Characterization of AHR2 and CYP1A Expression in Atlantic Sturgeon and Shortnose Sturgeon Treated with Coplanar PCBs and TCDD

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Abstract

Atlantic sturgeon and shortnose sturgeon co-occur in many estuaries along the Atlantic Coast of North America. Both species are protected under the U.S. Endangered Species Act and internationally on the IUCN Red list and by CITES. Early life-stages of both sturgeons may be exposed to persistent aromatic hydrocarbon contaminants such as PCBs and PCDD/Fs which are at high levels in the sediments of impacted spawning rivers. Our objective was to compare the PCBs and TCDD sensitivities of both species with those of other fishes and to determine if environmental concentrations of these contaminants approach those that induce toxicity to their young life-stages under controlled laboratory conditions. Because our previous studies suggested that young life-stages of North American sturgeons are among the more sensitive of fishes to coplanar PCB and TCDD-induced toxicities, we were interested in identifying the molecular bases of this vulnerability. It is known that activation of the aryl hydrocarbon receptor 2 (AHR2) in fishes mediates most toxicities to these contaminants and transcriptional activation of xenobiotic metabolizing enzymes such as cytochrome P4501A (CYP1A). Previous studies demonstrated that structural and functional variation in AHRs are the bases for differing sensitivities of several vertebrate taxa to aromatic hydrocarbons. Therefore, in this study we characterized AHR2 and its expression in both sturgeons as an initial step in understanding the mechanistic bases of their sensitivities to these contaminants. We also used CYP1A expression as an endpoint to develop Toxicity Equivalency Factors (TEFs) for these sturgeons. We found that critical amino acid residues in the ligand binding domain of AHR2 in both sturgeons were identical to those of the aromatic hydrocarbon-sensitive white sturgeon, and differed from the less sensitive lake sturgeon. AHR2 expression was induced by TCDD (up to 6-fold) and by three of four tested coplanar PCB congeners (3-5-fold) in Atlantic sturgeon, but less so in shortnose sturgeon. We found that expression of AHR2 and CYP1A mRNA significantly covaried after exposure to TCDD and PCB77, PCB81, PCB126, but not PCB169 in both sturgeons. We also determined TEFs for the four coplanar PCBs in shortnose sturgeon based on comparison of CYP1A mRNA expression across all doses. Surprisingly, the TEFs for all four coplanar PCBs in shortnose sturgeon were much higher (6.4 to 162 times) than previously adopted for fishes by the WHO.

Introduction

There are 27 species of sturgeons worldwide and each are threatened with decreases in abundance and in some instances extirpation. All sturgeons are included on the IUCN red list in which 17 are listed as "critically endangered" prompting claims in popular media that "sturgeons are more critically endangered than any other group of species" (IUCN 2010). Four reasons are typically advanced to explain reductions in their abundances: overharvest, habitat alteration, compromised water quality, and chemical pollution. There are two largely sympatric sturgeon species along the Atlantic coast of North America; Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* and shortnose sturgeon *Acipenser brevirostrum*. Atlantic sturgeon was listed as "endangered" and "threatened" under the U.S. Endangered Species Act (ESA) in 2012 (Federal Register 2012 a,b). In Canada it is designated as "threatened" by the Committee on the Status of Endangered Wildlife (COSEWIC). Shortnose sturgeon was listed as "endangered" throughout its U.S range under the ESA since 1973 and is designated as a species of "Special Concern" in Canada under COSEWIC. Populations of both Atlantic coast sturgeons inhabit estuaries that are contaminated with aromatic hydrocarbons such as polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins/furans (PCDD/Fs), but their effects on the abundances of sturgeon populations is unknown.

Both species are long-lived, up to at least 60 years of age (Dadswell 2006; Dadswell et al. 1984), reproduce initially at advanced ages (6-25 years), spawn intermittently thereafter at 1 to 5 year intervals (Smith 1985), and are only moderately fecund. This set of life-history characteristics may impede or delay the recovery of imperiled populations. Atlantic sturgeon is anadromous, juveniles remain resident within their natal estuaries for 2-6 years (Bain 1997), and undergo extensive and prolonged coastal migrations as subadults and adults (Erickson et al. 2011; Wirgin et al. 2015) including occasional forays to distant non-natal estuaries (Waldman et al. 2013; Wirgin et al. submitted). Shortnose sturgeon is amphidromous and is restricted to natal estuaries throughout its life history, although short excursions into coastal waters are sometimes observed at the geographical limits of their distribution (Wirgin et al. 2010; Fernandes et al. 2010; Wippelhauser et al. 2015). The two sturgeons are largely sympatric with spawning populations occurring from St. Johns River, Florida to the St. Lawrence River, Quebec (Atlantic sturgeon), and to the Saint John River, New Brunswick (shortnose sturgeon).

Early life-stages of fishes are often notoriously sensitive to aromatic hydrocarbon contaminants, although vulnerability among species varies (Elonen et al. 1998). Recent evidence suggests that sturgeons may be particularly susceptible to the toxic impacts of aromatic hydrocarbons such as coplanar PCBs, PCDD/Fs, and some polycyclic aromatic hydrocarbons (PAHs) (Chambers et al. 2012; Doering et al. 2014ab). Young life-stages in natural populations may be vulnerable because of the maternal transfer of lipophilic contaminants and the direct contact of bottom-dwelling embryos and larvae with contaminated sediments (Walker et al. 1991; Johnson et al. 1998). Hallmark early life-stage toxicities in fishes from exposure to aromatic hydrocarbons include compromised embryonic survivorship, altered hatching rates, pericardial and yolk-sac edemas, abnormal spinal curvature, impaired eye development, and a variety of craniofacial alterations (Chambers et al. 2012).

Studies in fishes have demonstrated that most, if not all, of these aromatic hydrocarbon-induced early life-stage toxicities result from impairment of cardiac structure and function (Incardona et al. 2004; Antkiewicz et al. 2005). The hearts of exposed young life stages are usually smaller, contain fewer myocardial cells, and have abnormal three-dimensional structure because of altered looping, ballooning, and incomplete rotation of the two chambers of their developing hearts (Carney et al. 2006; Singleman and Holtzman 2012). These aberrations typically impair cardiovascular function through atrial arrhythmia and slowing of the heart beat rate.

Studies have demonstrated that early life-stage toxicities in fishes are mediated through activation of the aryl hydrocarbon receptor (AHR) pathway (Antkiewicz et al. 2006). AHR is a ligand-activated cytoplasmic transcription factor that is complexed with two molecules of heat shock protein 90 and associated interacting protein (AIP). Contaminants that diffuse into the cytoplasm bind AHR which in turn releases its molecular chaperones and translocates to the nucleus where it dimerizes with its signaling partner, the aryl hydrocarbon receptor nuclear translocator (ARNT). Within the nucleus, AHR-ARNT complex recognizes and binds specific DNA motifs in the upstream regulatory regions of a variety of responsive genes in the AHR battery. Moreover, recent *in vitro* studies in mammalian cell lines point to an ever-expanding regulatory role of AHR in cell proliferation, differentiation, and pluripotency by unforeseen processes including alteration of chromatin dynamics and transcription of mobile genetic elements (reviewed in Mulero-Navarro and Fernandez-Salguero 2016).

The most studied of these AHR battery genes in fishes, cytochrome P4501A (CYP1A), is transcriptionally activated by AHR and is highly inducible by exposure to aromatic hydrocarbons. AHR pathway structure and function are conserved among vertebrate species with the exception that all fishes, and some avian species, exhibit two and more forms of the genes, AHR1 and AHR2 (Hahn et al. 1997), with AHR2 usually being more highly expressed across tissues and its protein being better than AHR1 at binding TCDD. Furthermore, TCDD (Prasch et al. 2003) and PAH (Van Tiem et al. 2011; Jayasundara et al. 2015) induced early life-stage cardiovascular toxicities in fishes usually require activation of AHR2, not AHR1.

Species of fishes and even some populations differ significantly in their sensitivities to early life-stage toxicities of TCDD and coplanar PCBs. For example, salmonids (bull trout *Salvelinus confluentus*, lake trout *S. namaycush*, and brook trout *S. fontinalis*) are among the most sensitive species, whereas zebrafish *Danio rerio* and northern pike *Esox lucius* are approximately three orders of magnitude less vulnerable (Elonen et al. 1997). Similarly, populations of Atlantic killifish *Fundulus heteroclitus* (Reid et al. 2016) and Atlantic tomcod *Microgadus tomcod* (Wirgin et al. 2011) from contaminated estuaries are orders of magnitude less sensitive to TCDD and coplanar PCBs induced early life-stage toxicities than conspecifics from cleaner locales.

Mechanistic studies have demonstrated that variation in sensitivity to TCDD or coplanar PCBs toxicity is often due to genetic variation in AHRs. In avian species, genetic polymorphisms within the ligand binding domain of AHR1 (Head et al. 2008; Farmahin et al. 2013) was the basis for variability in activation of the AHR pathway and decreased downstream toxicity. Genomic sequencing and transcriptome analyses demonstrated that genetic variation in multiple components of the AHR pathway contributed to a PCB-resistant phenotype in several populations of Atlantic killifish (Reid et al. 2016). Similarly, a two-amino acid deletion downstream of the AHR2 ligand binding domain impacting its affinity to TCDD was the basis of resistance to TCDD and coplanar PCBs toxicities in Atlantic tomcod (Wirgin et al. 2011).

We previously reported that both Atlantic sturgeon and shortnose sturgeon early life-stages were sensitive to TCDD or PCB126 at seemingly environmentally relevant doses with toxicities expressed by morphometric alterations, impaired eye development, and reduced duration of larval survivorship when exposed as embryos (Chambers et al. 2012). Furthermore, we demonstrated that CYP1A mRNA expression was dose-responsive and significantly induced

in Atlantic sturgeon and shortnose sturgeon larvae when exposed as embryos to TCDD or PCB126 at the lowest doses tested (Roy et al. 2011). In other sturgeons, Doering and colleagues fully characterized AHR1 and AHR2 and their expression in chemically treated white sturgeon *Acipenser transmontanus* and lake sturgeon *A. fulvescens* and found that AHR1 and AHR2 were approximately equally expressed in a variety of tissues suggesting a greater functional role for AHR1 in sturgeons than in other fishes (Doering et al. 2014a). They further evaluated the *in vitro* functionality of white sturgeon AHR1 and AHR2 in reporter gene assays in AHR-deficient mammalian cells and concluded that both sturgeon AHRs were activated by TCDD and coplanar PCBs (Doering et al. 2014b). They suggested that the cooperativity of AHR1 and AHR2 in activating toxicities may render sturgeons more sensitive than other fishes to toxicities from these contaminants.

