Developmental and Tissue-Specific Expression of AHR1, AHR2, and ARNT2 in Dioxin-Sensitive and -Resistant Populations of the Marine Fish Fundulus heteroclitus

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Fundulus heteroclitus is a well-characterized marine fish model for studying aryl hydrocarbon toxicity. The F. heteroclitus population in New Bedford Harbor (NBH), a Superfund site in southeastern Massachusetts, exhibits heritable resistance to the toxic effects of planar halogenated aromatic hydrocarbons (PHAHs), including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and polychlorinated biphenyls (PCBs). To investigate the role of the aryl hydrocarbon receptor (AHR) signal transduction pathway in PHAH resistance, we measured the relative levels of AHR1, AHR2, and ARNT2 mRNA in whole embryos at different developmental stages and in dissected tissues of adults, comparing expression of these genes in NBH fish with fish from a reference site (Scorton Creek, MA [SC]). Expression of both AHR1 and AHR2 mRNA increased during development, achieving maximum levels prior to hatching. Maximal embryonic expression of AHR1 was delayed relative to AHR2. Whole NBH and SC embryos exhibited no discernable differences in expression of these genes. As we have previously observed, adult SC fish expressed AHR2 and ARNT2 mRNA in all tissues examined, while AHR1 was expressed predominantly in brain, heart, and gonads. In contrast, AHR1 mRNA was widely expressed in NBH fish, appearing with unusual abundance in gill, gut, kidney, liver, and spleen. This AHR1 expression pattern was not observed in the lab-reared progeny of NBH fish, demonstrating that constitutive AHR1 expression in gill, gut, kidney, liver, and spleen is not a heritable phenotype. Furthermore, widespread AHR1 expression was not induced in reference-site fish by TCDD or PCB mixtures, suggesting that aberrant AHR1 expression is not simply a normal physiological response of contaminant exposure. These results identify ubiquitous AHR1 expression as an attribute unique to feral NBH F. heteroclitus, and they represent a first step in determining the regulatory mechanisms underlying this expression pattern and its possible role in TCDD resistance.

Key Words: AHR; ARNT; dioxins; PCBs; fish; Fundulus heteroclitus; resistance.

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Planar halogenated aromatic hydrocarbons (PHAHs), including polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) are ubiquitous contaminants of terrestrial, aquatic, and marine environments. These contaminants pose a particular hazard to fish, which are among the most sensitive vertebrate groups to PHAH toxicity, especially during early-life stages (Elonen et al., 1998; Toomey et al., 2000; Walker et al., 1991). Embryonic exposure to PHAHs results in edema, hemorrhage, slow growth, craniofacial abnormalities, and mortality (Henry et al., 1997; Spitsbergen et al., 1991; Walker et al., 1996). The timing and pathology of these effects suggests that the cardiovascular system is the most important target for toxicity (Guiney et al., 1997; Hornung et al., 1999).

Despite the exquisite sensitivity of fish to the toxic effects of PHAHs, some indigenous, non-migratory populations thrive in habitats contaminated with extremely high levels of these compounds. A well-characterized example of PHAH tolerance in feral fish is the Fundulus heteroclitus (killifish or mummiyoch) population found in New Bedford Harbor (NBH), a federal superfund site in southeastern Massachusetts. NBH sediment contains significant quantities of many contaminants, including heavy metals, PCDFs, and PCDDs, but the concentration of PCBs is remarkable, with total PCBs measured as high as 190,000 ppm at the source of contamination (Weaver, 1984). The NBH F. heteroclitus population exhibits heritable resistance to PCBs (Nacci et al., 1999), as well as TCDD and TCDF (Bello et al., 1998, 1999, 2000). This PHAH resistance is evident for both lethality and the prototypical sub-lethal response, induction of cytochrome P4501A (CYP1A) expression. Early life stages of NBH fish exhibited a 10- to 100-fold higher LC20 for PCB 126 than reference-site animals and a similar increase in the EC20 for induction of CYP1A enzyme activity (Nacci et al., 1999). Comparable trends were observed in lab-reared progeny for 2 generations (Nacci et al., 1999). Similarly, CYP1A was not inducible by high doses of TCDD or TCDF in field-caught NBH fish or their progeny, and primary hepatocytes isolated from NBH fish were 14-fold less sensitive to CYP1A induction by TCDD than hepatocytes isolated from fish collected in Scorton Creek (SC), an uncon-
taminated reference site (Bello et al., 1998; Bello, 1999; Bello et al., 2000).

