

STUDY OF REPRODUCTION BIOMARKERS IN COLUMBIA RIVER FISH SPECIES

Eugene P. Foster. Oregon Department of Environmental
Quality, Water Quality Division, 811 SW 6th Avenue
Portland, OR 97204. PH# (503)229-5358
FAX# (503)229-6124, EMAIL gfoster@teleport.com

Martin S. Fitzpatrick, Grant W. Feist, and Carl B. Schreck.
Oregon Cooperative Fishery Research Unit
Oregon State University, Department of Fisheries &
Wildlife, 104 Nash Hall, Corvallis, OR 97331

Introduction

A variety of hormones control reproduction and are necessary for proper development and ultimately species persistence. Estradiol-17B, the primary estrogen in female fish, stimulates ovarian development and vitellogenin synthesis while 11-ketotestosterone, the primary androgen in male fish, stimulates testicular development. Both are produced from testosterone. Recently, hormone mimics were detected in environmental samples. These compounds could potentially cause adverse effects to reproduction and development. Some of these compounds may be naturally occurring, such as phytoestrogens, but others are anthropogenically produced.

Environmental contaminant exposures were associated with reproductive effects via hormonal disruption, the mechanisms of which are not fully understood. Hormone mimics and endocrine disruption were associated with municipal sewage treatment plant (STP) discharges (Sumpter and Jobling, 1995) and bleached-kraft mill (BKM) effluent (McMaster et al., 1991; Munnkittrick et al., 1992; Van Der Kraak et al., 1992). Alkyl phenols found in STPs (Sumpter and Jobling, 1995), and sitosterol which was associated with BKM effluent (MacLatchy and Van Der Kraak, 1995) were estrogenic in laboratory studies. Alkyl phenols increased vitellogenin production in trout hepatocytes (Sumpter and Jobling, 1995) and increased vitellogenin production and inhibited testicular development in male rainbow trout (*Oncorhynchus mykiss*) (Jobling et al., 1996). Sitosterol decreased testicular growth as well as testosterone and 11-ketotestosterone production in goldfish (*Carassius auratus*) (MacLatchy and Van Der Kraak, 1995). In addition, BKM effluent masculinized female mosquitofish (*Gambusia affinis*) which was attributed to plant sterols contained in the effluent (Drysdale and Bortone, 1989; Bortone et al., 1989). Decreased reproductive success as measured by decreased fecundity, age to maturation, or survival of offspring can adversely affect fish populations (Houde, 1987).

Some Columbia River stocks of salmon and populations of sturgeon are experiencing reduced population declines. Snake River spring/summer chinook, Snake River fall chinook, and Snake River

sockeye salmon have been listed as threatened or endangered. White sturgeon in the Kootenai River system have been listed as threatened or endangered and white sturgeon populations have declined in portions of the main stem Columbia River.

Examination of potential endocrine disruption in these species is warranted because chemicals that cause endocrine disruption were detected in water, fish, and sediments. Chemicals identified as endocrine and/or reproductive disrupting (reviewed in Colborn et al., 1993) were detected in water collected from the Willamette River (a major tributary to the Columbia River) (Harrison et al., 1995) and fish and sediments collected from the Columbia River (ODEQ data). Endocrine and/or reproductive disrupting compounds such as atrazine, 2,4-D, metribuzin, trifluralin, and carbaryl were detected in water samples collected from the Willamette River while 2,3,7,8-tetrachlorodibenzo-p-dioxin, PCBs, DDT, and DDT metabolites were detected in fish and sediments collected from the Columbia River. In addition, the Columbia River receives STP and BKM effluent that may contain xenoestrogenic compounds such as alkyl phenols and sitosterol.

We are in the initial phase of a multi-year study investigating contaminant effects on the levels of reproductive hormones and gonadal development in fish from the Columbia River. The objectives of the study are to determine if chinook salmon (*Oncorhynchus tshawytscha*) or white sturgeon (*Acipenser transmontanus*) collected from the lower and upper Columbia River exhibit signs of reproductive dysfunction as compared to fish collected from a control area. A secondary objective is to determine if sturgeon can be sexed by plasma levels of sex steroids. In this study we measured levels of chlorinated pesticides and PCBs, plasma reproductive steroids, hepatic enzymatic activities, and stage of gonadal maturation. Presented are the results to date.

Methods

Adult chinook salmon samples were collected from fish that had returned to a lower and upper Columbia River fish hatcheries and an Oregon coastal river fish hatchery. The coastal river has no significant point sources of pollution. Adult white sturgeon samples were collected from commercially caught fish from the lower and upper Columbia River.

Skin on fillets were analyzed for chlorinated pesticides and PCBs by GC/MS according to USEPA NPDES method 608 and RCRA SW846 method 8080. Blood plasma samples were analyzed for estradiol-17B, testosterone, and 11-ketotestosterone by RIA (Fitzpatrick et al., 1996) and ELISA will be used to analyze for vitellogenin (Linares-Casenave, 1990). Liver tissues were collected and microsomes will be prepared by differential centrifugation (Carpenter et al., 1990) and will be analyzed for EROD activity (Prough et al., 1978) and cytochrome P450 1A content by Western immunoblot (Towbin et al., 1979). Gonadal tissues were collected, placed in formalin and will be prepared for stage of gonadal maturation and histological abnormalities.