The overall objective of these studies is to evaluate the sensitivity of both Atlantic coast sturgeons to environmentally relevant concentrations of PCBs and PCDD/Fs. As a first step in this process, we characterized AHR2 cDNAs in both Atlantic coast sturgeons and evaluated their expression in early life-stages of both species when exposed to graded doses of TCDD, four coplanar PCBs, and an environmentally relevant Aroclor mixture and in multiple tissues of juvenile shortnose sturgeon treated with graded doses of PCB126. Furthermore, we evaluated the relationship between AHR2 and CYP1A expression in treated sturgeons and used this opportunity to develop TEFs in shortnose sturgeon using CYP1A mRNA expression as a biomarker.

Methods

Cloning of AHR2 cDNAs

Total RNAs were isolated from one adult shortnose sturgeon and one adult Atlantic sturgeon each collected from the Saint John River, New Brunswick, Canada, using Ultraspec reagent (Biotexc, Houston, TX) as per the manufacturer's recommendations. Five hundred ng of random hexamers (Integrated DNA Technologies Inc., Coralville, IA) were added to 100 ng of total RNAs in 15 µl volumes and incubated at 75° C for 5 min. The mixture was chilled on ice and 10 µl of reverse transcriptase mix was added so that the final reaction mix contained 1X MMLV reaction buffer (Promega Life Science, Madison, WI), 20 U MMLV reverse transcriptase (Promega), 10 U RNasin RNase inhibitor (Promega), and 0.5 mM dNTPs (GE Healthcare Bio-

Sciences Corp. Piscataway, NJ). Reactions were incubated at 42° C for 1 h, products were denatured at 98° C for 5 min then chilled on ice.

White sturgeon *Acipenser transmontanus* AHR2 sequence was used to design primers (Table 1) to amplify Atlantic sturgeon and shortnose sturgeon AHR2 in RT-PCR reactions. Reactions were in 30 µl volumes that contained 0.5 µM of each forward and reverse primer, 0.2 mM dNTPs (GE Healthcare), 1X PCR buffer (Roche Molecular Systems, Indianapolis, IN), 1 U Taq DNA polymerase (Roche), 4 µl of cDNA products, and H_20 to volume. Cycling parameters were 95° C for 5 min, 35 cycles of 95° C for 15 s, 50° C for 15 s, 72° C for 60 s, and a final extension at 72° C for 7 min. RT-PCR products were purified with Qiagen MinElute PCR purification kits (Qiagen, Valencia, CA), sequenced, and compared with white sturgeon sequences. Derived sequences were used to develop additional Atlantic sturgeon or shortnose sturgeon-specific primers that were paired with white sturgeon AHR2 primers for subsequent use in RT-PCR. Using this approach, we were able to obtain the complete AHR2 coding and some 3' untranslated region sequences for both species.

We used a 5'/3' RACE Kit, 2nd Generation (Roche) to characterize the 5'-UTR of Atlantic sturgeon AHR2 mRNA. An anchor primer, STPANC, that contained a *Sal I* restriction site, and three gene-specific sturgeon primers were used (STAHRR437, STAHRR388, and STAHRR365) (Table 1). Reverse transcription was performed with STAHRR437 and poly A was added at the 3' end of the first strand cDNA with terminal transferase. Products were used in PCR with the anchor primer, STPANC, and STAHRR388 and an approximate 300 bp fragment smear was excised from a low melting point agarose gel. This DNA was then amplified with anchor primer STPANC and STAHRR365 that contained a *Sac I* site. Products were digested with *Sal I* (site present in the anchor primer) and *Sac I* (site present in the gene-specific primer) and were ligated to the pUC19 cloning vector. Ligation reactions were used to transform NEB 5-alpha competent cells (NE Biolabs, Ipswich, MA) and plated on IPTG/X-gal plates for white/blue color selection. White colonies were screened for inserts and four were sequenced to obtain the 5' end of the Atlantic sturgeon AHR2 mRNA sequence. One insert contained the sequence that matched with known AHR2 sequence in white sturgeon.

To characterize the 5'-UTR of shortnose sturgeon AHR2 mRNA, three primers were designed based on the 5' end of the Atlantic sturgeon gene (STAHRF191, STAHRF170 and STAHRF146). These primers in combination with previously determined shortnose sturgeon

specific primer STAHRR656 were used to amplify and sequence the 5'UTR of shortnose sturgeon AHR2 mRNA.

Phylogenetic analysis of sturgeons AHR2

A UPGMA phylogenetic tree was generated for AHR1 and AHR2 from fishes, birds, and mammals using MEGA 6.06-mac (Tamura et al. 2013). Sequences used and their Genbank accession numbers were: albatross AHR1 (BAC87795), cormorant AHR1 (BAD01477), quail AHR1 (ADI24459), chicken AHR1 (NP_989449), human AHR (L19872), mouse AHR (NP_038492), white sturgeon AHR1 (KJ420394), spiny dogfish AHR1 (AFR24092), Atlantic killifish AHR1 (AF024591), red sea bream AHR1 (BAE02824), Japanese medaka AHR1a (BAB62012), Japanese medaka AHR1b (BAB62011), zebrafish AHR1a (AF258854), goldfish AHR1 (ACT79400), albatross AHR2 (BAC87796), cormorant AHR2 (BAF64245), red sea bream AHR2 (ABI197788), Atlantic killifish AHR2 (FHU29679), rainbow trout AHR2a (NP_001117723), rainbow trout AHR2b (NP_001117724), Atlantic tomcod AHR2 (FJ215752), goldfish AHR2 (FJ554572), zebrafish AHR2 (AF063446), and white sturgeon AHR2 (KJ420395).

Sources of sturgeon embryos for gene expression analyses

Embryos of both sturgeon species were obtained from Acadian Sturgeon and Caviar, Inc., a commercial aquaculture facility in Saint John, New Brunswick, Canada. Their broodstock of shortnose sturgeon has been captive for several years and were originally collected from the Saint John River. Atlantic sturgeon broodstock from the Saint John River are collected annually by the same facility. Embryos of both species were transported at approximately 48 h post fertilization to the Howard Marine Science Laboratory of NOAA Fisheries, Sandy Hook, New Jersey, where they were reared and treated.

Chemical treatment of sturgeon embryos and larvae

In initial 2009 experiments, exposures of embryos and larvae to graded doses of TCDD and PCB126 were done as described in Roy et al. (2011). Twenty-five Atlantic sturgeon larvae 2 d post-hatch (dph) were waterborne exposed for 26-27 h in 50 ml of 1 part per thousand (ppt) artificial seawater in 100 ml glass beakers to graded doses of TCDD (nominal doses of 0.001

ppb, 0.01 ppb, 0.1 ppb, 1.0 ppb, and 10 ppb; AccuStandard, New Haven, CT; 99.1% purity) in acetone vehicle (0.05–0.1% acetone), PCB126 (nominal doses of 0.01 ppb, 0.1 ppb, 1.0 ppb, 10 ppb, 100 ppb, and 1000 ppb; AccuStandard; 99.7% purity) in 0.05–0.1% acetone, acetone alone, or H_20 alone. Immediately after exposures, larvae were harvested, snap frozen, and stored at -80°C until processing. Thirty shortnose sturgeon embryos 5 dp post-fertilization (dpf) were exposed exactly as described above, but after 26-27 h exposures were transferred to clean water where they were reared to hatch at ~ 10 dpf. Within 24 h of hatch, larvae were harvested, snap frozen, and stored at -80°C until processing.

Subsequent exposures were conducted in 2014 and 2015. In July 2014, 35 2 dpf Atlantic sturgeon embryos and in May, 2015, 25 1 dpf shortnose sturgeon embryos were waterborne exposed in 25 ml of 1-ppt artificial sea water in 100 ml glass beakers to graded doses of TCDD (nominal doses of 0.0005 ppb, 0.005 ppb, 0.05 ppb, 0.5 ppb, 5 ppb and 50 ppb (AccuStandard; 99.1% purity); PCB77 (nominal doses of 0.1 ppb, 1.0 ppb, 10 ppb, 100 ppb, 1000 ppb, and 10,000 ppb (AccuStandard; 99.7% purity); PCB81 (nominal doses of 0.1 ppb, 1.0 ppb, 10 ppb, 100 ppb, 1000 ppb, and 10,000 ppb (AccusStandard 99.8% purity); PCB126 (nominal doses of 0.1 ppb, 1.0 ppb, 10 ppb, 100 ppb, 1000 ppb, and 10,000 ppb (AccuStandard; 99.7% purity); PCB169 (nominal doses of 0.1 ppb, 1.0 ppb, 10 ppb, 100 ppb, 1000 ppb, and 10,000 ppb (AccuStandard; 99.0% purity); and an Aroclor mixture of Aroclor 1248 (40%), Aroclor 1254 (40%), and Aroclor 1260 (20%) (AccuStandard) in acetone vehicle or to acetone alone. The Aroclor mixture was designed to replicate the PCB mixture that was released into the upper Hudson River from 1947 to 1976.

Embryos were maintained in exposure water for 24 h at 15°C for Atlantic sturgeon or 12°C for shortnose sturgeon after which they were rinsed and transferred to 750 ml pyrex dishes with 500 ml of clean 1-ppt artificial seawater for rearing until hatch. Every 12 h dishes were cleaned of dead embryos, newly hatched larvae were removed, and an 80% percent water change was performed. Hatchlings were transferred and held alive in beakers for 24 h, snap frozen, and stored at -80° C until processing.

Treatment of juvenile shortnose sturgeon with PCB126

In 2009, hatchery-reared juvenile shortnose sturgeon of Connecticut River ancestry were i.p. injected as described in Roy et al. (2011) with graded concentrations of PCB126 (0.01 ppb, 0.1

ppb, 1.0 ppb, 10 ppb and 50 ppb (AccuStandard; 99.7% purity)), to compare tissue specific expression of AHR2 mRNA expression. Five fish per treatment group were injected with PCB126 in 25 µl of corn oil vehicle or with 25 µl of corn oil alone. After eight days of exposures, multiple tissues (blood, gill, heart, intestine, liver, and pectoral fin clip) were harvested, snap frozen, and stored at -80^{o}C until processing. A summary of all experimental exposures described above is provided in Table 2.

RNA isolations

In 2009, total RNA was isolated from three pools of five Atlantic sturgeon larvae each, individual shortnose sturgeon larvae, or approximately 50 mg of tissues from individual juvenile shortnose sturgeon. In 2014 and 2015, all RNA isolations from both Atlantic sturgeon and shortnose sturgeon were from individual larvae (n=6/treatment group). RNA isolations were conducted using Ultraspec reagent (Biotex, Houston, TX) as per the manufacturer's recommendations as described in Roy et al. (2011). RNA concentrations and purities were evaluated with a NanoDrop ND-1000 spectrophotometer (NanoDrop Products, Wilmington, DE). RNA concentrations of samples were adjusted to 100 ng/µl for subsequent procedures.

