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# Identification and expression of aryl hydrocarbon receptors (AhR1 and AhR2) provide insight in an evolutionary context regarding sensitivity of white sturgeon (*Acipenser transmontanus*) to dioxin-like compounds

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ABSTRACT

Sturgeons are ancient fishes, which are endangered in many parts of the world. Due to their benthic nature and longevity, sturgeon are at great risk of exposure to bioaccumulative contaminants such as dioxin-like compounds (DLCs). Despite their endangered status, little research has been conducted to characterize the relative sensitivity of sturgeons to DLCs. Proper assessment of risk of DLCs posed to these fishes therefore, requires a better understanding of this sensitivity and the factors that are driving it. Adverse effects associated with exposure to DLCs are mediated by the aryl hydrocarbon receptor (AhR). This study identified and characterized two distinct AhRs, AhR1 and AhR2, in white sturgeon (*Acipenser transmontanus*) for the first time as a first step in studying the relative sensitivities of sturgeons to DLCs. Furthermore, tissue-specific expression of both AhRs under basal conditions and in response to exposure to the model DLC, β-naphthoflavone (βNF), was determined. The sequence of amino acids of AhR1 of white sturgeon had greater similarity to AhRs of tetrapods, including amphibians, birds, and mammals, than to AhR1s of other fishes. The sequence of amino acids in the ligand binding domain of the AhR1 had greater than 80% similarity to AhRs known to bind DLCs and was less similar to AhRs not known to bind DLCs. AhR2 of white sturgeon had greatest similarity to AhR2 of other fishes. Profiles of expression of AhR1 and AhR2 in white sturgeon were distinct from those known in other fishes and appear more similar to profiles observed in birds. Expressions of both AhR1 and AhR2 of white sturgeon were greatest in liver and heart, which are target organs for DLCs. Furthermore, abundances of transcripts of AhR1 and AhR2 in all tissues from white sturgeon were greater than controls (up to 35-fold) following exposure to βNF. Based upon both AhRs having similar abundances of transcript in target organs of DLC toxicity, both AhRs being up-regulated following exposure to βNF, and both AhRs having greatest similarity to AhRs known to bind DLCs, it is hypothesized that both AhR1 and AhR2 of white sturgeon might mediate effects of DLCs in this species. Since current risk assessments are based on data derived largely from highly divergent fishes within the Salmonidae, presence of two functional AhRs in white sturgeon, one of which has greatest similarity to AhRs of birds, might have significant implications for the sensitivity of sturgeons to DLCs compared to other fishes.



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## 1. Introduction

Sturgeons (Acipenseridae) are ancient fishes with recognizable fossils of modern species dating back at least 65 million years (Wilimovsky, 1956). Today, sturgeons are endangered over much of their range, which has rendered them of great interest in context with ecological risk assessment. In the northwestern USA and British Columbia, Canada there is particular concern about declines of some populations of white sturgeon (*Acipenser transmontanus*), the largest freshwater species of fish in North America. These decreases in populations of white sturgeon have been attributed to several human activities, including pollution (Birstein, 1993; Coutant, 2004; Gisbert and Williot, 2002; Irvine et al., 2007; Luk'yanenko et al., 1999; Paragamian and Hansen, 2008; Scott and Crossman, 1973). Sturgeons are long-lived, sexual maturity is attained slowly, they spawn only intermittently, live in close association with sediments, and have a greater lipid content than numerous other fishes which increases the likelihood of bioaccumulation of lipophilic pollutants (Birstein, 1993). Dioxin-like compounds (DLCs), which include polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), coplanar polychlorinated biphenyls (PCBs), and certain polycyclic aromatic hydrocarbons (PAHs) are contaminants of concern because of their ability to bioaccumulate and because they can be persistent under certain conditions, such as in sediments (Birnbaum and DeVito, 1995). Some DLCs have been detected in white sturgeon at concentrations sufficient to warrant concern (Foster et al., 1999, 2001; Kruse and Scarnecchia, 2002; Kruse and Webb, 2006; MacDonald et al., 1997) given chronic effect levels in some species of fishes (Giesy et al., 2002; Rigaud et al., 2013). Due to their specific life history, white sturgeon could be particularly susceptible to the adverse effects of bioaccumulation of DLCs. However, little is currently known regarding the sensitivity of sturgeons or other ancient fishes to these contaminants.

