

Sensitivity and Tissue Specificity of Aryl Hydrocarbon Receptor Mediated Responses of White Sturgeon (*Acipenser transmontanus*)

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Background

- Worldwide many sturgeon species (Acipenseridae) have faced massive population declines with some species nearing extinction.
- These declines are attributed to human activities such as overfishing, construction of dams, habitat alteration, competition from introduced species, and pollution.
- Pollutants, including dioxins and PCBs, have contaminated sediments of some water bodies inhabited by sturgeon.
- Sturgeon are uniquely susceptible to bioaccumulation of lipophilic contaminants relative to other fish species because they:

- are extremely long lived
- have a higher lipid content
- are mature later
- spawn intermittently
- live in close association with sediments
- have a diet rich in benthic prey

- Although deformities and enzyme induction have been observed in sturgeon collected from some contaminated sites, little is known about sensitivity of sturgeon to dioxin-like chemicals.



Photo by Michael Gallacher

Figure 1.0: Juvenile White Sturgeon

Objectives:

- Investigation of EROD responsiveness in white sturgeon and how it compares to responsiveness of rainbow trout.
- Investigate tissue-specific responses of white sturgeon.
- Investigate evolutionary aspects of AhR mediated effects in white sturgeon

Methods

Biochemical and molecular endpoints were characterized in white sturgeon in order to better understand how sturgeon respond to AhR agonist.

Exposure Experiment: White sturgeon (*Acipenser transmontanus*) were injected intraperitoneally (i.p.) with one of three doses of β -naphthoflavone (β NF) dissolved in corn oil at 0mg/kg, 50mg/kg, or 500mg/kg. Rainbow trout (*Oncorhynchus mykiss*) were used as a previously characterized species. Tissue samples were collected three days following injection.

Endpoint: Ethoxyresorufin O-deethylase (EROD) activity was measured according methods of Kennedy & Jones, 1994. A primer against Cyp1A was designed from partial sequences cloned from white sturgeon. Primers against white sturgeon AhR and β -actin were designed by use of publically available sequences of white sturgeon (NCBI Accession # AY880254).

Statistical Test: Significance was determined using the Kruskal-Wallis test followed by the Mann Whitney U test. Statistical tests were conducted by use of SPSS 19 software (SPSS Inc., Chicago, IL, USA).

