THE EFFECTS OF CONTAMINANTS ON REPRODUCTION, EMBRYO DEVELOPMENT AND RELATED PHYSIOLOGICAL PROCESSES IN KOOTENAI RIVER WHITE STURGEON, *ACIPENSER TRANSMONTANUS* RICHARDSON.

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Thesis

This thesis of Gretchen O. Kruse, submitted for the degree of Master of Science with a major in Fisheries Resources and titled "The Effects of Contaminants on Reproduction, Embryo Development and Related Physiological Processes in Kootenai River White Sturgeon, *Acipenser transmontanus*", has been reviewed in final form. Permission, as indicated by the signatures and dates given below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

This study used biomarkers to evaluate the effects of environmental levels of organochlorine, organophosphate, organonitrate, and carbamate pesticides, polychlorinated biphenyls (PCBs) and metals in the aquatic system on Kootenai River white sturgeon, *Acipenser transmontanus*. The biomarkers that were used include tissue residue analysis, plasma steroid production and egg size in adult sturgeon, survival and contaminant uptake during incubation in embryos, red blood cell chromosome content and variability, liver histology, tissue residue analysis and acetylcholinesterase inhibition in juvenile sturgeon. Chemical residues were assessed in ovarian tissue from mature adult females, in whole-body tissue from juveniles, in incubated embryos, and in water and sediment samples from the river. Contaminant residues in ovarian tissue and river-bottom sediment (collected between 1997 and 1999) were compared with residues detected in samples collected between 1989 and 1991. Incubating white sturgeon embryos were exposed to different rearing media (water, sediment and suspended solids collected directly from the Kootenai River) to determine mortality rate and uptake of environmental contaminants. Blood samples were collected from adult and juvenile sturgeon to determine plasma steroid (testosterone, 11-ketotestosterone, and estradiol) levels, cholinesterase inhibition, and chromosomal DNA variability. Results from chemical residue analysis indicated that copper, zinc, iron, and the PCB aroclor 1260 were at levels that could adversely affect sturgeon reproduction as well as other aquatic

organisms and overall system productivity. Plasma steroid concentrations in Kootenai River sturgeon were comparable to those reported for other species of sturgeon. However, the significant negative correlations between testosterone production and bioaccumulated aroclor 1260 (Spearman; r = -0.729, r = -0.820), total organochlorine compounds (Spearman; r = -0.753) and zinc (Spearman; r = -0.652) suggest that males may experience decreased sperm production if they have bioaccumulated these contaminants at levels similar to those found in females. The significant positive correlation between the female hormone estradiol and DDT (Spearman; r = 0.893) also suggests potential feminization of male sturgeon that bioaccumulate DDT levels similar to those found in females. Zinc residues in sturgeon ovarian tissue were significantly (Mann-Whitney U test`; $P < 0.05$) higher than in samples taken between 1989 and 1991. River-bottom sediments were found to be a significant source of metal and PCB exposure for incubating white sturgeon embryos. Environmental levels of copper and PCB aroclor 1260 in the rearing media were associated with increased mortality (Spearman; r = 0.568) and decreased incubation time of sturgeon embryos. Results from liver histology, cholinesterase and DNA analyses in juveniles indicated that although juvenile sturgeon were experiencing low-level contaminant exposure, physiological functions were not limited or altered. The biomarkers used in this study indicate effects and are not a measure of cause. Therefore, it was concluded that embryonic, juvenile and adult life stages are potentially experiencing sublethal effects from contaminants in the Kootenai

River. The embryonic life stage appears to be the most susceptible to the effects of these contaminants. Although spawning may succeed, despite parental burden of contaminants, embryos potentially suffer significant mortality (in comparison with control conditions) as a result of additional exposure to contaminants in water and sediment.

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Introduction

The Kootenai River white sturgeon, a genetically and geographically distinct stock of the white sturgeon *Acipenser transmontanus* Richardson, was federally listed as an endangered species in September 1994 (US Fish and Wildlife Service 1994). The stock has been isolated from Columbia River basin stocks since the late Wisconsin glacial period, approximately 10,000 years ago (Apperson and Anders 1991).

The endangered species listing resulted from concern about a low population size as well as poor reproduction and recruitment within the stock. In 1995, the population size was estimated at only 1,469 adults and 87 wild juveniles (Paragamian et al. 1997). Although spawning has been documented (Paragamian et al. 1996), few embryos and larvae reach two years of age and recruit to the adult population. As of 1999, the population consisted of few fish younger than 20 years of age.

Several factors may have contributed to low recruitment. As early as 1891, riverbank diking and channelization were undertaken to control seasonal flooding of agricultural lands within the Kootenai River floodplain (Redwing Naturalists 1996). These habitat alterations have altered spring migration flows for sturgeon, and reduced in-river habitat suitability and productivity for sturgeon in the main stem lower Kootenai River (US Fish and Wildlife Service 1999). A second factor is the construction of Libby Dam in 1972, which resulted in a major

change of the river's natural hydrograph. Many scientists believe that a 50% reduction of spring flows is the primary cause of reproductive failure through its effects on fish migration and spawning habitat availability (US Fish and Wildlife Service 1999).

In addition to these physical habitat changes, introduction of xenobiotic or contaminating compounds in the Kootenai River system may increase stress and negatively impact reproductive processes, viability, survival and development of naturally-spawned sturgeon eggs. These effects have also been documented in other watersheds (Saunders 1969; Hall et al. 1989; Bickham et al. 1998). According to Wilcove et al. (1998) pollution is second only to habitat loss as a cause of endangerment for aquatic animals. Sappington (1995), found that chemical habitat and pollution correlated with 38% of species extinctions during the last 100 years.

Some efforts have been made to decrease contaminant loading in the Kootenai River through implementation and upgrading of treatment facilities that release contents into the river (Kootenai River Network 2000). Although Libby Dam has created a sink for many upriver contaminants, agriculture, mining, transportation, timber and recreational activities below Libby Dam continue to contribute contaminants to the lower river. As of 1999, there are no known large-scale contaminant sources within the system; however, in addition to historic loading, even minimal sources may further stress an already depleted sturgeon

population. Jensen (1971) reported that the effect of a five percent increase in mortality of age-0 brook trout (in a stressed population) would significantly decrease the fishery, and a 50% increase from one or more causes would eliminate the population altogether. Introduced contaminants in the Kootenai River may affect sturgeon in a similar manner.

The white sturgeon, a long-lived, bottom-dwelling species that represents the end of the food chain within its habitat (Detlaf et al. 1993) is therefore highly susceptible to exposure and bioaccumulation of contaminants. Factors that affect contaminant uptake in such a species are more complex than they are for pelagic organisms or those lower in the food chain (Anderson et al. 1987; Zaranko et al. 1997). Due to the wide physiological and geographical diversity within and among species, adequate surrogate populations or species are often difficult to find for use in evaluating the effects of contaminants on rare species (Rand and Petrocelli 1985). In addition, when monitoring an endangered species or stock for physiological effects of contaminants, the ability to conduct lethal experiments is very limited because of the need to avoid mortality.

Physiological 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). This approach incorporates tissue and environmental contaminant residue information with measurements of physiological functions in

organisms. This study combines multiple biomarker information from an individual species and, if possible, from multiple populations within the same species, a researcher can effectively form plausible conclusions about the effects of contaminants.

In 1991, the Idaho Department of Fish and Game, in cooperation with the Kootenai Tribe of Idaho, designed a study to determine if sturgeon eggs could successfully hatch in water from the Kootenai River. The eggs did not survive, but after a filtration and ultraviolet sterilization system was installed in 1999, incubation of sturgeon eggs in river water was successful (Ireland 1999).

A pilot contaminant study was also carried out in 1989, 1990 and 1991 to determine residue levels of several organochlorine pesticides, PCBs and metals in a small sample of Kootenai sturgeon testes and ovaries (Apperson 1991). Pesticide residues of DDD, DDE, DDT, endosulfan, toxaphene, chlordane and methoxychlor in Kootenai River white sturgeon ovarian tissue were all above published LD_{50} levels (lethal concentration at 50% mortality) for channel catfish *Ictalurus punctatus* (Johnson and Finley 1980). Detected levels of these pesticides have reportedly adversely affected reproduction in other fish species elsewhere (Johnson and Finley 1980: Jarvinen and Ankley 1999). Copper residues in ovarian tissue were also above levels shown elsewhere (Sorenson 1991) to affect hatching of fish eggs.

The present study employs biomarker research in order to evaluate potential effects of organochlorine, organophosphorous, organonitrate, and carbamate pesticides, polychlorinated biphenyls (PCBs) and metals on Kootenai River white sturgeon reproduction. The objectives of this study are: 1) Determine if bioaccumulated contaminants affect reproductive processes in adult sturgeon; 2) Determine contaminant uptake in developing embryos exposed to contaminants in Kootenai River sediment, suspended solids and water; 3) Assess contaminant residue bioaccumulation and its effects on physiological processes in juvenile sturgeon. The direct effects of bioaccumulated contaminant residues in adult sturgeon are discussed in chapter 1. Chapters 2 and 3 address the effects on embryonic and juvenile sturgeon.

Review of contaminant effects and biomarker research

Chemical compounds can enter into aquatic systems via application, drift or runoff from natural or disturbed areas (Spacie et al. 1995). The presence of one or more contaminants in an aquatic environment does not by itself constitute a hazard; rather, the bioavailability to aquatic organisms determines any impact they may have (Anderson et al. 1987). Factors that influence bioavailability of chemical compounds to organisms include: food chain location, biology and life stage, exposure duration, dose, route of exposure, water and sediment chemistry, and physical characteristics of the chemical compound (Heath 1995; Spacie et al. 1995).

Sediments and suspended solids can sequester and substantially reduce the bioavailability of many chemical compounds (McCarthy et al. 1991). Although some chemical compounds possess high sorption coefficients, many bound contaminants are capable of de-sorption over time and may be released back into the water column or to sediment-dwelling organisms at rates sufficient to exert adverse effects on resident biota (Birge et al. 1987). Bioavailable residues may be sequestered by sediments or suspended solid material, deposited on bottom sediment or taken up by organisms, where they are either detoxified or bioaccumulated. Bioaccumulated contaminants can be stored within liver, kidney, muscle, fat, ovaries, testes and other body tissues until further redistribution, depuration or metabolism (Heath 1995). Depending on specific chemical properties, these compounds can be reduced or altered over time (Spacie et al. 1995).

Bioaccumulated contaminants can cause a range of lethal and sublethal impacts, which can be expressed at the individual or population level (Heath 1995). Effects are manifested in embryonic, juvenile and adult fish through one or more of the following responses: death, stunted growth, physical deformities, immunosuppression or increased susceptibility to viral and bacterial diseases (Arkoosh et al. 1998), altered behavior (Saunders 1969), disruption of reproductive processes (Arcand-Hoy and Benson 1998), yolk sac deformities or delayed yolk absorption (Heath 1995). Frequently, contaminant concentrations in aquatic media (water, sediment, suspended solids) are not directly lethal but,

as Doudoroff and Katz (1957) pointed out, the ability to merely survive is not a reliable indication that a media is satisfactory.

In some instances, chemical exposure can increase resistance and tolerance to metal pollution (Sorenson 1991); however, fluctuating or constant high-level exposure can disrupt the processes that lead to resistance or tolerance (Rosenthal and Alderdice 1976; Stubblefield et al. 1999). The number and degree of additional stressors within a system can determine aquatic species' ability to detoxify or metabolize contaminants or to tolerate the effects of chemical insults (Adams et al. 1989).

A large volume of research data from contaminant studies on fish indicates that expression of effects is dependent upon life-stage (Skidmore 1965; Rosenthal and Alderdice 1976; McKim 1977; Heath 1995). For example, in adult fish, sublethal effects on reproduction or behavior may be expressed more than direct mortality because tolerance may be higher in adult fish than in younger fish. This region of sublethal effects lies between the zones of tolerance and resistance, where survival potential of an organism is reduced, indirectly at a later stage, as a consequence of stress exposure during earlier life stages or generations (Rosenthal and Alderdice 1976). Exposure to contaminants at levels considered sublethal in adult fish may result in direct mortality of younger fish. In juvenile fish, sublethal effects from contaminant exposure may be evident in

pathological changes or in changes to learned and inherent behavior (Heath 1995).

Some contaminants may effect the function of the endocrine system (Peterle 1991). The endocrine system regulates hormone-dependent physiologic functions that are necessary for survival and perpetuation of an organism. Chemicals that impact endocrine processes are called endocrine-disruptor (Ward 1998). These particular chemicals are capable of eliciting or inhibiting responses typically induced by hormonal or steroidal activities (Gillesby and Zacharewski 1998). Endocrine-disrupting compounds are generally lipophilic. These compounds are stored in fatty tissue and as a result, fish with higher body fat content can potentially store greater amounts of potential endocrine disrupting compounds than can fish with low body fat content (Peterson and Guiney 1979). Endocrine-disrupting agents may also interfere with production, release, transport, metabolism, binding, or elimination of the natural hormones and steroids (Peterle 1991; Arcand-Hoy and Benson 1998; Rolland et al. 1997). Some hormones and steriods are responsible for timing and rate of development in embryos, fry, juveniles, and adults. If production of hormones and steroids is disrupted, these processes can be effectively altered (Roberts et al. 1978).

The organochlorine compounds dimethyl-diphenyl-trichlorethane (DDT), polychlorinated biphenyls (PCBs) and their metabolites are classified as potential endocrine-disrupting contaminants (Gillesby and Zacharewski 1998). These

compounds are capable of potentially eliciting estrogenic activity, inducing gene transcription and sending incorrect messages to other organs. In addition to organochlorines, metals and some classes of pesticides may also affect reproduction in fish (Rolland et al. 1997). For example, organophosphate compounds strongly influence activity of brain enzymes such as acetylcholinesterase (AChE), a precursor to reproductive hormone and steroid production and regulation (Peterle 1991). Singh and Singh (1987) found that sublethal concentrations of the pesticides aldrin (0.017-0.85 ppm), malathion (2-4 ppm) and BHC (3-4 ppm hexachlorocyclohexane) in water significantly decreased reproductive hormone levels in air sac catfish (*Heteropneustes fossilis*). Kumar and Pant (1984) found that lead, copper and zinc caused reabsorption of oocytes from the ovaries of an Indian teleost fish. Reabsorption of oocytes indicates potential hormonal disruption within the endocrine system. However, without measuring endocrine and hormone function in these fish during the exposure period it is not possible to determine if contaminant exposure caused endocrine disruption and reabsorption of the eggs.

The effect of exposure and sensitivity of an embryo to a chemical compound can vary with developmental stage at the time of exposure. The effects of toxicant burden may be especially critical at the earliest life stages (McKim 1977). For example, in a study using several species of sturgeon, Lukyanenko (1980) found that resistance to organic and inorganic toxicants varied with embryonic age and the least tolerant phase occurred during early

gastrulation (the earliest phase of embryonic development). Birge et al. (1987), suggested that a deficiency of adipose tissue for sequestration of lipophilic contaminants during the early life stages may increase the vulnerability of embryonic and larval fish to contaminants.

Fertilized eggs may be highly sensitive to compounds that interfere with intermediary metabolism and energy production in the developing embryo (Rosenthal and Alderdice 1976). Some chemicals are passed on to the embryos through parental contribution and some are able to permeate the chorion during and after completion of the water hardening process (period after fertilization when the eggshell hardens and water is taken into the egg). Ankley et al. (1991) provided evidence that maternally transferred PCBs reduced hatching success in chinook salmon. Birge et al. (1987) stated that fish embryos and larvae accumulate high chemical residues from the environment over a relatively short period of time. Holcombe et al. (1976) showed that developing brook trout eggs readily accumulated lead from the rearing environment.

Exposure to certain contaminants during embryonic development may irreversibly influence responsive tissues as well as subsequent growth (Gillesby and Zacharewski 1998). Metal absorption can also affect water uptake during the water hardening process in fertilized eggs by binding to the mucopolysaccharide egg coating and altering permeability of the egg (Rosenthal and Alderdice 1976: Eddy and Talbot 1983). Eddy and Talbot (1983) found that

formation of the perivitelline fluid (water hardening) in fertilized salmon eggs was almost completely inhibited by the presence of sulfate and calcium as well as divalent metal ions such as zinc and magnesium.

Detlaff et al. (1993) found specific evidence indicating that sturgeon eggs and embryos are sensitive to several environmental contaminants, with some metals known to be toxic at very low concentrations. After fertilization, sturgeon eggs become adhesive and remain so during the water-hardening process, which may last for 1-3 hours (Conte et al. 1988). The adhesiveness of the eggs serves in attachment to rocky or cobble substrate, where eggs can be camouflaged and protected from predation or suffocation by shifting sediment. During this stage, suspended solids and sediment are also capable of adhering to the eggs, creating a potential source of contamination for the developing embryos.

Impacts of sorbed contaminants on fertilized eggs, embryos and larvae include mortality, delayed development and deformities. For example, Lemly (1993) found that selenium transferred in eggs from parents to the offspring caused edema, hemorrhaging, spinal deformities and death in several species of freshwater fish. Significant mortality also occurred in rainbow trout (*Oncorhynchus mykiss*) eggs that contained concentrations of methylmercury from 0.07 to 0.10 ppm (Heath 1987). Hazel and Meith (1970) found that water copper concentrations at 0.1-0.3 ppm decreased hatchability of king salmon

(*Oncorhynchus tshawytscha*) eggs. Crawford and Guarino (1976) found that when compared to controls, DDT treated eggs from killifish (*Fundulus* sp.) displayed marked reduction in early development rates. Gillespie and Baumann (1986) noted that offspring from female bluegills (*Lepomis macrochirus*) containing whole-body selenium concentrations of 16-18 ppm (30-38 ppm in ovaries) did not even survive to swim-up stage. In addition, Halter and Johnson (1974) found that PCB exposure resulted in early hatching of coho salmon (*Oncorhynchus kisutch*) eggs. The greatest impacts of early hatching include higher mortality and smaller larvae that are not yet physiologically capable of survival under sub-optimal or stressful conditions.

In addition to decreased survival and poor development of embryos, exposure to organochlorine compounds has also been related to long-term genetic and organ damage (Shugart 1990; Heath 1995). For example, contaminant exposure has been related to cross-linked or broken DNA strand mutations (Lloyd and Phillips 1998). Physiological function of organs can be disrupted if contaminants mimic or block proteins, enzymes and other chemicals necessary for cellular function. Although many of the techniques are relatively new to fisheries research, several methods are currently available to clarify the relationship between exposure to chemical compounds and effect on genetic expression and organ function.

Laboratory bioassays and other conventional laboratory methods of assessing contaminant effects on aquatic organisms generally lack ecological realism because of the many environmental factors that can influence stress responses at all levels of biological organization within the natural environment (Adams 1990). Historically, contaminant effects on aquatic systems have been assessed using primarily laboratory-simulated chemical tests on adult model or surrogate fish species (Peterle 1991). Endocrine processes in early life stages may be more sensitive to lower doses of certain chemicals than are their adult counterparts (Ward 1998). Therefore, the validity and relevance of traditional high-dose testing with adult animals is questionable in relation to effects on the endocrine system in early life stages. In the field it is also difficult to identify cause and effect because there are frequently a multitude of mutually correlated environmental factors which may be contributing to physiological alterations in aquatic organisms. Correlation analysis can be used to assess relationships between contaminants and physiological function. This type of analysis is also useful for indicating potential effects of contaminants on organisms; however, without direct measures of the effects, significant correlations do not indicate definite cause and effect. Repeatability of results can increase the validity of biomarker research and make it a valuable tool for assessing environmental effects of contaminants.

In cases of moderate contamination, it is very difficult to separate effects due to natural factors from those resulting from contaminant exposure (Chapman

et al. 1998). The multiple environmental and physiological factors affecting actions of contaminants within organisms have also made it difficult to interpret the meaning of tissue residue data. The recent focus on tissue-residue-based testing has made an attempt to address sublethal effects of individual contaminants or chemical mixtures from various matricies on specific target tissues (Jarvinen and Ankley 1999). This approach has assisted in the interpretation of chemical uptake, target organ bioaccumulation and sublethal effects on reproduction and growth. However, as with conventional high-dose testing, tissue-residue-based testing does not incorporate variability found in the natural environment.

One method of determining subtle physiological effects in the presence of environmental variability is through use of in-situ analysis of species-specific physiological bioindicators or biomarkers (Adams 1990). Mutual correlations among contaminants, compounded effects, spacial variability and tolerance differences among species make these biomarkers difficult to interpret independently. Therefore, biomarker research requires assessment of a suite of selected stress responses at several levels of organization in order to assess relationships between different classes of contaminants and physiological responses in organisms (Donaldson 1990; Cormier and Racine 1992; Travis 1993; Fossi and Leonzio 1994). Several examples of nonlethal biomarker research include: measurement of contaminant residues in biopsied tissue (Javinen and Ankley 1999), evaluation of blood plasma proteins and hormones

(Ward 1998), DNA adduct analysis for genetic deformities (Shugart 1990), monitoring of fecundity, fertilization and development rates (Ankley et al. 1998), and measurement of correlations among these factors and or contaminant residues in the surrounding environment. Although many of the methods used in biomarker research on fish are in their infancy, techniques are presently being developed and standardized at a fast enough pace that this type of research will likely soon become common-place in fisheries science.

Geography, Geology, Land Use, Contaminant Sources and Species Composition Within the Kootenai River Watershed

The Kootenai River basin is a transboundary river system, originating near Mt. Assiniboine in British Columbia, Canada (Figure 1; Kootenai River Network 2000). The river flows southeast for 247 km into Montana, USA, and into the 27 km-long Koocanusa Reservoir, impounded behind Libby Dam. From Libby Dam, the river flows westward another 106 km, into Idaho. It flows north at Bonners Ferry, toward British Columbia, Canada and into Kootenay Lake, 126 km from the Idaho/Montana border. The Kootenai River is the second largest tributary to the Columbia River in terms of runoff volume and the third largest in terms of watershed area, draining approximately 46,000 km^2 (Knudson 1994; Richards

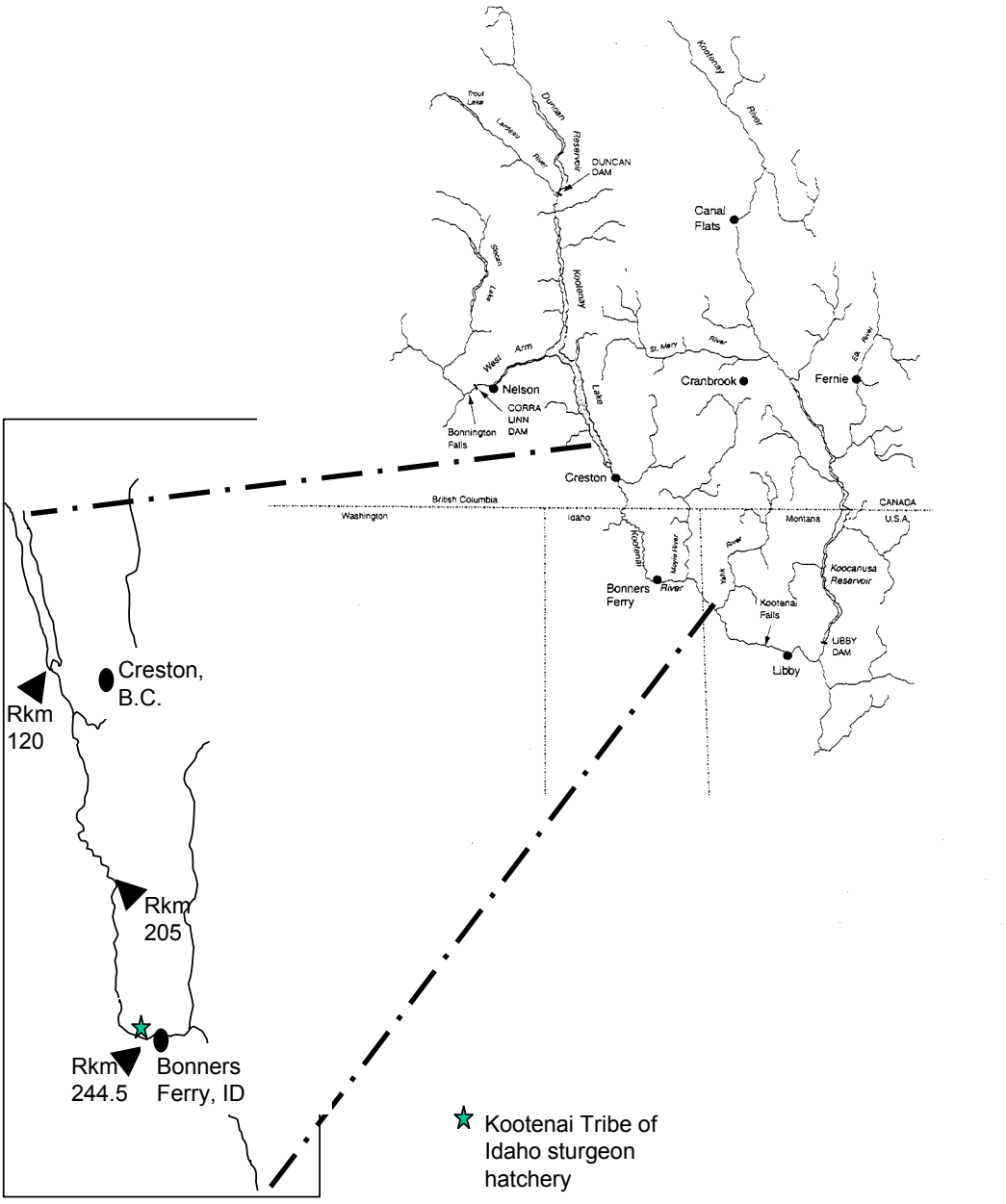

Figure 1. Study site map of the Kootenai River basin.

