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Assessment of bioaccumulated metal and organochlorine compounds in relation to physiological biomarkers in Kootenai River white sturgeon

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Summary

This study used biomarker research in order to determine potential physiological effects of bioaccumulated metal and organochlorine compounds in juvenile and adult Kootenai River white sturgeon (*Acipenser transmontanus*). Wild adult and hatchery-reared and released juvenile sturgeon were captured using hook and line, small-mesh multi-filament gillnets, setlines and angling gear. Biomarker parameters that were assessed include whole-body tissue residues, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), DNA chromosomal variability and liver histology in juveniles as well as ovarian tissue residues, plasma steroids, egg size and DNA chromosome variability in adults. Results from chemical residue analysis indicated that several metal and two organochlorine compounds were at levels that could potentially affect reproduction or other physiological functions in the sturgeon. The results also suggested a decrease in egg size and AChE levels as well as altered hormone production and increased genetic mutation due to bioaccumulated levels of the detected metal and organochlorine compounds. In conclusion, contaminants in the Kootenai River system are potentially creating additional stress (perhaps in the form of diminished function) on the white sturgeon population. However, further research in the form of laboratory testing, *in-situ* studies and long-term trend monitoring will better define any relationships between bioaccumulation of environmental contaminants and the decline in the Kootenai River white sturgeon population.

Introduction

The Kootenai River stock of white sturgeon (*Acipenser transmontanus*) was federally listed as an endangered species in 1994 (USFWS, 1994; Duke et al. 1999). One factor that may have contributed to low recruitment and a declining population is the introduction of xenobiotic or contaminating compounds into the Kootenai River system.

The Kootenai River is a trans-boundary (United States–Canada) river system that has undergone many physical changes in the past century from activities associated with agriculture, industry and urbanization (Kootenai River Network, 2000). All of these activities have, to some degree, contributed to contaminant loading within the basin. Little information has been collected about the contribution of contaminants from these activities; their specific effects on the Kootenai River system are largely unknown. Although the Kootenai River drainage has historically supported a fairly diverse cold-water fish fauna (Partridge 1983) many of the historically abundant populations have severely declined and several factors, including pollution, may be contributing to the declines.

Bioaccumulated contaminants (resulting from pollution) can cause a range of lethal and sublethal impacts on fish, which can be expressed as physiologic effects at the individual or population level and at various life stages (Heath 1995). The white sturgeon, a long-lived, bottom-dwelling species, represents the end of the food chain within its habitat (Dettlaff et al. 1993) and is therefore highly susceptible to exposure and bioaccumulation of contaminants. Factors that affect contaminant uptake and metabolism in such a species are more complex than for pelagic organisms or those lower in the food chain (Anderson et al., 1987; Zaranko et al., 1997).

When monitoring an endangered species or stock for physiologic effects of contaminants, the ability to conduct lethal experiments is very limited because of the need to avoid mortality. Physiologic biomarker research is an effective method for determining contaminant effects in aquatic ecosystems and in most cases can be conducted without the excessive mortality required by standard chronic and acute mortality tests (Ward, 1998). The biomarker approach incorporates tissue and environmental contaminant residue information with measurements of physiologic functions in organisms. Our study combines multiple pieces of biomarker information in order to assess potential relationships between bioaccumulation of metal and organochlorine compounds and physiologic parameters in adult and juvenile Kootenai River white sturgeon.

Materials and methods

Sample collection and tissue residue analysis

During the period 1997–2000, adult and juvenile Kootenai River white sturgeon were captured with rod-and-reel, setlines and small mesh multi-filament gill nets. Various tissue and body fluids samples were collected for biomarker analysis (Table 1). Thirty-four ovarian tissue samples were collected by biopsy (Conte et al. 1988) from female sturgeon representing developmental stages 2–4 (Tables 1 and 2), age 18–52 years, 141–222 cm fork length and weights ranging from 22–91.3 g. Blood samples from the ventral caudal vein of adult and juvenile sturgeon were drawn into heparanized vaccutainers and centrifuged to separate plasma from the red blood cells. Plasma samples were stored frozen until shipped to the prospective laboratories for analysis of plasma steroids, vitellogenin, acetylcholinesterase (AChE) or chromosomal DNA. Sperm samples collected during 2000 were hand-drawn by syringe from 14 wild, stage 9 (Tables 1 and 2) male sturgeons with 176–183 cm fork length (ages and weights for

Table 1
Analytical biomarker, type of tissue or fluid sampled, sex of sturgeon, number of samples collected (n), and range of fork lengths and weights for fish captured for contaminant assessment in Kootenai River white sturgeon, 1997–2000

	Sex of fish								
	Males			Females			Juveniles/immature		
Analytical biomarker (tissue type)	n	Range of fork lengths (cm)	Range of weights (g)	n	Range of fork lengths (cm)	Range of weights (g)	n	Range of fork lengths (cm)	Range of weights (g)
Egg size (ova)	–	–	–	15	152–201	34–80	–	–	–
Tissue*	14	153–174	NA	34	141–222	22–91	25	31–45	0.15–0.45
Chromosomal DNA (blood)	–	–	–	10	156–217	35–91	25	31–45	0.15–0.45
Vitellogenin (blood plasma)	29	119–208	11–61	–	–	–	–	–	–
Steroids (blood plasma)	19	127–187	10–51	22	102–222	8–59	5	102–163	8–32
Cholinesterase (blood and brain)	–	–	–	–	–	–	25	31–45	0.15–0.45
Histology (liver)	–	–	–	–	–	–	25	31–45	0.15–0.45

*Tissue types included sperm from males, ova from females and whole-body tissues from juvenile fish.
NA: data not available.

