COLUMBIA WHITE STURGEON CONSERVATION FISH CULTURE PROGRAM

KOOTENAY STURGEON HATCHERY

2004 Annual Report

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OVERVIEW

2004 was an extremely successful year for the Upper Columbia sturgeon culture program as we were able to spawn more adults than we had in the past years. Along with the adults spawning success we were able to raise and release over 12,000 yearlings from 6 Families. The year was not without its difficulties as this year we were not able to use the Bio-Oregon feed that we have used for the past 5 years because of the border being closed to beef products. We tried a number of different diets which were much drier than our previous diet and subsequently we had a harder time in getting the larvae onto feed and suffered a higher loss because of the drop out effect.

For 2004 we attempted to conduct the brood capture sessions in 2 time periods and to try to collect 4 females from each time period. One session was in April/May and the other in June. We were only able to come up with 2 females from the first session but did manage to get 6 in the June session. 8 adult female sturgeon along with 9 adult males were captured during the 2 brood collection sessions and transported to the Kootenay Sturgeon Hatchery near Ft. Steele.
These adults were held in 4 large 12’ circular ponds with the water temperatures as close as possible matching that of the Columbia River. Females were checked regularly to see when they would be ready to be induced to spawn. We were able to spawn 6 of the females and fertilized these eggs with milt from 4 separate males to create 6 Families. We were not as successful in being able to have all the males contribute this year as 5 of the males did not produce milt when spawning events happened at the hatchery.

Once again the Kootenay Sturgeon Hatchery provided eggs and larvae to the Columbia Basin Hatchery in Washington and they raised and released 2 families into the Columbia River in the USA portion. The Washington hatchery released 3,755 sturgeon from these 2 families.
We released a total of 12,748 yearling sturgeon averaging 48 grams each, making the grand total of 16,503. Each of these juveniles was scute marked and implanted with a PIT tag. Length and weight data for each tagged juvenile was collected prior to release along with its release location. A large release event took place at Castlegar on May 4th with 350 school kids and many volunteers helping to release the sturgeon. A smaller event took place with some more school kids at Trail on the same day when fish were released at Beaver Creek.

The FFSBC Health Lab once again tested for IPNV, WSHV1, WSHV2 and WSIV on a regular basis including testing adults and juveniles from each family. All results were negative.
There were a number of experiments and samples taken this year including an Incubation experiment carried out by Mark Tiley, CCRIFC testing white sturgeon larval and juvenile development and survival reared under fall and early winter Revelstoke reach temperature conditions. Milt was sent to Joe Cloud at the University of Southern Idaho for cryopreservation work. Samples from adults during brood capture, adults spawned at the hatchery and subsequent juveniles from each of the resulting families were sent to Kevin Kwak and Ron Hedrick at University of California, Davis, for PCR Diagnostic work. DNA samples were collected from all adults and from a portion of each family. Multiple spermiation induction of the captive males was once again conducted with the results being much poorer than in previous years.

In 2004 we once again had a relatively high number of deformities present in all of the families. These deformity rates ranged from 9 to 38%, (averaging 20%), with most of the deformities being misshapen or missing pectoral fins.

This Sturgeon Culture program continues to be very high profile with a large number of presentations being made during the year. There was the major release event staged by BC Hydro and the Action Planning Group. Terry Halleran of Westland TV filmed a great program that aired on the Knowledge Network. Presentations were made at the Roosevelt Forum in Washington and at the Northwest Fish Culture Conference in Victoria. A Sturgeon workshop was jointly hosted by the Freshwater Fisheries Society of B.C. and the Tribal sturgeon Hatchery at Bonners Ferry, Idaho with representatives from many agencies attending.

As always this Columbia Sturgeon culture program was once again a learning experience, just when we start to think we have these large beasts figured out something seems to go rather sideways. I guess it just keeps us on our toes.

BROOD CAPTURE

In this years brood capture we attempted to capture 4 females from an early session from April 26th to May 14th and then 4 females from the session from June 14th to 25th to see which capture time produced the best spawning results. We were only able to capture 2 females during the first session and did capture 6 females from the June session. We were able to spawn all 6 females caught during the June session and were unsuccessful in getting either of the early caught females to spawn.
During these sessions Ron Ek or Mickey Mc Donald participated for FFSBC and worked along with Bob Chapman (Golder Assoc) and either Matt Neufeld (WLAP) or Dean den Beisen (BC Hydro) or Sean Ord (BC Hydro). We made some changes to the brood capture and holding techniques that had been used in previous years by trying to capture brood by angling in addition to using the setlines and by holding the adults at the boat by their set line hook, (head up) rather than by tail noosing prior to conduction the maturity assessment.
The early session focused on the top section of the river near Castlegar with set lines being placed in Keenleyside Eddy, Kootenay Eddy, Bob's Hole and other sites in this area. 1 female and 4 males were caught in this area. One female was caught during this session at Waneta. (see Adult Sturgeon holding section)
Most of the last 2 weeks were spent down in the lower section of the river just above the US border at Waneta Eddy and Ft.Shepard Eddy. 5 females and 4 males were captured at these sites. One females was captured at Kootenay Eddy during the June session.(see Adult Sturgeon Holding section.)
The normal 30 hook set line was employed with chunks of Kokanee being used for bait.
The line would be set each afternoon and then left over night and pulled the next morning. 2 sizes of barbless halibut hooks were used on the line, sized 14 and 16.
A sample of eggs from each female captured were boiled and fixed in formalin on the boat so that initial PI calculations could be done back at the hatchery detailed information on brood capture procedures can be found in the "Upper Columbia River Adult White Sturgeon Capture, Transport and Handling Manual 2003 produced by Golder Assoc.)
Capture information including dates, size, location, PIT tag number and release dates are on the Adult Sturgeon Holding sheet following.

TRANSPORT

When an adult was captured that was determined to be maturing this year it was loaded into the sturgeon transport tank at the nearest access point to the river. The tank was filled with river water using a fire pump. To ease stress during transport and to help aid in healing small abrasions that happened during capture and handling salt was added to the transfer water to make a solution of 5 ppt. Oxygen is supplied to the tank through aeration stones that are recessed into the tank floor.

ADULT STURGEON HOLDING

2004

Sex	Pit Tag #	Weight kg	Captured	Location	Spawned	Released
Female	42647F5002	43	29-Apr	HLK	None	21-Jul
Female	7F7D3F700C	107	11-May	Waneta	None	15-Jul
Female	7F7D31051E	45	9-Jun	Waneta	16-Jun	17-Jun
Female	7F7D311727	95	11-Jun	Koot Eddy	11-Aug	6-Jul
Female	7F7D500107	45	16-Jun	Waneta	24-Jun	25-Jun
Female	7F7D494B31	39	18-Jun	Waneta	24-Jun	25-Jun
Female	7F7D31165E	59	18-Jun	Waneta	2-Jun	25-Jun
Female	7F7E6A3C3D	100	22-Jun	Waneta	29-Jun	6-Jul
Male	131473731A	48	30-Apr	HLK	None	24-Jun
Male	7F7D42061F	40	4-May	HLK	16-Jun	24-Jun
Male	7F7D49475D	34	4-May	HLK	16-Jun	18-Aug
Male	7F7E6A391D	41	5-May	HLK	None	24-Jun
Male	7F7E6A4A11	23	17-Jun	HLK	None	18-Aug
Male	7F7E687116	30	18-Jun	Waneta	24-Jun 29-Jun	Flowing Aug 13-18
Male	7F7D4E5E33	47	12-Jun	Waneta	24-Jun	Flowing Aug 13-18
Male	426465351D	41	22-Jun	Fort Shepard	None	Aug 13-18
Male	7F7D37534A	45	23-Jun	Waneta	24-Jun 29-Jun	Flowing Aug 13-18

SPAWNING

Of the 8 females that were transported and held at the Kootenay Sturgeon hatchery 6 were successfully induced to spawn. Each of the 8 females was checked regularly to determine their ripeness. (The procedure for these determinations was done as described in the "Broodstock Evaluation" section of the "Hatchery Manual for the White Sturgeon" University of California.)

COLUMBIA WHITE STURGEON SPAWNING SUMMARY

Female	Capture Date	Inject Date	Spawn Date	Male	Eggs	Neurolation%	Comments
42647F5002	29 Apr						Did not spawn
7F7D3F700C	11 May						Did not spawn
7F7D31051E	9 Jun						
		14 Jun	16-Jun	7F7D49475D	625ml	87%	4-5000 to Wash
				7F7D49475D	625ml	34%	
				7F7D42061F	625ml	0%	no fertilization
				7F7D49475D	500ml	60%	
7F7D311727	11 Jun	9 Aug	11 Aug	7F7D49475D	500ml	45%	
				7F7D49475D	500ml	18%	
				7F7D49475D	500ml	3%	Tiley experiment
7F7D500107	16 Jun	22 Jun	24 Jun	7F7E687116	740 ml	93%	
				7F7D4E5E33	740 ml	0%	no fertilization
7F7D494B31	18 Jun	22 Jun	24 Jun	7F7D37534A	700 ml	93%	
				7F7D4E5E33	700 ml	0%	no fertilization
7F7D31165E	18 Jun	22 Jun	24 Jun	7F7E687116	750ml	94%	
				7F7D37534A	750ml	0%	no fertilization
				7F7D4E5E33	750ml	97%	
7F7E6A3C3D	22 Jun	27 Jun	29 Jun	7F7E687116	500ml	94%	
				7F7E687116	500ml		Washington
				7F7D37534A	500ml		Washington
				7F7D37534A	500ml	93%	
				7F7D37534A	500ml	89%	
				7F7D37534A	500ml	0%	no fertilization

Family Matings

Family #1 – Female # 7F7D31051E X Male # 7F7D49475D
Family #2 – Female # 7F7D500107 X Male # 7F7E687116
Family #3 – Female # 7F7D494B31 X Male # 7F7D37534A
Family #4 – Female # 7F7D31165E X Male # 7F7D4E5E33
Family #5 – Female # 7F7D31165E X Male # 7F7E687116
Family #6 – Female # 7F7E6A3C3D X Male # 7F7E687116
Family #7 – Female # 7F7E6A3C3D X Male # 7F7D37534A
Family #8 – Female # 7F7E6A3C3D X Male # 7F7D37534A
+ Male # 7F7E687116
Family #9 – Female # 7F7D311727 X Male # 7F7D49475D

Columbia Spawning Matrix

The Columbia Sturgeon Conservation hatchery is set up to raise 6 individual families.
The breeding plan is to attempt to use as many spawning individuals as possible. It is impossible to know how many female sturgeon will spawn during the year so to try to ensure that we have as much genetic contribution from captured adults the following spawning matrix is attempted. If everything goes perfectly we would have 6 individual families mating 1 female to 1 male. If a lower number of females spawn then we would have up to 6 families some of which some would be ½ siblings.

Females	**Males**	
1st Female	4 Males	**#1**, #2, #3, #4
2nd female	2 Males	**#5**, #6
3rd Female	2 Males	**#3**, #4 (Replace the crosses using Males 3 & 4)
4th Female	2 Males	#1, **#2** (Delete Family from 1st Female using Male #2 (Delete the family from this cross using male #1)
5th female	2 Males	#5, **#6** (Delete Family from 2nd female using Male #5) (Delete family from this cross using male #6)
6th female	2 Males	**#4** and another to try. (Delete family from Female #3 using male #4) (And delete whatever 2nd cross from this mating)

The bold number in the male column is the remaining family.

Fish Health Testing Summary
Columbia White Sturgeon
2004 Brood

Virus Screening for Broodstock:

All Ovarian fluids and Milts of contributing adults were screened for the following viruses using standard tissue culture methods as described in Section X: Procedures for the Detection of Viruses as listed in the Canadian Fish Health Protection Regulations Manual of Compliance: *Note: Fluids were frozen at -20 for a period of time prior to testing*.