Results

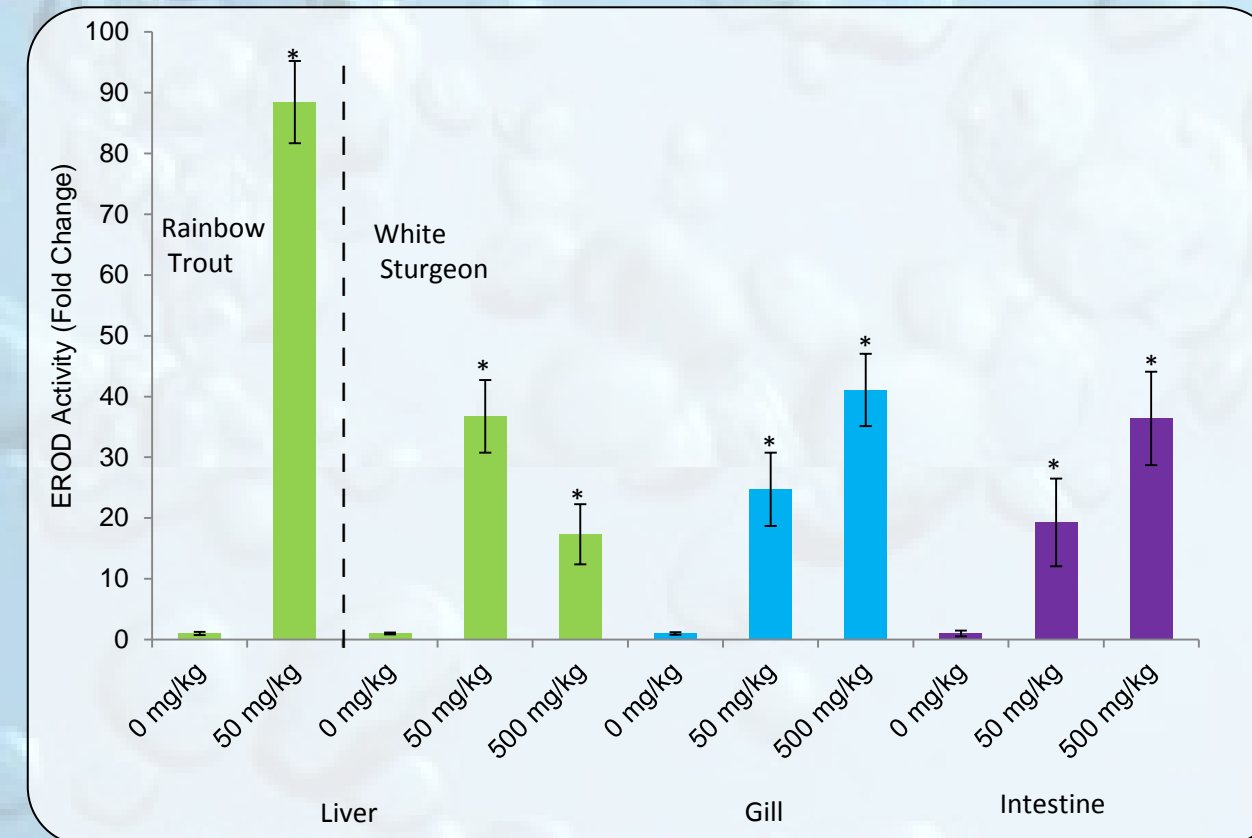


Figure 2.1: EROD activity in liver, gill, and intestine from white sturgeon (WS) and rainbow trout (RT) following 3-days of exposure to 50 or 500 mg/kg of β NF. Data are expressed as fold change over basal activity. EROD activity was non-detectable in gills and intestines from unexposed and exposed RT (Data not shown). Induction of EROD activity in gills and intestines from WS is based upon $\frac{1}{2}$ the detection limit due to non-detectable basal activity. * indicates a significant ($p \leq 0.05$) difference compared to basal activity.

- White sturgeon had moderate hepatic EROD responsiveness following exposure to β NF at either 50 mg/kg or 500 mg/kg compared to other fish species such as the rainbow trout (Table 1).
- EROD activity observed in gill and intestine of exposed white sturgeon was surprising. No publication was found presenting gill or intestinal tissue with equal or greater inducibility following i.p. injection as that observed in this study on white sturgeon. Similarly, no EROD activity was detected in rainbow trout gill or intestine in this study.

Species	Weight (g)	0 mg/kg	50 mg/kg	Induction	Source
Mudfish	110 - 560	64	337	5x	Gadagbui et al. 1996
Largemouth Bass	Avg. 40	739	2700	6x	Zhang et al. 1991
Mummichog	-	244	1386	6x	Klopper-Sams & Stegman, 1992
Nile Tilapia	52 - 85	80	485	6x	Gadagbui et al. 1996
Little Skate	Avg. 563	109	852	8x	Hahn et al. 1998
Northern Pike	-	480	4660	10x	Fortin & Celander, 1993
Dab	90 - 200	530	7070	13x	Fortin & Celander, 1993
Atlantic Salmon	75 - 125	50	1600	32x	Grosvik et al. 1997
White Sturgeon	12 - 27	13.9	510.6	37x	This study.
Tomlate	319 - 612	24	1312	55x	Stegman et al. 1997
European Eel	Avg. 25	24.7	1843	75x	Fenet et al. 1998
Brook Trout	-	25	1960	78x	Elkhus & Stegman, 1989
Rainbow Trout	9 - 24	9.6	849.4	88x	This study.
Rainbow Trout	100 - 200	80	7000	88x	Zhang et al. 2003
Brown Trout	Avg. 100	44	12480	284x	Fortin & Celander, 1993

Table 1: Comparison of hepatic EROD activity following i.p. injection of 50 mg/kg β NF in different species of fish (pmol/min/mg protein).

