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# Plasma Androgen Correlation, EROD Induction, Reduced Condition Factor, and the Occurrence of Organochlorine Pollutants in Reproductively Immature White Sturgeon (*Acipenser transmontanus*) from the Columbia River, USA

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**Abstract.** White sturgeon (*Acipenser transmontanus*) support an active fishery in the Columbia River, but there is poor reproductive success within the impounded sections. The poor reproductive success has been attributed to hydroelectric development; however, water pollution could be a significant factor. White sturgeon plasma, liver, and gonad samples were collected from four Columbia River locations and a California aquaculture facility. Total length and weight of the fish were measured, and plasma samples were analyzed for testosterone (T), 11-ketotestosterone (KT), 17β-estradiol (E2), and vitellogenin. Liver samples were analyzed for chlorinated pesticides and polychlorinated biphenyls, ethoxyresorufin-O-deethylase (EROD) activity and histopathology. Gonads were examined histologically to assess sexual maturity and characterize any lesions. Significant differences by location existed for p,p′-DDE, EROD activity, and condition factor. Plasma T was negatively correlated with p,p′-DDE in males and females, and plasma KT was negatively correlated in males. These data indicate that pollutants could be adversely affecting white sturgeon in the Columbia River basin.

Worldwide, sturgeon (*Acipenseridae*) are an important source of food and income, but sturgeon populations are imperiled due to overharvest, habitat loss, and pollution. The white sturgeon (*Acipenser transmontanus*) is an important species to the communities of the Pacific Northwest and supports an active fishery in the Columbia River. The white sturgeon fishery is the largest recreational fishery in the Columbia River basin in terms of effort, with an annual average of 158,000 angler trips (Melcher and Watts 1995). The annual average recreational harvest from the lower Columbia River (the area below Bonneville Dam) was 37,600 fish, and the commercial harvest for that area was 7,100 fish from 1984 through 1994 (Oregon Department of Fish and Wildlife and Washington Department of Fish and Wildlife 1995). Harvests are lower for the tribal and recreational fishery that operates in the impounded section of the Columbia River between Bonneville and McNary Dams. Despite the apparent success of the fishery, white sturgeon populations within the impounded sections of the Columbia River experience poor reproductive success (Beamesderfer *et al.* 1995).

White sturgeon are a long-lived species that take several years to reach sexual maturity. Sexual differentiation occurs at approximately 2 years of age in female white sturgeon raised in aquaculture facilities, and it may take another 1–5 years before vitellogenesis occurs (Doroshov *et al.* 1997). Wild fish have been reported to reach first sexual maturity at 10 to 12 years of age in males and 15 to 32 years of age in females (DeVore *et al.* 1995). After first sexual maturity, maturation and spawning occurs every 2–4 years (Chapman *et al.* 1996). The white sturgeon population in the lower Columbia River may be one of the most productive populations for this species (DeVore *et al.* 1995). However, the impounded sections of the Columbia River experience poor reproductive success, and the harvest rate on these populations may not be sustainable (Beamesderfer *et al.* 1995). Recently, the Kootenai white sturgeon, which occurs in a tributary to the Columbia River, was listed as threatened under the federal Endangered Species Act. The poor reproductive success of white sturgeon within the impounded sections of the Columbia River has been attributed to hydroelectric development, but water pollution could also be a significant factor. The life history and reproductive strategy of this species may make it particularly sensitive to the adverse effects of bioaccumulative pollutants.

The Columbia River receives effluent from point sources and runoff from nonpoint sources of pollution. Point sources of pollution discharging to the Columbia River include bleached-kraft pulp mills, aluminum smelters, and municipal sewage treatment plants. Nonpoint source runoff can come from for-

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ested and agriculture land, industrial areas, and urban areas. Hydroelectric facility and landfill releases could also be a factor. Persistent bioaccumulative compounds have been detected in the Columbia River, including chlorinated dioxins, chlorinated furans (Foster *et al*. 1999), chlorinated pesticides, and polychlorinated biphenyls (PCBs) (US EPA 1992) in fish tissue and sediment and organochlorines in water (McCarthy and Gale 1999). The accumulation of contaminants and their effects on white sturgeon reproduction in the Columbia River have not been investigated.

The objective of this study was to determine if persistent bioaccumulative pollutants, such as chlorinated pesticides and PCBs, were associated with adverse effects on the reproductive endocrine system of white sturgeon. Specifically, in a subset of samples we measured chlorinated pesticides, PCBs, and ethoxyresorufin-O-deethylase (EROD) activity in liver, and in all samples we measured plasma steroids and vitellogenin and examined livers and gonads histologically for evidence of pollutant-induced lesions in white sturgeon from the Columbia River. Results of these analyses were compared between the estuary and impounded sections of the river and with white sturgeon from a California aquaculture facility.

## Materials and Methods

### *Description of the Study Area*

The Columbia River basin comprises approximately 260,000 miles$^2$ 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. The reservoirs behind the hydroelectric dams are referred to as "pools."

### *Sample Location and Preservation*

White sturgeon from the commercial harvest at Young's Bay, Bonneville pool, the Dalles pool, John Day pool, and a commercial hatchery at University California Davis (UCD) were sampled in late winter and spring of 1997 (Figure 1). Fish from the Columbia River were sampled within 24 to 48 h of collection by gillnet, and fish from the hatchery were sampled immediately after removal from the tanks and killed with a blow to the head.

