Columbia River Project Water Use Plan
Columbia White Sturgeon Management Plan

Lower and Middle Columbia Rivers

Reference:

CLBWORKS-24 Mid Columbia Experimental Aquaculture (Year 5)

CLBWORKS-25 Mid Columbia Sturgeon Conservation Aquaculture (Year 1)

CLBWORKS-34 Lower Columbia Sturgeon Conservation Aquaculture Program (Year 5)

Lower Columbia River Adult White Sturgeon Monitoring Program: 2009 and 2010 Investigations Data Report

Study Period: June 2012 to May 2013

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COLUMBIA WHITE STURGEON CONSERVATION FISH CULTURE PROGRAM

KOOTENAY TROUT HATCHERY

2012 Annual Report

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1.0 EXECUTIVE SUMMARY

Juvenile White Sturgeon age classes are lacking in the Upper Columbia River population due to recruitment failure. Accordingly, conservation aquaculture has become a critical component of the Upper Columbia White Sturgeon Recovery Initiative with juveniles being released over the last decade into both the lower (since 2001) and Mid-Columbia (since 2007) River. Conservation fish culture and release of juvenile White Sturgeon into the Upper Columbia River as part of the Columbia Water Use Plan met release targets in 2012. Capture of adult White Sturgeon for use as broodstock in 2012 was conducted from June 6th through June 28th, with the capture effort spread throughout the river from Hugh Keenlyside Dam (HLK) to the Canadian/US border. Eight female and ten male sturgeon were captured and transported in a trailer-mounted transport tank to the Kootenay Trout Hatchery (KTH) for use in spawning events.

Six females and ten males were spawned during two spawning events July 4th and July 18th to produce 612,865 green eggs in 25 half sib families. The average neurulation percentage was 59% which produced an estimated total of 271,336 larvae.

In captivity, female egg maturation seemed to progress at a slower rate compared to previous years and the difference is assumed to be attributed to cooler temperatures in the lower Columbia at the time of capture. One female that was spawned on July 4th was found to contain the parasite *Polypodium hydriforme* within her gametes, representing the first observation for this parasite within the population. Subsequently her eggs were not used and she was returned to the river.

Tissue samples collected from the dorsal fin of all adults and offspring tested negative for reportable viruses: IPNV, WSHV1, WSHV2, and WSIV. Juvenile releases occurred in the spring of 2013, at approximately 9 months of age, on May 1st and 2nd. A total of 4,037 juvenile sturgeon were released into the lower Columbia River between HLK and the USA border. Release numbers and locations

included HLK Dam near Castlegar (n = 1400), Kootenay Eddy (n = 893), Genelle (n = 875), and Beaver Creek (n = 869). Average (± 1SD) weight (g) and length (cm) of released fish was 86.0 ± 31.4 g and 22.2 ± 3.7 cm, respectively. An additional 5,944 juvenile sturgeon were released April 30th and May 9th into the Mid-Columbia River at Shelter Bay Provincial Park downstream of Revelstoke area. Average (± 1SD) weight and length at release for these fish was 153.4 ± 56.6 g and 27.2 ± 3.1 cm, respectively. Size at release was larger compared to previous years and was to help address questions about survival following release into the Mid-Columbia.

School and public release events were tied in with the sturgeon releases at Beaver Creek, HLK and Shelter Bay near Revelstoke with participation by over 1,600 school children.

2.0 BACKGROUND

The population of White Sturgeon in the Canadian portion of the Columbia River has been undergoing recruitment failure for several decades (UCWSRI 2012). This was recognized as a critical issue for this population in the early 1990's and resulted in the establishment of the Upper Columbia White Sturgeon Recovery Initiative (UCWSRI) in 2001 and the population being listed as endangered under the Species at Risk Act (SARA) in 2006. The UCWSRI published a recovery plan in 2002 which provided direction for recruitment failure research, routine monitoring, and public awareness (UCWSRI 2002). Original estimates, based on annual levels of natural recruitment being insufficient for maintaining a self-sustaining population, suggested that the population would become functionally extinct by 2044 (UCWSRI 2002). Accordingly, intervention and monitoring were deemed essential to preclude extinction.

An integral part of the original recovery plan was the initiation of a conservation aquaculture program designed to provide artificial recruitment to the population and provide fish for research purposes. The conservation aquaculture program was

designed to support the population until such time as stock abundance/age structure and habitat conditions (including spawning, incubation and rearing flows and reservoir levels) can support a self-sustaining population. This program was initiated in 2001 and has stocked sub-yearling juvenile sturgeon annually into the lower Columbia River.

