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Gonad Organochlorine Concentrations and Plasma Steroid Levels in White Sturgeon (*Acipenser transmontanus*) from the Columbia River, USA

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Sturgeon are an important fishery resource world-wide, providing food and income through commercial, sport, and tribal fisheries. However, sturgeon populations are imperiled in many areas due to overharvest, habitat loss, and pollution. White sturgeon (*Acipenser transmontanus*) are found along the west coast of North America from San Francisco Bay, USA to British Columbia, Canada. The Columbia River, located in the Pacific Northwest USA, supports active commercial, sport, and tribal white sturgeon fisheries. The white sturgeon fishery in the Columbia River estuary is one of the most productive sturgeon fisheries in the world. Despite the success of the Columbia River estuary white sturgeon fishery, the populations within the impounded sections (i.e. behind the hydroelectric dams) of the Columbia River experience poor reproductive success (Beamesderfer et al. 1995). This poor reproductive success has been attributed to hydroelectric development, but water pollution could also be a significant factor. The bottom dwelling life history and late maturing reproductive strategy for this species may make it particularly sensitive to the adverse effects of bioaccumulative pollutants.

The Columbia River receives effluent from bleached-kraft pulp mills, aluminum smelters, municipal sewage treatment plants and runoff from agricultural, industrial, and urban areas. Bioaccumulative contaminants that have the potential for endocrine disruption have been detected in fish and sediments from the Columbia River (Foster et al. 1999). An integrated system of hormones control reproduction in vertebrates. Plasma steroids direct developmental events essential for reproduction. Disruption of endocrine control by contaminants has been linked to reproductive anomalies and failure in a number of vertebrate species (Guillette et al. 1996; Jobling et al. 1996). Because of this, it is important to understand if organochlorine compounds are accumulating in Columbia River white sturgeon and having an effect on their reproductive physiology.

The objective of this study was to determine if sturgeon from an impounded section of the Columbia River (where reproductive success has been low) had higher levels of bioaccumulative pollutants than sturgeon from the estuary (where reproductive success has been high) and if these compounds were associated with decreased plasma steroid levels. Specifically, we measured chlorinated pesticides and PCBs in the gonads and plasma steroids in white sturgeon from the Columbia River fishery.

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MATERIALS AND METHODS

The Columbia River basin comprises approximately 260,000 mi^2 with the mouth at the Pacific Ocean and headwaters in the Canadian Rockies. The basin extends into parts of Oregon, Washington, Idaho, Montana, and British Columbia. The area is characterized by river valleys, high desert, mountains and plateaus with arid conditions occurring in the east and heavy precipitation in the western portion of the basin. Land use within the basin includes agriculture, forestry, industrial, and urban areas with hydroelectric dams on the mainstem and tributaries.

White sturgeon from the sport harvest in the estuary and from the tribal fishery in The Dalles pool were sampled in late winter and spring of 1996. The reservoirs behind the hydroelectric dams are referred to as "pools". Gonads were collected from 7 white sturgeon (4 males and 3 females) from the estuary and 11 white sturgeon (all females) form The Dalles pool. Fish were sampled within 12 hr of capture in the estuary and within 24 to 48 hr of collection by gillnet in The Dalles pool. Total length (cm) was measured. Approximately 100g of gonad was collected for analytical chemistry and 1g of gonad was preserved in 10% buffered formalin for identification of sex and stage of maturation. All fish were reproductively immature with gonads characterized by a strip of reproductive cells surrounded by adipose tissue. Blood samples were collected from the caudal vein with a heparinized vacutainer, and plasma was prepared by centrifugation and subsequently stored at $-80^{o}C$.

Gonad samples were analyzed for chlorinated pesticides and PCBs (Table 1) were analyzed according to USEPA RCRA SW-846 method 3620 and 8080 with some modification (U.S. Environmental Protection Agency 1996, U.S. Environmental Protection Agency 1994). Homogenized gonad tissue (5 to 10g) was pulverized (via mortar & pestle) with approximately 10X wt of anhydrous sodium sulfate until a consistent, granular solid was formed. The sample was transferred to a 500 ml jar with teflon-lined lid, where surrogate spike (tetrachloro-m-xylene and dibutylchlorendate) and 250 ml dichloromethane were added. The jar was shaken at 30 rpm for 18 +/- 2 hr. The extract was filtered through Shark Skin (Schleicher & Schuell, Inc., Keene, NH) analytical paper and collected in a 500 ml Kuderna-Danish concentrator concentrated to ~1 ml. The extract was cleaned by gel permeation on a polystyrene/divinylbenzene column, eluted with dichloromethane, and collected in a Kuderna-Danish concentrator and concentrated by a solvent exchange with hexane to ~1 ml. Final cleanup was performed by eluting with 50% diethyl ether in hexane through a 1 g florisil cartridge. The final volume of 1.0 ml was transferred to an autosampler vial for analysis. Quantification and verification were performed on a Hewlett-Packard 5890 Series II gas chromatograph with dual electron capture detectors and J&W DB-608 and J&W DB-5 capillary column (0.53 mm x 30 m). Each sample extract was injected onto each column with individual analytes reported only if identified and quantified on both columns. Known standards and samples were compared based on concentration, peak shapes, and retention time offsets. Blanks, spike recoveries, and duplicates were performed with each batch of ten samples.