Total length (cm) and weight (kg) were measured and condition factor (CF) was calculated using the equation CF = (kg/cm$^3$) × 100,000. Blood samples were collected from the caudal vein with a heparinized vacutainer, and plasma was prepared by centrifugation and subsequently stored at −80°C. A portion of the anterior liver was removed, approximately 1 g was preserved in 10% buffered formalin for histopathology and the remainder was stored at −80°C for microsomal preparation and analytical chemistry. Approximately 1 g of gonad was preserved in 10% buffered formalin for identification of sex, stage of maturation, and lesions by histology. Condition factor was calculated, plasma steroids measured, and liver and gonad tissue examined histologically for all samples. Chemical analysis and EROD activity were measured on a subset of samples as chosen as described below.

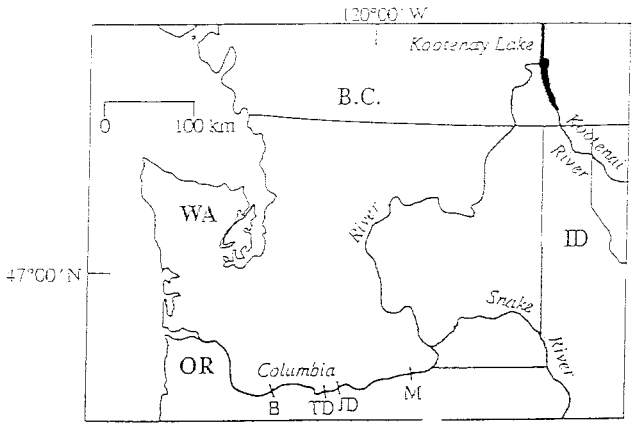


**Fig 1.** The Columbia River study area extends from near the mouth of the Columbia River (Young's Bay) upriver to Bonneville Dam (B), the Dalles Dam (TD), and John Day Dam (JD)

### *Chlorinated Pesticide and PCB Analysis*

Liver samples were analyzed for chlorinated pesticides and PCBs (Table 1). Chlorinated pesticides and PCBs were analyzed according to U.S. EPA RCRA SW-846 method 3620 and 8080 with some modification (US EPA 1996, 1994). Five to ten grams of homogenized liver tissue was pulverized (via mortar and pestle) with approximately 10× weight of anhydrous sodium sulfate until a consistent, granular solid was formed. The sample was transferred to a 500-ml jar with Teflon-lined lid, surrogate spike (tetrachloro-m-xylene and dibutylchlorendate) and 250 ml dichloromethane were added. The jar was shaken at 30 rpm for 18 ± 2 h. The extract was filtered through Shark Skin (Schleicher & Schuell, Inc., Keene, NH) analytical paper and collected in a 500 ml Kuderna-Danish concentrator and concentrated to ~ 1 ml. Extract cleanup was by gel permeation on a polystyrene/divinylbenzene column and 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 × 30 m). Sample extract was injected onto each column with each analyte reported 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 10 samples. Chemicals of interest were nondetectable in blanks. Average surrogate spike recoveries were 80% and duplicates were ± 20%. Toxic equivalency factors were used to calculate toxic equivalency concentrations (TECs) for the PCB congeners (Van den Berg *et al.* 1998).

### *Plasma Steroid Analysis*

Plasma samples were analyzed for testosterone (T), 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 (PBS) with gelatin (PBSG) at 1:1,000 for T (Endocrine Sciences, Tarzana, CA), 1:50,000

**Table 1.** Chlorinated pesticides and PCBs measured in sturgeon livers.

| 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 | |

for KT (courtesy Dr. A. P. Scott, Lowestoft Laboratory), and 1:60,000 for 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 interassay 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$^{-1}$, respectively.

### *Cytochrome P450 Content and EROD Activity*

Hepatic microsomes were prepared by differential centrifugation according to Carpenter *et al.* (1990) and stored at −80°C until use. Briefly, livers were minced in ice-cold buffer (0.1 M Tris-acetate, pH 7.4; 0.1 M KCl; 1 mM EDTA; 20 μM butylated hydroxytoluene; and 1 mM phenylmethylsulfonylfluoride) and homogenized in four volumes of the same buffer. The homogenate was centrifuged at 10,000 *g* for 30 min, and the resulting supernatant was centrifuged at 100,000 *g* for 90 min. The microsomal pellet was resuspended in buffer (0.1 M phosphate buffer, pH 7.25; 20% glycerol; and 1 mM EDTA). Microsomes were stored at −80°C until use. Total cytochrome P450 content was calculated from the spectral difference of CO *versus* CO-sodium dithionite reduced microsomes (Estabrook *et al.* 1972), and the results were used to confirm cytochrome P450 integrity by comparison of the P450 and P420 peaks. EROD activity was measured fluorometrically as described earlier (Prough *et al.* 1978; Burke and Mayer 1974). Microsomal protein content was measured according to Lowry *et al.* (1951).

### *Vitellogenin Assay*

Vitellogenin (Vtg) was measured in plasma by enzyme-linked immunosorbent assay (ELISA) (Linnares-Casenave *et al.* 1994) using white sturgeon antibody and purified Vtg that were a gift from Dr. S. Doroshov (University California-Davis). Briefly, 0.1 ml/well of vitellogenin in carbonate-bicarbonate buffer (pH 9.6) was added to a 96-well microtiter plate and incubated at 4°C for 16 h. The plate was rinsed with PBS-Tween (pH 7.5) and 0.1 ml/well of 1% bovine serum albumin in carbonate-bicarbonate buffer was added and incubated for 2 h then rinsed five times with PBS-Tween. Antibody (0.4 ml) was diluted 1:60,000 with phosphate buffer saline-tween (sodium) azide-polyethylene gylcol (PBSTA-PEG) (pH 7.5) added to 0.4 ml of Vtg standard or plasma and incubated in polypropylene microtubes for 18 h at room temperature. Then 0.1 ml/well of the preincubated standard or plasma was added to the precoated plates and incubated 2 h at room temperature and washed five times with PBS-Tween. Anti-rabbit immunoglobulin (IgG) (50 μl) conjugated to horseradish peroxidase (Sigma, St. Louis, MO) was diluted 1:30,000 and incubated 2 h at room temperature. Then 0.1 ml/well of 1 mg/ml of tetramethylbenzidine free base chromogen and hydrogen peroxide (0.006%) in 0.05 M phosphate citrate buffer was added and incubated for 30 min. The reaction was terminated with 50 μl/well of 2 M $H_2SO_4$ and the plates were read at 450 nm. The detection level for Vtg was 60 μg · ml$^{-1}$.

