

Julie A. Weis, OSB No. 974320  
Email: [jweis@hk-law.com](mailto:jweis@hk-law.com)  
HAGLUND KELLEY LLP  
200 SW Market Street, Suite 1777  
Portland, Oregon 97201  
Phone: (503) 225-0777  
Facsimile: (503) 225-1257

William K. Barquin, OSB No. 98180  
Email: [wbarquin@kootenai.org](mailto:wbarquin@kootenai.org)  
Kootenai Tribe of Idaho  
Portland Office  
1000 SW Broadway, Suite 1060  
Portland, OR 97205  
Phone: (503) 719-4496  
Facsimile: (503) 719-4493

Attorneys for Defendant-Intervenor  
Kootenai Tribe of Idaho

IN THE UNITED STATES DISTRICT COURT  
DISTRICT OF OREGON

**NATIONAL WILDLIFE FEDERATION,  
et al.,**

Plaintiffs,

v.

**NATIONAL MARINE FISHERIES  
SERVICE, et al.,**

Defendants.

Case No.: 3:01-cv-00640-SI

**DEFENDANT-INTERVENOR  
KOOTENAI TRIBE OF IDAHO'S  
SUPPLEMENTAL FILING IN SUPPORT  
OF JUNE 23, 2015 SUMMARY  
JUDGMENT HEARING**

In conformance with the Court's instructions to the parties at the June 23, 2015 summary judgment hearing in this case (the hearing), defendant-intervenor the Kootenai Tribe of Idaho (KTOI), a federally-recognized Tribe headquartered near the town of Bonners Ferry in Idaho's Kootenai River Valley, offers the following:

**PAGE 1 – KOOTENAI TRIBE OF IDAHO SUPPLEMENTAL  
FILING RE: JUNE 23, 2015 SUMM. J. HEARING**

HAGLUND KELLEY LLP  
200 SW Market Street, Suite 1777  
Portland, OR 97201  
(503) 225-0777 / (503) 225-1257 (fax)

**Map – Demonstrative Evidence**

Attachment A is the map of the Columbia River Basin used by the KTOI at the hearing to show the approximate location of the KTOI's headquarters in the upper Columbia River Basin below Libby Dam along the Kootenai River, and to illustrate the approximate relationship between that location and the mainstem Columbia River in the lower Columbia River Basin near Portland, Oregon. To the best of undersigned counsel's knowledge, the map is not included in the administrative record, although it has been used in this case previously, most recently at the pre-dam briefing for the Court's June 19, 2015 visit to Bonneville and The Dalles dams.

**Kootenai River White Sturgeon Conservation Aquaculture Paper – Administrative Record Document**

Attachment B is a 2002 publication of the American Fisheries Society Symposium describing the KTOI's Kootenai River white sturgeon conservation aquaculture program that was referenced in the KTOI's summary judgment brief (Docket No. 2010) at page 9 (original pagination). The document is in the administrative record for this case and is found as Document 82 (pages COE 001729-40) on the September 2008 DVD titled, "Administrative Record for U.S. Army Corps Engineers, Concerning the Operation and Maintenance of the Federal Columbia River Power System, Section B 06057 Administrative Record for CBD v USACE (Libby Dam)." The DVD's label references Libby Dam because the DVD contains the administrative record for the separate lawsuit styled Center for Biological Diversity v. U.S. Fish and Wildlife, Case No. CV 03-29 DWM (D. Mont.), which involved the Libby Dam operations component of the Federal Columbia River Power System (FCRPS) that was resolved by way of settlement in September 2008. As was discussed in a prior filing in this case, see Docket No.

1555 at pages 6-7 (original pagination), FCRPS operations assessed in the 2008 FCRPS Biological Opinion before this Court are consistent with the Libby Dam parties' agreed-upon path forward for Libby Dam and the Kootenai River white sturgeon.

RESPECTFULLY SUBMITTED this 29<sup>th</sup> day of June, 2015.

HAGLUND KELLEY LLP

By: /s/ Julie A. Weis

Julie A. Weis, OSB No. 974320  
Haglund Kelley LLP  
200 SW Market St., Suite 1777  
Portland, OR 97201  
Phone: (503) 225-0777  
Fax: (503) 225-1257  
Email: [weis@hk-law.com](mailto:weis@hk-law.com)

William K. Barquin  
Kootenai Tribe of Idaho  
Portland Office  
1000 SW Broadway, Suite 1060  
Portland, OR 97205  
Phone: (503) 719-4496  
Fax: (503) 719-4493  
Email: [wbarquin@kootenai.org](mailto:wbarquin@kootenai.org)

**CERTIFICATE OF SERVICE**

Pursuant to Local Rule Civil 100.13(c) and Fed.R.Civ.P. 5(d), I certify that on June 29, 2015, I caused the foregoing to be electronically filed with the Court's electronic filing system, which will generate automatic service upon all parties enrolled to receive such notice. The following will be manually served by first class U.S. mail:

Dr. Howard F. Horton, Ph.D.  
US Court Technical Advisor  
Professor Emeritus of Fisheries  
Oregon State University  
Department of Fisheries and Wildlife  
104 Nash Hall  
Corvallis, OR 97331-3803

Dated this 29<sup>th</sup> day of June, 2015.

/s/ Julie A. Weis

Julie A. Weis



American Fisheries Society Symposium 28:211-222, 2002  
 © 2002 by the American Fisheries Society

## Conservation Aquaculture: An Adaptive Approach to Prevent Extinction of an Endangered White Sturgeon Population

SUSAN C. IRELAND

*Kootenai Tribe of Idaho Fisheries Department,  
 Post Office Box 1269, Bonners Ferry, Idaho, 83805*

PAUL J. ANDERS

*University of Idaho, Aquaculture Research Institute,  
 Center for Salmonid and Freshwater Species at Risk, Moscow, Idaho, 83844, USA*

JOHN T. SIPLE

*Kootenai Tribe of Idaho Fisheries Department,  
 Post Office Box 1269, Bonners Ferry, Idaho, 83805, USA*

**Abstract.**—The white sturgeon population *Acipenser transmontanus* in the Kootenai River was listed as endangered by the U.S. Fish and Wildlife Service (USFWS) in 1994 due to postglacial isolation and the virtual lack of recruitment since 1974. The Kootenai River White Sturgeon Conservation Aquaculture Program was initiated to preserve genetic variability, begin rebuilding natural age-class structure, and prevent extinction while measures are identified and implemented to restore natural recruitment. The program is part of a comprehensive recovery strategy detailed in the USFWS recovery plan for the Kootenai River population of white sturgeon. A breeding plan, including culture methods to minimize potential detrimental effects of conventional stocking programs, has been implemented to guide recovery, population management, and the systematic collection and spawning of wild adults before they are lost from the wild breeding population. Between 1990 and 2000, 33 families were produced from the mating of 51 wild white sturgeon broodstock. Genetic analysis indicated that five mitochondrial control region length variants represented in the wild white sturgeon population were represented in similar frequencies in the wild white sturgeon broodstock. A total of 2,702 hatchery-reared white sturgeon were released into the Kootenai River between 1992 and 1999. White sturgeon juveniles approved for release had no diagnostic disease symptoms and less than or equal to 10% prevalence of endemic pathogens. A total of 398 hatchery-reared fish were recaptured in the wild (14.7% of 2,702 stocked; single recapture events) during the 1993–1999 sampling period. The Kootenai River Conservation Aquaculture Program is currently meeting its objectives of reducing the threat of population extinction by providing frequent year classes from native broodstock, representing inherent within-population genetic diversity in its broodstock and progeny, and minimizing the introduction of disease into the recipient wild population.

