KOOTENAI RIVER WHITE STURGEON INVESTIGATIONS AND EXPERIMENTAL CULTURE

Annual Progress Report FY 1991

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Setline and angling techniques were used to sample 56 white sturgeon _Acipenser transmontanus_ from the Kootenai River in 1991. Of those sampled, nine were recaptures from previous years of this study. A total of 382 white sturgeon were captured from March 1989 through October 1991. Fork lengths of white sturgeon in the sample ranged from 88-274 cm. Our data indicated there was a complete lack of recruitment of juveniles into the population. The youngest fish sampled was of the 1977 year class. The population was estimated at 880 individuals with a 95% confidence interval of 638 to 1,211. Annual mortality of white sturgeon since 1982 is 3.74%. Approximately 80% of the population was more than 20 years old and was reproductively mature. Surgical examination of 309 white sturgeon since 1989 indicated that approximately 7% of the female white sturgeon and 30% of the male white sturgeon are reproductive each year. The ratio of males to females was estimated at 1:1. White sturgeon sampled and released with and without surgical examination were recaptured at equal rates.

An ongoing sonic telemetry study has documented long distance movements by adults. White sturgeon regularly move across the British Columbia - Idaho border. White sturgeon seek out deep holes in the river or migrate to Kootenay Lake during late fall. During spring and early summer of both 1990 and 1991 reproductively mature white sturgeon moved from 15 to 110 km upriver and congregated within 10 km downriver from Bonners Ferry in areas of elevated water velocity. This behavior coincided with increasing discharge and water temperatures. Developing white sturgeon eggs were recovered from the river near Bonners Ferry on July 3, 1991.

Contamination of eggs by organochloride compounds were less in recent samples from the Kootenai River than in a single sample collected in 1982. White sturgeon eggs from the Kootenai River fish contained approximately one tenth the organochloride compounds of white sturgeon eggs from the lower Columbia River.

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TEXT

INTRODUCTION

The population of white sturgeon in the Idaho section of the Kootenai River was first surveyed in 1979 through 1982 (Partridge 1983). Recruitment of juvenile white sturgeon to the population appeared to be insufficient to sustain the harvest of adult fish, forcing a closure on harvest beginning in 1984.

When this study began, knowledge regarding habitat requirements of white sturgeon and our understanding of environmental influence on distribution, movement, spawning, and juvenile survival was insufficient to allow us to determine how development and management of the Kootenai River has impacted this species.

This project, authorized by the Northwest Power Planning Council (1987) and funded by Bonneville Power Administration (BPA), is an effort to identify environmental factors limiting the white sturgeon population in the Kootenai River and to recommend effective management actions to restore the wild population. Concurrently, BPA is providing the Kootenai Indian Tribe of Idaho with funding to develop an experimental white sturgeon culture facility on the Kootenai River to help evaluate the reproductive potential of the wild population and to culture fish to supplement this population. In November 1988, the Idaho Department of Fish and Game (IDFG) and Kootenai Tribe began working cooperatively to meet the goal of restoring this population.

DESCRIPTION OF STUDY AREA

Geography

The Kootenai River originates in Kootenay National Park, British Columbia (B.C.), flowing south into Montana, then turning northwest at Jennings, the site of Libby Dam, at river kilometer (rkm) 352.4 (Figure 1). Kootenai Falls, 50 km below Libby Dam, presents an impassable barrier to white sturgeon. As the river flows through the northeast corner of Idaho, a definite reach change occurs at Bonners Ferry. Upstream from town, the river has an average gradient of 0.6 m/km, with velocities higher than 0.8 m/s. Downstream from Bonners Ferry the river slows to an average gradient of 0.02 m/km, deepens, and meanders through the Kootenai Valley back into B.C., into the south arm of Kootenay Lake. The river leaves the lake through the west arm to a confluence with the Columbia River at Castlegar. A natural barrier at Bonnington Falls (and now a series of four dams) has isolated the Kootenai River white sturgeon from other populations in the Columbia River basin for approximately 10,000 years (Northcote 1973). The basin drains an area of 49,987 km^2 (Bonde and Bush 1975).

Development

Spring floods were common prior to commencement of operation of Libby Dam in 1972. Constructed by the U.S. Army Corps of Engineers, Libby Dam provides flood control and hydropower generation and is part of the BPA network. Dam operation drastically alters natural flow levels by storing water during spring runoff, discharging power peaking flows during late summer and fall, and drawing down the reservoir throughout winter. Corra Linn Dam effectively raises the mean level of Kootenay Lake 2.4 m, influencing the river level to Bonners Ferry.

To protect agricultural land between Bonners Ferry and Kootenay Lake, the riverbanks have been diked extensively since the 1920s, effectively removing most backwater and slough areas from the river system.

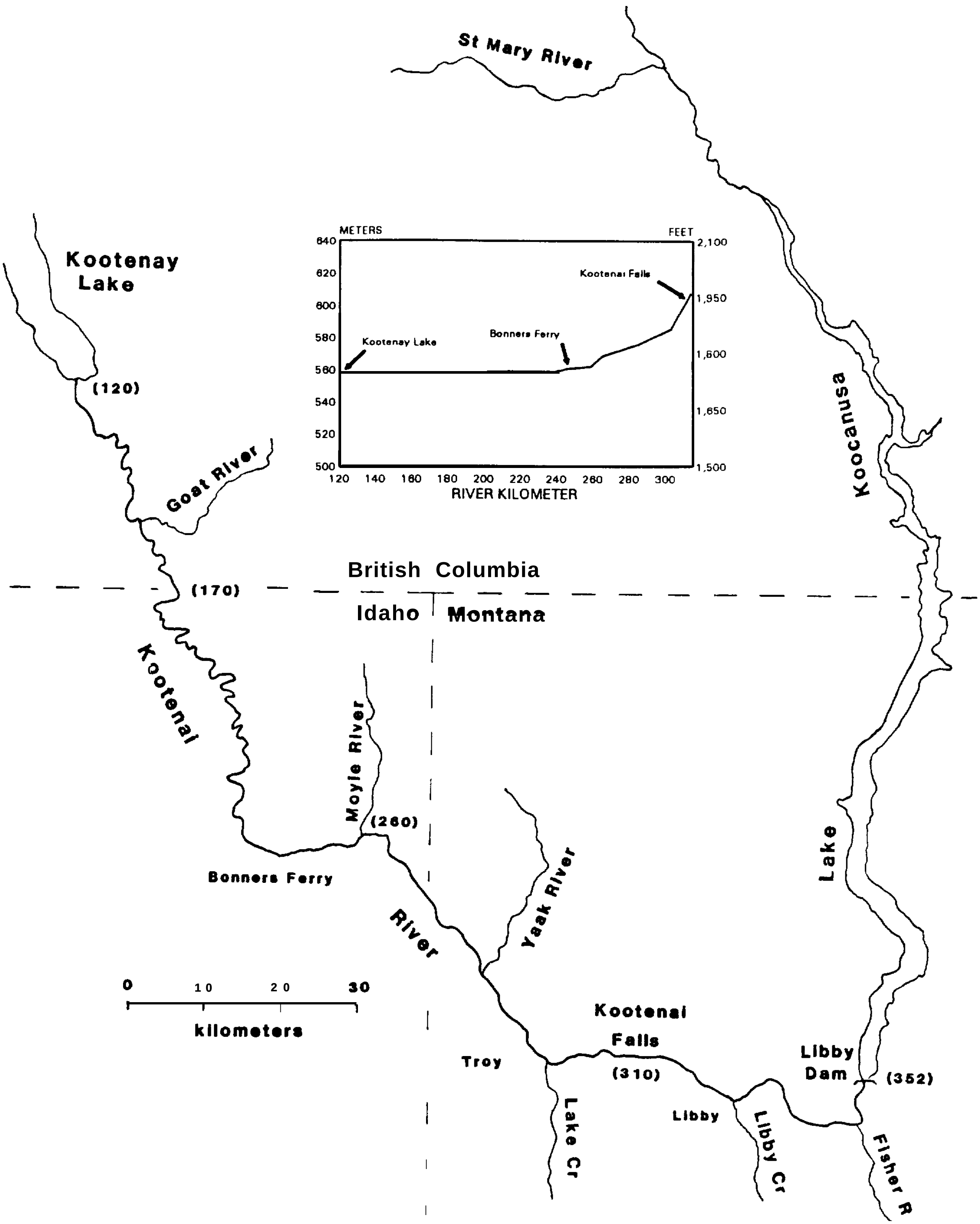


Figure 1. Map of the Kootenai River with a schematic of river gradient. Study area was from southern Kootenay Lake upriver to Kootenai Falls.

Contaminants

Prior to construction of Libby Dam, most point source pollution in the Kootenai River drainage came from a mine and fertilizer plant upriver on the St. Mary River (Bonde and Bush 1975). The ASARKO mine (copper and silver) on Lake Creek near Troy, Montana is the only current mining operation in the drainage below the dam. The closed Snowshoe Mine on Snowshoe Creek (tributary to Libby Creek) was reclaimed in 1989 due to water quality problems.

White Sturgeon Fishery

Idaho

Harvest of white sturgeon from the Kootenai River has been regulated in Idaho since 1944 when commercial fishing was prohibited. Increasingly restrictive statewide harvest limits and length restrictions were imposed over the years (Table 1). From 1944 through the mid-1970s, 10 to 20 fish were harvested per year; an estimated 43 to 50 white sturgeon/year were harvested from 1979 through 1982. The first and only prior investigation of fisheries resources on the lower Kootenai River was conducted from 1979 through 1982 by Partridge (1983). Partridge found that only 13% of his sample of white sturgeon were younger than age 15 and smaller than 92 cm total length, the legal size for harvest. Concluding this lack of recruitment of juveniles was limiting the fishery, harvest of white sturgeon was terminated in 1984.

Montana

Harvest of white sturgeon was not restricted in Montana prior to 1972 (Table 1). Harvest was restricted to two fish/year with a slot regulation of 102 to 183 cm total length for the next six years. Five to 18 white sturgeon were legally harvested annually during that period. The fishery was completely closed in 1979 (Graham 1981). Montana officials have declared white sturgeon a "species of special concern" due to the very small number (an estimated five fish) residing in the river in 1979.

British Columbia

White sturgeon harvest has been restricted in B.C. since 1952 (Table 1). From 1974 through 1989, anglers in B.C. were allowed to harvest one white sturgeon/year with a minimum length restriction of 1 m. Beginning in 1989 setlining for white sturgeon was prohibited, limiting method of harvest to angling. All white sturgeon harvest was prohibited in the Kootenai River beginning in 1990. Prior to this closure, 5 to 18 fish were harvested annually, and illegal harvest may have increased that estimate by 50% (Andrusak 1980). Since 1977, the B.C. Ministry of Environment tagged 180 white sturgeon at the mouth of the Kootenai River where it enters the south arm of the lake. Several of those fish were recaptured in Idaho (Andrusak 1980).

Table 1. A history of fishing regulations for white sturgeon in the Kootenai River.

Year	Idaho	Sturgeon fishing regulations Montana	British Columbia
1944	2 in possession; no yearly limit: no commercial harvest		
1948	1 setline; 1 in possession		
1949	1 setline; 1 in possession; 76 cm minimum size		
1952			setlines permitted; 1 per day; 92 cm minimum size
1955	1 Setline; 1 in possession: 102 cm minimum size		
1957	1 Setline; 2 per year; 1 in possession; 102 cm minimum size	setlines permitted for ling only	
1960	1 setline; 2 per year: 1 in possession; 92 - 183 cm length restriction		
1968		setline permitted for sturgeon February 15 through June 30	
1973		6 setlines with 6 hooks per line permitted February 15 through June 30; 2 per year; 102 - 183 cm length restriction	
1975		no setlines permitted; 2 per year: 102 - 183 cm length restriction	
1978			100 an minimum size
1979	2 per year; 1 in possession; 92-183 cm length restriction; permit required	closed	
1981			1 per year; 100 cm minimum size
1982			Sturgeon declared a game fish
1983	setlines prohibited; season: July 1 through December 31; 1 per year; 92-183 cm length restriction		
1984	catch and release only; open all year		
1989			setlines prohibited
1990			catch and release only

OBJECTIVES

1. Assess the status of white sturgeon in the Kootenai River between Kootenay Lake and Kootenai Falls with regard to distribution, population size, reproduction, and recruitment.
2. Describe weekly and seasonal movements of white sturgeon and describe the use frequency of physical habitat parameters, including depth, focal point velocity, temperature, and turbidity.
3. Determine gamete viability by experimental culture of white sturgeon from the Kootenai River.
4. Test experimental culture as a means of recruiting white sturgeon to the population.
5. Determine effects of pollutants on white sturgeon reproduction by measuring levels of contaminants in white sturgeon ova and offspring, and in river sediments.
6. Determine if Kootenai River white sturgeon are genetically different from other white sturgeon stocks by electrophoretic analysis.

METHODS

Population Status

Adult and Sub-adult White Sturgeon Sampling

Detailed methods for capture, handling, marking, and collecting habitat use information on sub-adult and adult white sturgeon are provided in Apperson and Anders (1990). All sonic transmitters placed on white sturgeon during 1991 were Sonotronics model ST-71-3 (65 mm x 18 mm; 8 g) equipped with 50-month batteries and a detection limit of up to 1 km.

White Sturgeon Egg and Larvae Sampling

Twelve mats of filter material (latex-coated animal hair) bolted to angle iron frames (62 x 75 cm) were used to collect white sturgeon eggs and, thereby, document spawning activity (McCabe and Beckman 1990). Substrate mats were deployed in the river from May 30 through July 12, 1991. Mats were set out in groups of three from rkm 245 to 255 in depths from 6 to 12 m, anchored with one or two 10-kg cement weights. Mats were checked weekly for eggs.

On June 3 and 4, 1991, Montana Department of Fish, Wildlife and Parks sampled the Kootenai River for white sturgeon eggs and larvae with a D-ring plankton net. On June 11 through June 13, a crew from the Columbia River Field Station of the U.S. Fish and Wildlife Service sampled the Kootenai River for white sturgeon eggs and larvae with a beam trawl. Detailed methodology and gear specifications are given by Parsley et al. (1989).

Habitat Utilization and Seasonal Movement

General habitat suitability indices and habitat parameter utilization frequency histograms generated for adult white sturgeon in the Kootenai River are discussed in Apperson and Anders (1991). During 1991 we monitored reproductively mature white sturgeon weekly from mid-April through July in an effort to identify timing of spawning related movement, timing of spawning, and habitats used for spawning.

Analysis of Contaminants

Levels of copper, zinc, lead, aluminum, strontium, and organochlorides in white sturgeon oocyte samples and in Kootenai River sediment samples were determined by Am Test, Inc. of Redmond, Washington. Metals were analyzed in accordance with Environmental Protection Agency (EPA) method 6010 (US EPA 1986) and organochlorides were analyzed according to Method PPB 12/83 (US EPA 1983). Copper, zinc, and strontium were chosen because previous water quality monitoring singled out these metals as being at potentially harmful levels (Bonde and Bush 1975). In 1990 and 1991 samples were examined for aluminum because high levels were found in preliminary well water quality tests at a proposed site for the culture facility adjacent to the river. Lead was examined because of its prevalence near silver and copper ore bodies that were being mined in the drainage. Organochlorides were measured because relatively high levels of DDT and its metabolites and polychlorinated biphenyl (PCB) were found in a white sturgeon ova sample in 1982 (IDFG).

RESULTS

Population Structure

Population Estimate and Abundance

Sample data from 1989 and 1990 were used to estimate the number of white sturgeon in the Kootenai River. The population was estimated at 880 individuals, with a 95% confidence interval of 638 to 1,211 (Apperson and Anders 1991). This translates to an average abundance of seven white sturgeon/km between Bonners Ferry and Kootenay Lake, with confidence interval of 5 to 10 white sturgeon.

A total of 382 white sturgeon were captured from March 1989 through October 1991. Catch per unit of effort of sampling is summarized by year, river section, and gear type in Appendix A. All but two fish were marked with Floy tags and 317 fish received Passive Integrated Transponder (PIT) tags. Fifty-six white sturgeon were recaptured, six of those were recaptured twice. Floy tags were lost from 11 white sturgeon (seven of those were lost during the year of tagging), but PIT tags were secure in all recaptured fish. With one exception, all white sturgeon were captured between rkm 244.5 (I rkm downstream from the Highway 95 bridge at Bonners Ferry) and the Kootenay Lake delta at rkm 120. In May 1989 one white sturgeon (105 cm fork length) was caught in Montana at rkm 310.5 (two rkm below Kootenai Falls). Using data from initial capture, fork length (FL) of white sturgeon in the sample ranged from 88 cm to 274 cm, with a mean of 150 cm (Figure 2). A weight-length relationship developed from 223 measurements was weight = (1.016×10^{-6})fork length$^{3.394}$ (Figure 3).