White Sturgeon Iridiovirus (WSIV)
White Sturgeon Herpes virus I (WSHI)
White Sturgeon Herpesvirus II (WSHII)
White Sturgeon Adenovirus (WSAV)

Infectious Hematopoietic Necrosis (IHNV)
Infectious Pancreatic Necrosis (IPNV)
Viral Hemorrhagic Septicemia (VHSV)

Cells lines used to determine presence/absence of virus included:

White Sturgeon Spleen (WSS-2)
White Sturgeon Skin (WSSK)
White Sturgeon Gill (WSG)

Rainbow Trout Gonad (RGT-2)
Epithelial Carp (EPC)
Chinook Salmon Embryo (CHSE-214)

All virus assays were run for a period of 28 days. No viruses or replicating agents were isolated from the reproductive fluids tested. See Table 1 for Broodstock results.

Case Number: 2004-1070 Columbia Sturgeon brood ovarian fluid
2004-1071 Columbia Sturgeon brood milt fluid

Client: Kootenay Sturgeon Hatchery-Ron Ek

Submission Dates: 2004-1070 July 5, 2004
Aug 13, 2004
2004-1071 July 8, 2004

Species: White Sturgeon

Stock: Columbia

Purpose: Broodstock Health screening

Tissue: Ovarian fluids, milt samples

Total Number Tested:

ovarian fluids =6	milt = 4
Tag # 7F7D31051E	# 7F7E687116
Tag # 7F7D500107	# 7F7D37534A
Tag # 7F7D494B31	# 7F7D4E5E33
Tag # 7F7D31165E	# 7F7D49475D
Tag # 7F7E6A3C3D	
Tag# 7F7D311727	

Results: All reproductive fluids were run on the following cell lines:
WSS-2, WSSK, WSG, EPC and CHSE-214

No viral agents or replicating filterable agents were detected.

Reproductive fluids were also tested for Infectious Pancreatic Necrosis (IPNV) using the PCR method. All tested samples were negative.

Juvenile Screening History:

A sub sample of 5-30 juveniles from all family groups were screened for the above listed viruses at 30 days and 60 days post hatch. Fish were processed according to Section X : Procedures for the Detection of Viruses as listed in the Canadian Fish Health Protection Regulations Manual of Compliance. All examined juveniles tested negative for the above listed viruses. Cell lines as listed for Broodstock testing were also used for juvenile testing. See Table 2:

Case Number:
2004-1085 Columbia Sturgeon Family 1
2004-1089 Columbia Sturgeon Family 2
2004-1090 Columbia Sturgeon Family 3
2004-1091 Columbia Sturgeon Family 4
2004-1092 Columbia Sturgeon Family 5
2004-1093 Columbia Sturgeon Family 6
2004-1094 Columbia Sturgeon Family 7

Client: Kootenay Sturgeon Hatchery-Ron Ek

Submission Dates:

Case	Date	Submission
2004-1085	July 29	30 day submission
	Sep 29	90 day submission
2004-1089	Aug 6	30 day submission
	Oct 1	90 day submission
2004-1090	Aug 6	30 day submission
	Oct 6	90 day submission
2004-1091	Aug 10	30 day submission
	Oct 6	90 day submission
2004-1092	Aug 10	30 day submission

	Oct 8	90 day submission
2004-1093	Aug 13	30 day submission
	Oct 8	90 day submission
2004-1094	Sep 15	30 day submission
	Nov 16	90 day submission

Species: White Sturgeon

Stock: Columbia

Results: No viral agents or replicating filterable agents were detected.

Comments: No cases were submitted from the Columbia group for diagnostics although fish submitted for the 90 day testing in late September early October it was noted had gone off feed.

No pathogen was isolated upon investigation however feeding behavioural change was attributed a change in feed diets which the hatchery was implementing at the time.

Sentinals

Case Number: 2004-1036 Sentinel fish pre-test
2004-1100 Columbia Sturgeon Sentinel fish

Client: Kootenay Sturgeon Hatchery-Ron Ek

Submission Dates: April 21, 2004 pre-test
Aug 25, 2004 30 day post testing all family groups

Species: Rainbow Trout

Stock: Gerrards

Purpose: Health screening

Tissue: 30 day testing =gill, spleen, pyloric ceaca, kidney

Total Number Tested: 2004-1036 pre-test =62 fish
2004-1099 30 day = 30 fish

Results: All samples were run on the following cell lines:
WSS-2, WSSK, WSG, EPC and CHSE-214

No viral agents or replicating filterable agents were detected.

DEFORMITIES

Deformity Summary

*Check done at time of sampling for release

Family	Site	1	2	3	4	5	Total	Total Released	% Deformed Released	% Deformed culled	Total % Deformed
1	Beaver	7	15	0	0	2	24	841	2.9%		
	HLK	38	66	5	0	3	112	909	12.3%		
	ttl	**45**	**81**	**5**	**0**	**5**	**136**	**1750**	**7.8%**	**2.70%**	**10.50%**
2	Beaver	101	105	16	0	0	222	1188	18.7%		
	HLK	118	211	39	0	0	368	1252	29.4%		
	ttl	**219**	**316**	**55**	**0**	**0**	**590**	**2440**	**24.2%**	**13.50%**	**37.70%**
3	Beaver	42	79	15	0	0	136	1065	12.8%		
	HLK	40	41	7	0	7	95	740	12.8%		
		82	**120**	**22**	**0**	**7**	**231**	**1805**	**12.8%**	**9%**	**21.80%**
4	Beaver	45	36	21	0	0	102	822	12.4%		
	HLK	84	75	27	0	2	188	993	18.9%		
	ttl	**129**	**111**	**48**	**0**	**2**	**290**	**1815**	**16.0%**	**6.60%**	**22.60%**
5	Beaver	20	27	13	0	7	67	1319	5.1%		
	HLK	36	77	32	0	12	157	1411	11.1%		
	ttl	**56**	**104**	**45**	**0**	**19**	**224**	**2730**	**8.2%**	**0.80%**	**9.00%**
7	Beaver	24	14	2	0	2	42	1098	3.8%		
	HLK	23	21	3	0	2	49	1110	4.4%		
	ttl	**47**	**35**	**5**	**0**	**4**	**91**	**2208**	**4.1%**	**11.70%**	**15.80%**
Total deformities		**578**	**767**	**180**	**0**	**37**	**1562**	**12748**			
% of deformities		**37.0%**	**49.1%**	**11.5%**	**0.0%**	**2.4%**			**12.2%**	**7.60%**	**19.80%**

Deformities Culled at Marking

Date	Family	Deformities culled 1	2	3	runt	total	%
14-Mar	1	16	20	12		48	2.70%
8-9 Mar	2	112	221	48		381	13.50%
10-Mar	3	54	90	29	8	181	9%
11-Mar	4	44	59	22	4	129	6.60%
7-8 Mar	5	2	17	4		23	0.80%
15-16 Mar	7	72	74	146		292	11.70%
						1054	7.60%

1= left pelvic
2 = right pelvic
3 = both pelvic fins
4 = skewed head
5 = abnormal tail

RELEASES

Release Summary - includes all fish (sonic as well)

Releases by site

	Family	#	Kgs	Mean Wt g	FLmm
Beaver Creek 4-May-05	1	841	52.5	62.4	196.2
	2	1188	50.3	42.3	187.7
	3	1065	50.4	47.3	182.6
	4	822	42.2	51.4	195.1
	5	1319	61.6	46.7	189
	7	1098	41.8	38.1	171.4
	total	**6333**	**298.8**	**47.2**	

	Family	#	Kgs	Mean Wt g	FL mm
HLK 4-May-05	1	909	62	68.2	199.5
	2	1252	55.1	44	189.8
	3	740	35.4	47.8	185
	4	993	46.7	47	191.2
	5	1411	62.6	44.3	187.8
	7	1110	44.3	39.9	174.1
	total	**6415**	**306.1**	**47.7**	

TOTAL:
12748 fish
604.9 kg

Releases by Family

Family	#	Kgs	Mean Wt g	Mean FL mm	Site
1	841	52.5	62.4	196.2	Beaver
1	909	62	68.2	199.5	HLK
	1750	**114.5**			
2	1188	50.3	42.3	187.7	Beaver
2	1252	55.1	44	189.8	HLK
	2440	**105.4**			
3	1065	50.4	47.3	182.6	Beaver
3	740	35.4	47.8	185	HLK
	1805	**85.8**			
4	822	42.2	51.4	195.1	Beaver
4	993	46.7	47	191.2	HLK
	1815	**88.9**			
5	1319	61.6	46.7	189	Beaver
5	1411	62.6	44.3	187.8	HLK
	2730	**124.2**			
7	1098	41.8	38.1	171.4	Beaver
7	1110	44.3	39.9	174.1	HLK
	2208	**86.1**			

Summary of All Washington Releases – by Site

Site	Release Date	Family	Number	Kgs	Mean FLmm	Mean Wt g	Deformities 1	2	3	4	5	7	8
NG/Marcus Flats	11-May-05	6	317	73.6	312.6	232	6	18	3	0	5	0	1
NG/Marcus Flats	24-Feb-05	6	333	39	244.3	117.2	0	13	6	0	3	0	3
NG/Marcus Flats	11-May-05	7	319	65.6	313.1	205.7	7	19	1	0	3	0	0
NG/Marcus Flats	24-Feb-05	7	392	47.5	254.4	121.1	7	18	0	0	4	0	1
			1361	**225.7**		165.8							
North Gorge	11-May-05	6	314	52	270.7	165.6	7	12	6	0	4	2	0
North Gorge	24-Feb-05	6	333	10.7	244.3	108.8	15	15	2	0	3	0	3
North Gorge	11-May-05	7	315	41.4	261.8	131.4	8	9	0	0	0	0	0
North Gorge	24-Feb-05	7	272	32.5	255.3	119.5	6	6	1	0	1	0	4
			1234	**136.6**		110.7							
Northport	11-May-05	6	272	47.8	284.1	175.7	8	13	4	0	2	0	0
Northport	24-Feb-05	6	230	27	243.0	117.5	8	8	6	0	1	0	3
Northport	11-May-05	7	321	45.7	266.2	142.3	3	6	0	0	2	0	0
Northport	24-Feb-05	7	337	43.8	262.9	129.9	6	10	0	0	2	0	0
			1160	**164.3**		141.6							
Total:			**3755**	**526.6**		140.2							

Summary by family

Site	Release Date	Family	Number	Kgs	Mean FLmm	Mean Wtg	Deformities 1	2	3	4	5	7	8
NG/Marcus Flats	11-May-04	6	317	73.6	312.6	232	6	18	3	0	5	0	1
NG/Marcus Flats	24-Feb-05	6	333	39	244.3	117.2	0	13	6	0	3	0	3
North Gorge	11-May-04	6	314	52	270.7	165.6	7	12	6	0	4	2	0
North Gorge	24-Feb-05	6	333	10.7	244.3	108.8	15	15	2	0	3	0	3
Northport	11-May-04	6	272	47.8	284.1	175.7	8	13	4	0	2	0	0
Northport	24-Feb-05	6	230	27	243.0	117.5	8	8	6	0	1	0	3
			1799	**250.1**		139.0							
NG/Marcus Flats	11-May-04	7	319	65.6	313.1	205.7	7	19	1	0	3	0	0
NG/Marcus Flats	24-Feb-05	7	392	47.5	254.4	121.1	7	18	0	0	4	0	1
North Gorge	11-May-04	7	315	41.4	261.8	131.4	8	9	0	0	0	0	0
North Gorge	24-Feb-05	7	272	32.5	255.3	119.5	6	6	1	0	1	0	4
Northport	11-May-04	7	321	45.7	266.2	142.3	3	6	0	0	2	0	0
Northport	24-Feb-05	7	337	43.8	262.9	129.9	6	10	0	0	2	0	0
			1956	**276.5**		141.4							
Total			**3755**	**526.6**		140.2							

Sonic Tags 11-May-05

Site / Family	Tags	Site / Family	Tags
Nancy Green/Marcus Flats	Acoustic tag ID 1817	Nancy Green/Marcus Flats	Acoustic tag ID 1823
Family 6	Acoustic tag ID 1825	Family 7	Acoustic tag ID 1828
	Acoustic tag ID 1815		Acoustic tag ID 1832
	Acoustic tag ID 1816		Acoustic tag ID 1818

SONIC TAGGING

A total of 31 juveniles were raised to between 115 and 252 grams and then implanted with a sonic tags. These fish were held another 3 weeks and then released with the other juveniles on May 4th.
15 of these tags were high frequency tags and 15 were low frequency tags. Each of these were split between the 2 release locations.