The Kootenai River white sturgeon *Acipenser transmontanus* population was listed as endangered in 1994 by the U.S. Fish and Wildlife Service under the U.S. Endangered Species Act (USFWS 1994). This transboundary population, occupying the Kootenay River and Kootenay Lake in British Columbia, Canada, and the Kootenai River in Idaho and Montana, has been in decline since the mid-1960s, due to limited or absent natural recruitment (Duke et al. 1999; USFWS 1999).

In 1995, the U.S. Fish and Wildlife Service convened a recovery team to identify and imple-

ment recovery strategies. Due to the transboundary nature of the white sturgeon population, the team included members with technical expertise from U. S. Fish and Wildlife Service, Idaho Department of Fish and Game, Kootenai Tribe of Idaho, Montana Fish, Wildlife and Parks, British Columbia Ministry of Environment, Land, and Parks, Canadian Department of Fisheries and Oceans, U.S. Army Corps of Engineers, University of Idaho, and Bonneville Power Administration. In cooperation with the agencies and the Tribe, the U.S. Fish and Wildlife Service prepared

a comprehensive recovery plan that was completed in 1999. The team concluded that recovering the species depended upon reestablishing natural recruitment, minimizing additional loss of genetic variability, and mitigating habitat impacts caused by the construction and operation of Libby Dam, and the loss of ecologically critical backwater and floodplain habitat and function.

The Kootenai River white sturgeon population is believed to have been isolated from other white sturgeon populations in the Columbia River Basin following post-Pleistocene recolonization approximately 10,000 years ago (Alden 1953; Northcote 1973). The population adapted to natural predevelopment conditions of the Kootenai system, which were characterized by frequently large spring freshets, an extensive large-river floodplain and delta marshland habitats in the downstream portions of the river upstream from Kootenay Lake (Figure 1). The flood-pulse model of large-river floodplain ecosystems (Junk et al. 1989) suggests that the mosaic of such habitats, as historically present in the Kootenai River, were valuable sources of nutrients required for system productivity and trophic stability. Modification of the Kootenai River by human activities including industrial and residential development, extractive land use practices, floodplain isolation by diking, and construction and operation of a hydropower dam drastically changed the river's natural thermograph and hydrograph (Partridge 1983; Anders 1991; Apperson and Anders 1991; Anders and Richards 1996; Duke et al. 1999; USFWS 1999; Anders et al. 2001, this volume). These changes not only altered white sturgeon spawning, incubation and rearing habitats, but also changed community structure and species composition across trophic levels, and resulted in depressed biological productivity (Anders and Richards 1996; Snyder and Minshall 1996; Paragamian and Kruse 1996; Paragamian et al. 1997; Anders et al. 2001, this volume).

#### *The Kootenai River White Sturgeon Population*

The size of the Kootenai River white sturgeon population *Acipenser transmontanus* was first estimated to be 4,000–6,000 individuals (Graham 1981). Using tag recovery data from 1979 through 1981, Partridge (1983) estimated population size to be 1,148 fish, confidence interval (CI) 95 907–1,503 (50–224 cm TL). In 1990, the population was estimated to include 880 individuals, CI 95 638–1,211 (88–274 cm TL; Apperson and Anders 1991).

The 1990 estimate was not statistically different from the previous estimate (Partridge 1983); however, these estimates were not directly comparable because they covered different geographic areas and employed different sampling protocols (Giorgi 1993). In 1997, the population was estimated to contain 1,468 individuals (CI 95 740–2197; Paragamian et al. 1997) composed predominantly of adult fish greater than 25 years of age.

Natural recruitment failure in this population was first reported in the early 1980s (Partridge 1983). During the mid- to late-1980s, limited or unsuccessful natural spawning was thought to be responsible for natural recruitment failure. Hypothesized causes of natural reproduction failure initially included postimpoundment thermal and physical habitat alterations (Anders 1991; Apperson and Anders 1991) and limited gamete viability due to exposure to contaminants in the river and its sediments (Apperson and Anders 1991). From 1991 through 1999, natural spawning of white sturgeon was confirmed in the Kootenai River in all years except 1992 by collections of hundreds of fertilized eggs and developing embryos from the Kootenai River under a range of postimpoundment hydrograph and thermograph conditions (Paragamian and Kruse 1996; Paragamian et al. 1997; USFWS 1999). Although successful natural spawning was documented during these years, natural recruitment has not been restored to date despite nearly a decade of augmented river discharge experiments intended to stimulate natural spawning and recruitment (Duke et al. 1999; USFWS 1999).

Substantial artificial alterations to the hydrograph and thermograph in the Kootenai River caused by Libby Dam were considered to be primary reasons for the Kootenai River white sturgeon's continuing lack of recruitment and declining numbers (Duke et al. 1999; USFWS 1999; Anders et al. 2001, this volume). The overall biological productivity of the Kootenai River downstream of Libby Dam has also been altered. Libby Dam blocks the open exchange of water, organisms, nutrients, and coarser organic matter between the upper and lower Kootenai River. Much of the Kootenai River has been channelized and stabilized from Bonners Ferry downstream to Kootenay Lake, resulting in reduced aquatic habitat diversity, altered flow conditions at potential spawning and nursery areas, and altered substrates in incubation and rearing habitats necessary for survival (Partridge 1983; Apperson and Anders 1991). Giorgi (1993) noted that the chronic