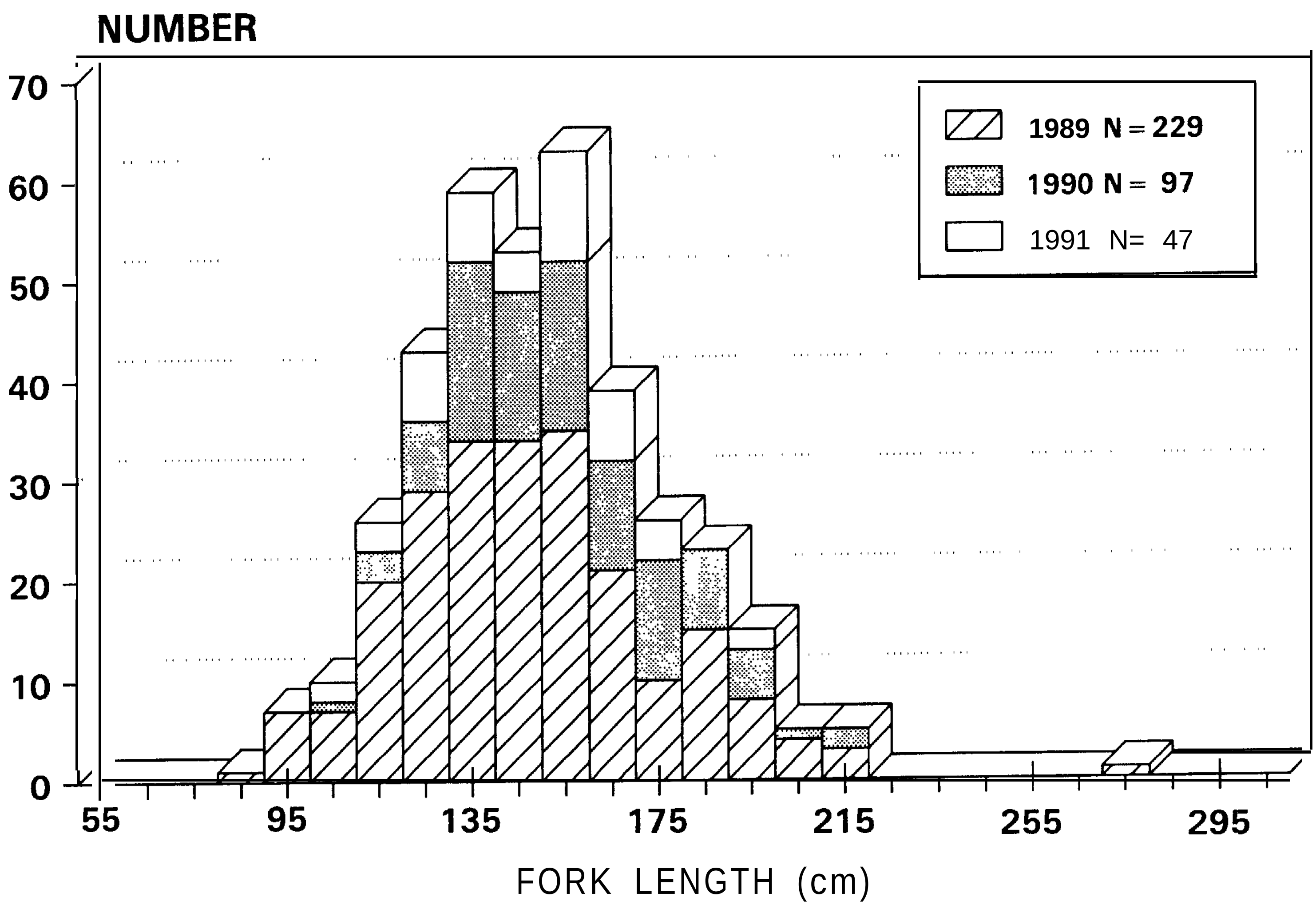


Figure 2. Length frequency of white sturgeon sampled from the Kootenai River, 1989 through 1991.

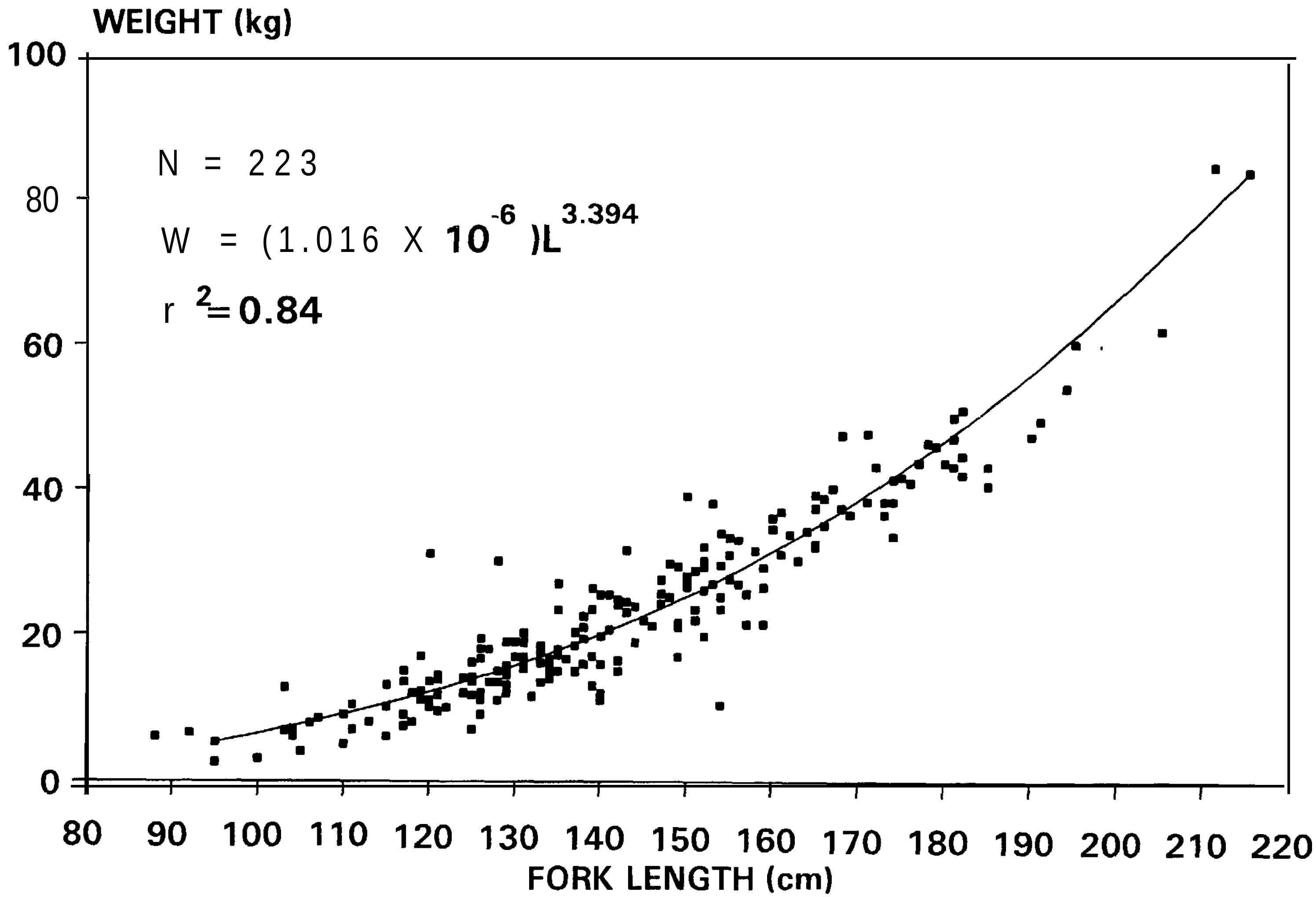


Figure 3. Weight-length relationship for white sturgeon sampled from the Kootenai River, 1989 through 1991.

Age and Growth

Average annual growth rate for 17 white sturgeon tagged in 1979 through 1982 and recaptured in 1989 through 1991 was 3.1 cm FL and 3.7 cm total length (TL) (Table 2). Those fish ranged from 100 to 158 cm FL when recaptured during the current study. A comparison of eight old and new samples of pectoral fin-ray sections showed discrepancies between estimated age differences and actual time between collection of samples of up to three years with a mean difference of 1.5 years (Table 3). Annuli discrepancies occurred on the outer edges of fin-ray sections.

Reproductive Potential

Maturity

A total of 309 white sturgeon was surgically examined to determine sex and stage of maturity. Twenty-two white sturgeon were surgically examined twice, with surgeries no less than one year apart. Eight males were identified by milt extraction without surgery. Of those surgically examined, 83% were positively sexed (Table 4). I surmise that refinement of our technique was responsible for the decrease in proportion of unsexable white sturgeon from 1989 to 1991, and the increase in non-reproductive males. Also, a factor was our disproportionate sampling during 1990 and 1991 in the section of river where reproductive fish concentrated. White sturgeon sampled and released with and without surgical examination have been recaptured at equal rates of 15%.

Lengths of white sturgeon at various reproductive stages is presented in Figure 4. The smallest ripe female in our sample was 135 cm FL and two females smaller than 130 cm FL were judged to be spent. White sturgeon of this size range in the Kootenai River are 19 to 29 years old. The smallest reproductive male was 115 cm FL and 23 years old.

Wild Spawning

D-ring net trawling-A total of 23 5-minute drift samples were made with the D-ring larval net on June 3 and 4 between rkm 245 and 258 (Bonners Ferry to Moyie River), but failed to collect white sturgeon eggs or larvae. Samples were collected at river depths of 2 to 9 m. Water temperature was 9 to 10°C and discharge was approximately 950 m^3/s (33,545 cfs) during sampling.

Beam net trawling-A repeat of the 1990 trawling effort by the U.S. Fish and Wildlife Service (Apperson and Anders 1991) failed to collect white sturgeon eggs or larvae. Trawling was conducted on June 11 through 13 at water temperatures of 10 to 11°C and discharge of approximately 1050 m^3/s (37,075 cfs) between rkm 230 and 260 (Shorty's Island to Moyie River).

Artificial substrate sampling-On July 3, 13 white sturgeon eggs were recovered on an artificial substrate at rkm 245, within 100 m downriver from the railroad bridge at Bonners Ferry. The eggs (preserved in 10% unbuffered formalin) were examined by the U.S. Fish and Wildlife Service's Willard Lab and determined to be approximately three days old and in various stages of development from yolk plug formation to closure of the neural tube. Water temperature was 14°C from June 27 through July 3; and discharge ranged from approximately 400 to 550 m^3/s (14,125 to 19,400 cfs) between June 29 and July 2. On July 3 through July 9 discharge increased to more than 900 (31,780) m^3/s.

Table 2. Growth rate of white sturgeon in the Kootenai River based on recaptured fish.

	First capture			Recapture			Mean annual growth	
Old tag number	Date[a]	Length[b] FL/TL (cm)	Weight (kg)	Date	Length FL/TL (cm)	Weight (kg)	Length[c] FL/TL (cm)	Weight[d] (kg)
03871	11/80	117/127	12.3	4/89	138/154	21.0	2.5/3.2	1.0
G0648	8/81	112/125	11.8	5/89	144/158	--	4.1/4.3	
00754	7/82	127/143	16.8	5/89	135/155	27.0	1.2/1.8	1.5
00652	8/81	92/102	5.9	9/89	130/145	19.0	4.8/5.4	1.6
00656	9/81	92/99	6.4	6/89	111/123	7.0	2.4/3.1	0.1
00663	9/81	129/145	17.7	6/89	155/178	--	3.3/4.2	
00699	5/82	106/121	10.0	7/89	140/160	12.0	4.7/5.4	0.3
00748	6/82	111/127	11.4	9/90	128/147	13.5	2.1/2.5	0.3
00717	6/82	69/79	2.2	8/89	100/112	3.0	4.3/4.6	0.1
00671	10/81	113/127	13.9	9/89	129/146	--	2.0/2.4	
00728	6/82	105/119	9.5	9/89	135/153	15.0	4.1/4.7	0.8
00753	7/82	118/125	12.3	9/89	135/143	27.0	2.3/2.5	2.0
03801	7/80	120/128	12.7	6/89	142/161	16.5	2.4/3.7	0.4
03817	7/80	1171130	12.3	7/82	---/136	13.6	---/3.0	0.7
03817	subsequent recapture			8/90	158/178	31.5	4.1/4.8	1.9
03715	5/80	101/112	7.3	5/81	---/117	8.6	---/5.0	1.3
03715	subsequent recapture			8/91	125/141	14.8	2.4/2.9	0.7
03944	5/81	107/115	9.1	6/91	129/143	15.8	2.2/2.8	0.7
03748	6/80	59/ 68	1.3	8/91	103/116	6.8	4.0/4.4	0.5

[a] Month/year
[b] FL = fork length; TL = total length
[c] FL: $N = 17$; $\bar{x} = 3.11$; $s = 1.11$
TL: $N = 19$; $x = 3.72$; $s = 1.11$
[d] Weight: $N = 16$; $\bar{x} = 0.87$; $s = 0.62$

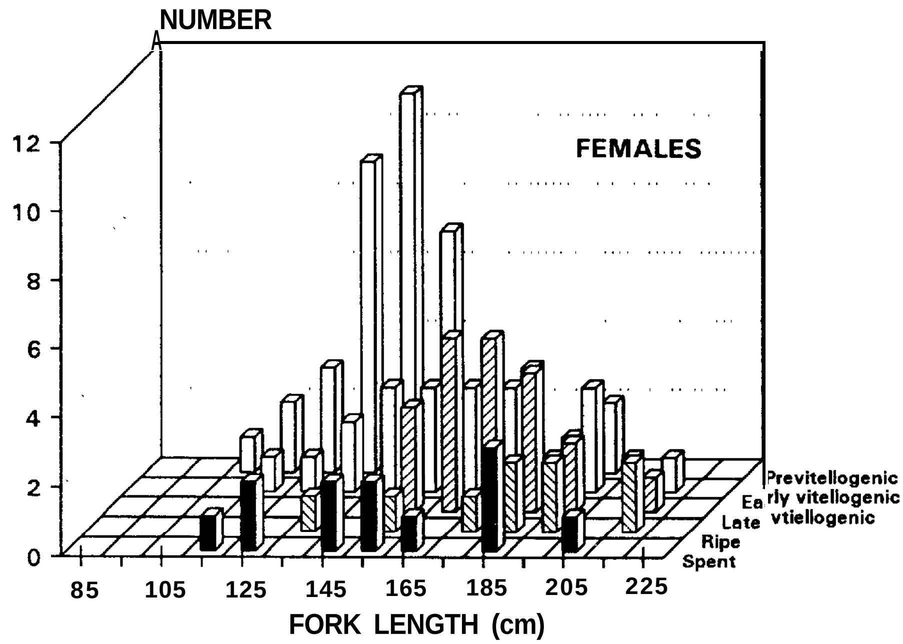
Table 3. Comparison of past and present estimated ages of individual white sturgeon in the Kootenai River.

Tag number	Past estimate Month/year	Age	Current estimate Month/year	Age	Estimated age difference	Actual years lapsed	Difference[a]
01085	8/81	15	6/89	24	9	8	1
01093	9/81	15	6/89	21	6	8	2
01114	7/80	16	6/89	24	8	9	1
01215	10/81	18	9/89	23	5	8	3
01027	8/81	19	5/89	26	7	8	1
01023	11/80	20	4/89	26	6	9	3
01543	5/80	17	8/90	27	10	10	0
01600	6/80	6	8/91	16	10	11	1

[a] Mean difference between estimated and actual age differences was 1.5 years.

Table 4. Sexual development of white sturgeon sampled in the Kootenai River, 1989 through 1991.

Categories of sexual development			Percent (number) of sample by year		
Category	Sex	Description of development	1989	1990	1991
0	unknown	gonad undifferentiated or not seen	31.7 (57)	13.5 (15)	6.1 (3)
1	Female	Previtellogenic: no visual signs of vitellogenesis; eggs present but have average diameter	13.9 (25)	11.7 (13)	8.2 (4)
2	Female	Early vitellogenic: eggs are cream to gray; average diameter 0.6 to 2.1 mm	6.7 (12)	7.2 (13)	4.1 (4)
3	Female	Late vitellogenic: eggs are pigmented and attached to ovarian issue; average diameter 2.2 to 2.9 mm	5.6 (10)	4.5 (5)	10.2 (5)
4	Female	Ripe: eggs are fully pigmented and detached from ovarian tissue; average diameter 3.0 to 3.4 mm	1.7 (3)	4.5 (5)	2.0 (1)
5	Female	Spent: gonads are flaccid and contain some residual fully pigmented eggs	3.3 (6)	0.9 (1)	2.0 (1)
6	Female	Previtellogenic with attritic oocytes: eggs present but have an average diameter <0.5 mm; dark pigmented tissue present that may be reabsorbed eggs	1.7 (3)	0	0
7	Male	Non-reproductive: testes with translucent smokey pigmentation	3.3 (6)	27.0 (30)	30.6 (15)
8	Male	Reproductive: testes white with folds and lobes	32.2 (58)	27.9 (31)	16.3 (8)
9	Male	Ripe: milt flowing: large white lobular testes	0	2.7 (3)	20.4 (10)



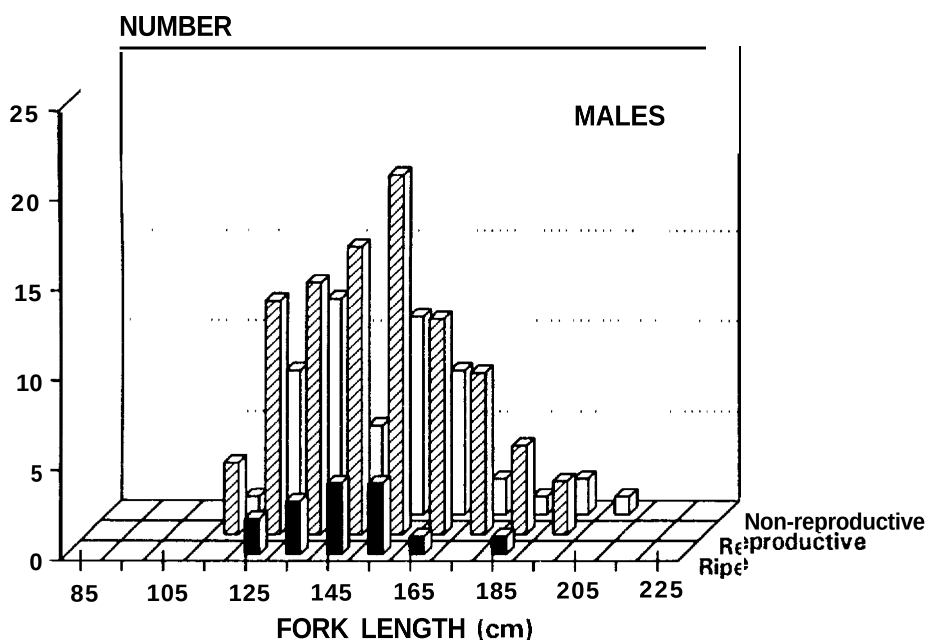


Figure 4. Length frequencies of reproductive stages of white sturgeon in the Kootenai River, 1989 through 1991. The spent female category includes previtellogenic fish with attritic oocytes.