Expression of AHR2 in early life-stages and tissues of sturgeons

We compared AHR2 expression among exposure groups of Atlantic sturgeon and shortnose sturgeon larvae and shortnose sturgeon tissues with the primers STAHRF197 and STAHRR270 (Table 1). ß-actin was amplified as the endogenous control using the primers Sturact60F and Sturact125R (Table 1). Reactions contained 1.5 µl of reverse transcriptase products, 1.5 µl of primer mix (100 nM final concentration of each primer) (Integrated DNA Technologies), and 3 µl of Power Syber Green PCR Master Mix (Applied Biosystems (ABI), Foster City, CA). PCR cycling parameters were 95°C for 10 min and 40 cycles of 95°C for 15 s and 60°C for 60 s in an ABI 7900 real-time PCR instrument.

Expression of CYP1A in early life-stages of chemically treated sturgeons

We also quantified and compared CYP1A expression in both species among the TCDD, PCB77, PCB81, PCB126, and PCB169 treatment groups to estimate Toxic Equivalency Factors (TEFs) for the four coplanar PCB congeners relative to TCDD using the primers StycypRT231F and StycypRT287R (Table 1) with ß-actin serving as the endogenous control. Reaction volumes and

conditions were identical to those described above for AHR2.

Statistical Analysis

We calculated mean fold induction of AHR2 and CYP1A using the relative comparison Ct method for real-time RT-PCR as described in Applied Biosystems' User Bulletin No. 2 (Livak and Schmittgen, 2001), and applied this to calculate ß-actin-normalized fold induction of CYP1A1 and AHR2 mRNA in the RT-PCR assays. The mean values of ΔΔCYP1A and ΔAHR2 for each treatment group were compared using a one-way ANOVA with a Tukey's post hoc test to determine the significance differences in response among toxicant groups and negative controls, and among different doses within the same chemical. The relationships between expression of CYP1A and AHR2 were evaluated for each of the four PCB congeners (PCB77, B81, PCB126 and PCB 169) and TCDD, individually and combined in both sturgeon species. The Pearson correlation coefficient and the *P*-values for testing the significance of the nonzero sample correlation coefficients were computed using **R** * V 3.4.2 (Venable et al. 2017).

TEFs were determined for shortnose sturgeon for the four coplanar PCBs based on EC_{20}, EC_{50}, and EC_{70} results for CYP1A expression calculated in GraphPad Prism 7.0c using the following equation; EC_{50}= ECF/ ((F/(100-F)) ^ (1/Hills Slope))

Where ECF is the concentration of chemical that gives a response F percent (20%, 50%, 70%) between the lowest and highest response and Hill Slope describes the steepness of the curves. Sensitivities of shortnose sturgeon larvae to each of the four coplanar PCBs in relation to TCDD were calculated through use of three points (EC_{20}, EC_{50}, and EC_{70}) on the dose-response curves for CYP1A expression for each chemical. TEFs were calculated by use of the formula

$$TEF = \frac{EC_{xx}\ TCDD}{EC_{xx}\ PCB\ congener}$$

Where EC_{XX} is the average of the concentration to induce EC_{20}, EC_{50}, and EC_{70} for each chemical in shortnose sturgeon larvae.

Results

Characterization and comparison of AHR2 among sturgeons

Based on previously determined white sturgeon AHR2 sequences, we designed sturgeon-specific primers to characterize the full-length coding sequence of AHR2 in both Atlantic sturgeon and

shortnose sturgeon. The Atlantic sturgeon AHR2 coding sequence was 3,258 nucleotides (nt) and coded for a 1,086 amino acid-long peptide that had a predicted molecular weight of 120.18 kDa. Shortnose sturgeon AHR2 coding sequence was slightly longer at 3,297 nt with a deduced coding sequence of 1,099 amino acids and a predicted molecular weight of 121.75 kDa.

We compared the AHR2 coding sequences of shortnose sturgeon, Atlantic sturgeon, lake sturgeon, white sturgeon, and Atlantic tomcod. Shortnose sturgeon AHR2 was slightly more similar to that of white sturgeon AHR2 (nucleotide similarity 98.4%; amino acid similarity 97.5%) than to Atlantic sturgeon AHR2 (nucleotide similarity 96.5%; amino acid similarity 95%), or lake sturgeon (nucleotide similarity 95.6%; amino acid similarity 91.8%) (Table 3). AHR2 sequences of all four sturgeons were approximately 65% similar (both nucleotide and amino acid) to AHR2 in Atlantic tomcod, a more phylogenetically distant taxon.

We compared the AHR2 ligand binding domain (LBD) of the two Atlantic coast sturgeons to that of the two previously characterized sturgeon species, white sturgeon and lake sturgeon, because of the possible mechanistic role of sequence variation in the LBD in conferring sensitivity to aromatic hydrocarbon contaminants (Fig 1). We found six variable amino acid sites when comparing the LBD among these sturgeons. At four of the sites, Atlantic sturgeon and shortnose sturgeon shared identical amino acid (AA) residues, but at AA319 and AA394 they differed. At AA388, which was previously shown to differ between lake sturgeon and white sturgeon and which was hypothesized to be the basis of differing sensitivities of those two species to PCDD/Fs and PCBs toxicities, we found that Atlantic sturgeon and shortnose sturgeon shared the Alanine-388 residue with the more sensitive white sturgeon compared to lake sturgeon.

Phylogeny of sturgeons AHR2 genes

A UPGMA dendrogram was generated from the deduced full-length shortnose sturgeon and Atlantic sturgeon amino acid sequences to determine the phylogenetic relationship between the two newly described AHR2 sequences and AHR1 and AHR2 sequences previously reported in the literature (Fig. 2). Two major clusters were detected; one containing human, mammalian, avian, and fish AHR1 and the second harboring avian and fish AHR2 sequences. The full-length amino acid sequences of shortnose sturgeon and Atlantic sturgeon AHR2s clustered well with previously characterized AHR2s of other fishes and marine birds and formed a clade distinct from that containing AHR1s in fishes and other vertebrates. Shortnose sturgeon AHR2 was

closest to white sturgeon AHR2 sequence as was indicated in the amino acid similarity table. As expected, the four sturgeon AHR2 sequences clustered closely indicative of their greater similarity than any other AHR2 sequences in fishes.

Expression of AHR2 in TCDD and PCB126 treated early life-stages of sturgeons

Initially, we quantified the expression of AHR2 in Atlantic sturgeon and shortnose sturgeon larvae treated with graded doses of TCDD and PCB126. We observed a dose-response relationship in Atlantic sturgeon larvae treated with TCDD with a maximum 6-fold induction at the highest dose used (10 ppb) (Fig. 3). PCB126 failed to elicit significant induction of AHR2 in Atlantic sturgeon larvae. We also observed significant induction of AHR2 mRNA in shortnose sturgeon larvae with increasing doses of TCDD (Fig. 4). Exposure to PCB126 induced AHR2 mRNA, but not significantly in shortnose sturgeon and the response did not vary with dose as was the case with TCDD.

Expression of AHR2 and CYP1A in Atlantic sturgeon early life-stages treated with four coplanar PCBs, TCDD, and an Aroclor mixture

Expression of AHR2 was quantified in Atlantic sturgeon larvae exposed as embryos to graded doses of the four coplanar PCBs, TCDD, and an environmentally relevant Aroclor mixture (Fig. 5). Expression of AHR2 was significantly increased by exposure to all four individual PCB congeners, TCDD, and Aroclor. In most cases, the enhanced expression was dose-responsive. For all compounds, greatest expression of AHR2 was observed at the highest or second highest dose tested. The magnitude of induction for all compounds was modest with greatest induction, approximately 5-fold, observed for PCB126. Surprisingly, the magnitude of induction with PCB126 even exceeded the 3-fold maximum with TCDD.

Expression of CYP1A mRNA was quantified in the same chemically treated Atlantic sturgeon larvae used to quantify expression of AHR2 mRNA (Fig. 6) allowing us to investigate the relationship between expression of AHR2 and CYP1A mRNAs. As expected, CYP1A mRNA was highly and significantly inducible by TCDD, all four coplanar PCBs, and the Aroclor mixture. Maximum induction elicited among PCB congeners, approximately 240-fold, was observed for PCB126 at the 1,000 ppb dose and exceeded maximum induction of 90-fold for TCDD at the highest dose tested, 50 ppb. Furthermore, maximum expression of CYP1A by PCB126 at the 10 ppb dose, 140-fold, exceeded that by TCDD (90-fold) at a comparable (but

higher dose) of 50 ppb. Significant dose-responsive expression was also detected in the Aroclor treated group with maximum 90-fold induction observed at the highest dose used (20 ppm).

In summary, the level of maximum expression for the four PCB congeners was in the sequence PCB126>PCB81>PCB77>PCB169. Among the four PCBs, significant induction, 45-fold, was observed for PCB126 even at the lowest dose tested (0.1 ppb), but not for the other three congeners. For PCBs 77, 81, and 169, significant induction was observed by the second lowest dose tested (1 ppb). Significant induction of CYP1A in the Aroclor mixture treated group did not occur until the third highest dose used (200 ppb).

Expression of AHR2 and CYP1A in shortnose sturgeon early life-stages treated with four coplanar PCBs, TCDD, and an Aroclor mixture

The pattern of AHR2 expression in chemically treated shortnose sturgeon was similar to that in Atlantic sturgeon (Fig. 7). The response to PCB126 was dose-responsive and maximum 4.5-fold induction occurred at the highest dose (10 ppm). AHR2 expression also increased 2.5-fold and 2.7-fold in PCB77 and PCB81-treated specimens, respectively, but was not dose-responsive as seen for PCB126-treated specimens. Increased expression was observed in TCDD-treated specimens but only to a maximum of 1.2-fold and that at an intermediate dose (1 ppb). Significant maximum 6-fold induction for the Aroclor mixture was observed at the two highest doses (2,000 and 20,000 ppm). Increased expression was not significant in the PCB169-treated group.

Significantly induced expression of CYP1A mRNA was observed in TCDD and all PCB-treated groups (Fig. 8). The lowest dose resulting in significant induction occurred at 0.1 ppb for PCB126 (31.6 fold), 100 ppb for PCB77 (76.8 fold), 10 ppb for PCB81 (68.1 fold), and 100 ppb for PCB169 (34.9 fold). Significant induction for TCDD first occurred at 0.01 ppb (43.7 fold). The sequence of lowest dose resulting in significant induction for the chemicals was PCB126>TCDD>PCB81>PCB77>PCB169. Significant induction in Aroclor-treated shortnose sturgeon was only observed at the highest dose (20,000 ppm).