1997). It descends approximately 3,000 m in elevation along its approximate 506-km length.

The Kootenai River basin undergoes substantial changes in terrain, geology and channel morphology from the headwaters to Kootenay Lake. Movement of materials and changes in geologic formations throughout the entire basin are predominantly affected by the definite seasonal rainfall patterns, snow accumulation, redistribution of soil materials and evaporation of soil moisture.

Terrain in the upper part of the basin between Mt. Assiniboine and Libby Dam consists of steep, rocky canyon walls (Snyder and Minshall 1996). With the exception of Lake Koocanusa, which is dominated by mud and silt substrate, substrate types in the upper river basin consist of large boulder, rock and cobble. In the middle river section between Libby Dam and Bonners Ferry, the river gradient levels out and the terrain transforms from steep canyon walls into braided stream channels. Dominant substrate types in this section consist of boulder and cobble. A majority of the land surrounding the upper and middle sections of the basin is forested. A 10-km stretch of land within the lower part of the middle section, between Crossport and Bonners Ferry, Idaho, also consists of intermittent agricultural land.

Below Bonners Ferry, the lower river section becomes a relatively deep and steep-banked meandering channel as it flows through the Purcell Trench

floodplain (Chugg and Fossberg 1980). Dominant substrate types in this area consist of sand and mud with intermittent and sparse cobble beds. Historically, the floodplain possessed marsh-type vegetation consisting of scattered trees and brush on higher parts of the landscape (Chugg and Fossberg 1980). Most of the floodplain between Bonners Ferry and Kootenay Lake has been converted to agricultural use. Terrestrial and aquatic sediment types within the floodplain system consist of deep alluvial soils that are readily transported or stored by natural and manipulated water flow patterns.

The Kootenai basin has undergone many physical changes in the past century, many of which are related to industrial, agricultural and urban development. Past and present land uses in the Kootenai River basin include hydroelectric power generation, mining and mineral extraction (coal, placer, and vermiculite), logging, lumber and pulp production, agriculture (grain and livestock production), urban development, recreation and transportation (Kootenai River Network 2000). All of these activities have, to some degree, contributed to contaminant loading within the basin.

Construction of Libby Dam and impoundment of Koocanusa Reservoir have potentially affected many components of the aquatic ecosystem, including contaminant levels within the lower Kootenai River. Reservoirs often create a settlement and storage area for metal and organochlorine compounds (Birge et al. 1987; Oak Ridge National Laboratory 1999). However, low oxygen levels in

deeper and colder portions of a reservoir may also enhance release of metal compounds from sediments and bedrock. Downstream from Libby Dam, hydroelectric power generation affects contaminant loading, redistribution and bioavailability through flushing, erosion and alteration of physical habitat (US Environmental Protection Agency 1999). Operation of mechanical and electrical equipment at Libby Dam is also a potential source for PCBs and other contaminants (Columbia Rivers United 1997).

Mining operations have been a part of the Kootenai River basin since the late 1800's (Georgi 1993). Some of the more prominent mining operations within the Kootenai River basin include Elkview, Fording River, Line Creek, Greenhills, Coal Mountain, North Star, Sullivan, St. Eugene, Placid Oil, Estella and King mines in British Columbia, the Sylvanite, Keeler, Goldflint, Keystone, ASARCO and W.R. Grace mines in Montana, and the Blue Joe Creek, Chief Joseph, Boulder Creek and Northern Star mines in Idaho (Kootenai River Network 2000; US Geological Survey 1999). Mining operations within Idaho produce primarily lead, zinc, copper, and silver, but also some gold, iron, nickel, cobalt, sulfur, thorium and uranium. The number of abandoned mines in the entire Kootenai River watershed is estimated at 10,000 (Kootenai River Network 2000). Large 'tailings dumps' are potentially substantial sources of metal pollution (Weatherley et al. 1980) because of their mechanical instability and surface slippage. Of 123 mines in Boundary County, Idaho, 54 (44%) are listed as 'status unknown' with regard to geologic stability (US Geological Survey

1999). The discharge and tailings piles at many of the abandoned mines are not monitored; some of them may be contributing significant amounts of heavy metal pollution to the Kootenai River system. The Cominco fertilizer plant was also operated from 1953 to 1987, at the Sullivan mine site, along the St. Mary River in British Columbia (a tributary to the Kootenai River). This fertilizer plant is considered to have been a significant point source for phosphorous loading within the Kootenai River (Kootenai River Network 2000).

Logging, lumber and pulp mill operations are a key economic component within the Kootenai River basin (Kootenai River Network 2000). Three of the most prominent forestry operations are Crestbrook Forest Industries pulp mill in Skookumchuck, British Columbia, Stimson Lumber Company and Champion International Inc. of Libby, Montana. Periodic monitoring indicates that logging and road building activities increase sediment loading in the streams and rivers (Kootenai River Network 2000). Pulp processing operations also typically result in discharge of toxins, such as chlorophenols and dioxins, which have been shown elsewhere to affect reproduction, growth and development in aquatic species (Ramamoorthy and Ramamoorthy 1997).

Agricultural operations within the lower watershed are another potential non-point source of contamination (Wilcove et al 1998; Tucker and Burton 1999). The primary agricultural area is between Bonners Ferry, Idaho and Kootenay Lake in British Columbia. The rich floodplain soils are used for growing grain,

fruit, forage and hops. A large portion of the land is also used for grazing cattle. Some of the effects of these agricultural operations include disturbance of riparian zones, increased and artificial loading of nutrients and suspended solids, pesticide and metal loading from crop applications, and runoff of improperly disposed chemicals. Much of the lower Kootenai River floodplain has also been diked in order to drain bottomlands for farming (Redwing Naturalists 1996). Diking has retained the river into a meandering trench and prevented suspended solids from distributing out onto the floodplain. The increase in suspended solids has potentially changed the physical substrate type within the main channel of the lower river from mixed rock, cobble and sand to primarily sand and mud (Duke et al. 1999). Channelization has also potentially impacted water quality, system productivity, spawning and rearing habitat, and contaminant bioavailability (Steiger et al. 1998).

Urban development, recreation, and transportation contribute contaminants to the Kootenai system through gas and oil discharge from boat motor exhaust, drainage ditch runoff, municipal discharge from treatment plants and accidental spillage (Kootenai River Network 2000). These activities are potential sources of hydrocarbons, metals, suspended solids, hormones and various other contaminants (Heath 1995).

Several municipalities' discharge treated wastewater effluent directly into the Kootenai River; these municipalities include Cranbrook, Kimberly, Fernie and

Creston in British Columbia; Libby, Troy and Eureka in Montana; and Bonners Ferry in Idaho. Only the Cranbrook operation uses secondary treatment; the other municipalities use septic tank or primary treatment facilities. Municipalities within the United States are required to operate under an National Pollutant Discharge Elimination System (NPDES) permit, issued by the US Environmental Protection Agency (USEPA; National Environmental Policy Act 42 U.S.C. 4321 *et seq*). Although this requirement is in place, updating of the permits to allow for improved standards is not always followed. For example, as of June 1999, the city of Bonners Ferry was still operating on an expired NPDES permit (Clean Water Act 33 U.S.C. 1251 *et seq.*) that was issued in 1991 (T. Davidson Personal Communication, US Fish and Wildlife Service, Spokane, Washington).

In addition to municipal discharge, recreation and transportation by boat, railroad and highway can also impact water quality. Specifically, fuels, lubricants and other chemicals associated with or transported by boats, rail cars and motor vehicles can be contributed to the aquatic system directly or indirectly through overland runoff. These chemicals can cause lethal or sublethal effects on aquatic organisms.

Little information has been collected about the contribution of contaminants from urban discharge, transportation and recreation; their specific effects on the Kootenai River system are largely unknown. Water quality standards in the Kootenai River basin are governed by USEPA and British

Columbia, Canada regulations (Knudson 1994). Other than data from recent monitoring efforts by the British Columbia Ministry of the Environment (Webber 1996) and the Kootenai Tribe of Idaho (Bauer 1999), little information is available about the actual contaminant residues in water, soil and organisms and their effects on aquatic biota. Most of the existing water quality information consists of data on temperature, pH, dissolved oxygen and suspended solids, along with residue data for several metals. Some information is also available on aquatic invertebrate diversity and sediment quality (K. Apperson Personal Communication, Idaho Department of Fish and Game, McCall, Idaho; Bauer 1999; Richards 1998). Recent data collection and modeling for the USEPA's Total Maximum Daily Load (TMDL) process has provided preliminary information pertaining to water quality for tributaries in the Idaho and Montana portions of the Kootenai River basin (State of Idaho Department of Environmental Quality 1998). The primary purpose of the TMDL process is not to classify large rivers, but rather to develop a list of streams that are impaired or threatened beyond minimum criteria for bull trout (*Salvelinus confluentus*) habitat. Therefore, the TMDL data is only indirectly applicable to the main stem of the Kootenai River. Pending the availability of funding sources, the Kootenai River Network and affiliated stakeholders are planning to address aquatic habitat quality issues along the main stem through a comprehensive water, sediment and biota monitoring program (Kootenai River Network 2000).

The Kootenai River drainage has historically supported a cold-water fish fauna consisting of bull trout, brook trout (*Salvelinus fontinalis*), kokanee (*Oncorhynchus nerka*), westslope cutthroat trout (*Salmo clarki spp*.), redband rainbow trout (*Oncorhynchus mykiss spp*.), mountain whitefish (*Prosopium williamsoni*), brown bullhead (*Ictalurus nebulosus*), yellow perch (*Perca flavescens*), pumpkinseed (*Lepomis gibbosus),* largemouth bass *(Micropterus salmoides)*, black crappie (*Pomoxis nigromaculatus*), lake chub (*Couesius plumbeus*), peamouth (*Mylocheilus caurinus),* northern squawfish *(Ptychocheilus oregonensis),* longnose dace (*Rhinichthys cataractae*), slimy sculpin (*Cottus cognatus*) torrent sculpin (*Cottus rhotheus*), redside shiner (*Richardsonius balteatus*), largescale sucker (*Catostomus macrocheilus*), longnose sucker (*Catostomus catostomus*), burbot (*Lota lota*) and white sturgeon (Partridge 1983). The white sturgeon was listed as an endangered species in 1994 (US Fish and Wildlife Service 1994). The bull trout was listed by the US Fish and Wildlife Service as a threatened species in 1998. As of 1999, the burbot is a species of special concern for which special interest groups are initiating the listing process.

Study area

The historic distribution of white sturgeon in the Kootenai River drainage includes the main stem river between Kootenai Falls in Montana and Kootenay Lake in British Columbia, Canada (Figure 1). Although sturgeon have been

reported in the river reach between Bonners Ferry and Kootenai Falls, Graham (1981) estimated that only one to five adult sturgeon regularly resided in this vicinity. For the present study, sample sites were located between river kilometers 244.5 (Ambush Rock) and 205 (Ferry Island). These sites were selected because they were within the white sturgeon spawning and pre-spawning staging areas and because the largest range of life stages in the white sturgeon were most likely to be exposed to contaminants within this 39.5-km area. Several ovarian tissue samples were also collected from adult sturgeon captured at the confluence of the Kootenai River and the south arm of Kootenay Lake.

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CHAPTER ONE:

Effects of Environmental Contaminants on Reproduction in White Sturgeon

Abstract

This study was designed to determine if bioaccumulated contaminants (organochlorine, organophosphate, organonitrate, and carbamate pesticides, metals and Aroclor PCBs) potentially affect sturgeon reproduction of the Kootenai River white sturgeon, *Acipenser transmontanus*. Successful reproduction in these fish depends on physiological processes that may be disrupted by actions of bioaccumulated contaminants. Ovarian tissue, blood plasma and eggs were collected from female sturgeon to evaluate correlations between steroid levels (testosterone, 11-ketotestosterone and estradiol) and tissue residues or egg size as well as changes in bioaccumulation over the past 10 years. Water and sediment samples were also collected to determine baseline environmental residue levels. Organophosphate, organonitrate or carbamate pesticides did not bioaccumulate in adult sturgeon oogonia during early spring. Several metal and organochlorine compounds were detected in ovarian tissue, eggs, sediment and water. Zinc, DDT and Aroclor 1260 are bioaccumulated from the aquatic environment at levels that could potentially affect hormone production in adult sturgeon. Zinc concentrations were significantly higher in recently collected ovarian tissue samples than in previously collected samples (Mann-Whitney U test; $P = 0.001$), suggesting an increase in bioavailable zinc. Lead, copper and DDT concentrations were not significantly different between previous and recent sampling periods, indicating no significant change in bioavailability or metabolism of these compounds. Number of eggs

per milliliter correlated with higher zinc (Spearman; r = -0.780) and total organochlorine (Spearman; r = -0.706) concentrations, indicating that higher zinc and total organochlorine concentrations related to larger egg size. Although egg size appears to increase with higher zinc and organochlorine concentrations, the negative impacts on steroid production and embryonic development may offset any advantages of increased egg size. Plasma steroid concentrations in Kootenai River white sturgeon were comparable to ranges found in other populations of sturgeon; however, there were significant correlations between plasma steroids and ovarian tissue concentrations of zinc, Aroclor 1260, DDE, DDT and total organochlorines. Water concentrations of total iron, zinc, manganese and the PCB Aroclor 1260 exceeded suggested environmental background levels and in some cases also exceeded EPA freshwater quality criteria. Sediment concentrations of copper and zinc were not significantly different between previous and recent sampling periods, indicating no significant change in environmental concentrations. Biomarker research merely indicates the potential for effects so it is difficult to determine whether or not these sublethal bioaccumulated concentrations are independently reducing reproductive capabilities in white sturgeon. Therefore, more extensive field and laboratory studies should be designed to attempt to reproduce these findings and to determine if these compounds are actually affecting egg production and survival.

Introduction

Successful reproduction in fish depends on integrated activities of steroids, hormones and proteins in the brain, pituitary gland, liver and gonads (Gillesby and Zacharewski 1998). These endocrine system activities and their products can potentially be inhibited or induced by adult exposure to and bioaccumulation of environmental contaminants. The fatty nature of ovarian tissue lends itself to high bioaccumulation of potential endocrine-disrupting compounds. These compounds can in turn be transported to embryos or redistributed to the adult body during the high-energy use period of oogenesis (Ankley et al. 1991; Heath 1995; Miller 1993). Although body size and lipid content also affect uptake and metabolism for some compounds, age or life span of adults can also affect rate and extent of contaminant bioaccumulation and metabolism (Heath 1995). An older individual of a long-lived species has a longer period of exposure and potential bioaccumulation than a younger individual or a shorter-lived species.

The Kootenai sturgeon is a long-lived, bottom-feeding and relatively sedentary species (Scott and Crossman 1973). These characteristics potentially allow for substantial bioaccumulation and storage of contaminants associated with food chain organisms, water and sediment (Weatherley et al. 1980; Delisle et al. 1975). Kootenai sturgeon become reproductive between 12 and 15 years old and typically reproduce until death. A sturgeon may reproduce for up to 40

years, often at intervals of 2-5 years, potentially producing many successive generations (Detlaff et al. 1993; Doroshov et al. 1997). Although males can spawn yearly, a single cycle of oogenesis in female sturgeon may extend up to five years. During oogenesis, stored fat is the primary energy source for the female and its developing oogonia. Because fat is also a storage area for many lipophilic organochlorine contaminants, redistribution of these stored compounds can occur during oogenesis rendering them bioavailable for incorporation by oogonia and for disruption of endocrine functions in the adult (Heath 1995; Petersen and Kristensen 1998; Ungerer and Thomas 1996). Bioaccumulation and redistribution of contaminants in adult fish may result (directly or indirectly) in genetic alteration, decreased fertility, reduced egg and gonad development, disruption of timing and migration for spawning events, and disruption of the endocrine system and hormonal processes that normally cue these physiological functions (Arcand-Hoy and Benson 1998; Heath 1995).

Plasma steroids and hormones produced within the endocrine system play a major role in reproductive functions. In most fish species, plasma sex steroid concentrations are sexually distinctive and correlated with maturity status (Chieffi and Pierantoni 1987; Cuisset et al. 1994; Doroshov et al. 1997). Therefore, under normal physiological conditions, male, female and immature sturgeon at different stages of sexual development should display different levels of sex steroids. Under stress-induced conditions inherent to contaminant exposure, concentrations of these steroids may be altered in a manner that depends on the

properties of the contaminant (Gillesby and Zacharewski 1998). For example, compounds that induce estrogenic activities may result in feminization of males (Gillesby and Zacharewski 1998). In contrast, exoestrogenic compounds inhibit normal estrogenic activities and so may reduce egg development and production or alter spawning behavior in females(Gillesby and Zacharewski 1998).

Levels of plasma steroids and several other variables can therefore be used to monitor the potential effects of contaminant-associated stress on the endocrine status in reproductive adult fish (Adams 1990; Ankley et al. 1998). Other variables that can be monitored include levels of and changes in environmental and tissue contaminant concentrations, egg size in spawning females, and relationships between these variables. Bioaccumulated contaminants can disrupt physiological functions in the ovaries and they can also affect development rate and size of eggs, which can, in turn, affect development, and growth of resulting embryos (Heath 1995).

The objectives for this portion of the study were: 1) to determine baseline concentrations of selected contaminants in ovarian tissue of mature female sturgeon, 2) to determine baseline plasma steroid concentrations of testosterone, 11-ketotestosterone and 17β-estradiol in Kootenai River white sturgeon at different stages of sexual development, 3) to determine if plasma steroid concentrations in Kootenai River white sturgeon differ from concentrations in other sturgeon species, 4) to determine if egg size and plasma steroid

concentrations correlate with contaminant concentrations in ovarian tissue, 5) to determine if present contaminant concentrations in ovarian tissue are significantly different from those detected in samples collected during a previous sampling effort (1989-1990), and 6) conduct a pilot study to determine baseline environmental contaminant concentrations within water and substrate samples taken from sturgeon spawning and staging areas.

Materials and Methods

Ovarian Tissue

Ovarian tissue samples were collected from 34 adult sturgeon, captured between kilometers 215 and 120. This area extends from Rock Creek in the Kootenai River to its confluence at the south end of Kootenay Lake. During 1997, 1998 and 1999, fish were captured with conventional rod and reel gear and 45.7 m setlines equipped with baited 14/O circle hooks. With the exception of four fish captured during August, 1998, all samples were collected directly prior to spawning, between the months of March and June. Capture location, date, length and weight were recorded for each fish. The 34 ovarian tissue samples were collected from fish representing developmental stages 2-4, ages 18-52, 141-222 cm fork length and weights ranging from 22 to 91.3 kg (Appendix 2).

A five-cm incision was made in the abdominal wall of all sturgeon, approximately 25 cm forward of the pelvic fin, in order to observe gonad tissue to

determine sex and developmental stage of each fish. Developmental stages of male and female sturgeon were classified according to Table 1. Fifteen grams of ovarian tissue was biopsied from all female sturgeon at developmental stages 2, 3 and 4.

All 34 ovarian tissue samples were frozen and stored from 1-6 months until analyzed for organochlorine pesticides, Aroclor 1200 series PCB's, metals (arsenic, cadmium, copper, iron, mercury, lead, selenium and zinc; see Appendix 1 for methods) and percent lipid content. Selection of contaminants for analysis was based on historic data availability as well as presence, application and bioaccumulative potential of contaminants within the lower Kootenai River system. The first 17 samples were also analyzed for organophosphate, organonitrogen and carbamate pesticides (Appendix 1). Because organophosphate, organonitrogen and carbamate pesticides are highly volatile, do not generally bioaccumulate and were not present in the first 17 samples, the analyses for these pesticides were deleted from the remaining samples. Data were tested for normality of distribution with the expression $2(SE)\pm K$ (where SE is the standard error and K is a measure of kurtosis), and homogeneity of variances (Cochran's C-test; Kirk 1995).

Results were compared with available ovarian tissue contaminant burden data collected from Kootenai River white sturgeon in 1989, 1990 and1991 (Comparable analytical methods applied: Apperson and Anders 1991). The null

Table 1. Stage definition of sexual development in white sturgeon (Apperson and Anders 1991).

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

hypothesis was that concentrations of contaminants in ovarian tissue were not significantly different between earlier (1989-91) and later (1997-99) sampling periods (Mann-Whitney U test; $P < 0.05$). Data points were rank transformed and analyzed with the non-parametric Mann-Whitney U test because of non-normal distribution and heterogeneity of variances,

In 1997, 1998 and 1999, mature stage-4 eggs (Table 1) were taken during spawning from female sturgeon used as broodstock at the Kootenai Tribal sturgeon hatchery. Egg size was measured as number of eggs per ml. Eggs were also tested for organochlorine, organophosphate, organonitrogen and carbamate pesticides, Aroclor 1200 series PCBs and the eight metals (Appendix 1). The Spearman test was applied to rank transformed data to test the null hypothesis that contaminant concentrations and size of eggs were not significantly correlated ($\alpha = 0.05$).

Plasma Steroids

For plasma steroid analysis, blood samples were drawn from 46 sturgeon (19 mature males, 22 mature females and 5 immature and non-reproductive sturgeon of unknown sex) captured during March and April of 1998 and 1999, two to three months prior to spawning. Sampling sites were located between river kilometers 205 and 215. Captured fish ranged from 102 to 222 cm fork length, 8.1 to 80.25 kg weight and 30 to 49 years old (Appendix 2). Blood was

drawn from the ventral caudal vein into heparanized vaccutainers. Samples were either centrifuged or settled in a refrigerator to separate plasma from the red blood cells. Plasma was frozen and shipped to Oregon State University for analysis of testosterone, 11-ketotestosterone and 17β-estradiol by competitive binding radioimmunoassay methods (RIA: Fitzpatrick et al. 1986). Plasma aliquots were extracted with diethyl ether, the dried extracts reconstituted in assay buffer, and then portions of reconstitute were incubated with the appropriate tritium labeled steroid in the presence of the polyclonal antisera specific to that steroid. Bound and free steroids were separated by centrifugation after incubation with dextran charcoal, and an aliquot was decanted for scintillation spectrophotometry. Concentrations of steroids were calculated for the extracts using a linear regression of counts versus known concentrations of steroid from a standard curve. Plasma steroid concentrations (ng/ml$\pm$standard error) were measured in duplicate to estimate concentrations of testosterone, 11-ketotestosterone and 17β -estradiol.

Data were tested for normality of distribution with the expression 2(SE)$\pm$K (where SE is the standard error and K is a measure of kurtosis), and also tested for homogeneity of variances (Cochran's C-test; Kirk 1995). Data did not display a normal distribution or homogeneity of variances so the nonparametric Kruskal-Wallace and Mann-Whitney U-tests were applied to the rank transformed data to test the null hypotheses that plasma steroid concentrations were not significantly different ($P < 0.05$) between fish of different sex, developmental stage, capture

location, capture date and age. The null hypotheses that plasma steroid concentrations and ovarian tissue contaminant burden in vitellogenic female sturgeon were not related were tested with Spearman rank correlation analyses ($P < 0.05$). Ranges of plasma steroid concentrations in Kootenai River white sturgeon were compared with data for wild Columbia River and hatchery-reared stocks of white sturgeon (*Acipenser transmontanus*) as well as Siberian (*Acipenser baerii*) and Atlantic (*Acipenser oxyrinchus*) sturgeon (Chapman 1989; Cuisset et al. 1994; Doroshov et al. 1997; Fitzpatrick et al. 1996; Webb et al. 1999). Although all of the wild sturgeon populations used for comparison have potentially been exposed to varying types and concentrations of contaminants, values were compared for similarities among fish at similar stages of maturity. The null hypothesis that the ratio of estradiol:11-ketotestosterone was not significantly different between males and female Kootenai River white sturgeon was also tested (Mann-Whitney U test; $P < 0.05$).

Water and Sediment

Surface water samples and river-bottom substrate samples were collected at eight sites in the lower Kootenai River between river kilometers 205 and 244.5, during mid-June, 1999. Water samples were drawn within 1.5 m of the surface, using a LaMotte sampler. A Ponar Dredge was used to collect a single sediment sample from the top 15 cm of substrate at each site. Dredge samples from each site were sub-divided into five subsamples for individual contaminant analyses

(Appendix 1). Using a wooden spoon, sub-samples were placed in certified glass jars and chilled at 4^{o}C until shipped later the same day to the lab for analysis. Samples were analyzed for pH, temperature, conductivity, turbidity and dissolved oxygen, sediment total organic carbon, metals, Aroclor 1200 series PCBs, and organochlorine, organophosphate, organonitrate and carbamate pesticides (Appendix 1). Data were tested for normality of distribution with the expression 2(SE)$\pm$K (where SE is the standard error and K is a measure of kurtosis), and homogeneity of variances (Cochran's C-test; Kirk 1995). Data did not display normal distributions or homogeneity of variances so they were rank transformed for non-parametric statistical analyses. Data were available for copper and zinc concentrations during earlier (1989-91) and later (1999) sampling periods so the non-parametric Mann-Whitney U test ($P < 0.05$) was used to test the null hypothesis that copper and zinc concentrations in sediment had not changed significantly.