Acknowledgements

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Results

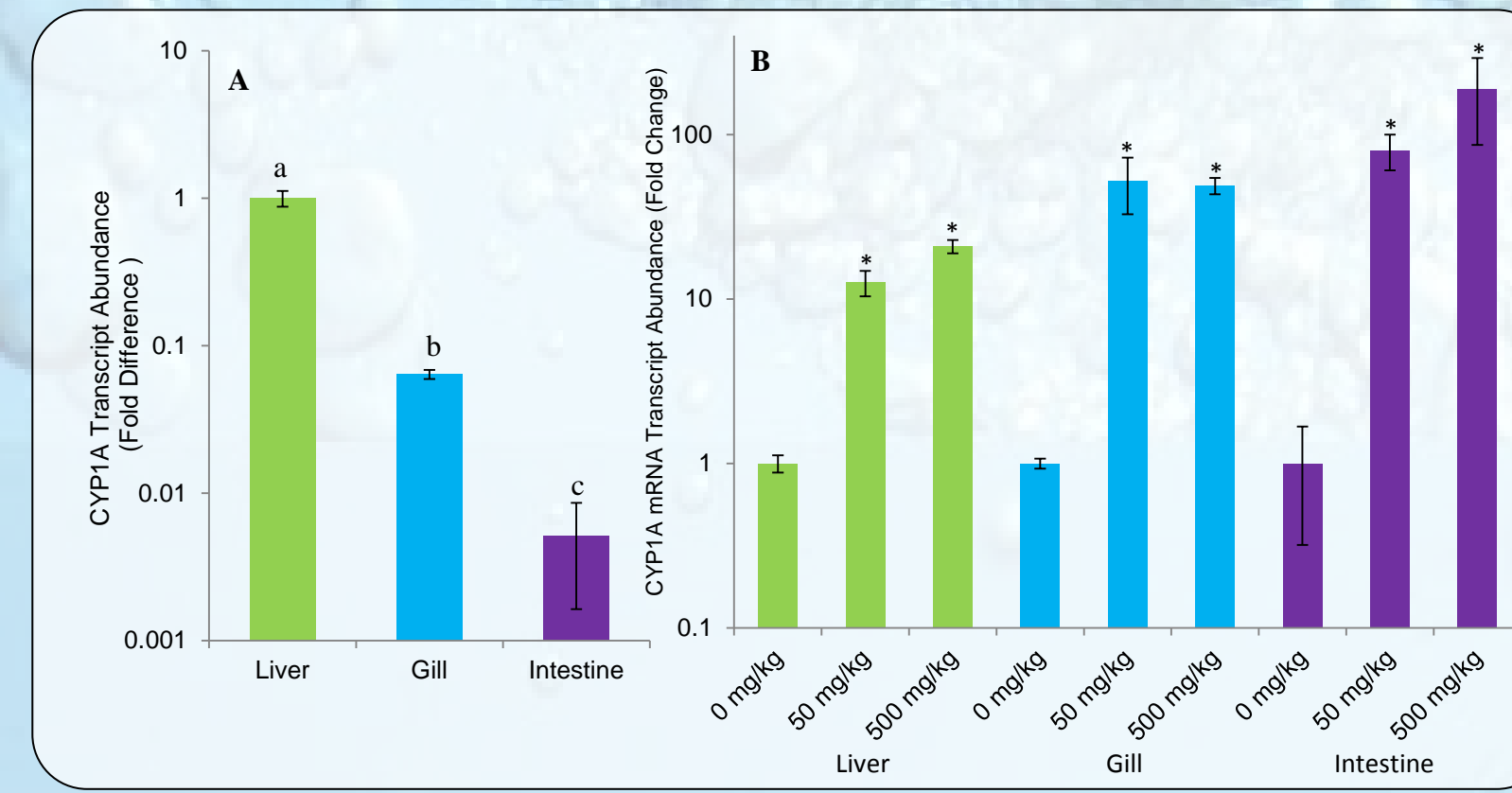


Figure 2.2:

A: Comparison of basal transcript abundance of Cyp1A among tissues in white sturgeon. Different letters indicate significant differences ($p \leq 0.05$).

B: Transcript abundance Cyp1A among liver, gill, and intestine in white sturgeon following 3-days exposure to 50 and 500 mg/kg of β NF. * indicates a significant ($p \leq 0.05$) difference compared to basal mRNA abundance.