Contaminants Analysis

Detectable levels of aluminum, copper, lead, zinc, and strontium were found in oocyte samples from white sturgeon in the Kootenai River along with detectable levels of PCB's and pesticides (DDD, DDE, DDT, and Dieldrin) (Table 5; Appendix B). Levels of PCB's and pesticides in three oocyte samples from the lower Columbia River were approximately 10-fold higher than levels found in the Kootenai River oocyte samples.

River sediment samples collected in 1989 contained 1.62 to 12.8 µg/g copper and 22.4 to 70.6 µg/g zinc. Metal concentrations were considerably higher at the downriver site. No organochlorides were found in river sediments (Appendix B4).

Movement and Habitat Use

From June 1989 through October 1991, ultrasonic transmitters were attached to 34 adult white sturgeon that were captured throughout the Kootenai River downriver from Bonners Ferry (Table 6; Figure 5). Analyses of seasonal habitat use and movement patterns of white sturgeon tracked through 1990 are reported by Apperson and Anders (1991).

Spawning Related Movement

Six reproductively mature white sturgeon (three males and three females) were located weekly from April through July 1991 to continue to obtain information on spawning related movements. By May all six white sturgeon moved upriver from Shorty's Island (rkm 231) and moved between Shorty's Island and rkm 243 (2 km downriver from Bonners Ferry) through July (Figure 6; Appendix C). These fish remained congregated in the same areas as during 1990, and where all brood fish were collected in 1991, including 10 ripe males. Water velocities at transmittered white sturgeon locations during that time period ranged from 0.3-0.6 m/s in 1990, and 0.4-0.8 m/s in 1991.

From July through October 1991, transmitters were attached to five female white sturgeon that will be reproductively mature within two years. Transmitters remained active on two adult male white sturgeon that were tagged earlier in 1991.

DISCUSSION

Population Structure and Reproductive Potential

The pattern of the past and present length frequencies of white sturgeon in the Kootenai River is very similar, but a 40 cm shift toward larger fish was noted (Figure 7a). Lengths of 417 white sturgeon sampled from the Kootenai River in 1980 through 1982 ranged from 50 cm TL to 224 cm TL, with a mean TL of 122 cm (Partridge 1983). It appears from this and from growth rate data that the same individuals sampled 10 years ago have grown, with virtually no recruitment of juveniles into the population. Our sample of 374 white sturgeon collected since 1989 included only eight fish that were younger than the 1972 year class. Estimated ages of these fish by five analysts varied by zero to two years. I believe that our sample accurately represented the population. Partridge (1983)

Table 5. A comparison of contaminants found in oocytes of white sturgeon from the Kootenai and Columbia rivers, 1989 through 1991.

Contaminant	Kootenai River Range of concentrations	Kootenai River Sample size	Columbia River Range of concentrations	Columbia River Sample size
PCB's (µg/kg)[a]				
Arochlor - 1260	ND[b] -330	12	ND -3,741	3
Pesticides (µg/kg)[a]				
Heptachlor	ND	12	ND - 112	3
Aldrin	ND	12	ND - 77	3
				3
pp-DDD	ND --660	12	ND 505-12,099	3
pp-DDT	ND - 96	12	ND - 214	3
Metals (µg/g)[a]				
Zinc	1.39 - 32.8	17		0
Copper	1.18 - 3.2	18	--	0
Aluminum	1.2 -200	8	--	0
Lead	ND - 1.6		--	0
Mercury	ND	2	--	0
Cadmium	ND	2	--	0
Strontium	ND - 0.32	5	--	0

[a] Reported on a "wet weight basis"; where necessary dry weight was converted by multiplying by percent total solids.
[b] ND = not detected; detection limits varied with quantity of each sample; refer to Appendix B for detailed report of analyses.

Table 6. White sturgeon tagged with ultrasonic transmitters in the Kootenai River, 1989 through 1991.

Sonic code	Sex (stage)[a]	Total length (cm)	Initial capture		Last location	
			Date	River km	Date	River km
249	M(8)	158	05/17/89	237.7	11/07/90	215.9
258[b]	F(2)	155	05/17/89	234.5	10/07/91	236.3
276	M(8)	141	05/17/89	234.5	10/10/90	215.5
267	F(1)	123	05/31/89	225.1	10/11/90	120.0
294	M(8)	164	06/07/89	203.6	09/17/90	215.5
285	M(8)	169	06/09/89	199.5	07/24/90	140.0
339	F(2)	171	06/13/89	192.0	09/31/89	120.0
357[c]	M(8)	146	06/14/89	193.1	11/07/90	192.2
2228[d]	F(5)	215	06/14/89	193.1	11/06/90	212.5
366[a]	M(8)	164	06/14/89	191 .o	04/25/90	211.2
348	M(8)	185	06/14/89	190.0	10/25/90	215.5
384	Unknown	151	06/23/89	154.2	06/21/90	230.0
2255[d]	F(2)	170	06/22/89	163.0	10/30/90	120.0
2246[d]	F(4)	207	06/28/89	140.0	11/06/90	209.7
456	M(8)	156	06/28/89	138.7	10/30/90	120.0
465[f]	F(3)	220	07/20/89	205.5	10/30/90	120.0
375	F(3)	215	07/20/89	213.2	11/07/90	216.2
2264[d]	F(2)	143	08/31/89	228.7	04/11/91	228.6
2237[d]	F(6)	136	09/06/89	216.0	04/19/91	215.5
447	M(8)	170	09/26/89	215.2	04/10/91	120.0
88	F(3)	196	04/11/90	225.0	06/02/91	120.0
10579	M(9)	152	05/30/90	230.0	06/26/91	174.0

[a] Refer to Table 4 for definitions of reproductive stages for sturgeon.
[b] Replaced with transmitter #366 on 07/07/90 at rkm 232.6.
[c] Not moved since 07/18/89; suspect fish lost transmitter.
[d] Two-year transmitter.
[e] Taken to netpen on 04/25/90; released without transmitter on 07/17/90.
[f] Fish recaptured and transmitter removed on 08/29/91 at rkm 225.1.
[g] Transmitter not coded.

Table 6. Continued.

Sonic code	Sex (stage)[a]	Total length (cm)	Initial capture Date	Initial capture River km	Last location Date	Last location River km
555[h]	F(4)	236	06/29/90	204.0	04/25/91	115.0
97[i]	F(3)	181	06/24/90	204.0	05/07/91	120.0
2273	F(2)	214	09/26/90	129.8	10/07/91	224.7
2327	F(4)	212	11/01/90	213.5	06/29/91	120.0
284	M(8)	181	04/03/91	225.1	09/12/91	120.0
248	M(9)	192	06/01/91	240.5	10/07/91	120.0
347	F(3)	201	07/11/91	231.0	10/01/91	209.6
266[j]	F(5)	170	08/11/91	241.0	09/12/91	120.0
2435	F(2-3)	175	08/27/91	121.0	10/07/91	210.5
293	F(2)	185	08/27/91	121.0	09/12/91	120.0
338	F(3)	198	08/29/91	225.1	08/29/91	225.1
876[g]	F(3)	184	10/25/91	215.5	10/25/91	215.5

[h] Fish did not move since 09/18/90; suspect she died; was held in netpen from 06/08-06/29/90

[i] Released from netpen; originally captured at rkm 231.3 on 06/03/90.

[j] Released from hatchery, 1991 brood fish.

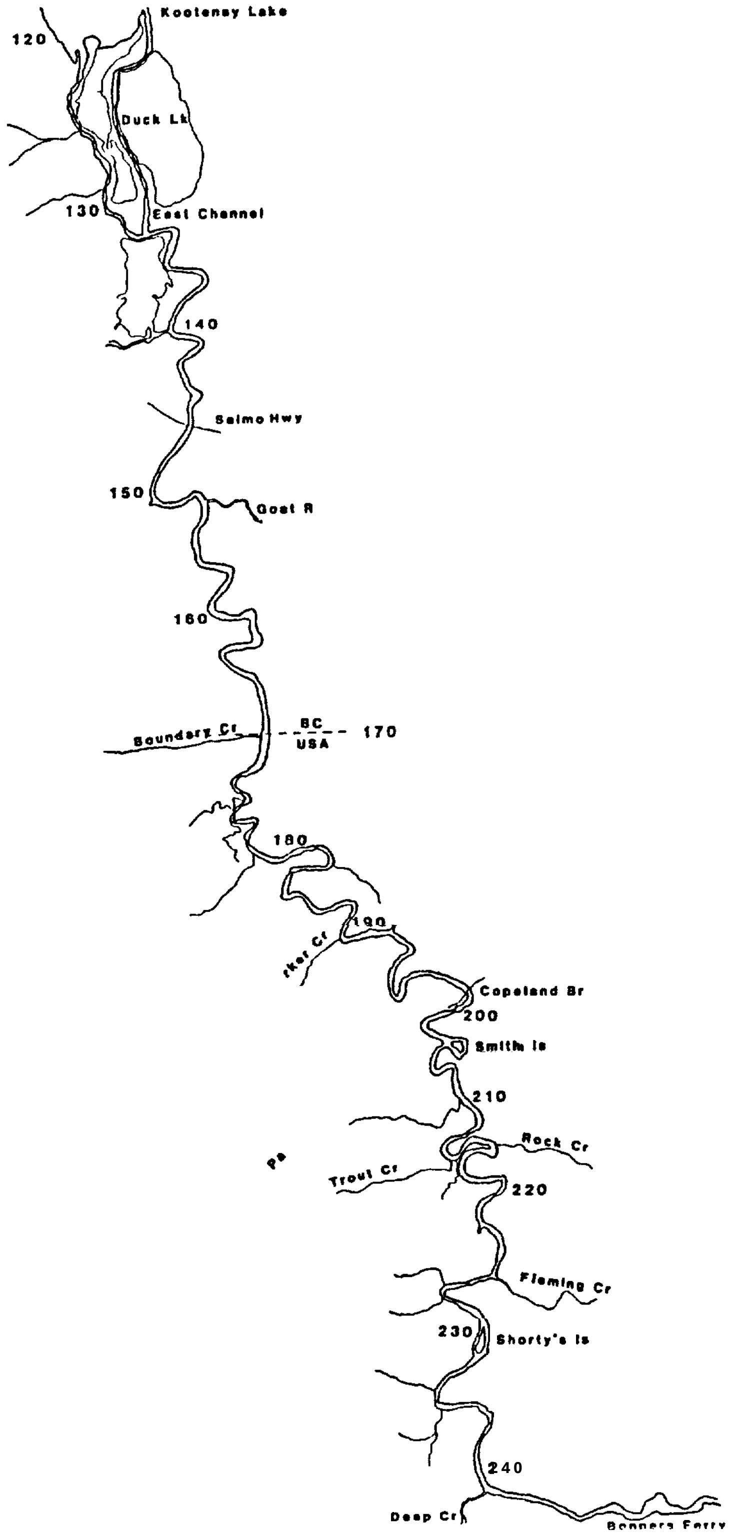


Figure 5. The lower Kootenai River. Numbers refer to river kilometers from the Columbia River.

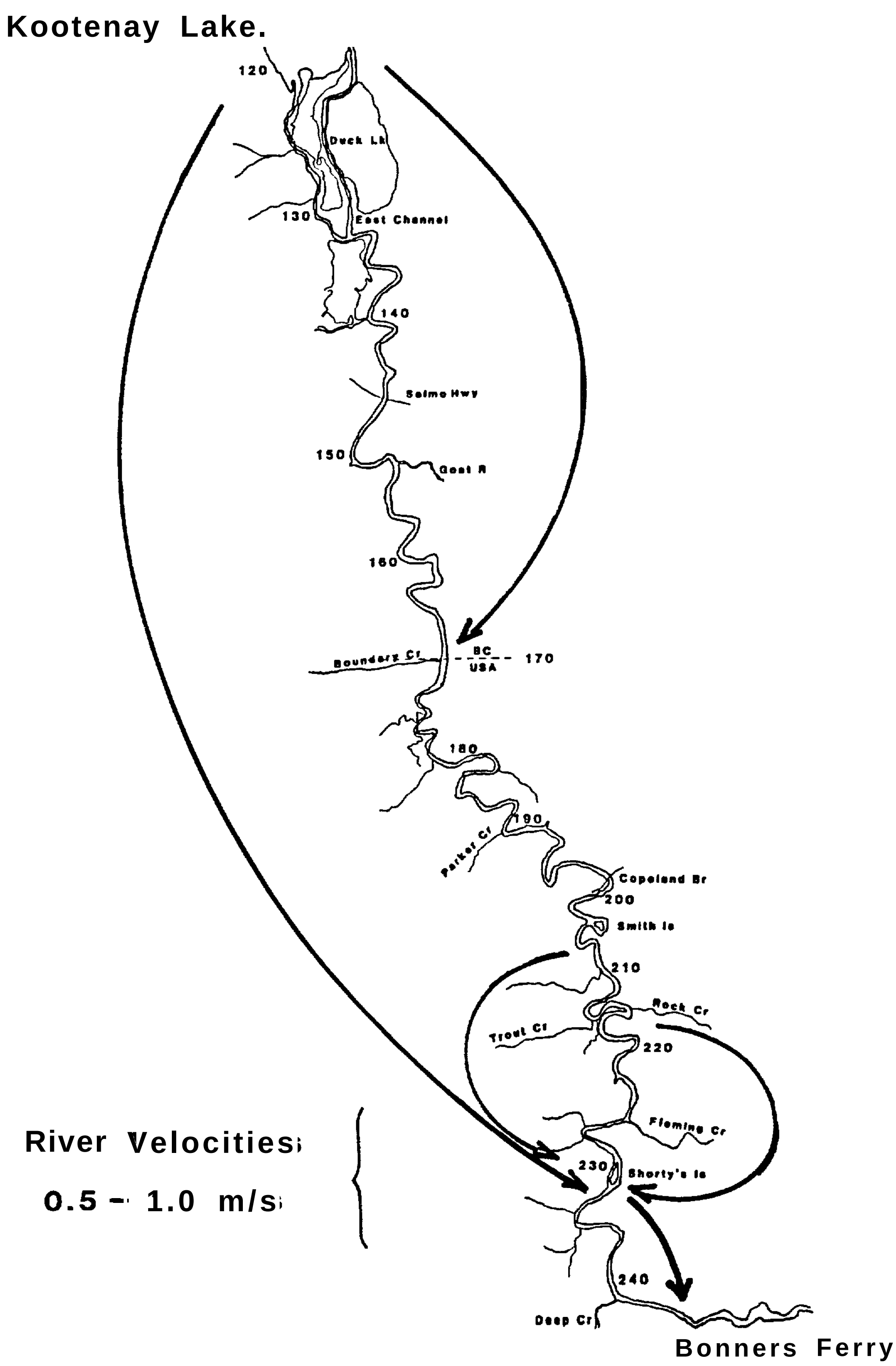
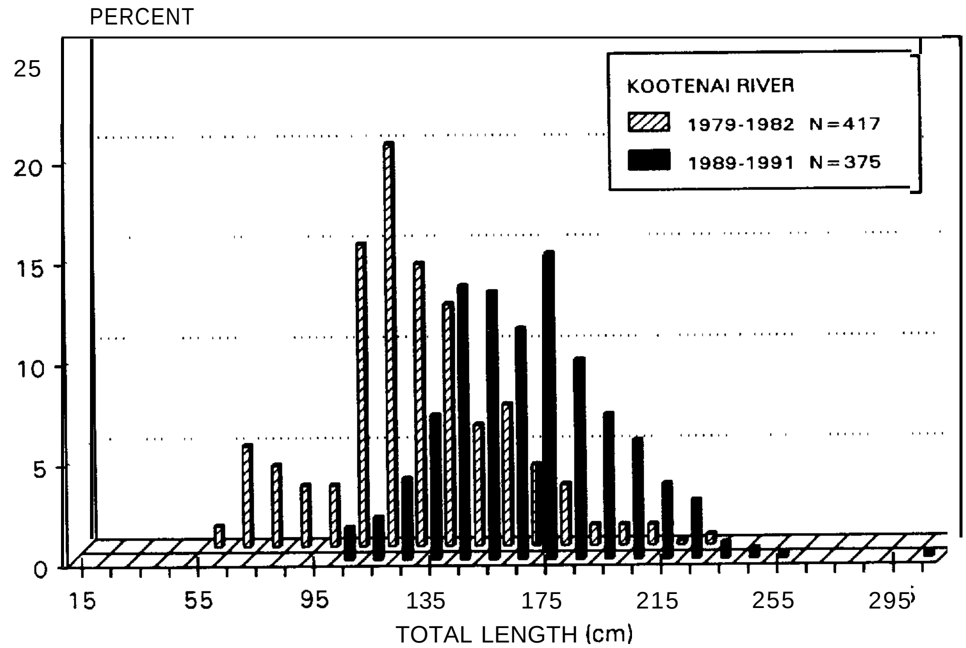


Figure 6. Observed upriver spring migrations of reproductively mature white sturgeon in the Kootenai River, 1990 and 1991.