								Sonic Tags			
Fish	Pitag1	Release Date	Rkm	FLmm	WTg	Family	Def	Serial #	Freq	ID Code	Coded Pinger
1	985120016089479	4-May-05	HLK	280	127	5		7768F	69.0	1836	V8SC-2H-R04K
2	985120019245107	4-May	HLK	320	204	5		7762F	69.0	1830	V8SC-2H-R04K
3	985120019243477	4-May	HLK	330	216	2		7766F	69.0	1834	V8SC-2H-R04K
4	999000000472531	4-May	HLK	300	153	2		7756F	69.0	1824	V8SC-2H-R04K
5	985120019260822	4-May	HLK	315	241	3		7754F	69.0	1822	V8SC-2H-R04K
6	985120019233420	4-May	HLK	325	208	2		7752F	69.0	1820	V8SC-2H-R04K
7	985120019115534	4-May	HLK	295	173	2		7758F	69.0	1826	V8SC-2H-R04K
8	985120019237827	4-May	Beaver Cr.	310	217	1		7753F	69.0	1821	V8SC-2H-R04K
9	985120019259996	4-May	Beaver Cr.	290	156	5	3	7761F	69.0	1829	V8SC-2H-R04K
10	985120019259380	4-May	Beaver Cr.	265	141	3		7769F	69.0	1837	V8SC-2H-R04K
11	985120019165441	4-May	Beaver Cr.	310	177	2	1	7751F	69.0	1819	V8SC-2H-R04K
12	985120013433618	4-May	Beaver Cr.	300	165	5		7765F	69.0	1833	V8SC-2H-R04K
13	985120019218049	4-May	Beaver Cr.	295	195	1		7763F	69.0	1831	V8SC-2H-R04K
14	985120019279548	4-May	Beaver Cr.	310	241	3		7767F	69.0	1835	V8SC-2H-R04K
15	985120019255444	4-May	Beaver Cr.	260	115	5		7759F	69.0	1827	V8SC-2H-R04K
16	985120016091596	4-May	Beaver Cr.	315	203	2	2	7737F	69.0	1805	V8SC-2L-R04K
17	985120019115653	4-May	Beaver Cr.	320	215	5		7745F	69.0	1813	V8SC-2L-R04K
18	985120013436658	4-May	Beaver Cr.	295	163	5		7741F	69.0	1809	V8SC-2L-R04K
19	985120019119769	4-May	Beaver Cr.	320	183	2		7735F	69.0	1803	V8SC-2L-R04K
20	999000000167177	4-May	Beaver Cr.	315	205	4		7742F	69.0	1810	V8SC-2L-R04K
21	985120019257507	4-May	Beaver Cr.	320	215	1		7743F	69.0	1811	V8SC-2L-R04K
22	985120013470800	4-May	Beaver Cr.	330	252	5		7733F	69.0	1801	V8SC-2L-R04K
23	999000000164830	4-May	HLK	310	214	3		7739F	69.0	1807	V8SC-2L-R04K
24	999000000458623	4-May	HLK	290	145	2		7740F	69.0	1808	V8SC-2L-R04K
25	985120019117911	4-May	HLK	285	174	4		7732F	69.0	1800	V8SC-2L-R04K
26	985120013471786	4-May	HLK	290	187	1		7746F	69.0	1814	V8SC-2L-R04K
27	985120013439038	4-May	HLK	315	234	1		7736F	69.0	1804	V8SC-2L-R04K
28	985120013463594	4-May	HLK	305	183	2		7738F	69.0	1806	V8SC-2L-R04K
29	985120019234795	4-May	HLK	265	119	5		7744F	69.0	1812	V8SC-2L-R04K
30	985120019233408	4-May	HLK	290	203	1		7734F	69.0	1802	V8SC-2L-R04K

Presentations and Meetings - 2004

June & August, 2004 – Terry Halleran of Westland TV here to film sturgeon program. In June, he attended a spawning where he filmed egg and milt collection as well as fertilization, deadhesion, disinfection and incubation of sturgeon eggs. In August, he filmed the release of an adult back into the Columbia.

October 26, 2004 – First Annual White Sturgeon Program Meeting attended by Bryan Ludwig, Ray Billings, Laird Siemens, Ron Ek, Tim Yesaki and Sherry Guest at the FFSBC Fish Health Unit in Nanaimo, BC.

November 16, 2004 – Ron Ek presented at the Lake Roosevelt Forum Conference in Spokane, WA.

Nov 17 & 18, 2004 – Ron Ek and Bryan Ludwig attended the Upper Columbia White Sturgeon Recovery Initiative - Recovery Team meeting in Spokane, WA.

December 10, 2004 – Ron Ek did a presentation on the Kootenay and Columbia White Sturgeon programs at the KSH at the North West Fish Culture Conference in Victoria, BC.

Columbia White Sturgeon 2004
Research Projects

Sturgeon Culture Workshop:
A sturgeon culture workshop was jointly held at KSH in Fort Steele and at the Kootenai Tribal Hatchery in Bonners Ferry, Idaho. Many representatives from various agencies attended.
(Minutes in Appendix 2)

Cryopreservation Samples:
Aug 25th -150 mls of milt from Male # 42647D276C sent to Joe Cloud, University of Southern Idaho fro cryopreservation work. It arrived in good shape on Aug 27th and was frozen in 2 sized straws. They found 30% motility rate in thawed sample, which was very good. These samples are stored at the University.
(See Appendix 3)

Washington Hatchery:
On June 29, Brian Lyons from the Moses Lake Hatchery in Washington received fertilized eggs from Families 6 and 7 (taken 29 June) as well as larvae from Family 1 (taken 16 June).
Family #1 (Female # 7F7D31051E X Male # 7F7D49475D) 5,000 larvae
Family #6 (Female # 7F7E6A3C3D X Male # 7F7E687116) 500ml fertilized eggs
Family #7 (Female # 7F7E6A3C3D X Male # 7F7D37534A) 500ml fertilized eggs

DNA Sampling:
Thirty DNA samples (fins) were removed from each family group and preserved in ethanol. Samples to Steve McAdam (WLAP).
DNA samples also removed from the adults.

Larvae and Juvenile Rearing Experiment:
Mark Tiley (CCRIFC) obtained larvae from KSH to conduct an experiment to determine growth and survival of white sturgeon over wintered in the Arrow Reservoir at Revelstoke.
(See Appendix 4)

PCR Diagnostic Approaches to the Detection of the White Sturgeon Iridovirus (WSIV) as Developed at the University of California, Davis:
Fin, mucous and/or gonad material was collected from broodfish holding at KSH and sent to Kevin Kwak and Ronald Hedrick to aid in this project.
(See appendix 5)

APPENDIX 1

Female Holding/Spawning Summaries

FEMALE: 7F7D494B31

#	ID	Captured Location	Date	Size LBS	KGS	Cm
B31	7F7D494B31	Waneta	18-Jun	86	39	

Egg Biopsies

Date	Egg Size	Mean PI	Comments	Progesterone Assay		
				Control	Prog #1	Prog #2
capture 18 Jun	3.4mm	0.091		27%	100%	100%

Injections - LHRH

Date	Initial Dose Date	Initial Dose Amount	Resolving Dose Date	Resolving Dose Amount	Egg Release Date	Egg Release Time	Spawn Date	Spawn Time
22-Jun	22-Jun 9:30 PM	0.39 ml	23-Jun 9:30 AM	0.70 ml	24-Jun		24-Jun	2:30 PM

Spawning

Date	Male	Upweller	weight of eggs	
24-Jun-04	34A	2	700ml	fertilize at 3:10 pm
	E33	3	700 ml	incision and vent egg removal

FEMALE: **7F7E6A3C3D**

#	ID	Captured Location	Date	Size LBS	KGS	Cm
C3D	7F7E6A3C3D	Waneta	22-Jun	220	100	

Egg Biopsies

Date	Egg Size	Mean PI	Comments	Progesterone Assay		
capture				Control	Prog #1	Prog #2
22-Jun	3.7 mm	0.082		0%	100%	100%

Injections - LHRH

Date	Initial Dose Date	Initial Dose Amount	Resolving Dose Date	Resolving Dose Amount	Egg Release Date	Egg Release Time	Spawn Date	Spawn Time
	27-Jun 815pm	1	27-Jun 815 am	1.8 ml	29-Jun	630 am	29-Jun	830 am

Spawning

Date	Male	Upweller	weight of eggs
29-Jun	116	1	500 ml
	116	Wash	500 ml
	34A	Wash	500 ml
	34A	3	500 ml
	34A	5	500 ml
	34A	8	500 ml

FEMALE: 7F7D31051E

#	ID	Captured Location	Date	Size LBS	KGS	Cm	Release
51E	7F7D31051E	Waneta	June 9/04	100	45		

Egg Biopsies

Date	Egg Size	Mean PI	Comments	Progesterone Assay		
capture				Control	Prog #1	Prog #2
Jun-09	3.3 mm	0.055				
Jun-11	3.3 mm	0.062		0%	88%	100%

Injections - LHRH

Date	Initial Dose Date	Amount	Resolving Dose Date	Amount	Date	Egg Release	Spawn Date	Time
14-Jun	14-Jun 8pm	0.45 ml	15-Jun 8:00 AM	0.81 ml	16-Jun	6:00 AM	16-Jun	9:00 AM

Spawning

Date	Male	Upweller	weight of eggs	
16-Jun	475D	1	625 ml	first eggs taken
16-Jun	475 D	2	625 ml	last eggs taken
16-Jun	61F	4	625 ml	
16-Jun	475D	5	500 ml	

FEMALE: 7F7D500107

#	ID	Captured Location	Date	Size LBS	KGS	Cm
107	7F7D500107	Waneta	16-Jun	100	45	

Egg Biopsies

Date	Egg Size	Mean PI	Comments	Progesterone Assay		
capture				Control	Prog #1	Prog #2
16 Jun	3.4mm	0.058		0%	71%	95%

Injections - LHRH

	Initial Dose		Resolving Dose		Egg Release		Spawn	
Date	Date	Amount	Date	Amount	Date	Time	Date	Time
22-Jun	22-Jun 9:30 PM	0.45 ml	23-Jun 9:30 AM	0.81 ml	24-Jun	10:am	24-Jun	1:00 PM

Spawning

Date	Male	Upweller	weight of eggs	
24-Jun	116	7	740 ml	
	E33	8	740 ml	fertilize at 1:25 pm

FEMALE: 7F7D31165E

#	ID	Captured Location	Date	Size LBS	KGS	Cm
65E	7F7D31165E	Waneta	18-Jun	130	59	

Egg Biopsies

Date	Egg Size	Mean PI	Comments	Progesterone Assay		
capture				Control	Prog #1	Prog #2
18 Jun	3.4mm	0.055		0%	82%	100%

Injections - LHRH

	Initial Dose		Resolving Dose		Egg Release		Spawn	
Date	Date	Amount	Date	Amount	Date	Time	Date	Time
22-Jun	22-Jun 8pm	0.59 ml	23-Jun 8:00 AM	1.06 ml	24-Jun	7:20 AM	24-Jun	9:30 AM