Table 2
Stage definition of sexual development in white sturgeon (Apperson and Anders, 1991)

Category/sex	Description of development
Females	
1	Previtellogenic: No visual signs of vitellogenesis; eggs present but have average diameter <0.5 mm
2	Early vitellogenic: Eggs colored cream to gray; average diameter 0.6–2.1 mm
3	Late vitellogenic: Eggs are pigmented and attached to ovarian tissue; average diameter 2.2–2.9 mm
4	Ripe: Eggs are fully pigmented, detached from ovarian tissue and ready for ovulation; average diameter 3.0–3.4 mm
Males	
7	Non-reproductive: Testes with translucent smoky pigmentation
8	Reproductive: Testes white with folds and lobes

these males were unavailable). During 1999, whole body tissue, blood, liver and brain tissue samples were collected from 25 4-year-old, hatchery reared juvenile sturgeons (released into the Kootenai River system in 1997). Sampled juvenile sturgeon ranged between 31 and 45 cm fork length and 0.15–0.45 g in weight. All egg, sperm and whole-body tissue samples were stored frozen from 1 to 6 months until analyzed by US Environmental Protection Agency (EPA) analytical methods for organochlorine pesticides, Aroclor 1200 series polychlorinated biphenyl (PCB)s and metals (US EPA, 2000). Additional data were recorded from each fish, including capture location, length, weight, and age (if ages were available from pectoral fin ray sections).

Selection of contaminants for tissue residue analysis was based on historic data availability as well as presence, application and bioaccumulative potential of contaminants within the lower Kootenai River. Contaminant residue data were tested for normality of distribution with Cochran's C test (Kirk, 1995).

Egg size analysis

During the period 1997–2000, mature stage-4 (Table 2) ova were taken during spawning from 15 female sturgeon used as broodstock at the KTOI hatchery (Table 1). Egg size was measured as number of eggs per ml. Spearman rank correlation analysis was used to test the null hypothesis that contaminant concentrations and size of eggs were not significantly correlated ($\alpha = 0.05$).

Plasma steroid analysis

Plasma samples from 22 female, 19 male and five immature and non-reproductive sturgeons were analyzed for testosterone, 11-ketotestosterone and 17β-estradiol as an indication of potential disruption to reproductive processes (Table 1). Steroid analysis was conducted at Oregon State University by competitive binding radioimmunoassay (RIA) methods (Fitzpatrick et al., 1996). Plasma steroid data were tested for normality of distribution (2SE + K) and homogeneity of variances (Cochran's C test; Kirk, 1995). Data did not display a normal distribution or homogeneity of variance, therefore the nonparametric Spearman rank test was applied to test the null hypotheses that plasma steroid concentrations were not significantly correlated with ovarian tissue contaminant concentrations ($\alpha = 0.05$). The ratio of 17β-estradiol to 11-ketotestosterone (E2/11-kt ratio) was calculated for male and female sturgeon to assess sexual dimorphism. The null hypothesis that the E2/11-kt ratio was not significantly different between male and female was also tested (Mann–Whitney U test; $P < 0.05$).

Vitellogenin analysis

Plasma samples from 29 male sturgeons were shipped to Oregon State University for enzyme immunoassay (EIA) analysis of vitellogenin concentrations as an indicator of potential exposure to estrogenic substances (Linares-Casenave et al., 1994; Heppell et al., 1999; Table 1). Vitellogenin levels

in male Kootenai River white sturgeon were compared with those found in other populations of sturgeon from outside of the Kootenai River drainage.

Chromosomal DNA analysis

Plasma samples from 10 adult and 25 juvenile sturgeons were shipped to International Ecogen in North Vancouver, BC, Canada for genetic analysis by flow cytometry (Easton, 1997; Table 1). A known volume of human lymphocytes added to chicken red blood cells was used as an internal control. The mean channels for DNA content for the sturgeon blood and the human lymphocytes were established in the instrument's most sensitive range and maintained throughout. The subsequent data were standardized and analyzed with Elite Software (Coulter Corp., Miami, FL) to produce the mean channel and full peak coefficient of variation (CV) values (degree of variation of DNA content within the population of cells). Coefficient of variation difference (CVD) values were then calculated by subtracting the CV value of the sturgeon red blood cells from that of the control population of chicken erythrocytes. The CVD values indicated the net variation between DNA content in sturgeon and control samples. CV data were not normally distributed and displayed heterogeneity of variances; therefore, the non-parametric Mann–Whitney U test ($P < 0.05$) was used to test the null hypotheses of no significant differences among CV values found in the adult sturgeon, juvenile sturgeon and the control populations. Blood cells were also analyzed for chromosome aberrations and evolution of new cell lines. Organochlorine pesticide and metal tissue concentrations in female ovarian tissue and juvenile whole-body tissue were correlated with rank transformed CVD values to assess correlations between chromosomal DNA variability and tissue contaminant burdens.