CONSERVATION AQUACULTURE

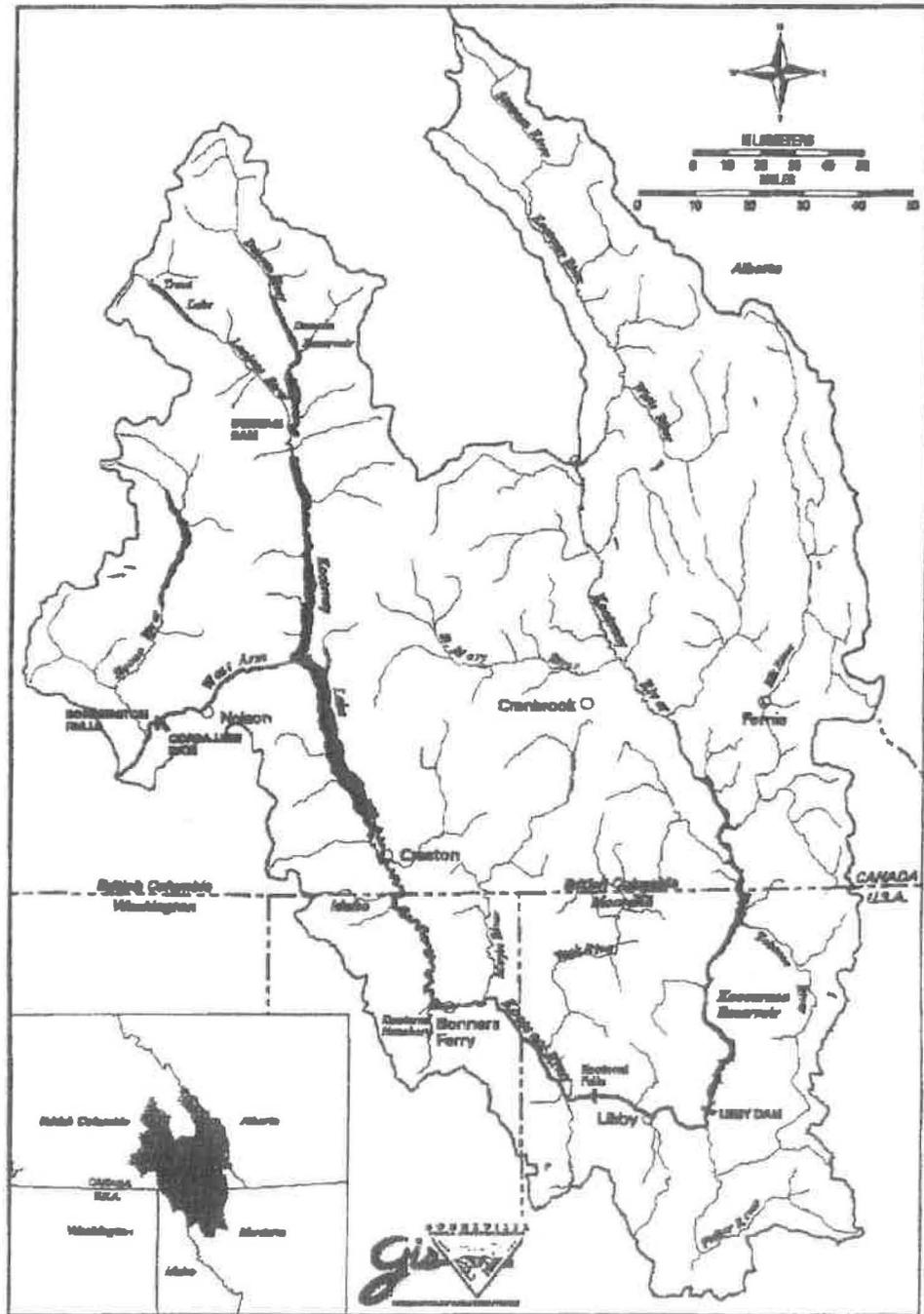


Figure 1. Map of the Kootenai/yc Basin and location of the Kootenai White Sturgeon Conservation Aquaculture Facility near Bonners Ferry, Idaho.

effects on wild sturgeon spawning in "chemically polluted" water and rearing over contaminated sediments, in combination with bioaccumulation of contaminants in the food chain, was possibly reducing the successful reproduction and early-age recruitment to the Kootenai River white sturgeon population. Due to postdevelopment anthropogenic changes to the Kootenai River ecosystem, early life mortality factors such as embryo and larval suffocation and predation, larval and fingerling food limitation or starvation, or first overwintering mortality may have affected the survival of early life stages of the white sturgeon population (Anders et al. 2001, this volume).

#### *Conservation Aquaculture Program Rationale*

Rationale supporting conservation aquaculture involves the fact that human development, resource use, and population growth contribute to degradation and loss of aquatic ecosystems and endemic fish populations (Anders 1997, 1998). The damming of a river is a cataclysmic event in the life of riverine ecosystems (Gup 1994). By changing the flow of water, sediment, nutrients, and biota, dams interrupt and alter most of a river's important ecological processes (Ligon et al. 1995; Sparks 1995). These events associated with the impoundment of a river provide strong rationale for a carefully designed conservation aquaculture program, especially in a case like the Kootenai River, where ecosystem alteration appears to be at least partially responsible for decades of failed natural recruitment of white sturgeon. Furthermore, when a river's physical foundation is disrupted, even the most insightful research programs aimed at restoring natural recruitment may fail (Ligon et al. 1995). Given this scenario, conservation aquaculture programs can provide a "population safety net."

Restoration mandates by the federal Endangered Species Act (ESA) are focused on natural populations and the ecosystems upon which they depend (Flagg et al. 1995). Nevertheless, the USFWS recognizes that conservation of ESA listed species may be facilitated by artificial means, while factors impeding population recovery are rectified (Hard et al. 1992; USFWS 2000). Hard et al. (1992) also suggested that restoration of depleted populations may be hindered by the lack of suitable numbers of naturally produced juveniles needed for restoration, even if factors impeding recovery could be immediately corrected. Avise (1994) suggested that species exhibit de-

creased reproduction (and recruitment) at low population densities for a number of nongenetic reasons, due to lack of social interaction necessary for breeding, difficulties in finding mates, and other density-dependent ecological factors collectively known as the "Allee effect" (Andrewartha and Birch 1954). Furthermore, when populations are small in size, the possibility of extinction through stochastic events becomes of great concern, namely a phenomenon known as the "extinction vortex" (Gilpin and Soule 1986). Further rationale supporting conservation aquaculture programs is that they can provide protection from the above hazards of small population size and associated deleterious effects of reduced within-population genetic diversity.

The recovery team's review of the demographic status of the Kootenai River white sturgeon population strongly suggested use of conservation aquaculture to maintain adequate population size and genetic variability to enable population persistence until repeated natural recruitment could be restored by alternative adaptive management approaches.

