	565	958	505	1255	1998	1237	806	722	486
	TEQ	6	8	3	5	3	6	10	6	4	4	2
Di-ortho PCBs												
Total Di-orthos[l]	ng/g	9554	11 531	5081	8164	4793	10 916	27 311	14 960	13 834	13 338	6094

[a] 2,3,7,8 tetrachlorodibenzo-*p*-dioxin.
[b] Sum of polychlorinated dibenzo-dioxins including tetra-, penta-, hexa-, hepta-, and octa-chlorinated dibenzo-dioxins
[c] 2,3,7,8 tetrachlorodibenzofuran.
[d] Sum of polychlorinated dibenzo-furans including tetra-, penta-, hexa-, hepta-, and octa-chlorinated dibenzo-furans
[e] Sum of all 18 coplanar polychlorinated biphenyls including di-, tri-, tetra-, penta- and hexa- chlorinated biphenyls
[f] 3,3',4,4'-tetrachlorobiphenyl.
[g] 3,4,4',5-tetrachlorobiphenyl.
[h] 3,3',4,4',5-pentachlorobiphenyl
[i] 3,3',4,4',5 5'-hexachlorobiphenyl.
[j] Sum of all 46 mono-ortho polychlorinated biphenyls including tetra-, penta-, hexa-, and hepta-, chlorinated biphenyls
[k] Subset of 8 mono-ortho PCBs for which TEF's are available 2',3,4,4',5-PeCB (PCB 123), 2,3',4,4',5-PeCB (PCB 118), 2,3,4,4',5-PeCB (PCB 114), 2,3,3',4,4'-PeCB (PCB 105), 2,3',4.4',5,5'-HxCB (PCB 167), 2,3,3',4,4',5-HxCB (PCB 156), 2.3,3',4,4',5'-HxCB (PCB 157) and 2,3,3',4,4',5,5'-HpCB (PCB189).
[l] Sum of all 141 di-ortho polychlorinated biphenyls including di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, and nona, and deca-chlorinated biphenyls

mdownriver sites extending from New York City (RM 0) to Yonkers, NY, (RM 17) and much lower levels were observed in fish from RM 37 to RM 82.

The ratios of total PCDDs to total PCDFs expressed on a wet weight basis differed between tomcod from the Newark Bay complex and tomcod from the main stem of the Hudson River. A similar phenomenon was observed, when considering the ratios of 2,3,7,8-TCDD to 2,3,7,8-TCDF on a wet basis between the two sites. The ratio of total PCDDs:PCDFs in tomcod from the Newark Bay complex was 1.5–1.9, whereas concentrations of PCDFs exceeded those of PCDDs in tomcod at eight of nine sites in the main stem of the Hudson River (mean PCDDs:PCDFs ratio = 0.66). This suggests that sources of PCDDs and PCDFs to the Newark Bay complex are probably different than those to the main stem of the Hudson River.

Surprisingly, hepatic wet weight concentrations of coplanar and mono-*ortho* PCBs were also very high in young-of-the-year tomcod from the Newark Bay complex and burdens and coplanar congener profiles were similar to those in tomcod in the most contaminated samples (RM 17 to RM 37) from the main stem of the Hudson River. Within the Hudson River, levels of PCBs were relatively low in tomcod from the New York City area (RM 1 to RM 10), levels increased in the Yonkers, NY, area (RM 17), peaked in the area of Haverstraw Bay (RM 37), and then decreased linearly upriver with the lowest levels observed in tomcod at the most upriver site sampled (RM 82). At all sites within the Hudson River estuary, including Newark Bay, hepatic concentrations of PCB 77 far exceeded concentrations of other congeners known to induce CYP1A1 expression. In Newark Bay, PCB 77 constituted 87–88% of coplanar PCBs congeners known to induce CYP1A1 and among Hudson River sites PCB 77 contributed 87–91%. At all sites, PCBs contributed a very small percentage of TEQs because of the relatively low TEFs recommended for use in fish studies (0.005–0.00005) compared with those for humans/mammals and birds (Van den Berg et al., 1998).