In mammals, most (if not all) biological effects of PHAHs are mediated by the aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor belonging to the basic/helix-loop-helix/Per-ARNT-Sim (bHLH-PAS) family of proteins (reviewed in (Hankinson, 1995; Schmidt and Bradfield, 1996)). The AHR acts in concert with the aryl hydrocarbon nuclear translocator (ARNT), another bHLH-PAS protein that can also serve as the dimerization partner for several other bHLH-PAS transcription factors (e.g., Ema et al., 1997; Huang et al., 1993; Sogawa et al., 1995; Wang et al., 1995). The AHR:ARNT complex binds xenobiotic response elements (XREs), specific nucleotide sequences in the enhancer regions of target genes, causing alterations in the rate of transcription.

F. heteroclitus expresses 2 highly divergent AHR genes: AHR1, expressed primarily in heart, brain, and gonad, and AHR2, which is widely expressed (Karchner et al., 1999). Although the transcriptional properties of these proteins are not yet resolved, both forms bind TCDD and interact with XREs in a ligand- and ARNT-dependent fashion (Karchner et al., 1999). Second, we detect only one ARNT gene in F. heteroclitus, an ARNT2 ortholog that, unlike its mammalian counterpart (Drutel et al., 1996; Hirose et al., 1996), is widely expressed (Powell et al., 1999). Finally, F. heteroclitus (and numerous other fish species) express only one CYP1A ortholog, which shares sequence characteristics of both forms of the related mammalian enzymes, P450IA1 and 1A2 (Morrison et al., 1995, 1998).

Capitalizing on recent advances in the understanding of AHR signal transduction components in F. heteroclitus, we sought to test the overall hypothesis that alterations in this pathway underlie the heritable PHAH resistance observed in the NBH population. To begin this effort, we have measured the expression of AHR1, AHR2, and ARNT2 mRNA in multiple tissues and life stages of F. heteroclitus from New Bedford Harbor and Scorton Creek, an uncontaminated reference site. Our findings suggest that while specific differences in the expression of AHR1 exist in NBH fish, they are neither directly heritable in laboratory-reared NBH progeny nor inducible in SC fish by exposure to contaminants similar to those in the NBH environment.

**MATERIALS AND METHODS**

**Fish collection and maintenance.** Fundulus heteroclitus were collected from Scorton Creek, Massachusetts and New Bedford Harbor, Massachusetts using minnow traps. They were maintained in aquaria at 20°C in flowing seawater and fed Tetramin staple flake and minced krill (Mid-Jersey Pet Supply).

**Spawning and maintenance of F. heteroclitus embryos.** F. heteroclitus spawn on a semi-lunar cycle (Taylor, 1984). Gravid fish were collected in June 1999 from Scorton Creek on the day of the new moon. Eggs from 6 female fish (approximately 200 per clutch) were stripped into separate petri dishes; twenty eggs from one clutch were immediately frozen in liquid nitrogen. The gonad of one male fish was removed and minced, and the material was distributed between the six clutches of eggs. Eggs were incubated with minced testis for 5 min, then disinfected by rinsing with 0.1% H2O2 in sterile-filtered sea water (Marking et al., 1994). In our experience, this brief H2O2 treatment substantially increases the survival of the embryos. Development proceeded at room temperature in sterile sea water (changed daily) on a gently rotating orbital shaker under a 12-h light:dark regimen. Pools of 15–20 embryos were randomly selected and frozen with liquid N2 at various times points during development. Following the hatch, fry were moved to beakers and fed freshly hatched brine shrimp once daily while collections of 15–20 animals continued.

Eggs from NBH fish were collected during the summer of 1999 from breeding pads (Scotch-Brite) placed in an aquarium containing animals collected in 1998. These embryos were allowed to develop under conditions similar to the SC fish. To insure the validity of comparisons of gene expression between the different populations, embryos were examined microscopically and sorted by stage according to the guidelines of Armstrong and Child (1965). To study the heritability of gene expression patterns observed in feral F. heteroclitus from NBH, we propagated this population in the laboratory for 2 generations. Embryos were collected from breeding pads in aquaria containing feral NBH fish collected in 1994 and 1995. The progeny (NBH F1 generation) were raised as described above and similarly bred after 2 years of growth. These progeny (NBH F2 generation), hatched in 1996 (4 fish) and 1998 (13 fish), were raised to adulthood in the laboratory (Bello, 1999) prior to dissection in January 2000.