A



B

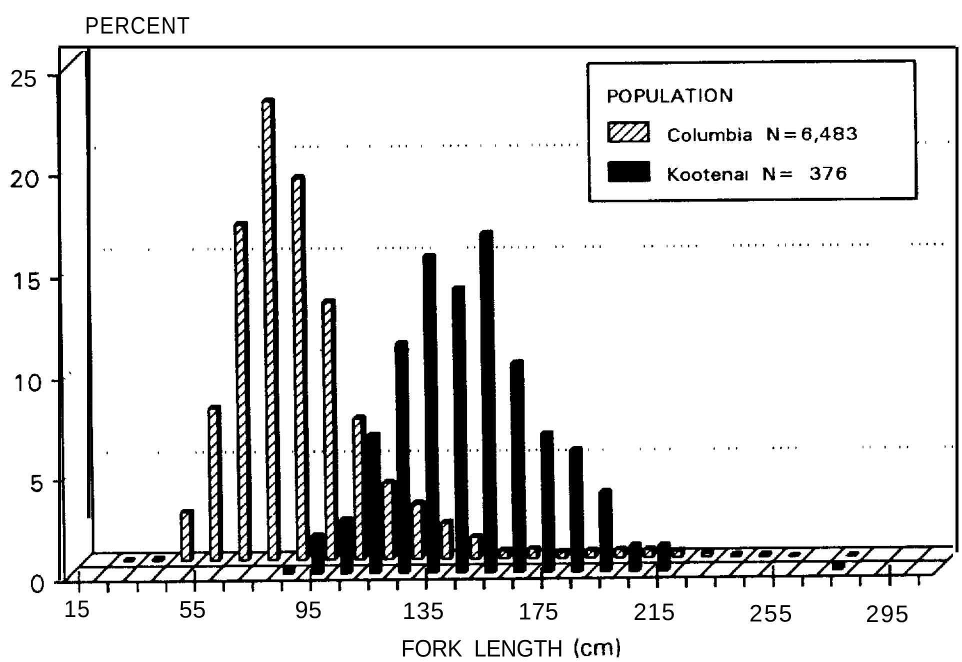


Figure 7. A) Comparison of past (Partridge 1983) and current length frequencies of white sturgeon in the Kootenai River.
B) Comparison of current length frequencies of white sturgeon sampled by setline in the Kootenai River and lower Columbia River (Beamsderfer et al. 1990).

observed a relatively strong year class of white sturgeon produced in 1974. One to five of the white sturgeon in our sample may have been of the 1974 year class. Recruitment of white sturgeon to setline gear has been evaluated by the Oregon Department of Fish and Wildlife in the lower Columbia River (Beamsderfer et al. 1989). Gear identical to IDFG's fully recruited white sturgeon >90 cm FL and did catch fish as small as 50 cm with all hook sizes (Figure 7b).

A comparison of population estimates made in 1982 (Partridge 1983) and in 1990 (Apperson and Anders 1991) show a decline in number of fish from 1,194 (with a 95% confidence interval of 907 to 1,503) to 880 (with a 95% confidence interval of 638 to 1,211) would indicate an overall annual mortality rate of 0.0374 for adult white sturgeon. The average abundance of 5 to 10 white sturgeon/km in the Kootenai River is comparable to the eight white sturgeon/rkm of similar-sized fish found in the Middle Snake River (Cochnauer 1983). However, in addition to adults, juveniles (60 to 91.5 cm TL) were found in the Middle Snake River at an average abundance of 18 white sturgeon/rkm. Similarly, in Hells Canyon of the Snake River, five white sturgeon/rkm were found that were >91.5 cm TL and 20 white sturgeon/rkm were found that were 46 to 91.5 cm TL (Lukens 1984).

Approximately 80% of the population is over 20 years old and is reproductively mature. Approximately 7% of the female white sturgeon and 30% of the male white sturgeon in the Kootenai River are reproductive each year. With a 1:1 sex ratio, this means 22 to 42 females may attempt to spawn each year; and 96 to 182 males may attempt to spawn. IDFG does not know what minimum spawning population is required to sustain the Kootenai River white sturgeon population, or at what age individual reproductive potential declines. I can estimate that with zero recruitment, the number of Kootenai River white sturgeon may decline to less than 500 individuals within 15 years, and to 100 individuals within 55 years based on the current annual mortality rate. It is imperative that recruitment of juvenile white sturgeon occurs during the next 10 years to ensure maintenance of the wild population.

Extensive aging of white sturgeon from fin-ray sections was recently conducted with lower Columbia River fish (Beamsderfer et al. 1989). Fin-ray sections were read independently by at least two people, and identical ages were assigned 35% to 62% of the time for fish 15 to 25 years of age and declined for older fish. However, most discrepancies were only one or two years. As we recapture fish marked with Oxytetracycline we hope to validate our aging techniques, if only to develop degrees of confidence for estimating age within given age groups. Refinement of aging techniques will assist with determination of both mortality rates and ages of reproductive maturity.

Wild Spawning

Typically, the operation of Libby Dam drastically alters seasonal downriver discharge by storing natural spring runoff, providing more constant flows throughout the year, and providing late summer power peaking flows (Figure 8a). Spring discharge in the lower river in 1990 and 1991 was atypical for the post Libby Dam period. Increasing and high flows coincided with increasing temperature through June in both 1990 and 1991, instead of discharge declining through late spring as occurred in 1989 and most prior years under normal dam operation (Figure 8). The river channel at Shorty's Island was constricted and had slightly increased velocities approaching 0.6 m/s. Water velocities where the white sturgeon eggs were recovered were estimated at 0.8 to 1.0 m/s (from prior measurements at similar discharges). These velocities in the Kootenai River were toward the lower end of ranges of white sturgeon spawning velocities found in the lower Columbia. Mean water column velocities measured in white sturgeon spawning areas below the lower three Columbia River dams ranged from 0.5-2.8 m/s (Miller et al. 1991). White sturgeon in the lower Columbia River key into increasing discharge with increasing temperature for spawning. Water

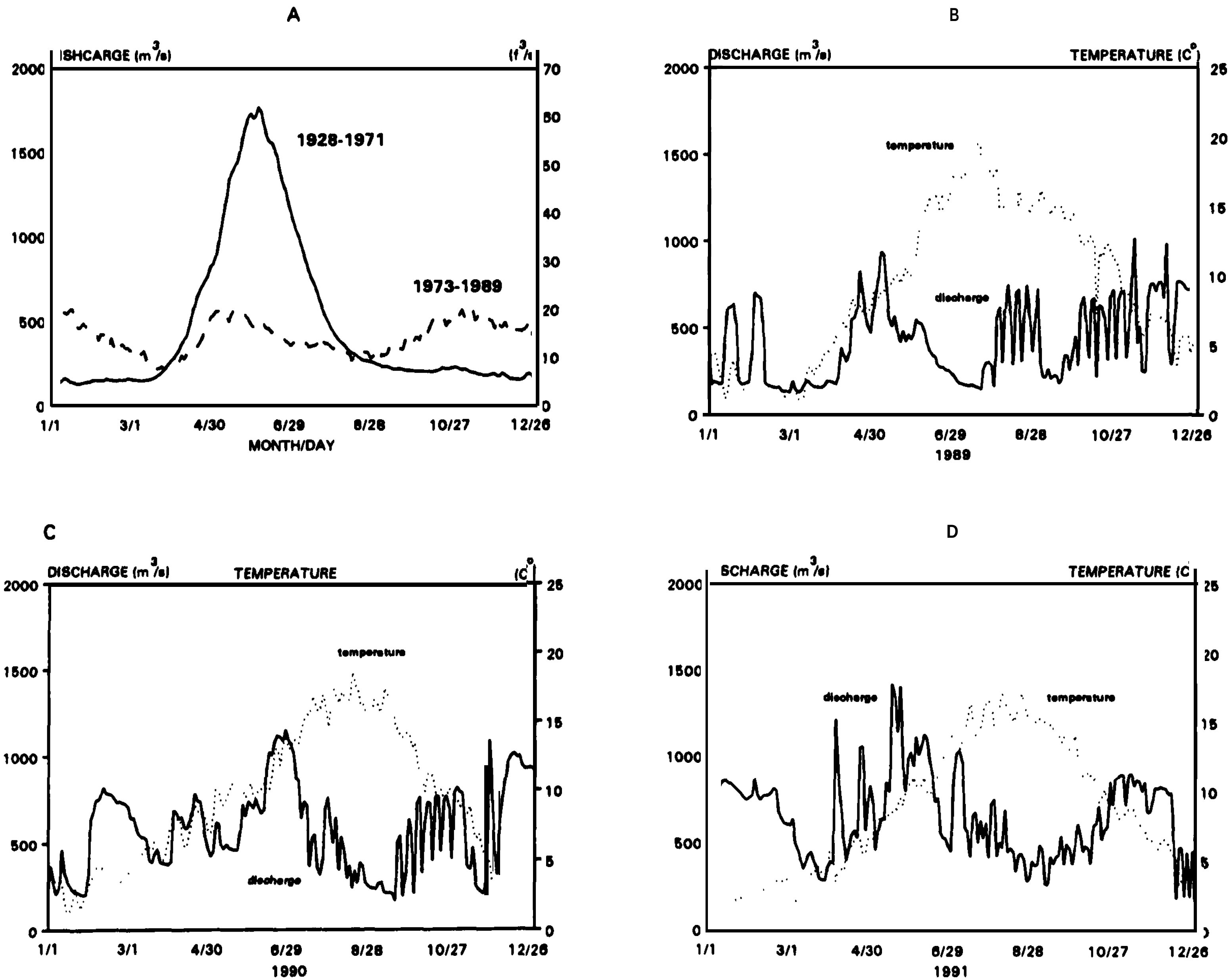


Figure 8. A) Historical discharge to the lower Kootenai River. B - D) Mean daily discharge and minimum daily temperature at Porthill, Idaho, 1989 through 1991.

temperatures during the spawning period have not been altered dramatically by the operation of Libby Dam (Figure 9).

During the spring of 1991, six transmittered and reproductively mature white sturgeon moved from 15 to 110 km upriver, congregating in the 10 km section of the river described above. White sturgeon movements were very similar during 1990. Timing of migrations in both years coincided with an increase in discharge in the lower river from approximately 700 to more than 1,200 m^3/s (24,700 cfs to more than 42,300 cfs), and an increase in water temperature from 8 to 14°C. Flows in 1974, the only year since Libby Dam that appreciable white sturgeon production occurred, exceeded 1,000 m^3/s (35,000 cfs) during most of the spawning season. I believe a manipulation of the regulated discharge through Libby Dam to approximate more natural spring flows would enhance white sturgeon reproduction in the Kootenai River. Any flow conditions less than the 1974 regime with a peak of less than 1,000 m^3/s should be considered as experimental. Figure 10 depicts a recommended discharge at Bonners Ferry.

No white sturgeon eggs were collected while trawling in 1991. Water temperatures during trawling were cooler than 12°C. Water temperatures of 14 to 17°C are considered optimal for white sturgeon spawning in the lower Columbia River (Miller et al. 1991). Trawl efforts in 1990 also did not detect any white sturgeon eggs. In 1990 sampling occurred during the last week in June when white sturgeon began dispersing from the Shorty's Island area. More intensive trawling and sampling with artificial substrate throughout the spawning period will be necessary to positively document spawning. Such sampling is planned for 1992.

White Sturgeon Enhancement

Given that the Kootenai River white sturgeon is a population with the potential for reproduction, but with an inherent reduced genetic variability (Setter and Brannon 1990), we must exercise caution as supplementation efforts are begun. With the facilities to use gametes from a limited number of brood fish, the number of offspring should be restricted for stocking into the river. Incorporating mortality rates of various age groups from the Snake River populations (Cochnauer 1983; Lukens 1984), we estimated that an annual stocking of 7,000 to 10,000 yearling white sturgeon would be required to maintain the current numbers of adults in the population. With stocking from Kootenai Falls to Kootenay Lake, a distance of 186 km, densities would be 38 to 54 white sturgeon/km. Approximately 60 km of that river section has marginal habitat for white sturgeon. Experimental stocking of white sturgeon in the Middle Snake River below Bliss Dam was at densities of approximately 18 white sturgeon/km in 1989 (Patterson et al. 1992). After evaluation of these initial supplementation efforts in both the Snake and Kootenai rivers stocking densities may be refined.

Hatchery supplementation of Kootenai River white sturgeon without natural reproduction is a high risk activity. A hatchery program may be unable to maintain the population's genetic diversity and productivity because very limited numbers of broodstock can be collected and spawned annually. Experimental culture is being used to isolate factors currently limiting recruitment and to provide limited recruitment to temporarily preserve the population. The success of this white sturgeon supplementation program is unknown because no evaluation of hatchery-reared white sturgeon released in the wild has been previously conducted. Survival of Snake River fish stocked as one year olds has been documented. Ryman and Laikre (1991) discuss that such "supportive breeding" of a population can result in a reduced "effective population" when the contribution of offspring from a limited number of captive parents outnumber wild reproduction, even though there may be a gain in total number of offspring. Specific risks of continued experimental culture of this population with a limited number of brood fish should be thoroughly examined and weighed against any benefits.

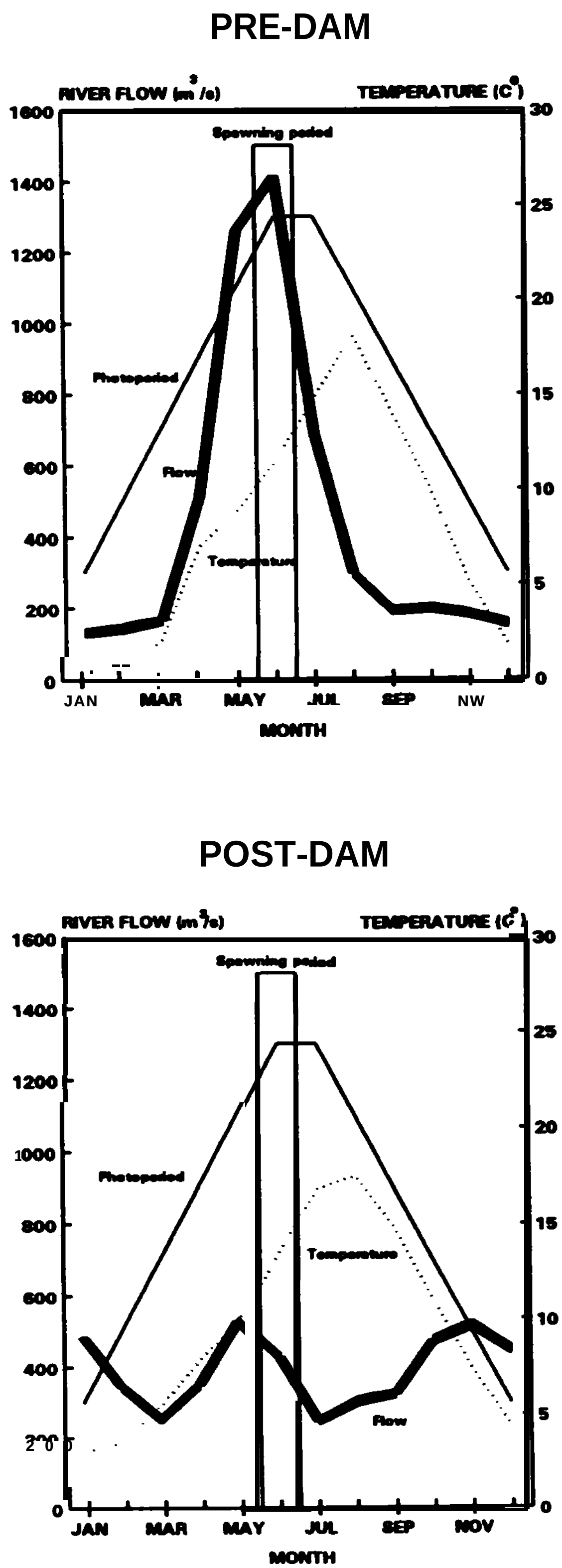


Figure 9. Mean monthly flow, water temperature, and relative photoperiod in the Kootenai River at Porthill, Idaho before and after the operation of Libby Dam.