Results

Ovarian Tissue

Ovarian tissue contained detectable concentrations of the metals arsenic, cadmium, copper, iron, lead, selenium, and zinc (Table 2). There were also detectable levels of the organochlorines DDE, DDT, dieldrin, aldrin and the PCB Aroclor 1260 (Table 3). Organophosphate, organonitrate and carbamate

Table 2. Total number of samples, number and percent of total samples with detectable residues, range and mean (±S.D) concentrations of metals detected in Kootenai River white sturgeon ovarian tissue, collected 1997-1999. (n=33)

Contaminant	Number of samples (% of total)	Concentration (ppm)	
		Range	Mean± S.D.
Arsenic	17 (52)	0.09 – 1.20	0.31 ± 0.29
Cadmium	19 (58)	0.001 – 0.94	0.06 ± 0.21
Copper	30 (91)	0.58 – 6.90	2.39 ± 1.56
Iron	33 (100)	15.0 – 56.0	28.0 ± 9.94
Lead	18 (55)	0.05 – 0.88	0.18 ± 0.20
Selenium	33 (100)	0.55 – 12.0	1.76 ± 2.02
Zinc	33 (100)	19.0 – 170	37.0 ± 29.1

Table 3. Total number of samples, number and percent of total samples with detectable residues, range and mean (±S.D.) concentrations of lipids and organochlorine compounds detected in Kootenai River white sturgeon ovarian tissue, collected 1997-1999. (n=34)

Contaminant	Number of samples (% of total)	Concentration (ppb)	
		Range	Mean± S.D.
DDE	34 (100)	48.0 – 1800	296 ± 372
DDT	12 (35)	22.0 – 88.0	44.3 ± 18.4
Aroclor 1260	25 (74)	160 – 1300	460 ± 288
Dieldrin	1 (3)	49.0	---
Aldrin	5 (15)	53.0 – 98.0	77.0 ± 16.8
Lipids (%)	33 (97)	2.30 – 23.7	9.7±4.67

pesticides were not detected in ovarian tissue above method detection limits (Appendix 1).

The null hypothesis stating that ovarian tissue contaminant concentrations were not significantly different between previous and present sampling periods was rejected for zinc (Figure 1). Zinc (Mann-Whitney U test; P = 0.001) concentrations were significantly higher in samples collected between 1997 and 1999 than in samples collected between 1989 and 1990 (Appendix 3, Table 2). Copper, lead, and DDE concentrations were not significantly different between the two periods.

The null hypothesis that egg size and tissue concentrations of metals and organochlorine compounds were not significantly correlated was rejected for zinc (Spearman; r = -0.780) and total organochlorines (Spearman; r = -0.706; Figure 2). The relationship between egg size and total organochlorine concentrations appeared to be driven by one data point. When this outlier (point greater than 2 standard deviations from the mean) was removed from the analysis, there was not a significant relationship between egg size and ovarian tissue concentrations of total organochlorines. For nine large female sturgeon, (161-201 cm fork length and 37-80 kg weight) analyzed for contaminant concentrations and egg size (Appendix 2), larger egg size (ie. fewer eggs per ml) showed a significant inverse correlation with increasing zinc (Spearman; r = -0.780) and total organochlorine (Spearman; r = -0.706) concentrations (Table 4). Eggs from eight of the nine

Figure 1. Comparison of zinc (a), lead (b) and copper (c) concentrations in Kootenai River white sturgeon ovarian tissue collected during two sampling periods (1989-1991 and 1997-1999; Mann-Whitney U test; $P < 0.05$). Error bars indicate standard deviation from the mean. Outliers are indicated with an asterisk or open circle.

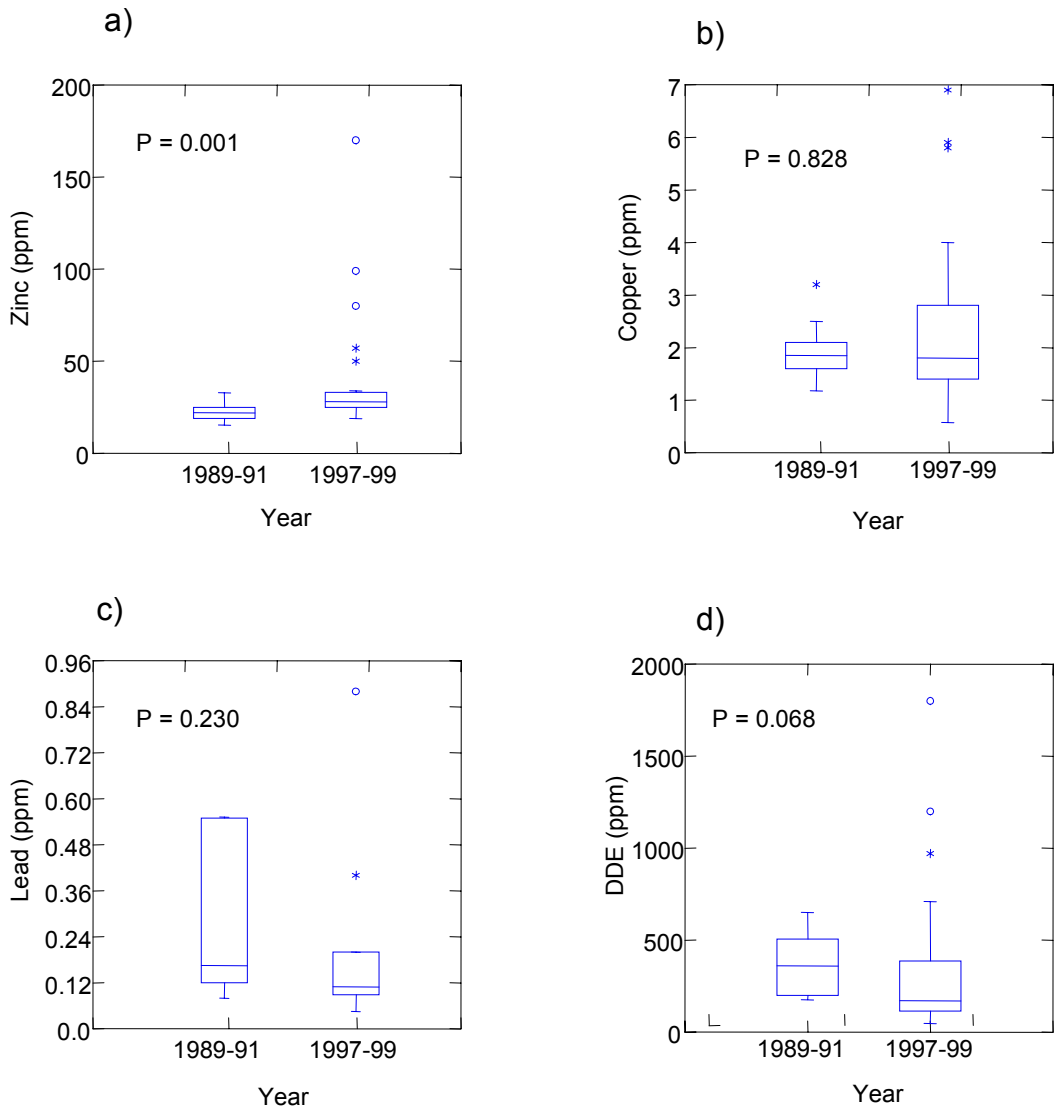


Figure 2. Correlations between number of eggs per milliliter and zinc (a) and total organochlorines (b) detected in Kootenai River white sturgeon ovarian tissue, 1997-1999 (Spearman test; α=0.05). All females were used for conservation aquaculture at the Kootenai Tribal sturgeon hatchery. Large dot indicates points that are greater than 2 standard deviations from the mean and strongly influence the r value. Brackets indicate 95% confidence intervals.

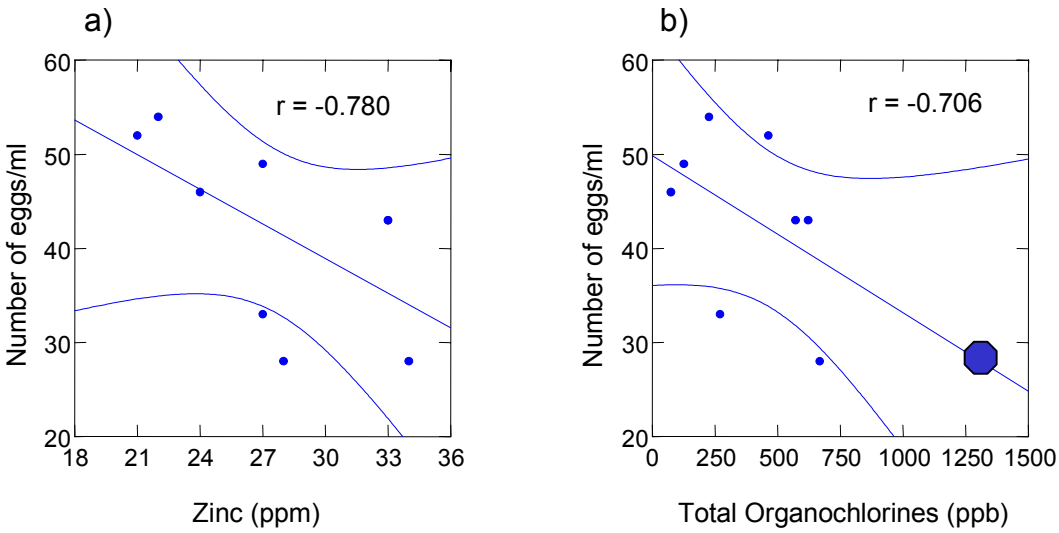


Table 4. Spearman Rank correlations (r value) between plasma steroid biomarkers 17∃-estradiol (E2), 11-ketotestosterone (11-kt),Testosterone (T) and contaminants for vitellogenic female Kootenai River white sturgeon. n = 13

Contaminant	Correlation coefficients (r)				
	E2	11-kt	T	E2:11-kt ratio	Number of eggs/ml
Arsenic	0.607	-0.607	-0.536	0.750	0.500
Cadmium	0.024	-0.381	-0.429	0.210	0.493
Copper	0.446	0.260	-0.042	-0.100	0.545
Iron	-0.474	-0.438	-0.349	0.110	-0.154
Lead	0.443	0.299	0.084	-0.036	0.841
Selenium	0.308	-0.044	-0.055	0.290	0.650
Zinc	-0.432	-0.652*	-0.536	0.338	-0.780*
DDE	0.377	-0.550	-0.407	0.611*	-0.513
DDT	0.893*	-0.286	-0.000	0.464	-0.655
Aroclor 1260	-0.041	-0.820*	0.729*	0.733*	0.000
Total organochlorines	0.258	-0.753*	-0.500	0.791*	-0.706*

* indicates significant correlations (α = .05)

females contained zinc and eggs from all nine contained one or more organochlorine compounds.

Plasma Steroids

Plasma steroid concentrations in Kootenai River white sturgeon were comparable to concentration ranges found in Atlantic, Siberian, Columbia River and domestic white sturgeon at similar stages of sexual development (Tables 5-7). Fish were grouped as mature reproductive males or females, and immature or non-reproductive sturgeon. In order to display variability within and among species, comparisons were made between ranges of concentrations, rather than mean concentrations.

The null hypothesis that plasma steroid concentrations differed between fish of different size, capture location, date and age was rejected (Kruskal-Wallace; $P < 0.05$). However, in several cases, sturgeon of different sex and developmental stage showed significantly different plasma steroid concentrations (Kruskal-Wallace and Mann-Whitney U tests; $P < 0.05$; Figure 3-5).

Male Kootenai River white sturgeon possessed significantly higher concentrations of testosterone than female (Mann-Whitney U; $P = 0.001$) or immature (Mann-Whitney U; $P = 0.002$) sturgeon, respectively. Levels of 11-ketotestosterone were also significantly higher (Mann-Whitney U; $P = 0.002$) in

Table 5. Comparison of ranges of testosterone concentrations (ng/ml) in immature and non-reproductive and sexually reproductive male and female Kootenai, Columbia River and cultured white sturgeon, Atlantic sturgeon and Siberian sturgeon (Chapman 1989; Cuisset et al. 1994; Doroshov et al. 1997; Fitzpatrick et al. 1996; Webb et al. 1999).

	Male	Female	Immature/ non-reproductive
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	---

Table 6. Comparison of ranges of 11-ketotestosterone concentrations(ng/ml) in immature and non-reproductive and sexually reproductive male and female Kootenai River and Columbia River white sturgeon and Siberian sturgeon (Chapman 1989; Cuisset et al. 1994; Fitzpatrick et al. 1996).

	Male	Female	Immature/ non-reproductive
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

Table 7. Comparison of ranges of estradiol concentrations (ng/ml) in sexually reproductive Kootenai River, Columbia River and cultured white sturgeon, Atlantic sturgeon and Siberian sturgeon (Chapman 1989; Cuisset et al. 1994; Doroshov et al. 1997; Fitzpatrick et al. 1996; Webb et al. 1999).

	Male	Female
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

Figure 3. Concentrations of plasma testosterone (a), 11-ketotestosterone (11-kt; b) and estradiol (c) in male, female and immature Kootenai River white sturgeon, 1998. Error bars indicate standard deviation from the mean. Different letters above boxes indicate significantly different treatment means within males or females (Kruskal-Wallace and Mann-Whitney U tests; $P < 0.05$).

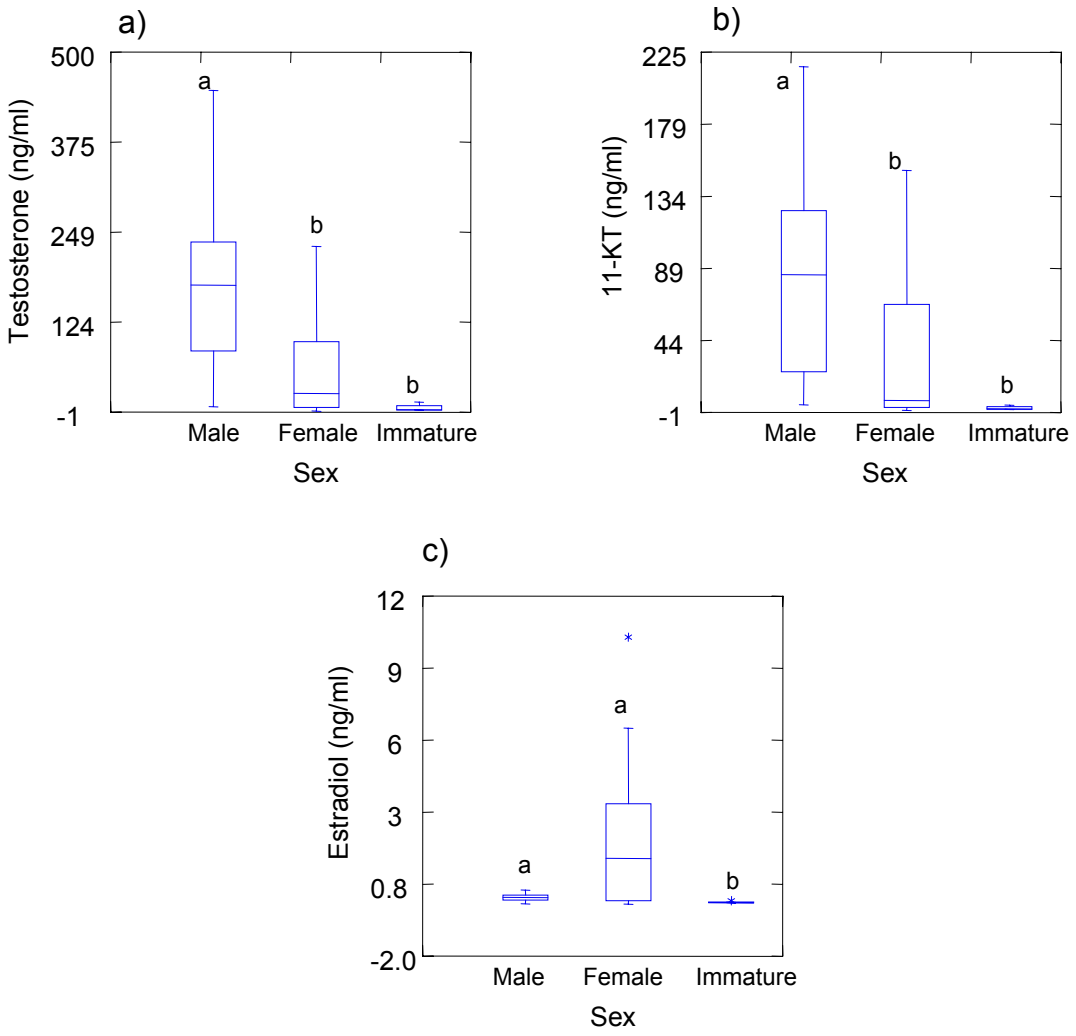


Figure 4. Concentrations of plasma estradiol (a), testosterone (b) and 11-ketotestosterone (11-kt; c) in male (M) and female (F) Kootenai River white sturgeon at different stages of sexual maturity, 1998. Error bars indicate standard deviation from the mean. Different letters indicate significantly different treatment means within males or females (Kruskal-Wallace and Mann-Whitney U tests; $P < 0.05$).

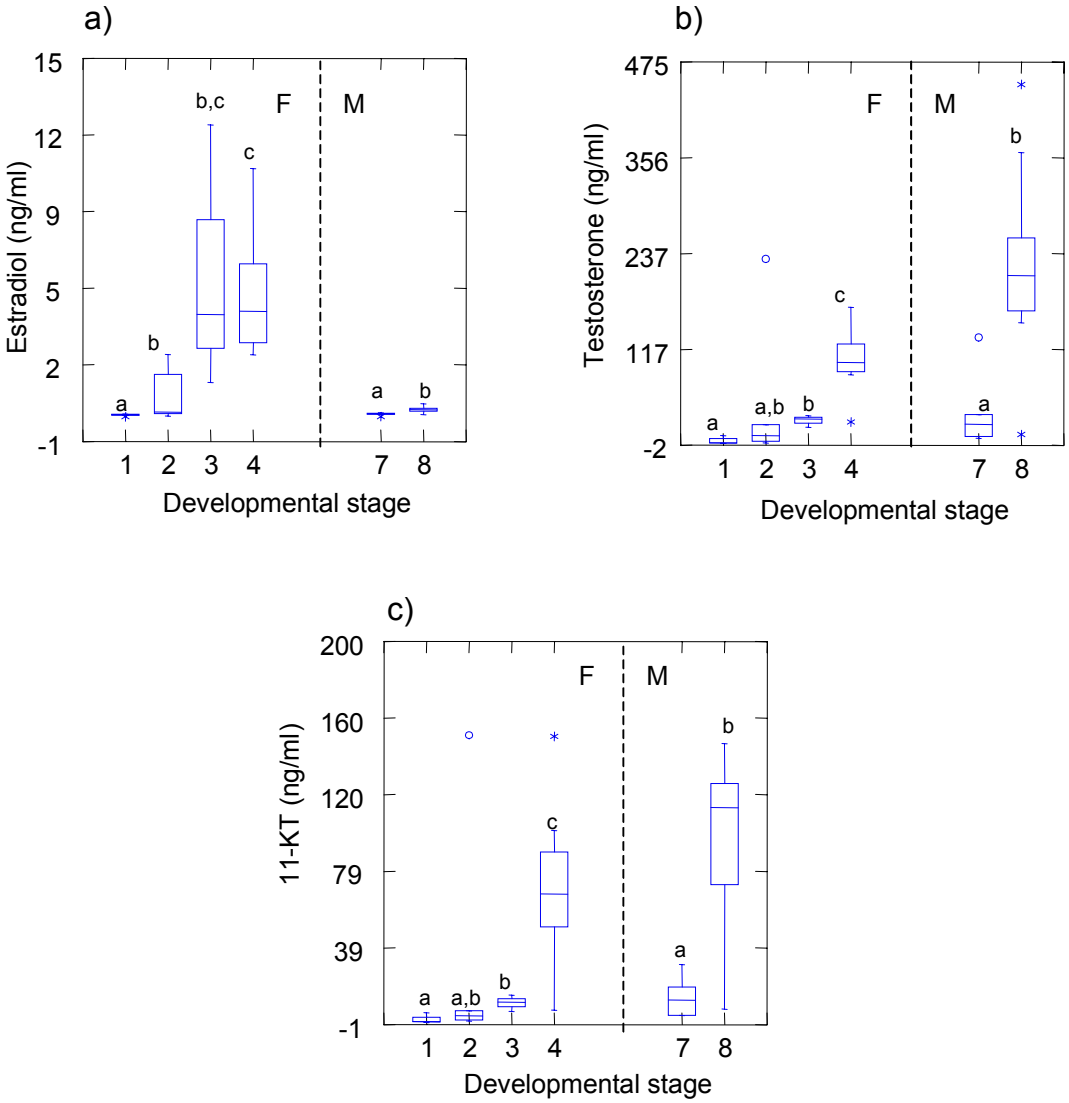
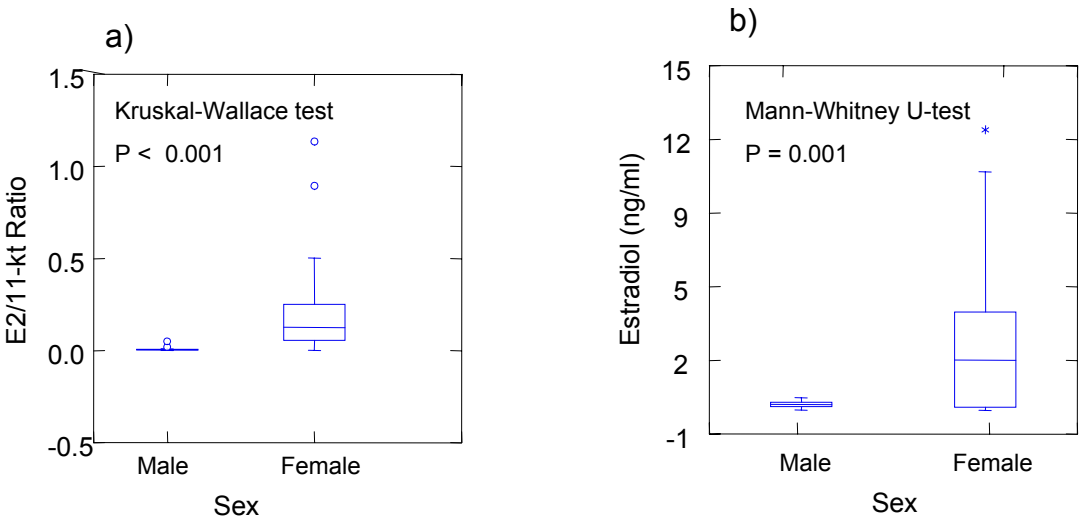


Figure 5. Plasma estradiol (a) concentrations and the estradiol:11-ketotestosterone ratio (E2/11-kt ratio; b) in male and female Kootenai River white sturgeon, 1998. Estradiol concentrations do not include data from stage 1 females. Error bars indicate standard deviation from the mean. (Kruskal-Wallace and Mann-Whitney U tests; $P < 0.05$)



male than in female (Mann-Whitney U; P = 0.003) or immature (Mann-Whitney U; P = 0.002) sturgeon, respectively. Mature reproductive male sturgeon at a later stage of development (stage 8; Table 1) 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 early reproductive stage 7 males.

The ratio of 17β -estradiol to 11-ketotestosterone (E2/kt ratio) was significantly higher in female (0.218) than male (0.008) sturgeon (Mann-Whitney U test; P < 0.001). Ratios ranged from 0.025 – 0.503 in females and from 0.002 – 0.047 in males.

Mature reproductive female and immature non-reproductive sturgeon did not possess significantly different testosterone (Mann Whitney U; P = 0.081) and 11-ketotestosterone (Mann Whitney U; P= 0.088) concentrations. Mature reproductive females possessed significantly higher (Mann Whitney U; P = 0.012) concentrations of 17β-estradiol than immature fish. There was no significant difference (Mann Whitney U; P = 0.087) between 17β-estradiol concentrations in mature reproductive male and female sturgeon at all stages of development; however, 17β-estradiol concentrations were significantly higher (Mann Whitney U; P = 0.001) in late vitellotgenic stage 2-4 female sturgeon than in male sturgeon.

The null hypothesis that plasma steroid concentrations and ovarian tissue contaminant concentrations were not significantly correlated was rejected in several cases (Figure 6, 7). Plasma testosterone concentrations showed a significant inverse correlation (Spearman; r = -0.729) with ovarian tissue concentrations of the PCB Aroclor 1260 (Table 4). Significant inverse correlations also existed between plasma 11-ketotestosterone and ovarian tissue concentrations of Aroclor 1260 (Spearman; r = -0.820), zinc (Spearman; r = -0.652) and total organochlorines (Spearman; r = -0.753; Table 4). One data point for zinc and one for 11-ketotestosterone drove the significant correlation between 11-ketotestosterone and zinc. This relationship was no longer significant when these outliers (points greater than 2 standard deviations from the mean) were removed from the analysis. A significant positive correlation (Spearman; r = 0.893) existed between 17β-estradiol and DDT concentrations in ovarian tissue (Table 4).). One data point for zinc and one for 11-ketotestosterone drove the significant correlation between estradiol and DDT concentrations zinc. This relationship was no longer significant when these outliers (points greater than 2 standard deviations from the mean) were removed from the analysis. The E2/11-kt ratio in female sturgeon showed a significant positive correlation with ovarian tissue concentrations of DDE (Spearman; r = 0.611), Aroclor 1260 (Spearman; r = 0.733) and total organochlorines (Spearman; r = 0.791; Table 4). The E2/11-KT ratio increase in male and female Kootenai sturgeon significantly correlated to decreasing 11-ketotestosterone concentrations (Spearman; r = -0.476)

Figure 6. Significant correlations between plasma steroid concentrations (testosterone; 11-ketotestosterone; and 17β-estradiol) and the PCB Aroclor 1260 (a, b), total organochlorines (c), zinc (d) and DDT(e) detected in Kootenai River white sturgeon ovarian tissue, 1998 (Spearman test; α=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.