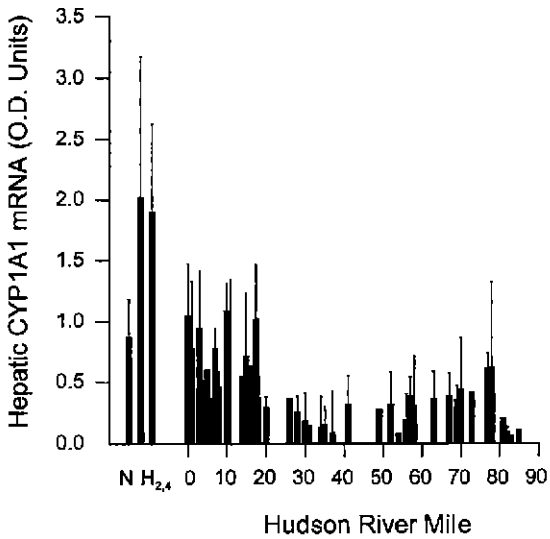

Fig. 3. Relative hepatic CYP1A1 mRNA levels (integrated optical density units) in young-of-the-year Atlantic tomcod from Newark Bay (N), Hackensack River Miles 2 and 4 ($H_{2,4}$), and 39 sites in the main stem of the Hudson River between River Miles (RM) 0 (the Battery at the southern tip of Manhattan Island) and RM 85 (Kingston, NY). Vertical lines indicate 95% confidence intervals for the 32 samples of $N = 5$–30.

3.2. Levels of CYP1A1 mRNA expression

There was considerable variation in hepatic CYP1A1 mRNA levels among juvenile tomcod samples from the Hudson River estuary (Fig. 3). Hepatic CYP1A1 mRNA expression was highest in tomcod from the Newark Bay complex with fish from the two sites in the Hackensack River exhibiting the highest levels of CYP1A1 mRNA among all locales surveyed. Levels of CYP1A1 were approximately two-fold higher in fish from the Hackensack River than in the sample from the apex of Newark Bay. Lowest levels of CYP1A1 mRNA were observed in tomcod from the most upriver sites in the Hudson River. For comparison, levels of CYP1A1 mRNA in tomcod from the two Hackensack River sites were 23–34 times higher than in tomcod from Kingston, NY (RM 82–85).

Over all sites within the main stem of the Hudson River, there was a weak but significant decrease in hepatic CYP1A1 mRNA moving upriver from RM 0 to RM 85 ($\ln(\text{CYP1A1 mRNA}) = -0.422 - 0.013$ (RM); $R^2 = 0.114$;

$F_{(1402)} = 51.53$; $P < 0.001$). Significant differences in mean CYP1A1 mRNA were detected among Hudson River sites at which at least 5 individuals were sampled ($F_{(28\,339)} = 8.12$, $P < 0.001$) which relate to the following pattern. CYP1A1 mRNA levels were highest near the mouth of the Hudson River (RM 0), were quite variable moving upriver to RM 18 and then dropped to RM 37. Further upriver, levels increased up to RM 78 but then dropped sharply from RM 81 to RM 85 to levels comparable to that at RM 37.

3.3. Relationship between hepatic CYP1A1 mRNA and concentrations of HAHs

Hepatic burdens of the halogenated aromatic hydrocarbons-PCDDs, PCDFs, and PCBs expressed in units of TCDD TEQs were a significant, but weak predictor of hepatic CYP1A1 mRNA in the juvenile tomcod caught at the 11 sites throughout the lower Hudson River estuary including the Newark Bay complex (ln(CYP1A1 mRNA) = $-1.617 + 0.263$ ln(TEQs); $R^2 = 0.155$; $F_{(1,\ 135)} = 47.62$; $P < 0.0005$) (Fig. 4). Furthermore the relationship between contaminants and CYP1A1 mRNA expression was significantly non-linear even after log transformation of both variables ($F_{(9,\ 135)} = 13.81$; $P < 0.0005$). Within the main stem of the Hudson River the relationship between HAH contaminants and CYP1A1 mRNA expression was significantly non-linear ($F_{(7,\ 97)} = 15.64$; $P < 0.0005$) with no significant slope to a straight line forced through the points (ln(CYP1A1 mRNA) = $-1.355 + 0.191$ ln (TEQs); $R^2 = 0.012$; $F_{(1,\ 97)} = 2.60$; $P > 0.10$). Rather, CYP1A1 mRNA levels appeared to increase with hepatic HAHs to a level of approximately 30–40 TEQs and then declined somewhat as contaminant levels increased to 85 TEQs — the highest level observed in the main stem Hudson River (Fig. 4).