Cholinesterase analysis

Brain and blood samples from 25 juvenile sturgeons were collected (Table 1) and analyzed for AChE and butyrylcholinesterase (BChE) at the Institute of Environmental and Human Health at Texas Technological University, Lubbock, Texas according to preparation and incubation methods cited in Ellman et al. (1961) and Gard and Hooper (1993). The null hypothesis of no significant Cholinesterase (ChE) inhibition related to carbamate or organophosphate pesticide exposure was tested by comparing the activity of pre-incubation and post-incubation samples (Student's t-test; $P < 0.05$). The null hypotheses of no significant correlations between ChE concentrations and absolute activity, capture date, weight, fork length and contaminant concentrations in juvenile sturgeon were also tested using either the Pearson test for normally distributed data with homogeneous variances or the Spearman test for nonnormally distributed data with heterogeneous variances ($P < 0.05$).

Liver histology

Livers from 25 juvenile sturgeons were shipped to the USFWS Fish Health Laboratory in Orofino, Idaho (Table 1). Individual liver samples were sliced into gross cross-sections, placed into cassettes, and processed in a Tissue-Tek 2 processor for approximately 12 h. Processed sections were vacuum processed to eliminate all air voids in the tissue, embedded in paraffin and thin sectioned. Two sections from each liver were stained and viewed for abnormalities or cell damage that may have been related to contaminant exposure. Frequency of abnormalities was expressed as the number of livers with the abnormality divided by the total number of livers analyzed.

Results

Adult sturgeon

Ovarian tissues contained detectable concentrations of the metals arsenic, cadmium, copper, iron, lead, selenium and zinc (Table 3). There were also detectable levels of the organochlorines DDE, dichlorodiphenyltricholoethane (DDT), aldrin and the PCB Aroclor 1260 (Table 3). The observed ovarian tissue concentrations of the PCB Aroclor 1260, DDE, copper and zinc in this study occurred at levels shown elsewhere to inhibit reproduction in other fish species (Monosson, 1997; Thomas and Kahn, 1997; Jarvinen and Ankley, 1999). Sturgeon sperm samples contained detectable levels of the metals arsenic, copper, iron, lead, selenium and zinc (Table 3).

The null hypothesis that egg size and egg tissue concentrations of metals and organochlorine compounds from female broodstock sturgeon were not significantly correlated was rejected for selenium (Spearman rank test; $r = 0.731$; Fig. 1). This relationship appeared to be driven by one data point. However, removal of this outlier (point >2 standard deviations from the mean) still resulted in a significant correlation ($r = 0.678$).

The null hypothesis that plasma steroid concentrations differed between male and female sturgeons was rejected (Kruskall–Wallis; $P < 0.05$). Male sturgeons possessed significantly higher concentrations of testosterone (Mann–Whitney U; $P \leq 0.002$) and 11-ketotestosterone (Mann–Whitney U; $P \leq 0.003$) than female or immature sturgeons. Mature reproductive male sturgeons at stage 8 development (Table 2) also possessed significantly higher concentrations of 17β-estradiol (Mann–Whitney U; $P = 0.005$), testosterone (Mann–Whitney U; $P = 0.003$) and 11-ketotestosterone (Mann–Whitney U; $P = 0.003$) than stage 7 males. The mean ratio of E2/11-kt was significantly higher in female (0.128) than in male (0.008) sturgeons (Mann–Whitney U; $P < 0.001$).

The null hypothesis that plasma steroid concentrations and ovarian tissue contaminant concentrations were not significantly correlated was rejected in several cases (Table 4). The relationship between ovarian concentrations of zinc and 11-ketotestosterone and the relationship between DDT and estradiol appeared to be driven by one data point. These relationships were no longer significant when these outliers were removed from the analysis (Kruse, 2000).

Plasma vitellogenin concentrations in 28 mature male sturgeons ranged from 0.042–2.049 $\mu g/ml^{-1}$. The mean plasma vitellogenin concentration was 0.612 ± 0.517 $\mu g/ml^{-1}$.

The null hypotheses of no significant correlation between DNA chromosomal CVD values and iron (Spearman rank test; $r = 0.862$) or selenium (Spearman rank test; $r = -0.742$) concentrations in adult ovarian tissue were rejected (Fig. 2). The relationship between CVD values and selenium was driven by one data point which, when removed, made the relationship nonsignificant (Kruse, 2000).