### *Liver and Gonad Histology*

Samples of liver and 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 examined by light microscopy. In addition to verifying sex and stage of reproductive maturation (Conte *et al.* 1988), gonad tissue was examined for evidence of histopathology.

### *Statistics*

Location differences were detected using one-way analysis of variance (ANOVA) and least significant difference multiple comparison test. Data were log transformed when variances between locations were significantly different using Cochran's C test or Bartlett's test ($p < 0.05$). When variances were different after log transformation, Kruskal-Wallis and Mann-Whitney U tests were used for detecting location differences. Regression was used to examine the association between organochlorines, plasma steroids, EROD activity, and condition factor. Linear regression was used to examine the relationship between liver lipid content and organochlorine concentration. Nondetects for chlorinated pesticide were treated as zero and the assay detection limit was used for steroids.

## Results

White sturgeon were collected from Young's Bay (females n = 2, males n = 6), Bonneville pool (female n = 14, males n = 10), The Dalles pool (females n = 13, males n = 16), John Day pool (females n = 14, males n = 6), and the California hatchery (females n = 6, males n = 6) for a total of 93 sturgeon sampled. Columbia River white sturgeon lengths and weights ranged from 117 to 149 cm and 7.8 to 18.0 kg, respectively. White sturgeon from the California aquaculture facility lengths and weights ranged from 78 to 91 cm and 2 to 4.1 kg, respectively. Columbia River white sturgeon ages were estimated to range from ~ 10 to ~ 20 years old (Tracy and Wall 1993). California hatchery fish were ~ 2 years old. All fish used in

this study were reproductively immature and had sexually differentiated gonads.

### *Chlorinated Pesticides and PCBs*

Thirty-two liver samples were analyzed for chlorinated pesticides and PCBs. The samples analyzed were randomly selected after stratifying based on location and low, medium, and high EROD activity for all locations. The most commonly detected chlorinated pesticides, DDE, DDD, and DDT, were found in 32, 30, and 24 of the liver samples analyzed, respectively. Concentrations of DDE were not different between river locations, but all river locations were higher than UCD fish (Table 2). Levels of DDE were higher in males than females (data not shown). The other chlorinated pesticides found in sturgeon liver were endosulfan I detected in five fish, heptachlor detected in two fish, heptachlor epoxide detected in one fish, and δ-BHC detected in two fish (data not shown). There were no other chlorinated pesticides detected or differences between locations.

Ten fish had detectable levels of one or more PCB congeners. The PCB congeners 77, 105, 114, 126, and 167 were detected at low concentrations and infrequently. No differences were found between locations for PCB congener concentrations, total PCBs (Table 2), or TECs. There were no correlations between percent lipid in liver and chlorinated pesticide or PCB concentrations.

### *Condition Factor*

Condition factors were compared for Columbia River locations only ($n = 71$). California hatchery fish were not included in this comparison because age and feeding would be expected to have an effect on condition factor. Female sturgeon collected at Bonneville Pool had a lower condition factor than those collected from other locations, but no differences were observed for males between locations (Table 3). There was no correlation detected between condition factor and organochlorine concentrations (data not shown).

### *Plasma Steroids*

Mean plasma steroid levels ranged from 0.77 $ng \cdot ml^{-1}$ to 1.14 $ng \cdot ml^{-1}$ for KT and 0.91 $ng \cdot ml^{-1}$ to 1.67 $ng \cdot ml^{-1}$ for T in females and 2.33 $ng \cdot ml^{-1}$ to 30.09 $ng \cdot ml^{-1}$ for KT and 4.41 $ng \cdot ml^{-1}$ to 37.7 $ng \cdot ml^{-1}$ for T in males. Plasma E2 was usually below detection levels, which is consistent for reproductively immature white sturgeon (Webb *et al.* 2001). In general, plasma androgen levels were higher in California hatchery fish than Columbia River fish (Table 4). Plasma levels of T and KT were negatively correlated with DDE. Plasma T and KT were negatively correlated with DDE in Columbia River males using reciprocal Y regression as the best fit model from an alternative models analysis (Figures 2 and 3). Plasma T levels in Columbia River females were negatively correlated with DDE using linear regression as the best fit model from an alternative models analysis (Fig. 4). The correlations between T and KT and DDE were improved when the California hatchery fish were included in the analysis (Table 5) because these fish had higher levels of T and KT and lower concentrations of DDE (Table 2 and Table 4). Correlations between KT and DDD in females and for T and KT with total PCBs in both sexes were significant because of nondetectable concentrations of KT in females and nondetectable concentrations of total PCBs in both sexes (data not shown). Plasma steroid levels showed no other significant correlations with other chlorinated pesticides, PCBs, EROD activity, or condition factor.

### *Hepatic EROD Activity*

Hepatic EROD activities were measured in a subset ($n = 67$) of the sturgeon sampled due to acceptable tissue availability. Sturgeon from Bonneville and The Dalles pool had higher hepatic EROD activity than sturgeon from John Day pool and UCD (Table 6). John Day pool sturgeon had higher EROD activity than UCD sturgeon. Mean EROD activity for Young's Bay sturgeon was not different from other locations. EROD activity was not correlated with PCB concentration, condition factor, or plasma steroids (data not shown).