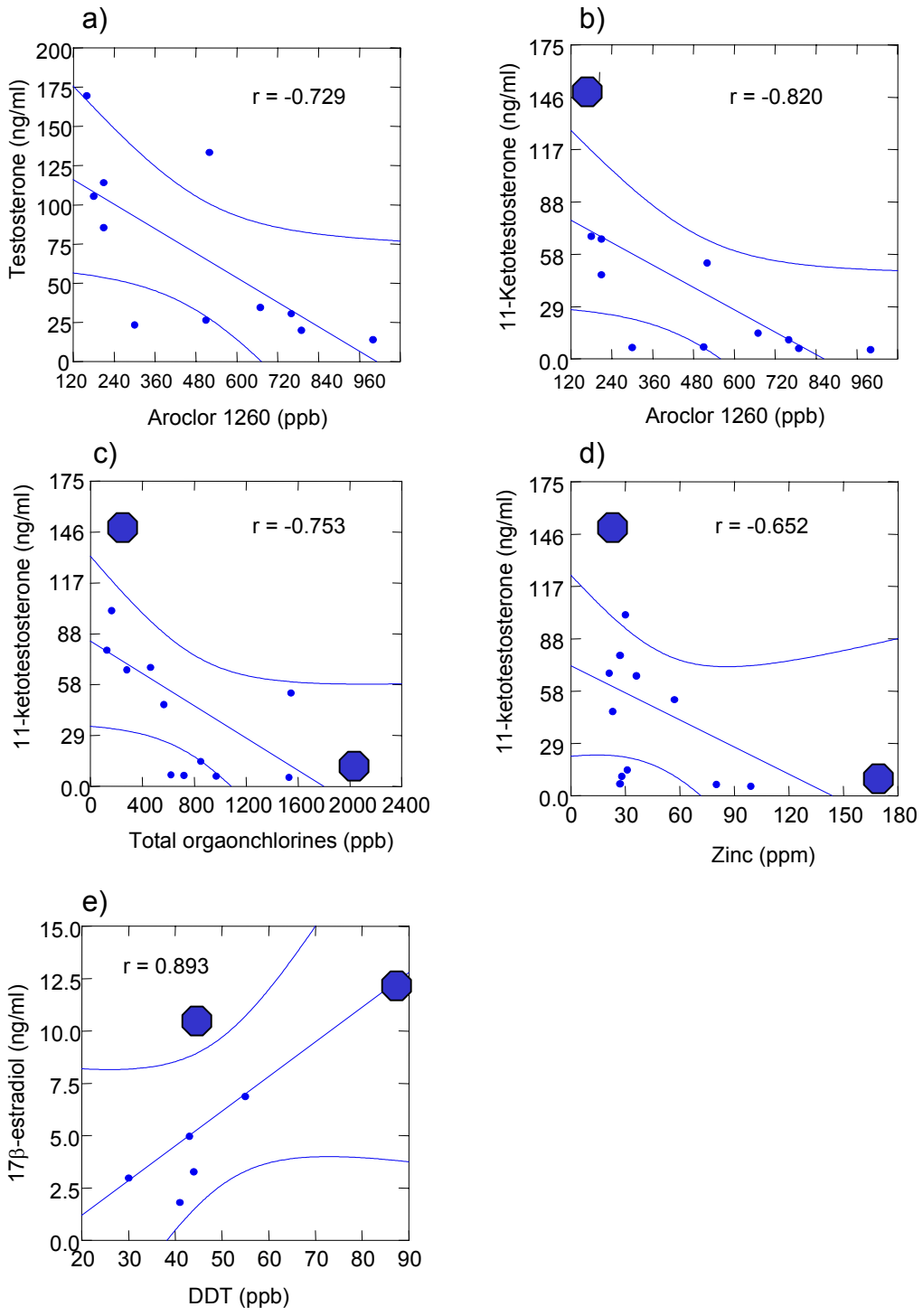
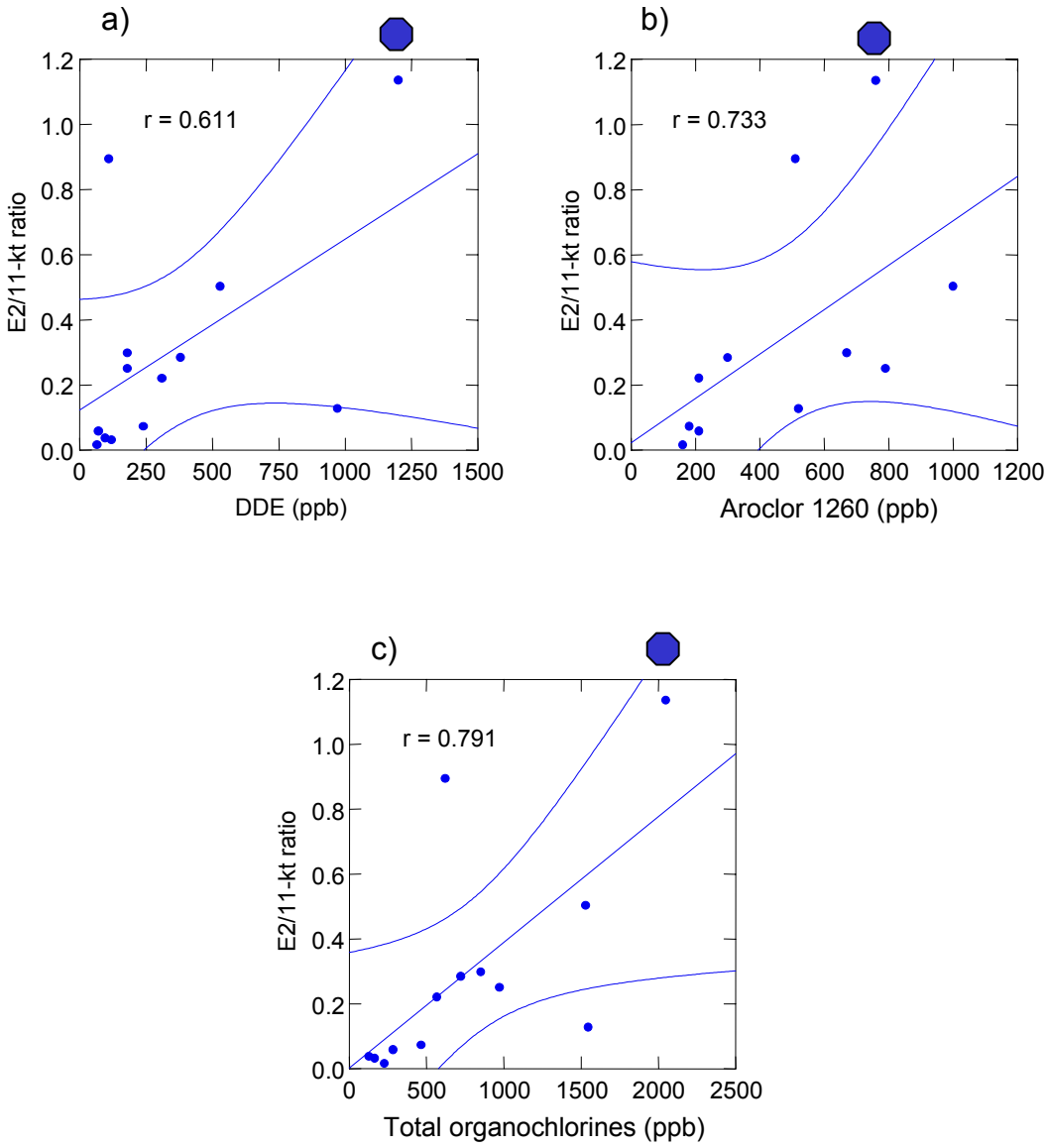


Figure 7. Significant correlations between the ratio of plasma steroid estradiol to 11-ketotestosterone (E2/11-kt ratio) and DDE (a), the PCB Aroclor 1260 (b), and total organochlorines (c) detected in Kootenai River white sturgeon ovarian tissue, 1998 (Spearman test; α=0.05). Large dot indicates points that are greater than 2 standard deviations from the mean and strongly influence the r value. Brackets indicate 95% confidence intervals.



Water and Sediment

All water samples contained detectable residues of iron, manganese, zinc and one sample contained the PCB Aroclor 1260 (Table 8). All eight of the sediment samples contained detectable levels of cadmium, copper, iron, lead, manganese, mercury and zinc (Table 9). Sediment and water samples did not contain organochlorine, organophosphate, organonitrate or carbamate pesticide residues above method detection limits (Appendix 1).

The null hypotheses of no significant difference between copper and zinc concentrations in sediment samples collected during 1989-91 and samples collected in 1999 were not rejected. Therefore, copper (Mann-Whitney U test; P = 0.142) and zinc (Mann-Whitney U test; P = 0.057) concentrations in river-bottom sediment were not significantly different between earlier (1989-1991) and later (1999) samples.

Discussion

Absence of detectable organophosphate, organonitrate and carbamate pesticide residues in ovarian tissue, water and sediment indicates that high levels of these compounds were not present during the time period that samples were collected. Water and sediment samples were collected during the season of heavy agricultural application of pesticides so if concentrations were high in the aquatic media, they likely would have been detected.

Table 8. Range and mean concentrations of metals and total organic carbon detected in eight sediment samples taken from river kilometer 205.0-244.5 in the Kootenai River, Idaho.

Variable (mg/kg)	Concentration	
	Range	Mean±st. deviation
Cadmium	0.10 – 0.60	0.39±0.19
Copper	2.90 – 14.00	9.41±4.07
Iron	240 – 4,600	3068±1392
Lead	11.0 – 34.0	23.1±7.68
Manganese	95.0 – 300	204±74.7
Mercury	0.01 – 0.02	0.02±0.01
Zinc	44.0 – 85.0	67.0±14.8
Total Organic Carbon	680 – 21,000	12,035±7925

Table 9. Range and mean concentrations of metals detected in eight surface water samples taken from river kilometer 205.0-244.5 in the Kootenai River, Idaho.

Variable	Concentration	
	Range	Mean ±st. deviation
Iron (mg/l)	0.48 – 1.00	0.07±0.19
Manganese (mg/l)	0.01 – 0.03	0.02±0.01
Zinc(mg/l)	0.01 – 0.22	0.04±0.07
PCB Aroclor 1260 (ug/l)	---	0.40[a]
pH	6.71 – 7.50	7.10±0.31
Temperature (^{o}C)	11.9 – 12.9	12.4±0.39
Conductivity (mS/Cm)	0.17 – 0.19	0.18±0.01
Turbidity (ntu)	12.2 – 55.1	27.0±14.35
Dissolved Oxygen (mg/l)	10.1 – 12.4	11.2±0.93

[a] The PCB Aroclor 1260 was only detected in one of the eight water samples

Detection of metal and organochlorine compounds in Kootenai River white sturgeon ovarian tissue indicates that environmental exposure has resulted in bioaccumulation of these compounds above method detection limits. The lack of a significant difference in ovarian tissue concentrations of copper, lead, DDE and DDT indicates that the bioaccumulated amounts of these compounds (in sturgeon) have not decreased in the past 10 years. The presence of significantly higher zinc concentrations in ovarian tissue samples collected during 1997,1998 and 1999 and samples collected during 1989, 1990 and 1991 indicates that bioaccumulation of zinc has increased within the past 10 years. The increase in zinc residues may be the result of either increased contaminant loading in the system or re-distribution of bound particles into a more bioavailable state (Birge et al. 1987). It is likely that zinc mines in the basin (37 percent respectively of all registered mines in the Idaho portion of the Kootenai River drainage; US Geological Survey 1999) are the primary contributors of these metals to the Kootenai River. Runoff during the spring or other periods of high precipitation can result in contaminant distribution and leaching from the mine sites. These factors have, therefore, potentially intensified bioavailability of zinc.

The lack of a significant difference in zinc and copper concentrations in river-bottom sediment samples collected during earlier and later sampling periods indicates that concentrations of these metals (in sediments) is neither increasing nor decreasing. Contaminant concentrations in Kootenai River sediments are measured as total metal concentrations indicative of the amount of

metal potentially bioavailable to organisms at some point (Chapman et al. 1998). In comparison with the Wisconsin interim criteria for sediments, levels of metals in Kootenai River sediments are acceptable for freshwater aquatic life (Wisconsin Department of Natural Resources 1985); however, depending upon geology, stream morphology, organism speciation and many other factors considered when developing the criteria, this comparison may or may not be valid.

Concentrations of total iron, zinc and manganese in Kootenai River water exceeded environmental background levels suggested by Forstner and Wittmann (1979). Mean iron concentrations for all samples exceeded EPA freshwater quality criteria of 0.18-0.32 mg/l (at 50 and 100 mg/l hardness). I was unable to locate published literature describing the impacts of high iron and manganese concentrations on fish, but several studies indicate that concentrations of zinc in Kootenai River water are high enough to physiologically affect. Although the mean concentration of total zinc in water was only 0.037 ± 0.074 mg/l, water from the confluence of Myrtle Creek (rkm 234.5) contained 0.22 mg/l zinc, which exceeds EPAs freshwater life criteria of 0.088 mg/l zinc (based on 70 mg/l water hardness; Bauer 1999). In addition, the mean concentration of zinc in all water samples exceeded criteria for freshwater life in British Columbia, Canada (at 70mg/L hardness; Bauer 1999).

Although toxicity of zinc varies with water hardness and pH (higher toxicity at lower hardness and pH), results of comparable studies indicate that

concentrations of zinc in Kootenai River water may be negatively impacting fish reproduction, feeding, habitat selection and system productivity. According to Pierson (1981) sexual maturity was the most sensitive indicator of zinc toxicity in the guppy (*Poecilia reticulata*) when after 70 days at 0.173 mg/l water-borne zinc, significantly fewer females had matured. McFarlane and Franzin (1978) reported reduced spawning success and reduced egg and larval survival in zinc-exposed white suckers (*Catostomus commersoni*). In addition, Brungs (1969) cited that exposure to 0.18 mg/l zinc in water (200 mg/l hardness) reduced spawning frequency in fathead minnows (*Pimephales promelas*) by 83%. Of the fathead minnows that did spawn, egg production was reduced by 50% after exposure to 0.088 and 0.042 mg/l water-borne zinc. According to Little et al. (1993), water concentrations of zinc $\geq$0.02 mg/l caused decreased feeding activity in rainbow trout (*Oncorhynchus mykiss*). Hansen et al. (1999) also showed that significant habitat avoidance occurred in rainbow trout exposed to test water containing 0.005 mg/l zinc (98-104 mg/l hardness; pH 7.85-8.3). If zinc input increases during high spring flows (which also trigger spawning migration in sturgeon), spawning adult sturgeon may avoid otherwise ideal spawning substrate in order to avoid high levels of zinc.

Pratt (1990) found that zinc levels as low as 0.0042 mg/l (100 mg/l water hardness) decreased primary productivity and biomass of protein and chlorophyll in laboratory mesocosms. In the Kootenai River, a zinc-induced reduction in primary production and reduced production of lower food-chain organisms would

affect the higher food-chain organisms such as sturgeon. Therefore, it is imperative that true sublethal values for zinc are determined in relation to other parameters in the Kootenai River and that the chronic and acute criteria are met if sublethal reproductive effects on sturgeon are to be avoided or minimized.

Data from water samples collected throughout the lower Kootenai River by the Kootenai Tribe of Idaho during 1997-1998 (Bauer 1999) indicate much lower concentrations of zinc (0.00065-0.00456 mg/l) than the samples collected for this study. Bauer (1999) suggests that because these levels are below EPA criteria, water concentrations of zinc are not toxic to fish; however, contamination can occur easily when sampling for metals, resulting in inflated concentrations. Therefore, I recommend an expanded study to validate the contaminant concentrations in water and sediment within the lower Kootenai River.

Although the PCB Aroclor 1260 was only detected in one water sample out of eight (and none of the sediment samples), the detected concentration (0.4 ug/l) was 40 times the EPA and Idaho allowable levels for freshwater criteria (0.014 ug/l). Polychlorinated biphenyls are only slightly soluble in water so the probability of detecting it in water is low unless high amounts are sorbed to suspended solids in the water or there is a nearby source of input. Bioconcentration factors for PCBs between water and aquatic organisms have been reported in the order of 10^5-10^6 (Roberts et al. 1978). Based on these numbers, the PCB bioaccumulation risk for Kootenai River fish could potentially

be in the range of 40,000-400,000 ppb. Defoe et al. (1976) found that water concentrations of Aroclor 1260 at 0.002 ug/l resulted in tissue concentrations of 500 ppb. This calculates to a bioconcentration factor of 250,000. Roberts et al. (1978) reported fish mortality and reduced phytoplankton species diversity and growth in water containing 0.1-1.0 ppb PCBs. The exact source of PCBs in the Kootenai River is unknown. One possible source, however, is from leaching of PCB-based lubrication in transformers and hydraulic units that were produced and installed at Libby Dam (approximately 100 km upriver of sample sites) prior to the ban on PCBs. Although it is likely that several units have been replaced since the PCB ban became effective, some of the PCB-containing equipment may still be in operation. In addition, leaking lubricants may have contaminated the areas around this equipment. This area could be a long-term source of PCB leachate due to the poor ability of PCBs to degrade in aerobic conditions.

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). Mean PCB concentrations in sturgeon ovarian tissue measured 460 ppb, exceeding both New York (130 ppb) and International Joint Commission (100 ppb) fish consumption regulations for piscivorous wildlife (US Environmental Protection Agency 1992; International Joint Commission United States and Canada 1999). Mean ovarian tissue DDE concentrations of 296 ppb also exceeded the New York criteria for piscivorous wildlife (200 ppb). The

organochlorine pesticides aldrin and dieldrin were banned approximately 20 years ago. Aldrin rapidly metabolizes to dieldrin (Kahn et al. 1979) so aldrin would not have expected at detectable levels in the ova. Its presence suggests that either sturgeon have a slower metabolism than other fish species, or that aldrin is still being contributed to the aquatic system through some source.

The significant positive correlations between tissue burdens of both zinc and total organochlorines and the size of broodstock eggs indicates that higher zinc and total organochlorine concentrations may be associated with larger egg size. Although larger egg size provides larval fish with a physiological advantage at hatching, zinc and organochlorine compounds (at levels observed in Kootenai River white sturgeon embryos) can have negative impacts on development, growth, behavior and survival of larvae (Heath 1995). The negative impacts on growth and survival may potentially outweigh the positive effects of larger egg size.

Based on comparisons with data from other species of sturgeon, ranges of plasma steroid concentrations in Kootenai River white sturgeon are not extreme. There appears to be wide variability between species and populations depending on environmental factors as well as migration and timing of the reproductive cycle. Comparison of concentration ranges (rather than means) provided for this variability while still making it possible to categorize and compare plasma steroids in reproductive and non-reproductive sturgeon.

Concentrations of plasma estradiol in Kootenai River white sturgeon were within the ranges of those found in Atlantic (*Acipenser oxyrinchus*), Siberian (*Acipenser baeri*) and Columbia River and domestic cultured white sturgeon (Chapman 1989; Fitzpatrick et al. 1996; Doroshov et al. 1997; Webb et al. 1999; Cuisset et al. 1994). The Kootenai River white sturgeon had estradiol concentrations closest to those found in Atlantic sturgeon. Blood samples from the Kootenai River sturgeon were collected prior to cessation of final vitellogenesis, so the similarity of estradiol concentrations between species is most likely the result of sample collection timing (Van Eenennaam et al. 1996). Steroid concentrations generally peak within the few months prior to spawning (Doroshov et al. 1997); however, migration timing can also determine when peak steroid production occurs. When comparing Atlantic, Siberian and white sturgeon just prior to spawning, Van Eenennaam et al. (1996) found similar plasma sex steroid concentrations among the species. Compared to wild white sturgeon however, the female Atlantic sturgeon possessed higher concentrations of estradiol. Van Eenennaam et al. (1996) suggested that final ovarian vitellogenesis occurred later in spring in the spring-migrating Atlantic sturgeon than in the white sturgeon that generally migrated into the pre-spawning and staging areas during fall. The white sturgeon in their study had, therefore, most likely passed peak vitellogenesis and steroid production at the time of sampling.

The higher concentrations of testosterone and 11-ketotestosterone in male than in female sturgeon observed in this study was expected because these steroids are primarily associated with males during spermatogenesis (Chieffi and Pierantoni 1987). Testosterone concentrations in male fish remain fairly constant throughout the annual cycle (Campbell et al. 1976) and are typically much higher than concentrations in females. However, because testosterone and 11-ketotestosterone are pre-cursors to additional hormones associated with oogenesis, these steroids have been found to fluctuate in pre-spawning females during the reproductive cycle (Fitzpatrick et al. 1996; Doroshov et al. 1997).

The significantly higher 17β-estradiol to 11-ketotestosterone ratio (E2/11-kt ratio) in female than in male Kootenai River white sturgeon is consistent with ratios found in common carp (*Cyprinus carpio*) collected from waters throughout the United States (Goodbred et al. 1997). The E2/11-kt ratio is useful in evaluating steroid production differences between male and female fish (Goodbred et al. 1997); the ratio determines sexual dimorphism, sexual characteristics and developmental functions. The ratios should be different between males and females and when compared with ratios from other fish, extreme E2/11-kt values are useful indicators of potential endocrine disruption. The mean E2/11-kt ratio in female Kootenai River white sturgeon was approximately five times lower than ratios found in female carp (Goodbred et al. 1997); however, the ratio in male Kootenai River white sturgeon was comparable

to those found in carp. These results indicate that sexual dimorphism between male and female Kootenai River white sturgeon is not as definitive as in the carp. The significant correlation between E2/11-kt ratios and decreasing 11-ketotestosterone levels indicates that depressed production of 11-ketotestosterone is determining the ratio rather than increased production of estradiol, as was also found by Goodbred et al. (1997) in the carp study.

There were no significant differences in steroid concentrations among female Kootenai River white sturgeon at different stages of ovarian development; however, the general trend showed increasing concentrations of all steroids in females during later stages of ovarian development. Of twelve females tested, the single stage-4 female possessed the highest concentration of all three plasma steroids. Fitzpatrick et al. (1996) also found that mature female sturgeon in the Columbia River system possessed higher concentrations of 17β-estradiol than non-reproductive females.

The lack of significant differences in 17β-estradiol concentrations between mature male and female sturgeon is consistent with results found by Fitzpatrick et al. (1998) for white sturgeon at different stages of maturity. The lack of differences may be a result of the long reproductive cycle (3-5 years) in female sturgeon. Fish in the early phases of maturation and the reproductive cycle often have lower levels of 17β-estradiol that increases as the fish matures and oocytes develop. Kagawa et al. (1981) and Bangalore et al. (1978) found that within 1-2

months prior to the spawning period, 17β-estradiol concentrations were extremely low in immature but peaked in mature female white-spotted char (*Salvelinus leucomaenis*) and freshwater catfish (*Heropneustes fosilis*). Twenty-three percent of the females in this study, and a large number of females in the Columbia River white sturgeon study, were early vitellogenic (stage 1) females which would not be expected to spawn for 2-3 years; therefore, one would expect to find lower concentrations of 17β-estradiol in these fish than in fish with more developed ovaries. After the stage 1 females were removed from the analysis, significantly higher 17β-estradiol concentrations were found in females than in males.

Although in some instances the significant correlations were driven by a couple of data points, results from this study suggest potential inhibition of the androgens, testosterone and 11-ketotestosterone, and induction of 17β-estradiol resulted from bioaccumulated concentrations of zinc, DDT and the PCB Aroclor 1260. Several metals and organochlorine compounds (at concentrations comparable to those found in this study) have been found to significantly inhibit steroid synthesis and metabolism in fish. Weatherly et al. (1980) believes that stress symptoms and impaired reproduction (possibly related to disturbance of steroid secretion) may be detected in fish long exposed to sublethal levels of zinc. Allen-Gil et al. (1993) found a significant inverse correlation between body burden of metals and plasma testosterone concentrations in feral Arctic grayling (*Thymallus arcticus*). Freeman and Sangalang (1977) found that hormone

production and steroidogenesis were altered in fish after exposure to PCB compounds and Freeman and Idler (1975) noted an increased breakdown of testosterone by gonads of PCB-exposed brook trout (*Salvelinus fontinalis*). Sivarajah et al. (1978) also found that PCB injections caused depressed androgen production in rainbow trout (*Salmo gairdneri*) and carp (*Cyprinus carpio*). The observed decrease in 11-ketotestosterone with increasing E2/11-kt ratio and the increase in E2/11-kt ratio with increased organochlorine concentrations further suggests the idea that the androgen 11-ketotestosterone may be depressed by bioaccumulated organochlorine compounds.