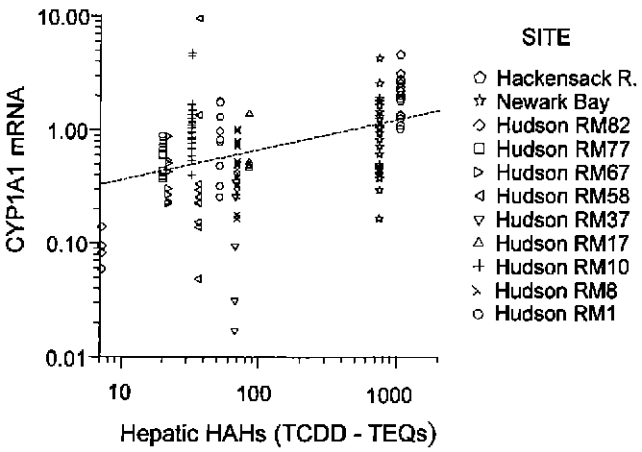

Fig. 4. Relationship between hepatic halogenated aromatic hydrocarbons (HAH), dioxins, furans and PCBs in units of 3,3′4,4′-tetrachlorodibenzo-*p*-dioxin Toxic Equivalency Quotients (TCDD TEQs) and CYP1A1 mRNA expression in livers of juvenile tomcod from the lower Hackensack River, Newark Bay, and rivers miles (RM) 1 to 82 in the main stem of the Hudson River The dotted line represents a linear regression through 4–30 CYP1A1 mRNA expression measures at each of the 11 sites assayed for hepatic HAH contaminants (ln(CYP1A1 mRNA) = $-1.617 + 0.263$ ln (hepatic HAHs); $R^2 = 0.155$; $F_{(1,\ 135)} = 47\ 62$; $P < 0.0005$, $N = 146$).

4. Discussion

The hepatic concentrations of TCDD in juvenile tomcod from the Newark Bay complex in this study are among the highest ever observed in a natural population. At 897 and 655 pg/g TCDD (ww), livers of juvenile tomcod from the Hackensack River and Newark Bay, respectively, are actually somewhat more contaminated than livers of adult tomcod sampled from sites in the Hackensack River in December 1996 (554 and 208 pg/g TCDD (ww) for males and females, respectively, (Courtenay et al., 1999)). Hepatic levels of TCDD in juvenile tomcod from the main stem of the Hudson River were much lower and ranged from 0 to 28 pg/g (ww) with highest levels in tomcod from downriver sites extending from New York City to Yonkers, NY. Hepatic TCDD burdens in juvenile tomcod from the main stem of the Hudson River were also very similar to levels recorded in adult tomcod collected in recent years (11–47 pg/g (ww)) (Courtenay et al., 1999 Roy et al., 2001). For comparison, the hepatic level of TCDD in female tomcod from the pristine Margaree River, Nova Scotia, was 1 and 38 pg/g in female tomcod from the Miramichi River, New Brunswick, at which a bleached kraft pulp and paper mill operates (Courtenay et al., 1999).

These results suggest that halogenated aromatic hydrocarbon burdens are accumulated by Hudson River tomcod and come to equilibrium with the local environment early on in life as the juveniles sampled in this study had been spawned only 5–7 months earlier. In fact, the temporal window of exposure needed to incur such high tissue burdens of aromatic hydrocarbons must be narrow, because young-of-the-year tomcod do not settle to the benthic strata and do not begin to feed independently until mid April–May of their first year (Klauda et al., 1988). Studies have demonstrated the teratogenicity and lethality of TCDD and dioxin-like compounds to young life stages of several species of freshwater fish at TEQs that are similar to those observed in this study of young-of-the-year tomcod (Walker et al., 1991 Elonen et al., 1998). This suggests that possible damage may be occurring to young life stages of tomcod in the Hudson River from exposure to these compounds.

Levels of total PCBs, total coplanar PCB congeners, and individual coplanar PCB congeners that induce CYP1A1 in fish were much higher in tomcod from all sites in the Hudson River estuary compared with tomcod from elsewhere. For example, levels of hepatic coplanar PCB congeners (18 congeners) exceeded 100 000 pg/g in young-of-the-year tomcod from Newark Bay and Hudson River RM 37 compared with a mean of 182 pg/g in five pools of livers (4–5 livers per pool) from adult female tomcod from the Miramichi River, NB (Roy et al., 2001). However, despite these high tissue burdens, PCBs contributed very little to the TEQs calculated for tomcod from the Hudson River estuary because of the low TEFs for PCB congeners recommended for use in fish (Van den Berg et al., 1998). It should be noted that these fish TEFs are almost exclusively derived from early life stage mortality studies conducted on a single species, rainbow trout *Oncorhynchus mykiss*. Given the magnitude of interspecific variation in CYP1A1 inducibility in fish, including Hudson River taxa (Wirgin et al., 1996), and in early life stage teratogenicity and mortality (Walker et al., 1991; Elonen et al., 1998), it would be prudent to conduct studies on additional ecologically and phylogenetically diverse taxa to increase confidence in these values.