### *Liver and Gonad Histology*

Compared to the UCD sturgeon, livers from Columbia River sturgeon had an increased incidence of mononuclear inflammatory cells, increased incidence of eosinophilic granular cells around blood vessels and bile ducts, and increased number and size of macrophage centers. There was no evidence of bile duct hyperplasia, adenofibrosis or dysplasia of bile ducts, swollen or vacuolated hepatocytes, megalocytosis, or parasites.

Gonadal tissue from Columbia River sturgeon showed evidence of intersex in one fish, irregular egg cell walls in three fish, and striated muscle in the ovary of five other sturgeon. No other irregular gonad development was observed in Columbia River fish, and no gonadal lesions were seen in UCD fish.

### *Plasma Vtg*

Vtg was measured in plasma of all sturgeon sampled ($n = 93$). Low levels of Vtg ($> 60$ $\mu g \cdot ml^{-1}$ but $< 100$ $\mu g \cdot ml^{-1}$) were detected in one male and three females from Bonneville pool, five males and four females from The Dalles pool, two males and six females from the John Day pool, and six males and four females from UCD; Vtg was not detected in fish from Young's Bay. Vtg levels were not correlated with chlorinated pesticides, PCBs, or EROD and were not different between locations (data not shown).

## Discussion

Columbia River white sturgeon exhibit differential reproductive success with reduced production within the impounded sections ("pools") of the river and greater production in the unimpounded lower portion of the river. The most recent

**Table 2.** Average concentrations (SE) of DDE, DDD, DDT, and PCB congeners (mg $kg^{-1}$ wet weight) in liver of white sturgeon from Young's Bay (YB), Bonneville Pool (BON), The Dalles Pool (TD), John Day Pool (JD), and a California hatchery (UCD)

| Location | n | DDE | DDD | DDT | PCB-77 | PCB-105 | PCB-114 | PCB-126 | PCB-167 | Total PCB | Lipid(%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| YB | 4 | 1.21[a] | 0.10 | 0.02 | < 0.0001 | 0.0170 | 0.0085 | 0.0157 | < 0.0001 | 0.0412 | 12.4 |
| | | (0.63) | (0.05) | (0.01) | na | (0.0130) | (0.0085) | (0.0157) | na | (0.0265) | (2.0) |
| BON | 6 | 0.73[a] | 0.18 | 0.02 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | 15.1 |
| | | (0.13) | (0.06) | (0.00) | na | na | na | na | na | na | (1.7) |
| TD | 10 | 0.89[a] | 0.08 | 0.02 | 0.0002 | 0.0050 | < 0.0001 | < 0.0001 | 0.0010 | 0.0062 | 11.4 |
| | | (0.27) | (0.03) | (0.00) | (0.0002) | (0.0034) | na | na | (0.0010) | (0.0042) | (1.2) |
| JD | 8 | 0.79[a] | 0.15 | 0.02 | 0.0025 | 0.0004 | < 0.0001 | < 0.0001 | < 0.0001 | 0.0029 | 10.2 |
| | | (0.29) | (0.05) | (0.01) | (0.0017) | (0.0004) | na | na | na | (0.0019) | (1.2) |
| UCD | 4 | 0.01[b] | 0.00 | 0.00 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | NA |
| | | (0.01) | (0.00) | (0.00) | na | na | na | na | na | na | |

Dissimilar letter within column denotes difference. DDE location difference identified by one-way ANOVA ($p < 0.02$) and least significant difference multiple comparison test ($p < 0.05$) after log transformation. NA = not analyzed, na = not applicable, nondetects were treated as zero.

**Table 3.** Mean condition factor (SE) for female and male immature white sturgeon from the Columbia River

| Location | n | Female | n | Male |
|---|---|---|---|---|
| Young's Bay | 2 | 0.602[a] | 6 | 0.617 |
| | | (0.050) | | (0.042) |
| Bonneville Pool | 14 | 0.500[b] | 10 | 0.535 |
| | | (0.011) | | (0.015) |
| The Dalles Pool | 13 | 0.557[a] | 16 | 0.549 |
| | | (0.012) | | (0.013) |
| John Day Pool | 14 | 0.539[a] | 6 | 0.556 |
| | | (0.013) | | (0.020) |

Dissimilar letter within column denotes difference using one-way ANOVA ($p < 0.005$) and least significant difference multiple comparison ($p < 0.05$).

**Table 4.** Average concentrations (ng $ml^{-1}$) of plasma testosterone (T) and 11-ketotestosterone (KT) in male and female white sturgeon

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| Location | n | T | KT | n | T | KT |
| YB | 6 | 14.7[ab] | 7.7[a] | 2 | 0.9[a] | 0.82[ab] |
| | | (5.7) | (3.4) | | (0.1) | (0.01) |
| BON | 10 | 8.2[a] | 3.2[ab] | 14 | 0.9[a] | 0.77[bc] |
| | | (2.7) | (1.0) | | (0.1) | (0.03) |
| TD | 16 | 5.9[a] | 3.0[ab] | 13 | 0.7[a] | 0.77[c] |
| | | (1.1) | (0.6) | | (0.1) | (0.02) |
| JD | 6 | 4.4[ab] | 2.3[b] | 14 | 1.1[a] | 0.82[bc] |
| | | (1.6) | (0.8) | | (0.2) | (0.05) |
| UCD | 6 | 37.7[b] | 30.1[c] | 6 | 1.7[b] | 1.14[a] |
| | | (2.7) | (7.0) | | (0.2) | (0.14) |