Despite the presence of contaminant concentrations exceeding regulations and the documented effects of these concentrations on other aquatic species, it is difficult to determine whether or not environmental and bioaccumulated concentrations are independently reducing reproductive capabilities in white sturgeon. Although several of the significant correlations were driven by a couple of data points, analysis between individual contaminants in ovarian tissue, plasma steroid concentrations and egg size did, however, indicate that specific contaminants may potentially create additional stress on the reproductive capabilities of the Kootenai River white sturgeon. The uptake and effects of these compounds have the potential to contribute to additional stress on the sturgeon that could further decrease their chances for successful reproduction. The combination of bioaccumulated compounds may also be resulting in cumulative effects (Alabaster and Lloyd 1982; Peterle 1991);

however, other than grouping organochlorine compounds for analysis, cumulative effects were not addressed in this study.

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CHAPTER TWO:

Effects of Contaminant Uptake on

Survival of White Sturgeon Embryos

Abstract

This experiment was designed to determine if exposure to contaminants associated with water, sediment or organic matter in the rearing media disrupt growth, physical development, survival or gender of embryonic Kootenai River white sturgeon, *Acipenser transmontanus*. Sturgeon eggs from one female were collected during artificial propagation, fertilized with sperm from one male and divided into three treatments to determine uptake of organochlorine pesticides, Aroclor 1200 series PCBs and metals during exposure to different rearing and de-adhesion media (sediment, suspended solids, unfiltered and filtered sterilized river water). River-bottom sediment (SED treatment), suspended solids (UFW treatment) and unfiltered river water contributed significantly higher concentrations of metals to embryos than Fuller's Earth and filtered river water (FE treatment). Uptake of the organochlorine pesticide DDE (Kruskal-Wallace; P = 0.962) was not significantly different among the three treatments. The PCB Aroclor 1260 was not detected in embryos from the UFW treatment. Although PCB levels were higher in embryos from the SED treatment than from the FE treatment, they were not significantly different (Kruskal-Wallace; P = 0.052). All of the eggs from the UFW treatment died within one week. The total mortality was likely associated with bacterial and fungal growth on incubating eggs and may be attributed to poor de-adhesion properties and the high level of organic matter in the suspended solids. Fungal growth was not associated with embryos in the SED or FE treatments; however, sediment coated eggs displayed

significantly higher mortality (20.6%) than eggs coated with Fuller's earth (12.6%; Mann-Whitney U test; P = 0.036). Mortality was significantly correlated with concentrations of copper (Spearman; r = 0.568) and Aroclor 1260 (Spearman; r = 0.800) detected in eggshell-associated de-adhesion media and embryo body burden within the SED, FE and UFW experimental treatments. It was concluded that contact with river-bottom sediments (in comparison with contact to river water or suspended solids) can potentially increase exposure of incubating embryos to metals. Exposure to copper or Aroclor 1260 in the rearing media is also potentially decreasing survival and incubation time of white sturgeon embyros.

Introduction

Exposure of a fish egg or sperm to contaminants during fertilization and early development can result in disruption of developmental processes that establish gender, determine growth and development, and ensure reproductive capabilities in successive generations (Raloff 1994; McKim 1994; Heath 1995). Although elimination of contaminants via egg and sperm release may be advantageous to the adult fish (Guiney et al. 1979), the inherited burden can adversely impact progeny (Heath 1995). The degree of contaminant bioaccumulation from parental stock coupled with environmental exposure can determine survival rate of progeny and eventual juvenile recruitment within a population (Hall et al. 1993).

The adhesive nature and permeability of white sturgeon, *Acipenser transmontanus*, eggs following fertilization (Detlaff et al. 1993) increases their susceptibility to contaminants associated with water, sediment and organic matter as total organic carbon. The key to correctly identifying the hazard posed by contaminants in an aquatic ecosystem is through evaluation of the bioavailability potential for contaminants (Chapman et al. 1998). Embryonic bioaccumulation of contaminants may be influenced by any material that binds to the adhesive egg shell and by the concentrations in the water sorbed during water hardening (Rodgers et al. 1987; McKim 1994).

The primary spawning habitat for the Kootenai River white sturgeon consists of sand and silt substrate (Paragamian and Kruse 1996); therefore, fertilized sturgeon eggs become coated with sediment and suspended or settled organic matter during the adhesive phase of incubation. Adhered organic matter and sediment may potentially increase contaminant exposure for embryos because they play a major role in transport, activity and persistence of contaminants (Rodgers et al. 1987). Incubating eggs may accumulate contaminant residues from several sources including parents, water, suspended organic solids and sediments. Such accumulation may be significant in rivers such as the Kootenai where contaminants have been shown to be contributed from several sources, including mining and mineral extraction, agriculture, logging and lumber processing, recreation, hydroelectric power generation, urban development and transportation (Kootenai River Network 2000). Continual redistribution of these contaminants by fluctuating flows and benthic organisms increases their redox potential and renders them repeatedly bioavailable to organisms.

The objectives of this chapter were to: 1) determine if contaminants were passed on to, sorbed by or adhered to developing Kootenai River white sturgeon embryos during production, fertilization, and exposure to sediment, river water and suspended solids; 2) determine if contaminant uptake/adhesion varies with different rearing media (suspended solids, sediment, unfiltered and filtered

sterilized river water; 3) determine if there are any relationships between concentrations of sorbed and adhered contaminants and survival of the embryos.

Materials and Methods

In order to determine uptake of contaminants into developing embryos from exposure to river-bottom sediment, suspended solids and river water, fertilized white sturgeon eggs were incubated in three treatment groups. To prevent fertilized eggs from sticking together they were mixed with a designated de-adhesion media (sediment, suspended solids or Fuller's Earth) for 2-3 hours until adhesiveness ceased. The first treatment group contained fertilized eggs that were de-adhesed with suspended solids from the river water column and reared in unfiltered river water (UFW). A second treatment group (also used as the control) contained eggs that were de-adhesed with Fuller's Earth and reared in filtered river water (FE). Eggs from the third treatment group were de-adhesed with river-bottom sediment and reared in filtered river water (SED).

Surface sediment and suspended solids that were used for de-adhesion was collected from sturgeon spawning areas in the Kootenai River (rkm 229-230), during June, 1999. Sediment was collected with a Ponar dredge and suspended solids were collected with a 80-μm mesh plankton net. Sub-samples of the sediment and suspended solids were removed from the dredge and net

with a wooden spoon, placed in certified sterile glass jars and stored in the refrigerator at 0^{o}C for 2 days.

White sturgeon eggs were taken from a single female during spawning for conservation aquaculture at the Kootenai Tribal experimental sturgeon hatchery (rkm 242). The number of eggs per ml was counted and eggs were placed in a beaker to a volume of 250 ml, fertilized with sperm from one male sturgeon and separated into three treatment groups of approximately 83 ml each. All of the eggs were then de-adhesed according to methods in Conte et al. (1988), using suspended solids, Fuller's Earth or river-bottom sediment. At the beginning of the de-adhesion process, one 10-g sample was taken from each treatment group to establish baseline contaminant concentrations in the fertilized eggs and their prospective de-adhesion media.

Following de-adhesion, eggs from each of the three treatment groups were subdivided into eight rearing containers per group (approximately 10 g of eggs per container), for a total of 24 containers. Eggs from the SED and FE treatments were reared in ultraviolet light sterilized river water filtered through an AMIAD automatic filter to remove particles larger than 10 µm. Eggs from the UFW treatment were reared in unfiltered water that was pumped directly from the Kootenai River.

The holding tank was covered with black plastic to maintain darkness. Water temperatures were allowed to fluctuate with in-situ river water temperatures during de-adhesion and during rearing. Inflow temperature was recorded daily. Jars were checked daily and dead or dying embryos were counted, removed, placed into sample jars and frozen for contaminant analysis. Basic water quality parameters (including alkalinity, total dissolved solids, total suspended solids, ammonia, nitrate+nitrite, ortho-phosphorous, calcium, copper, magnesium, manganese and zinc) were recorded one day each during June and July as part of standard procedure for hatchery operations (Appendix 4).

Within 13 days following fertilization, the majority of the surviving eggs had begun to hatch and all remaining embryos and larvae were counted, removed from containers and frozen for contaminant analysis. Total egg mortalities for individual containers were determined by dividing the total number of dead eggs by the initial total number of eggs in each jar. Embryo samples were tested for organochlorine pesticides, Aroclor 1200 series PCBs and eight metals (Appendix 1).

This study was designed to test the two null hypotheses that contaminant uptake and embryo mortality did not significantly differ among treatments (Kruskal-Wallace; Mann-Whitney U test; $P < 0.05$). A third hypothesis was that contaminant concentrations in embryos did not significantly correlate with embryo mortality (Spearman; $\alpha = 0.05$). Data were tested for normality of distribution

with the expression 2(SE)±K (where SE is the standard error and K is a measure of kurtosis), and homogeneity of variances (Cochran's C-test; Kirk 1995). Data displayed heterogeneity of variances and were not normally distributed so non-parametric statistical analyses were used to test the hypotheses.

Results

Lipid concentrations in eggs and embryos from all treatment groups and the baseline samples ranged from 3.0-9.0 % (Table 1). These concentrations were not significantly different among treatments (Kruskal-Wallace; P = 0.519).

The null hypothesis was rejected, indicating that contaminant uptake was significantly different among treatments (Table 1). The embryos reared in the SED treatment sorbed higher concentrations of most metals and organochlorine compounds than embryos from the FE and UFW treatments. Based on contaminant testing of sediment samples from the lower Kootenai River, concentrations of cadmium, copper, iron, lead and zinc were all lower in embryos from this experiment than in sediment samples. Concentrations of iron and zinc were higher in embryos from this experiment than in water samples collected from the same lower Kootenai River locations as sediment samples.

Eight metals were detected in eggs and embryos from this experiment (Table 1). Uptake of arsenic (Kruskal-Wallace; P = 0.041), cadmium (Kruskal-Wallace; P = 0.032), copper (Kruskal-Wallace; P = 0.001), iron (Kruskal-Wallace;

Table 1. Treatment, percent mortality, contaminants detected, number of samples contaminants were detected in and percent of total samples, concentration range, mean and standard deviation by group for white sturgeon eggs from UFW, FE, SED treatments and baseline samples. The difference in number of decimal places for concentration range and mean indicate variation in method detection limit. Asterisks and letters indicate significant differences between treatment and pairwise means respectively, for individual contaminants (Kruskal-Wallace and Mann-Whitney U tests; $P < 0.05$).

Treatment (Mortality)	Contaminant	Number of samples (% of total sample)	Concentration range	Mean concentration (SD)
UFW (100%)				
	Arsenic (ppm)	8 (100)	0.26-0.97	0.42 (0.25) *b
	Cadmium (ppm)	8 (100)	0.01-0.03	0.02 (0.01) *a,b
	Copper (ppm)	8 (100)	1.30-2.50	1.96 (0.38) *a
	Iron (ppm)	8 (100)	540-1200	881 (205) *a,b
	Magnesium (ppm)	8 (100)	400-860	630 (141) *a,b,c
	Lead (ppm)	8 (100)	1.00-3.00	1.78 (0.62) *a,b
	Selenium (ppm)	8 (100)	0.46-1.00	0.68 (0.20)
	Zinc (ppm)	8 (100)	12.0-24.0	18.4 (3.74)
	PCB (ppb)	0 (0)	<52	<52
	DDE (ppb)	6 (75)	39.0-60.0	48.3(7.12)
	Lipid (%)	8 (100)	5.80-9.00	7.06 (1.28)
FE (12.6%)				
	Arsenic (ppm)	2 (25)	0.17-0.29	0.23 (0.09) *d
	Cadmium (ppm)	3 (38)	0.01-0.01	0.01 (0.001) *a,d
	Copper (ppm)	8 (100)	0.79-1.50	1.15 (0.22) *a,d,e
	Iron (ppm)	8 (100)	16.0-140	38.30 (41.4) *a,d,e
	Magnesium (ppm)	8 (100)	96.0-170	137 (24.7) *a,d,e
	Lead (ppm)	6 (75)	0.08-0.63	0.24 (0.21) *a,d
	Selenium (ppm)	8 (100)	0.48-0.94	0.71 (0.17)
	Zinc (ppm)	8 100)	9.50-18.0	19.9 (2.93)
	PCB (ppb)	3 (38)	92.0-100	95.7 (4.01)
	DDE (ppb)	8 (100)	34.0-71.0	51.6 (13.6)
	Lipid (%)	8 (100)	3.90-7.70	5.96 (1.35)
SED (20.6 %)				
	Arsenic (ppm)	8 (100)	0.36-0.82	0.61 (0.15) *b,d
	Cadmium (ppm)	8 (100)	0.002-0.04	0.03 (0.01) *b,d
	Copper (ppm)	8 (100)	1.90-2.20	2.01 (0.10) *d
	Iron (ppm)	8 (100)	17.0-2800	1889 (843) *b,d
	Magnesium (ppm)	8 (100)	810-1400	1050(185) *b,d
	Lead (ppm)	8 (100)	1.80-3.30	2.71 (0.51) *b,d
	Selenium (ppm)	8 (100)	0.63-0.89	0.74 (0.08)
	Zinc (ppm)	8 (100)	22.0-26.0	24.0 (1.20)
	PCB (ppb)	4 (50)	120-160	140 (23.1)
	DDE (ppb)	8 (100)	38.0-65.0	47.8 (9.51)
	Lipid (%)	8 (100)	4.60-8.70	6.60 (1.37)

Table 1 continued. Treatment, percent mortality, contaminants detected, number of samples contaminants were detected in and percent of total samples, concentration range, mean and standard deviation by group for white sturgeon eggs from UFW, FE, SED treatments and baseline samples. The difference in number of decimal places for concentration range and mean indicate variation in method detection limit. Asterisks and letters indicate significant differences between treatment and pairwise means respectively, for individual contaminants (Kruskal-Wallace and Mann-Whitney U tests; $P < 0.05$).

Treatment (Mortality)	Contaminant	Number of samples (% of total sample)	Concentration range	Mean concentration (SD)
Baseline				
	Arsenic (ppm)	2 (67)	0.56-0.72	0.64 (0.11) *
	Cadmium (ppm)	3 (100)	0.002-0.08	0.04 (0.04) *
	Copper (ppm)	3 (100)	1.90-2.80	2.30 (0.46) *[e]
	Iron (ppm)	3 (100)	290-1900	1196 (824 *[e]
	Magnesium (ppm)	3 (100)	860-1200	1000 (178) *[c,e]
	Lead (ppm)	3 (100)	0.29-5.80	2.93 (2.76) *
	Selenium (ppm)	3 (100)	0.28-1.20	0.79 (0.47)
	Zinc (ppm)	3 (100)	18.0-27.0	22.7 (4.51)
	PCB (ppb)	2 (67)	110-130	120 (14.1)
	DDE (ppb)	3 (100)	29.0-60.0	47.0 (16.1)
	Lipid (%)	3 (100)	3.00-9.10	5.23 (3.36)

P = 0.001), magnesium (Kruskal-Wallace; P < 0.001) and lead (Kruskal-Wallace; P = 0.002) were significantly different among the treatment groups and the baseline (Table 1, Figure 1). All of these metals were at their highest concentrations in the baseline samples and the SED treatment, followed by concentrations in the UFW and FE treatments respectively. Concentrations of selenium (Kruskal-Wallace; P = 0.787) and zinc (Kruskal-Wallace; P = 0.052) were not significantly different among treatment groups.

Two organochlorine compounds (DDE and Aroclor 1260) were detected in eggs and embryos from this experiment (Table 1). Concentrations of the organochlorine compound DDE were not significantly different between the treatment groups (Kruskal-Wallace; P = 0.962; Table 1). Although DDE was present in all treatment groups, the organochlorine PCB Aroclor 1260 was not detected above 360 ppb in eggs and embryos from the UFW treatment. There was no significant difference between Aroclor 1260 concentrations when comparing the SED, FE and baseline samples (Kruskal-Wallace; P = 0.052). However, concentrations of Aroclor 1260 in SED and FE treatments did differ significantly from each other (Wilcoxon rank-sum test; P = 0.008).

The null hypothesis stating no significant difference in mortality rates between treatments was rejected. All of the eggs from the UFW treatment died within the first 7 days (Table 1). Percent mortality was significantly higher in the

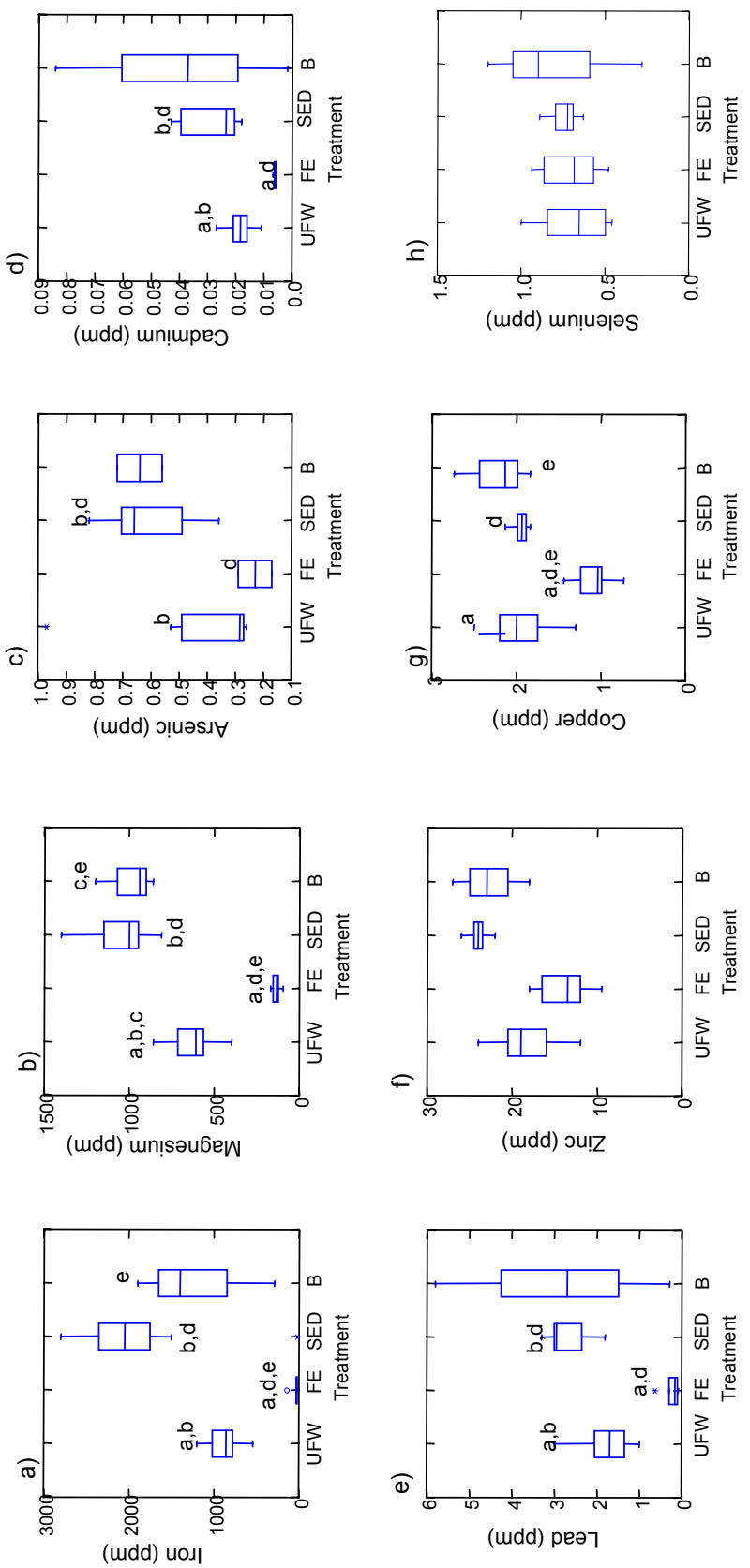


Figure 1. Metals detected in Kootenai River white sturgeon eggs from sediment (SED), Fuller's earth (FE), unfiltered water (UFW) and baseline (B) treatments. Letters indicate significant pairwise differences between treatments (Kruskal-Wallace and Mann-Whitney U tests; $P < 0.05$).

eggs and embryos from the SED treatment (20.6%;6-36 %) than from the FE treatment (12.6%; 10-16 %; Mann-Whitney U test; P = 0.036; Figure 2).

The null hypothesis stating no significant correlation between contaminant concentrations of copper or Aroclor 1260 and embryo mortality was rejected. Percent mortality for individual replicates within all three treatment groups showed a significant positive correlation with copper (Spearman; r = 0.568) and with the PCB Aroclor 1260 (Spearman; r = .800; Figure 3). However, no other significant correlations were found between other contaminant concentrations and embryo mortality.

Discussion

Rejection of the three test hypotheses for this experiment indicates that uptake of some metals and other contaminants as well as survival of embryos is related to rearing media. The significantly higher concentrations of arsenic, cadmium, copper, iron, magnesium and lead in embryos from the SED treatment than from the UFW and FE treatments indicates that in comparison with water and suspended solids, river-bottom sediment may be a more significant route for uptake of these metals. In support of these findings, Ramamoorthy and Ramamoorthy (1997) stated that although metals also partition into organic material in the water column, they are more likely associated and bound with sediments. Because baseline samples were collected following de-

Figure 2. Percent mortality in Kootenai River white sturgeon eggs from Fuller's Earth (FE) and sediment (SED) treatments (Mann-Whitney U test; $P < 0.05$). Error bars indicate standard deviation from the mean.

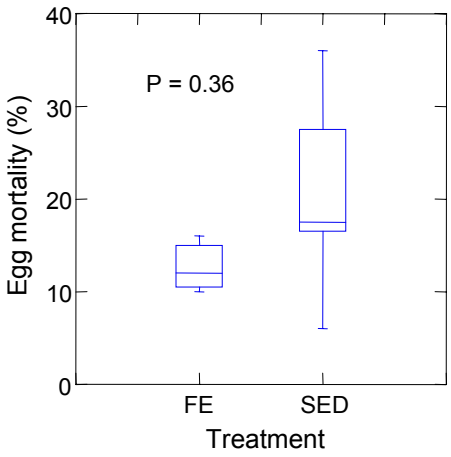
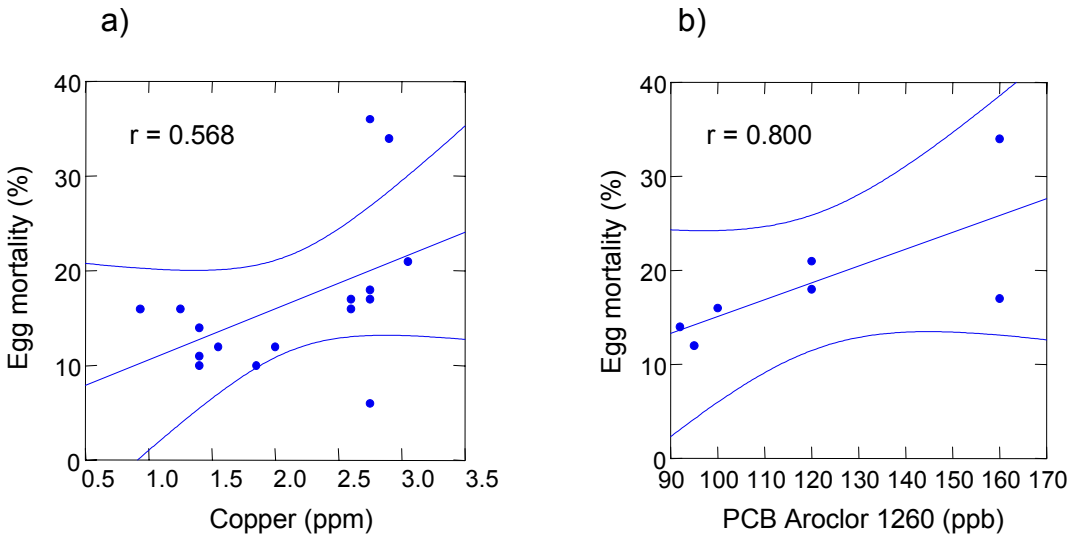


Figure 3. Correlations between copper (a) and the PCB Aroclor 1260 (b) concentrations and percent mortality of white sturgeon eggs and embryos from Fuller's Earth and sediment coated treatments (Spearman test; α=0.05). Brackets indicate 95% confidence intervals.



adhesion, high levels of metals in the baseline samples are likely associated with levels in de-adhesion media, weighted heavily by sediment-associated levels. However, contaminant levels in water do play a role in sediment toxicity because according to Spacie et al. (1995) contaminant uptake from sediments was affected by the sediment-water mixing that generally occurs near the substrate surface. Dual exposure to contaminants in sediment, suspended solids and water (in the UFW and SED treatments) resulted in higher uptake by embryos in these treatments in comparison to embryos in the FE treatment which were only exposed to contaminants in water.

The presence of Aroclor 1260 in some of the FE and SED samples, but not in any of the UFW samples or in the control from the UFW treatment, indicates that uptake of this PCB most likely took place during the later stages of development after the UFW eggs died. Research by Peterson and Kristenson (1998) suggests that embryos with a longer incubation period have higher uptake of lipophilic substances such as PCBs. The significant difference between Aroclor 1260 concentrations in SED and FE treatments suggested that sediment was a greater source of PCB uptake for the embryos than water. Although most research indicates that PCB uptake by aquatic organisms is higher from water than from other media, comparison of relative concentrations in sediment and water indicates that sediments generally contain higher concentrations and are more frequently the dominant source for bioavailability and uptake than water (Reynoldson 1987). Although the PCB Aroclor 1260 was not detected in

sediment samples collected from the lower Kootenai River it was detected in water (refer to Chapter 2).