Table 1. Chlorinated pesticide and PCB analyte list.

Chlorinated Pesticide	PCB (IUPAC)
α-BHC	Aroclor 1221
β-BHC	Aroclor 1232
δ-BHC	Aroclor 1242
γ-BHC (lindane)	Aroclor 1248
Heptachlor	Aroclor 1254
Heptachlor epoxide	Aroclor 1260
Aldrin	3,3',4,4',-tetrachlorobiphenyl (77)
Dieldrin	3,4,4',5-tetrachlorobiphenyl (81)
Endrin	2,3,3',4,4'-pentachlorobiphenyl (105)
Endrin ketone	2,3,4,4',5-pentachlorobiphenyl (114)
Endrin aldehyde	2,3',4,4',5-pentachlorobiphenyl (118)
Endosulfan I	2',3,4,4',5-pentachlorobiphenyl (123)
Endosulfan II	3,3',4,4',5-pentachlorobiphenyl (126)
Endosulfan sulfate	2,3,3',4,4',5-hexachlorobiphenyl (156)
p,p'-DDE	2,3,3',4,4',5'-hexachlorobiphenyl (157)
p,p'-DDD	2,3',4,4',5,5'-hexachlorobiphenyl (167)
p,p'-DDT	3,3',4,4',5,5'-hexachlorobiphenyl (169)
p,p'-Methoxychlor	2,3,3',4,4',5,5'-heptachlorobiphenyl (189)
Chlordane	
Toxaphene	

Chemicals of interest were nondetectable in blanks. Average surrogate spike recoveries were 80% and duplicates were +/- 20%.

Plasma samples were analyzed for testosterone (T), 11-ketotestosterone (KT), and 17β-estradiol (E2) by radioimmunoassay according to Fitzpatrick et al. (1993) as modified from Fitzpatrick et al. (1986). Briefly, 100-µl aliquots were extracted with 20 vols diethyl ether. The antisera were diluted with phosphate buffered saline with gelatin (PBSG) at 1:1000 for T (Endocrine Sciences, Tarana, CA), 1:50,000 for KT (courtesy Dr. A.P. Scott, Lowestoft Laboratory), and 1:60,000 for the E2 (courtesy Dr. G. Nischwender, Colorado State University) assays. [^{3}H]steroids were diluted in PBSG to obtain ~15,000 (T and E2) and ~10,000 (KT) dpm/100 µl. The extraction efficiency was 85.8% to 86.8% for T, 84.2% to 84.3% for KT, and 73.0% to 80.6% for E2. The assay was validated by demonstrating that serial dilutions of samples were parallel to the standard curve for each steroid. Intra- and inter-assay coefficients of variation were less than 5% and 10%, respectively. Detection limits for T, KT, and E2 were 0.3, 0.8, and 0.3 ng/ml, respectively. Samples of gonad were preserved in 10% buffered formalin and embedded in paraffin. Sagital 10 µm serial sections were placed on slides, stained with hematoxylin and eosin, and were examined by light microscopy. Sex and stage of reproductive maturation were determined according to Conte et al. (1988).

Location differences for organochlorines, steroids, lipid content, and total length were detected using student's t-test ($p < 0.05$). Differences between DDE, DDD, and DDT concentrations were determined by one-way analysis of variance (ANOVA) and least significant difference (LSD) multiple comparison ($p < 0.05$). Regression analysis was used to examine the relationship between lipid content, total length, organochlorines, and plasma steroids.

RESULTS AND DISCUSSION

White sturgeon are a long lived species and omnivorous bottom feeders that could make them susceptible to bioaccumulating high levels of chlorinated pesticides and PCBs. The compounds DDT, DDD, and DDE were detected in 17, 17, and 18 of the samples, respectively. Concentrations of DDE were greater than DDD and DDT (Table 2). There were two other chlorinated pesticides detected. Heptachlor epoxide and endosulfan I were each detected in four samples at concentrations <0.5 mg/kg (data not shown).

Table 2. Average concentration (SE) of DDE, DDD, and DDT (mg/kg wet-wt) in gonad of white sturgeon from the Columbia River.

Location	n	DDE	DDD	DDT	Lipid(%)	Total Length(cm)
Estuary	7	1.45^{a*}	0.19^{b*}	0.07^{b}	37.8^{*}	115.3^{*}
		(0.34)	(0.08)	(0.02)	(8.2)	(2.5)
The Dalles	11	4.38^{c}	0.65^{d}	0.09^{d}	54.5	135.4
		(0.45)	(0.05)	(0.02)	(2.0)	(4.8)

Dissimilar letter within row denotes difference using one-way ANOVA and LSD multiple comparison ($p < 0.05$). * Estuary significantly different from The Dalles using student's t-test ($p < 0.05$). Estuary Lipid(%) n=5.