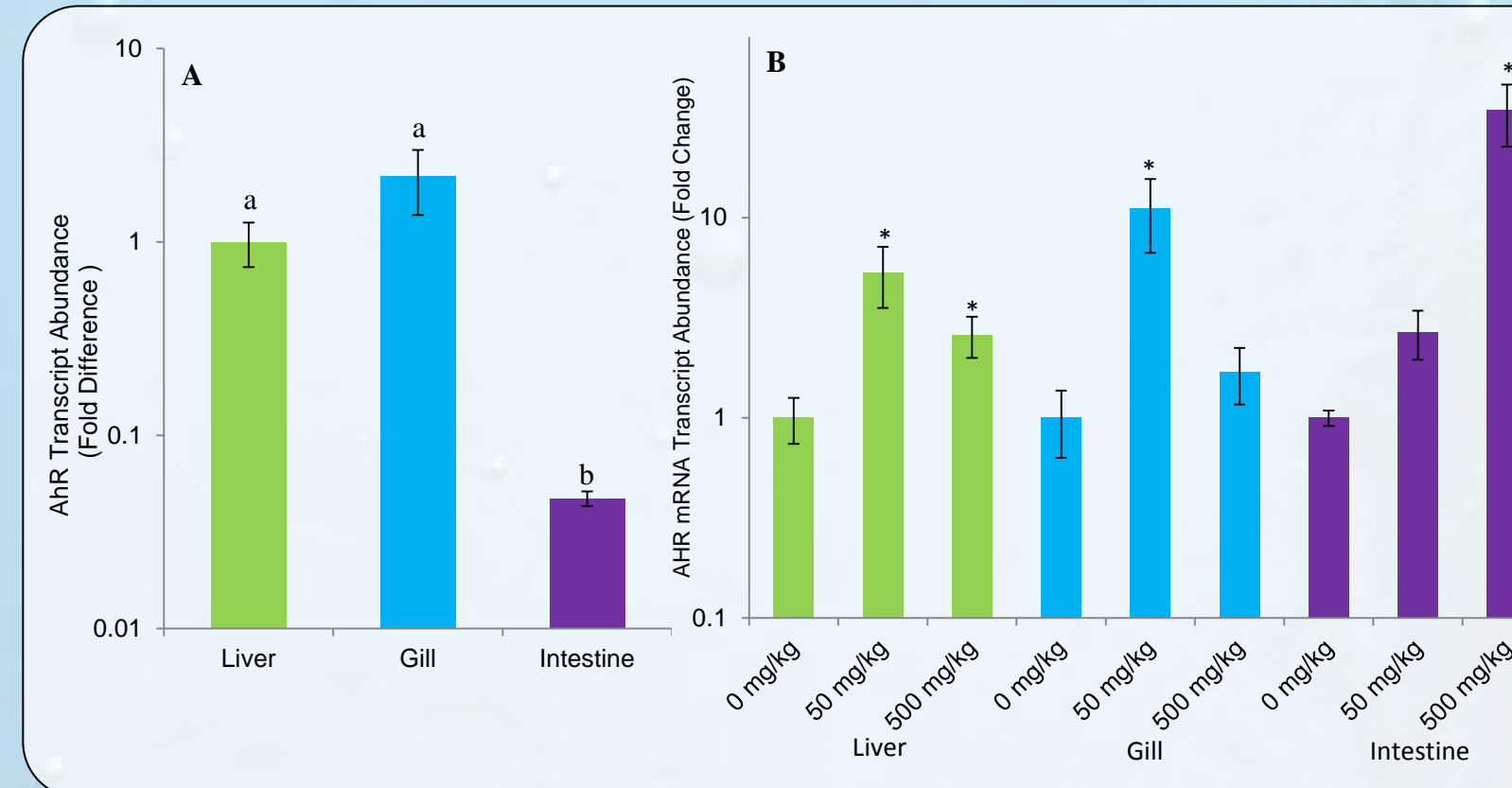


Figure 2.3:

A: Comparison of basal transcript abundance of AhR among tissues of white sturgeon. Different letters indicate significant differences ($p \leq 0.05$).

B: Transcript abundance of AhR among liver, gill, and intestine in white sturgeon following 3-days exposure to 50 or 500 mg/kg of β NF. * indicates a significant ($p \leq 0.05$) difference compared to basal transcript abundance.

- Although liver expressed greater basal Cyp1A and AhR transcripts, gill and intestine exhibited greater induction following exposure.
- Greater induction in AhR transcript abundance was observed in white sturgeon than has been observed for other fishes.

References

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 Aluru, N, Vuori, K, Vijayan, MM. (2005). Comp. Biochem. And Physio. C, 141, 40-49.
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Results

- Autoregulation of AhR transcript abundance observed in white sturgeon appears to coincide with that previously reported for rainbow trout (Aluru et al, 2005), however it differs from that of some other vertebrates (Jonsson et al, 2011).
- Comparisons with other fishes suggests that responsiveness of the AhR pathway in sturgeon might be different from other species thus a phylogenetic tree was developed (Fig. 3).
- The white sturgeon AhR clusters closely with those of other cartilaginous fish species, however it differs from sequences of salmonids and most teleost fishes.