Spawning

Date	Male	Upweller	weight of eggs	
24-Jun	116	6	750 ml	1010 am fertilization
16-Jun	34A	5	750 ml	
16-Jun	E33	4	750 ml	

FEMALE: 7F7D311727

#	ID	Captured Location	Date	Size LBS	KGS	Cm
727	7F7D311727	Koot Eddy	11-Jun	210	95	

Egg Biopsies

Date	Egg Size	Mean PI	Comments	Progesterone Assay		
capture				Control	Prog #1	Prog #2
11 Jun	3.2mm	0.177		0%	0%	0%
27 Jun		0.151		0%	0%	0%
20 July		0.132		0%	0%	0%
9 Aug		0.128		0%	0%	0%

Injections - LHRH

	Initial Dose		Resolving Dose		Egg Release		Spawn	
Date	Date	Amount	Date	Amount	Date	Time	Date	Time
	9-Aug 8:00 PM	0.95 ml	10-Aug 8:00 AM	0.89	11-Aug	6:00 AM	11-Aug	930 am

Spawning

Date	Male	Upweller	weight of eggs
11-Aug	75D	2	500 ml
	75D	3	500 ml
	75D	Tiley exp	500 mls divided into 3 experimental upwellers on 10'C

FEMALE: 7F7D3F700C

#	ID	Captured Location	Date	Size LBS	KGS	Cm
00C	7F7D3F700C	Waneta	11-May	235	107	

Egg Biopsies

Date	Egg Size	Mean PI	Comments	Progesterone Assay Control	Prog #1	Prog #2
capture				Control	Prog #1	Prog #2
11 May	3.4mm	0.161				
14 June		0.108				
23 June		0.122		0%	0%	0%
23 July		0.131		0%	0%	0%

Injections - LHRH

	Initial Dose		Resolving Dose		Egg Release		Spawn
Date	Date	Amount	Date	Amount	Date	Time	Date

Spawning

Date	Male	Upweller	weight of eggs

FEMALE: 42647F5002

#	ID	Captured Location	Date	Size LBS	KGS	Cm
5002	42647F5002	HKL	29-Apr	95	43	

Egg Biopsies

Date	Egg Size	Mean PI	Comments	Progesterone Assay Control	Prog #1	Prog #2
capture				Control	Prog #1	Prog #2
29-Apr	3.3	0.200				
14-Jun		0.156				
5-Jul		0.152		11%	10%	5%

Injections - LHRH

	Initial Dose		Resolving Dose		Egg Release		Spawn
Date	Date	Amount	Date	Amount	Date	Time	Date
No inject							

Spawning

Date	Male	Upweller	weight of eggs
No Spawn			

APPENDIX 2

Proceedings of

Sturgeon Culture Workshop

March 8 and 9, 2004

Locations:	Kootenay Trout Hatchery Fort Steele, BC [March 8]	Kootenai Tribe of Idaho Bonner's Ferry, ID [March 9]

Chair: Ron Ek

1) BROOD CAPTURE

i) Techniques

- Angling preferred over set lines, less stress on fish
- Setlines should be run as often as possible, every 2-3 hours
- Fish captured on setlines should be attached to the boat head up utilizing the setline hook rather than tail up using a tail noose.

ii) Timing

- Fish captured later (closer to spawning time) are more successfully spawned than fish captured earlier.
- In 2004, FFSBC will capture 4 early females and 4 late females to compare results.

iii) Stress

- Minimize stress as much as possible.
- Hatchery candidates should be placed into holding tubes or the transport tank as soon as possible after capture.
- Handling fish major contributor to stress.
- Stress is the main contributor to attritic egg

iv) Transport

- Salt used in transportation tank at a concentration of 10 ppt.

v) Sampling

- A variety of samples are collected from the adults depending upon the requirements. They include weights, lengths, DNA, sperm, ovarian, eggs, blood, mucous, urine, etc.
- Need a co-ordinator of collected DNA samples.
- Have to know which samples are dependent on each other.
- For hatchery candidates wait until spawning time to collect as many samples as that can be done at that time.
- Again, sampling requires handling the fish which adds to stress. The least handling the better.

2) BROOD HOLDING

i) Containers

- Adults are being held in a variety of containers.

  10, 12, 15, and 20 ft. circulars
  10 ft. wide raceways
  24' x 8' "tomato tanks"

ii) Temperatures

- Match river temperatures where adults captured.
- Most successful brood spawning occurs when the holding water mimics the donor river water temperatures.

iii) Flows

- Provide and maintain adequate O2 levels in brood holding containers.
- Create a flow , KTH uses 10 lpm per fish,
- More a water quality than a water quantity issue minimizes stress.

iv) Feeding Adults

- Either live fish or fresh killed fish are fed, depending upon what works.
- Putting fish that aren't feeding into containers with fish that are
- For short term holding (3-4 months) fish don't need to eat but it is good to offer them feed.

- Malaspina feed a commercial diet , fill partially frozen squid using a sausage-stuffer

.

3) BROOD RELEASES

- KTH try to release adults as close to where they are captured.
- KTOI release right at hatchery dock.
- Make sure all incisions are healed prior to release.

4) OTHER BROOD ISSUES

i) Drug Treatments

- Topical applications of iodophor and nitrofurazone are used on adults.
- Surface boils have been observed with OTC use.
- If you don't have to administer drugs, don't.

ii) External Tagging Observations

- Some swelling observed on dorsals fins from dorsal applied loop tags.
- Floy tags inserted in pectoral fins okay after 4-5 years.
- Floy tags inserted in scutes(drill scute first) okay after 4-5 years.

5) SPAWNING

i) Polarization Index (PI) And GVBD Checks

- Goal is to reduce number of checks, maximum of 2 – 3 samples.
- On early caught fish do first check when first captured and do second check when late fish are captured.
- Last check should be week before spawning.
- Very rare to have a low PI and no GVBD .
- Motic digital microscope very good for doing PI and GVBD checks, accurate measurements can be done with the computer and pictures can be sent via e-mails.
- Progestrone tests 16hours at 16 degrees C, hold to +/- one degree.

ii) Sutures

- Chromic gut sutures recommended for cold water sites, PDS sutures for warm water sites
- Chromic gut for external use, PDS for internal use.
- Some use of staples and tissue glue.
- Wicking effect a concern with chromic gut.
- Incisions made to one side of midline.

iii) Hormone Injections

- Hormone used is GNRHA
- Maximum injection per site 1 ml.
- Usually inject .5/.6 ml. per injection site.
- Dosages used for females: KTH - 50ug/kg.
  KTOI - 100ug/kg.
  UC Davis – 20ug/kg.
  College Southern Idaho 50ug/kg.
- Doesn't take much if fish in right condition.
- Injections can be done underwater, less stressful.

iv) Spawning Techniques

- Caesarean is used in most industry situations.
- KTH and KTOI use hand expression as they don't require a lot of eggs for conservation goals.
- College of Southern Idaho use caesarean when some fish can't be hand spawned.
- The more ovarian fluid the easier the fish is to spawn.

v) Egg Inventory Methods

- Volume pre- fertilization.
- Weight pre- fertilization.
- Volume Post de-adhesion

vi) Fertilization

- Eggs spawned into bowls
- Eggs either fertilized right away or kept up to one hour in bowl in flowing trough water to keep same temperature.
- Milt collected in 50 ml. syringes , placed in zip lock
- bags for better O2 exchange.
- Work ongoing on cryopreservation

.

vii) Multiple Spermiation

- Males have been used 3-4 times per year.
- KTH procedure for re-using males is :
  Males that are to be used are put in the same container. Males are injected and water temperature is increased to 14 degrees. Milt is stored at 4 degrees. Probably works well as water temp. increase is a short b

6) INCUBATION

i) Incubators

- Modified MacDonald jars
- Upwellers than to modified MacDonald jars.
- Load 800 to 1200 mls. per jar
- KTH in 2004 will use a combination of upwellers and modified MacDonald jars and compare results.

ii) Temperatures and Flows

- Eggs incubated from 13 – 15.5 degrees.
- Flows kept low, don't break the horizontal plane til neuralation.
- After neuralation flows can be increased substantially

ii) Egg Development

- Eggs checked daily, the more sampling the more information.
- Use percent neuralation rate for your hatch rate.
- Industry uses a 80% hatch success and a 80% onto feed rate.
- KTH doing some pictures on egg development and Malaspina has done video on egg development.

iii) Hatching Containers

- KTH and College of SI hatch into troughs
- UC Davis – 4 ft. diameter tanks
- KTOI – 4 ' or 8' rectangular tanks
- Malaspina use 2x1 meter tanks with net pens in tank, easier to move larvae

7) REARING

i) Containers and Densities

- Various container shapes and sizes used, mostly circulars to start with.
- No standard, use whatever works.
- The key is to develop good husbandry techniques, handle fish as little as possible.
- No standard for densities in a container.
- KTH uses a maximum of 15 gr./l of water.

- College of S.I. uses pounds per sq. ft.
  Low density - .5 lbs./sq.ft

Medium density – 1.5 lbs/sq.ft.
High density - > 1.5 lbs/sq.ft.

- Generally densities are based on a 2 dimensional scale.
- 10 gram fish use 10 kg/ m2 of water
- 100 gram fish use 20 kg/ m2 of water
- White sturgeon under a production situation utilize the whole water column.
- Have to be careful don't have to low a density, no interaction.

ii) Flow Rates

- Most monitor DO to ensure adequate flow rates.
- KTH monitors DO and uses a flow rate of .75kg.fish per litre per minute.

iii) Feed

- Semi moist or moist best diet to start fish on.
- Stay conservative on feed sizes, better to stay on smaller size.
- Some switch from moist to dry once fish bigger.
- Switch from moist to dry diet mainly for financial savings.
- Notice "blip" in morts if switching from one brand of feed to another.

iv) Temperatures and Sampling (Fish Size)

- KTH – rear at 14-15 degrees til fish reach 25 grams than temper water to slow growth. Sample fish size every 30 days, grade by "eyeball" when required.
- KTOI – rear on river water, no temperature control. Don't sample fish size, but grade by "eyeball".
- College of S.I. – rear at 13-15 degrees, sample 1 lb. + fish 60 day cycle, 30 day cycle if experiment.
- UC Davis - rear at 18-19, temperature depends on group of fish. Around 20 degrees for farm production fish (different fish than wild). Sample fish size generally every 60 days.
- Malaspina – can manipulate temperature to 20+ degrees

8) MARKING , RELEASE & RECAPTURE

i) Marking

- KTOI pit tag and scute before release.
- KTH pit tag and scute before release.
- Kootenai River sturgeon use 125 kHz pit tags.
- Columbia River sturgeon use 134 kHz pit tags.
- KTOI and KTH use different scute removal patterns.

- KTOI remove one scute for marking (2004).
- KTH remove two scutes for marking, the first one is a landmark.
- Different methods of marking are being looked at, coded wire tag, calsene

ii) Releases

- Release strategies are not "set in stone".
- Different release strategies concerning size of fish, numbers of fish, timing and location of releases are being considered.
- As more data is collected more informed decisions can be made regarding releases.

iii) Recaptures

- Juvenile fish are captured mainly by entanglement in gill nets.
- KTOI uses 1 ½ inch square gill nets
- Washington uses 2 inch stretch gill nets.

9) OTHER

- Farms found when grading off leaders they ended up with a sex split of 50% males and 50% females.
- Farms don't have an issue with WSIV, its all part of doing business.
- WSIV is in Kootenai River, good fish husbandry is paramount to keeping WSIV out of hatchery.
- PCR detection tests for WSIV are being looked at.
- Malaspina is getting funding to develop a Sturgeon Research Center, a couple of years down the road but great news.