Juvenile sturgeon

Whole-body tissue from 25 juvenile sturgeons contained detectable concentrations of several inorganic metal compounds and two samples contained the organochlorine

Table 3
Contaminants tested for, number and percent of total samples with detectable residues, and range and mean (± SD) concentrations of metal and organochlorine compounds detected in Kootenai River white sturgeon ova (collected 1997–2000), sperm (collected 2000) and juvenile whole-body tissue (collected in 1999)

Contaminant (concentration unit)	Number of fish sampled (% of total number of fish sampled)	Range	Mean (± SD)
Ova			
Arsenic (ppm)	20 (51)	0.070–1.2	0.287 (0.274)
Cadmium (ppm)	19 (49)	0.001–0.94	0.064 (0.214)
Copper (ppm)	36 (92)	0.58–6.9	2.245 (1.462)
Iron (ppm)	39 (100)	14–56	26.308 (10.000)
Lead (ppm)	19 (49)	0.045–0.88	0.169 (0.191)
Selenium (ppm)	39 (100)	0.240–12	1.549 (1.191)
Zinc (ppm)	39 (100)	17–170	34.231 (27.506)
DDE (ppb)	33 (85)	48–1800	301.455 (376.730)
DDT (ppb)	12 (31)	22–88	44.250 (18.415)
Aldrin (ppb)	5 (13)	53–98	77 (16.837)
Aroclor 1260 (ppb)	25 (64)	160–1300	460 (288.401)
Total Organochlorines (ppb)	33 (85)	58–2473	674.394 (595.250)
Sperm			
Arsenic (ppm)	8 (57)	0.040–0.090	0.049 (0.017)
Copper (ppm)	1 (7)	0.140	–
Iron (ppm)	5 (36)	0.220–6.300	1.494 (2.688)
Lead (ppm)	4 (29)	0.050–0.290	0.120 (0.114)
Selenium (ppm)	2 (14)	0.040	0.040 (0)
Zinc (ppm)	14 (100)	0.090–0.880	0.245 (0.232)
Juvenile whole-body tissue			
Zinc (ppm)	25 (100)	9.900–24.000	17.276 (3.778)
Cadmium (ppm)	8 (32)	0.011–0.052	0.022 (0.014)
Lead (ppm)	5 (20)	0.570–1.800	0.952 (0.505)
Cobalt (ppm)	7 (28)	0.080–0.200	0.113 (0.043)
Nickel (ppm)	11 (44)	0.230–3.700	0.760 (1.005)
Manganese (ppm)	25 (100)	0.520–6.300	1.758 (1.189)
Iron (ppm)	25 (100)	0.300–350.000	31.880 (68.180)
Chromium (ppm)	17 (68)	0.160–1.300	0.445 (0.336)
Aluminum (ppm)	25 (100)	2.600–42.000	11.776 (11.090)
Copper (ppm)	25 (100)	0.290–17.000	2.395 (4.265)
Arsenic (ppm)	25 (100)	66.000–400.000	215.840 (87.126)
Selenium (ppm)	25 (100)	0.470–0.940	0.714 (0.113)
Mercury (ppm)	25 (100)	0.260–0.450	0.326 (0.045)
DDE (ppb)	2 (8)	67.000–77.000	72.000 (7.071)

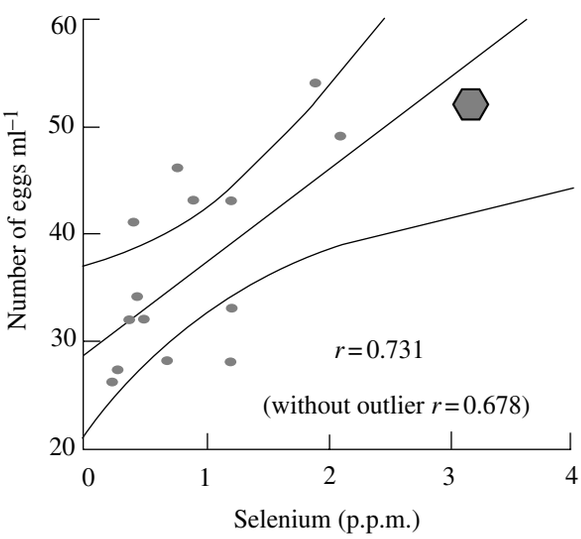

Fig. 1. Correlation between number of eggs per milliliter and selenium detected in broodstock Kootenai River white sturgeon ovarian tissue, 1997–2000 (Spearman rank test; $\alpha = 0.05$). Brackets indicate 95% confidence intervals. Large dot indicates an outlier that is greater than 2 standard deviations from the mean

pesticide, DDE (Table 3; Kruse, 2000). Detected levels of lead, aluminum, copper, selenium, and mercury were comparable with levels found related to reduced survival and growth in other fish species (Jarvinen and Ankley, 1999).