Dissimilar letter within column denotes difference using Kruskall-Wallis ($p < 0.005$) and Mann Whitney U-test ($p < 0.05$) for male T, one-way ANOVA ($p < 0.001$) and least significant difference ($p < 0.05$) for log-transformed male KT, Kruskall-Wallis ($p < 0.05$) and Mann Whitney U-test ($p < 0.05$) for female T, and Kruskall-Wallis ($p < 0.001$) and Mann Whitney U-test ($p < 0.05$) for female KT data. SE is in parentheses. Young's Bay (YB), Bonneville pool (BON), The Dalles pool (TD), John Day pool (JD), and a California hatchery (UCD).

estimates of mature-sized white sturgeon vary widely between the areas of our study: 8,500 individuals in the free flowing portion, 600 in the Bonneville pool, 900 in The Dalles pool, and 500 in the John Day pool (Beamesderfer *et al.* 1995; DeVore *et al.* 1995). In addition, sturgeon in the Bonneville pool have been reported to be slower growing, with females maturing at smaller sizes and older ages than sturgeon from other Columbia River locations (Beamesderfer *et al.* 1995). This is consistent with our results showing lower average condition factor for females from Bonneville pool than other locations. The cause of the sex-specific reduction of condition factor is unknown but could be due to poor habitat and forage base within the Bonneville Pool and the greater energetic demand on females during ovarian maturation or sex-specific interference by contaminants on normal physiologic function and metabolism.

The life history of white sturgeon may leave them particularly vulnerable to the effects of bioaccumulative pollutants. As opportunistic bottom feeders, these fish frequently come into contact with sediments that could contain sediment-sorbed hydrophobic pollutants, such as chlorinated pesticides and PCBs. These contaminants have been detected in sediments from the Columbia River, and these contaminants could be ingested incidentally during normal feeding or contained in food items and bioaccumulated. In addition, white sturgeon are a long-lived species that historically reached 80 years of age, lengths of 20 ft, and weight as high as 1,800 pounds (Wydoski and Whitney 1979). The long life span would allow for increased opportunity for exposure and bioaccumulation of contaminants.

The reproductive strategy of white sturgeon may also put this species at risk from bioaccumulative pollutants. Spawning is a major excretory route for females that have accumulated hydrophobic pollutants that are resistant to metabolism (Gundersen *et al.* 1998). These compounds are deposited in the eggs via maternal transfer, reducing the female's body burden but exposing the developing embryo. White sturgeon have delayed sexual maturation as compared to many other species of fish: Columbia River female white sturgeon become sexually mature between 15 and 32 years of age (DeVore *et al.* 1995). This reproductive strategy allows for a long period of accumulation of contaminants prior to first spawning, which could adversely affect the adult reproductive cycle or the development of off-

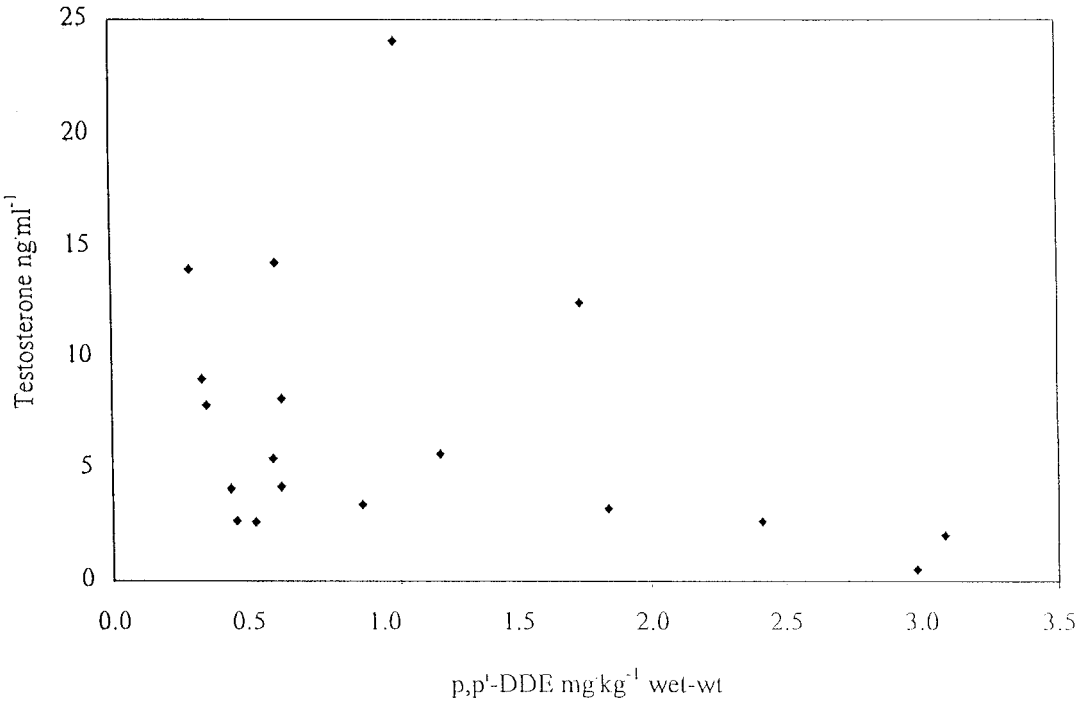


**Fig 2.** Columbia River male white sturgeon plasma testosterone (T) versus liver p,p′-DDE (DDE) (n = 18). Reciprocal-Y regression: $T = 1/0.004 + 0.269.*$ DDE. Correlation coefficient = 62.5%, $r^2 = 39.1\%$, $p < 0.006$