Code:		
	KTH	Kootenay Trout Hatchery
	KTOI	Kootenai Tribe of Idaho
	FFSBC	Freshwater Fisheries Society of BC
	UC-Davis	University of California at Davis
	CSI	College of Southern Idaho
	OTC	oxytetracycline
	GVBD	germinal vesicle break down
	DO	dissolved oxygen
	WSIV	white sturgeon iridovirus

Mickey McDonald March 12, 2004 file: sturgeon workshop

APPENDIX 3

Conservation Sturgeon Culture Research: Cryo Review

Cryopreservation of fish sperm is used for:

1) mating of non-synchronous fish (fish culture tool)
2) gene conservation (stock rebuilding)
3) preservation of specific lines (aquaculture)

Sturgeon sperm cryopreservation involves:

- harvesting the sperm – as usual, gonadotropins to induce spermiation
- preparing the sperm for freezing – adding cryprotectant and extenders
- 3-step freezing process – must be slow enough to allow for water to diffuse into the extra cellular spaces and fast enough to protect the intracellular components from the increased concentration of salts
- and storage at –196°C.

Expected Results:

- The highest quality sperm must be used in order to optimize fertilization rates of cryopreserved sturgeon sperm. Fertilization rates will range between 25-50% of non-frozen control sperm fertility (pers. comm. J. Cloud, University of Idaho).

Application for white sturgeon recovery:

- Fish Culture Tool: Sperm production was not limited in the first year of brood capture and spawning. In fact, some males were induced to spermiate a number of times. Therefore, non-synchronous gamete production was not an issue in year 1. If the induction of spermiation in mature males becomes problematic in the future, cryopreservation may be required for the mating of non-synchronous fish.
- Gene conservation: If the number of mature adults becomes a limiting factor in the future, cryopreservation must be considered. Sperm (male genetic component) can be frozen and held in perpetuity until needed.

Conservation Sturgeon Culture Research: Cryopreservation Update

Location: Hill Creek Hatchery (year 1), Kootenay Sturgeon Hatchery (year 2-)

Description:
The original objective for cryopreservation work was to freeze milt early in the spawning season, from running males caught in the wild, and then use those frozen samples to fertilize a small sub-sample of eggs from the captive females. This would allow us to test the efficacy of the cryopreservation within the same spawning season. However, timing and logistics did not allow for this to happen.

In year 1, we were successful in cryopreserving sturgeon milt from one captive, wild-caught, male in collaboration with Prof. Joe Cloud, University of Idaho. This occurred late in the spawning season when no eggs were available to conduct viability crosses. A large volume of milt was shipped to the University of Idaho by two methods: courier (8 days) and by car (8 hours). The courier samples were held up by customs and arrived a week later (smelly!).

The couriered samples were used in experiments to assess the cryopreservation and thawing protocols. The milt samples were frozen in two different sized straws (0.25 and 5.0 ml). Results were poor. No motility was observed upon thawing. In the past, acceptable motility rates after thawing have been obtained with the 0.25 ml straws. However, the volume of the straws makes them difficult to work with, especially when fertilizing large batches of sturgeon eggs. As a result, Joe Cloud has been experimenting with freezing milt in the large volume straws.

In year 2, we had planned to test the viability of a portion of the cryopreserved milt. However, after further discussions, Joe Cloud recommended that we hold off on any crosses with cryopreserved sturgeon milt until the freezing/thawing protocol could be refined.

Prof. Cloud was not satisfied with the results from their recent tests with large volume (5.0 ml) straws and was experimenting with different protocols. They conducted cryopreservation trials with concentrated Kootenay River white sturgeon sperm cells. The sperm cells were centrifuged to 10 times concentration and then frozen in 0.5 ml straws. These straws were subjected to 3 different freezing protocols. These samples were thawed and tested for motility. No motility was observed. If motility was observed, the thawed sperm was to be crossed with viable eggs.

This past year, we planned to send fresh milt samples down to the University of Idaho for long-term preservation (gene conservation). Any surplus milt was to be used in further cryopreservation trials. We planned to send samples from the 6 wild caught males that were in captivity. We also hoped to send milt from wild caught males that were not taken into captivity.

A decision was made to drive the sperm samples down to the University of Idaho (UI) because of past border issues. The sperm was harvested early in the morning by KSH staff. Only one male produced sperm of sufficient quality and density. The samples arrived at UI in the early afternoon. Upon arrival motility was assessed and determined to be “better than anything previously seen.” UI staff usually work with sturgeon sperm that is 3-4 days old. Forty 0.5 ml straws from male “BC 75D04” were cryopreserved and placed in the UI long-term storage facility. Prof. Cloud was

impressed with the sperm motility and decided to thaw some samples the next day to assess post-thaw motility. Post-thaw motility was determined to be 5-10%. According to Prof. Cloud, these results are sufficient to fulfill the gene conservation objective because fertilization would occur with this sperm.

Next year, more sperm samples will be driven down to UI for cryopreservation and long-term storage. Discussions about shipping some of the cryopreserved sperm to KSH in order to thaw and cross with small egg groups have already taken place with Prof. Cloud. To accomplish this task, we will need to rent? or buy a dry-shipper (~4-5K).

APPENDIX 4

White sturgeon larval and juvenile development and survival reared under fall and early winter Revelstoke Reach temperature conditions

Data Progress Report from July 28, 2004 to September 11, 2004
Prepared for the Upper Columbia White Sturgeon Project Management Sub-committee

Mark Tiley, CCRIFC Hydro Impacts Biologist

Introduction

In 2003 CCRIFC was successful in determining successful hatching of white sturgeon embryos (from two spawning events) in the mid-Columbia River adjacent to Revelstoke Golf Course, approximately 6 river kilometres downstream of Revelstoke Dam. Embryo hatching occurred on August 19 and between August 27 and 29, 2003. The late emergence of larval sturgeon in combination with peak summer water temperatures, generally not exceeding 12°C between late August to mid September, may inhibit or signficantly reduce overwintering survival of Arrow Reservoir white sturgeon larvae and juveniles. CCRIFC therefore submitted proposals to conduct overwintering experiments to monitor mortality and growth under contolled and ambient Revelstoke Dam tailrace temperature conditions. CCRIFC was successful in obtaining research funding from the Endangered Species Recovery Fund (ESRF) and from BC Hydro to conduct experiments to determine growth and survival of juvenile white sturgeon hatched in late summer and under mid Columbia River temperature conditions. The two null and alternative hypotheses that were to be tested were as follows:

1. H_o: Juvenile white sturgeon are capable of successfully overwintering when exposed to ambient Revelstoke tailrace temperature conditions.

   H_a: Juvenile white sturgeon are unable to successfully over winter when exposed to ambient Revelstoke tailrace water temperature conditions.

2. H_o: Juvenile white sturgeon overwintering survival does not increase with increased length of 12°C growing season.

   Ha: Juvenile survival white sturgeon overwintering survival increases with increased length of 12°C growing season.

Unexpected early spawning of white sturgeon broodstock in the Kootenay Sturgeon Hatchery and the failure of efforts to spawn a captive female sturgeon in August 2004 has resulted in an inability to test number 2 null and alternative hypotheses. Consequently, we are proposing new hypotheses which could be tested to serve as an alternative study which should provide a significant opportunity for learning in regards to juvenile white sturgeon growth and overwintering survival under ambient Revelstoke Tailrace conditions. Mortality, temperature and growth data have been collected from the test juveniles that have been rearing in Revelstoke Dam since July 28, 2004. The following progress report summarizes observed juvenile mortality, temperature and growth data spanning July 28 2004 to September 11, 2004 which forms the basis of CCRIFC's recommendation to continue research with the families currently being reared in tanks within Revelstoke Dam.

Site location

The switchgear building basement of Revelstoke Dam was considered to be the best location for the experiment based on water availability, space, low noise level, low human traffic and accessibility.

To determine whether Raw Header Water temperature was comparable to that of Revelstoke Dam penstock temperature, temperature loggers were deployed to measure both sources at hourly intervals. Figure 1 below provides a graphic comparison between Revelstoke Dam raw water for domestic use and penstock temperature. Mean difference in temperature between domestic water and penstock water between June 18 and June 23 was 0.24°C. In 2003, Revelstoke Dam tailrace temperature was observed to be nearly identical to temperatures observed at downstream temperature monitoring stations located at the Revelstoke Golf Course and as far downstream as the west bank of the Columbia downstream and on the opposite bank to the Illecillewaet River mouth. It was therefore concluded that Revelstoke Dam raw water was suitable for conducting proposed temperature experiments.

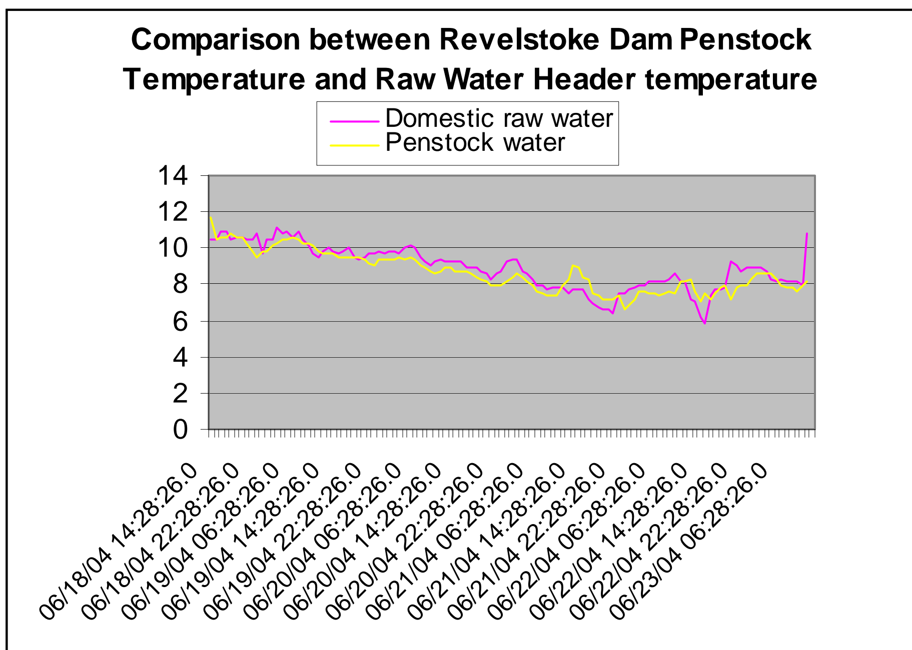


Figure 1. Comparison between Revelstoke Dam raw domestic water temperature and penstock water temperature from June 18, 2004 to June 23 at 1028 hrs.

Transportation

In response to the recommendation to establish feeding regime, flow rate and rearing maintenance protocols and schedules using live "test fish" prior to conducting actual experiments, juvenile white sturgeon produced in June 2004 from female C3D, male 34A and male 116 were transported from the KootenayTrout Hatchery (KTH) to Revelstoke Dam on July 28, 2004. The juvenile sturgeon were approximately 21 mm total length (TL) and weighed 0.07g on average by July 28. It was originally anticipated that these initial families transported to Revelstoke Dam would be sacrificed once an acceptable routine rearing sytem had been developed and families produced from captive adults in August became available.

A total of 2811 juveniles were placed in aerated coolers at 15.5^{o}C. 15.5^{o}C is currently the temperature in which white sturgeon juveniles are reared at the KTH. Each half-sibling family was maintained in separated coolers from 0800 hrs Pacific Time, arriving at Revelstoke at 1630 hours Pacific Time. No food was provided until the juveniles had been tranferred into the awaiting circular experimental tanks. Water temperature for each cooler was monitored continually by digital thermometer. Ice was placed in each cooler every 30 minutes at which time cooler temperature was recorded. The frequent addition of ice was intended to gradually reduce temperatures from 15.5^{o}C to as close to 10°C -11°C as possible. Temperatures of 10°C -11°C were observed for several days within in experimental tanks receiving inflow from the raw water header (elevation 553. 97 metres or 1817.50 ft) of Revelstoke Dam. Table 1 below summarizes mortality immediately following transport into experimental tanks 1 to 8.

Table 1. Total and percent mortality at the time of transfer from coolers to experimental tanks on July 28, 2004.