Expression of AHR2 in tissues of PCB126 treated juvenile shortnose sturgeon

Expression of AHR2 was quantified in blood, fin clips, gill, heart, intestine, and liver of juvenile shortnose sturgeon i.p. injected with graded doses of PCB126 and sacrificed after 8 d (Fig. 9). We did not observe a dose-response in any tissues and only liver and blood showed significant

induction of AHR2 mRNA. Three-fold induction was observed in liver, but only at the highest dose used (50 ppb) and 6-fold induction was detected in blood at an intermediate dose (1.0 ppb).

Relationship between AHR2 and CYP1A expression in Atlantic and shortnose sturgeon

A significant positive relationship between expression of AHR2 and CYP1A was observed in both sturgeons (Table 4). In Atlantic sturgeon, this direct relationship occurred for all five chemicals individually (r=0.37 to 0.56) (P=0.05 to P=0.001) with a mean across all five chemicals of r=0.43 (P=0.01) (Supplementary Fig. 1). In shortnose sturgeon, we found significant positive correlations between AHR2 and CYPIA for four of five chemicals (r=0.48 to 0.66) with the exception being PCB169 (r=0.21). The mean correlation between AHR2 and CYP1A expression in shortnose sturgeon for all five chemicals was r=0.50 (P= 0.05) (Supplementary Fig. 2).

Identification of TEFs in shortnose sturgeon

Toxic Equivalency Factors were determined relative to gene expression for TCDD in shortnose sturgeon for the four coplanar PCBs based on the average of their EC_{20}, EC_{50}, and EC_{70}. TEFs for the four PCBs were 0.032 (PCB126), 0.004 (PCB81), 0.002 (PCB77), and 0.008 (PCB169). In summary, the *in vivo* TEFs derived for shortnose sturgeon in this study ranged from 6.4 (PCB126) to 162 (PCB169) higher than those adopted for fishes (primarily based on *in vitro* studies in rainbow trout juveniles) by the WHO and reported in Van den Berg et al. (1998) (Table 5).

Discussion

Many studies have demonstrated that young life-stages of fishes are highly sensitive to toxicities from aromatic hydrocarbon contaminants; however, there is considerable variability among species (Elonen et al. 1998), and even populations (Yuan et al. 2006; Wirgin et al. 2011; Reid et al. 2016), in their sensitivities to these contaminants. Where sturgeons lie on the continuum of vulnerabilities is largely unknown although recent reports suggest that their young life-stages may be among the more sensitive of fishes to toxicities from PCBs, TCDD, and PAHs (Chambers et al. 2012; Doering et al. 2014b; Doering et al. 2015). Previous studies in a variety of vertebrate taxa have shown that variation in AHR sequence, within and immediately

downstream of the ligand binding domain, correlate well with vulnerabilities to toxicities from exposure to TCDD and coplanar PCBs (Karchner et al. 2006; Cohen-Barnhouse et al. 2011; Wirgin et al. 2011) and maybe even in sturgeons (Doering et al. 2014ab, 2015). As an initial step in evaluating AHR2 functionality in Atlantic sturgeon and shortnose sturgeon, we characterized AHR2 structure and expression in both North American Atlantic coast sturgeon species.

Phylogenetic analysis revealed that we successfully isolated and characterized AHR2 in both Atlantic coast sturgeons. Nucleotide and amino acid sequence similarity between Atlantic and shortnose sturgeon was very high (95-97%) as was their similarity to AHR2 as previously characterized for the Pacific Coast white sturgeon (95-98%) and for inland lake sturgeon (91-96%) (Doering et al. 2014). In contrast, nucleotide and amino acid AHR2 similarity between these two Atlantic coast sturgeons and distantly related Atlantic tomcod *Microgadus tomcod* (Roy and Wirgin 1997) was much lower at approximately 65%. In the UPGMA dendrogram, the deduced amino acid sequence of putative AHR2s of Atlantic sturgeon, shortnose sturgeon, white sturgeon, and lake sturgeon clustered tightly within the clade that contained the AHR2 sequences of other fishes confirming that we had characterized AHR2 in these two sturgeons.

Doering et al. (2015) hypothesized that variation in sensitivities between white sturgeon and lake sturgeon to PCDD/Fs and PCBs-induced toxicities results from specific amino acid substitutions within the LBD of AHR1 and AHR2. They empirically tested this hypothesis in reporter gene assays for white sturgeon and lake sturgeon with transfected AHR1 and AHR2 proteins. These assays detected no difference in AHR1 activity between species, but AHR2 was activated at significantly lower concentrations of PCDD/F and PCBs in white sturgeon than in lake sturgeon. A comparison of amino acid sequences within the LBD of white and lake sturgeons revealed four substitutions but only the substitution at amino acid 388 (Ala-388 in white sturgeon; Thr-388 in lake sturgeon) affected the three-dimensional structure of the LBD which is presumed to bind AHR agonists and therefore would influence the toxic vulnerabilities to these contaminants. In comparison, we found a total of six variable sites within the LBD of the four sturgeon species. At each of these sites, one sturgeon species differed from the other three. At the putative key AA388 site in AHR2, both Atlantic sturgeon and shortnose sturgeon shared the Ala-388 residue with the more sensitive white sturgeon suggesting that AHR2 is

likely more sensitive to activation in Atlantic sturgeon and shortnose sturgeon as well as white sturgeon than in lake sturgeon.

There are conflicting reports in the literature on the i*n vivo* inducibility of AHRs in chemically treated or environmentally exposed organisms including fishes. For example, Meyers et al. (2003) reported that AHR2 was inducible by β-naphthoflavone (βNF) in Atlantic killifish *Fundulus heteroclitus* from Kings Creek, Virginia, but not in the PAH-resistant population in the nearby and PAH-contaminated Elizabeth River. Inducibility of AHR2 in these populations was correlated with inducibility of CYP1A by βNF and sensitivity to early life-stage toxicities by PAHs. In the most germane study to ours, Doering et al. (2014a) reported that AHR2 was inducible by βNF (50 and 500 ppm) in liver (6-fold), gill (11-fold), and intestine (35-fold) of laboratory-exposed juvenile white sturgeon. In contrast, Andreasen et al. (2002) reported an absence of AHR2 mRNA induction in zebrafish *Danio rerio* larvae treated with 500 ppb TCDD. Similarly, Roy and Wirgin (1997) reported an absence of hepatic AHR2 induction in PCB77 or B[a]P treated Atlantic tomcod from the Hudson River and the cleaner Miramichi River, New Brunswick.

We evaluated the inducibility of AHR2 in Atlantic sturgeon larvae treated as embryos with graded doses of TCDD, four coplanar PCBs, and an Aroclor mixture. We found that AHR2 was significantly inducible but only at low levels by all four PCB congeners, Aroclor mix, and TCDD in Atlantic sturgeon treated as embryos and sacrificed 8-10 d later after hatch or as larvae that were sacrificed within 24 h of hatch. Exposure of larvae to 10 ppb TCDD resulted in the greatest induction of AHR2 (6-fold) of all the chemical treatments. Induction of AHR2 with the four coplanar PCBs in Atlantic sturgeon larvae was modest and usually dose-responsive with maximal induction ranging between 1.7-fold (PCB 169) and 4.7-fold (PCB126). AHR2 expression with the Aroclor mixture was also dose-responsive with a maximum 2.8-fold induction. For all PCB congeners, levels of maximum AHR2 induction followed the relative TEFs for these compounds reported for fishes in Van den Berg et al. (1998).

We also investigated the magnitude and dose-responsive of AHR2 induction in shortnose sturgeon larvae treated as embryos with the same chemicals as Atlantic sturgeon and sacrificed after hatch. For three of the four PCBs (exception of PCB169), there was a trend of modestly increasing AHR2 expression with dose. Once again, maximum induction was seen for PCB126

(4-fold) and 2.5-fold and 2.8-fold induction were observed for PCB77 and PCB81, respectively. There was little evidence, of AHR2 induction in shortnose sturgeon treated with the Aroclor mix.

Surprisingly, there was no evidence of dose-responsive AHR2 induction in tissues of shortnose sturgeon juveniles i.p. injected with 50 ppm PCB126 and sacrificed after 7 d except in liver at the highest dose (50 ppb) and in blood at an intermediate dose (1 ppb). Previously, we had demonstrated significant induction of CYP1A mRNA in these tissues of the same specimens at the 50 ppb dose in liver, heart, gill, and intestine, but not in blood or fin clips (Roy et al. 2011). Thus, in these tissues of shortnose sturgeon there was little correspondence between levels of AHR2 and CYP1A mRNA expression.

In summary, we found significant dose-responsiveness, but with only modest levels of AHR2 induction for PCBs in early life-stages of both sturgeons, but little evidence for that in tissues of older specimens. In the absence of empirical data, it is impossible to say whether the difference in AHR2 inducibility between earlier life-stages and juveniles was due to lower levels of contaminants in target tissues of juveniles than in earlier life-stages or reduced sensitivity of juveniles to the toxic effects of these chemicals.

Because we hypothesized that levels of AHR2 expression were an important determinant of the inducibility of genes in the AHR battery, we also quantified levels of CYP1A mRNA in this study in the same specimens for which AHR2 expression was measured. As expected, CYP1A was highly inducible in both sturgeon species for all chemical treatments. For example, CYP1A mRNA was significantly inducible in TCDD (20-fold) and PCB126-treated (40-fold) Atlantic sturgeon at the lowest doses tested, 0.0005 ppb and 0.1 ppb, respectively. Furthermore, CYP1A expression was significantly induced initially in PCB81 and PCB77 at the 1 ppb dose. Maximum induction for these chemicals ranged from 240-fold with PCB126 to 30-fold with PCB169.

Similar results for inducibility were obtained with shortnose sturgeon for which initial significant induction was observed at the 0.01 ppb level for TCDD (9.4 fold) and 0.1 ppb for PCB126 (31.6 fold). The lowest doses at which significant induction of CYP1A occurred in shortnose sturgeon were 1 ppb (PCB81, 8.6-fold), 10 ppb (PCB77, 7.1 fold), and 100 ppb (PCB169, 34.9 fold) which were at an least order of magnitude higher doses than those that initiated induction in Atlantic sturgeon. This suggests that shortnose sturgeon may be an order of magnitude less sensitive than Atlantic sturgeon to coplanar or TCDD induced toxicities.