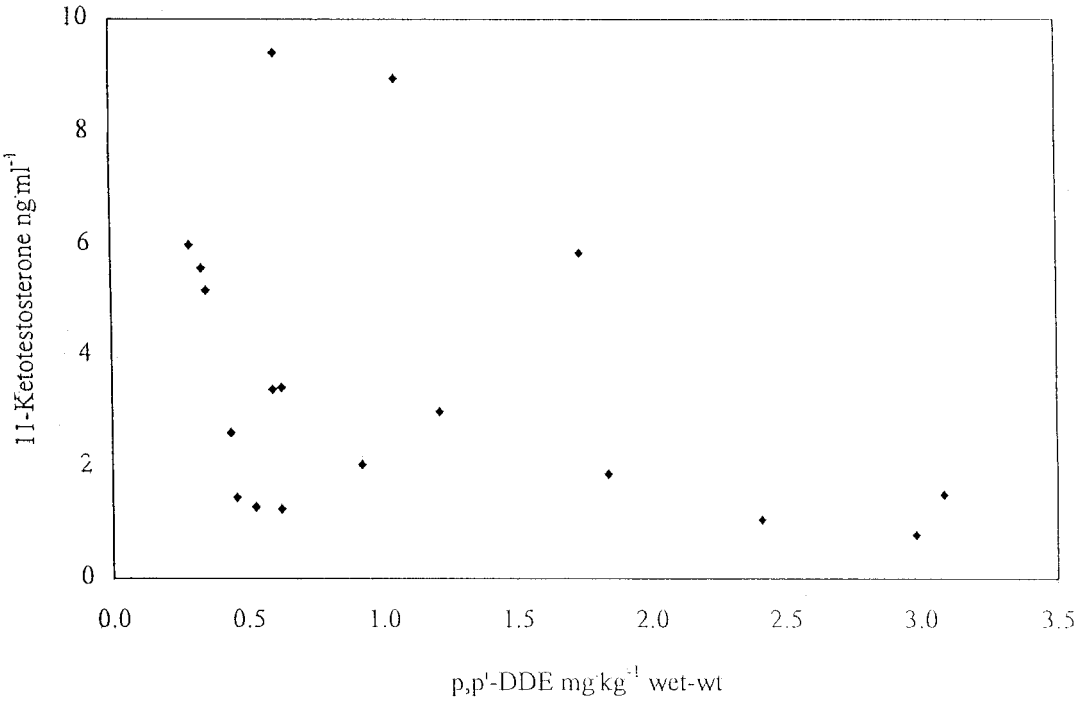


**Fig 3.** Columbia River male white sturgeon plasma 11-ketotestosterone (KT) versus liver p,p′-DDE (DDE) (n = 18). Reciprocal-Y regression: KT = 1/0.234 + 0.208 * DDE. Correlation coefficient = 58.2%, $r^2 = 33.9\%$, $p < 0.020$

spring due to maternal transfer of bioaccumulative pollutants. In addition, female white sturgeon may only spawn every 2–4 years (Doroshov *et al.* 1997), which would allow for additional accumulation between spawnings.

The chlorinated pesticide DDT and its metabolites, DDE and DDD, were detected frequently and in the highest concentrations of the organochlorines measured. Detection of these compounds was expected based on results from studies of the

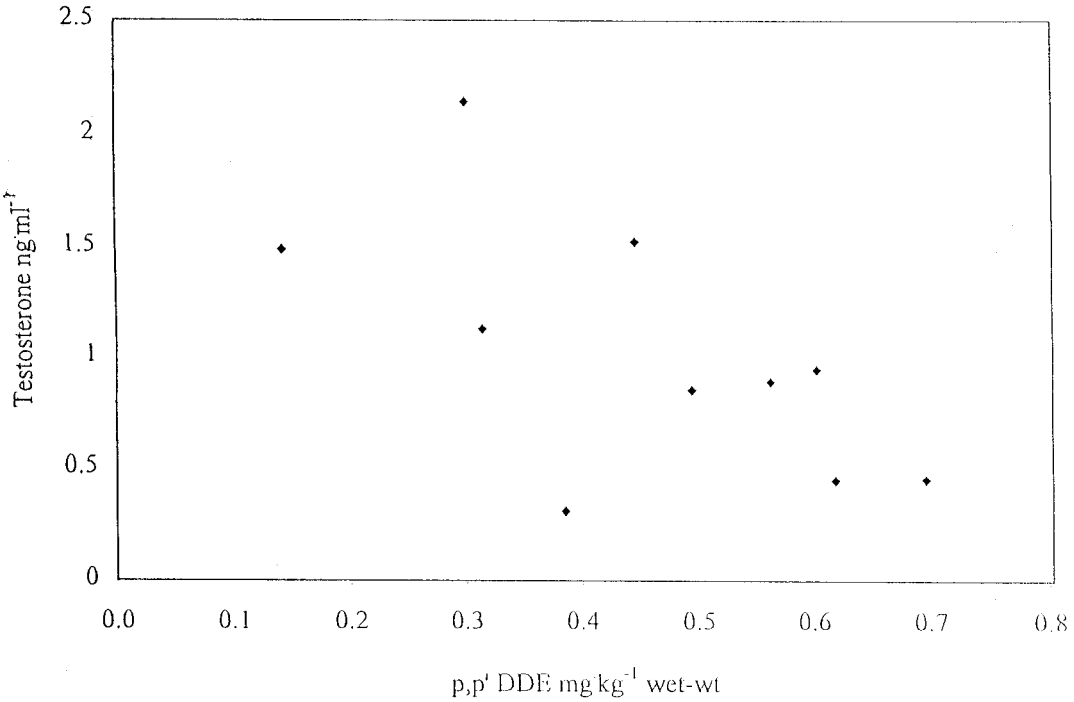


**Fig 4.** Columbia River female white sturgeon plasma testosterone (T) versus liver p,p′-DDE (DDE) (n = 10). Linear regression: $T = 1.96 + (-2.08) * \text{DDE}$. Correlation coefficient = 62.5%, $r^2 = 39.1\%$, $p < 0.055$