#### *History of Conservation Aquaculture Program*

To address concerns of gamete viability and possible negative effects of exposure to water- and sediment-borne contaminants, experimental breeding of wild Kootenai River white sturgeon broodstock was initiated in 1990. This operation resulted in the first successful artificial propagation of wild Kootenai River white sturgeon (Apperson and Anders 1991). Based on a breeding plan incorporating population genetic concerns (Kincaid 1993), progeny from wild broodstock were successfully produced and reared in the Kootenai White Sturgeon Conservation Aquaculture Facility located in Bonners Ferry, Idaho (Figure 1) in 1991, 1992, 1993, 1995, 1998, and 1999 to address concerns of increasing demographic and genetic risks to a nonrecruiting population.

Objectives of the Kootenai River Conservation Aquaculture Program were to 1) reduce the threat of population extinction by providing annual or near-annual year-class production from native broodstock, 2) maintain inherent within-population genetic diversity and mimic wild population haplotype or genotype frequencies in hatchery broodstock and progeny, and 3) minimize the introduction of disease into the wild population.

## CONSERVATION AQUACULTURE

215

## Methods

*Broodstock collection and holding*

White sturgeon broodstock used in the Kootenai River Conservation Aquaculture Program were captured by angling or setlining. Male and female broodstock were captured from February through May in areas containing prespawning aggregations confirmed by ten years of ongoing radio and ultrasonic telemetry studies (Paragamian et al. 1997). Annual collection of gravid females from these areas, and subsequent spawning of these fish in the hatchery, suggested that fish spawning throughout the entire spawning season congregated simultaneously in the same areas. Thus, the broodstock sampling regime incorporated spawners from the duration of the spawning run. Furthermore, the timing of spawning in the hatchery approximated the range of estimated spawning dates in the wild during most years.

To identify potential broodstock in the field, all captured fish were biopsied to determine sex and gonad maturation stage according to criteria by Conte et al. (1988). Every fish collected was weighed and measured (TL, FL), checked for recapture, and if not recaptured, marked with an individually numbered Floy tag and injected with an individually coded PIT tag. Following sex determination and gonad development examination, potential broodstock were directly transferred from a water-filled stretcher to an oxygenated tank for immediate transfer to the hatchery by truck. Upon identification by PIT tag number, male and female broodstock recaptured in the wild (previously spawned in the hatchery) were weighed, measured, and immediately released. All recaptured male and female broodstock that contributed to surviving progeny groups were never spawned more than once.

Final gonadal maturation occurred in the Kootenai River White Sturgeon Conservation Aquaculture Facility, where potential broodstock were held separately or with one or two other fish of the same sex in circular fiberglass tanks (3 m diameter × 1.2 m deep). An external standpipe maintained water level at approximately 1.14 m inside the tank. Water exchange was provided at 10–15 volumes/d and dissolved oxygen was maintained at more than or equal to 5.0 mg/L, as recommended by Conte et al. (1988). Broodstock were held in Kootenai River water pumped into the hatchery and fed live juvenile rainbow trout *Oncorhynchus mykiss*.

*Spawning and Rearing*

Breeding matrices and protocols were developed to maximize effective population number and minimize chances of future poststocking inbreeding in the wild (Kincaid 1993). Biopsied ovarian follicles from all potential female broodstock held in the hatchery were evaluated to estimate timing of final maturation. Germinal vesicle breakdown (GVDB assay, Conte et al. 1988) and oocyte polarization index (PI; Van Eenennaam et al. 1996) were examined at least twice for at least 20 eggs from each female broodfish prior to spawning. Selection criteria for female broodstock were greater than or equal to 80% GVDB and less than or equal to 0.10 PI values. All selected female broodstock received two injections of synthetic gonadotropin-releasing hormone LHRHa at a total dose of 0.1 mg/kg body weight: an initial dose (10%) and a resolving dose (90% of total dose) (Conte et al. 1988). Males did not receive LHRHa injections, with the exception of two males that were experimentally injected during 1997. From 1990 through 1996, all male broodstock were brought to the hatchery, where sperm was extracted. During 1997, and in subsequent years, sperm samples were collected from naturally milting males in the field, often up to several days before fertilization, and held in plastic bags in ice-filled coolers, with O<sub>2</sub> replacement occurring every 12 h. A minimum water-activated motility period of 2 min, verified under a dissecting microscope, as well as a high ratio of activated to nonactivated sperm, were required to designate viable sperm samples (Conte et al. 1988).

Prior to 1993, eggs were removed by cesarean surgery (Conte et al. 1988). During 1993 and all subsequent years, eggs were removed by hand stripping to minimize postspawning stress experienced by the broodstock (Ireland 1999). Use of this hand-stripping technique also enabled earlier release of postspawned broodstock back into the river, and reduced the chance for disease or infection during postsurgery recovery, which took up to several months. Eggs were collected within 48 h after injection of the LHRHa resolving dose, after onset of oviposition, characterized by several hundred eggs visible on the bottom of the spawning tank. Eggs were fertilized, volumetrically quantified, de-adhered with Fuller's Earth, and incubated in modified MacDonald hatching jars (13 L capacity, round bottom cylinders, 50 cm tall, and 20 cm in diameter; Conte et al. 1988). Each MacDonald jar received 5,000–25,000 fertilized eggs. A recent partnership with the British Colum-

bia Ministry of Fisheries (BCMF) has provided a "fail-safe" facility for the Kootenai River white sturgeon conservation aquaculture program at the Kootenay Sturgeon Hatchery near Fort Steele, B.C. as a back-up measure to minimize the risk of catastrophic loss at either facility. Starting in 1999, approximately 5,000–20,000 fertilized, disinfected eggs from up to five families were shipped to the BCMF Kootenay Sturgeon Hatchery in Fort Steele, B.C., for incubation and rearing.

Adequate water flow through the hatching jars was maintained to provide a gentle rolling of the eggs, allowing oxygen to reach all eggs in each jar. Upon hatching, fry swam up and exited the MacDonald jars with the effluent water and were deposited directly into rectangular fiberglass fry collection tanks (1.2 m × 0.56 m × 0.31 m deep). Eggshells were siphoned daily from the fry collection tanks. Upon completion of hatching, all fry within a family were transferred to a larger fiberglass rearing tank for grow-out (2.1 m × 0.56 m × 0.31 m deep). Larval and fingerling densities were maintained below 0.8–1.0 kg of fish/m<sup>3</sup> of water as a precaution against density-dependent, stress-induced disease outbreaks (LaPatra et al. 1996). All families and half-sib families were reared separately until release. Fish held beyond the age of one year were transferred to large circular fiberglass tanks (3–4.5 m in diameter) and reared in densities below 1.0 kg/m<sup>3</sup>.