The null hypotheses that the intrinsic variability of chromosomal DNA in either the juvenile (Mann–Whitney U; $P = 0.026$) or adult (Mann–Whitney U; $P = 0.002$) sturgeon red blood cells did not differ significantly from the control blood cell populations was rejected. The null hypothesis that intrinsic variability of chromosomal DNA in juvenile and adult sturgeon red blood cells was not significantly different (Mann–Whitney U; $P = 0.511$) was not rejected. No significant relationships existed between whole-body tissue contaminant concentrations and CVD values in juvenile sturgeon (Kruse, 2000).

AChE and BChE were detected in blood and brain samples from juvenile Kootenai River white sturgeon (Table 5; Kruse, 2000). The null hypotheses of no significant ChE inhibition related to organophosphate (Student's t-test; $P = 0.09$) and carbamate (Student's t-test; $P = 0.12$) pesticide exposure was not rejected. Significant inhibition of AChE and BChE did not occur in the fish used for this study.

The null hypothesis that ChE concentration and activity did not correlate with tissue concentrations of metals was rejected (Fig. 3). Tissue concentrations of chromium showed a significant negative correlation with blood serum BChE (Spearman rank test; $r = -0.662$) and AChE (Spearman rank test; $r = -0.630$) activity. Tissue concentrations of lead negatively correlated with blood serum BChE activity (Spearman rank test; $r = -0.900$). Increasing tissue concentrations of aluminum correlated with decreasing brain BChE activity (Spearman rank test; $r = -0.423$).

Table 4
Spearman rank correlations (*r* value) between plasma steroid biomarkers 17β-estradiol (E2), 11-ketotestosterone (11-kt), Testosterone (T) and contaminants for vitellogenic female Kootenai River white sturgeon (n = 13)

Contaminant (n)	Correlation coefficients (*r*)			
	E2	11-kt	T	E2/11-kt ratio
Arsenic (7)	0.607	− 0.607	− 0.536	0.750
Cadmium (8)	0.024	− 0.381	− 0.429	0.210
Copper (12)	0.446	0.260	− 0.042	− 0.100
Iron (13)	− 0.474	− 0.438	− 0.349	0.110
Lead (8)	0.443	0.299	0.084	− 0.036
Selenium (13)	0.308	− 0.044	− 0.055	0.290
Zinc (13)	− 0.432	− 0.652*	− 0.536	0.338
DDE (13)	0.377	− 0.550	− 0.407	0.611*
DDT (7)	0.893*	− 0.286	− 0.000	0.464
Aroclor 1260 (11)	− 0.041	− 0.820*	0.729*	0.733*
Total organochlorines (13)	0.258	− 0.753*	− 0.500	0.791*

*Indicates significant correlations ($\alpha = 0.05$).

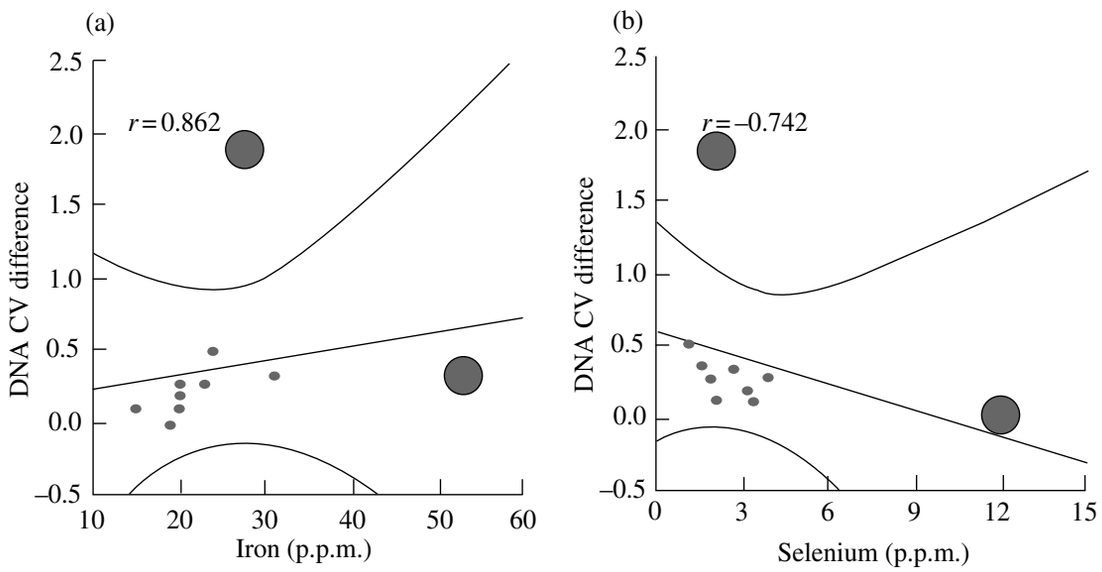

Fig. 2. Correlations between the coefficient of variation (CV) difference in adult Kootenai River white sturgeon (calculated by subtracting the CV in adult sturgeon from the CV in the control blood cell population) and ovarian tissue concentrations of iron (a) and selenium (b; Spearman's test; $\alpha = 0.05$). Large dot indicates points that are greater than 2 standard deviations from the mean and strongly influence the *r* value. Brackets indicate 95% confidence intervals