Although construction and operation of dams have been implicated in the decline of white sturgeon in the Columbia River, the mechanisms responsible for recruitment failure have been difficult to ascertain with certainty (Gregory and Long 2008). During development of the Columbia River WUP, this uncertainty made it difficult for the WUP Consultative Committee (CC) to develop response measures to address sturgeon declines. The conservation aquaculture program, as delivered under the Columbia River WUP, is divided between two areas of the Columbia River, the lower Columbia River and the Mid Columbia River. The program goals differ between the two areas. Under operational parameters of the Columbia River Treaty, adequate flows treatments before and during spawning, incubation and drift phases of the life cycle were not seen as feasible in the lower Columbia River downstream of HLK. The CC therefore agreed to a plan which included monitoring to assess trends in population dynamics, research into juvenile habitat use and survival, and an assessment of the feasibility of different management responses. However, it was deemed impossible to deliver this plan without releases of hatchery reared juvenile sturgeon as wild juvenile age classes were lacking and a project (CLBWORKS#34 - Lower Columbia River White Sturgeon conservation aquaculture program) was initiated to provide for dependable financial resources for the maintenance of the aquaculture program for the duration of the Columbia WUP.

In the Mid Columbia River there were more uncertainties (e.g. biological, operational etc.) during the development of the WUP and the CC report (BC Hydro

2005) recognized several possible long term directions for the Mid Columbia program including:

- Initiate a conservation aquaculture program for development of an Arrow Lakes Reservoir failsafe population.
- Develop a self-sustaining (in the long term) population in a Kinbasket Reservoir/upper Columbia River recovery area.
- Initiate a conservation aquaculture program for development of a Kinbasket Reservoir failsafe (non-reproducing) population.

The CC recommended that the conservation aquaculture strategy for this program be robust enough to allow for the determination of whether or not wild production is possible and where recovery efforts would be best directed in either the Mid Columbia or Kinbasket. The White Sturgeon management plan for the Mid Columbia divided the operational delivery of aquaculture into two projects. CLBWORKS#24, Mid Columbia White Sturgeon Experimental Aquaculture, provides for delivery during the first four years of the program (2008-2011). During this time, the focus will be on providing for larval and sub-yearling juvenile releases designed to assist with monitoring habitat selection and use, and early survival. The second phase of the work under project CLBWORKS#25, Mid Columbia White Sturgeon Conservation Aquaculture, was to be directed following a technical review of the entire Mid Columbia White Sturgeon management plan. The results of a review in 2012 were to continue with conservation aquaculture program (2012-2018) in the Mid Columbia and assess optimal size at release (survival / temperature / growth relationships) by releasing larger sized juveniles compared to those released from 2008-2012.

Though the programs for the lower and Mid Columbia Rivers differ in implementation and design, they share several overarching objectives. The overall objectives of the Columbia River White Sturgeon conservation aquaculture program include:

- The capture, transportation between the Columbia River and KTH, care and breeding of mature adult sturgeon at targeted numbers of 10 females and 10 males to provide for an annual objective of eight genetically distinct families or secondarily subfamilies. Adults are to be returned to the Columbia River upon completion of spawning.
- The successful incubation and rearing of approximately equal numbers of healthy juveniles from each family or subfamily bred in a given year targeting an annual release in the fall of the brood year or subsequent spring of a total of up to 12,000 sub-yearling sturgeon to facilitate stock rebuilding and research needs. Stocking targets are established through the TWG.
- The annual marking and tagging of all fish according to protocols, including scute removal to designate brood year, Passive Integrated Transponder (PIT) tagging, sonic tagging and other tagging as may be required of both broodstock adult and juvenile sturgeon.
- Annual participation in public awareness and educational activities including but not necessarily limited to release events, school events, public events, open houses workshops etc.
- Provision of research, testing and pilot programs exploring techniques for improved efficiencies and an ability to provide for broader genetic diversity of released stock.

3.0 BROOD CAPTURE

The procedures for broodstock capture follow those reported in earlier Annual reports and are described in detail (BC Hydro 2011). This report is restricted to data collected from adult White Sturgeon selected as suitable broodstock candidates. Information on all fish caught and released is described in other Columbia River Water Use Plans (BC Hydro 2011).