No evidence was found from other studies to indicate that concentrations of arsenic, cadmium, iron, magnesium, lead or DDE observed in tissues from this study reduced embryonic survival in fish; however, concentrations of Aroclor 1260 and copper in the SED, FE and UFW treatments fell within ranges shown in several other studies to disrupt normal embryonic survival and development in other fish species. Although the significant relationship between PCB Aroclor 1260 concentrations and mortality appears to have been driven by two data points, Halter and Johnson (1974) and Roberts et al. (1978) both reported results from studies in which embryonic and early life stage mortality were increased by low-level exposure to PCBs. A study on Sheepshead Minnow (*Cyprinodon variegatus*) by Hansen et al. (1975) showed that concentrations of the PCB Aroclor 1254 greater than 5 ppb in fertilized eggs correlated with reduced survival of offspring. Concentrations of Aroclor 1260 in white sturgeon embryos from this study ranged from 34-160 ppb. In another study, low survival and a high rate of deformities (70%) of rainbow trout (*Oncorhynchus mykiss*) embryos occurred following exposure to two lesser chlorinated PCBs, Aroclor 1242 and 1254 (US Environmental Protection Agency 1980). Higher chlorinated PCBs are generally more toxic than those with fewer chlorine atoms (Roberts et al. 1978) and the Aroclor 1260 detected in the Kootenai River sturgeon eggs contained more chlorine atoms than those used in the study on trout. Stalling and Mayer (1972)

and Ankley et al. (1991) also found significant correlations between embryo mortality and PCB residues in Atlantic (*Salmo salar*) and chinook (*Oncorhynchus tshawytscha*) salmon.

The significant positive correlation between copper concentrations and embryo mortality suggests that copper is reducing survival and decreasing incubation time of white sturgeon eggs. Embryos from the SED treatment in this study began hatching 4 days earlier than the embryos in the FE treatment. The early-hatching embryos in this experiment also burst their yolk sacs during hatching. Similar findings were reported by Scudder et al. (1988) who found that copper decreased incubation time for fathead minnows (*Pimephales promelas*). The resulting larvae were physiologically pre-mature and showed less activity than unexposed larvae. Continuous embryonic exposure to contaminants produces changes in developmental rate during different stages. It can also result in decreased incubation time and pre-mature larvae that are incapable of many normal physiological functions. These pre-mature larvae tend to feed less and spend a great deal of time resting on the substrate where they are exposed to additional contaminants (Heath 1995; Borsuk 1998). One hypothesized reason for early hatching in copper-exposed embryos is quiescence (Sorenson 1991). It is thought that normal shivering and twitching distributes enzymes throughout the perivitelline space; however, quiescence prevents normal distribution of these enzymes and they build up near the head region, allowing more rapid degeneration of the chorion at this point and accelerated hatching.

High early mortality in the UFW treatment is most likely accounted for by fungal and bacterial growth that began within the first three days of incubation. Exposure to fungal growth during early gastulation can result in total mortality of sturgeon eggs (J. Siple Personal Communication Kootenai Tribe of Idaho, Bonners Ferry). Mortality rates were significantly lower in the SED and FE treatments and as fungus appeared, embryos in the UFW treatment died. Therefore, the organic matter in the suspended solids used for de-adhesion and the organic material in the unfiltered river water were likely the primary sources of bacteria and fungi.

The results of this study indicate that contaminants associated with adhered sediments and suspended solids may have a greater impact on embryonic survival and development than contaminants associated with water. Although Birge et al. (1987) and other authors believe that developing embryos are protected from impacts of external contaminants, there is also evidence to the contrary. Research by Scudder et al. (1988) indicated that the chorion is variably permeable to copper at specific developmental stages. Although embryos appear to be less sensitive than fry to copper, copper is more toxic to small fish than larger ones and any changes to egg permeability during embryogenesis allow for increased entry of copper into the chorion (Sorenson 1991). Eddy and Talbot (1983) found that the water hardening process in Atlantic salmon (*Salmo salar*) eggs could be inhibited or prolonged by the presence of

divalent metal ions. Therefore, exposure of the developing embryo to contaminants in rearing media may be prolonged and uptake increased because the hard, protective and otherwise relatively impermeable chorion is not promptly formed. According to McKim and Benoit (1974) uptake of aqueous copper by brook trout embryos magnified up to five times from baseline exposure levels. Although copper concentrations in sturgeon embryos from this experiment were lower than concentrations found in sediment samples, they were higher than concentrations found in water from the Kootenai River.

The objectives of this section focused on contaminant uptake during embryonic development and the relationships between resulting contaminant burdens and embryo mortality. One of the difficulties with this type of study is determining the proportion of contaminant that is actually incorporated into the eggshell from that which is attached to the outside of the shell or associated with the specific de-adhesion medium. Although the water-hardening process may imbibe contaminants into the egg, some contaminants inevitably remain bound with the outer shell or de-adhesion medium where they are not necessarily bioavailable to the developing embryo (Rosenthal and Alderdice 1976). Rate of contaminant uptake varies greatly between the embryonic and larval phases (Lukyanenko 1980) so it was necessary in this experiment to remove the larvae at or immediately prior to hatch when the chorion was still intact. As a result, the contaminant residues in eggs from this experiment include imbibed and adhesed concentrations. It has not been possible to rear viable eggs without any de-

adhesion medium (J. Siple Personal Communication, Kootenai Tribe of Idaho, Bonners Ferry) so the best measure of effects is a comparison among treatments of contaminant concentrations and mortality rates.

In conclusion, the bioavailable portions of the PCB Aroclor 1260 and copper in the Kootenai River system appear to be related to mortality in white sturgeon embryos. However, more controlled studies would be needed to establish an effect. Although the mortality rate in relation to contaminant exposure is not excessive, it may be an additional stress on viable reproduction in this sturgeon population. Based on the high mortality in the eggs coated with suspended solids and reared in unfiltered river water, it is recommended to address the effects and taxonomy of fungal and bacterial growth on fertilized sturgeon eggs in situ.

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CHAPTER THREE:

Tissue Residues and Effects of Bioaccumulated Contaminants on Physiological Parameters in Juvenile White Sturgeon

Abstract

This study was designed to determine the potential effects of bioaccumulated organochlorine, organophosphate, organonitrate, or carbamate pesticides, Aroclor 1200 series PCBs and metals on juvenile Kootenai River white sturgeon, *Acipenser transmontanus*. Juvenile hatchery-reared sturgeon that had been in the river for two years were captured to collect blood and whole-body tissues for chemical residue, cholinesterase, chromosomal DNA analysis and liver histology. Blood was also drawn from 10 adult female sturgeon to compare DNA variability between juveniles and adults. Tissues contained detectable levels of several metals and one organochlorine pesticide, DDE. Zinc concentrations showed a significant negative correlation with weight (Spearman; r = -0.455). Significant brain or blood cholinesterase inhibition resulting from exposure to organophosphate and carbamate pesticides did not occur. Increasing chromium levels correlated with lower blood AChE activity (Spearman; r = -0.630), suggesting decreased AChE activity with higher bioaccumulated levels of chromium. Increasing tissue chromium (Spearman; r = -0.630) and lead (Spearman; r = -0.900) levels correlated with lower blood serum butyrlcholinesterase (BChE), suggesting decreased AChE production in fish with high levels of bioaccumulated chromium and lead. Aluminum levels significantly correlated with lower brain BChE levels (r = -0.423), suggesting decreased AChE production with higher bioaccumulated levels of aluminum. The intrinsic variability of chromosomal DNA in juvenile (Mann-Whitney U-test; $P < 0.002$) and

adult (Mann-Whitney U-test; P = 0.026) sturgeon red blood cells significantly differed from the control blood cell population (chicken erythrocytes); however, variability between juvenile and adult sturgeon was not significantly different (Mann-Whitney U-test; P = 0.511). The intrinsic variability of chromosomal DNA in adult sturgeon correlated with ovarian tissue iron (Spearman; r = 0.862) and selenium levels (Spearman; r = -0.742). These results suggest that higher levels of bioaccumulated selenium may prevent or slow the rate of genetic damage but higher levels of bioaccumulated iron may actually increase genetic damage. Genetic analysis also indicated that one juvenile was a triploid, while one juvenile and an adult had an additional blood cell population. Liver histology suggested low-level contaminant exposure resulting in lymphocyte aggregation, increased melanin and focal necrosis. It was concluded that organophosphate, organonitrate and carbamate pesticides are not bioaccumulated in tissues and do not affect chlolinesterase activity; therefore, they may not be an immediate hazard to the health of juvenile Kootenai River sturgeon. It was also concluded that low-level exposure to chromium, lead, aluminum and iron are potentially resulting in slight alterations to cholinesterase production/activity and DNA integrity. Liver damage was not severe; however, a longer exposure period could potentially result in more severe damage.

Introduction

Survival of a fish beyond the embryonic and larval phases of life is not necessarily an indication that prior contaminant exposure did not affect the organism (Rosenthal and Alderdice 1976). Despite contaminant exposure, adult spawning may succeed and embryos may develop to hatch. However, histopathological alterations and changes in feeding, growth and developmental behavior in juveniles at later stages of life may potentially result from induced habitat avoidance, improper production of hormones and proteins, or genetic deformities in the sperm and eggs from which they originated (Rosenthal and Alderdice 1976).

Much like adult fish, juvenile fish can bioaccumulate significant concentrations of contaminants through dietary and dermal exposure (Heath 1995). Juvenile Kootenai River white sturgeon (*Acipenser transmontanus*) are potentially at high risk from both of these exposure routes because they live on the substrate and feed primarily on sediment-dwelling invertebrates (Paragamian et al. 1997). Biomagnification (uptake through diet) of metals and organochlorines may be especially high because these compounds sequester into sediments and organic matter, and are easily and inadvertently consumed by bottom-feeding organisms (Zaranko et al. 1997).

Bioaccumulation and biomagnification of sublethal levels of chemical compounds in young organisms may create stress and often result in retarded growth and development or abnormal behavior (Adams 1990; Heath 1995). Physiological tools such as histology, analysis of changes to genetic structure and monitoring of enzyme levels in relation to tissue contaminant levels have been used to assess the impacts of contaminant exposure on immature and non-reproductive organisms (Shugart 1990; Webber and Spieler 1994).

Cholinesterase, (ChE) a neurotransmitter enzyme, produced in the brain and circulated throughout the body (Cobb and Hooper 1994; Heath 1995), plays an important role in regulation of nerve impulse transmission at cholinergic synapses. Cholinesterase hydrolyzes neurotransmitter enzymes such as acetylcholine, thereby preventing it from accumulating around the synapse. Cholinesterase activity can occur in the form of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Although AChE is present in all fish, BChE is unique to the more primitive fishes such as Chondrosteans (Tong-Yuh Huang, Institute of Environmental and Human Health, Texas Tech University, Personal Communication).

The extent of ChE inhibition has been used to diagnose fish suffering from poisoning as a result of exposure to organophosphate and carbamate pesticides as well as metals (Rao and Rao 1984). Suppression of AChE and/or BChE activity and buildup of ChE at nerve and neuromuscular synapses, following

exposure to metal and organophosphate pesticide contaminants, can result in nerve damage, muscular contraction, paralysis, or death (Heath 1995; Ramamoorthy and Ramamoorthy 1997; Weber and Spieler 1994). Sublethal effects include depressed activities and behavior related to feeding, reproduction and self-defense. Separate determination of AChE and BChE strengthen the ChE evaluation method because BChE is particularly sensitive to organophosphate pesticides (Cobb and Hooper 1994).

Bioaccumulation and biotransformation of chemical compounds may also lead to cellular alterations (Monod et al. 1998). Genotoxic agents can break or cross-link chromosomes, resulting in unequal distribution among the daughter cells produced during natural cell division (LGL Limited 1999). The extent of chromosomal aberrations can be assessed through observation of DNA integrity and content within blood cells (Shugart 1990). Although chromosome formation is highly species-specific, an understanding of variability among species can make DNA damage detection a powerful tool for assessing genotoxic properties of environmental pollutants (Kohn 1983).

Because the liver is the primary organ for biotransformations of organic and probably also inorganic compounds, it can be of key importance when considering the action of toxic chemicals on fish (Heath 1995). The liver serves many functions and potential effects of contaminants are numerous. A wide variety of chemicals have been shown to cause lesions (focal and hepatocellular

alterations) in the livers of fish, indicating potential carcinogenesis or disruption of normal liver function (Heath 1995; Hawkins et al. 1995). Although neither methods for quantification of lesions nor a direct relation of liver lesions to chemical concentrations have been definitively established (Moore and Myers 1994), the presence or absence of lesions, tumors and other abnormalities may prove useful for indicating damage due to tissue contaminant burden.

This chapter focuses on the effects of contaminants on juvenile sturgeon. As with adult sturgeon, in order to determine the potential effects of contaminant exposure, it was necessary to couple tissue analysis with biomarker research (Fossi et al. 1994). The objectives of this chapter were to: 1) determine whole-body tissue concentrations of organochlorine, metal, PCB 1200 series Aroclors, organophosphate and carbamate compounds; 2) assess brain and blood acetylcholinesterase concentrations for potential disruption by organophosphate and carbamate pesticides; 3) determine the degree of genetic chromosome damage in relation to contaminant burden, through chromosomal DNA analysis; and 4) analyze liver tissue for assessment of damage or histopathological changes that may have resulted from contaminant exposure.

Materials and Methods

Twenty-five 4-year-old, hatchery-reared juvenile white sturgeon (released into the Kootenai River system in 1997) were collected in sinking multifilaent

gillnets, between river kilometers 203.5 and 234.4 (Paragamian et al. 1996). Sampling took place between July 1 and August 3, 1999. Capture and retention of juvenile sturgeon was permitted pursuant to application of a Section 10 permit for sampling of an endangered species (Endangered Species Act 16 U.S.C. 1531 *et seq*).

After sturgeon were removed from the net, a 2-ml sample of blood was drawn from the caudal vein for DNA and acetylcholinesterase analysis. An additional 10 blood samples from wild adult sturgeon and one duplicate set from a wild juvenile were collected for DNA analyses during adult sampling in the spring of 1999. The adult fish used for DNA analysis were also analyzed for ovarian tissue contaminant burden and plasma steroid concentrations (Chapter 1). After the blood was drawn, juvenile sturgeon were sacrificed for removal of liver and brain tissues. The remaining portions of carcass and viscera were divided equally for chemical analysis tests. Samples were stored on ice for 1-6 hours until they could be prepared, fixed or frozen for shipping to the laboratories for analysis. Tissue contaminant burden, DNA and ChE data were tested for normality of distribution with the expression $2(SE)\pm K$ (where SE is the standard error and K is a measure of kurtosis), and homogeneity of variances (Cochran's C-test; Kirk 1995).

Tissues

Whole-body tissues from the 25 juvenile sturgeon were processed for chemical residue analysis of 17 inorganic compounds, Aroclor 1200 series PCBs, organochlorine, organophosphate and carbamate pesticides and lipid content (Appendix 1). Selection of contaminants for analysis was based on historic data availability as well as presence, application and bioaccumulative potential of contaminants within the lower Kootenai River. Due to individual laboratory protocols, method detection limits and contaminants selected sometimes varied from detection limits that were set for adult ovarian tissue. Because data were not normally distributed and possessed heterogeneity of variances, they were rank transformed to test the null hypothesis that whole-body tissue concentrations of contaminants were not significantly correlated to fish weight or fork length (Spearman; $P < 0.05$).

Cholinesterase

Brain and blood samples were analyzed for cholinesterase at the Institute of Environmental and Human Health at Texas Tech University, Lubbock. Brain samples were weighed and homogenized (10-fold dilution, w/v, in 0.05 M tris buffer, pH 7.4) on ice in a glass Wheaton homogenizing tube equipped with a Teflon-coated pestle attached to an overhead stirrer. Approximately 10 strokes

were used to complete the homogenization. The samples were then diluted another 5-fold with the same buffer, giving a 50-fold overall dilution. Blood samples were diluted 5-fold in the 0.05 tris buffer (pH 7.4) and assayed. Cholinesterase (ChE) activities were measured in brain and serum samples using the method of Ellman et al. (1961), as modified by Gard and Hooper (1993). Modifications enabled use of a SPECTROmax 96-well spectrophotometric plate reader (Molecular Devices Corporation, Palo Alto, California) used in conjunction with a computer equipped with Softmax software (Molecular Devices Corporation). The substrate for the reaction was acetylthiocholine (AThCh). The spectrophotometer was set in a kinetic mode and measured absorption at 412 nm for three minutes with readings taken at thirteen second intervals with a zero second lag phase. Acetylcholinesterase (AChE) was differentiated from butyrylcholinesterase (BChE) by a five-minute pre-incubation with the specific BChE inhibitor, iso-OMPA (tetraisopropyl pyrophosphoramide, $1X10^{-3}$ M final concentration, in the incubation phase). Samples were run in triplicate at 25 ^{o}C. Cholinesterase activities were converted from optical density units/minute to μmoles AThCh hydrolyzed per minute (or "units") per ml plasma or g brain using the extinction coefficient, 13,600 $cm^{-1}M^{-1}$. Blood and brain BChE activity was calculated as the difference between total ChE and AChE acitivity.

Reactivation analysis of cholinesterase enzymes was performed to test for the presence of organophosphate-inhibited cholinesterases. Cholinesterase

enzymes were treated with 2-PAM (2-pyridinealdoxime methochloride) in order to bind and remove inhibiting organophosphates at the active site. Cholinesterase (AChE & BChE) activities were measured in both the fresh and incubated blood homogenates and analyzed for a post-incubation activity increase.

Diluted brain samples were divided into three 500 μl aliquots. One of the aliquots was assayed immediately for absolute ChE activity and maintained on ice. The other two aliquots were used for 2-PAM reactivation. One of the aliquots was spiked with 2-PAM (10 μl, $1X10^{-4}$ M) and the other with an equal volume of de-ionized water. These samples were incubated in a water bath at 25 ^{o}C. After 1 hour, sub-samples were removed from the incubating material and assayed for ChE activity.

The null hypothesis of no significant ChE inhibition related to carbamate pesticide exposure was determined by comparing the activity of the pre-incubation sample with that of the 2-PAM post-incubation samples (Student's T-test; $P < 0.05$). Spontaneous reactivation to a level greater than 20 percent higher than the pre-incubation sample was taken as indication that the sample contained carbamate-inhibited ChE.

An upper-tailed Student's t-test was then used to test the null hypothesis of no significant ChE inhibition related to organophosphate pesticide exposure. Mean activities of the 2-PAM and the non-2-PAM incubated samples were

compared to determine if there was a significant activity increase. An increase of at least five percent after 2-PAM incubation was considered significant and the sample was assumed to contain organophosphate-inhibited ChE.

The null hypotheses of no significant correlations between cholinesterase concentrations and absolute activity, capture date, weight, fork length and contaminant concentrations in juvenile Kootenai River white sturgeon were also tested using either the Pearson test for normally distributed data with homogeneous variances or the Spearman test for non-normally distributed data with heterogeneous variances ($P < 0.05$).

Genetics

Blood samples were shipped to International Ecogen in North Vancouver, B.C., Canada for genetic analysis by flow cytometry. A 100-µl sample of blood from each fish was fixed in citrate buffer. All blood samples were stored at –80^{o}C until analyzed. Prior to analysis, frozen blood cells were thawed rapidly at 37^{o}C and then placed on ice. They were washed twice in phosphate buffered saline (PBS) and the cells were counted in order to adjust the numbers to the optimum level for the staining protocol ($2X10^{6}$ cells per ml). A known volume of standard human lymphocytes was then added to chicken red blood cells (BioSure Controls, Grass Valley, California) as an internal control. Separate tubes of

chicken erythrocyte nuclei and human lymphocytes were stained at the same time as external controls.

The cells were fixed in 1.0 ml 0.5% paraformaldehyde for 10 minutes at 4^{o}C. The cells were then centrifuged and the supernatant removed. Membrane perforation was achieved by the addition of 1.0 ml 0.1% Triton X-100 (Pheoenix Flow Systems, San Diego, California) for 3 minutes at 4^{o}C followed by centrifugation and removal of the supernatant. The RNA, which if present would take up the flourescent dye and result in a false DNA reading, was removed by the addition of 0.1 ml of 1.0 mg/ml RNAse followed by incubating for 20 minutes at 37^{o}C. The cells were then proportionately bound to the nuclear DNA. The samples were allowed to stand for 1 hour in total darkness at 4^{o}C. Cells from each specimen were passed through a fine insulin syringe and then filtered through a 47 micron screen (Phoenix Flow Systems, San Diego, California) to remove any cell debris and any unbroken cell clumps. Prior to running of the sturgeon blood samples the instrument was calibrated, using DNA Check Beads (Coulter Corp., Miami, Florida) followed by human lymphocytes and chicken erythrocyte nuclei.

All thirty-six samples (11 adult and 25 juvenile) were processed on a Epic Elite Flow Cytometer (Coulter Corp., Miami, Florida) at a rate of about 150 cells per second, using an argon laser (488 nanometers). Duplicate blood samples

from an additional deformed wild juvenile were analyzed by flow cytometry but results were not included in statistical analysis.

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, Florida) 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 calculated by subtracting the CV value of the sturgeon red blood cells from the control population of chicken erythrocytes. The CVD values indicated the net variation between DNA content in sturgeon and control samples. Data were not normally distributed and displayed heterogeneous variances so non-parametric statistical analyses were used. The null hypotheses of no significant difference between CV values in adult or juvenile sturgeon and the CV values in the control samples were tested with a Mann-Whitney U-test ($P < 0.05$). The null hypothesis that CV values in adult and juvenile sturgeon were not significantly different was also tested with a Mann-Whitney U-test ($P < 0.05$). Blood cells were also analyzed for chromosome aberrations and evolution of new cell lines.

Organochlorine compounds and metals were measured in adult ovarian tissue (Chapter 1) and in juvenile whole-body tissue. The Spearman test ($P <$

0.05) was applied to rank transformed CVD and tissue contaminant burden data to assess correlations between chromosomal DNA variability and tissue contaminant concentrations.

Liver Histology

The 25 juvenile sturgeon livers were fixed in Davidson's Solution until processing. Individual liver samples were sliced into gross cross-sections, placed into cassettes and set into a Tissue-Tek 2 Processor for approximately 12 hours. The processor further fixed the tissue, stopped all enzyme activity, dehydrated the tissue to remove water and infiltrated sections with paraffin. Processed sections were placed into a vacuum infiltrator to eliminate all air voids in the tissue and were then transferred to a Tissue-Tek 2 Embedding Center, blocked in liquid paraffin and hardened for thin sectioning. An American Optical microtome was used to cut several 5-µn tissue sections of each liver. Thin sections were placed on a 45^{o}C water bath to settle out wrinkles. Two sections from each liver were chosen and placed on slides. The slides were placed in a warm oven for 1 hour, and stained (1 section from each liver with hemotoxin/eosin and 1 with giemsa). Sections were cover-slipped 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

Tissues

Whole-body tissue contained detectable concentrations of several inorganic compounds and two samples contained the organochlorine pesticide, DDE (Table 1). The null hypothesis that tissue zinc concentrations were not correlated to weight was rejected (Spearman; r = -0.455; Figure 1). This relationship was driven by one data point that was greater than 2 standard deviations from the mean. Removal of this outlier resulted in a non-significant relationship between weight and whole body zinc concentrations. Organophosphate and carbamate pesticides were not detected in whole-body tissue from juvenile sturgeon.

Cholinesterase

Acetylcholinesterase and BChE were detected in blood and brain samples from juvenile Kootenai River white sturgeon (Table 2). It appeared that as the percent of AChE in the blood increased, the percent of BChE decreased (Spearman; r = -1.00; Figure 2); however, absolute activity of AChE and BChE increased concurrently in blood serum (Spearman; r = 0.721). The null hypotheses of no significant ChE inhibition related to organophosphate

Table 1. Total number of samples, percent of total samples with parameter measurement, mean, standard deviation and range of values for each parameter (genetic chromosomal variability; CV difference, size, age, whole-body lipid and whole-body contaminant concentrations) in 25 hatchery-reared (broodyear 1995) juvenile Kootenai River white sturgeon, released in 1997 and captured between river kilometers 205.0 and 234.4 during July, 1999.