PCDDs, PCDFs, PCBs, and other DLCs share structural similarities and bind with relatively high affinity to the aryl hydrocarbon receptor (AhR) (Giesy et al., 1994). Following ligand binding, the AhR heterodimerizes with the aryl hydrocarbon nuclear translocator (ARNT) allowing binding to consensus dioxin responsive elements on DNA, resulting in pleiotropic expression of a suite of biotransformation enzymes and regulating all known effects of exposure to DLCs (Okey, 2007). Activation of AhR-mediated pathways causes a range of adverse effects in vertebrates, including hepatotoxicity, immune suppression, reproductive and endocrine impairment, teratogenicity, carcinogenicity, and loss of weight (Kawajiri and Fujii-Kuriyama, 2007). Some fishes, such as salmonids, are among the vertebrates of greatest sensitivity to adverse effects from exposure to DLCs (Johnson et al., 1998; Walker et al., 1991). Despite the potential for adverse effects of DLCs to sturgeons, little is known about the sensitivity of these and other ancient fishes to these chemicals. Sturgeons and some other ancient fishes, including sharks, rays, and skates share similar molecular responses to DLCs with regards to cytochrome P450 enzymes that are consistent with the more modern teleost fishes (Agradi et al., 1999; Doering et al., 2012; Hahn et al., 1998; Roy et al., 2011). Toxicity studies conducted with sturgeons have found them to be among the most sensitive fishes to adverse effects of other environmental pollutants, such as endocrine disrupting chemicals and metal ions (Dwyer et al., 2005; Vardy et al., 2011, 2012). This evidence justifies the hypothesis that sturgeons could be sensitive to DLCs, which together with their great risk of exposure, warrants further investigations into the AhR signalling pathway of sturgeons.

Knowledge of the specific structure of the AhRs of sturgeons is important because it has been shown that in birds sensitivity to DLCs is related to the sequence of amino acids of the ligand binding domain of the AhR (Karchner et al., 2006; Farmahin et al., 2012, 2013; Head et al., 2008). However, a similar relationship between species sensitivity to DLCs and the sequence of amino acids of the AhR has not yet been established for fishes (Doering et al., 2013). To establish such relationships, a better understanding of the AhR in different species of fishes would be required, with the ultimate goal of allowing the prediction of the sensitivity of species of concern, such as some species of sturgeons, to DLCs. Therefore, objectives of this study were to identify full-length amino acid sequences of AhRs expressed in different tissues of white sturgeon and to determine their tissue-specific expression under basal conditions and in response to exposure to a model DLC. This work will supplement current knowledge on evolutionary aspects of the AhR pathway among ancient fishes and allow for a better understanding of the mechanisms by which sturgeons respond and their sensitivity to exposure to DLCs.

## 2. Materials and methods

### 2.1. Fish

Juvenile white sturgeon (*A. transmontanus*), ranging in mass from 12 to 27 g (approximately 1.5 years of age) were randomly selected from an in-house stock reared from eggs acquired from the Kootenay Trout Hatchery (Fort Steele, BC, Canada). White sturgeon were maintained in separate 712 L tanks under flow-through conditions at approximately 12 °C and fed frozen bloodworms (Hagen, Montreal, QC, Canada) at approximately 2% of their body weight daily.

### 2.2. Exposure protocol

The protocol for exposing juvenile white sturgeon has been described previously (Doering et al., 2012). Briefly, twenty-one individuals were injected intraperitoneally (i.p.) with one of three doses ($n = 7$) of beta-naphthoflavone (βNF purity >98%; Sigma–Aldrich, Oakville, ON, Canada) dissolved in corn oil. Doses used were 0 mg βNF/kg-bw (0 mM), 50 mg βNF/kg-bw (46 mM), and 500 mg βNF/kg-bw (460 mM). Three days following injection, all fish were euthanized by overdose of tricaine methanesulfonate (MS-222, Sigma–Aldrich). Livers, gills, and intestines were collected from fish exposed to βNF and immediately snap-frozen in liquid nitrogen. Brains, hearts, livers, gills, stomachs, intestines, spleens, head kidney, and muscle were collected from control fish for basal expression studies and immediately snap-frozen in liquid nitrogen.

### 2.3. Identification and sequencing of AhRs in white sturgeon

Full-length AhR1 and AhR2 genes had not yet been identified for sturgeons. However, a fragment of 609 nucleotides from an AhR-like gene of white sturgeon was available online (Accession #: AY880254.1). Additional nucleotide fragments of AhR-like genes were identified in a library of the transcriptome of liver from white sturgeon that was generated by paired-end sequencing by use of the *Illumina* HiSeq 2000 platform (*Illumina*, San Diego, CA, USA). Methods used for *Illumina* paired-end transcriptome sequencing have been described previously (Tompsett et al., 2013; Wiseman et al., 2013). Full-length cDNA sequences for each AhR were acquired by use of rapid amplification of cDNA ends–polymerase chain reaction (RACE–PCR). cDNA was synthesized by use of the SMARTer RACE cDNA Amplification Kit (Clontech, Mountain View, CA, USA) and RACE–PCR was performed by use of the Advantage 2 PCR Kit (Clontech), both according to the protocol provided by the manufacturer. Gene-specific RACE–PCR primers for white sturgeon AhR1 and AhR2 were designed by use of Primer3 software (Table 1; Rozen and Skaletsky, 2000) according to the protocol provided by the manufacturer and synthesized by Invitrogen (Burlington, ON,