#### *Genetic Inventory*

White sturgeon possess a series of length variants in the control region of their mitochondrial genome that have been used to identify maternal lineage. This length variation arises as a consequence of a gain or loss of 1–5 perfectly repeated tandem 78–82 base-pair sequences (Buroker et al. 1990; Brown 1992; Brown et al. 1996). Frequencies of these length variants were recently reported for 113 wild white sturgeon from the Kootenai system (Kootenai River  $N = 66$ ; Kootenay Lake  $N = 47$ ; Anders and Powell 1998). Length variant frequencies were subsequently determined for 54 wild broodstock brought to the Kootenai River Conservation Aquaculture Facility from 1997 through 1999 (see Powell and Anders 1999 for DNA isolation and PCR protocols). A Monte Carlo simulation for chi-square tests that employed 1000 bootstrap resampling iterations (Roff and Bentzen 1989) was used to statistically compare length variant frequencies of the 113 wild fish with those of the 54 broodstock from the same wild (source) population. Preliminary

results indicated that mtDNA length variants in the control region of progeny generally exhibit the same patterns as maternal parents. However, exceptions to strict maternal inheritance have been observed (University of Idaho, Center for Salmonid and Freshwater Species at Risk, unpublished data). These findings will be summarized and published following systematic examination of a sufficient number of parent-progeny groups to quantify deviation from strict maternal inheritance. Currently, observations of deviation from strict maternal inheritance of mtDNA length variants do not appear to jeopardize use of this marker for legitimate haplotype frequency comparisons between the wild population of Kootenai River white sturgeon and the subset of broodstock spawned in the hatchery. The legitimate use of this marker for such comparisons will be reevaluated upon completion of this length variant inheritance study.

#### *Disease Testing*

From 1992 through 1996, white sturgeon produced in the Kootenai River Conservation Aquaculture Program were periodically tested for the presence of white sturgeon iridovirus (WSIV); testing was mandatory when disease mediated fish loss occurred in the hatchery. From 1997 through 1999, all broodstock and at least thirty progeny from each spawning year were annually tested for the presence of pathogens. Disease testing included parasitology, bacteriology, virology and histopathology examinations. Since 1997, ovarian fluid and male and female gametes were also sampled and tested for viral pathogens (e.g. WSIV and *Herpes* viruses 1 and 2). Disease testing results were reviewed by relevant state, provincial, federal and tribal management agencies. Generally, fish with no diagnostic signs of disease symptoms and less than or equal to 10% prevalence of endemic pathogens were approved for release. (LaPatra et al. 1999).

#### *Release Strategies*

Prior to 1999, all releases of hatchery-reared Kootenai River white sturgeon were experimental, to assess growth, survival, and habitat use of juveniles in the wild. Hatchery-reared white sturgeon juveniles were measured (TL, FL), weighed, tagged with a PIT tag, and scutes were removed for identification of the year-class in case of tag loss (e.g., the ninth left lateral and the eighth right lateral scutes were removed from juveniles from the 1998 year-class). Due to current limitations of

## CONSERVATION AQUACULTURE

217

permanent marking technologies for juvenile white sturgeon, all fish were PIT-tagged and released at weight greater than 20 g. In order to determine poststocking survival and potential genetic contribution to the next generation, family and year-class identifications were included in data records for each fish. Since 1999, with the completion of the Recovery Plan for the White Sturgeon in the Kootenai River (USFWS 1999), the White Sturgeon Conservation Aquaculture Program has become fully implemented, following preservation stocking strategies outlined in the breeding plan (Kincaid 1993). The stocking goal was 1,000 fish per family at the age of 15–24 months to produce an estimated 4–10 adults that will survive to breeding age. Stocking rates were based on an empirically based series of estimated annual survival rates for white sturgeon during an 18-year poststocking period (Kincaid 1993).

#### Monitoring Program

A monitoring program was implemented in 1993 to annually recapture hatchery-reared white sturgeon juveniles in the Kootenai River, using experimental mesh gill nets, hoop nets, and angling (Marcuson et al. 1995; Paragamian et al. 1997; Ireland 1999). Mark-and-recapture techniques were used to estimate annual growth and survival of hatchery-stocked white sturgeon in the Kootenai River. An ultrasonic telemetry study was implemented in 1999 to determine juvenile white sturgeon habitat use in relation to depth, velocity,

substrate and cover. Average poststocking survival rates for the first year and condition factors ( $W_p$ ; Beamenderfer 1993) for each release group are currently being estimated.

#### Results

A total of 377 broodstock were captured from 1990 through 1999, of which 51 were spawned (17 females, 34 males), producing 33 families, including half-sibling families (Table 1). Fertilization and hatching rates ranged from 6% to more than 99% and 1% to 73%, respectively.

A total of 2,702 hatchery-reared white sturgeon were released into the Kootenai River on eight separate occasions between 1992 and 1999 (Table 2). Fish were released at six sites between Bonners Ferry and the Canadian border. Age at release ranged from 1 to 4 years and average length at release ranged from 22.9 to 56.5 cm (TL). A total of 398 hatchery-reared fish were recaptured (14.7%) in the wild following release, using all collection methods during the 1993–1999 sampling period (Table 2). A total of 481 recapture events occurred between 1993 and 1999, including individuals recaptured more than once. Preliminary annual survival estimates for each release group ranged from 77% to 99% for the period beginning in the year after release. Annual growth of hatchery-reared juvenile white sturgeon released into the Kootenai River and subsequently recaptured averaged 5.27 cm (FL).

Table 1. Numbers of wild white sturgeon broodstock spawned and families produced at the Kootenai Tribal Hatchery from 1990 through 1999.

Year	Females	Males	No. of families produced
1990	1	1	1
1991	1	3 <sup>a</sup>	1
1992	1	3 <sup>a</sup>	3
1993	1	2	2
1994	0	0	0 <sup>c</sup>
1995	2	4	4
1996	1	2	2 <sup>d</sup>
1997	3	5	6 <sup>e</sup>
1998	3	6	6
1999	4	8	8
Total	17	34	33

a. Sperm from 3 males pooled.

b. Eggs fertilized separately with sperm of each male.

c. No white sturgeon handled, due to ESA listing.

d. No survivors to age at release; hatching success 1% due to low gamete quality.

e. No survivors to age at release; hatching success > 80%; larvae died shortly after hatch due to equipment failure.

218

IRELAND, ANDERS, AND SIPLE

Table 2. Release and recaptures of hatchery produced white sturgeon juveniles released in the Kootenai River in Idaho and Montana between 1992 and 1999.