**HAH Exposure Regimens**

**Chemicals.** TCDD was obtained from Ultra Scientific (N. Kingstown, RI). Aroclor 1254 and Aroclor 1242 were kindly supplied by Dr. J. Stegeman (Woods Hole Oceanographic Institution).

**Larvae.** Duplicate groups of 20 SC larvae (8 days following hatch) were exposed to water-borne TCDD (2 nM or 20 nM) or acetone vehicle in beakers of sterile-filtered seawater for 24 h. This dose (Tanguay et al., 1999) and exposure period (Hahn and Stegeman, 1994) were associated with substantial induction of CYP1A and/or AHR genes in other fish species. Each group was frozen in liquid N, before RNA isolation.

**Adult fish.** SC fish (7–13 g) were treated with a single intraperitoneal injection of TCDD [10 ng/g (Abnet et al., 1999a)] in corn oil (5 μl/g) or with corn oil alone (3 groups of 5 fish for each treatment). Following injection, fish were held without feeding for 24 h in static seawater with abundant aeration, and were subsequently sacrificed and dissected (Hahn and Stegeman, 1994). Additional SC fish (3 groups of 5 fish each) were similarly injected with a mixture of PCBs (200 μg total PCBs/g) resembling the congener distribution found in sediment and animal tissues collected from New Bedford Harbor [65% Aroclor 1254, 35% Aroclor 1242 (Elskus et al., 1994; Pruell et al., 1990)] and held for 5 days in continuously flowing seawater, before dissection. This dose and time period were calculated to result in a PCB body burden similar to feral NBH fish held in the laboratory for 6 months (see Discussion section). Tissues from each group of 5 fish were pooled for RNA isolation.
Dissected tissues from untreated and PHAH-treated fish were held at 4°C overnight in RNA-Later solution (Ambion, Austin, TX) and subsequently frozen. Total RNA was isolated from frozen tissues, eggs, embryos, fry, and larvae using RNA Stat-60 Reagent (Tel-Test, Inc.). RNA concentration was determined spectrophotometrically, and its integrity was verified on formaldehyde agarose gels (data not shown). Total RNA (1 μg) was reverse transcribed using Omniscript reverse transcriptase (Qiagen, Valencia, CA) primed by random hexamers (PE Applied Biosystems) Aliquots of the reactions (4.5 μl; cDNA from 225 ng total RNA) were used in semi-quantitative PCR reactions with AmpliTaq Gold (PE Applied Biosystems) and primers (0.15 μM each; Life Technologies, Inc.) specific for AHR1, AHR2, ARNT2, CYP1A, or β-actin (Table 1). The primer pairs amplifying AHR1, AHR2, and ARNT2 spanned boundaries between exons, eliminating the possibility of amplification of contaminating genomic DNA, and reactions omitting the addition of reverse transcriptase were used as negative controls (data not shown). All primers amplified products of similar length to minimize differences in the efficiency of amplification. The linear range of detection for the PCR products was determined by varying the number of cycles between 25 and 44 in 3-cycle increments (data not shown). Optimal amplification conditions for AHR1, AHR2, ARNT2, and CYP1A were: 95°C/10 min; 31 cycles of 95°C/15 s, 60°C/30 s; and 72°C/7 min. Linear amplification of CYP1A required 25 cycles only. Equal fractions of the PCR reactions were electrophoresed on 2.5% agarose gels prepared with equal quantities of agarose (Fisher Scientific) and low-melt agarose (NuSieve GTG; FMC BioProducts). Gels were stained in an ethidium bromide solution for 30 min and de-stained in water for 15 min. Band intensities were quantified using a ChemiImager 4000 low light imaging system (Alpha Innotech) with automatic background subtraction. Values were expressed as a percentage of maximum integrated density for each product or as a ratio to the intensity of the β-actin band amplified from the same sample.