Family Code Name	Female X Male Cross	Trough Number	Number of fish obtained	Number of Mortalities Post transport	Percent Mortality	Starting time for transfer into experimental tanks
X	F C3D X M 34A	3	908	51	5.6	1700
Y	F C3DX M 116	1	987	14	1.4	1830
Z	F C3D X M 116 & 34A	5	916	43	4.7	1900

At the time juveniles were ready to be transferred to white circular potable water tanks, temperatures holding family Y, X and Z had fallen to 11.6^{o}C, 13.6^{o}C and 12.3^{o}C respectively. The total holding volume of the potable water tanks is 680 litres with a 1.38meter (m) diameter and a height of 0.6 m. Temperatures of the circular tanks ranged between 10.5^{o}C and 10.9^{o}C upon arrival to the switchgear-building basement. It was therefore necessary to further reduce cooler water temperatures by gradually adding Revelstoke Dam domestic raw water. Temperatures were carefully monitored to ensure that temperature change did not exceed 1°C every 30 minutes. The inflow to Tank 5 was shut off and allowed to heat to 12.8^{o}C by 1900 hours, the same temperature as the cooler holding the remaining family X juveniles. Once the juveniles had been transferred into tank 5, the inflow was turned back on. Inflow was set at approximately 6 litres (L) per minute (min), generally creating velocities of no more than approximately 0.05m/second (s).

Mortality

Mortality was high overall over the first two days following transportation and transfer into circular tanks. However, mortality was variable, ranging from 133 mortalities in Tank 7 (family X) to 27 mortalities observed in tank 4 (family Z) by July 30. Mortality fell dramatically between July 31 and August 3. It is highly probably that the initial high mortality was a result of the transportation and/or the temperature acclimation process. Ron Ek indicated that we transferred the juvenile fish at a vulnerable stage in their development and that we could suffer a high rate of mortality. Ron indicated that embryos, yolk sac larvae or older, more robust juveniles are much more suited to transportation. Figures 2 and 3 below illustrate combined daily mortality for all operating experimental tanks.

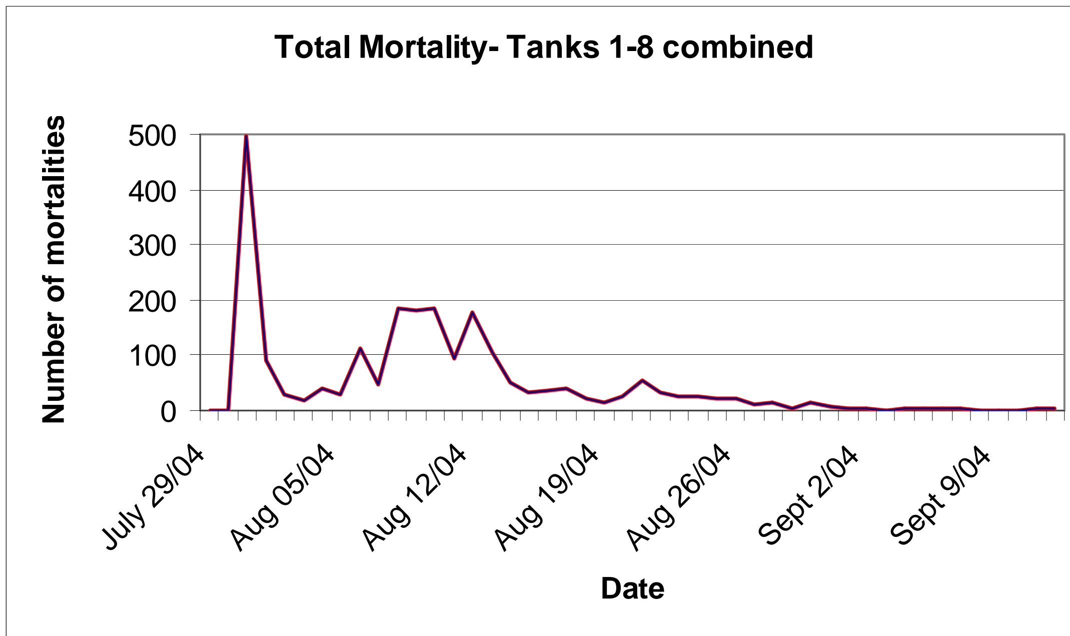


Figure 2. Tanks 1-8 combined daily mortality from July 29, 2004 to September 11, 2004.

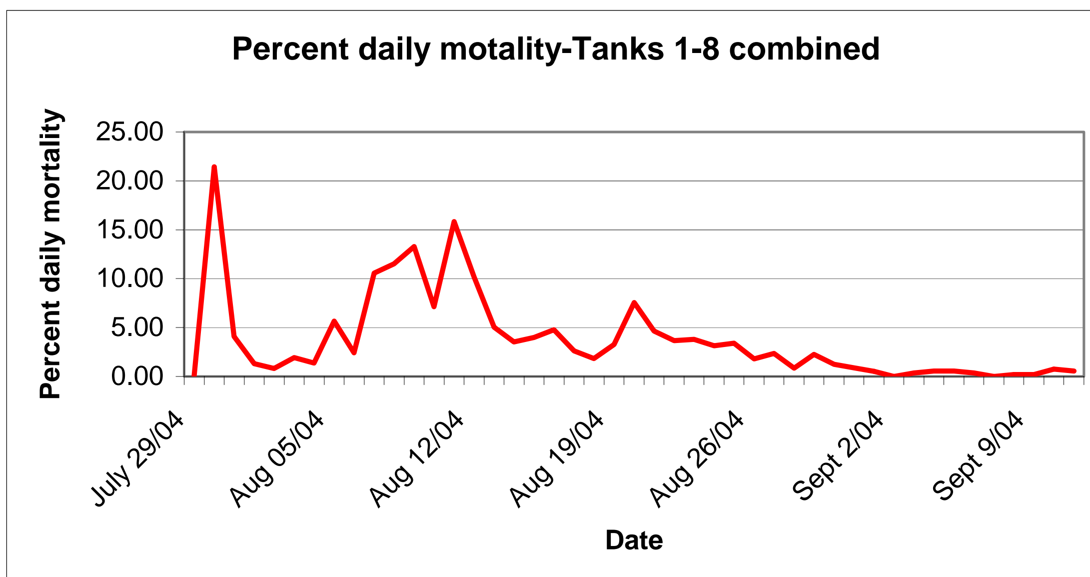


Figure 3. Combined percent daily mortality for tanks 1-8 from July 29, 2004 to September 11, 2004.

Combined mortality increased to over 10 percent/day for much of the second week of August. The causes for this increase in mortality are unknown but some potential causes are as follows:

1. Delayed physiological effects of transportation/temperature acclimation
2. Low water temperatures
3. Adjustment from trough environs to circular tanks and associated changes in fish density, flow, feed distribution, etc.
4. Change in feeding schedule
5. Tank inflow arrangement and flow rate

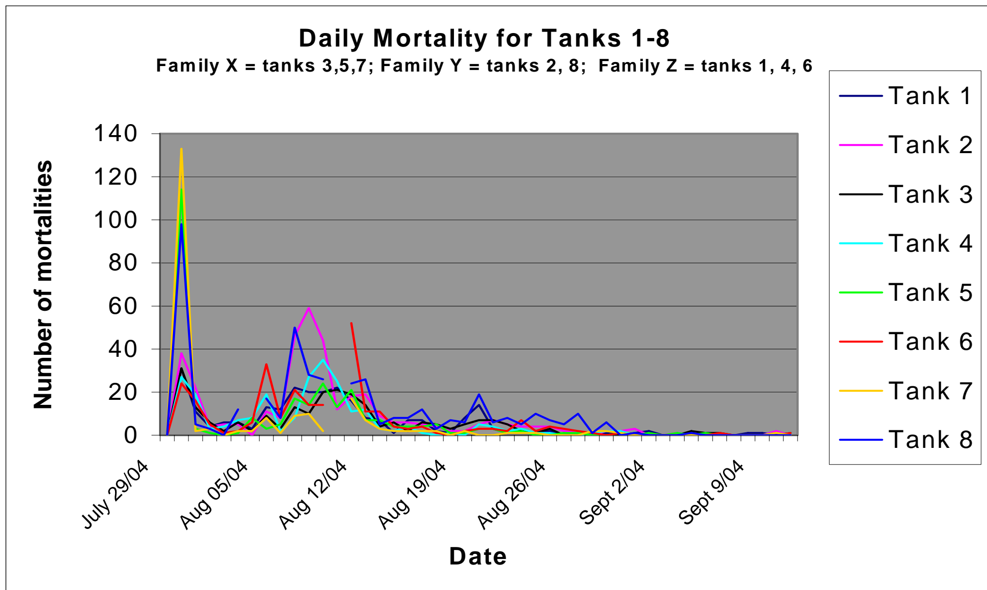
It should be mentioned that for the first week or two, significant numbers of juveniles were observed swimming along the sides of the tanks, possibly in search of food. The KTH would lower the water level within troughs and sprinkle food along the sides. The food would stick to the side of the trough after refilling. While some feed is available along the sides of circular tanks, by far the majority of feed settles on the bottom. It's possible that a significant portion of the juveniles had become conditioned to feeding on the sides of troughs. Such a conditioning may have lead to their gradual weakening and eventual mortality.

The Kootenay Trout Hatchery had provided the test fish with hand feeding (approximately 6 times per day) in addition to belt feeding prior to transportation. CCRIFC is only capable of providing 19 to 20 hours of belt feeding for a given day. To compensate, feed equivalent to at least 3% of fish biomass was provided each hour initially. As it is uncertain as to how long the feed remains recognisable as food as the juvenile sturgeon appear to feed exclusively by taste and/or smell, larger amounts of feed per fish biomass (20g to 30g feed/day/tank) have been provided. Differences in feed availability associated with feeding technique and rearing environment may have contributed to the observed higher mortality during the second week of August.

A minority of juveniles would swim upside down beneath the water surface film or where the water surface met the edge of the tank. A few juveniles in tank 1 were observed to have had air bubbles trapped within their intestines and gradually died over a period of days. Tank 1 had an inflow that entered above the surface film and created some bubbling; however several other tanks had the same inflow configuration.

Tanks 5 and 7 had an inflow that entered below the water surface that resulted in feed accumulating on the surface film. Both tanks 5 and 7 had a high mortality rates initially and therefore the inflow was changed immediately so that the inflow entered from above the water surface. Mortality for both tanks 5 and 7 appears to be comparable to other tanks since the modification to the inflows. The juveniles that were transferred to tanks 7 and 5 were the last to leave the coolers and therefore were food depraved the longest on the day of transportation.

Figures 4 and 5 illustrate daily and percent mortality for each tank. All three families appear to follow a similar trend in mortality, not including the first two days following transportation, with mortality rate peaking around August 12, and gradually tapering off to 0% to 5% mortality rates by the end of August. Tank 8 (family Y) is the only exception, as it tended to have comparatively higher mortality over the last two weeks of August. This tank did not have circular flow as did the rest of the tanks until the end of August. The inflow in tank 8 was more similar to the KTH inflow design and so was maintained in this configuration. This tank also tended to have a higher flow rate and a greater algal build-up. Once circular flow was provided, mortality decreased.