Results

Chlorinated Pesticide and PCBs

All fish except one had detectable levels of p,p-DDE which was the only chlorinated pesticide or PCB detected with the exception of p,p-DDD and heptachlor epoxide which were detected in one other fish. Average p,p-DDE levels were significantly higher for female fish collected from the upper river than the coastal hatchery fish hatchery while p,p-DDE levels for all fish collected at the lower river hatchery were greater than the coastal river hatchery fish (Table 1).

Table 1. Average p,p-DDE concentrations in adult chinook salmon collected from fish hatcheries in the lower and upper Columbia River and coastal river.

Location	Male	Female	Total
Coastal	5.25 (2.5)	5.00 ^a (0.7)	5.12 ^a (1.2)
Lower	11.62 (2.2)	10.56 ^{ab} (1.6)	11.06 ^b (1.3)
Upper	4.67 (0.9)	15.00 ^b (5.0)	9.83 ^{ab} (3.2)

(SE)

Dissimilar letter denotes difference between stations.

Reproductive Hormones

Average testosterone levels for female chinook salmon from the lower river were higher than males from the same area or females from the upper river and the coastal river hatcheries (Table 2). Also, male chinook salmon from the lower river had higher levels of testosterone than males collected from the upper river and the coastal river hatcheries (Table 2). Estradiol levels for female chinook salmon from the lower river were higher than females from the upper river and the coastal hatcheries (Table 2). Female estradiol levels were higher than males for each location (Table 2). 11-ketotestosterone levels were higher in chinook salmon from the lower river than the fish from the upper river and the coastal river hatchery while males had higher levels than females at all locations (Table 2).

Testosterone levels in female white sturgeon from the lower river were less than the levels in males from the same location and lower than females from the upper river (Table 3). Estradiol

levels in female white sturgeon from the upper river were higher than males from the same location and higher than the levels in females from the lower river (Table 3). 11-ketotestosterone levels in male and female white sturgeon from the lower river were lower than the levels in fish from the upper river (Table 3).

Table 2. Average testosterone, estradiol-17B, and 11-ketotestosterone in adult chinook salmon collected from fish hatcheries in the lower and upper Columbia River and coastal river.

Location	T		E2		KT	
	Male	Female	Male	Female	Male	Female
Coastal	46.3 ^{b1} (2.3)	110.3 ^{2b} (10.7)	0.6 ¹ (0.1)	1.6 ^{b2} (0.2)	154.5 ^{b1} (8.6)	15.6 ² (0.9)
Lower	75.5 ^{a1} (7.8)	221.2 ^{a2} (10.5)	0.5 ¹ (0.1)	1.9 ^{a2} (0.1)	212.5 ^{a1} (13.8)	24.6 ² (0.7)
Upper	47.4 ^{b1} (4.2)	118.0 ^{b1} (11.6)	0.6 ¹ (0.1)	1.3 ^{b2} (0.1)	147.6 ^{b1} (8.6)	17.4 ² (1.2)

(SE)

T = testosterone; E² = estradiol-17B; KT = 11-ketotestosterone. Dissimilar letter denotes difference between stations. Dissimilar number denotes within station difference between sexes.

Table 3. Average testosterone, 17 beta estradiol, and 11-ketotestosterone in white sturgeon collected from the lower and upper Columbia River.

Location	T		E2		KT	
	Male	Female	Male	Female	Male	Female
Lower	14.2 ^{a1} (4.8)	1.3 ^{a2} (0.2)	0.74 ^{a1} (0.1)	0.6 ^{a1} (0.1)	11.9 ^{a1} (4.5)	0.6 ^{a2} (0.3)
Upper	23.3 ^{a1} (3.9)	27.4 ^{b1} (12.9)	0.70 ^{a1} (0.1)	5.6 ^{b2} (2.8)	54.2 ^{b1} (13.3)	49.8 ^{b1} (22.3)

(SE)

T = testosterone; E² = estradiol-17B; KT = 11-ketotestosterone. Dissimilar letter denotes difference between stations. Dissimilar number denotes within station difference between sexes.

Discussion

The current study was designed to investigate both the effects of environmental contaminants on the chinook salmon and white sturgeon reproductive systems via reproductive hormones and the potential for using plasma reproductive hormones for sexing white sturgeon. The study is in the initial phases and only limited data are available at this time.

The chlorinated pesticide results showed an increase in p,p-DDE levels for fish collected at both the lower and upper Columbia River fish hatcheries as compared to the control fish collected from coastal river fish hatchery. However, the levels of p,p-DDE detected are probably not of toxicological significance.

The plasma steroid results showed that the chinook salmon collected from the lower river fish hatchery had higher steroid levels than the fish from upper river and coastal river hatcheries. Completion of the scheduled analysis should help to understand the significance of this finding.

There were differences between lower river and upper river white sturgeon steroid levels. These differences were confounded by collection date and stage of gonadal maturation. Completion of gonadal histology would help identify differences due to location. The difference in steroid levels between male and female sturgeon collected from the lower river appears promising for identification of sturgeon sex with plasma hormones. However, the variability in plasma hormones between sexes for the upper river sturgeon was troubling, primarily the lack of differentiation between sexes for 11-ketotestosterone. Again, the completion of scheduled analysis should help determine the utility of this approach.

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