**RESULTS**

**Developmental Expression of AHR1, AHR2, and ARNT2 Transcripts**

To determine the temporal pattern of expression of AHR1, AHR2, and ARNT2 mRNAs during normal F. heteroclitus development, we collected mature eggs and embryos of SC fish at time points from before fertilization to 4 days post-hatch. Total RNA was extracted and the relative expression of these genes was measured at each time point using semi-quantitative RT-PCR. Transcripts of AHR1, AHR2, and ARNT2 were detected throughout F. heteroclitus embryonic development. However, in eggs and early embryos, transcripts of all 4 genes, including the β-actin standard, were found at very low abundance (data not shown). To determine if PHAH-resistant NBH fish exhibit distinct patterns of AHR and ARNT mRNA expression during early development, we measured the relative abundance of these mRNAs at a subset of developmental stages. Approximately 20 NBH embryos were collected at stages surrounding 2 distinctive events: the onset of circulation (stage 25) and the hatch (stage 34). Expression levels of AHR1, AHR2, and ARNT2 were determined relative to β-actin expression. Expression of these genes during development of NBH F. heteroclitus was not substantially different from that detected in SC embryos. We observed the characteristic increase of all 3 transcripts between the earlier and later stages (Figs. 2a–2d).

**Aberrant Tissue Distribution of AHR1 mRNA in Feral NBH Fish**

The tissue-specific expression of AHR1, AHR2, and ARNT2 mRNAs in SC and NBH fish was analyzed in isolated tissues of PHAH-resistant and sensitive adult F. heteroclitus. We detected AHR2 and ARNT2 expression in all tissues of both SC and NBH fish. As we have previously observed in SC fish, AHR1 was expressed predominantly in the brain, heart, and ovary (Fig. 3; Karchner et al., 1999). However, in NBH fish, AHR1 expression was unusually widespread, detected in all tissues examined (Fig. 3).

**Aberrant AHR1 Expression in NBH Fish Is Not Directly Heritable**

We next sought to determine if the unusual, ubiquitous pattern of AHR1 expression in NBH fish is genetically transmissible. The relative abundance of AHR1, AHR2, and ARNT2 mRNAs was measured in tissues dissected from laboratory-reared F2 progeny of fish collected from New Bedford Harbor. The expression pattern of AHR1 was identical to the pattern seen in SC fish (Fig. 4). Thus, constitutive, ubiquitous AHR1 expression is not directly inherited from feral NBH fish.
Expression of AHR1 and AHR2 Is Unresponsive to HAH Exposure

The failure of ubiquitous AHR1 expression to be genetically transmitted to NBH progeny fish suggests that the pattern observed in feral NBH fish may represent environmentally induced, rather than constitutive, expression. This hypothesis raises the possibility that widespread AHR1 expression might be inducible in SC fish by exposure to similar contaminants. To test this hypothesis, we exposed SC fish to several PHAHs relevant to the NBH environment.

TCDD exposure induces expression of mRNA for AHR2 in zebrafish embryos (Tanguay et al., 1999) and AHR2α and AHR2β in adult rainbow trout kidney (Abnet et al., 1999a). To determine the effect of TCDD exposure on the expression of AHR1 and AHR2 mRNA in *F. heteroclitus* at an early life stage, we exposed groups of 20 SC larvae (8 days post-hatch) to water-borne TCDD for 24 h. As measured in whole larvae, neither AHR1 nor AHR2 was induced by 2 or 20 nM TCDD when band intensities were normalized to β-actin expression. CYP1A expression, however, was strongly induced at both concentrations, confirming the uptake of TCDD and the ability of our RT-PCR method to detect TCDD-induced changes in gene expression (Fig. 5).

Gene expression in larvae was measured in pools of whole animals. Since the differences in AHR1 expression between the SC and NBH fish are primarily distinguished by their tissue distribution, it is possible that observation of whole animals obscured any AHR1 induction. Thus, we next exposed adult SC fish (3 groups of 5 fish) to a single dose of TCDD (10 ng/g) via intraperitoneal injection, dissecting 8 tissues after 24 h and measuring the relative expression of AHR1, AHR2, and CYP1A mRNA in each. Compared to fish injected with vehicle alone, there was no apparent induction of either AHR1 or AHR2 in any tissue, and the distribution of AHR1 mRNA was not altered as a result of exposure. As expected, CYP1A mRNA was strongly induced in all 8 tissues (Fig. 6).