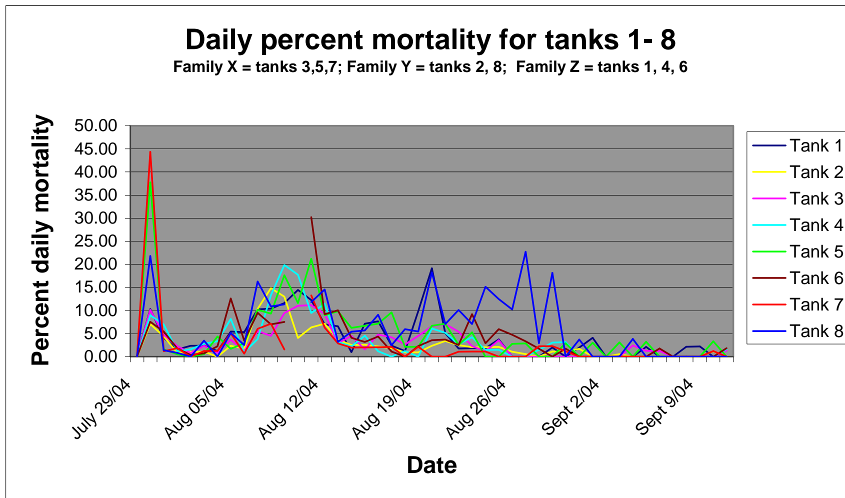


Figure 4. Daily mortality for tanks 1 to 8 from July 29, 2004 to September 11, 2004.

Figure 5. Percent daily mortality rate for tanks 1 to 8 from July 29, 2004 to September 11, 2004.

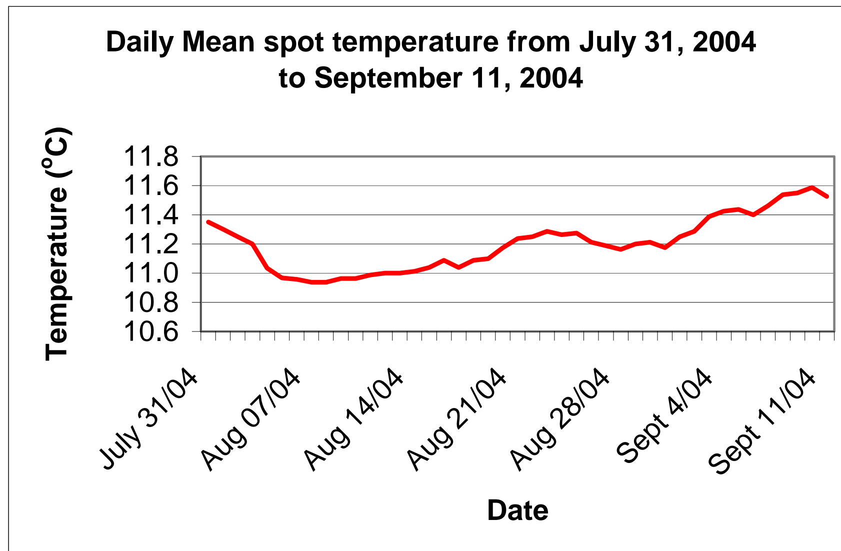


Figure 6. Daily mean spot temperature from July 31, 2004 to September 11, 2004.

The increase in mortality in the first week of August corresponds to a very slight decline in temperature to below 11°C. Conversely, the gradual decrease in mortality corresponds to a very slight increase in temperature of approximately 0.6°C. Figure 6 summarizes temperature data collected during feeding and cleaning maintenance schedules. Temperature logger data indicate a very low temperature range of approximately 11°C from August 25 to end of the September 11 sample period with temperature seldom reaching or exceeding 12°C. According to the KTH, 11°C is about the threshold at which white sturgeon juveniles will grow. It is therefore possible that the increase in temperature by approximately 0.6°C, with temperatures being maintained to above 11°C could have contributed to the observed decline in mortality thereafter. It is difficult to consider the slight temperature shifts as a significant contributor to the observed changes in mortality rate, but the data does support the stated 11°C threshold for white sturgeon growth. Such growth is likely a critical factor in the early life history development and survival of juvenile white sturgeon.

Growth and Condition

The size of individual fish increased from 0.07grams (g) as of July 28, 2004 to between 0.1g and approximately 0.5g. 0.3g fish were common at the end of this sample period. There has been no apparent sign of “curly fin” disease to date, although assessment of very small fish (20mm to 25mm TL) under microscope has not yet been conducted.

Recommendations

The mortality rate appears to have stabilized since late August to <1% overall.
The number of surviving juveniles by the end of the sample period was 574 individuals (figure 7).

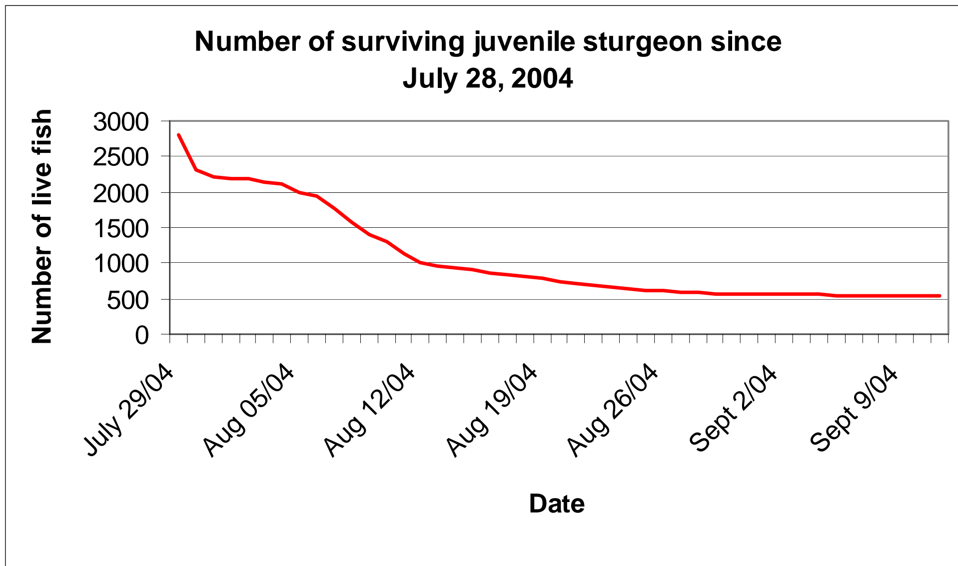


Figure 7. Number of surviving juvenile white sturgeon since transportation to Revelstoke Dam from the Kootenay Trout Hatchery on July 28, 2004.

Table 2 summarizes the remaining fish as of September 11, 2004 which may be used for future experiments.

Table 2. Number of surviving fish in tanks 1 to 8 by September 11, 2004.

Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6	Tank 7	Tank 8
Family Z	Family Y	Family X	Family Z	Family X	Family Z	Family X	Family Y
62	121	95	64	28	46	92	66

The number of surviving juveniles for family X, Y and Z was 215, 187 and 172 respectively. The survival rate for families X, Y and Z as of September 11, 2004 was 23.7%, 18.9% and 18.8% respectively.

In consideration that temperatures in the Mid Columbia River downstream of Revelstoke Dam have likely peaked, it is highly recommended that the experiment continue to determine mortality rate as water temperature declines. The first hypothesis could then be retained:

1. H_o: Juvenile white sturgeon are capable of successfully overwintering when exposed to ambient Revelstoke tailrace temperature conditions.

   H_a: Juvenile white sturgeon are unable to successfully over winter when exposed to ambient Revelstoke tailrace water temperature conditions.

Completion of the water heating system has been postponed pending PMSC decisions. In order to adequately test hypothesis 2, two or three replicates of three treatments would be necessary. There are no longer enough surviving juveniles to provide suitable statistical power. Furthermore, additional fish from the KTH would not be suitable for testing hypothesis 2 as such juveniles would have by now had approximately 12 weeks of rearing in approximately 15.5^{o}C water temperature conditions. It is recommended that temperature manipulation experiments involving heated water be considered in the future but not for this year.

However, we recommend that surplus KTH fish from female C3D and males 116 and 34A, which have presumably grown to considerably larger sizes than the juveniles reared at approximately 11°C (0.1g to 0.5g) be used to determine the significance of size and condition in overwintering survival. Thus, an alternative hypothesis that could be tested is as follows:

3. H_o: Juvenile white sturgeon overwintering survival rate does not increase with increased size and body condition.

   Ha: Juvenile survival white sturgeon overwintering survival rate increases with increased size and body condition.

We believe that significant learning could be obtained through further monitoring of mortality and growth. We thank you for your time.

For further questions and comments please contact Mark Tiley, CCRIFC Hydro Impacts Biologist, at 250-837-2154; mark.tiley@telus.net, or Bill Green, CCIFC Director at 250-417-3474; ccrifc@cyberlink.bc.ca

APPENDIX 4

PCR Diagnostic Approaches to the Detection of the White Sturgeon Iridovirus (WSIV) as Developed at the University of California, Davis

Kevin T. Kwak and Ronald P. Hedrick
November 10, 2003

Introduction

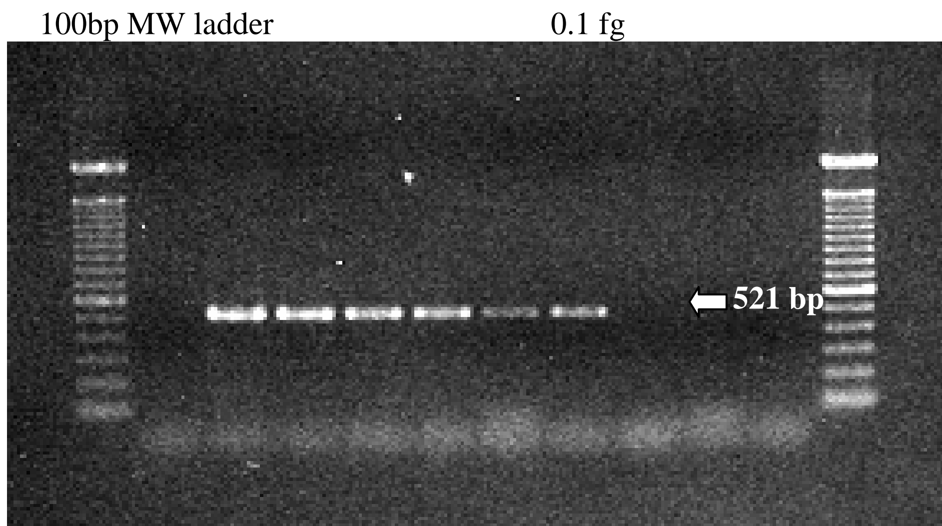
The following is a short description of a recently developed and laboratory tested polymerase chain reaction (PCR) assay to detect the white sturgeon iridovirus (WSIV). This information is being provided to culturists and biologists working with white sturgeon populations with the aim to broaden test validation to naturally occurring and other captive white sturgeon by more extensive field tests. To date the test has been effective in detecting WSIV DNA target sequences in experimentally-exposed juvenile sturgeon in the laboratory and 3 yr-old subadults naturally exposed to the virus in a commercial farm setting. With the help of others working with white sturgeon, it is now necessary to apply the PCR assay to broodstocks and natural populations at other locations where WSIV is known or suspected. A brief description of the test development and initial laboratory trials are followed by some recommendations for collection, storage and shipment of samples from the field or facility to our laboratory for analysis. We look forward to participating with you to validate this test and to learn more of the distribution and biology of this important viral pathogen of white sturgeon.

Development of the PCR Assay

The initial DNA sequences for WSIV were obtained by purifying virus grown in white sturgeon cell cultures (WSS-2). Purified virus preparations were subjected to DNA extraction and the DNA cut with restriction enzymes. The resulting viral genome fragments were ligated into plasmids and subsequently sequenced. The sequences of one fragment revealed a homology to the major capsid protein (MCP) gene found in other iridoviruses (e.g. lymphocystis and ranaviruses). The DNA sequence found in this fragment became the focus for the development of a PCR assay for WSIV. That the sequences from this fragment indeed represented a major structural polypeptide of the virus were obtained later when polypeptides of the virus were separated on polyacrylamide gels and the amino acid sequences of peptides from those proteins were obtained. The peptide sequences corresponded precisely to DNA coding regions from the original cloned and sequenced viral genome DNA fragment. The gene that is the target for the new PCR codes for a 58 kD protein (531 amino acids) that represents one of 3 major proteins found in the virus.