Table 5
Brain and blood acetylcholinesterase (AChE) and buturylcholinesterase (BChE) activity and percent of sample in 25 hatchery-reared and released (hatched in 1995 and released in 1997) juvenile Kootenai River white sturgeon captured during July, 1999

	Brain				Blood			
	AChE		BChE		AChE		BChE	
Concentration	Amount in sample (%)	Activity (Units)	Amount in sample (%)	Activity (Units)	Amount in sample (%)	Activity (Units)	Amount in sample (%)	Activity (Units)
Mean concentration (± SD)	47.7 (8.29)	2.00 (0.70)	52.3 (8.31)	2.25 (0.62)	7.58 (1.93)	0.028 (0.012)	92.4 (1.93)	0.329 (0.068)
Range	32.3–64.7	1.12–2.57	35.3–67.9	1.07–3.25	4.47–13.0	0.014–0.059	87.0–95.5	0.223–0.508

The 25 livers from juvenile sturgeons did not display extreme cell damage or abnormalities. Of the abnormalities noted, 20 (80%) of the samples contained small areas of lymphocytic aggregations, 24 (96%) contained higher melanin than other samples, eight (32%) showed focal necrosis and one (4%) contained higher levels of fatty tissue than the other samples.

Discussion

Detection of metal and organochlorine compounds in Kootenai River white sturgeon ovarian tissue, sperm and whole-body tissue indicates that environmental exposure has resulted in bioaccumulation of these compounds above method detection limits. Although no published literature specific to effects of