Parameter	Number of samples (% of total)	Mean (±st. deviation)	Range
CV difference	25 (100)	0.35 (0.42)	0.06 – 2.10
Fork length (cm)	24 (96)	37.9 (3.56)	31.0 – 45.0
Weight (kg)	23 (92)	0.26 (0.08)	0.15 – 0.45
Lipid (%)	25 (100)	3.58 (1.09)	1.40 – 6.00
Phosphorous (ppm)	25 (100)	5352 (1668)	2200 – 8600
Zinc (ppm)	25 (100)	17.3 (3.78)	9.90 – 24.0
Cadmium (ppm)	8 (32)	0.02 (0.01)	0.01 – 0.05
Lead (ppm)	5 (20)	0.95 (0.51)	0.57 – 1.80
Cobalt (ppm)	7 (28)	0.11 (0.04)	0.08 – 0.20
Nickle (ppm)	11 (44)	0.76 (1.01)	0.23 – 3.70
Manganese (ppm)	25 (100)	1.76 (1.19)	0.52 – 6.30
Iron (ppm	25 (100)	31.9 (68.2)	0.30 – 350
Chromium (ppm)	17 (68)	0.45 (0.34)	0.16 – 1.30
Magnesium (ppm)	24 (96)	285 (47.3)	200 – 360
Aluminum (ppm)	25 (100)	11.8 (11.1)	2.60 – 42.0
Vanadium (ppm)	24 (96)	0.16 (0.06)	0.08 – 0.26
Copper (ppm)	25 (100)	2.40 (4.27)	0.29 – 17.0
Calcium (ppm)	25 (100)	7397 (3496)	23.0 – 14000
Potassium (ppm)	25 (100)	2068 (238)	1600 – 2600
Sulfur (ppm)	25 (100)	2080 (206)	1800 – 2500
Sodium (ppm)	25 (100)	1696 (219)	1400 – 2100
Arsenic (ppb)	25 (100)	216 (87.1)	66.0 – 400
Selenium (ppm)	25 (100)	0.71 (0.11)	0.47 – 0.94
Mercury (ppm)	25 (100)	0.33 (0.05)	0.26 – 0.45
DDE (ppb)	2 (8)	72.0 (7.07)	67.0 – 77.0

Figure 1. Correlation between weight and whole body tissue concentrations of zinc in 25 hatchery-reared (brood year 1995) and released (1997) Kootenai River white sturgeon captured in July, 1999 (Spearman test; α = 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.

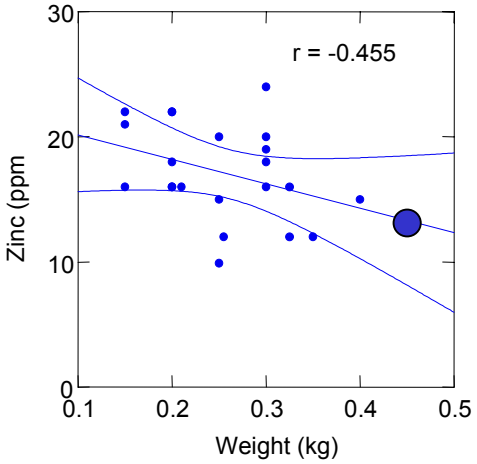
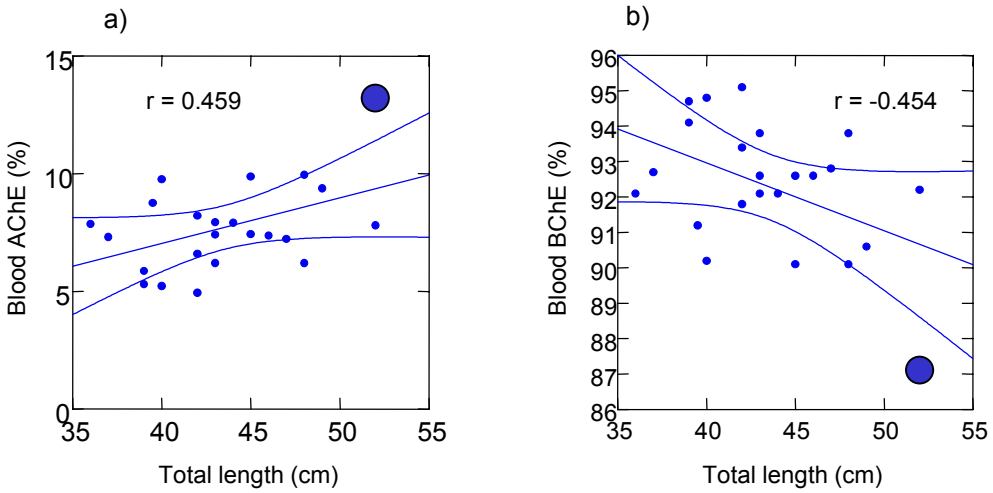


Table 2. Brain and blood acetylcholinesterase (AChE) and buturylcholinesterase (BChE) activity and percent of sample in 25 hatchery-reared (brood year 1995) and released (1997) juvenile Kootenai River white sturgeon captured during July, 1999.

Concentration	Brain				Blood			
	AChE		BChE		AChE		BChE	
	Amount in sample (%)	Activity (Units)	Amount in sample (%)	Activity (Units)	Amount in sample (%)	Activity (Units)	Amount in sample (%)	Activity (Units)
Mean concentration (± standard deviation)	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

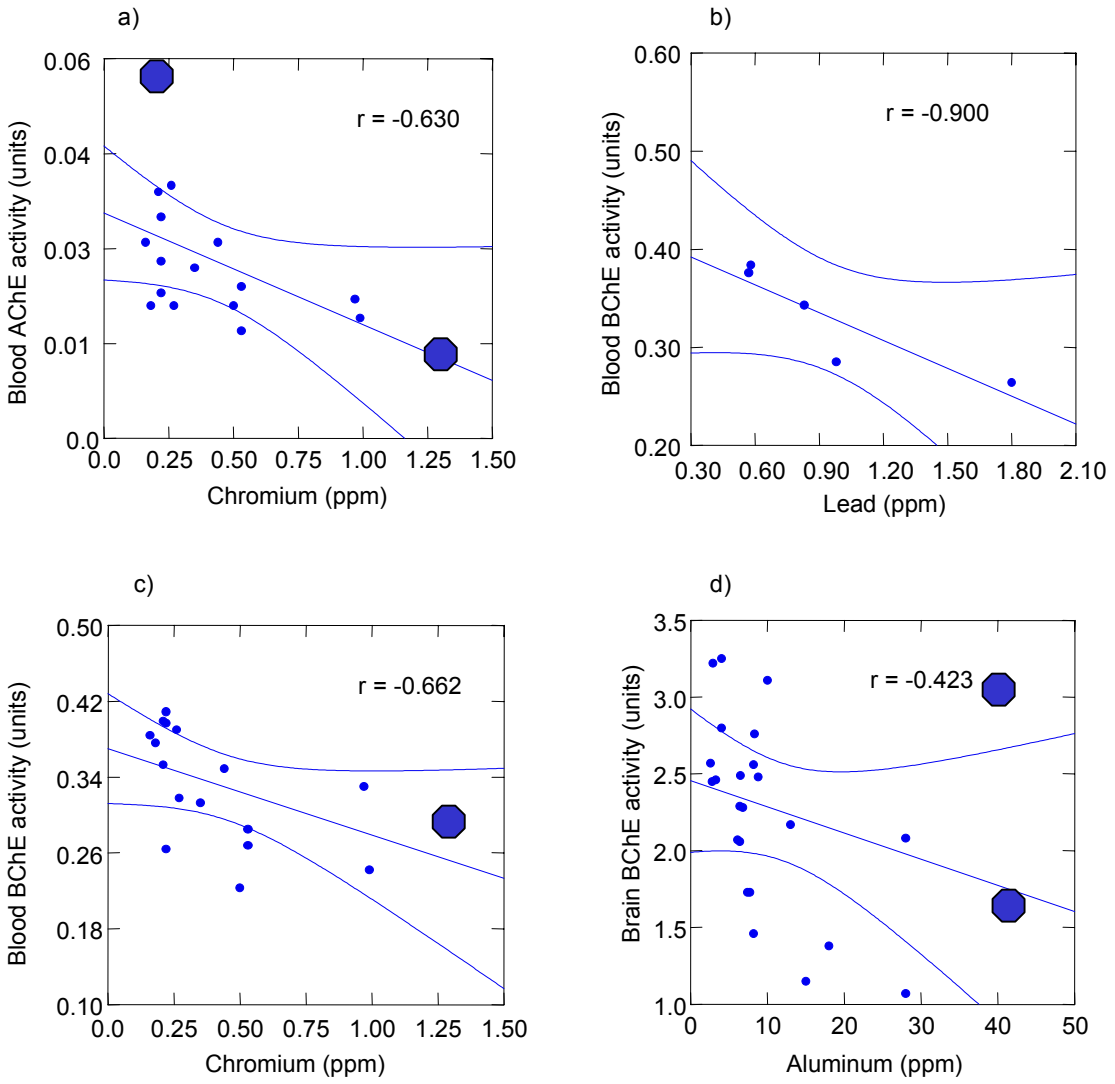
Figure 2. Correlations between total lenth (cm) and blood plasma acetylcholinesterase (AChE; a) and butyrylcholinesterase (BChE; b) levels in hatchery-reared (brood year 1995) and released (1997) juvenile Kootenai River white sturgeon captured during July, 1999 (Pearson rank correlation; α = 0.05). Large dot indicates points that are greater than 2 standard deviations from the mean and strongly influence the r value. Brackets indicate 95% confidence intervals.



(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 of no significant correlation between blood ChE concentrations and fish total length was rejected. Blood AChE concentrations showed a significant positive correlation with total length (Pearson; r = 0.459). Blood BChE concentrations showed a significant negative correlation with total length (Pearson; r = -0.454). Both of these correlations were weighted by one high data point (greater than 2 standard deviations from the mean) for both BChE and AChe activity. When these outliers were removed, the null hypothesis was not rejected.

The null hypothesis that ChE concentration and activity did not correlate with tissue concentrations of metals was rejected (Figure 3). Tissue concentrations of chromium showed a significant negative correlation with blood serum BChE (Spearman; r = -0.662) and AChE (Spearman; r = -0.630) activity. Tissue concentrations of lead negatively correlated with blood serum BChE activity (Spearman; r = -0.900). Increasing tissue concentrations of aluminum correlated with decreasing brain BChE acitivity (Spearman; r = -0.423). Relationships between the metals chromium and aluminum and ChE activites appear to have been driven by a few outliers (points greater than 2 standard deviations from the mean). Removal of these outliers did not change significance of the correlations (in some cases it resulted higher r values).

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



Genetics

The null hypotheses that the intrinsic variability of chromosomal DNA in either the juvenile (Mann-Whitney U-test; P = 0.026) or adult (Mann-Whitney U-test; P = 0.002) sturgeon red blood cells did not differ significantly from the control blood cell populations was rejected (Figure 4). The null hypothesis that intrinsic variability of chromosomal DNA in juvenile and adult sturgeon red blood cells were not significantly different (Mann-Whitney U-test; P = 0.511) was not rejected. Removal of outliers (points greater than 2 standard deviations from the mean) from the analysis did not change significance of the test.

The 10 adult sturgeon used for genetic analysis represented a range of weights, length, and ages (Table 3). They also possessed variable levels of contaminant residues in biopsied ovarian tissue. The null hypotheses of no significant correlation between CVD values and iron or selenium concentrations in adult ovarian tissue were therefore rejected (Figure 5). The CVD values for adult sturgeon showed a significant positive correlation with iron (Spearman; r = 0.862) and a significant negative correlation with selenium (Spearman; r = -0.742). The significant correlations for iron and selenium were potentially driven by one outlier (point greater than 2 standard deviations from the mean) for each metal and an outlier for DNA CV difference. If the outliers were removed from the data set, the correlation between selenium and CVD values in adults became

Figure 4. Variability of chromosomal DNA content (expressed as coefficient of variation or CV) in juvenile (a ,c) and adult (b, c) Kootenai River white sturgeon blood cells as compared to a control population of blood cells (Mann-Whitney U-test; P < 0.05).

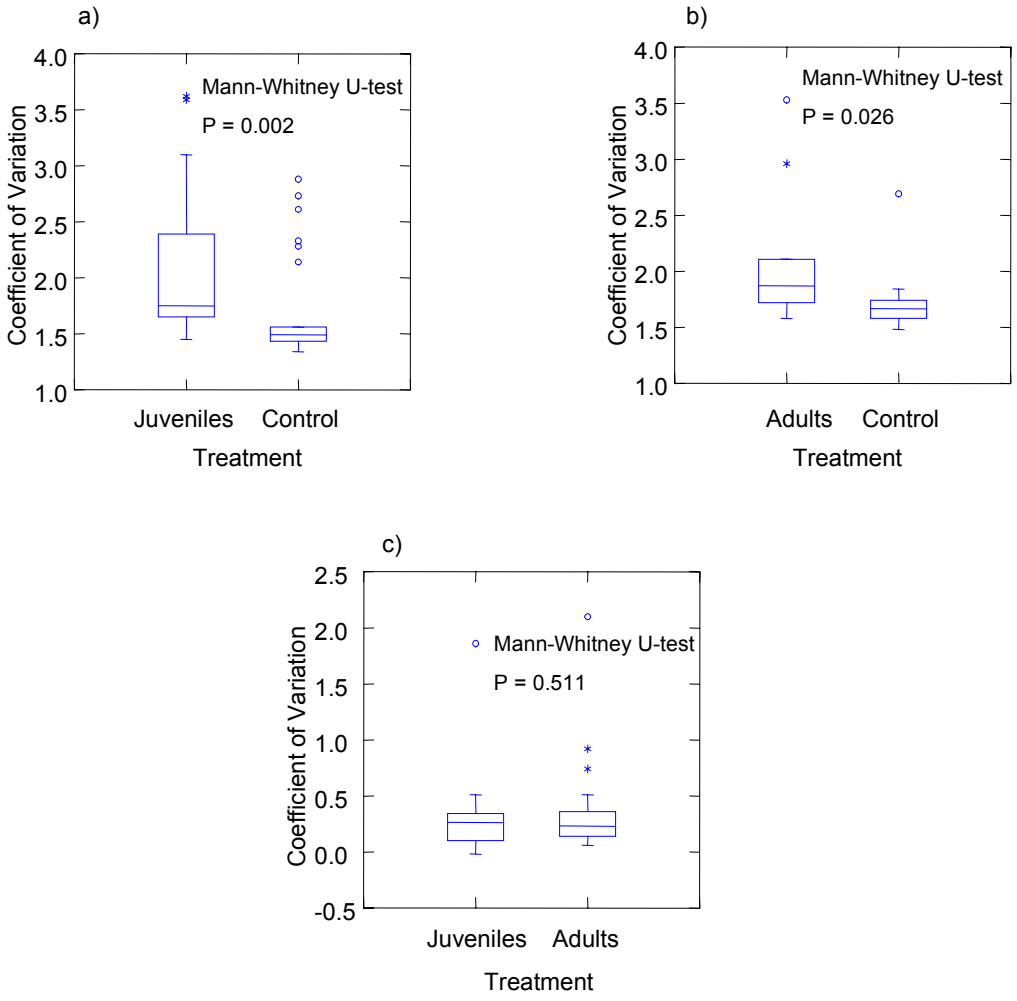
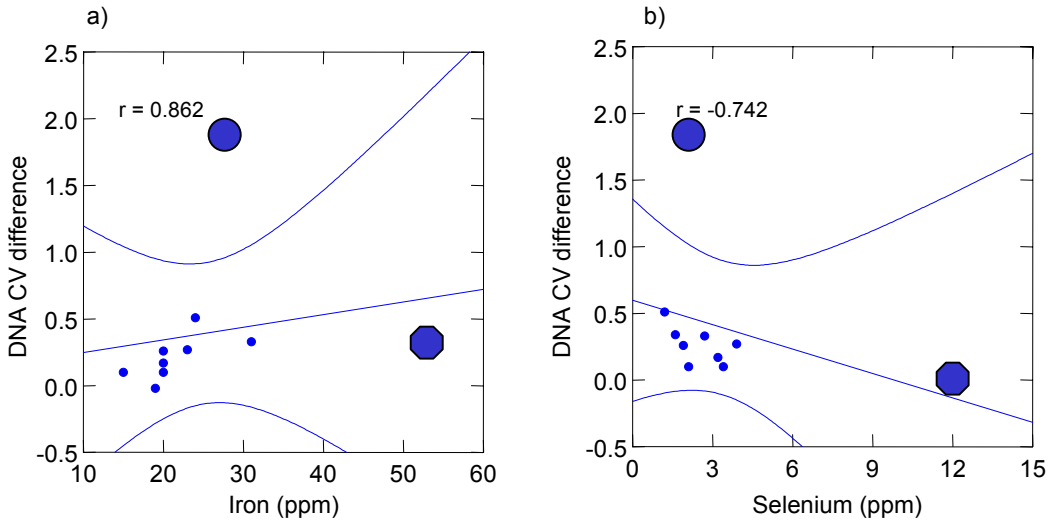


Table 3. Total number of samples, percent of total samples with parameter measurement, mean, standard deviation and range of values for each parameter (genetic chromosomal variability;CV difference, size, age, ovarian lipid and ovarian tissue contaminant concentrations) in 10 adult female Kootenai River white sturgeon captured between river kilometers 205 and 215.7 during March, 1999.

Parameter	Number of samples (% of total)	Mean (±standard deviation)	Range
CV difference	10 (100)	0.39 (0.55)	-0.02 – 1.86
Fork length (cm)	10 (100)	182 (19.5)	156 – 217
Weight (kg)	10 (100)	5.53 (17.8)	29.3 – 91.3
Age (years)	8 (80)	40.0	30.0 – 52.0
Lipid (%)	10 (100)	13.81 (3.83)	11.0 – 23.7
Arsenic (ppm)	4 (40)	0.43 (0.29)	0.19 – 0.80
Cadmium (ppm)	4 (40)	0.04 (0.04)	0.01 – 0.10
Copper (ppm)	8 (80)	4.35 (1.72)	1.70 – 6.90
Iron (ppm)	10 (100)	25.2 (10.7)	15.0 – 53.0
Lead (ppm)	4 (40)	0.23 (0.12)	0.11 – 0.40
Selenium (ppm)	10 (100)	3.38 (3.15)	1.20 – 12.0
Zinc (ppm)	10 (100)	26.9 (4.63)	21.0 – 36.0
DDE (ppb)	10 (100)	419 (592)	66.0 – 1800
DDT (ppb)	6 (60)	52.2 (20.5)	30.0 – 88.0
PCB Aroclor 1260 (ppb)	8 (80)	414 (249)	160 – 760

Figure 5. 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 test; α = 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.



non-significant but the correlation between iron and CVD values remained significant.

Of the 10 adult and 25 juvenile sturgeon blood samples analyzed by flow cytometry, one juvenile was a triploid, while another juvenile and an adult had an additional blood cell population with 27.6% and 27.4% respectively of DNA of the standard red blood cells. The juvenile had 16.7% haploid cells in the blood while the adult had 49.1% haploid cells.

Liver Histology

The 25 livers from juvenile sturgeon did not display extreme cell damage or abnomalities. Of the abnormalities noted, twenty (80%) of the samples contained small areas of lymphocytic aggregations, 24 (96%) contained higher melanin than other samples, 8 (32%) showed focal necrosis and 1 (4%) contained higher levels of fatty tissue than the other samples.

Discussion

Some contaminants compartmentalize into specific areas of the body and metals are often permanently sequestered into bony structures (C. Schmidt Personal Communication, US Geological Survey Columbia, Missouri); therefore, it is difficult to determine the proportion of bioavailable contaminants from whole-

body residue measurements. It is thus unadvisable to base an evaluation of the effects of contaminants on physiological parameters by relying strictly on whole-body tissue residue data.

In this study, the higher zinc concentrations were associated with lower weight of juvenile sturgeon. A possible explanation for this correlation is that as a fish grows, the proportion of the body weight consisting of tissues that accumulate and retain the greatest amount of zinc (ie. organs and gut) decreases (C. Schmidt Personal Communication, US Geological Survey Columbia, Missouri).

Although the relationships appear to be driven by a couple high data points, the results of the cholinesterase study suggest that larger fish possess higher levels of AChE and lower levels of BChE. Under normal conditions, increasing length generally accompanies increasing age or maturity. Gard and Hooper (1993) found an age-dependant variability of ChE activity in avian brains; therefore, a length-dependent variability of ChE activity could have been expected in sturgeon as well.

Organophosphate or carbamate pesticides were not detected in whole-body tissue from juvenile Kootenai River white sturgeon so if residues are present in the aquatic system, they did not bioaccumulate in tissues. In addition, ChE inhibition resulting from exposure to organophosphate or carbamate

pesticides was not detected in blood plasma or brain tissue. In conclusion, these pesticides, which are applied to agricultural lands within the lower Kootenai River, do not appear to be present at levels that inhibit acetylcholine hydrolysis in juvenile sturgeon. Although organophosphate and carbamate pesticides can break down within hours of application (Miyamoto et al. 1979) the rate of application is highest during the summer, which is when the fish were collected for this study. If these pesticides were present at extremely high levels in the aquatic system, residues may have been detected in juvenile sturgeon tissues or in the water samples analyzed for the study in Chapter 1. It is likely that riverbank diking (which controls runoff) and careful application of pesticides to agricultural lands is preventing these products from entering the river at high enough concentrations to affect the sturgeon.

The significant negative correlations between blood serum BChE/AChE and whole-body metal concentrations indicate potential suppression of activity in these enzymes with increasing 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 normal stimuli (Weber and Spieler 1994).

The intrinsic variability of red blood cell chromosomal DNA in juvenile and adult sturgeon differed significantly from the control blood cell population. The

detected differences are likely due to an inappropriate selection of a control blood cell population. In this study, chicken erythrocytes were used as the control population. According to Birstein et al. (1993) the difference in chromatin 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.

Although one juvenile sturgeon was a triploid and an additional juvenile and adult possessed approximately one-quarter the amount of DNA found in the control red blood cells, these slight variations in chromosomal DNA appear to be a common occurrence within Acipenseriformes. According to Birstein et al. (1997), Polydontidae (paddlefish) and Acipenseridae (sturgeon) originated from a tetraploid ancestor with variable DNA content. Recent research by Birstein et al. (1997) and Blacklidge and Bidwell (1993) cited triploid, tetraploid and octaploid occurrences in several species of sturgeon. Birstein et al. (1997) also cited one instance where a closely related species, the paddlefish (*Polyodon spathula*), possessed only one set of chromosomes. Van Eenennaam et al. (1996) cited several instances and forms of polyploidy in white sturgeon, following temperature shock to embryos. It is probable that natural occurrence of polyploidy in sturgeon (rather than DNA damage due to contaminant exposure) explain the triploid chromosomes found in the two juvenile and one adult Kootenai River white sturgeon.

The presence of a correlation between 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. Easton (1997) showed that an increase in the variation may be dose-dependent. Selenium is considered to be an antioxidant that destroys free radicals, preventing them from damaging cells and molecules; however, it is unknown whether or not it also plays a role in repairing damaged cells and molecules. The strong negative relationship between selenium and CVD values was heavily weighted by one high data point for selenium so no firm conclusion 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 exposure. Un-bound 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. Depending upon the physiological binding site for iron (within the organism), DNA mutations may be expressed as cross-links or broken strands (Lloyd and Phillips 1998). Some genotoxic chemicals such as metals, also act by blocking the DNA repair capability within a cell, thus enabling breakage to go unrepaired (International Ecogen 1999).

Although the flow cytometry method is not conclusive in determining genetic mutations, the CVD values for the deformed wild juvenile sturgeon did not indicate gross abnormal chromosomal expression. The physical deformities in this fish may have been caused by other exogenous factors during or following the embryonic period rather than by genetic mutation within the parents.

Liver histology indicated small areas of lymphocytic infiltration or aggregation that may have been caused by infectious agents of low virulence. These associated viruses may or may not cause significant cellular injury. Although the presence of lymphocytic aggregations is fairly common and probably normal, they can also result from toxic agents capable of causing minor cellular damage within the liver tissue. Cytoplasmic inclusions 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). Leland (1983) also showed that exposing brown trout to copper and zinc resulted in swollen cells and aggregate granules in the liver. It is unclear whether these granules were due to aggregation for excretion or the result of normal sequestration of abnormally high levels of otherwise essential trace elements. Whether the factor(s) causing aggregations in the sturgeon livers are infectious or toxic, exposure has not resulted in a more vigorous inflammatory reaction.

The sturgeon liver samples also contained slightly abnormal levels of melanin. Melanin is a semiconductor that has been found to associate with

manganese (Committee on Biologic Effects of Atmospheric Pollutants 1973). Increased melanin in fish livers sometimes occurs with increasing age or with increasing melanomacrophage activity that is induced by agents such as low level toxic exposure or pollutants (J. Morrison, US Fish and Wildlife Service Olympia Fish Health Center, Washington). All of the liver samples were taken from young fish (4 years old) so contaminant exposure, rather than age, is a more probable cause of increased melanin. Focal necrosis (death of cells) in the sturgeon livers can be a result of improper fixing of the sample; however, Heath (1995) noted that focal necrosis can also result from exposure to pollutants. Although this study indicated only slight liver damage in juvenile sturgeon, these fish were exposed to environmental conditions in the Kootenai River for only two years. A longer exposure period may result in more severe damage. Therefore, this portion of the study should be duplicated in the future.

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Conclusion

Agricultural pesticides (organophosphates, organonitrates and carbamates) were not detected in water, sediment or tissue samples from the lower Kootenai River and do not appear to disrupt acetylcholinesterase production in juvenile white sturgeon. However, existing levels of inorganic metals and organochlorine compounds may potentially be affecting reproduction and they are also potentially impacting DNA expression and physiological integrity of the liver in younger sturgeon. The results of this study also suggest that river-bottom sediments may increase exposure of embryonic sturgeon to copper and Aroclor 1260, causing higher embryonic mortality.

Tissue concentrations of zinc appear to have increased within the past 10 years, indicating a potential increase in bioavailability of this metal in the aquatic system. Tissue concentrations of copper, lead and DDE and river-bottom sediment levels of copper and zinc are not significantly different from levels in samples collected 10 years ago, suggesting that tissue and sediment-associated concentrations of these metals has neither increased nor decreased.