**Table 5.** Regression analysis of T and KT versus DDE using data from all locations or Columbia River fish only

| Data Used | Regression | Correlation (%) | $r^2$ (%) | p-Value |
|---|---|---|---|---|
| Male T versus DDE | | | | |
| All Locations | Reciprocal-Y | 64.8 | 42.0 | <0.003 |
| Columbia River | Reciprocal-Y | 62.5 | 39.1 | <0.006 |
| Male KT versus DDE | | | | |
| All Locations | Reciprocal-Y | 63.9 | 40.9 | <0.003 |
| Columbia River | Reciprocal-Y | 58.2 | 33.9 | <0.020 |
| Female T versus DDE | | | | |
| All Locations | Linear | 73.2 | 53.5 | <0.007 |
| Columbia River | Linear | 62.5 | 39.1 | <0.055 |

Reciprocal-Y: $Y = 1/(a + bX)$. Linear: $Y = a + bX$.

**Table 6.** White sturgeon mean liver EROD activitity for Columbia River and a California hatchery (UCD)

| Location | n | EROD Activity (pmol resorufin min$^{-1}$ · mg protein$^{-1}$) |
|---|---|---|
| Young's Bay | 2 | 138.1[abc] (124.6) |
| Bonneville | 15 | 137.8[a] (23.0) |
| The Dalles | 22 | 133.5[a] (26.1) |
| John Day | 18 | 46.0[b] (13.7) |
| UCD | 10 | 10.8[c] (1.8) |

Dissimilar letter denotes difference using Kruskall-Wallis ($p < 0.001$) and Mann Whitney-U test ($p < 0.05$). SE in parentheses.

Yakima River Basin. The Yakima River is a major tributary of the Columbia River that discharges upstream from our study area and has high levels of DDT and metabolites in fish and sediments (Rinella *et al.* 1999). However, total and congener PCBs were infrequently detected and were at low concentrations, which was unexpected because PCBs have been used in electric transformers and the hydroelectric dams operating on the Columbia River were suspected of using and potentially releasing PCBs to the river. In this study, liver tissue was analyzed for chlorinated pesticides and PCBs because it is the major site for enzymatic induction in fish and an important organ for biotransformation of xenobiotics and reproductive steroids. But major storage sites for hydrophobic contaminants resistant to metabolism are usually lipid depot tissues, such as muscle, fat deposits, and gonads. Higher concentrations of these compounds would be expected in gonads and other lipid depot tissues.

Reproduction for vertebrates depends on an integrated system of tissues and biomolecules that act in concert to bring about reproductive functions at appropriate stages of development and environmental conditions. Hormones provide the communication between the tissues that direct developmental events essential for reproduction, such as gonadal differentiation and maturation (Redding and Patino 1993). Gonad growth is associated with steroid secretion, which may act directly on the gonads, as in androgen stimulation of testicular recrudescence, or on other tissues, as in estrogen stimulation of liver Vtg production, which then is incorporated into the growing oocytes (Redding and Patino 1993). The female sturgeon reproductive cycle consists of a previtellogenic phase with low levels of androgens and estrogens, followed by vitellogenesis and increased levels of androgens and estrogens, and finally oocyte maturation with decreasing levels of androgens and estrogens (Doroshov *et al.* 1996). T and KT are produced in both female and male sturgeon and probably serve an important role in the reproductive cycle of both sexes.

Gonadal development can be used for identifying the stage of reproductive maturation in white sturgeon (Conte *et al.* 1988). Although Columbia River sturgeon were larger than the California hatchery fish, they were at the same stage of reproductive maturation based on gonad histology and these groups of fish were expected to have similar levels of plasma steroids. However, Columbia River sturgeon had lower levels of plasma androgens than the California hatchery fish, which may be due to natural variability at this stage of gonadal development, warmer rearing water, faster maturation at the California hatchery, or other exogenous factors, such as organochlorine residues.

Environmental levels of DDT caused poor reproductive success in white croaker (*Genyonemus lineatus*) (Hose *et al.* 1989) and developmental abnormalities in winter flounder (*Pseudopleuronectes americanus*) (Smith and Cole 1973) but less is known about the effects of DDE and DDD on fish reproduction and development. Studies with rainbow trout (*Oncorhynchus mykiss*) showed *o,p′*-DDT to induce Vtg production, but *o,p′*-DDE was not estrogenic (Donohoe and Curtis 1996). In the current study, T and KT were negatively correlated with liver DDE concentrations for males, and T was negatively correlated with DDE in females. These correlations suggest an antiandrogen effect by DDE, but other factors could be affecting the correlations, such as subtle differences in maturity. A general antisteroid effect cannot be ruled out, as nearly all samples had E2 levels below detection limits; however, the captive population from California with the lowest DDE levels also had very low E2 concentrations. Correlation patterns were still present with removal of the California hatchery fish from the data set (Table 5). Other studies have reported depressed testosterone levels in juvenile alligators collected from an area contaminated with dicofol, DDT, DDD, and DDE (Guillette *et al.* 1996) and fish collected from an area affected by the discharge of bleached kraft pulp mill effluent had depressed androgen levels (Munkittrick *et al.* 1992). Interference with steroidogenesis may be one mechanism for contaminant-related plasma androgen reduction (Guillette *et al.* 1995), but other mechanisms, such as induction of excretory pathways, may be important.