PCBs, not TCDD, comprise nearly all of the contaminant load in NBH fish, and certain ortho-substituted PCBs have been shown to increase AHR-like binding activity in rat hepatic cytosols (Denomme et al., 1986; Landers et al., 1991). Thus, we sought to determine if exposure to an environmentally relevant mixture of PCBs could alter the expression pattern of *F. heteroclitus* AHR1 or AHR2. Adult SC fish (3 groups of 5) were treated with a single ip dose (200 µg/g) of a PCB mixture resembling the congener distribution observed in New Bedford Harbor sediments and animal tissues (65% Aroclor 1254, 35% Aroclor 1242; Elskus et al., 1994; Pruell et al., 1990). Fish were held for 5 days before dissection. We detected no changes in the expression or distribution of mRNA for AHR1 or AHR2 (Fig. 7). As expected, however, CYP1A mRNA was strongly induced in all tissues, confirming the uptake of the PCB mixture and the overall responsiveness of the endogenous AHR signaling pathway to this chemical mixture.

DISCUSSION

Heritable resistance to PHAHs in the chronically exposed *F. heteroclitus* population of New Bedford Harbor has been characterized for both lethality (Nacci et al., 1999) and biochemical responses (Bello et al., 1998, 1999, 2000; Nacci et al., 1999). Since such responses are mediated by the AHR signaling pathway in mammalian models, this study compared the expression of genes encoding components of this pathway in NBH fish and PHAH-sensitive fish from an uncontaminated reference site, examining both adult tissues and early life stages.

Developing fish embryos are particularly sensitive to the toxic effects of PHAH compounds (Elonen et al., 1998; Tooney et al., 2000; Walker et al., 1991), and the ability of even transient, waterborne exposure, immediately following fertilization, to induce CYP1A expression implies that functional AHR is present from the earliest stages of development (Binder et al., 1985; Cantrell et al., 1996; Guiney et al., 1997; Tanguay et al., 1999; Toomey et al., 2000). Our studies in *F. heteroclitus* correlate well with those in zebrafish showing low levels of ARNT2 (Wang et al., 1998), AHR1 (Wang et al., 1998), and AHR2 (Tanguay et al., 1999) expression in the early stages, with subsequent increases as development progresses. We show here that AHR1 reaches its maximal expression level approximately 48 h later than AHR2, roughly correlating with the appearance of the tissues in which AHR1 is predominantly expressed in adult animals (Fig. 1). Notably, although the developing vasculature is an important target of PHAH toxicity (Guiney et al., 1997; Hornung et al., 1999), we observed no increases in AHR1 or AHR2 expression associated with the onset of circulation (Figs. 1 and 2). Similarly, we observed no differences in gross expression of AHR1 or AHR2 following the hatch (Figs. 1 and 2), although PHAH-induced lethality typically occurs after hatching (Elonen et al., 1998; Henry et al., 1997; Spitsbergen et al., 1991; Walker and Peterson, 1991; Wisk and Cooper, 1990) and *F. heteroclitus*...
embryos exhibit a hatch-dependent increase in both basal CYP1A activity and sensitivity to CYP1A induction by PCBs (Binder et al., 1985).

We observed no gross differences in the expression of AHR1, AHR2, or ARNT2 mRNA in the offspring of TCDD-resistant NBH fish at the embryonic stages surrounding the onset of circulation and hatching (Fig. 2). However, AHR1 mRNA was clearly more widely expressed in adult NBH fish than in their SC counterparts, although there was no obvious increase in expression of this gene in the subset of tissues in which it is ordinarily found at high levels (heart, brain, ovary) (Fig. 3). The failure to observe increased gross expression in the NBH embryos could result from inadequate sensitivity of the RT-PCR assay to discern modest differences in whole-body expression, resulting from more substantial increases in a subset of individual tissues. This shortcoming could be addressed by in situ hybridization or in situ RT-PCR approaches. Alternatively, the apparent lack of increased AHR1 expression in these embryos could reflect the inability of this trait to be genetically transmitted. Consistent with this notion, the ubiquitous pattern of AHR1 expression seen in feral NBH fish (Fig. 3) was not observed in their adult F2 progeny (Fig. 4).