The sex ratio of reproductive white sturgeon in the Kootenai River appears to be 3:1 males to females. Capture ratios during broodstock collection efforts were also 3: 1. Capture rates during sampling to estimate the population were 2:1 to 1:1 (Apperson and Anders 1991). The spawning program has been limited by capture of female broodstock. It, therefore, is advisable to schedule future female broodstock collections for late summer through fall and in sections of the river where capture rates have been highest. Female fish may be more vulnerable to our gear at these times of year.

contaminants

Concentrations of copper found in white sturgeon oocytes potentially present the most severe contaminant effect on reproductive success. Water copper levels of only 9 µg/l appeared to inhibit yolk uptake in larval white sturgeon (Joel Van Eenennaam, University of California at Davis, personal communication). Copper levels in the Kootenai River at Porthill ranged from 2-12 µg/l (1983-1986, USGS records).

Nothing is known regarding the toxicity of zinc to white sturgeon. Current water quality criteria to protect freshwater aquatic life is 47 µg/l as a 24-hour average (U.S. Environmental Protection Agency 1984). Concentrations of dissolved zinc in the Kootenai River at Porthill ranged from 9-19 µg/l from 1983 through 1986.

Levels of PCBs in recent egg samples from the Kootenai River ranged from not detectable to 0.33 µg/g, lower than the 0.47 µg/g of PCBs found in one egg sample in 1982 (IDFG files). PCBs were absent from eggs used in culture. Levels of DDT complex residues in Kootenai River white sturgeon oocytes (0.016 to 0.1 µg/g) were well below the level found in the egg sample in 1982 (0.45 µg/g).

Concentrations of organochlorides required to affect white sturgeon reproduction and egg and fry survival are not known, and scant information is available regarding contaminant levels in other populations of white sturgeon. Organochlorides will accumulate in tissues with high lipid content, therefore, reproductive organs will have higher concentrations than skeletal muscle. Rainbow trout Oncorhvnchus mykiss eggs contaminated with 0.090 µg/g of a DDT complex and 2.70 µg/g of PCBs (as Aroclor 1242) resulted in 60-702 of the fry developing deformities and 752 cumulative mortality 25 days after hatching (Hogan and Brauhn 1975). A concentration of PCB (as Aroclor 1260) in water of 0.002 µg/l resulted in a tissue residue in fathead minnow Pimephales promelas of 0.5 µg/kg (Nebeker 1976). More highly chlorinated PCB mixtures are bioaccumulated in lipids of fish than the lower chlorinated compounds (i.e., PCB as Aroclor 1260 is more chlorinated than Aroclor 1242). Water concentration of 1.8 µg/l PCB (Aroclor 1254) caused a 50% reduction in fathead minnow reproduction.

Successful reproduction does occur in the lower Columbia River, where ova samples from three white sturgeon in 1991 contained chlorinated compound levels (DDE: 3.5 to 4.9 µg/g; PCB: <5.4 to 8.7 µg/g) up to lo-fold that of levels found in Kootenai River white sturgeon. Two white sturgeon from an earlier Columbia River sample contained 0.16 and 1.45 µg/g PCB (as Aroclor 1254); 0 and 0.47 µg/g DDT; 0 and 0.71 µg/g DDD; and 0.07 and 1.75 µg/g DDE (Bosley and Gately 1981). Continued analyses of white sturgeon eggs from known viable populations should help identify tolerance levels for egg viability.

RECOMMENDATIONS

1. Take immediate steps to provide habitat for wild sturgeon spawners with natural spring and summer flows. Treat any flows less than natural as

experimental and evaluate enhancement of spawning success, embryo survival, and ultimately, juvenile recruitment relative to various flow patterns. AI-I initial minimum flow recommendation is found in Figure 10.

2. Continue experimental culture of Kootenai River white sturgeon only to evaluate habitat use and survival of juvenile white sturgeon stocked in the river. Use a maximum of 7,000 age 1 offspring from Kootenai River broodstock as an interim guideline until genetic risks are assessed. All hatchery offspring should be permanently marked with a PIT tag for individual identification.

3. Determine genetic risks to the wild population of supplementation of Kootenai River white sturgeon. Guidelines for broodstock management and stocking should be developed to protect the wild population.

4. Broodstock collection should be conducted during late summer, and adults held through winter at the experimental facility. Mature female white sturgeon were captured less frequently relative to males during spring than other seasons.

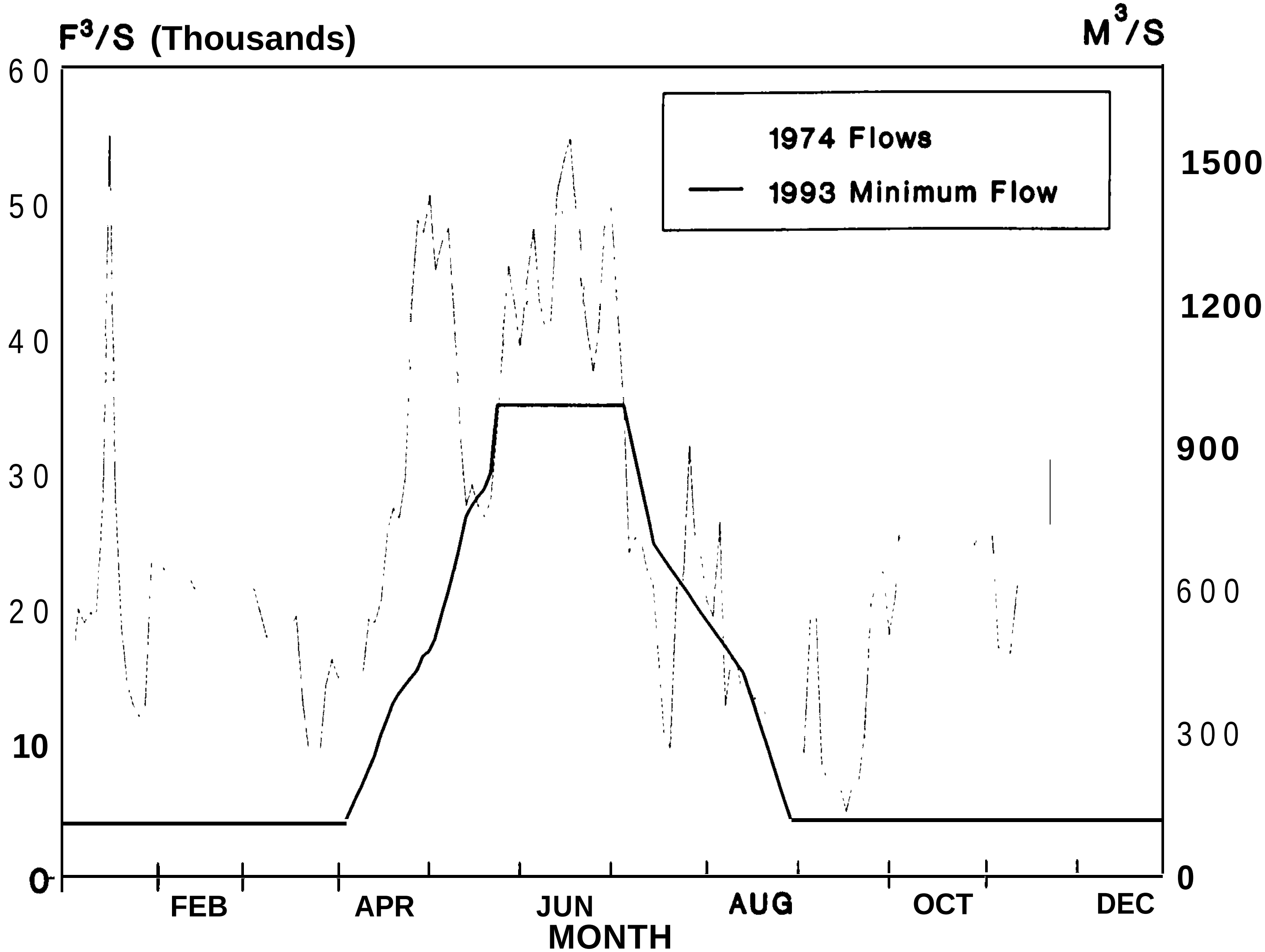


Figure 10. Recommended minimum discharge in the Kootenai River at Bonners Ferry to enhance white sturgeon spawning.

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A P P E N D I C E S

Appendix A. Capture of white sturgeon, Kootenai River, 1989 through 1991.

Year	River section	Large setlines[a] Setline hours	Large setlines[a] Number sturgeon captures	Small setlines[b] Setline hours	Small setlines[b] Number sturgeon captures	Angling Rod hours	Angling Number of sturgeon captures
1989	British Columbia	4,803	53	545	0	23	9
1989	Idaho (lower river)[c]	8,095	173	675	2	90	15
1989	Idaho (upper river)[d]	690	0	0	0	6	0
1989	Montana	1,487	2	0	0	1	0
1990	British Columbia	3,515	47	247	0	56	6
1990	Idaho (lower river)	8,630	83	279	0	54	0
1990	Idaho (upper river)	67	0	0	0	0	0
1990	Montana	0	0	0	0	0	0
1991	British Columbia	145	10	47	0	10	3
1991	Idaho (lower river)	2,574	31	0	0	209	13
1991	Idaho (upper river)	0	0	0	0	0	0
1991	Montana	0	0	0	0	0	0

[a] large setline = six hooks, two each of size 12/0, 14/0, and 16/0
[b] small setline = 10 hooks, size 10/0
[c] downriver from Bonners Ferry
[d] upriver from Bonners Ferry

Appendix B1. Contaminant levels found in white sturgeon oocyte samples, Kootenai River, 1989.

Floy tag number:	1147	1154	1156	1157	1169	1172	1177	1243	----
Fork length (cm):	187	135	165	186	198	184	191	170	---
<u>PCB's (µg/kg)</u>[a]									
Arochlor-1260	73[b]	73[b]	73[b]	300	330	<50	200	310	230
<u>Pesticides (µg/kg)</u>[a]									
Alpha BHC	<4.0	<4.0	x4.0	<3.5	<3.5	<3.5	<3.5	<3.5	<3.5
Lindane	<4.0	<4.0	<4.0	<3.5	<3.5	<3.5	<3.5	<3.5	<3.5
Heptachlor	<3.0	<3.0	<3.0	<3.5	<3.5	<3.5	<3.5	x3.5	<3.5
Aldrin	<4.0	x4.0	<4.0	<3.5	<3.5	<3.5	<3.5	<3.5	<3.5
Endosulfan I	<5.0	<5.0	<5.0	<12	<12	<12	<12	<12	<12
DDE	100[b]	100[b]	100[b]	650	440	176	360	190	570
DDD	<10	<10	<10	<10	44	6	40	28	61
DDT	<20	<20	<20	<10	96	33	86	73	90
delta BHC	----	----	----	<7.3	<7.3	<7.3	<7.3	<7.3	<7.3
Toxaphene	<1,000	<1,000	<1,000	<200	<200	<200	<200	<200	<200
Chlordane	<100	<100	<100	<50	<50	<50	<50	<50	<50
Methoxychlor	<50	<50	<50	<50	<50	<50	<50	<50	<50
<u>Metals (µg/g)</u>[a]									
Copper	1.72	1.85	2.24	1.62	1.87	2.50	1.18	1.31	1.32
Zinc	32.8	22.3	23.1	21.5	23.3	18.8	20.4	21.6	15.3

[a] Reported on a "wet weight" basis; % total solids not reported
[b] Composite of three egg samples (1147, 1154, and 1156)

Appendix B2. Contaminant levels in white sturgeon eggs, embryos, and larvae from one spawning pair[a], Kootenai River, 1990.

	Unfertilized eggs	Fertilized eggs	Embryos	Larvae
PCB's (µg/kg)[b]				
Arochlor 1016	<120	<70	<70	<70
Arochlor 1221	<480	<270	<270	<260
Arochlor 1232	<120	<70	<70	<70
Arochlor 1242	<120	<70	<70	<70
Arochlor 1248	<120	<70	<70	<70
Arochlor 1254	<120	<70	<70	<70
Arochlor 1260	<120	<70	<70	<70
Pesticides (µg/kg)[b]				
Alpha BHC	<7	<4	<4	<4
Lindane	<7	<4	<4	<4
Heptachlor	<5	<3	<3	<3
Aldrin	<7	<4	<4	<4
Beta BHC	<10	<5	<5	<5
Delta BHC	<12	<7	<7	<7
Heptachlor Epoxide	<7	<4	<4	<4
Endosulfan I	<10	<5	<5	<5
p,p'-DDE	<10	6	6	6
Dieldrin	16	10	10	9
Endrin	<12	<7	<7	<7
p,p'-DDD	22	17	23	20
Endosulfan II	<7	4	5	<4
p,p'-DDT	<24	<13	<13	<13
Endrin Aldehyde	<24	<13	<13	<13
Endosulfan Sulfate	<19	<11	<11	<10
Methoxychlor	<48	<27	33	<26
Toxaphene	<1,400	<800	<800	<800
Chlordane	<120	<70	<70	<70
Metals (µg/g)[c]				
Copper	1.66	0.716	0.664	0.402
Zinc	17.8	3.69	4.6	1.39
Aluminum	24	7.9	0.100	4.6
Lead	0.552	0.322		0.080

[a] Progeny of floy tag numbers 1290 and 1248; samples other than unfertilized eggs were from mortalities.

[b] Reported on an "as received" basis.

[c] Reported on a "wet weight" basis.

Appendix B3. Metal levels found in white sturgeon oocyte samples, Kootenai River, 1990.

Floy tag number: Fork length (cm):	1090 211	1530 165	1551 196	1565 214	9999 ---
Moisture (%)	65.5	58.2	55.3	60.5	64.9
Metals (µg/g)[a]					
Copper	2.1	2.5	3.2	1.6	2.0
Zinc	22.0	32.0	31.0	27.0	25.0
Lead	0.12	0.08	<0.01	0.16	1.6
Aluminum	1.2	1.7	5.3	1.6	3.9
Strontium	0.20	0.29	0.32	<0.16	0.20

[a] Reported on an "as received" basis.

Appendix B4. Contaminant levels found in Kootenai River sediments, 1989.

River kilometer:	239	215	179	165	126
Total solids (%)	80.7	78.7	73.6	72.8	59.5
PCB's (µg/kg)[a]					
Arochlor-1260	<50	<50	<50	<50	<50
Pesticides (µg/kg)[a]					
Alpha BHC	<3.5	<3.5	<3.5	<3.5	<3.5
Lindane	<3.5	<3.5	<3.5	<3.5	<3.5
Heptachlor	<3.5	<3.5	<3.5	<3.5	<3.5
Aldrin	<3.5	<3.5	<3.5	<3.5	<3.5
Endosulfan I	<12	<12	<12	<12	<12
DDE	<3.5	<3.5	<3.5	<3.5	<3.5
DDD	<10	<10	<10	<10	<10
DDT	<10	<10	<10	<10	<10
Delta BHC	<7.3	<7.3	<7.3	<7.3	<7.3
Toxaphene	<200	<200	<200	<200	<200
Chlordane	<50	<50	<50	<50	<50
Methoxychlor	<50	<50	<50	<50	<50
Metals (µg/g)[b]					
Copper	2.85	4.82	2.93	1.62	12.8
Zinc	22.4	47.4	41.4	51.7	70.6

[a] Reported on an "as received" basis.
[b] Reported on a "dry weight" basis.

Appendix B5. Contaminant levels found in white sturgeon oocytes and larvae, Kootenai River, 1991.