This wide range of CYP1A expression levels in both sturgeons for all PCBs allowed for a robust test of the effects of AHR2 levels on expression of genes in the AHR battery. We found a significant relationship between AHR2 and CYP1A levels (P=0.01) for all four PCBs and TCDD in Atlantic sturgeon and four of five chemicals in shortnose sturgeon (exception being PCB169). It has yet to be determined if increased levels of AHR2 regulate the magnitude of induction of CYP1A and other AHR battery genes in these treatment groups.

Toxicity Equivalency Quotients (TEQs) provide resource managers with a useful quantitative measure of the total toxicity of environmental matrices (i.e. sediments and biota) and assume that the toxicity of each chemical in a matrix is additive and is mediated by the AHR pathway. Calculation of TEQs require that the actual concentration of each AHR agonist in the matrix be empirically determined and that its TEF is known. TEFs for PCDD/Fs and coplanar PCBs have been internationally adopted for broad taxonomic groups; e.g., mammals, birds, and fishes (Van den Berg et al. 1998), but it is recognized that the sensitivities of the various taxa within each of these broad groups may vary widely. For example, within the fishes, it was empirically demonstrated that the vulnerabilities of salmonids and zebrafish to TCDD-induced early life-stage toxicities vary by greater than an order of magnitude (Elonen et al. 1998). Because of their imperiled status and earlier studies in sturgeons (Doering et al. 2014b, 2015; Chambers et al. 2012), we hypothesized that the TEFs for coplanar PCBs in sturgeons are greater than in most other fish taxa. This was confirmed here as the TEFs for each of the four coplanar PCBs was from almost one to more than two orders of magnitude greater than those reported for fishes in Van den Berg et al. (1998). Our empirical results are consistent with those of Eisner et al. (2016) who reported that the TEF for PCB126 in lake sturgeon (but not white sturgeon) was also an order of magnitude greater than that accepted by the WHO.

We demonstrate that AHR2 is inducible in sturgeons by low levels of coplanar PCBs and TCDD, that AHR2 and CYP1A expression levels are significantly correlated, and that TEFs for PCBs in sturgeons may be substantially higher than reported for most other fish taxa. In the absence of empirical, congener-specific measurements of PCBs and PCDD/Fs levels in environmentally exposed sturgeons from estuaries such as the Hudson River and Delaware River, however, it is problematic to relate our treatment levels to those to those to which sturgeon populations are environmentally exposed in impacted spawning rivers. Furthermore, we have only characterized AHR2 in these sturgeons, whereas, previous investigations in white

sturgeon Doering et al. 2014ab suggest that cooperativity between AHR2 and AHR1 in activating *in vitro* gene expression may enhance the vulnerability of sturgeons to aromatic hydrocarbon toxicity. Similar studies have yet to be completed in Atlantic Coast sturgeons.

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Figure Captions

Fig. 1. A comparison of the amino acid sequence of the Ligand Binding Domain (LBD) of AHR2 among four North American sturgeon species; Atlantic sturgeon, shortnose sturgeon, lake sturgeon, and white sturgeon. Deduced peptide sequences are from residues 291 to 397 of all species. Asterisks denote amino acid residues that are shared among all four species.

Fig. 2. Phylogenetic analyses of 28 full-length AHR1 and AHR2 amino acid sequences in vertebrate species. An unrooted UPGMA tree with bootsrap values was generated based on the genetic distance between each taxon and implemented in MEGA 6.06-mac (Tamura et al. 2013). AHR1and AHR1b in Japanese medaka and AHR2a and AHR2b represent duplicated forms of these two AHR genes in these taxa. Genetic distance is represented on the x-axis (See text for Genbank accession numbers).

Fig. 3 Mean (+/- SE) fold induction of AHR2 mRNA compared to acetone vehicle control in 2 d post-hatch Atlantic sturgeon larvae waterborne exposed to graded doses of TCDD (0.001 to 10 ppb) and PCB126 (0.01 to 1000 ppb) for 26-27 h and immediately sacrificed.

Fig. 4 Mean (+/- SE) fold induction of AHR2 mRNA compared to acetone vehicle control in shortnose sturgeon larvae waterborne exposed for 26-27 h as embryos to graded doses of TCDD (0.001 ppb to 10 ppb) and PCB126 (0.01 ppb to 1000 ppb). After exposure, embryos were transferred to clean water where they were maintained until hatch (7-10 days post fertilization) and sacrificed.

Fig 5: Mean (+/- SE) fold induction compared to acetone vehicle control of AHR2 mRNA expression determined by quantitative RT-PCR in Atlantic sturgeon larvae exposed as embryos for 24 h to graded doses of TCDD, four coplanar PCBs, and an environmentally relevant Aroclor mixture and sacrificed as larvae 24-36 h post-hatch. The mean values of the ΔΔAHR2 for each chemical and dose were compared using one-way ANOVA with a Tukeys post hoc test to determine the statistical significance of differences in response between doses. Different letters over the bars represent significant differences in response between doses. Asterisks represent a significant difference compared to the acetone vehicle control.

Fig. 6: Mean (+/- SE) fold induction compared to acetone control vehicle of CYP1A mRNA expression determined by quantitative RT-PCR in Atlantic sturgeon larvae exposed as embryos for 24 h to graded doses of TCDD, four coplanar PCBs, and an environmentally relevant Aroclor mixture and sacrificed as larvae 24-36 h post-hatch. The mean values of the ΔCYP1A for each chemical and dose were compared using a one-way ANOVA with a Tukeys post hoc test to test for differences in response between treatments. Different letters over the bars represent significant differences in response between dose treatments. Asterisks represent a significant difference compared to the acetone control vehicle ($P < 0.05$).

Fig. 7: Mean (+/- SE) fold induction compared to acetone control vehicle of AHR2 mRNA induction determined by quantitative RT-PCR in shortnose sturgeon larvae exposed as 1 day-old embryos for 24 h to graded doses of TCDD, four coplanar PCBs, and an environmentally relevant Aroclor mixture, transferred to clean water, and sacrificed 24-36 h post-hatch. Asterisks

represent significant differences from the acetone control vehicles while letters represent significant differences between doses using a one way ANOVA and Tukey's post hoc analysis ($P < 0.05$).

Fig. 8: Mean (+/- SE) fold induction compared to acetone control vehicle of CYP1A mRNA expression determined by quantitative RT-PCR in shortnose sturgeon larvae exposed as 1 day-old embryos for 24 h to graded doses of TCDD, four coplanar PCBs, and an environmentally relevant Aroclor mixture, transferred to clean water, and sacrificed 24-36 h post-hatch. Asterisks represent significant differences from the acetone control vehicles while letters represent significant differences between doses using a one way ANOVA and Tukey's post hoc analysis ($P < 0.05$).

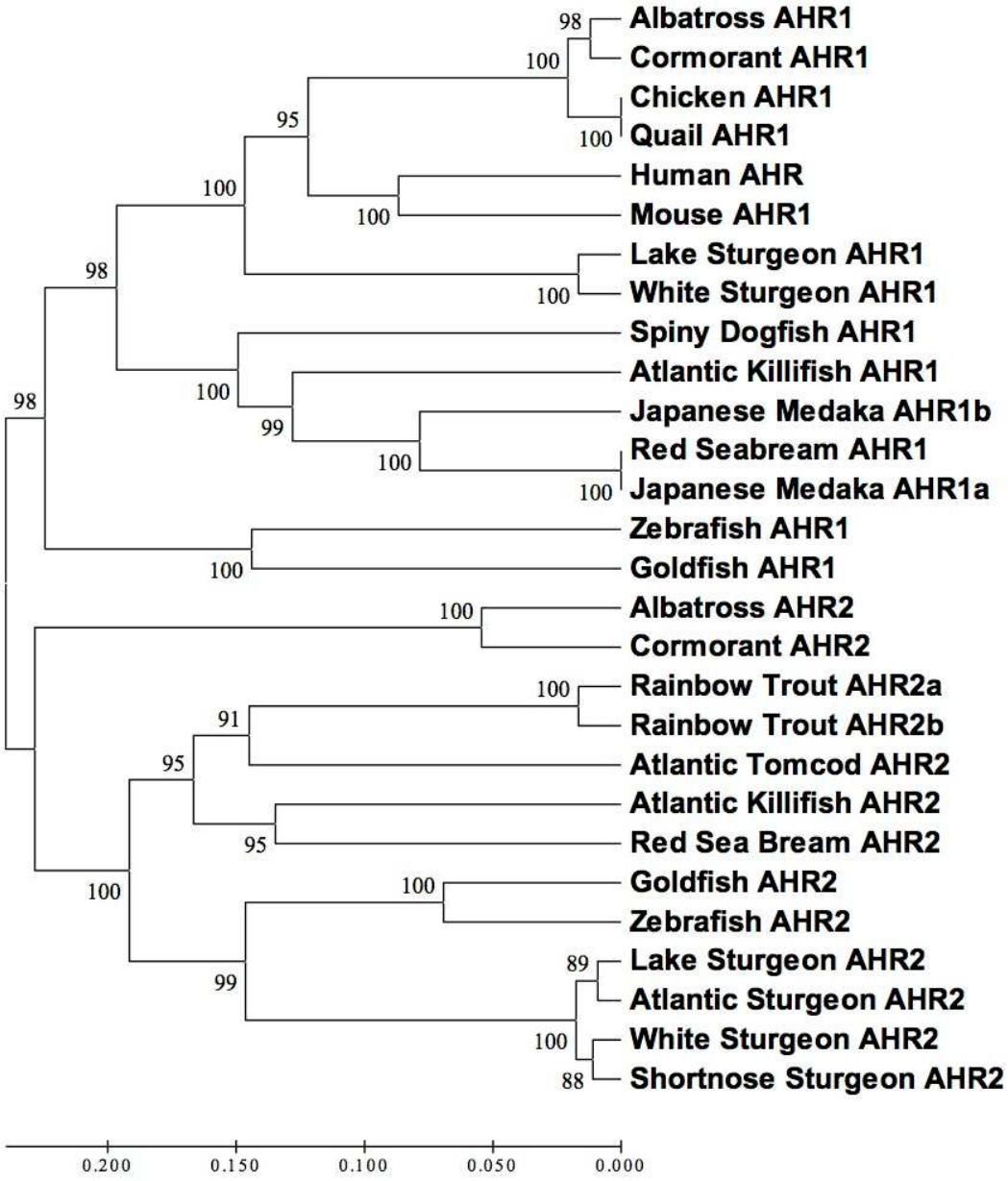
Fig. 9: Mean (+/- SE) fold induction of AHR2 mRNA expression compared to corn oil controls in six different tissues of juvenile shortnose sturgeon of Connecticut River origin that were hatchery bred, reared, and i.p. injected with graded doses of PCB126 (0.01 ppb to 50 ppb) in corn oil vehicle and sacrificed 8 d after treatment.

```
White Sturgeon         TLIFQTKHKL DFTPMGCDTR GKVVLGYTDT GLCMRGTGYQ FIHAADMMHC ADNHVRMIKT
Atlantic Sturgeon      TLIFQTKHKL DFTPMGCDTR GKVVLGYTDT ELCMRGTGYQ FIHAADMMHC ADNHVRMIKT
Shortnose Sturgeon     TLIFQTKHKL DFTPMGCDTR GKVVLGYTET ELCMRGTGYQ FIHAADMMHC ADNHVRMIKT
Lake Sturgeon          TLIFQTKHKL DFTPTGCDTR GKAVLGYTDT ELCMRGTGYQ FIHAADMMHC ADNHVRMIKT
                       ********** **** ***** ** ***** *  ********* ********** **********

White Sturgeon         GESGLTVFRL LTKNGSWVWV QANARLIYKG GRPDFIVARQ RALTNEE
Atlantic Sturgeon      GESGLTVFRL LTKNGSWVWV QANARLIYKG GRPDFIVARQ RALTNEE
Shortnose Sturgeon     GESGLTVFRL LTKNGSWVWV QANARLIYKG GRPDFIVARQ RALSNEE
Lake Sturgeon          GESGLTVFRL LTKNGSWVWV QANARLIYKG GRPDFIVTRQ RALTNEE
                       ********** ********** ********** ******* ** *** ***
```