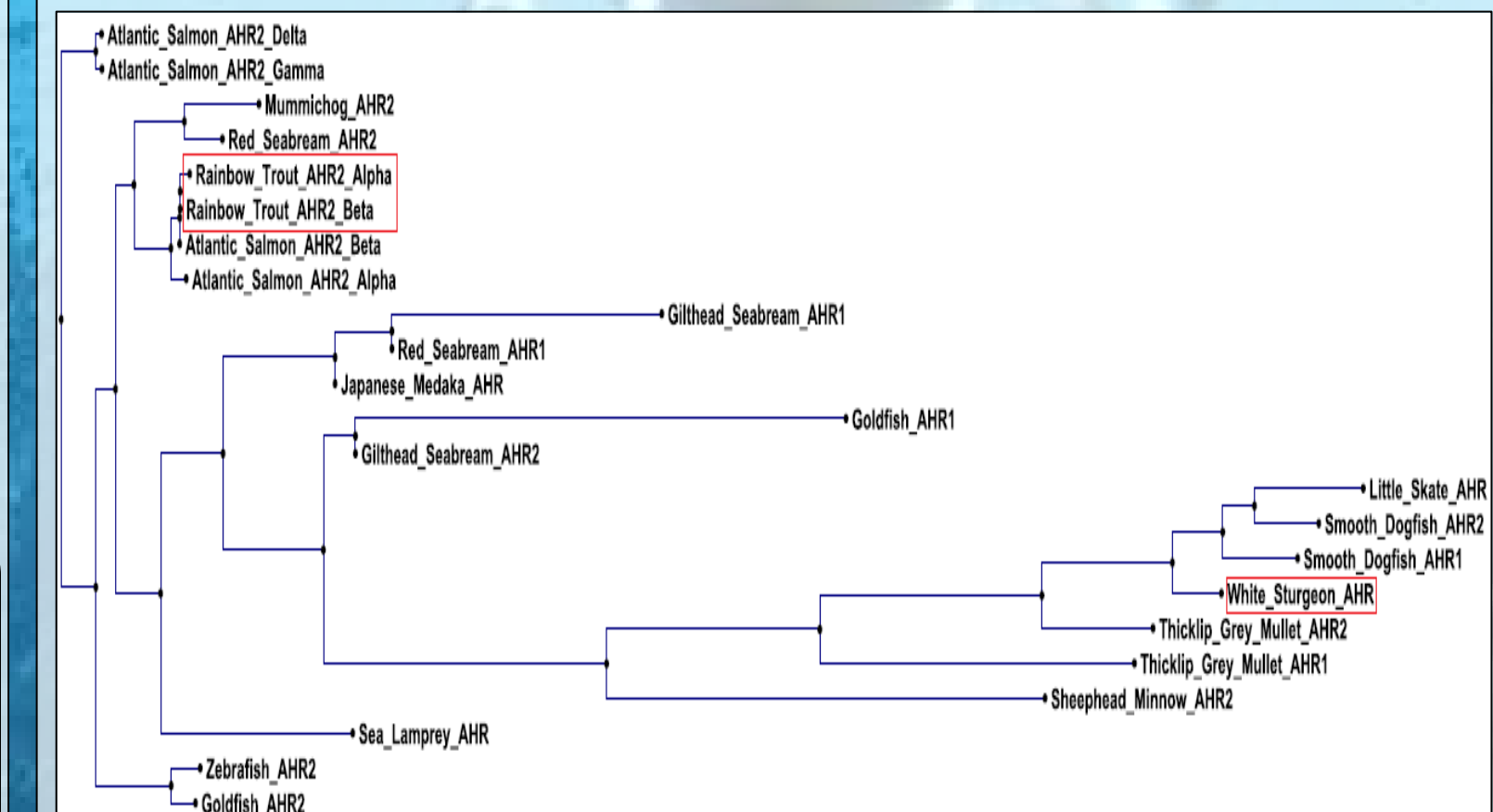


Figure 3.0: Maximum likelihood phylogenetic tree comparing the white sturgeon AhR nucleotide sequence to AhR sequences of other fish species.

Conclusion

- Results of this study demonstrated that white sturgeon are responsive to AhR agonists and may be among the more sensitive fish species with regard to inducibility of Cyp1A.
- Although the liver is the main location of these responses, other organs such as the gill and intestine have the capacity to respond as evidenced by induction of EROD activity and both Cyp1A and AhR transcript abundance.
- AhR dynamics could be both species and tissue-specific in piscine systems and are present in ancient fishes.
- It is currently unclear whether AhR autoregulation has an impact on species or tissue sensitivity to AhR agonists.

Ongoing Research

- Development of an AhR agonist dose-response in white sturgeon utilizing hepatocyte, intestine, and gill primary cultures.
- Continue work on AhR dynamics in white sturgeon studying other concurrent AhR isoforms.
- Investigate responsiveness of other sturgeons to AhR agonists.