	Oocyte sample floy tag number 01494	01582	Larvae sample[a]
Total solids (%)	32.0	31.0	6.5
PCB's (µg/kg)[b]			
Arochlor[c]	<82	<81	<220
Arochlor 1221	<330	<320	<900
Pesticides (µg/kg)[b]			
Alpha-BHC	<4.9	<4.9	<14
Lindane	<4.9	<4.9	<14
Heptachlor	x3.3	<3.2	<9
Aldrin	<4.9	<4.9	<14
Beta-BHC	<6.6	<6.5	<18
Delta-BHC	<8.2	<8.1	<22
Heptachlor Epoxide	<4.9	<4.9	<14
Endosulfan I	<6.6	<6.5	<18
pp-DDE	<6.6	210	<18
Dieldrin	<6.6	<6.5	<18
Endrin	<8.2	<8.1	<22
pp-DDD	<8.2	<8.1	<22
Endosulfan II	<4.9	<4.9	<14
pp-DDT	<16	<16	<45
Endrin Aldehyde	<16	<16	<45
Endosulfan Sulfate	<13	<13	<36
Methoxychlor	<33	<32	<90
Toxaphene	<990	<970	<2,700
Chlordane	<82	<81	<220
Metals (µg/g)[d]			
Aluminum	200	130	200
Cadmium	CO.02	CO.03	CO.02
Copper	2.0	1.4	2.0
Mercury	CO.007	<0.015	CO.007
Lead	0.55	0.17	0.55
Zinc	18	19	18

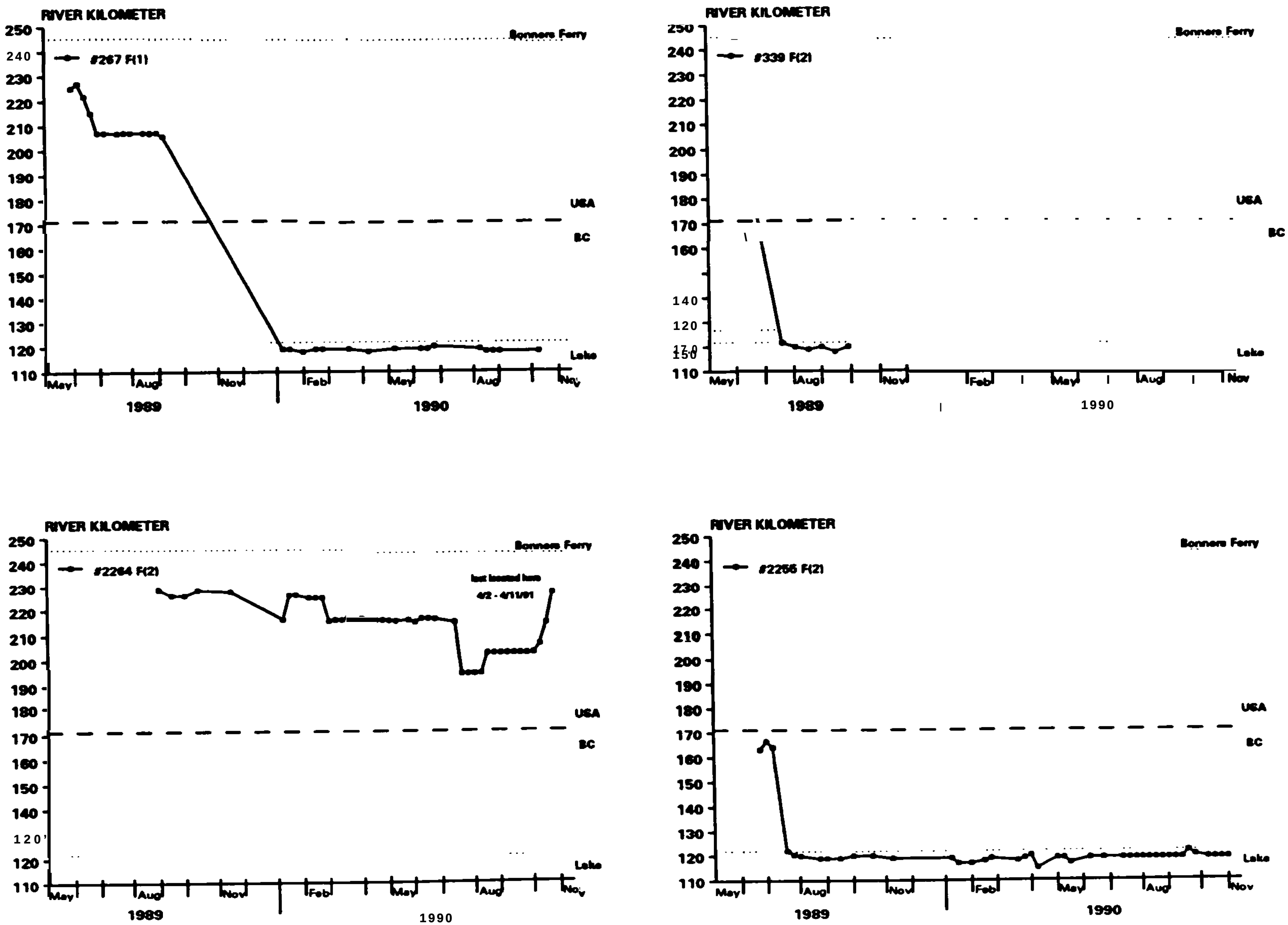
[a] Progeny of floy tag number 1494 (female); and tag numbers 1495, 1499, and 1189 (males).
[b] Reported on a "wet weight" basis.
[c] Detection for each PCB as Arochlor 1016, 1232, 1242, 1248, 1254, 1260.
[d] Reported on an "as received basis".

Appendix B6. Contaminant levels found in white sturgeon oocytes Columbia River, 1991.

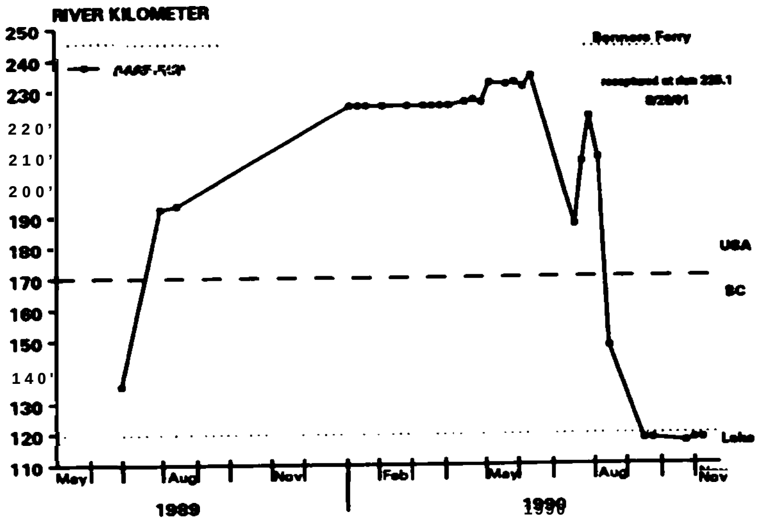
Tag number:	JD70243	JD70659	JD70366
Fork length (cm):	223	237	272
Total solids (%)	43	43	51
PCB's (µg/kg)[a]			
Arochlor[b]	<5,400	<2,500	<1,500
Arochlor 1221	<22,000	<10,000	<6,000
Arochlor 1260	<5,400	8,700	7,300
Pesticides (µg/kg)[a]			
Alpha-BHC	<330	<150	<180
Lindane	<330	<150	<90
Heptachlor	<220	260	<60
Aldrin	<330	180	<90
Beta-BHC	<430	<200	'<120
Delta-BHC	<540	<250	<150
Heptachlor Epoxide	<330	<150	<90
Endosulfan I	<430	<200	<120
pp-DDE	3,500	3,600	4,900
Dieldrin	<430	<200	<120
Endrin	<540	<250	<150
pp-DDD	<1,300	<1,200	2,100
Endosulfan II	<330	<150	<90
pp-DDT	<1,100	<500	420
Endrin Aldehyde	<1,100	<500	<300
Endosulfan Sulfate	<870	<400	<240
Methoxychlor	<2,200	<1,000	<600
Toxaphene	<65,000	<30,000	<18,000
Chlordane	<5,400	<2,500	<1,500

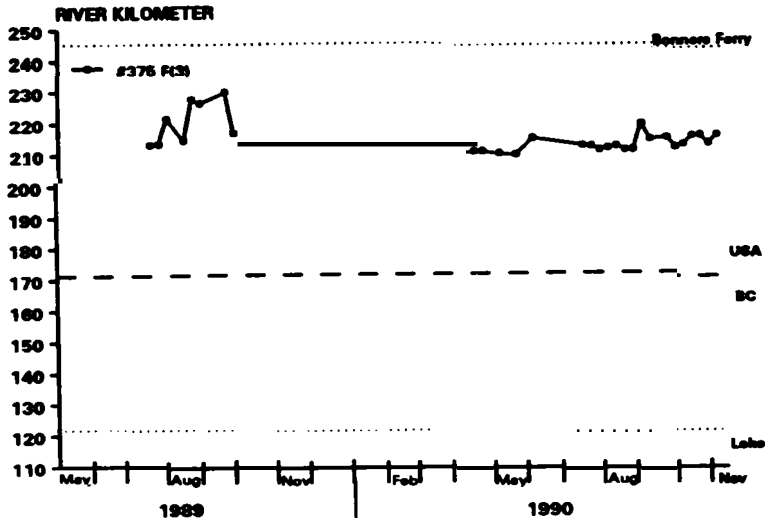
[a] Reported on a "dry weight" basis.
[b] Detection for each PCB as Arochlor 1016, 1232, 1242, 1248, 1254.

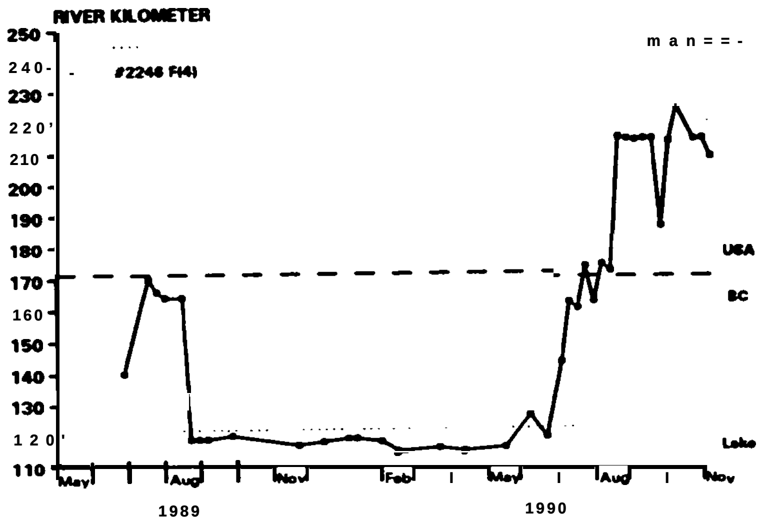
Appendix C. Movements of white sturgeon tagged with ultrasonic transmitters in the Kootenai River, 1989 through 1991.

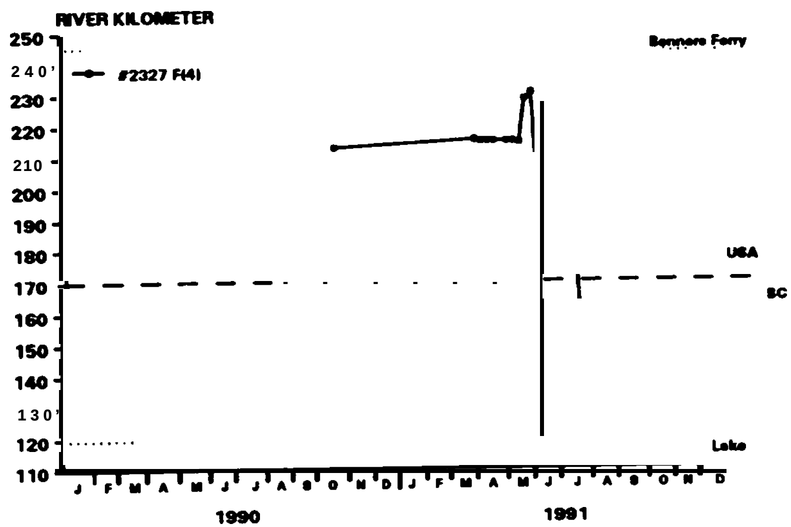


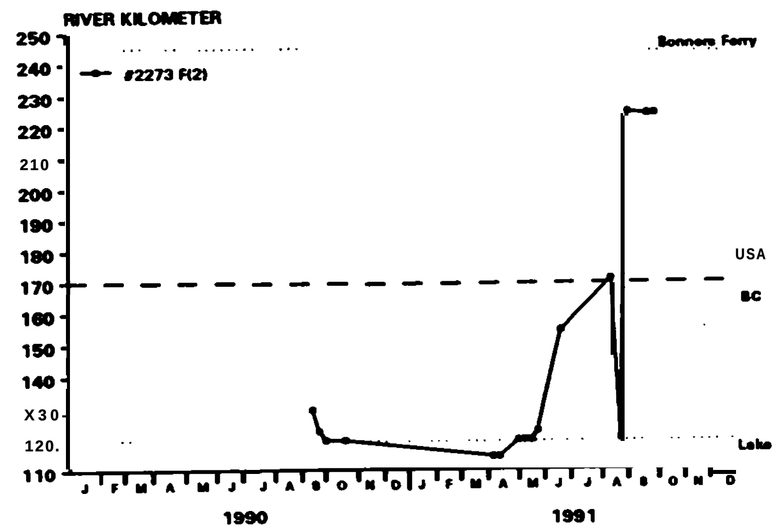
Appendix C. (continued)








RIVER KILOMETER
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
#2327 F(4)
Bonners Ferry
USA
BC
Lake
J F M A M J J A S O N D J F M A M J J A S O N D
1990
1991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240
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160
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140
X30
120.
110
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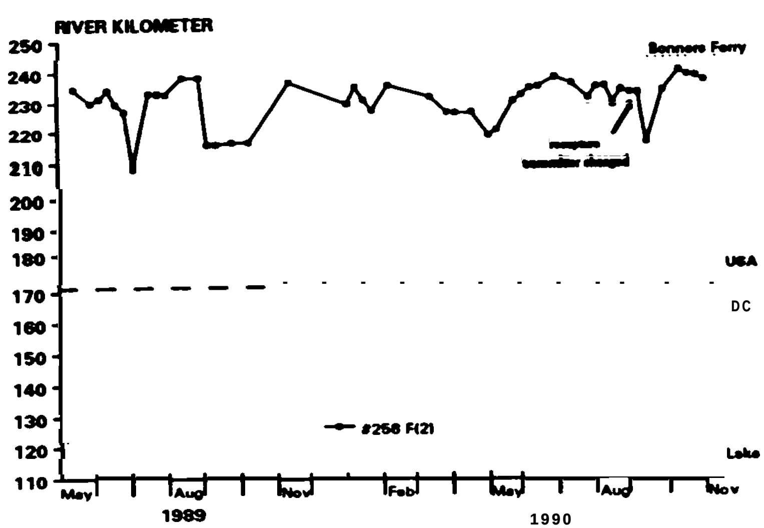
Bonners Ferry
USA
BC
Lake
J F M A M J J A S O N D J F M A M J J A S O N D
1990
1991


RIVER KILOMETER
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160
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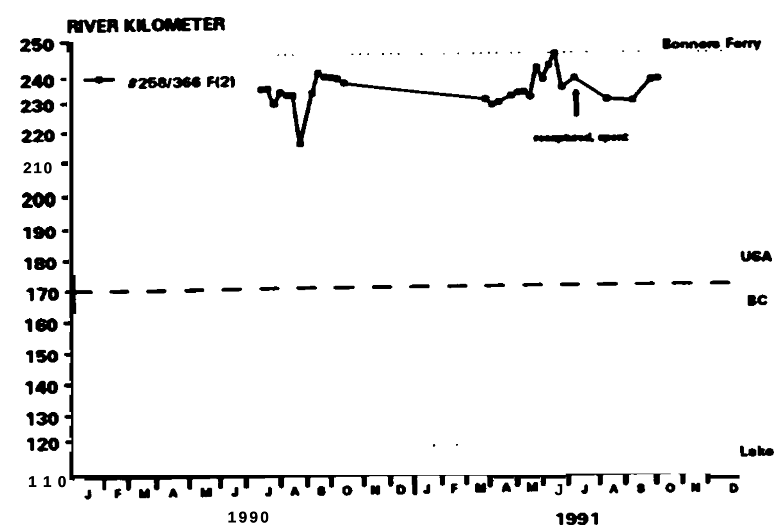
110
Bonners Ferry
USA
DC
Lake
#256 F(2)
May
Aug
Nov
Feb
May
Aug
Nov
1989
1990