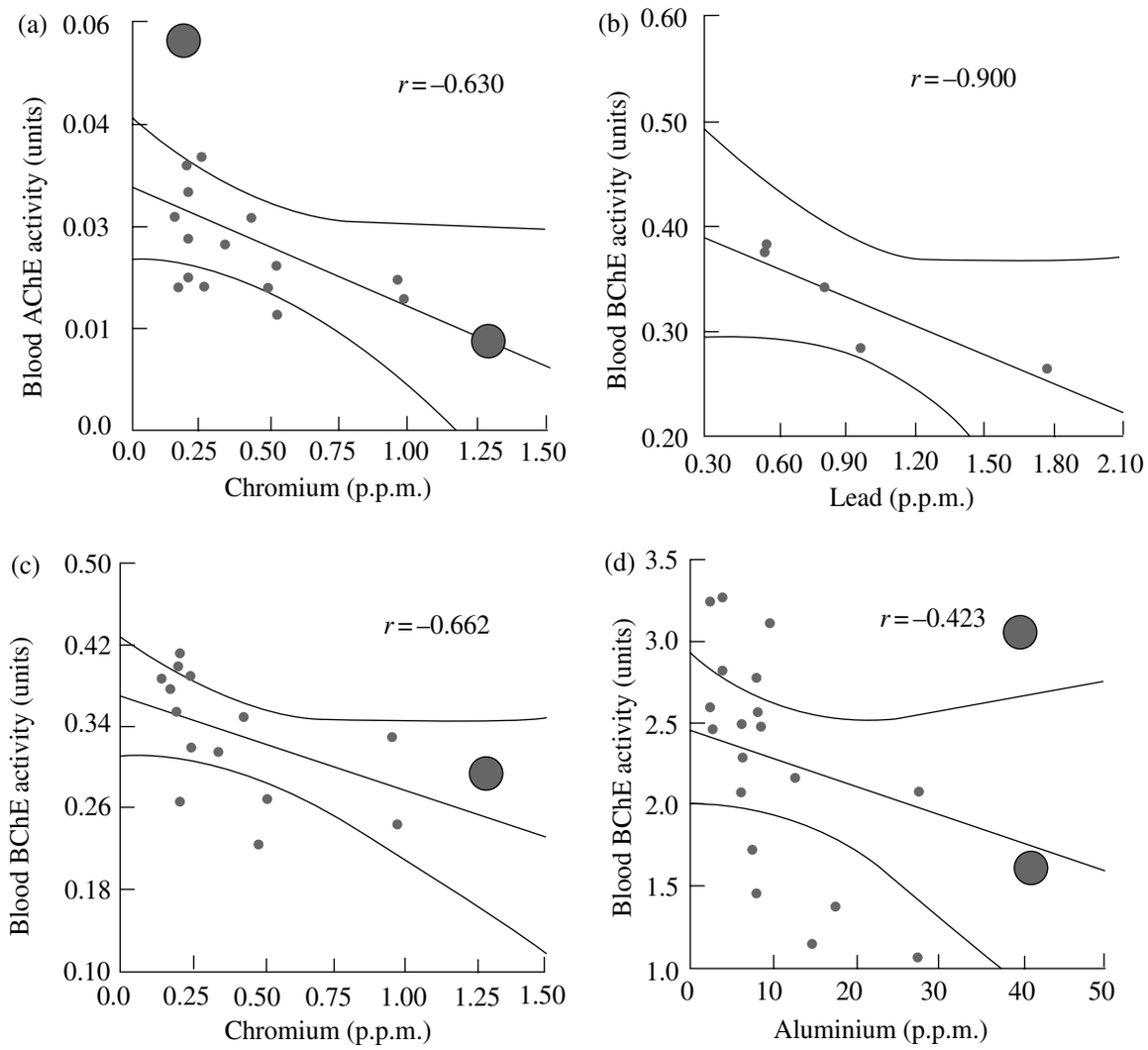

Fig. 3. Correlations between blood acetylcholineresterase (AchE; a) and butyrylcholinesterase (BchE; b,c,d) and whole body tissue concentrations of lead, aluminium and chromium in 25 hatchery-reared (brood year 1995) and released (1997) juvenile Kootenai River white sturgeon captured in 1999 (Spearman test; $\alpha = 0.05$). Large dots indicate points that are greater than 2 standard deviations from the mean and strongly influence their value. Brackets indicate 95% confidence intervals

similar levels of metals and organochlorine compounds on sturgeons was currently available, results from studies using surrogate species indicate that the potential exists for sublethal effects on physiological functions.

Higher levels of metals in ova (than sperm) from broodstock fish indicate that ova depurate more metals from the adult body and potentially contribute more metals to offspring than sperm. Differences between concentrations in ova and sperm may be the result of differences in physiology. Lower lipid content as well as higher production and turnover rate of sperm allow for substantially lower bioaccumulation rates (for many anthropogenic compounds) than with ova, which take several years to develop and contain higher levels of lipids (Heath, 1995).

The significant correlation between egg size and selenium concentration in ova indicates potential reduction in egg size with increased bioaccumulation of selenium. Although it is also considered an essential mineral, there is a fine line between beneficial and toxic levels of selenium (Lemly, 1997) and selenium bioaccumulation has been associated with fish reproductive failure (Cumbie and Van Horn, 1978). Excess selenium is deposited in the yolk of developing eggs where it can retard growth and development of the egg through depression of protein synthesis.

When compared with ratios from other fish, plasma steroid levels and E2/11-kt values can be useful indicators of potential endocrine disruption. The higher concentrations of testosterone and 11-ketotestosterone in male than in female sturgeon observed in this study were expected because these steroids are primarily associated with male reproductive function (Chieffi and Pierantoni, 1987). Plasma steroid levels in Kootenai River white sturgeon were also comparable to levels found in other populations of sturgeon (Chapman, 1989; Fitzpatrick et al., 1996; Doroshov et al., 1997; Webb et al., 1999; Table 6). Under normal conditions E2/11-kt ratios should be different between males and females and the significant difference between ratios in Kootenai River sturgeon is consistent with ratios found in common carp (*Cyprinus carpio*; Goodbred et al., 1997).

Although in some instances the significant correlations were driven by one or two data points, results from this study suggest potential inhibition of the androgens, testosterone and

	Male	Female	Immature/ non-reproductive
Testosterone			
Kootenai River WS	6.32–446.79	0.48–169.60	1.93–12.94
Columbia River WS	5.80–73.70	0.29–196.70	0.10–109.50
Cultured WS	–	36.00–355.00	0.25
Atlantic sturgeon	0.03–54.70	0.10–125.90	–
Siberian sturgeon	880.00	25.00–90.00	–
11-ketotestosterone			
Kootenai River WS	3.85–215.83	0.18–150.81	0.95–3.51
Columbia River WS	4.40–339.80	0.00–161.20	0.00–106.00
Siberian sturgeon	–	–	0.63–177.50
Estradiol			
Kootenai River WS	0.04–0.57	0.03–10.40	–
Columbia River WS	0.10–1.20	0.10–23.7	–
Cultured WS	–	0.68–5.10	–
Atlantic sturgeon	0.16–1.79	0.06–7.80	–
Siberian sturgeon	1.00–44.50	1.00–100.00	–

Table 6
Comparison of ranges of testosterone, 11-ketotestosterone and estradiol concentrations (ng ml^{-1}) in immature and nonreproductive and sexually reproductive male and female Kootenai, Columbia River and cultured white sturgeon, Atlantic sturgeon and Siberian sturgeon (Chapman, 1989; Cuisset et al., 1994; Fitzpatrick et al., 1996; Doroshov et al., 1997; Webb et al., 1999)

11-ketotestosterone, and induction of 17β-estradiol resulted from bioaccumulated concentrations of zinc, DDT and the PCB Aroclor 1260. Several metal and organochlorine compounds (at concentrations comparable to those found in this study) have been associated with inhibited steroid synthesis and metabolism in fish (Weatherley et al., 1980; Freeman and Idler, 1975; Freeman and Sangalang, 1977; Sivarajah et al. 1978; Allen-Gil et al., 1993). The observed decrease in 11-ketotestosterone with increasing E2/11-kt ratio and the increase in E2/11-kt ratio with increased organochlorine concentrations suggests that the androgen 11-ketotestosterone may be depressed by bioaccumulated organochlorine compounds.