Given the magnitude of upriver sources and down river transport of PCBs, one might have predicted a gradient in hepatic PCB levels in tomcod with Hudson River river miles. In fact, that is exactly what was reported for PCB levels in the fillets of white perch *Morone americana* and yellow perch *Perca flavescens* collected between Hudson River RM 143.5 and RM 25.8 (Farley and Thomann, 1998). Given the known upriver range of young-of-the-year tomcod in the Hudson River to at least Albany, NY, (RM 140) (Klauda et al., 1988), it would be informative in future studies to increase upriver coverage to further evaluate the possibility of an association between hepatic PCB levels and river miles. It is also somewhat surprising that hepatic PCB burdens and congener profiles were similar between young-of-the-year tomcod from the Newark Bay complex and the most contaminated Hudson River sites. The absence of a gradient in hepatic PCB burdens with river miles and the similarity of congener profiles between tomcod from Newark Bay and the Hudson River suggests that down river municipal sources may be primarily responsible for the elevated tissue burdens of PCBs observed in tomcod in this study. It is also possible that young-of-the-year tomcod from the Hudson River exhibit more movement than earlier assumed.

We observed significant spatial heterogeneity of CYP1A1 mRNA expression in juvenile tomcod from among collection sites in the Hudson River estuary. Not unexpectedly, CYP1A1 expression was highest in tomcod from the Newark Bay complex in which levels of sediment-borne PCDDs and PCDFs are the highest in the estuary (Bopp et al., 1998) and at which levels of PCBs and PAHs are also very high (Huntley et al., 1993 Wenning et al., 1994). Significant differences in CYP1A1 mRNA expression in tomcod were observed among collection sites in the main stem of the Hudson River. In this case, differences in levels of CYP1A1 mRNA expression did not correspond to hepatic levels of TEQs or individual classes of halogenated aromatic hydrocarbons. For example, CYP1A1 expression was lowest in tomcod from RM 37, a site at which hepatic levels of TEQs were among the highest. Conversely,

CYP1A1 expression was generally highest at down river sites in the New York City area, locales at which hepatic TEQs were low or moderate.

Based on the lack of correspondence between hepatic halogenated aromatic hydrocarbon contaminants and CYP1A1 levels observed in this study, we hypothesize that CYP1A1 expression levels in the Hudson River tomcod population may primarily reflect exposure to PAHs or other compounds not measured in this study. It is also possible that levels of CYP1A1 expression may reflect a balance between induction by exposure to PAHs and inhibition of expression by exposure to halogenated aromatic hydrocarbons (Courtenay et al. 1999). Unfortunately, because of their rapid metabolism, we were unable to quantify hepatic PAH levels in this study. However, evidence supportive of this assertion includes the following. First, CYP1A1 mRNA was not significantly inducible in adult tomcod collected from the Hudson River, extensively depurated in clean laboratory water, and i.p. injected under controlled laboratory conditions with TCDD, PCB 77, PCB 126, or PCB 169. In contrast, CYP1A1 mRNA was highly inducible in tomcod from the Hudson River that were i.p. injected with B[aP or β-NF (Wirgin et al., 1992; Courtenay et al., 1999). Second, in earlier studies, CYP1A1 mRNA expression declined rapidly in adult tomcod that were collected from the Hudson River, transferred to clean water and sacrificed after 4, 8, 19, 120 and 480 h (Kreamer et al., 1991). This rapid clearance of hepatic CYP1A1 mRNA is characteristic of exposure of tomcod to PAHs (Courtenay et al., 1999). In contrast, induction and clearance of hepatic CYP1A1 mRNA was much more prolonged in PCB 77 or TCDD-treated adult tomcod (Courtenay et al., 1993, 1999). Third, levels of bile metabolites of PAHs were significantly higher in tomcod from the Hudson River than in tomcod from elsewhere (Wirgin et al., 1994). Fourth, levels of hepatic DNA adducts, an index of cumulative lifetime exposure to bulky PAHs, were significantly higher in tomcod from the Hudson River than in fish from elsewhere (Wirgin et al., 1994).