RIVER KILOMETER
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240
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160
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130
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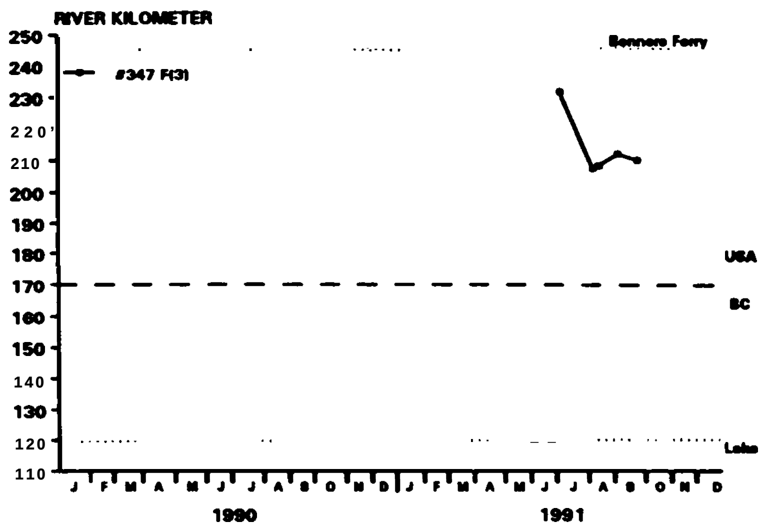
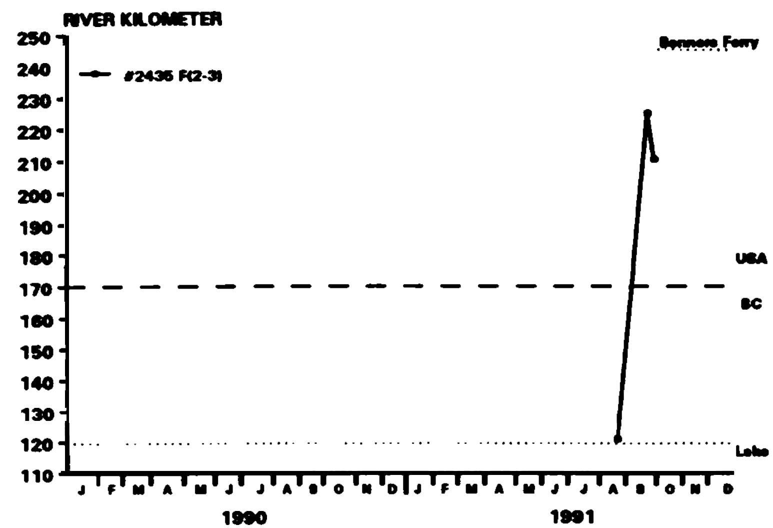
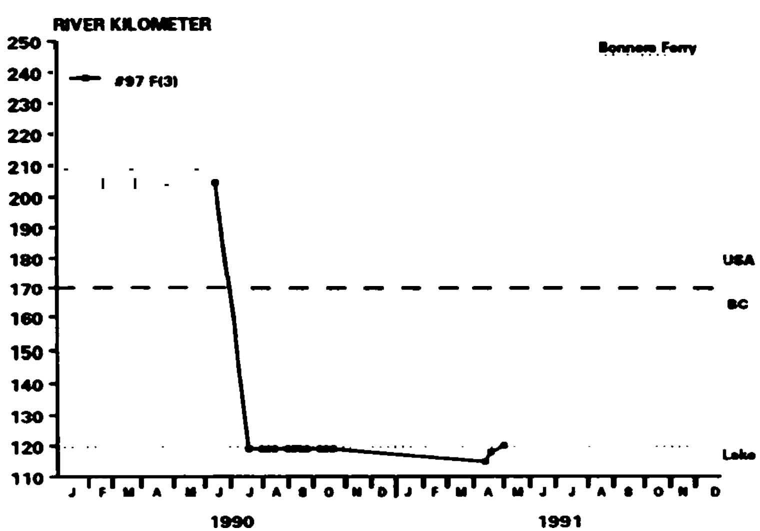
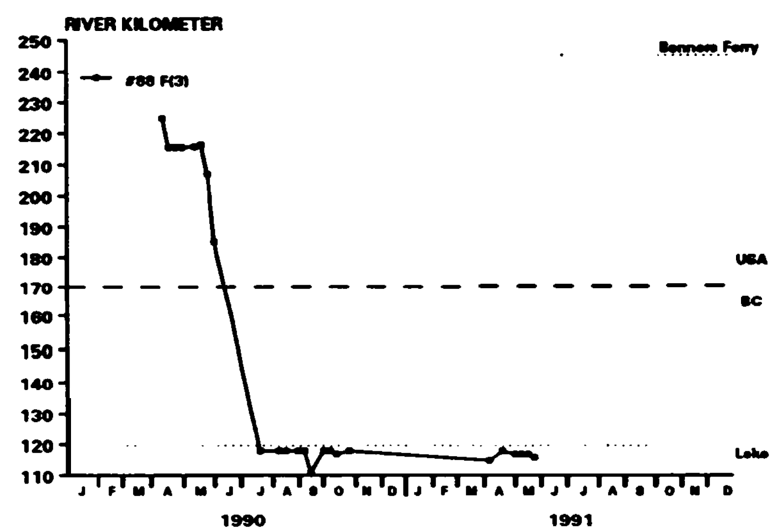
110
#256/366 F(2)
Bonners Ferry
USA
BC
Lake
J F M A M J J A S O N D J F M A M J J A S O N D
1990
1991

Appendix C. (continued)









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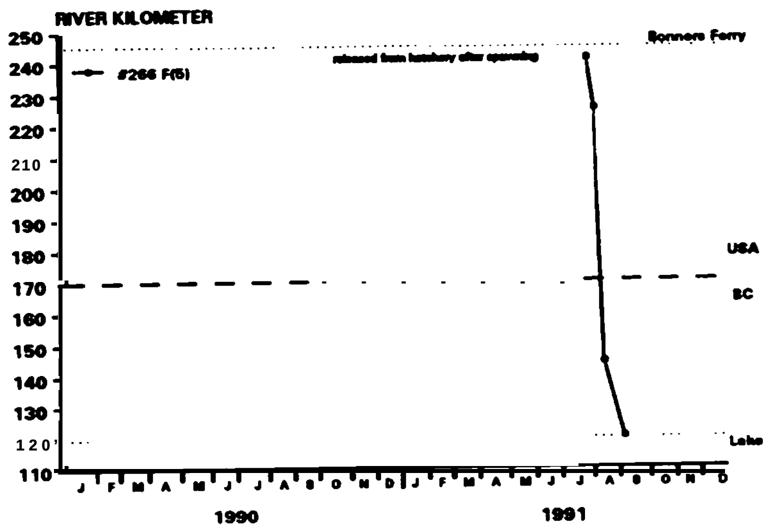

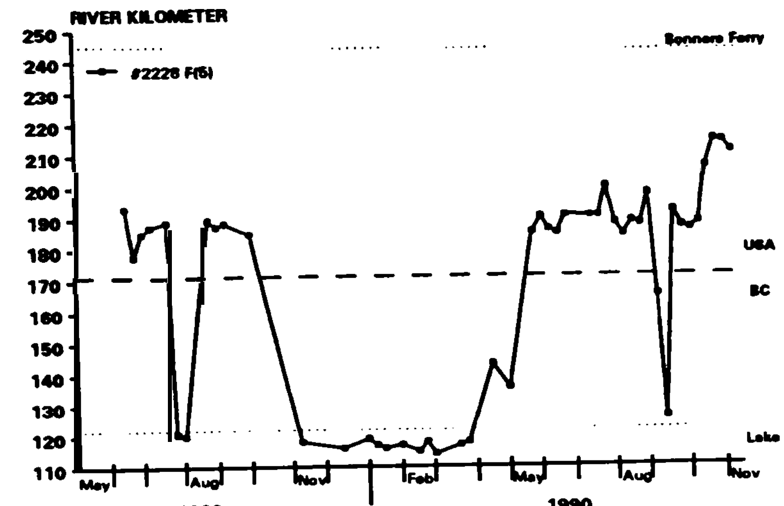

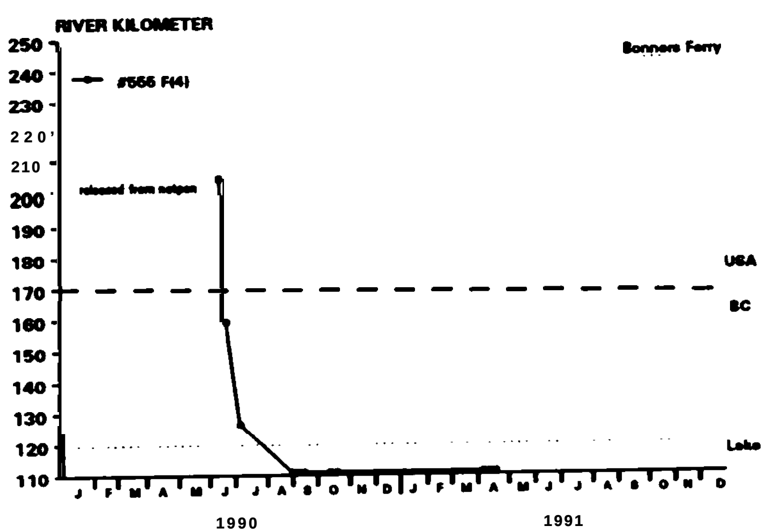

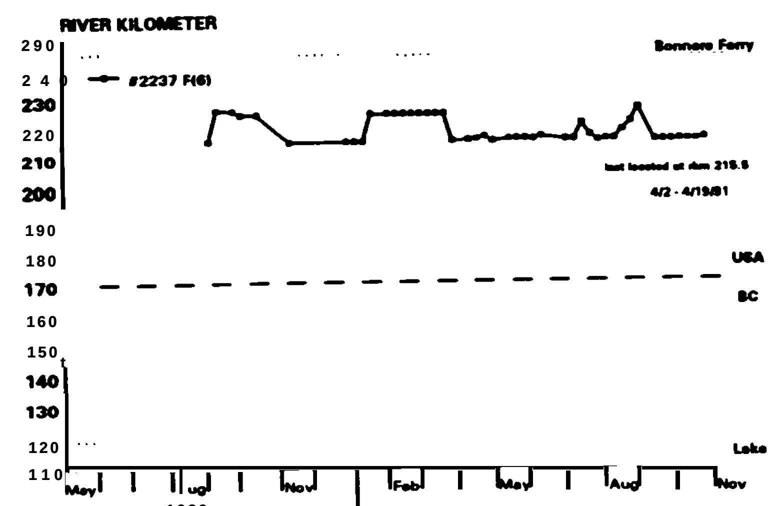

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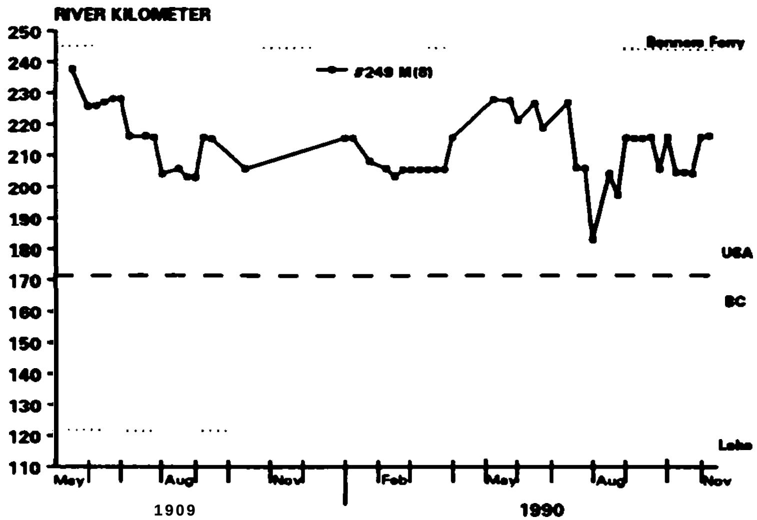
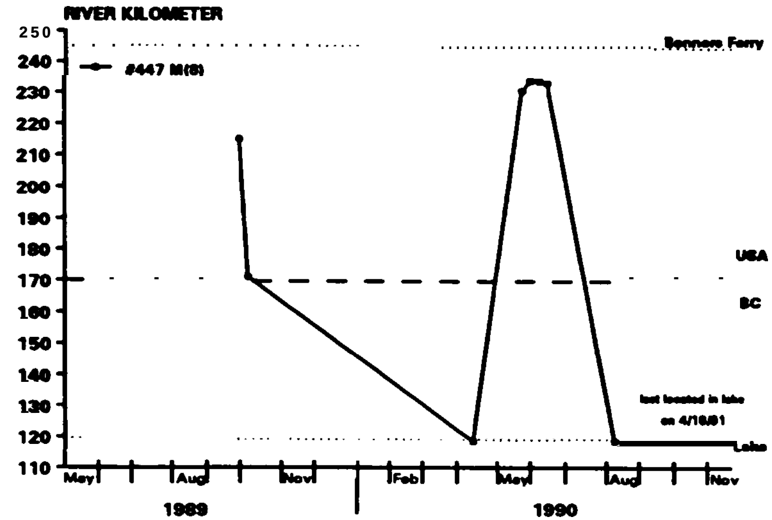
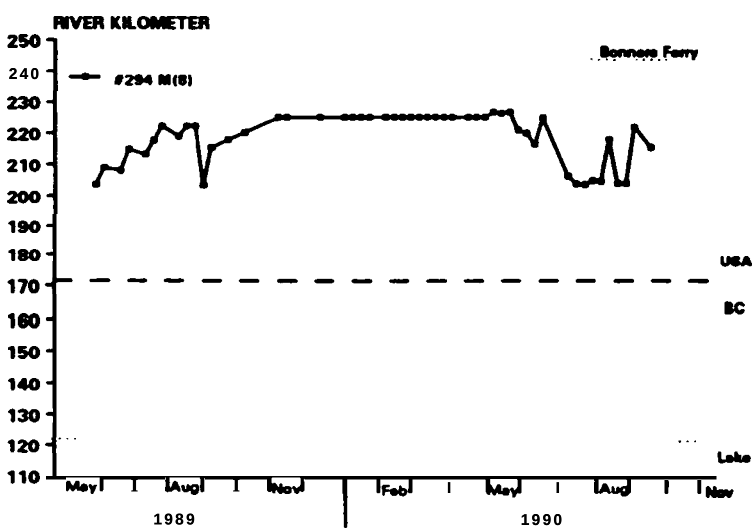
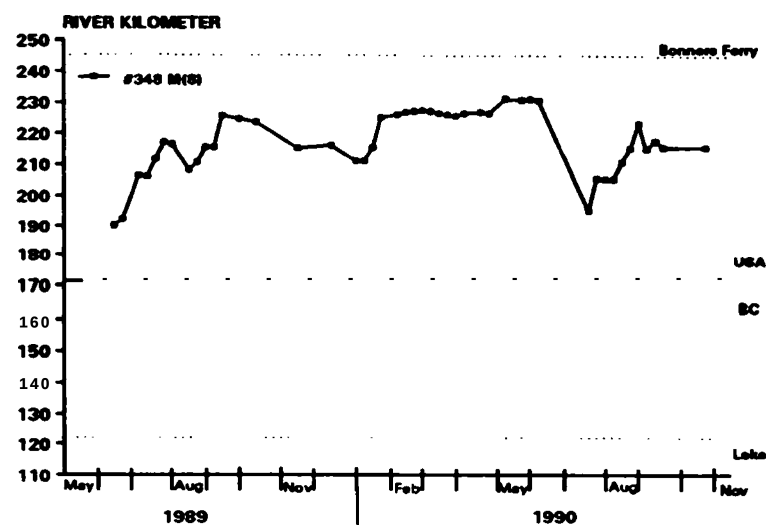
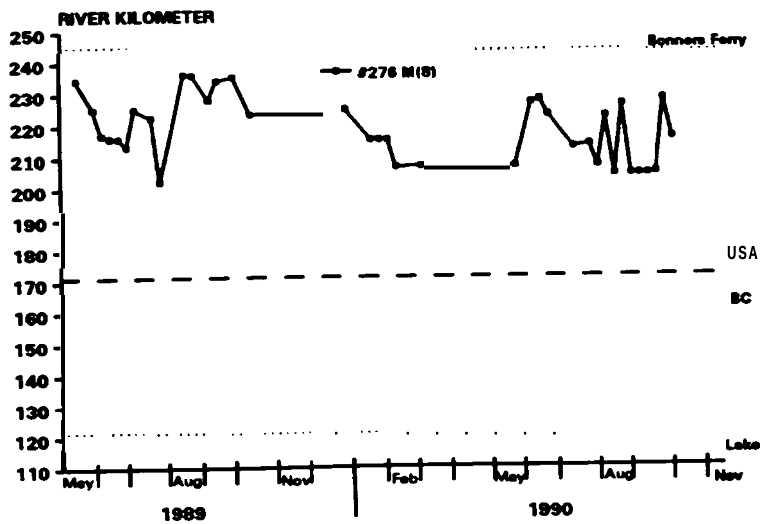