Male fish have a vitellogenin gene that is not usually expressed, but male fish of several species, not exposed to endocrine disrupting compounds, have been documented to have low concentrations (≤ 1000 $\mu g/ml^{-1}$) of vitellogenin present (Copeland and Thomas, 1988; Goodwin et al., 1992). If estrogenic compounds were inducing abnormal vitellogenin production in male Kootenai River white sturgeon, higher levels of vitellogenin would have been expected.

The presence of a significant correlation between DNA chromosomal CVD values and selenium and iron concentrations in adult ovarian tissue indicates that these two metals may have contributed to the degree of chromosomal DNA variability in the adult sturgeon analyzed. Selenium is necessary for proper formation and functioning of glutathione peroxidase, which is a major cellular antioxidant that destroys free radicals, preventing them from damaging cells and molecules (Lemly, 1997). Without adequate selenium, normal metabolic operations break down (Lemly, 1997), thereby potentially contributing to DNA mutations. The strong negative relationship between selenium and CVD values was heavily weighted by one high data point for selenium. Therefore, although it is possible that selenium protects DNA from damage by free radicals, no suggestions about its effect on DNA can be made without further research. The positive correlation between CVD values and iron content, however, suggest a possible increase in DNA mutation due to iron bioaccumulation. Unbound iron can function as a free radical (side products of metabolism that can damage organs and functions at the molecular and cellular level; Passwater, 1999). Free iron that has been liberated from iron-containing proteins can liberate other oxygen-related compounds to produce radicals that mutate DNA proteins, thereby increasing the variability of chromosomal DNA expression.

The intrinsic variability of red blood cell chromosomal DNA in juvenile and adult Kootenai River white sturgeon differed significantly from the control blood cell population of chicken erythrocytes. The detected differences are likely due to an inappropriate selection of a control blood cell population. According to Birstein et al. (1997), the difference in chromatic structure within cells of phylogenentically distant animals can influence and even change the results of DNA measurements. The lack of a significant difference between CV values in juvenile and adult sturgeon indicates that significant chromosomal mutations did not occur between the two generations.

Cholinesterase inhibition resulting from exposure to organophosphate or carbamate pesticides was not detected in blood plasma or brain tissue from juvenile sturgeon in this study. Therefore, these pesticides, which are applied to agricultural lands within the lower Kootenai River, were not present at levels that inhibited acetylcholine hydrolysis. However, the significant negative correlations between blood serum BChE/AChE and whole-body metal concentrations indicate potential suppression of these enzymes with increasing bioaccumulated concentrations of aluminum, chromium and lead. Nemcsok et al. (1984) also demonstrated inhibition of AChE activity in rainbow trout and carp exposed to a divalent cation, copper. These effects could, in turn, impact behavioral performance in the sturgeon by suppressing neuron sensitivity or abnormal stimuli (Weber and Spieler, 1994).

Liver histology results indicated only slight liver damage in juvenile sturgeon. Small areas of lymphocytic infiltration or aggregation were noted and although the presence of these aggregations is frequently common and likely normal, they can also result from toxic agents capable of causing cellular damage within the liver tissue virulence (J. Morrison, pers. comm.). Cytoplasmic inclusions were also noted and seem to be a fairly common occurrence with fish that have been exposed to either metals or organic xenobiotics such as organochlorine pesticides (Patton and Couch, 1984). It is unclear whether the granules detected in Kootenai River white sturgeon livers were due to aggregation for excretion of contaminants or the result of normal sequestration of abnormally high levels of otherwise essential trace elements (J. Morrison). The sturgeon liver samples contained slightly high levels of melanin (J. Morrison pers. comm.) which may be associated with increasing melaomacrophage activity induced by agents such as low level toxic exposure or pollutants such as managanese or with age (Committee on Biologic Effects of Atmospheric Pollutants, 1973; J. Morrison pers. comm.).

However, all of the liver samples were taken from young fish (4 years old), indicating that contaminant exposure, rather than age, could be a potential cause of increased melanin. Focal necrosis (death of cells) in the sturgeon livers can be a result of improper fixing of the sample or with exposure to pollutants (Heath, 1995). The fish used in this study were exposed to environmental conditions in the Kootenai River for only 2 years; a longer exposure period may result in more severe damage.

In conclusion, existing levels of inorganic metals and organochlorine compounds may potentially impact reproduction, AChE production, DNA expression and physiological integrity of Kootenai River white sturgeon. Results from this study indicate that contaminants in the Kootenai River system are potentially creating additional stress (perhaps in the form of diminished physiological function) on the white sturgeon population at various life stages. Therefore, duplication of significant results (from this study) and long-term trend monitoring are recommended in order to determine if a consistent pattern is observed among biomarker and contaminant level relationships.

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