Metabolism of steroids by the liver is important for maintenance of plasma steroid concentrations. In this study, the negative correlation of plasma androgen levels with liver DDE concentrations could be due to increased clearance from the induction of steroid-metabolizing enzymes. Some mammalian liver cytochrome P450 enzymes are inducible by DDE (You *et al.* 1999), which could cause a decrease in plasma androgen levels; however, work with rainbow trout (Addison *et al.* 1977) suggested that fish are refractory to DDE induction of cytochrome P450. Cytochrome P450 3A1 (CYP3A1), which functions as testosterone 6β-hydroxylase, was induced in rats exposed to DDE (You *et al.* 1999) and could be the orthologue of rainbow trout CYP3A27 (Lee *at al.* 1998) but induction of CYP3A27 has not been reported (Buhler and Wang-Buhler 1998). Cytochrome P4501A has been the only inducible P450 reported for fish, but recently Haasch *et al.* (1998) reported the induction of CYP2M1 and CYP2K1 in channel catfish (*Ictalurus punctatus*) and bluegill (*Lepomis macrochirus*), demonstrating that P450s not inducible in some species of fish may be inducible in others. The modulation of aromatase activity or steroidogenesis may also affect plasma androgen levels. Steroid-metabolizing enzymes and steroidogenesis were not measured in this study and the mechanism for DDE reduction of plasma androgens in sturgeon is unknown.

In mammalian systems, DDE is an androgen receptor antagonist (Kelce *et al.* 1995) that can adversely affect sexual development in male rats (Loffler and Peterson 1999). Androgens play an important role in reproduction for mature fish, but it is unknown what effect reduction of androgen levels in immature fish would have on sexual development, maturation, and eventual reproductive success. Reproductive steroids may be important in the maintenance of differentiated gonads and may influence somatic growth. Natural and synthetic androgens have been used in aquaculture to cause phenotypic masculinization of genotypic females (*e.g.*, Green *et al.* 1997) and estrogens were used to feminize males (*e.g.*, Hopkins *et al.* 1979). Xenoestrogen discharge to surface waters have had the unintended consequences of feminizing male fish (Folmar *et al.* 1996). In the current study, only a limited vitellogenic response was observed in a small number of males, suggesting that xenoestrogenic exposure is not an important factor in the studied populations.

A marker of CYP1A induction, EROD activity, was elevated in sturgeon collected from the Bonneville and The Dalles locations but was not correlated with TECs or total or congener PCB concentrations. CYP1A is induced by exposure to arylhydrocarbon receptor (Ah-R) agonists, although other mechanisms exist (Sadar *et al.* 1996). Coplanar organic compounds, such as 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans, non- and mono-ortho-substituted PCBs, and polycyclic aromatic hydrocarbons (PAHs) are Ah-R agonists that induce CYP1A. Chlorinated dioxins and furans have been detected in fish and sediments collected from the Columbia River (Foster *et al.* 1999) and could have contributed to hepatic EROD induction, but these organochlorines were not measured in this study. Our data suggest that EROD induction was not due to PCBs, but induction could be from exposure to chlorinated dioxins, chlorinated furans, or PAHs.

The Ah-R agonists, such as coplanar PCBs and chlorinated dioxins and furans, are carcinogenic and can cause reproductive and developmental effects (Peterson *et al.* 1993). Studies with lake sturgeon (*Acipenser fulvescens*) reported a correlation of EROD activity with bile duct proliferation and peripor-

tal fibrosis (Rousseaux *et al.* 1995), but in this study EROD activity was not correlated with liver lesions, plasma steroids, or condition factor. This could indicate that induction of EROD occurred at lower levels than those required to cause effects on the endpoints or induction occurred through a non-Ah-R pathway.

Modest hepatic lesions, more accentuated in Columbia River fish, were primarily inflammatory. Although bacterial septicemia can result in hepatitis, no etiology for the inflammatory changes was identified histologically. The more intense response in wild fish versus those raised in a hatchery situation could reflect the more controlled environment of the latter, with less exposure to infectious agents. Hepatocellular and bile ductular degenerative and proliferative changes, more typical of toxic insult, were not seen.

Changes in normal gonad histology and altered tissue structure could change the normal physiological function of reproductive organs, adversely affecting reproduction. Intrusion of striated muscle into ovarian tissue has been reported for sturgeon species collected from the Caspian Sea and was attributed to pollution (Romanov and Sheveleva 1993). In this study we observed striated muscle in the ovarian tissue from five fish. The effects of the striated muscle intrusion on these fish future reproductive function is unknown.

In summary, the chlorinated pesticides DDE, DDD, and DDT were frequently detected in livers of Columbia River white sturgeon with concentrations for DDE > DDD > DDT. But organochlorine concentrations for the upriver locations were not significantly different from the estuary area, indicating similar levels of organochlorine contamination in the estuary or the lack of difference may also be due to the small sample size from Young's Bay. These sample areas comprise large geographic areas, and sturgeon within these areas may be differentially exposed to contaminants leading to some fish having very low levels while others may have very high levels. Additional data are needed from these areas to better understand the organochlorine variability within the sample areas. Condition factor for females from Bonneville pool were lower than females from other locations. Plasma T and KT versus DDE for males and T versus DDE for females were negatively correlated. Hepatic EROD activity was elevated in sturgeon from Bonneville and The Dalles pools but was not correlated with PCBs. Gonad histology from several fish showed irregular ovarian cell walls and intrusion of striated muscle into the ovary.

These data indicate contaminant effects on plasma androgen concentrations and the induction of liver enzymes. Contaminants may also be involved with reduced condition factor and altered gonad development, but the organochlorines measured were not correlated with either of these endpoints. The contribution of contaminant stress to the poor reproductive success of Columbia River white sturgeon is unknown, but remains a distinct possibility. However, other factors, such as nutritional status and habitat quality, may be more important for the reproductive success of white sturgeon in the Columbia River above Bonneville Dam. These multiple stressors may be working in combination to cause decreased white sturgeon reproduction. Work is ongoing to evaluate the role contaminants may have on the reproductive success of white sturgeon in the Columbia River.

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