Figure 1

Figure 2


Albatross AHR1
Cormorant AHR1
Chicken AHR1
Quail AHR1
Human AHR
Mouse AHR1
Lake Sturgeon AHR1
White Sturgeon AHR1
Spiny Dogfish AHR1
Atlantic Killifish AHR1
Japanese Medaka AHR1b
Red Seabream AHR1
Japanese Medaka AHR1a
Zebrafish AHR1
Goldfish AHR1
Albatross AHR2
Cormorant AHR2
Rainbow Trout AHR2a
Rainbow Trout AHR2b
Atlantic Tomcod AHR2
Atlantic Killifish AHR2
Red Sea Bream AHR2
Goldfish AHR2
Zebrafish AHR2
Lake Sturgeon AHR2
Atlantic Sturgeon AHR2
White Sturgeon AHR2
Shortnose Sturgeon AHR2
0.200
0.150
0.100
0.050
0.000

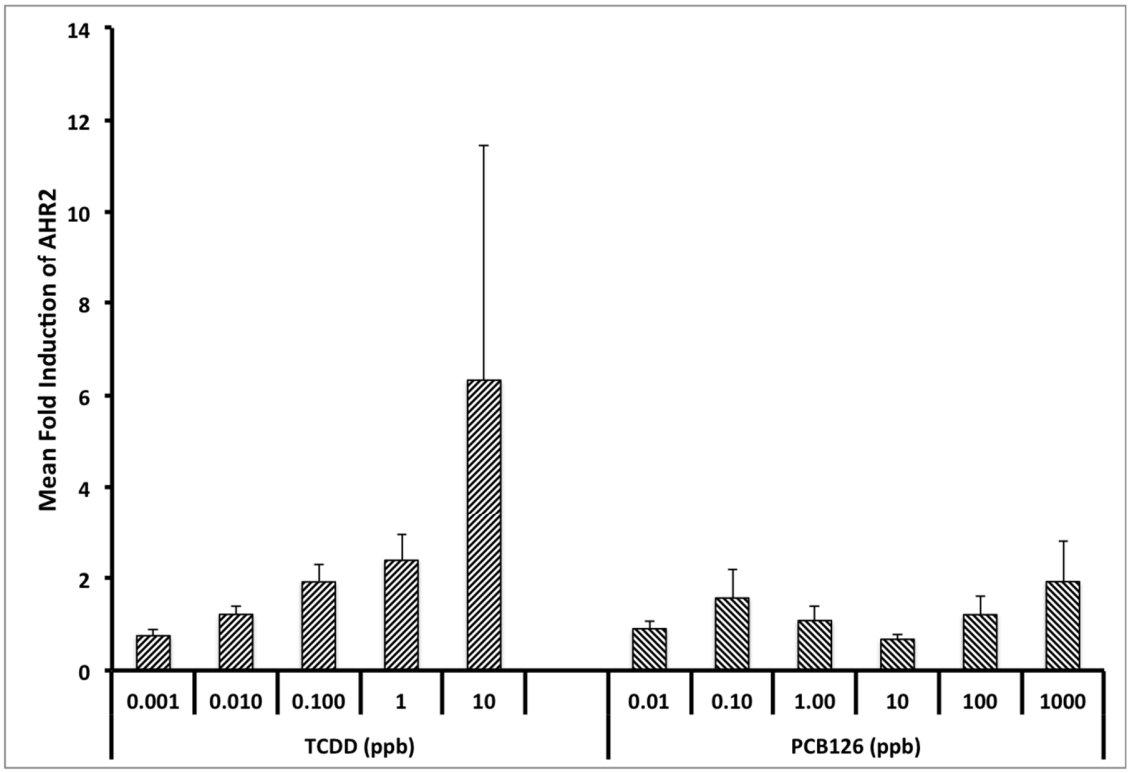

Mean Fold Induction of AHR2
14
12
10
8
6
4
2
0
0.001
0.010
0.100
1
10
0.01
0.10
1.00
10
100
1000
TCDD (ppb)
PCB126 (ppb)

Figure 3

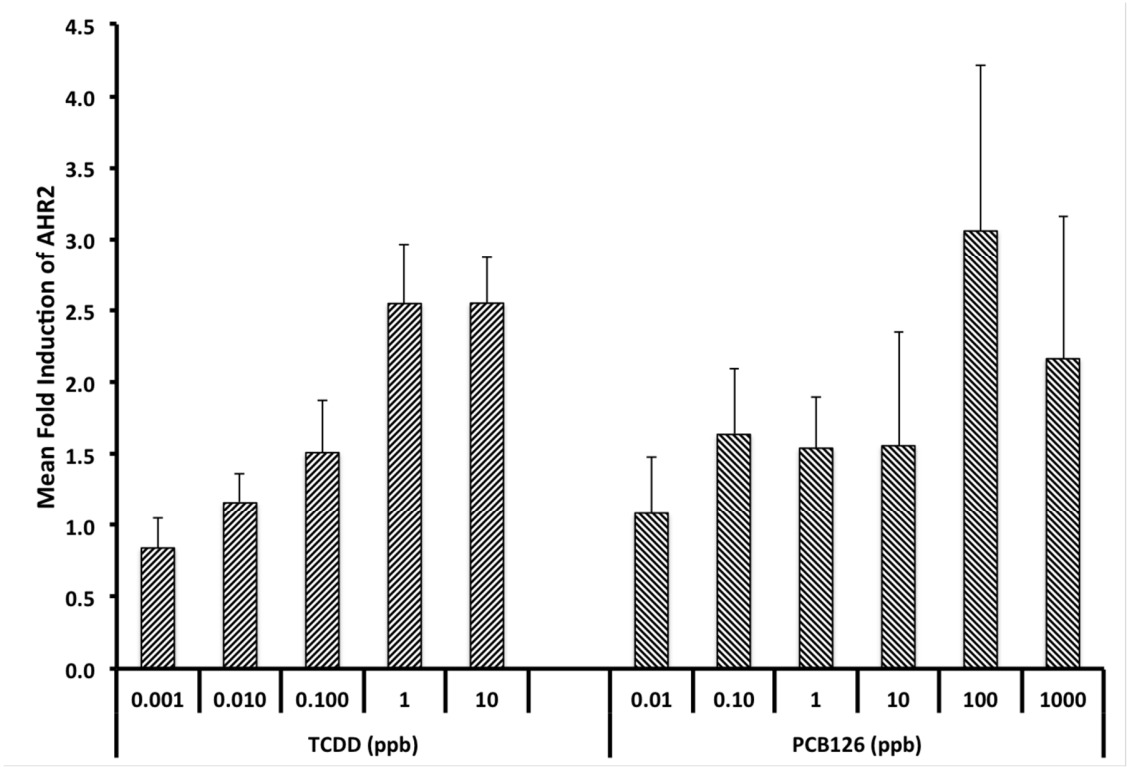

Mean Fold Induction of AHR2
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.001
0.010
0.100
1
10
0.01
0.10
1
10
100
1000
TCDD (ppb)
PCB126 (ppb)

Figure 4

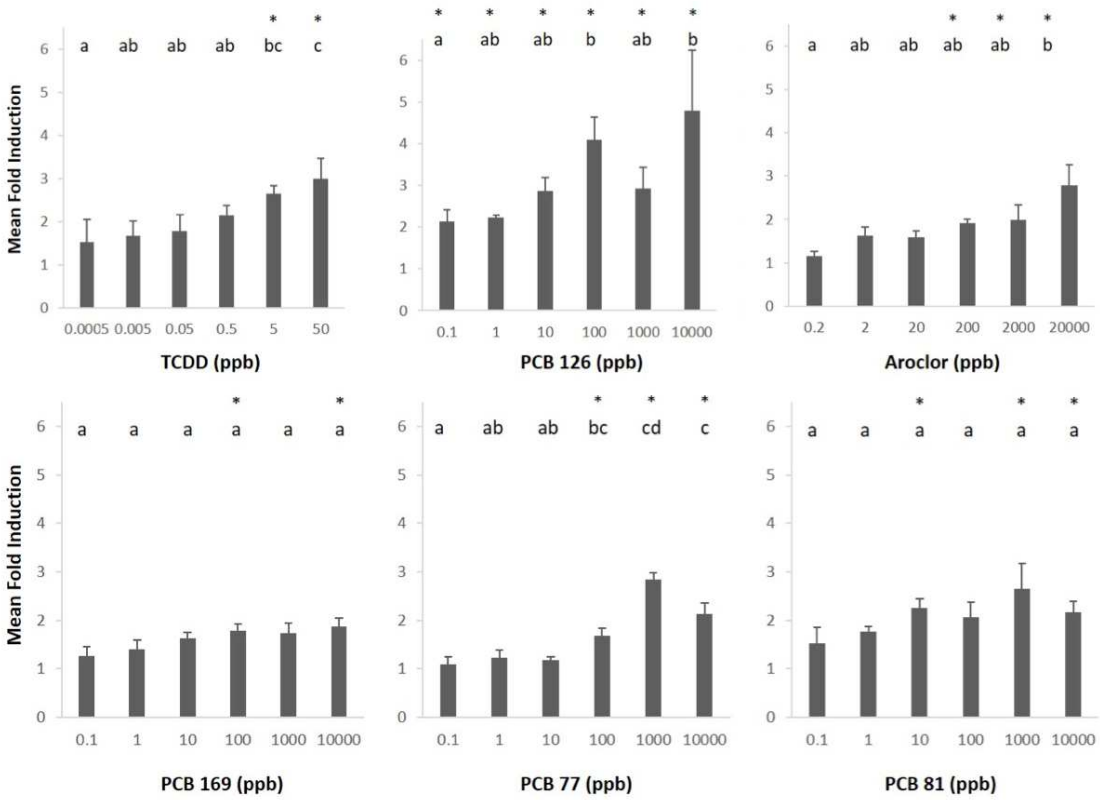

Mean Fold Induction
a ab ab ab bc c
0.0005 0.005 0.05 0.5 5 50
TCDD (ppb)
a ab ab b ab b
0.1 1 10 100 1000 10000
PCB 126 (ppb)
a ab ab ab ab b
0.2 2 20 200 2000 20000
Aroclor (ppb)
Mean Fold Induction
a a a a a a
0.1 1 10 100 1000 10000
PCB 169 (ppb)
a ab ab bc cd c
0.1 1 10 100 1000 10000
PCB 77 (ppb)
a a a a a a
0.1 1 10 100 1000 10000
PCB 81 (ppb)