Primers were chosen from regions corresponding to optimal sites within the 1,596 base pairs (bp) representing the gene for the 58 kD protein by computer programs such that the amplified product would be 521 bp in length. A matrix approach was used to optimize the reagents and thermal cycling conditions for the PCR. The range of detection was evaluated by diluting plasmid containing the target sequences in 100 ng of DNA from uninfected white sturgeon tissues. Detection limits ranged to as low as 0.1 femtograms of plasmid DNA (Figure 1). The ability of the PCR to discriminate WSIV from other iridoviruses was tested by examination of DNA obtained from cell cultures infected with epizootic hematopoietic necrosis virus (EHNV), frog virus 3 (FV 3), red seabream iridovirus (RSIV), white sturgeon adenovirus (WSAV), and white sturgeon herpesvirus 2 (WSHV-2). Amplification of a 521 bp was observed from WSIV infected cell cultures but not the DNA extracted from any of the other iridovirus infected cell cultures.

Figure 1. Detection of target at limiting dilutions of a plasmid bearing the WSIV DNA sequences diluted into 100 ng of white sturgeon DNA.



Preliminary Trials with the WSIV PCR

Both experimentally infected juvenile white sturgeon and naturally infected subadult white sturgeon were used to evaluate the newly developed PCR. In these trials most samples collected were from sacrificed fish. However, we did explore non-lethal samples from a few larger juveniles and certain samples taken from the subadult fish from the commercial farm could have been obtained non-lethally (the fish were being processed for the meat market).

Juvenile trials

In the first trial, 3 groups of 50 juvenile sturgeon (2.35 g) were maintained under identical conditions in flow through 19 C well water in the laboratory. One group was designated as the control group and received a mock virus exposure to 400ml of tissue culture medium (MEM) without virus. The second group was exposed to a low dose ($10^{2.73}$ $TCID_{50}$/ml) and the third group to a higher dose ($10^{3.73}$ $TCID_{50/ml}$) of WSIV grown in cell culture. After a 1 h static bath challenge, water flow to the aquaria was resumed. At 28 days post exposure, samples were collected from 50 fish from each group for PCR analysis. Each fish had the head cut into two sections one of which was used for PCR analysis (entire half head including gills) and the second for histological evaluation. Results from the first trial indicated that nearly all fish, including controls were positive by PCR for WSIV DNA. All fish in the high dose and all but one fish in the low dose and 3 fish in the control group were positive for WSIV. Histological evidence of infection was observed in all groups including the control group. The stock group from which the white sturgeon juveniles were drawn for this experiment broke with WSIV infection approximately 3 months later. We therefore concluded that WSIV was present in these fish (including the controls) prior to the onset of the study. This study did demonstrate that the WSIV PCR could detect fish with WSIV well prior to the onset of an outbreak (e.g. in the stock fish).

In the second trial, fish from Trial 1 that were not sampled but maintained for an additional 8 months were again examined by PCR. Ten fish with and average weight of 322 g from each exposure group (high, low

and control) were assayed for the presence of WSIV using PCR. The fish were killed and a section of the gill, nasal rosette, snout, pectoral fin, and gonad removed for DNA extraction and PCR assay. The epidermis from the snout region (this was a flap cut from the ventral portion of the snout) was found positive for WSIV DNA in fish from all three treatment groups (Table 1, Figure 2). Samples from gill, gonad, nasal rosette and pectoral fin were also positive in some fish. In certain fish, no evidence of WSIV DNA was detected.

Figure 2. Tissues from white sturgeon juveniles testing positive (+) for WSIV DNA. Fish were approximately 11 months in age and survivors from the initial high prelevance exposure group. A total of 10 fish were examined. Samples 51-gill, 52-nasal rosette, 52-snout, and 53-snout yielded a positive signal for WSIV DNA.

+ WSIV MW

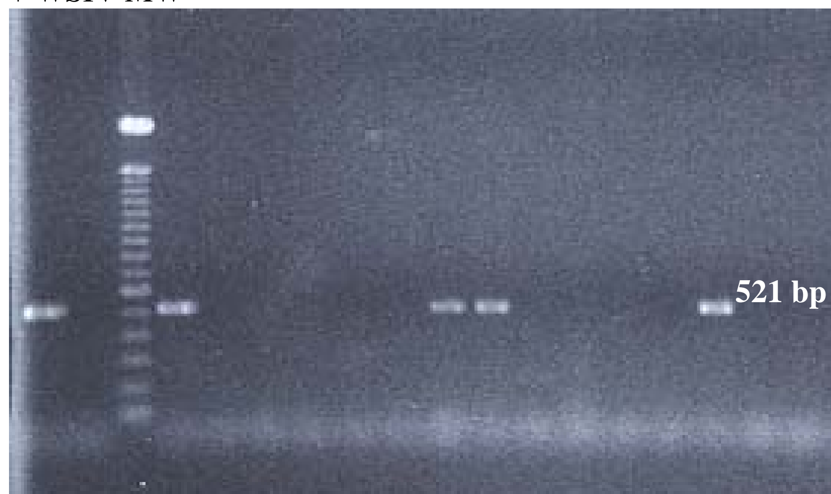


Table 1. Tissues from juvenile white sturgeon testing positive (+) for the presence of WSIV DNA using a newly developed PCR assay at 9 months following experimental exposures to WSIV.

Treatment	Gill	Nasal rosette	Snout	Pectoral fin	Gonad
High 51	+				
52		+	+		
53			+		
54					
55					
56					+
57		+			
58			+		
59					
60			+		
Control 51					
52			+		
53					

54			+		
55					
56			+		
57					
58					
59					
60					
51					
52					
53					
Low 54					
55	+				
56					
57				+	
58					
59					
60			+		+

Non-lethal sampling

A total of 4 fish (1.2 kg) remaining in the low dose exposure group 16 months following initial WSIV exposures were sampled non-lethally. Samples from each fish included a biopsy punch (6 mm) from an area of the ventral aspect of the snout anterior to the barbels, a small section of peripheral gill filaments, and a scraping of mucus from the region of the pectoral girdle. The results of the PCR assays indicated that 2 of the four biopsies of the ventral snout were positive for WSIV DNA while no evidence of viral DNA was found from the mucus or the gill samples.

Sampling of juvenile white sturgeon with no signs of disease

A group of 20 stock juvenile white sturgeon (1.4 kg) obtained from a commercial farm with no signs of disease nor from a group experiencing disease at the farm were examined for the presence of WSIV DNA. In this study samples were limited to 4 fin tissues, mouth, gill and barbels. A total of 8 of 20 fish from this group had a least one tissue positive by PCR testing (Table 2). The gill and dorsal fin were found positive in four of the 8 positive fish (I'm not really sure what you wanted to say here. But the positive tissues of gill and dorsal fin were in four different fish). All other tissue sites were only detected as positive in one fish.

Table 2. Tissues from white sturgeon juveniles testing positive (+) for WSIV DNA. Fish were approximately 6 months in age and from a commercial production facility. A total of 20 fish were examined. Only those fish testing positive are shown as all other fish were negative.

Fish no.	dorsal fin (# -1)	caudal fin (# -2)	anal fin (# -3)	pectoral fin (# -4)	mouth (# -5)	gill (# -6)	barbel (# -7)
11	+						
17		+					
18		+			+		
20				+	+		
22	+			+			
24							+
25						+	

29						+	

Subadult sampling from a commercial farm

To test the new PCR assay on older white sturgeon naturally exposed to WSIV, fish were obtained from a local farm producing white sturgeon for the meat and caviar market. Samples were taken from fish approximately 3 years in age, deemed to be prospective males by biopsy, that were being processed for the meat market. Tissues from a total of 7 sites on each of 30 fish were examined. These sites included gonad, mucus, barbel, gill, nasal rosette, epithelium anterior of the barbels (ventral snout), and pectoral fin (Table 3). Mucus was found to be positive from a total of 4 fish sampled and was the preferred site of virus detection in this limited trial. The mucus was collected by scraping in the area of the ventral surface of the fish between the pectoral fins (pectoral girdle region). In two of the 7 fish that were positive by PCR viral DNA was detected in more than one tissue. Of interest and potentially related to the presumed vertical transmission of the virus, gonad from two fish was found to harbor viral DNA. The fish examined in this study had no signs of disease and were healthy in their outward appearance.

Figure 3. Detection of WSIV DNA in tissues taken from 3 yr old white sturgeon collected from a commercial production facility. This gel shows the results of sampling of mucus from 30 fish.

+ - MW Mucus Samples 1-30

Table 3. Tissues from white sturgeon subadults testing positive (+) for WSIV DNA. Fish were approximately 3 years in age and from a commercial production facility. A total of 30 fish were examined. Only those fish testing positive are shown as all other fish were

Fish No.	Mucus	Barbel	Gill	Nasal rosette	Snout	Pectoral fin	Gonad
4							+
5	+						
9	+						

14							
16	+						
17	+	+	+			+	
24		+				+	+

Conclusions and Goals of Future Sampling Efforts

These preliminary results with juvenile and subadult white sturgeon with the newly developed WSIV PCR are sufficiently encouraging to warrant extended testing to field samples from both captive and wild populations of white sturgeon. With that in mind, we are asking biologists, pathologists, and culturists with access to white sturgeon to collect and send samples to our laboratory for PCR testing. The goals of this testing will be three-fold: 1) to aid in validation of the test by comparing results obtained from the PCR with histological analyses conducted on tissues collected from the same fish, 2) to determine if the virus is prevalent in captive broodstocks and progeny from those broodstocks and 3) to aid in our general understanding of the distribution and biology of the virus in naturally occurring populations of white sturgeon.

Recommendations for Sample Collection, Storage and Shipment

Below we provide information on the types of samples that may be sent to the laboratory for analysis. In all cases we ask that you coordinate the sample collection and shipping **by contacting us first**. Contact information can be found at the bottom of the document.

Types of samples to collect

Small fish (< 5 cm FL) – in these cases the samples will have to be lethal as we will process portions of the head and fins for PCR. Therefore, ship fish as frozen on ice and bagged separately for different groups. Choose only moribund or very freshly dead fish for analysis and freeze quickly. Parallel sets of fish fixed in 10% neutral buffered formalin (NBF) for at least 48 h for histological analysis should be shipped as well. We recommend draining off the formalin, rinsing with 70% ethyl alcohol and then shipping moist with alcohol in tightly sealed whirlpack bags (double bagged and clearly labelled).

Juveniles – If the fish are of sufficient size to obtain non-lethal samples the following tissues are recommended: 1) mucus scraped from the ventral surface (emphasizing area anterior to the barbels and the pectoral girdle) with a dull blade (we use a weighing spatula – the idea is to get mucus and some epidermis but not ulcerate the fish), 2) punches (6 mm) or small sections (v-cuts with scissors) of the pectoral and caudal fins. Place replicate punches in 10% NBF and treated for shipment as mentioned above. Mucus should be placed into a small screw cap vial. We use cryovials designed for freezing in liquid nitrogen. They hold about 2 ml and have a sealing gasket on the screw cap lid. The same vials can be used for fin punches. Both should be frozen at -20C or – 70C where possible. If lethal sampling is conducted, distal elements of the gill filaments can also be included and treated as fin punches. We should emphasize that these need not be large samples and they should easily fit in the 2 ml vial.

Adults – For the most part these should be non-lethal samples. Tissues to collect are: 1) punches from the pectoral fin, 2) barbels, 3) mucus from the pectoral girdle region, and 4) gonad. Parallel punches and barbels should be frozen for PCR and fixed in 10% NBF for histology as described above. Mucus should be collected and stored as mentioned above. Gonad, preferably stroma of ovary or testes or differentiated

gametes (eggs and sperm) can be placed into cyrovials and frozen as well. A parallel piece of the same gonad should be placed in 10% NBF and treated for shipment as mentioned above.

Contact Information

Please contact our laboratory well ahead of any sampling planned so that we can discuss the shipment of samples or any questions you may have regarding their collection and storage.

Call or e-mail:

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