Three mechanisms, individually or in concert, may be invoked to explain the modulation of CYP1A1 inducibility in fish from contaminated sites. First, chronic exposure to persistent compounds such as PCDDs, PCDFs, and PCBs may deplete the cellular pool of AhR and therefore, decrease CYP1A1 inducibility in lipophilic tissues such as liver, which accumulate these contaminants. Evidence supportive of this assertion has been generated *in vitro* in a variety of rodent cells (Pollenz, 1996) and in vivo in multiple tissues in rats exposed to TCDD (Pollenz et al., 1998). Depletion of AhR in cells chronically challenged with TCDD was associated with decreased inducibility of CYP1A1 reporter gene constructs (Pollenz et al., 1998). Furthermore, it was demonstrated that *in vitro* exposure of mouse hepatoma cells to TCDD induced ubiquitination of AhR protein and subsequent proteosome pathway-mediated degradation of AhR within several hours of exposure (Ma and Baldwin, 2000). In fact, hepatic TEQs observed in tomcod from throughout the lower Hudson River estuary approach contaminant levels used to down-regulate AhR expression in rodents and thus, it is conceivable, that environmental exposures of tomcod may deplete AhR to levels that are insufficient to activate CYP1A1 transcription. This assumes that CYP1A1 expression is mediated exclusively through the AhR pathway, a concept that has been debated for some time (Sterling et al., 1994)

Second, studies with killifish *Fundulus heteroclitus* from highly contaminated estuaries suggest that genetic adaptation to aromatic hydrocarbon-induced toxicity may occur in impacted populations. F_1 and F_2 embryos and larvae of parents collected from PCB-contaminated New Bedford Harbor, MA, were 'profoundly less sensitive' to PCB 126 and 3-methyl cholanthrene induced lethality, independent of maternal contaminant transfer, than progeny of parents from reference sites. F_1 and F_2 embryos of parents from New Bedford Harbor also exhibited reduced EROD activity compared with embryos from parents collected from reference sites (Nacci et al., 1999). Reduced CYP1A1 protein expression was also observed in the larval F_1 offspring of killifish collected from Newark Bay and challenged with

Aroclors 1248 and 1262 (Elskus et al., 1999). Although the molecular bases for genetic adaptation in these populations have not been determined, we reported allelic variation in tomcod AhR that maps to the 3′ transactivation domain (TAD) of the AhR (Roy and Wirgin, 1997), a region in rodent AhR that has been demonstrated to serve as a site-specific degron for ubiquitination (Ma and Baldwin, 2000).

Third, epigenetic mechanisms related to pollution exposure may be operative to modulate CYP1A1 transcription. The Hudson River estuary, similar to other highly polluted aquatic ecosystems, is contaminated with complex mixtures of xenobiotics, including metals (nickel and chromium) (Squibb et al., 1991) known to methylate CpG islands in the 5′ regulatory region of inducible genes (Klein and Costa, 1997), such as CYP1A1 (Shen and Whitlock, 1989). Methylation of these sites in gene promoters has been shown to inhibit their enhancer function, and thereby diminish their transcriptional activity. While this mechanism to modulate gene expression has been extensively investigated and described *in vitro* in metal-treated mammalian cells its existence in animals exposed in vivo to environmentally relevant concentrations of these xenobiotics has yet to be explored.

Ratios of hepatic PCDDs to PCDFs differed dramatically between tomcod from the main stem of the Hudson River and Newark Bay. Two implications can be drawn from these results. First, because of the persistence of PCDDs and PCDFs in lipophilic tissues such as liver, these compounds can be used as chemical signatures to track cohorts of fish. These results which are consistent with our earlier collected data from the same sites (Courtenay et al., 1999) suggest that tomcod from the Hudson River and Newark Bay constitute separate unit stocks. Emigration of tomcod from Newark Bay to the main stem of the Hudson River is highly unlikely, however, the possibility of the reverse movement cannot be ruled out. Second, the major sources of PCDDs and PCDFs to the main stem of the Hudson River and Newark Bay are most probably distinct and the physical transport of PCDDs and PCDFs from Newark Bay to the lower Hudson River through the Kill Van Kull is probably minimal.

Unlike controlled acute laboratory treatments, natural populations from highly polluted aquatic ecosystems are chronically exposed for many generations to mixtures of aromatic hydrocarbons and other contaminants. Little is known of the acute effects of mixtures of pollutants on CYP1A1 expression and only recently have the impacts of chronic exposure on the development of genetic or physiological resistance to toxicants been proposed. Specimens that have been used in controlled laboratory experiments usually are either from hatchery-derived stocks or from natural populations from pristine environments. It is clear that fish from contaminated populations often do not respond, as do their conspecifics with naïve backgrounds. Impaired inducibility of CYP1A1 is probably a common phenomenon in fish from highly impacted populations as recent studies with killifish (Prince and Cooper, 1995; Van Veld et al., 1996; Hahn, 1998; Nacci et al., 1999) and European sea flounder *Platichthys flesus* (Besselink et al., 1998) have shown. In all instances with killifish, reduced inducibility of CYP1A was accompanied by resistance to overt lethality from exposure to aromatic hydrocarbons. It is clear that extensive laboratory calibration experiments need to be performed before CYP1A1 expression can be used as a biomarker in natural populations from highly contaminated locales.

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