Figure 5

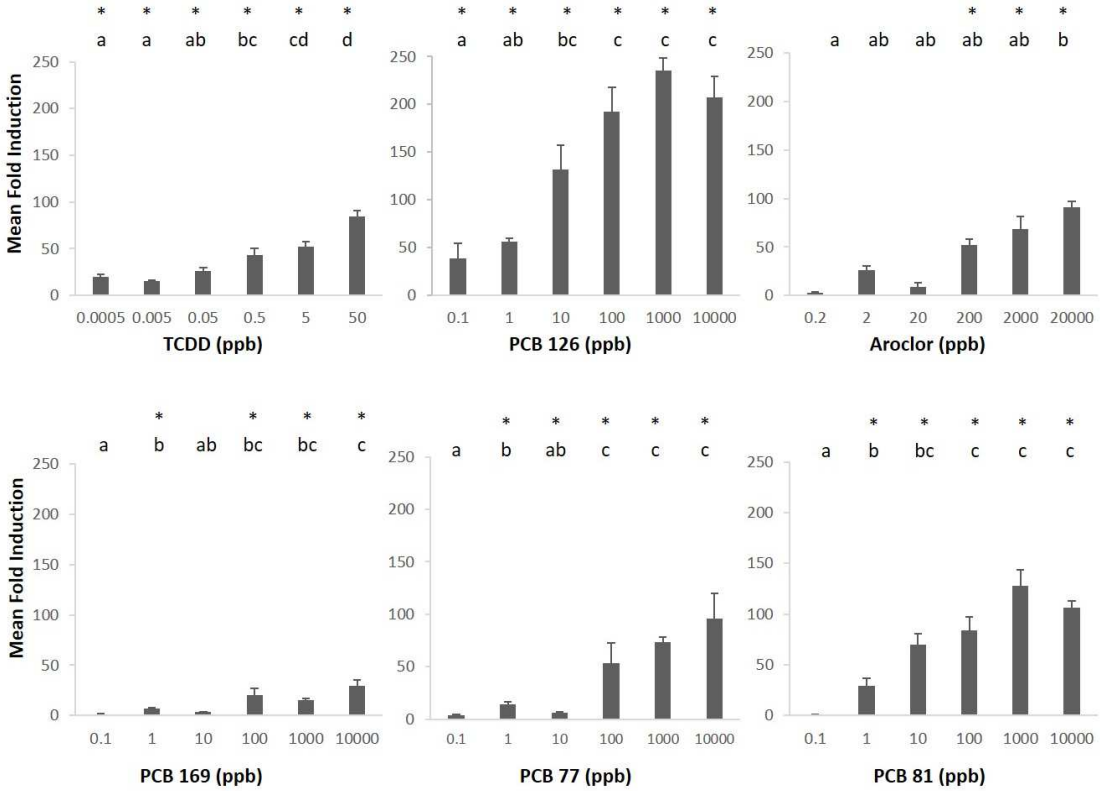

Mean Fold Induction
TCDD (ppb)
0.0005 0.005 0.05 0.5 5 50
a a ab bc cd d
PCB 126 (ppb)
0.1 1 10 100 1000 10000
a ab bc c c c
Aroclor (ppb)
0.2 2 20 200 2000 20000
a ab ab ab ab b
PCB 169 (ppb)
0.1 1 10 100 1000 10000
a b ab bc bc c
PCB 77 (ppb)
0.1 1 10 100 1000 10000
a b ab c c c
PCB 81 (ppb)
0.1 1 10 100 1000 10000
a b bc c c c

Figure 6

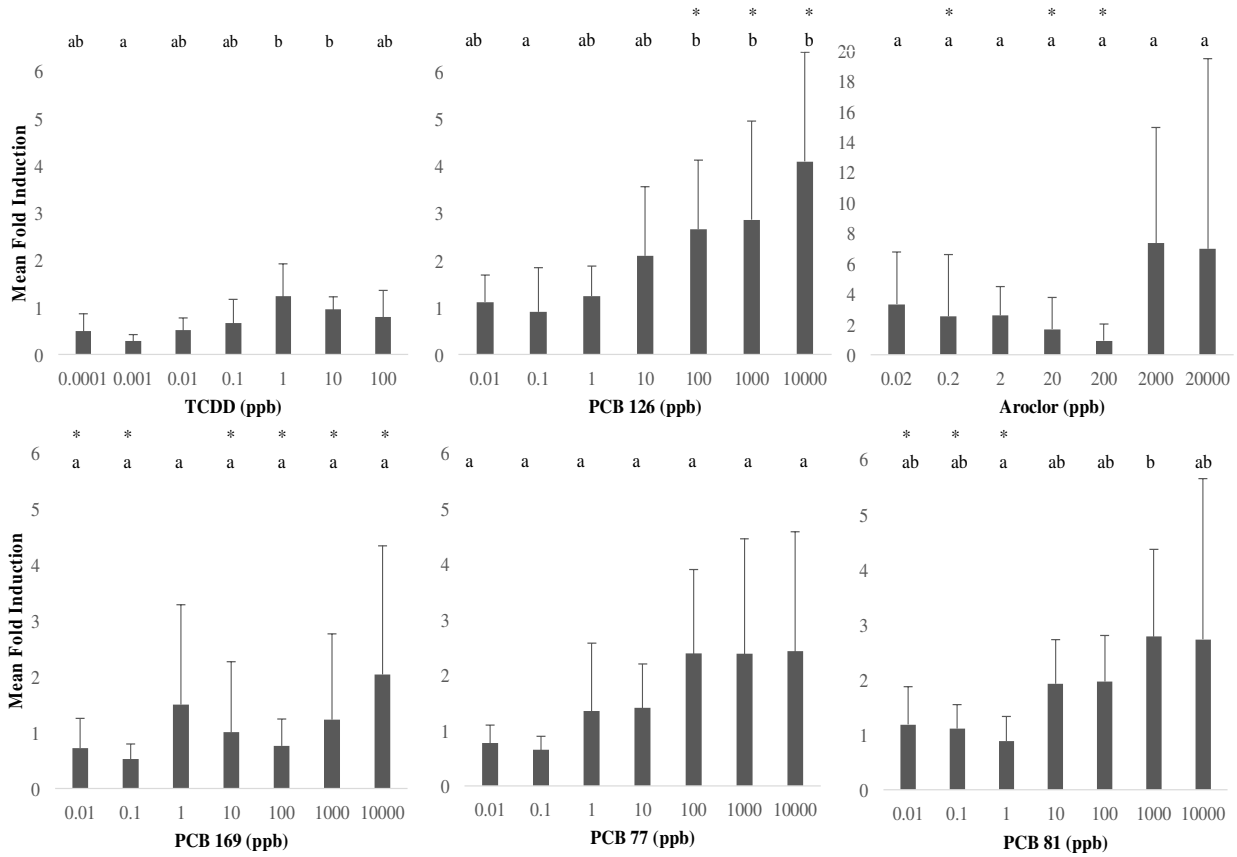

Mean Fold Induction
ab a ab ab b b ab
0.0001 0.001 0.01 0.1 1 10 100
TCDD (ppb)
ab a ab ab b b b
0.01 0.1 1 10 100 1000 10000
PCB 126 (ppb)
a a a a a a a
0.02 0.2 2 20 200 2000 20000
Aroclor (ppb)
Mean Fold Induction
a a a a a a a
0.01 0.1 1 10 100 1000 10000
PCB 169 (ppb)
a a a a a a a
0.01 0.1 1 10 100 1000 10000
PCB 77 (ppb)
ab ab a ab ab b ab
0.01 0.1 1 10 100 1000 10000
PCB 81 (ppb)

Figure 7

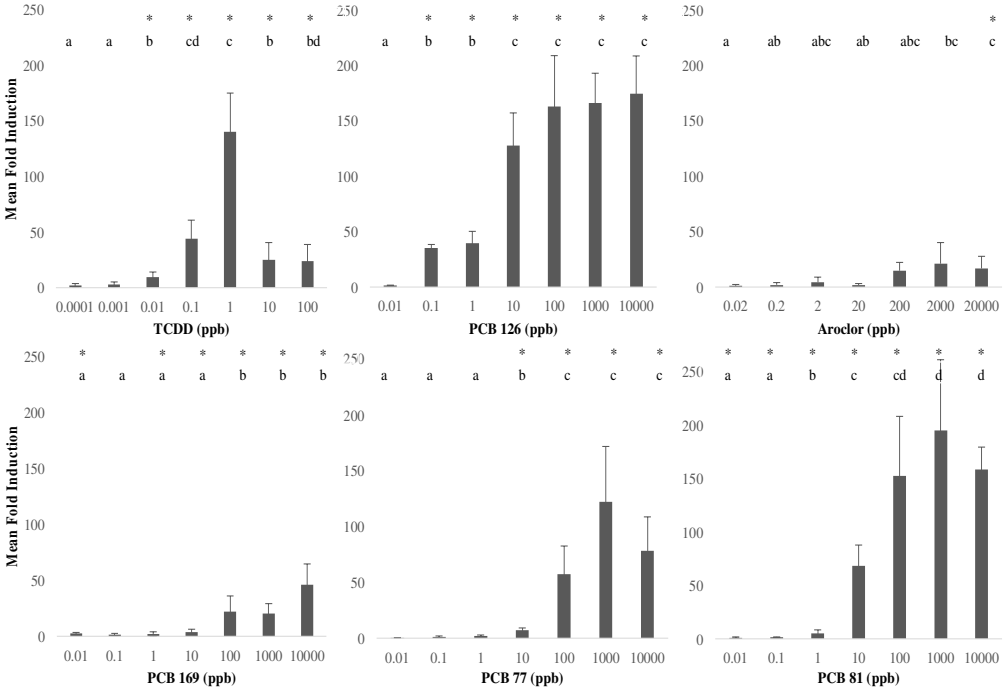

Mean Fold Induction
TCDD (ppb)
PCB 126 (ppb)
Aroclor (ppb)
PCB 169 (ppb)
PCB 77 (ppb)
PCB 81 (ppb)