The parent compound, DDT, can be metabolized to DDE by biological dehydrochlorination and to DDD by reductive dechlorination. Higher concentrations of the metabolite DDE than the parent compound suggest that the source of these compounds were from historical uses. The chlorinated pesticide DDT was used extensively in the agricultural areas of the Yakima River Basin, a tributary to the Columbia River upstream of our study area. These chlorinated pesticides were frequently detected in samples from the Yakima River basin (Rinella et al. 1999).

There were higher concentrations of DDE and DDD in gonads of white sturgeon from The Dalles pool than the estuary. Total length and lipid content were also greater for sturgeon from The Dalles pool than the estuary (Table 2). These relationships hold when only female fish were analyzed. Both total length and lipid content could be important covariables affecting organochlorine concentration. However, regressions for chlorinated pesticides versus total length were not significant with low r^2 values (Table 3). The regressions for chlorinated pesticides versus lipid content had significant p-values ($p < 0.05$) but low r^2 values

(Table 3) and location differences for lipid normalized DDE, DDD, and DDT were the same as for the wet-wt data. This analysis of the data suggests that location was a significant factor for DDE and DDD concentrations.

Table 3. Results of regression analysis for DDT, DDD, and DDE versus total length and lipid content for Columbia River white sturgeon.

	Total Length		Lipid Content	
Chemical	r^2	p-value	r^2	p-value
DDT	0.4%	0.80	24.8%	0.05
DDD	18.1%	0.08	32.6%	0.02
DDE	9.5%	0.21	25.9%	0.04

The higher concentrations in The Dalles pool sturgeon may be a result of proximity to the mouth of the Yakima River which is contaminated with DDT, DDD, and DDE (Rinella et al. 1999). However, other factors such as age may still be an important variable and should be examined in subsequent investigations. White sturgeon in the estuary may also have reduced exposure as they are able to access the ocean which could have lower concentrations of these compounds than the riverine environment.

There were two PCB congeners that were detected infrequently at low concentrations, and were not different with location (Table 4). This differs from studies in the Ohio River drainage looking at another long lived species, the paddlefish (*Polyodon spathula*), in which PCBs were a significant contaminant in the gonads (Gundersen et al. 1998). Based on concentration, our data indicate that DDE, DDD, and DDT could be contaminants of concern in Columbia River sturgeon. However, other contaminants not measured in this study, such as chlorinated dioxins and furans and polycyclic aromatic hydrocarbons, could also be important contaminants in white sturgeon.

Plasma testosterone and 11-ketotestosterone were significantly higher in males than females (Table 5) but there were no differences with location (data not shown). Higher plasma androgen levels in males than females is consistent with gender identification research conducted with Columbia River white sturgeon (M. Webb *personal communication*). Plasma 17β-estradiol was not detected in samples (data not shown). Male plasma testosterone was positively correlated with DDE (r^2 >99%, p-value <0.01). These correlations may be an artifact of one data point influencing the regression for females and a small number of samples for males (n = 4). These regression results were different from data which showed a negative correlation between plasma androgen levels and liver p,p'-DDE concentrations in male and female white sturgeon (E. Foster *unpublished data*).

The gonads are the site of steroid production and disruption of steroidogenesis,

Table 4. Average concentration (SE) of PCB congeners and total PCBs (mg/kg wet-wt) in white sturgeon from the Columbia River.

		PCB Congener					
Location	n	#105	(D)	#157	(D)	Total PCBs	(D)
Estuary	7	0.02 (0.01)	(4)	0.02 (0.01)	(4)	0.05 (0.02)	(5)
The Dalles	11	0.01 (0.01)	(3)	0.02 (0.01)	(9)	0.03 (0.01)	(9)

(D) = number of detections.

via interference with enzymes important in steroid production, would be expected to reduce plasma steroid levels. Mature male brook trout (*Salvelinus fontinalis*) exposed to cadmium had testes with hemorrhagic necrosis and reduced *in vitro* androgen production (Sangalang and O'Halloran 1973). Environmental levels of DDT were associated with poor reproductive success in white croaker (*Genyonemus lineatus*) (Hose et al. 1989). However, less is known about the effects of DDE and DDD on fish reproduction and development.

Table 5. Average concentration (SE) of plasma testosterone and 11-ketotestosterone (ng/ml) in male and female white sturgeon from the Columbia River.

Sex	n	testosterone	11-ketotestosterone
Male	4	4.94[a] (1.72)	6.74[a] (2.98)
Female	14	0.95[b] (0.24)	1.16[b] (0.15)

Dissimilar letter within column denotes difference using student's t-test ($p < 0.05$).

This study reports on gonad chlorinated pesticide and plasma steroid levels in white sturgeon from the Columbia River. The organochlorines DDT, DDD, and DDE were detected frequently in samples from the estuary and The Dalles pool. Higher levels of these compounds were found in white sturgeon gonad samples from The Dalles pool than the estuary. There was no significant difference between locations for plasma steroids. Additional studies are needed to better characterize the association between gonad contaminant and plasma androgen levels, location and age differences, and to determine if other bioaccumulative contaminants are present in white sturgeon.

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