Year class	Number released	Mean TL (mm) at release (S.D.)	Mean W (g) at release (S.D.)	Release season and year	Number (%) recaptured <sup>a</sup>
1990	14	455	321	Summer 1992	54 (25.2) <sup>b</sup>
1991	200	255	64.4	Summer 1992	—
1992	91	—	—	Fall 1994	41 (45)
1995	1,076	229 (27)	47 (16)	Spring 1997	295 (15) <sup>c</sup>
1995	891	343 (43)	147 (61)	Fall 1997	—
1995	99	408 (70)	283.3 (136.8)	Summer 1998	6 (6)
1995	25	565 (71)	805.8 (276.4)	Summer 1999	2 (<1)
1998	306	261 (42)	79.5 (44.4)	Fall 1999	—
Total	2,702				398 (14.7)

a. Percent recaptured during 1993-1999 sampling period for each release year (Excluding multiple recapture events).

b. Includes 1990 and 1991 year class.

c. Includes 1997 spring and fall release.

#### Genetic Inventory

Five mitochondrial control region length variants were observed among 113 fish surveyed from the wild population in the Kootenai River and Kootenay Lake (Table 3). Analysis of the 54 Kootenai River broodstock indicated that all five length variants found in the wild population were also present in the broodstock sample group (Table 3). Haplotype (length variant) frequency distributions of the wild and broodstock sample groups were not significantly different ( $0.975 < P < 0.9$ ,  $df = 4$ ,  $N = 167$ ; Table 3).

#### Discussion

The Kootenai River Conservation Aquaculture Program is currently meeting its objectives of reducing the threat of population extinction by providing frequent year classes from native broodstock, representing inherent within-population genetic diversity in its broodstock, and minimiz-

ing the introduction of disease into the recipient wild population.

#### Population Management

From 1992 through 1999, 2,702 juvenile white sturgeon, representing 33 families (including half-sibling families; Table 1), artificially propagated at the Kootenai River White Sturgeon Conservation Aquaculture Facility from native broodstock, were released into the Kootenai River. These releases have added white sturgeon year classes to the population to counteract the demographic and genetic risks to a declining population currently lacking natural recruitment. High annual survival rates (77–99%) estimated for juvenile white sturgeon released at ages 1–4 into the Kootenai River suggest that early life mortality factors affecting YOY or younger life stages may be limiting natural recruitment in the wild Kootenai River white sturgeon population. However, our current inability to monitor growth, condition, and survival of these early life stages (posthatch larvae to YOY)

Table 3. Comparison of mtDNA control region length variant frequency between 113 wild Kootenai River white sturgeon and 54 Kootenai Hatchery broodstock from the same population. Percent of samples having each length variant is indicated in parentheses.

Length variant (copy number)	Wild population (n=113)	Kootenai hatchery broodstock (n=54)
LV-01	54 (47.8)	26 (48.1)
LV-02	35 (31.0)	14 (25.9)
LV-03	11 (9.7)	6 (11.1)
LV-04	6 (5.3)	3 (5.6)
LV-05	7 (6.2)	5 (9.3)

## CONSERVATION AQUACULTURE

219

due to their absence in the wild population has limited our understanding of early life mortality factors in the river.

In the breeding plan designed to preserve the genetic variability of the Kootenai River white sturgeon population (Kincaid 1993), the term "preservation stocking" was used to indicate that the preservation of genetic variability is the primary objective of the conservation aquaculture program. However, genetic variability, population, and effective population size ( $N_e$ ) are inexorably linked. Thus, gradual expansion of the wild white sturgeon population, in the presence of failed natural recruitment, is a secondary, yet important objective of the program, relative to population persistence and the maintenance of effective population size. Rather than a specific set of culture techniques, the Kootenai White Sturgeon Conservation Aquaculture Program involves an adaptive suite of approaches that prioritize the preservation of an endangered white sturgeon population and its locally adapted genotypes, phenotypes, and behaviors (Anders 1998), as recommended by Kincaid (1993).

#### *Genetic Inventory*

Representing a wild population's genetic diversity and variation in a subset of broodstock is critical to the long-term success of hatchery programs. Failure to restore wild populations using conservation aquaculture programs may have arisen from under- or over-representing a subset of a wild populations' specific genotypes or haplotypes or from other selection pressures (Hindar et al. 1991; Waples 1991; Waples and Teel 1990). Such failures may have occurred due to design oversight or logistical or economic constraints.

Although not a comprehensive population assessment, our genetic analysis (mtDNA control region length variant analysis) provided an efficient, low-cost technique to monitor genetic diversity and variation of native broodstock relative to that of the wild (source) population. The relative simplicity and low cost of this analysis makes it possible to genetically type wild broodstock prior to spawning. Access to this genetic information can provide hatchery managers, biologists, and geneticists with the opportunity to develop spawning matrices to reduce or eliminate unintended mating of closely related broodstock.

Implementation of this analytical technique can also help mimic natural within-population genetic diversity and variation, and theoretically improve fitness of progeny groups. Although

length variant heteroplasmy (the coexistence of different mtDNA length variants in the same individual) was initially thought to be very uncommon, it is becoming more commonly observed, due in part to increased sensitivity provided by the polymerase chain reaction (PCR), and more robust research. In the majority of cases, heteroplasmy is observed as a direct result of variable numbers of tandem repeats (VNTRs) or adjacent to the control region (Hunt et al. 1998). Heteroplasmy has been observed in numerous taxa, including white sturgeon (Buroker et al. 1990; Hunt et al. 1998). Heteroplasmy has been observed in Kootenai River white sturgeon (Powell and Anders 1999), and is being investigated to evaluate the use of control region length variants as a valid population marker. Future genetic research should include the use of bi-parentally inherited nuclear markers (RFLPs and microsatellites) at population, broodstock, and progeny levels to further resolve relevant population genetic signal, and to address responses of the wild population to continued operation of the Kootenai White Sturgeon Conservation Aquaculture Program.

#### *Animal Health Management*

A primary goal of any aquaculture program is to minimize introduction and transmission of pathogens in cultured and native populations. Available scientific information should be used to develop conservation and management strategies that minimize the transmission of disease from cultured fish to native populations and the potential severity of disease in the native population (LaPatra et al. 1999). Although asymptomatic infection may be widely distributed within and among wild populations, maintenance of optimal rearing conditions (e.g. optimal rearing densities, temperature regimes, water quality conditions) can reduce or prevent stress-induced outbreaks of disease in the hatchery setting. Development, refinement, and strict implementation of the Program's disease testing protocols for white sturgeon produced in the Kootenai River White Sturgeon Conservation Aquaculture Facility should continue to minimize potential disease outbreaks and disease transmission risks to the wild population. Recent Kootenai Hatchery upgrades completed in 1999 (new water intake system, increased water temperature control for incubation and hatching, sediment filtration systems, pathogen control (UV sterilization), and added rearing capacity) contributed to increased hatching success and survival of early life stages, and minimized

disease outbreak and fish loss (Ireland 1999). High fertilization, development, and hatching rates in 1999 and 2000 may be indicative of future benefits to be provided from these extensive hatchery upgrades. The addition of a "fail-safe" facility and collaboration with the biologists and culturists in British Columbia, Canada, also helps to ensure success of the program.