Although contaminant levels may not completely prevent spawning in adult sturgeon the egg size, timing of embryonic incubation and survival of embryos are potentially affected by bioaccumulated contaminants. Plasma steroid levels in Kootenai River white sturgeon were comparable to those detected in other

populations of sturgeon; however, the inhibition of male androgen steroids by zinc and Aroclor 1260, and induction of the female hormone estradiol by DDT, suggests potential feminization and lowered sperm production in the male Kootenai River white sturgeon. Therefore, I recommend duplication of significant results (from this study) in order to determine if a consistent pattern is observed among biomarker and contaminant level relationships.

Of the compounds detected in different aquatic media, copper, zinc, DDT, the PCB Aroclor 1260 and possibly iron are of greatest concern for their effects on the sturgeon and other aquatic species. Detected levels of these compounds have been shown to cause decreased nutrient productivity in aquatic systems as well reduced fecundity and habitat avoidance in aquatic species. Levels of several metals and the PCB Aroclor 1260 exceed EPA freshwater criteria for aquatic life and results from this study indicates that some of these compounds may reduce reproductive fitness in the sturgeon. Therefore, contaminants in the Kootenai River system are potentially creating additional stress (perhaps in the form of diminished function) on the white sturgeon population at various life stages. I recommend further research into contaminant discharge from mining, agricultural and hydroelectric operations in order to determine the exact source(s) of metal and organochlorine contaminants and implement remedial efforts to reduce contaminant loading in the Kootenai River system.

Appendices

Appendix 1. Sample type, collection method and date, test analyte, laboratory and applicable EPA test method for contaminant analysis of Kootenai River sturgeon tissue, water and sediment samples.

Sample type	Collection method	Collection date	Test analyte category and individual compounds	Contract Laboratory	EPA Method	Sample detection limits* *	Spike Recovery (%)
Ovarian tissue	Biopsy	1997-1999	**Polychlorinated biphenyls (PCBs)**	AMTEST Inc., Redmond WA	8080/3050/ 3040	Aroclor 1221: 120-1600 ppb All others: 58-400 ppb	99.9-118.3
			Aroclor 1221				
			Aroclor 1232				
			Aroclor 1242				
			Aroclor 1248				
			Aroclor 1254				
			Aroclor 1260				
			Organochlorine pesticides	AMTEST Inc., Redmond WA	8080/3050/ 3040		56-129
			a-BHC			5.1-24 ppb	
			Lindane			5.1-24 ppb	
			Heptachlor			3.8-18 ppb	
			Aldrin			5.1-24 ppb	
			B-BHC			7.6-36 ppb	
			d-BHC			8.9-42 ppb	
			Heptachlor epoxide			5.1-24 ppb	
			Endosulfan I			7.6-36 ppb	
			pp-DDE			7.6-36 ppb	
			Dieldrin			7.6-36 ppb	
			Endrin			8.9-42 ppb	
			pp-DDD			8.9-42 ppb	
			Endosulfan II			5.1-24 ppb	
			pp-DDT			18-84 ppb	
			Endrin Aldehyde			18-84 ppb	
			Endosulfan Sulfate			14-66 ppb	
			Methoxychlor			33-160 ppb	
			Toxaphene			800-4800 ppb	
			Chlordane			84-400 ppb	
			Metals				
			Arsenic	AMTEST Inc., Redmond WA	7060	.1 ppm	67.7-119
			Cadmium		7131	.00 ppm	
			Copper		6010	.1-.2 ppm	
			Iron		6010	.5-1.0 ppm	
			Mercury		7471	.1 ppm	
			Lead		7421	.1 ppm	
			Selenium		7740	.1 ppm	
			Zinc		6010	.1-.2 ppm	

Appendix 1 continued. Sample type, collection method and date, test analyte, laboratory and applicable EPA test method for contaminant analysis of Kootenai River sturgeon tissue, water and sediment samples.

Sample type	Collection method	Collection date	Test analyte category and individual compounds	Contract Laboratory	EPA Method	Sample detection limits*	Spike Recovery (%)
Ovarian tissue	biopsy	1987-1989	**Organochlorogen pesticides**	AMTEST Inc. Redmond WA	8140		53-113
			Butylate			.1- 2 ppm	
			Chlorpropham			.2- 3 ppm	
			Hexazinone			.2- 4 ppm	
			Metalachlor			.2- 4 ppm	
			Metribizin			.1- 2 ppm	
			Prometryn			.1- 2 ppm	
			Propazine			.1- 2 ppm	
			Simazine			.1- 2 ppm	
			Terbutryn			.1- 2 ppm	
			Triadimefon			.2- 3 ppm	
			Organophosphorous pesticides	AMTEST Inc. Redmond WA	8140		29-89
			Azinphosmethyl			.24-1.0 ppm	
			Chlorfenvinphos			.1- 4 ppm	
			Chlorpyrifos			.07- 3 ppm	
			Demeton			1.0 ppm	
			Diazinon			.09- 2 ppm	
			Dimethoate			.1- 4 ppm	
			Disulfoton			.2 ppm	
			Ethion			.08- 3 ppm	
			Fenthoathion			.08- 5 ppm	
			Fenthion			.5 ppm	
			Fonofos			.07-1.5 ppm	
			Isofenphos			.18- 8 ppm	
			Malathion			.07- 5 ppm	
			Methidathion			.1- 3 ppm	
			Methyl-parathion			.08- 2 ppm	
			Parathion			.07- 2 ppm	
			Phorate			.07- 2 ppm	
			Phosalone			.1- 5 ppm	
			Phosmet			.12- 5 ppm	
			Terbufos			.1-1.0 ppm	
			Tetrachlorvinphos			.11- 23 ppm	

Appendix 1 continued. Sample type, collection method and date, test analyte, laboratory and applicable EPA test method for contaminant analysis of Kootenai River sturgeon tissue, water and sediment samples.

Sample type	Collection method	Collection date	Test analyte category and individual compounds	Contract Laboratory	EPA Method	Sample detection limits*	Spike Recovery (%)
Ovarian tissue			Carbamate pesticides	ANTEST Inc., Redmond WA	8140		66-132
			Aldicarb Sulfoxide			1.0 ppm	
			Aldicarb Sulfone			1.0 ppm	
			Oxamyl			1.0 ppm	
			Methomyl			1.0 ppm	
			3-OH Carbofuran			0.6 ppm	
			Aldicarb			1.0 ppm	
			Propoxur			.3-.6 ppm	
			Carbofuran			.3-.6 ppm	
			Carbaryl			.3-.6 ppm	
			Methiocarb			.3-.6 ppm	
Fertilized eggs/ embryos	Spawning	1998	**Polychlorinated biphenyls (PCBs)**	ANTEST Inc., Redmond WA	8080/3550/ 3540		69-94.6
			Aroclor 1221			55-360 ppb	
			Aroclor 1232			55-91 ppb	
			Aroclor 1242			55-91 ppb	
			Aroclor 1248			55-91 ppb	
			Aroclor 1254			55-91 ppb	
			Aroclor 1260			55-360 ppb	
			Organochlorine pesticides	ANTEST Inc., Redmond WA	8080/3550/ 3540		64-127
			a-BHC			13-22 ppb	
			Lindane			13-22 ppb	
			Heptachlor			9.9-16 ppb	
			Aldrin			14-22 ppb	
			B-BHC			20-33 ppb	
			d-BHC			23-38 ppb	
			Heptachlor epoxide			13-22 ppb	
			Endosulfan I			20-33 ppb	
			pp-DDE			23-33 ppb	
			Dieldrin			20-33 ppb	
			Endrin			23-38 ppb	
			pp-DDD			23-38 ppb	
			Endosulfan II			13-22 ppb	
			pp-DDT			46-76 ppb	
			Endrin Aldehyde			46-76 ppb	
			Endosulfan Sulfate			36-60 ppb	

Appendix 1 continued. Sample type, collection method and date, test analyte, laboratory and applicable EPA test method for contaminant analysis of Kootenai River sturgeon tissue, water and sediment samples.

Sample type	Collection method	Collection date	Test analyte category and individual compounds	Contract Laboratory	EPA Method	Sample detection limits*	Spike Recovery (%)
Fertilized eggs/ embryos	Spawning	1999	**Organochlorine pesticides**	AMTEST Inc., Redmond WA	8080/3580/ 3640		54-127
			Methoxychlor			99-140 ppb	
			Toxaphene			2600-4400 ppb	
			Chlordane			220-350 ppb	
			Metals	AMTEST Inc., Redmond WA			
			Arsenic		7060	.1 ppm	
			Cadmium		7131	.02 ppm	
			Copper		6010	.1 ppm	
			Iron		6010	.5 ppm	
			Lead		7421	1.0 ppm	
			Mercury		7421	.1 ppm	
			Selenium		7740	.1 ppm	
			Zinc		6010	.1 ppm	
Juvenile body tissue	Gillnet sacrifice	June/July 1999	**Polychlorinated biphenyls (PCBs)**	University of Idaho, Analytical Sciences Laboratory, Moscow ID			78.9-100.2
			Aroclor 1260		8081	0.1 ppm	
			Organochlorine pesticides		8081	0.05 ppm	85.7-100.3
			a-BHC				
			g-BHC				
			b-BHC				
			Heptachlor				
			d-BHC				
			Aldrin				
			Heptachlor epoxide	University of Idaho, Analytical Sciences Laboratory, Moscow ID			
			Chlordane				
			Endosulfan I				
			4,4' DDE				
			Dieldrin				
			Endrin				
			4,4' DDD				
			Endrin Aldehyde				
			Endosulfan Sulfate				

Appendix 1 continued. Sample type, collection method and date, test analyte, laboratory and applicable EPA test method for contaminant analysis of Kootenai River sturgeon tissue, water and sediment samples.

Sample type	Collection method	Collection date	Test analyte category and individual compounds	Contract Laboratory	EPA Method	Sample detection limits*	Spike Recovery (%)
Juvenile body tissue	Gillnet sacrifice	June/July 1999	**Organochlorine pesticides**	University of Idaho, Analytical Sciences Laboratory, Moscow ID	8081	0.05 ppm	85.7-100.3
			Methoxychlor				
			Toxaphene				
			Metals				
			Molybdenum			0.5 ppm	
			Phosphorus			0.62 ppm	
			Zinc			0.325 ppm	
			Cadmium			0.37 ppm	
			Lead			0.5 ppm	
			Cobalt			0.376 ppm	
			Nickel			0.22 ppm	
			Manganese			0.028 ppm	
			Iron			0.03 ppm	
			Chromium			0.15 ppm	
			Magnesium			0.043 ppm	
			Aluminum			0.78 ppm	
			Copper			0.075 ppm	
			Calcium			0.23 ppm	
			Potassium			0.056 ppm	
			Sulfur			9 ppm	
			Sodium			4.2 ppm	
			Selenium			1.4 ppm	
			Arsenic		ICP, Hydride	0.005 ppm	
			Mercury		ICP Hydride	11 ppb	
					ICP, cold vapor	0.005 ppm	
			Organophosphorous pesticides			5 ppb	
				Washington State University Environmental Quality Lab, Richland WA			

Appendix 1 continued. Sample type, collection method and date, test analyte, laboratory and applicable EPA test method for contaminant analysis of Kootenai River sturgeon tissue, water and sediment samples.

Sample type	Collection method	Collection date	Test analyte category and individual compounds	Contract Laboratory	EPA Method	Sample detection limits*	Spike Recovery (%)
Juvenile body tissue	Gillnet sacrifice	June/July 1999	**Carbamate pesticides**	Washington State University, Environmental Quality Lab, Richland WA		2 ppb	
Sediment	Ponar Dredge	June 1999	**Polychlorinated biphenyls (PCBs)** Aroclor 1016 Aroclor 1221 Aroclor 1232 Aroclor 1242 Aroclor 1248 Aroclor 1254 Aroclor 1260	CDI Analytical Laboratories, Everett WA	8082 MOD	0.1 mg/kg	61-110 %
			Organochlorine pesticides a-BHC g-BHC b-BHC Heptachlor d-BHC Aldrin Heptachlor epoxide Chlordane Endosulfan I 4,4' DDE Endosulfan II 4,4' DDT Dieldrin Endrin	CDI Analytical Laboratories, Everett WA	8081	Toxaphene 0.5 mg/kg Chlordane 0.02 mg/kg All others 0.01 mg/kg	61-110 %

Appendix 1 continued. Sample type, collection method and date, test analyte, laboratory and applicable EPA test method for contaminant analysis of Kootenai River sturgeon tissue, water and sediment samples.

Sediment	Ponar Dredge	June 1999	**Organochlorine pesticides** 4,4' DDD Endrin Aldehyde Endosulfan Sulfate Methoxychlor Toxaphene	CCI Analytical Laboratories, Everett WA	8081	Toxaphene 0.6 mg/kg Chlordane 0.02 mg/kg All others 0.01 mg/kg	81-110 %
			Metals Arsenic Cadmium Copper Iron Lead Mercury Selenium Zinc Manganese	CCI Analytical Laboratories, Everett WA	Mercury 7471 All others 6010	0.6 mg/kg 0.1 mg/kg 0.1 mg/kg 0.3 mg/kg 1.6 mg/kg .025 mg/kg 1.0 mg/kg 0.2 mg/kg .8 mg/kg	
			Organonitrogen pesticides Butylate Chlorpyrifon Hexazinone Metolachlor Metribuzin Prometryn Propazine Simazine Terbutryn Triadimefon	APPL, Fresno CA	8141A MOD		
			Organophosphorous pesticides	Washington State University Environmental Quality Lab, Richland WA CCI Analytical Laboratories, Everett WA	9089	.3 ppb	

Appendix 1 continued. Sample type, collection method and date, test analyte, laboratory and applicable EPA test method for contaminant analysis of Kootenai River sturgeon tissue, water and sediment samples.

Sample type	Collection method	Collection date	Test analyte category and individual compounds	Contract Laboratory	EPA Method	Sample detection limits*	Spike Recovery (%)
Sediment	Ponar Dredge	June 1999	**Carbamate pesticides**	Washington State University, Environmental Quality Lab, Richland WA		0.7 ppb	
			Total Organic Carbon	CCI Analytical Laboratories, Everett WA			
Water	LaMotte sampler	June 1999	**Polychlorinated biphenyls (PCBs)**	CCI Analytical Laboratories, Everett WA	8082 MOD		61-110 %
			Aroclor 1016				
			Aroclor 1221				
			Aroclor 1232				
			Aroclor 1242				
			Aroclor 1248				
			Aroclor 1254				
			Aroclor 1260				
			Organochlorine pesticides	CCI Analytical Laboratories, Everett WA	8081		61-110 %
			a-BHC				
			g-BHC				
			b-BHC				
			Heptachlor				
			d-BHC				
			Aldrin				
			Heptachlor epoxide				
			Chlordane				
			Endosulfan I				
			4,4' DDE				
			Dieldrin				
			Endrin				
			4,4' DDD				
			Endosulfan II				
			4,4' DDT				
			Endrin Aldehyde				
			Endosulfan Sulfate				
			Methoxychlor				
			Toxaphene				

Appendix 1 continued. Sample type, collection method and date, test analyte, laboratory and applicable EPA test method for contaminant analysis of Kootenai River sturgeon tissue, water and sediment samples.

Sample type	Collection method	Collection date	Test analyte category and individual compounds	Contract Laboratory	EPA Method	Sample detection limits*	Spike Recovery (%)
Water	LaMotte sampler	June 1999	**Metals**	DCI Analytical Laboratories, Everett WA			
			Arsenic		6010	0.03 mg/l	
			Cadmium		6010	0.005 mg/l	
			Copper		6010	0.006 mg/l	
			Iron		6010	0.01 mg/l	
			Lead		6010	0.06 mg/l	
			Managanese		7471	0.0005 mg/l	
			Mercury		6010	.0002 mg/l	
			Selenium		6010	0.05 mg/l	
			Zinc		6010	0.007 mg/l	
			Organonitrogen pesticides	APPL, Fresno CA	8141A NCD		
			Butylate				
			Chlorpropham				
			Hexazinone				
			Metalachlor				
			Napropamide				
			Prometryn				
			Prometone				
			Simazine				
			Terbutryn				
			Triadimefon				
Water	LaMotte sampler	June 1999	**Organophosphorous pesticides**	APPL, Fresno CA		0.1 ppb	
			Carbamate pesticides	Washington State University, Environmental Quality Lab, Richland WA		25 ppt	
				Washington State University, Environmental Quality Lab, Richland WA			

Appendix 2. Sex, developmental stage, age, capture date, capture location (rkm), fork length (cm) and weight (kg) for all male, female, sexually immature adult and juvenile sturgeon captured during 1997-1999 for steroid and ovarian tissue contaminant analysis, egg size, genetic assessment, acetylcholinesterase and liver lesion analysis.

PIT tag number	Sex	Sexual develop-ment stage	Age[1]	Capture date	Capture location (rkm)	Fork length (cm)	Weight (kg)
7F7D0A3E78	Male	8	35	3-24-98	208.0	176	39.6
7F7D371E5A	Male	8	30	3-24-98	215.3	160	29.3
7F7DOF6D0C	Male	8	32	3-25-98	215.4	186	48.2
7F7D364D37	Male	8	31	3-25-98	208.0	157	31.5
7F7D364BF4	Male	8	39	3-25-98	215.4	140	10.4
7F7F117D29	Male	7	34	3-10-98	215.5	148	23.3
7F7D337121	Male	8	26	3-2-98	208.0	184	45.0
7F7F403D5A	Male	8	36	3-19-98	213.3	173	38.3
7F7D3F695A	Male	8	33	3-17-98	215.0	151	27.9
7F7D3F630F	Male	8	26	3-26-98	215.3	165	23.0
7F7D0A3F53	Male	7	24	3-12-98	215.1	141	20.3
7F7F443059	Male	7	32	3-18-98	213.3	176	37.4
7F7D0F3553	Male	7	--	3-24-98	207.1	127	9.9
7F7D374C53	Male	7	30	3-24-98	213.9	153	28.4
---	Male	8	24	3-2-98	208.0	187	51.3
7F7D0A4510	Male	8	27	3-3-98	215.7	142	17.3
7F7D11322E	Male	8	--	3-6-98	215.4	146	26.3
7F7F120C23	Male	8	30	3-9-98	215.4	179	43.3
7F7D420D3F	Male	8	30	3-17-98	215.1	154	25.7
7F7D0A2B0F	Immature	--	25	3-24-98	208.0	114	11.3
7F7D321936	Immature	--	25	3-24-98	204.0	122	8.1
7F7D36420F	Immature	--	16	3-11-98	213.3	102	8.3
7F7D311728	Immature	--	30	3-24-98	208.0	155	31.5
7F7D381D76	Immature	--	32	3-26-98	215.5	163	19.8
7F7D337471	Female	4	27	4-22-97	215.7	176	--
7F7E45642C	Female	4	--	4-21-97	215.5	161	--
7F7D264945	Female	4	53	3-18-97	215.6	196	60.0
7F7D3F7075	Female	4	--	5-20-97	215.0	190	--
7F7F442665	Female	4	21	3-17-97	215.6	171	49.0
7F7F403C57	Female	3	--	3-18-97	203.6	144	22.0
7F7E36616D	Female	4	18	3-19-97	215.5	141	23.0
7F7DE3A4C	Female	4	--	3-4-97	215.2	159	37.0
7F7D363A25	Female	4	45	3-11-97	215.0	195	47.0
7F7D372F69	Female	3	--	3-3-98	205.0	147	30.3
7F7D430024	Female	2	31	3-16-98	215.5	169	--
7F7F420733	Female	4	49	3-18-98	215.4	222	59.0
7F7F12113D	Female	2	--	3-11-98	214.9	153	32.0
7F7BOE6A0B	Female	4	--	5-26-98	234.3	188	--
7D7E650808	Female	4	--	5-5-98	227.0	201	80.0
7F7DOF650B	Female	4	--	4-30-98	227.5	184	49.0
7F7F42770E	Female	4	--	5-7-98	231.5	166	43.0
7F7F137D73	Female	4	39	3-1-99	205.0	170	49.3
7F7D39514C	Female	3	--	3-8-99	---	156	29.3

[1] Ages were verified by only one reader

Appendix 2 continued. Sex, developmental stage, age, capture date, capture location (rkm), fork length (cm) and weight (kg) for all male, female, sexually immature adult and juvenile sturgeon captured during 1997-1999 for steroid and ovarian tissue contaminant analysis, egg size, genetic assessment, acetylcholinesterase and liver lesion analysis.

PIT tag number	Sex	Sexual develop-ment stage	Age[1]	Capture date	Capture location (rkm)	Fork length (cm)	Weight (kg)
7F7E68765A	Female	4	43	3-16-99	205.0	200	35.0
7F7D394A00	Female	4	52	3-16-99	205.2	217	91.3
7F7D305915	Female	4	32	3-17-99	215.6	188	55.3
7F7D421A17	Female	3	47	3-17-99	215.7	192	64.3
7F7D434C78	Female	4	--	3-18-99	215.6	167	45.0
7F7D43322E	Female	4	38	3-25-99	215.6	182	48.0
7F7D100942	Female	3	--	8-25-98	122.0	174	45.4
7F7D0D6802	Female	2	--	8-25-98	122.0	157	23.2
7F7F12172E	Female	3	--	8-25-98	122.0	190	--
7F7D0B6C20	Female	3	--	8-25-98	122.0	170	41.3
7F7D372420	Female	4	43	3-24-99	215.6	208	80.3
7F7D11261A	Female	4	34	3-1-99	205.0	176	49.3
7F7D372463	Female	4	30	3-17-99	205.4	156	35.0
7F7D113654	Female	4	38	3-24-99	205.6	187	53.0
7F7D344C5A	Female	4	45				
504E113715	Juvenile	--	4	7-1-99	227	35	0.33
505E62344D	Juvenile	--	4	7-15-99	215.7	37	0.30
505E6F3F15	Juvenile	--	4	7-15-99	215.7	35	0.21
504E2B3E3B	Juvenile	--	4	7-15-99	215.7	37	0.30
504E692605	Juvenile	--	4	7-15-99	215.7	31	0.15
504E5F3166	Juvenile	--	4	7-15-99	215.7	45	0.30
50515E4953	Juvenile	--	4	7-15-99	215.7	38	0.20
505A000821	Juvenile	--	4	7-15-99	215.7	42	0.30
50620B2526	Juvenile	--	4	7-15-99	215.7	40	0.25
50497A7771	Juvenile	--	4	7-15-99	215.7	35	0.15
504E0F335F	Juvenile	--	4	7-15-99	215.7	39	0.20
504E63447B	Juvenile	--	4	7-1-99	225	34	--
50617D515A	Juvenile	--	4	7-15-99	215.7	45	0.40
504E624910	Juvenile	--	4	7-15-99	215.7	40	0.35
504E5A5213	Juvenile	--	4	7-15-99	215.7	41	0.20
504E174360	Juvenile	--	4	7-15-99	215.7	37	0.15
504E2F072F	Juvenile	--	4	7-16-99	225	35	0.25
504F78435A	Juvenile	--	4	7-16-99	225	32.5	0.20
504E5D5561	Juvenile	--	4	7-7-99	205	38	0.33
504D37354E	Juvenile	--	4	7-7-99	205	42	0.45
504E62165C	Juvenile	--	4	7-7-99	205	--	--
504E676F25	Juvenile	--	4	7-7-99	205	36	0.30
504E654410	Juvenile	--	4	7-9-99	234.4	37	0.20
504E005400	Juvenile	--	4	7-15-99	215.7	38	0.25
504F745765	Juvenile	--	4	7-15-99	215.7	40	0.26

[1] Ages were verified by only one reader

Appendix 3. Total number of samples, percent of total samples with detectable residues, range and mean concentrations of metals (reported in parts per million), organochlorine pesticides and polychlorinated biphelyls (reported in parts per billion) detected in Kootenai River white sturgeon ovarian tissue, collected 1989-1991.

Contaminant	Number of Samples (% of total)	Concentration range	
		Range	Mean± Standard Deviation
Copper (ppm)	17 (100)	1.18 - 3.2	1.886±.578
Zinc (ppm)	17 (100)	15.3 - 32.8	370.857±191.063
Lead (ppm)	7 (41)	0.08 - 1.6	22.994±5.115
DDE (ppb)	7 (41)	176.0 – 650.0	.462±.540
DDT (ppb)	5(29)	33.0 – 96.0	75.6±25.265

Appendix 4. Water quality parameters from the Kootenai Tribal hatchery during June and July 1999. Water samples were taken from the intake point in the river (unfiltered) and from the hatchery head tank (filtered).

Parameter (mg/l)	Intake		Head tank	
	June	July	June	July
Alkalinity (mg/l CaCO3)	42	74	42	66
Total Dissolved Solids (mg/l)	61	84	64	85
Total Suspended Solids (mg/l)	10	4.3	<1.1[1]	<1.0[1]
N-Ammonia (mg/l)	0.032	0.021	0.044	<0.010[1]
Nitrate+Nitrite (NO2+NO3) (mg/l)	0.067	0.45	0.076	0.11
Ortho-Phosphorous (mg/l)	<0.004[1]	<0.004[1]	<0.004[1]	<0.004
Calcium (mg/l)	13.3	22.1	13.2	22.5
Copper (mg/l)	0.002[1]	0.002[1]	0.002[1]	0.002
Magnesium (mg/l)	3.65	6.09	3.54	6.11
Manganese (mg/l)	0.010	0.006	0.002	0.001[1]
Zinc (mg/l)	0.009	0.006	0.004[1]	0.004[1]

[1] Undetected at reported detection limit