The exposure history of the NBH F2 progeny fish differed dramatically from NBH fish collected from the field and their embryonic offspring. Not only did these fish lack the chronic, lifelong environmental exposure to high levels of HAHs, they were also spawned from laboratory-reared F1 fish and thus experienced little developmental exposure via maternal deposition. In contrast, despite 6 months of depuration under clean laboratory conditions, NBH feral fish may yet have harbored a substantial PCB body burden. Published measurements of PCB concentrations in freshly collected NBH *F. heteroclitus* range from 198 μg/g to 2340 μg/g (dry weight of whole carcasses) (Lake et al., 1995), consistent with our own measurements [272 ± 36 μg/g for decapitated, eviscerated carcasses only (Bello, 1999)]. Total PCBs are eliminated from live fish with a half-life of approximately 4.5 months (Elksus et al., 1995).
1999; Lake et al., 1995). Thus, even if NBH fish are held for 6 months in the laboratory before analysis of gene expression, they could retain a PCB body burden of between 66 mg/g to 780 mg/g (dry weight), 2 to 3 orders of magnitude higher than the PCB concentration in *F. heteroclitus* from uncontaminated sites [0.2 mg/g; (Bello, 1999; Elskus et al., 1999). Based on estimates derived from data relating to uptake and elimination kinetics of complex PCB mixtures injected into *F. heteroclitus* (Elskus et al., 1999), we project that the dose of 200 μg/g might produce a PCB body burden on the order of 100 μg/g dry weight, lower than freshly collected NBH fish but well within the range of projected body burdens of NBH fish depurated for 6 months. Successful uptake of the PCBs was confirmed by the robust induction of CYP1A mRNA expression. However, AHR1 expression was not increased in any tissue and may have been diminished in ovary (Fig. 7).

While it is clear that acute PHAH exposure does not induce AHR1 mRNA expression in SC fish, we cannot eliminate a role for contaminants in producing the characteristic widespread expression pattern in the NBH population. Feral NBH fish experience chronic contaminant exposure from the earliest life stages, and this exposure pattern may affect AHR1 expression differently from the acute exposure regimens used in this study. Furthermore, despite the lack of direct heritability of widespread AHR1 expression in lab-reared NBH progeny fish, it remains possible that the capability for such induction is a population-specific, heritable phenotype. PHAH exposure studies in NBH offspring and fully depurated feral fish will be required to test this hypothesis, and fish stocks are being raised for this purpose. It is tempting to speculate that NBH-specific AHR1 inducibility could involve different transcriptional regulatory elements in the promoter or enhancer region of the AHR1 gene. A similar mechanism of population-specific gene expression has been extensively characterized for the lactate dehydrogenase-B (*Ldh-B*) gene of *F. heteroclitus*, in which
conserved differences in the promoter and upstream regulatory elements largely account for adaptive differences in the expression of this enzyme in geographically distinct populations (Segal et al., 1996; Crawford et al., 1999; Schulte et al., 2000).

Additional *F. heteroclitus* populations in different polluted habitats also exhibit resistance to toxicity of local organic contaminants, including TCDD and PCBs (Newark, NJ: Elskus et al., 1999; Prince and Cooper, 1995a,b); and polycyclic aromatic hydrocarbons (Elizabeth Island, VA: Van Veld and Westbrook, 1995; Vogelbein et al., 1996). Populations of other fish species chronically exposed to PCBs also exhibit reduced induction of CYP1A (Virgin et al., 1992; Forlin and Celander, 1995). It is not known if any of these populations share the aberrant AHR expression pattern observed in NBH fish.

The physiological and toxicological significance of the multiple AHRs found in many fish species is not yet understood. Comparison of the endogenous response with transfection-based reporter assays of AHRs from rainbow trout implies that a single AHR, AHR2α, may account for nearly all of the CYP1A induction elicited by the PHAHs in this system (Abnet et al., 1999b), despite the ability of both AHR2α and AHR2β to bind TCDD and interact with XREs *in vitro* (Abnet et al., 1999a). In *F. heteroclitus*, both AHR1 and AHR2 bind TCDD, and both interact with XREs in a ligand- and ARNT-dependent fashion (Karchner et al., 1999). Evolutionally, the amino acid sequence of AHR1 is most closely related to mammalian AHRs (Karchner et al., 1999), which singularly mediate the molecular response to HAH exposure. However, AHR1 mRNA is nearly undetectable in many tissues that exhibit PHAH-inducible CYP1A expression, implying that AHR2 is capable of mediating this response. The transcriptional capabilities of these proteins have not been directly resolved, and their individual *in vivo* functions are not known. Thus, it is unclear what contribution, if any, ubiquitous AHR1 expression...
in NBH fish may make to the physiology of PHAH resistance. Studies are underway to characterize the in vivo function of AHR1 and its potential role in acquired PHAH resistance in *Fundulus heteroclitus* from New Bedford Harbor.

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