The Kootenai River Conservation Aquaculture Program is currently meeting its objectives of reducing the threat of population extinction by providing frequent year-classes from native broodstock, representing inherent within-population genetic diversity in its broodstock, and minimizing any introduction of disease into the wild population. The occasional failures of annual natural recruitment may be a natural phenomenon in sturgeon populations. However, the absence of natural recruitment during the past 25 years is a prescription for imminent population extinction. Until suitable habitat conditions are reestablished in the Kootenai River ecosystem to increase white sturgeon survival past the egg/larval stage and restore natural recruitment in the wild white sturgeon population, the Kootenai River White Sturgeon Conservation Aquaculture Program, through careful monitoring, review, and implementation, will continue to protect this unique endangered population from extinction.

#### Acknowledgments

This project is supported by Bonneville Power Administration (Contract DE-B179-88B193743, Project 198806400). The authors wish to acknowledge the past and present Kootenai Tribal Fisheries Program staff (Larry Aitken, Robert Aitken, Eric Wagner, Dennis David, Ralph Bahe, Chris Lewandowski, Charlie Holderman, Jean Bahe, Gary Aitken, Sr., and Ron Tenas) for their dedication to the conservation aquaculture program and the recovery of white sturgeon. We also wish to thank the following people for their contribution to this project: Terry Patterson, College of Southern Idaho; Serge Doroshov, Joel Van Eenennaam, and Joseph Groff, University of California, Davis; Allan Scholz, Eastern Washington University; Scott LaPatra, Clear Springs Foods; Steve Duke, Robert Hallock, Toni Davidson and Kathy Clemens, U.S. Fish and Wildlife Service; Vaughn Paragamian, Virginia Wakkinen, Vint Whitman, Ned Homer, and Keith Johnson, Idaho Department of Fish and Game; Rick Westerhof, Scott Bettin, and Charlie Craig, Bonneville Power Ad-

ministration; Jeff Laufle, Army Corps of Engineers; Jay Hammond and Colin Spence, British Columbia Ministry of Environment; Don Peterson, Bryan Ludwig, Tim Yesaki, Laird Siemens, and Ron Ek, British Columbia Ministry of Fisheries; Ernie Brannon, Madison Powell, Ron Hardy, Mike Caster, Mike Peterson, Joseph Cloud, Will Young, and Dennis Scarnecchia, University of Idaho; Brian Marotz, Steve Dalbey, and Greg Hoffman, Montana Fish, Wildlife, and Parks; Michael Parsley and Timothy Counihan, U.S. Geological Survey, Biological Resources Division; Paul Klatt, J-U-B Engineers, Inc.; and Gretchen Kruse, Free-Run Aquatics. The authors also wish to thank Steve Duke, Serge Doroshov, and Bernie May for their thorough and constructive comments that greatly improved the manuscript.

#### References

- Alden, W. C. 1953. Physiography and glacial geology of Western Montana and adjacent areas. Geological Survey Professional Paper 231. U.S. Government Printing Office.
- Anders, P. J. 1991. White sturgeon (*Acipenser transmontanus*) movement patterns and habitat utilization in the Kootenai River system, Idaho, Montana and British Columbia. Master's thesis. Eastern Washington University.
- Anders, P. J. 1997. The need for objective assessment of conservation aquaculture. *Fisheries* 22(5):46.
- Anders, P. J. 1998. Conservation aquaculture and endangered species: Can objective science prevail over risk anxiety? *Fisheries* 23(11):28-31.
- Anders, P. J., and D. L. Richards. 1996. Implications of ecosystem collapse on white sturgeon (*Acipenser transmontanus*) in the Kootenai River, Idaho, Montana, and British Columbia. Pages 27-40 in S. Doroshov, F. Blinkowski, T. Thuemeler, and D. MacKinlay, editors. Culture and Management of Sturgeon and Paddlefish Symposium Proceedings. Physiology Section, American Fisheries Society, Bethesda, Maryland.
- Anders, P. J., and M. S. Powell. 1998. Preliminary report of mitochondrial DNA diversity and variation of white sturgeon (*Acipenser transmontanus*) from the Columbia River Basin. Report F in Effects of mitigative measures on productivity of white sturgeon populations in the Columbia River downstream from McNary Dam, and Determine the status and habitat requirements of white sturgeon in the Columbia and Snake Rivers upstream from McNary Dam. Annual Progress Report to the Bonneville Power Administration. BPA Project 198605000.
- Andrewartha, G., and L. C. Birch. 1954. The distribution and abundance of animals. University of Chicago Press, Chicago, Illinois.