Figure 8

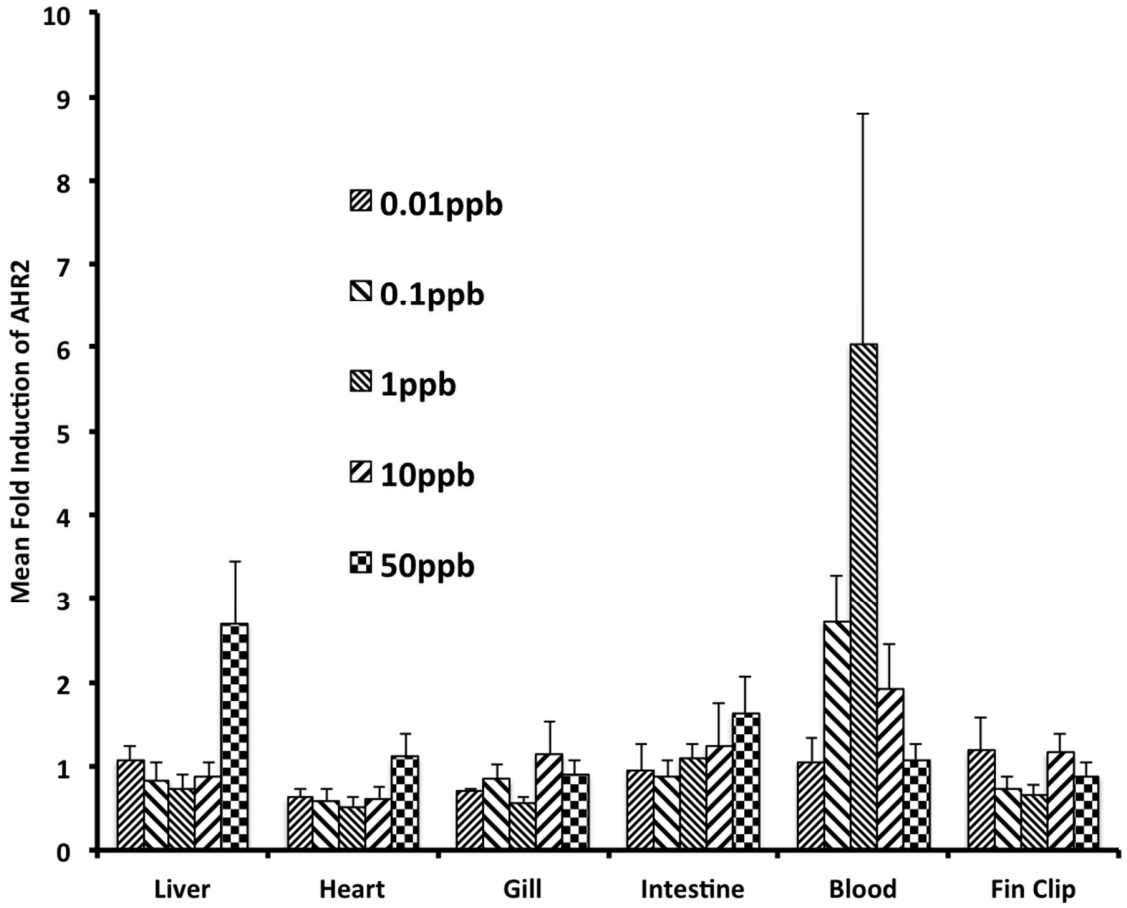

Mean Fold Induction of AHR2
10
9
8
7
6
5
4
3
2
1
0
0.01ppb
0.1ppb
1ppb
10ppb
50ppb
Liver
Heart
Gill
Intestine
Blood
Fin Clip

Figure 9

Table 1. Primers used to amplify Atlantic sturgeon and shortnose sturgeon AHR2

Forward primers for RT-PCR		Reverse Primers for RT-PCR	
STAHRF613	GGTGGTCACAGCTGAAGG	STAHRR1157	CCACCTTCCCTCTGGTGTC
STAHRF608	GTGCTGGTGGTCACAGC	STAHRR947	GAAAGCCAGAAGAGTTGT
STAHRF927	TTGACAACTCTTCTGGCT	STAHRR1220	ACTGATACCCGGTGCCTC
STAHRF1312	GACCAAGAATGGAAGCTG	STAHRR1222	GAACTGATACCCGGTGCC
STAHRF1235	GATATGATGCACTGTGCAG	STAHRR1843	CTCCCAGTGCTTTAGCTC
STAHRF1571	GATCCCAACTCTCTTCTG	STAHRR2033	CTCCAATGGAATCCATC
STAHRF1793	AATGGAAATGACCTCCAA	STAHRR2189	GGTTTAGATCCATCTGGG
STAHRF1972	GGGAAAGATAATGGTCAG	STAHRR2414	GTTCCATGTTGTCTTGGG
STAHRF2223	GCCCAACCAAGTTGGAATG	STAHRR2617	GGGCTGCCCCATGTTGG
STAHRF2354	CTGCCAATCTGAACACCC	STAHRR2861	GAAGGTGGTGCTGTTTTG
STAHRF2528	CATGGGCAACCACAGATAC	STAHRR3105	GGGGTCTGATAGATCTTG
STAHRF3028	GTTGGCTCAGAGCAATCAG	STAHRR3282	GGCTTCGTCTGGTAGTAG
STAHRF3237	CCAACCAAATCCCCCTCC	STAHRR3495	GGAAAATATGTGTTTCCG
STAHRF3184	CCCCCAACCAAAGCCCC	STAHRR3532	CTAGTAATCACAGCAGTT
STAHRF3401	CTCCGCAACCAGGCCATCC	STAHRR3669	CTGAGGGTGAAACTGTC
STAHRF2645	CAGAAGTCCCAGCTGATGC	STAHRR3755	GACTCCTTTCAAATACTTG
STAHRF2759	GTGCCCATGTCGGCCCAG	STAHRR3856	CATTAGTTCATCCAGCC

Primers used for 5'UTR		Primers used for Real Time RT- PCR	
STAHRR388	GAGAACGGCAGCAGGCTGG	STAHRF197	TCATGCAAAGTGGCAGCAA
STAHRR437	GCTGAGCCGCAGCACAGACAG	STAHRR270	TTCGGGCGGAATGAGTTG
STAHRR365	CTTGTCGAGCTCAGAGTT	Sturact60F	CATTGTCACCAACTGGGATGAC
STAHRF191	GAAGAAACTTTACTAG	Sturact125R	ACACGCAGCTCATTGTAGAAGGT
STAHRF170	GAAAATTGCGTGCAATG		
STAHRF146	CTCCGGGTCTGATCGC		
STAHRR656	GGTAGGTGACGAATAG		
STPANC	GACCACGCGTATCGATGTCGAC		

Table 2.

Summary of experimental treatments in which gene expression was measured in Atlantic sturgeon and shortnose sturgeon in this study. dpf=days post-fertilization and dph=days post hatch

Year	Species	Life-Stage Treated	Treatment Chemicals	Age When Treated	Exposure Time	Age When Sampled	Tissues Analyzed	Results In Figure #
2009	Atlantic Sturgeon	Larvae	TCDD, PCB126	2 dph	26-27 h	3 dph	Whole body	Fig. 3
2009	Shortnose Sturgeon	Embryos	TCDD, PCB126	5 dpf	26-27 h	1 dph	Whole body	Fig. 4
2009	Shortnose Sturgeon	Juveniles	PCB126	1-year-old	8 d	1-year and 8 d	Blood, gill, fin, heart, intestine, liver	Fig. 9
2014	Atlantic Sturgeon	Embryos	TCDD, PCB77, PCB81, PCB126 PCB169, Aroclor Mix	2 dpf	24 h	1 dph	Whole body	Fig. 5, 6
2014	Shortnose Sturgeon	Embryos	TCDD, PCB77, PCB81, PCB126 PCB169, Aroclor Mix	1 dpf	24 h	1 dph	Whole body	Fig. 7, 8

Table 3.

Nucleotide and amino acid sequence similarity of AHR2 among four North American sturgeon species and Atlantic tomcod.

Nucleotide Similarity

	Shortnose sturgeon	**White sturgeon**	**Lake sturgeon**	**Atlantic tomcod**
Atlantic sturgeon	96.5%	96.0%	93.9%	65.6%
Shortnose sturgeon		98.4%	95.6%	65.2%
White sturgeon			95.7%	64.7%
Lake sturgeon				64.8%

Amino Acid Similarity

	Shortnose sturgeon	**White sturgeon**	**Lake sturgeon**	**Atlantic tomcod**
Atlantic sturgeon	95.0%	95.1%	92.4%	65.3%
Shortnose sturgeon		97.5%	91.8%	65.5%
White sturgeon			89.8%	65.4%
Lake sturgeon				56.0%

Table 4.

Correlation between expression of AHR2 and CYP1A in Atlantic sturgeon and shortnose sturgeon for TCDD, PCB77, PCB81, PCB126, and PCB169

Species	Chemical	*r*	*P* value
Atlantic Sturgeon	TCDD	0.56	0.0004
	PCB77	0.57	0.0003
	PCB81	0.34	0.0520
	PCB126	0.46	0.0045
	PCB169	0.42	0.0123
Shortnose Sturgeon	TCDD	0.58	0.0009
	PCB77	0.56	0.0003
	PCB81	0.66	0.0001
	PCB126	0.48	0.0027
	PCB169	0.21	0.2283

Table 5.

A comparison of Toxic Equivalency Factors (TEFs) for fishes adopted by the World Health Organization[1] to those empirically derived in shortnose sturgeon in this study

PCB Congener	TEFs WHO[1]	TEFs Shortnose Sturgeon (this study)	Times Higher than WHO TEFs
PCB77	0.0001	0.002	21
PCB81	0.0001	0.004	44
PCB126	0.005	0.032[2]	6.4
PCB169	0.00005	0.008	162

[1] Reported in Van den Berg et al. (1998)
[2] TEFs for PCB126 reported by Eisner et al. (2016) based on CYP1A expression in liver explants from juvenile white sturgeon and lake sturgeon were 0.005 and 0.04, respectively