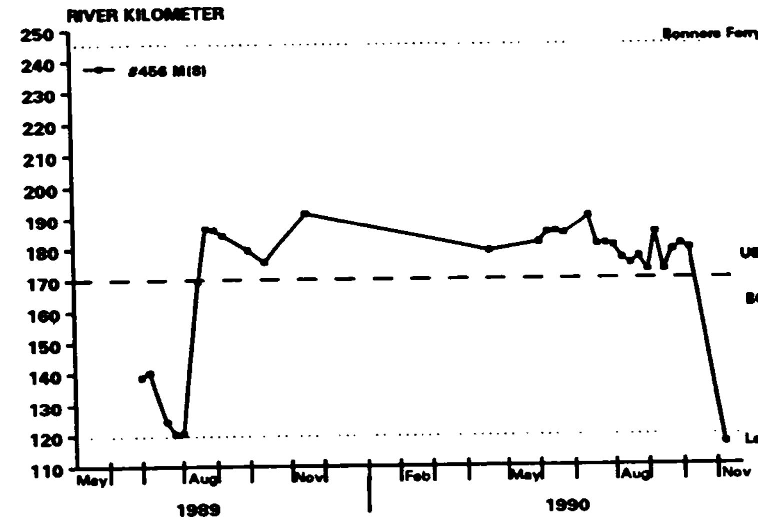


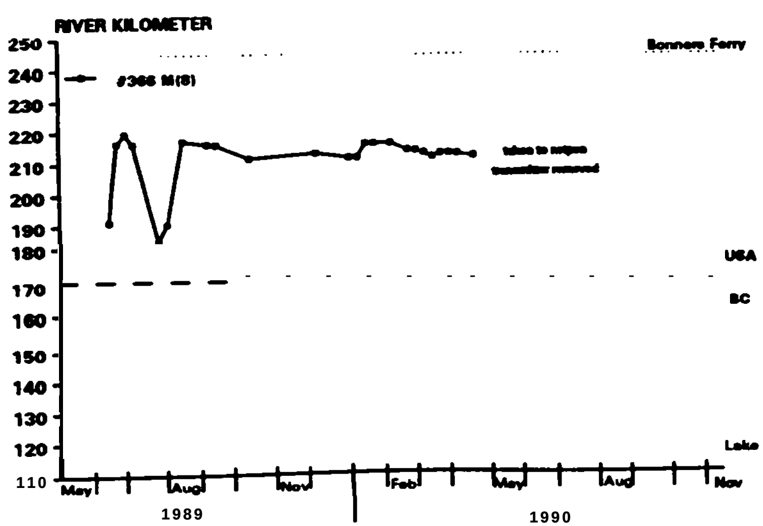


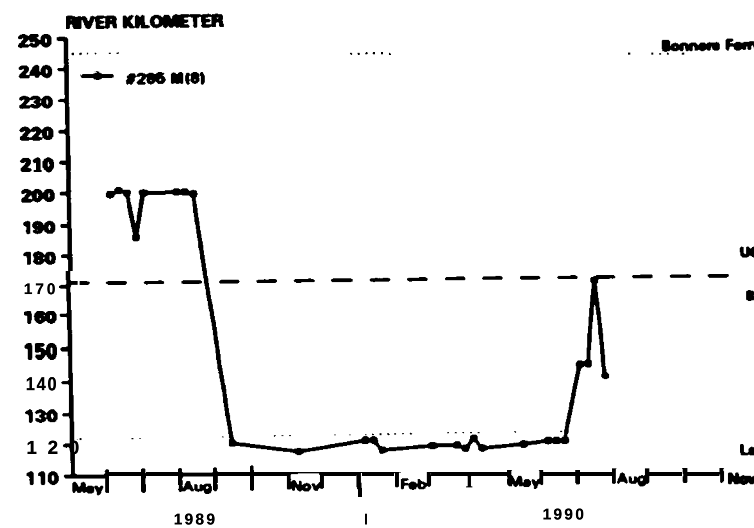


Appendix C. (continued)









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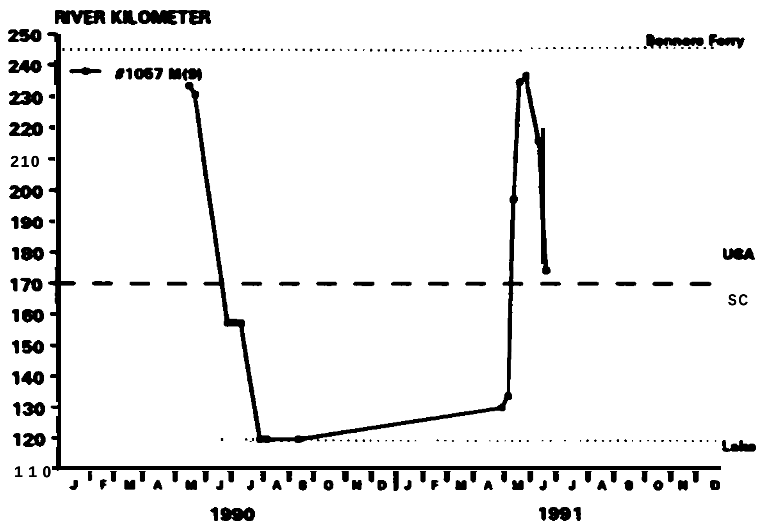

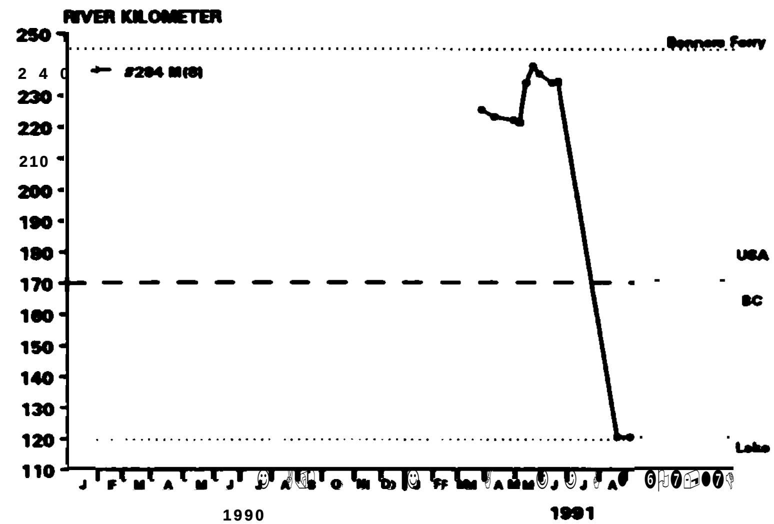

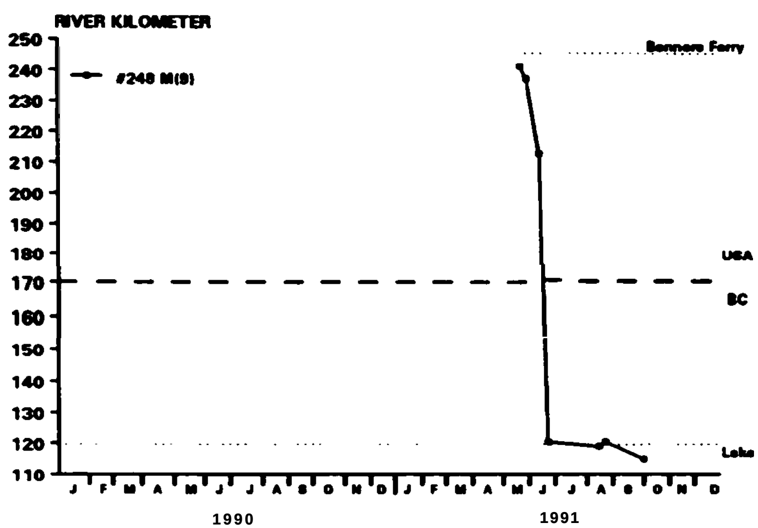

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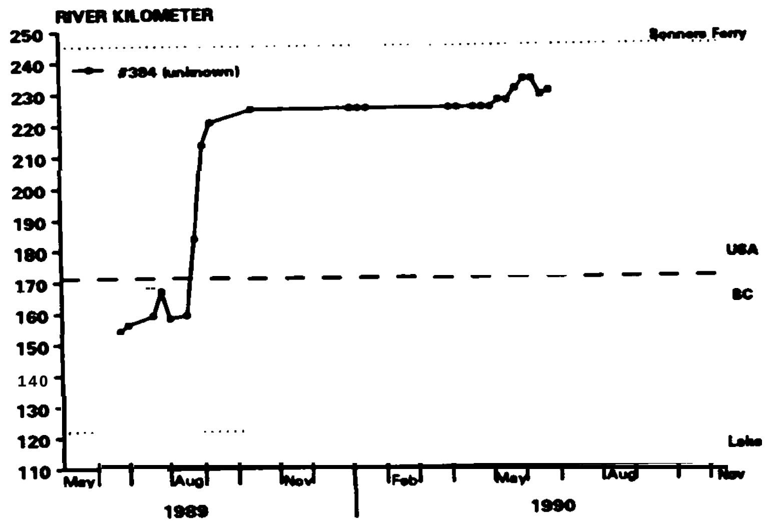

Appendix D. Cryopreservation of sperm from white sturgeon: a progress report by R. Schneider and J.G. Cloud, Department of Biological Sciences, University of Idaho, Moscow.

Cryopreservation of Sperm from White Sturgeon:
A Progress Report

R. Schneider and J. G. Cloud
Department of Biological Sciences
University of Idaho
Moscow, Idaho 83843

INTRODUCTION

Cryopreservation of spermatozoa represents a solution to the problem of asyncrony in spawning times between male and female broodstock. In the past, if a female was ready to spawn and a ripe male was unavailable, the eggs would be lost. With cryopreserved sperm, eggs can be fertilized when no male is available. Likewise with endangered species or stocks of fish, cryopreservation of sperm represents a potential means of preserving the genetic diversity currently present in the populations.

Several variables need to be considered when cryopreserving cells. The rate at which the cells are cooled greatly affects survivability by two mechanisms: (1) intracellular ice crystal formation and (2) increased intracellular salt concentrations. When freezing a cell, the water present in that cell must be drawn out as it is cooled. If this dehydration is not accomplished and ice crystals are formed intracellularly, these ice crystals can damage the cell. Opposing this cellular dehydration is the cytoplasmic concentration of salts as water is drawn out. Increased salt concentrations in the cytoplasm can cause denaturation of cellular proteins and result in a reduction in cell viability. Since decreased temperatures inhibit denaturation of proteins, the cells must be cooled quickly to avoid protein damage. With these two factors in mind, cells must be frozen slow enough to allow the removal of water and fast enough so that the increased salt concentrations do not denature intracellular proteins. Thawing the cells also presents a problem; the cells must be thawed in a manner to successfully reverse these processes.

One cell that appears to be able to survive the freezing and thawing process is the sperm cell. Sperm from a number of fish species have been successfully cryopreserved (reviewed by Harvey, 1990). Rainbow trout is one species in which sperm has been successfully cryopreserved; average fertility of cryopreserved rainbow trout sperm can range from 50 to 80% of control, nonfrozen sperm depending on the method of cryopreservation and the quality of semen that was used (Stoss, 1983). To date, sperm from white sturgeon has not been successfully frozen.

MATERIALS AND METHODS

1. Gamete Handling:

Semen was obtained from commercial and state hatcheries and shipped to the University of Idaho on ice at 4°C. Motility of the sperm was assessed visually; only semen with greater than 50% motile sperm were used. The sperm were frozen in a medium containing 300 mM glucose (pH6), 7.5% DMSO (dimethyl sulfoxide) and 10% chicken egg yolk (Stoss and Holtz, 1981). Three parts of the freezing solution was added slowly to one part semen. The sperm/freezing media mixture was then drawn into 0.5 ml straws. Both ends of the straw was sealed; the resultant straws were frozen on dry ice or in liquid nitrogen vapor.

Straws frozen on dry ice were placed directly on a solid block of dry ice for 15 minutes. Following this time interval, the straws were plunged into liquid nitrogen. The straws remained in liquid nitrogen for a minimum of 15 minutes before they were tested in fertility or motility assays.

Straws were also frozen with liquid nitrogen vapor using a computer-controlled freezing chamber. Upon reaching -90°C in the freezing chamber, the straws were plunged into a container of liquid nitrogen and remained at this temperature until assayed.

2. Assays

Straws were thawed by placing them into a container of water at 4°C for 90 seconds. Sperm motility was visually estimated immediately following thawing. Fertility of trout sperm was measured by adding thawed semen to 200 eggs. The resultant embryos were incubated in a Heath incubator. Sperm were considered viable if they fertilized eggs and the resultant embryos developed to the eyed state.

3. Fluorescent Staining and Flow Cytometry

10 ul of 6 mM carboxyfluorecein diacitate (CFDA) was mixed with 0.5 ml of semen. The solution was allowed to equilibrate for 15 minutes at 4°C. The sample was then centrifuged at 500xg for 5 minutes. The supernatant was decanted and the soft pellet was resuspended in Cortland's salt solution. A portion of the sample was used for fluorescent microscopy. The remaining cells were counted and were analyzed for fluorescence. Live cells contain esterase that alter CFDA; the resultant carboxyfluorcein is fluorescent and membrane impermeable.

RESULTS

Sperm from four sturgeon males had 80% or greater motility at the time of freezing. All semen was frozen on dry ice followed by immersion in liquid nitrogen. The motility of the frozen/thawed semen is summarized in Table 1; the motility of frozen/thawed sperm from all males was reduced over control. However, there was a substantial number of motile sperm. Our attempt to use the frozen sperm to fertilize eggs was not successful; because the eggs did not develop following fertilization with fresh semen, no useful information was obtained.

Table 1. Motility of Sturgeon Sperm following Freezing and Thawing

Male	Pre-Freeze Motility	Post Freeze/Thaw Motility
1	80%	50%
2	80%	
3	> 90%	
4 '	> 90%	

Because rainbow trout gametes are available to us throughout the year, we used rainbow trout sperm as a model system to examine new procedures or protocols.

In our first study on the rates of freezing the cooling rates were compared from 9°C/min to 81°C/min in liquid nitrogen vapor. Although there appears to be a large variation among treatment groups with different replicates that may be due to the sequence in which we froze the various samples, there is no apparent difference in the average fertility between cooling rates (Table 2). This data also shows that fertility of frozen/thawed sperm ranged between 59 to 68.5% of the control, nonfrozen sperm.

The figure is a sample of the data that we have developed using CFDA as a live-dead stain. As shown in the figure we can clearly identify cells that are dead from those that are viable by this criterion.

CONCLUSIONS

Based on our present results on sperm motility post-freezing, we presently have an adequate protocol for the cryopreservation of sturgeon sperm. This forthcoming year we will improve our freezing method and obtain actual fertility data for our frozen sperm.

Table 2. Effect of Freezing Rate on the Fertility of the Sperm

Treatment Group	% Eye-Up[a,b] (mean ± SEM)	% of Control
Control	93.8 ± 2.0	
9°C/min	64.3 ± 7.4	68.5
27°C/min	55.3 ± 8.9	59.0
81°C/min	60.8 ± 4.5	64.8

[a]Development to eye-up was used as a measure of successful fertilization.

[b]Represents a mean of four replicates with 200 eggs fertilized with 125 ul of frozen/thawed sperm per replicate.

References

Harvey, B. 1990. Germplasm Conservation in Aquaculture. In: The Preservation and Value of Genetic Resources. G.H. Orians, G.M. Brown, W.E. Kunin and J. Swierzbinski, eds. University of Washington Press, pp. 32-38.

Stoss, J. 1983. Fish Gamete Preservation and Spermatozoan Physiology. In: Fish Physiology, vol. 9B. W.S. Hoar, D.J. Randall and E.M. Donaldson, eds. Academic Press, New York, pp. 305-350.

Stoss, J. and Holtz, W. 1981. Cryopreservation of Rainbow Trout (Salmo gairdneri) Sperm. II. Effect of pH and Presence of a Buffer in the Diluent. Aquaculture. 25:217-222.

Appendix D. (continued)

BECTON DICKINSON LYSYS II Version 1.0 11/90

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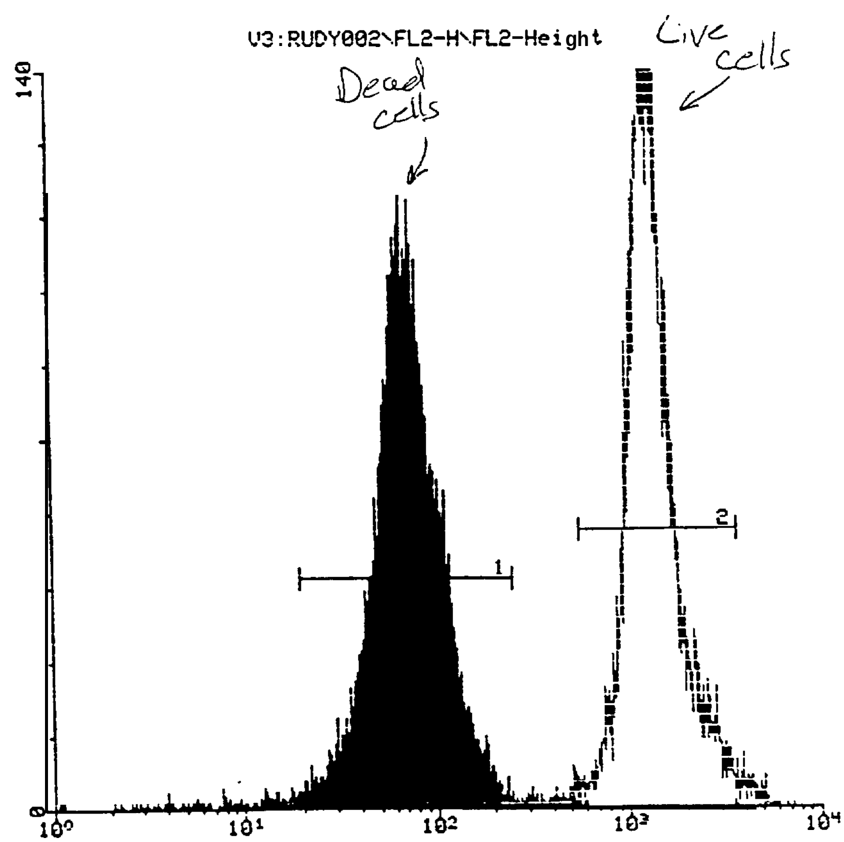

V3:RUDY002\FL2-H\FL2-Height

--- Arithmetic Histogram Statistics for V3:RUDY002 ---

Parameter FL2-H FL2-Height Ungated

M	Left,Right	Events	%	Peak	PkChl	Mean	Medi
0	1.00, 9910	10000	100. eO	116	66.71	77.88	7[illegible]
1	19.80, 250	9812	98.11	116	66.71	76.03	71
2	567, 3752	3	0.03	1	577.72	1573.49	57i