## CONSERVATION AQUACULTURE

221

- Apperson, K. A., and P. J. Anders. 1991. Kootenai River white sturgeon investigations and experimental culture. Annual Progress Report FY1990. Idaho Department of Fish and Game. Report prepared for Bonneville Power Administration, Contract No. DE-A179-88BP3497; Project No. 198806500. Portland, Oregon.
- Avise, J. C. 1994. Molecular markers, natural history, and evolution. Chapman Hall Publishing, New York.
- Beamesderfer, R. C. 1993. A standard weight ( $W$ ) equation for white sturgeon. *California Fish and Game* 79(2):63-69.
- Brown, J. R. 1992. Mitochondrial DNA length variation and heteroplasmy in populations of white sturgeon (*Acipenser transmontanus*). *Genetics* 132:221-228.
- Brown, J. R., K. Beckenbach, A. T. Beckenbach, and M. J. Smith. 1996. Length variation, heteroplasmy and sequence divergence in the mitochondrial DNA of four species of sturgeon (*Acipenser*). *Genetics* 142:525-535.
- Buroker, N. E., J. R. Brown, T. A. Gilbert, P. J. O'Hara, A. T. Beckenbach, W. K. Thomas, and M. J. Smith. 1990. Length heteroplasmy of sturgeon mitochondrial DNA; an illegitimate elongation model. *Genetics* 124:157-163.
- Conte, F. S., S. I. Doroshov, P. B. Lutes, and M. E. Strange. 1988. Hatchery manual for the white sturgeon (*Acipenser transmontanus*) with application to other North American Acipenseridae. Publication 3322. Publications Division, Agriculture and Natural Resources, University of California, Oakland.
- Duke, S., and eleven coauthors. 1999. Recovery plan for the Kootenai River white sturgeon (*Acipenser transmontanus*). *Journal of Applied Ichthyology* 15(1999):157-163.
- Flagg, T. A., C. V. W. Mahnken, and K. A. Johnson. 1995. Captive broodstocks for recovery of Snake River sockeye salmon. Pages 81-90 in H. L. Schramm, Jr. and R. G. Piper, editors. *Uses and effects of cultured fishes in aquatic ecosystems*. American Fisheries Society, Symposium 15, Bethesda, Maryland.
- Gilpin, M. E., and M. E. Soule. 1986. Minimum viable populations: processes of species extinction. Pp. 19-34 in M. E. Soule, editor. *Conservation biology: the science of scarcity and diversity*. Sinauer and Associates, Sunderland, Massachusetts.
- Giorgi, A. 1993. The status of the Kootenai River white sturgeon. Report prepared for the Pacific Northwest Utilities Conference Committee. September 1993.
- Graham, P. J. 1981. Status of white sturgeon in the Kootenai River. Montana Department of Fish Wildlife and Parks. Kalispell, Montana.
- Gup, T. 1994. Dammed from here to eternity: dams and biological integrity. *Trout* 35:14-20.
- Hard, J. J., R. P. Jones, Jr., M. R. Delarm, and R. S. Waples. 1992. Pacific salmon and artificial propagation under the Endangered Species Act. NOAA Technical Memorandum. NMFS, NWFSC-2.
- Hindar, K., N. Ryman, and P. Utter. 1991. Genetic effects of cultured fish on natural populations. *Canadian Journal of Fisheries and Aquatic Sciences* 48:945-957.
- Hunt, D. L., L. E. Whipple, and B. C. Hyman. 1998. Mitochondrial DNA variable number tandem repeats (VNTRs): utility and problems in molecular ecology. *Molecular Ecology* 7:1441-1455.
- Ireland, S. C. 1999. Kootenai River white sturgeon studies and conservation aquaculture. Kootenai Tribe of Idaho. Annual report prepared for the Bonneville Power Administration, Contract DE-B179-88BI93743, Project 198806400, Portland, Oregon.
- Junk, W. J., P. B. Bayley, and R. E. Sparks. 1989. The flood pulse concept in river floodplain systems. *Canadian Special Publication of Fisheries and Aquatic Sciences* 106:110-127.
- Kincaid, H. 1993. Breeding plan to preserve the genetic variability of the Kootenai River white sturgeon. Final Report to Bonneville Power Administration, U.S. Fish and Wildlife Service. Project 93-27. Contract Number DE-A179-93B002886. Portland, Oregon. Also Appendix D in U.S. Fish and Wildlife Service. 1999. Recovery plan for the Kootenai River population of the white sturgeon (*Acipenser transmontanus*). Region 1, USFWS, Portland, Oregon.
- LaPatra, S. E., and six coauthors. 1996. Preliminary evidence of sturgeon density and other stressors on manifestation of white sturgeon iridovirus disease. *Journal of Applied Aquaculture* 6(3):51-58.
- LaPatra, S. E., S. C. Ireland, J. M. Groff, K. M. Clemens, and J. T. Siple. 1999. Adaptive disease management strategies for the endangered population of Kootenai River white sturgeon. *Fisheries* 24(5):6-13.
- Ligon, F. K., W. E. Dietrich, and W. J. Trush. 1995. Downstream ecological effects of dams. *BioScience* 45(3):183-192.
- Marcusen, P., V. Wakkinen, and G. Kruse-Malle. 1995. Kootenai River white sturgeon investigation. Idaho Department of Fish and Game. Annual Report prepared for Bonneville Power Administration. Contract No. DE-B179-88BP43497; Project No. 198806500, Portland, Oregon.
- Northcote, T. G. 1973. Some impacts of man on Kootenay Lake and its salmonids. Great Lakes Fisheries Commission, Technical Report, No. 2.
- Paragamian, V. L., and G. Kruse. 1996. Kootenai River white sturgeon (*Acipenser transmontanus*) spawning characteristics and habitat selection post Libby Dam. Pages 41-48 in S. Doroshov, F. Binkowski, T. Thuemeler, and D. MacKinlay, editors. *Culture and Management of Sturgeon*

- and Paddlefish Symposium Proceedings. Physiology Section, American Fisheries Society, Bethesda, Maryland.
- Paragamian, V. L., G. Kruse, and V. Wakkinen. 1997. Kootenai River white sturgeon spawning and recruitment evaluation. Idaho Department of Fish and Game. Annual Report prepared for Bonneville Power Administration. Contract No. DE-B179-88BP43497; Project No. 198806500, Portland, Oregon.
- Partridge, F. 1983. River and stream investigations. Idaho Department of Fish and Game, Federal aid to fish and wildlife restoration, Project F-73-R-5, Subproject IV, Study IV: Kootenai River Fisheries Investigations.
- Powell, M. S., and P. J. Anders. 1999. Assessing genetic variation among Columbia Basin white sturgeon populations. Annual Progress report to the Bonneville Power Administration. BPA Project 99-22.
- Roff, D. A., and P. Bentzen. 1989. The statistical analysis of mitochondrial DNA polymorphisms:  $X^2$  and the problem of small samples. *Molecular Biology and Evolution* (6):539-545.
- Snyder, E. B., and G. W. Minshall. 1996. Ecosystem metabolism and nutrient dynamics in the Kootenai River in relation to impoundment and flow enhancement for fisheries management. Annual Report. Stream Ecology Center, Idaho State University, Pocatello, Idaho.
- Sparks, R. E. 1995. Need for ecosystem management of large rivers and their floodplains. *Bioscience* 45(3):168-182.
- USFWS (U.S. Fish and Wildlife Service). 1994. Endangered and threatened wildlife and plants; determination of endangered status for the Kootenai River population of white sturgeon-Final Rule. Federal Register 59(171):45989-46002. (September 6, 1994).
- USFWS (U.S. Fish and Wildlife Service). 1999. Recovery plan for the Kootenai River population of the white sturgeon (*Acipenser transmontanus*). Region 1, USFWS, Portland, Oregon.
- USFWS (U.S. Fish and Wildlife Service). 2000. USFWS policy regarding controlled propagation of species listed under the Endangered Species Act. Federal Register 65(183):56916-56922 (September 20, 2000).
- Van Eenennaam, J. P., S. I. Doroshov, and G. P. Moberg. 1996. Spawning and reproductive performance of domestic white sturgeon (*Acipenser transmontanus*). Pages 117-122 in S. Doroshov, F. Binkowski, T. Thuemeler, and D. MacKinlay, editors. Culture and Management of Sturgeon and Paddlefish Symposium Proceedings. Physiology Section, American Fisheries Society, Bethesda, Maryland.
- Waples, R. 1991. Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. *Canadian Journal of Fisheries and Aquatic Sciences* 48(Supplement 1):124-133.
- Waples, R. S., and D. J. Teel. 1990. Conservation genetics of Pacific Salmon I. Temporal changes in allele frequency. *Conservation Biology* 4(2):144-156.