Fish were caught and transported to the KTH sturgeon facility from June 6th to June 28th, 2012. In total, eight female fish with mature eggs and ten male fish that were either flowing with milt or appeared to have mature gonads were selected as broodstock in 2012 collection efforts. Table 1 shows the information on all adults

taken to the hatchery including sex, PIT tag number, capture location, date, length, weight, spawn date and date of release.

Table 1: Information for adult White Sturgeon selected as broodstock candidates and transported to the Kootenay Trout Hatchery spring 2012. Koot Eddy: Kootenay eddy; RKM: river kilometre measured from the HLK dam.

Sex	Pit Tag #	Weight kg	Length cm	Capture Date	Capture Location	RKM	Spawn Date	Release Date
F	985161000180641	46	174	Jun-06	Koot Eddy	10.5	July 18	July 20
F	7F7D381E45	76	195	Jun-08	Koot Eddy	10.5	July 18	July 20
F	985161000056632	55	188	Jun-11	Waneta	56	July 18	July 23
F	7F7D222A69	78	202	Jun-20	Genelle	24	July 18	July 24
F	1F3B207115	105	229	Jun-21	Waneta	56	July 18	July 23
F	985120022620878	50	182	Jun-22	Waneta	56	July 4	July 6
F	7F7D4D174C	77	209	Jun-22	Waneta	56	July 4	July 6
F	426505161B	69	202	Jun-25	Koot Eddy	10.5	July 4	July 10
M	42381E6117	60	191	Jun-06	Koot Eddy	10.5	July 4/18	July 19
M	4204067834	40	175	Jun-06	Koot Eddy	10.5	July 4	July 19
M	7F7D1C4C51	62	197	Jun-06	Dock Set	3.5	July 18	July 25
M	4239441550	45	175	Jun-11	Waneta	56	July 18	July 26
M	985120022605171	56	186	Jun-11	Waneta	56	July 18	July 26
M	985121005923954	43	177	Jun-18	HLK	0.1	July 18	July 25
M	7F7D4F6A1F	49	181	Jun-21	Ft Shepard	52.3	July 4	July 10
M	42616E411F	43	178	Jun-22	Waneta	56	July 4	July 11
M	7F7D4F723C	56	184	Jun-26	Rialto	0.2	July 18	July 24
M	4239436C4E	90	212	Jun-28	Waneta	56	July 4	July 11

3.1 Brood Capture History

High site fidelity to one habitat has been described for adult White Sturgeon in the lower Columbia River (BC Hydro 2011) and highlights the importance of spatially balancing broodstock sampling. This ensures a subset of the adult population is not predisposed to capture more than others. With the exception of a single female, all adult females from the lower Columbia River and selected as broodstock candidates, had been captured and tagged previously. One of the females had been captured seven times (Table 2). Tables 2 and 3 present the capture history for each individual selected as a broodstock candidate in 2012. Of the male broodstock selected, four were first time captures and one of the males had been captured 10 times (Table 3).

Adult movements in the river are known to be highest during the spring and summer months (BC Hydro 2011; UCWSRI 2012). A subset of adults had previously been captured upstream near HLK (0.1 rkm), but were captured in spawning condition downstream at Waneta Eddy (56 rkm) in 2012. Conversely, no adults in spawning condition that had been captured previously near the Waneta area were captured upstream.

Table 2: Broodstock capture history for adult female White Sturgeon caught in the lower Columbia River in 2012 and spawned at the KTH. Koot Eddy: Kootenay eddy; Mat. Code: and established maturation code used to describe the maturation state of the gonad; RKM: river kilometre measured from the HLK Dam.

Sex	PIT TAG #	Station Name	RKM	Capture Date	Mat. Code	Length (cm)	Weight (kg)
F	985161000180641	Koot Eddy	10.5	Jun-06-12	F4	174.0	46.0
F	7F7D381E45	Koot Eddy	10.5	Jun-08-12	F4	195.0	69.0
	7F7D381E45	HLK	0.1	May-27-93		120.0	15.0
	7F7D381E45		5.7	Nov-14-95		131.0	18.6
	7F7D381E45		0.2	May-16-97		131.5	18.2

F	985161000056632	Waneta	56	Jun-11-12	F4	188.0	55.0
	985161000056632		52.4	Aug-26-09		178.0	52.6
F	7F7D222A69	Genelle Eddy		Jun-20-12	F4	202.0	78.0
	7F7D222A69	Waneta	56	Jul-14-92		173.0	47.3
	7F7D222A69	Waneta	56	Jun-26-02		200.0	82.5
F	1F3B207115	Waneta	56	Jun-21-12	F4	229.0	100.0
	1F3B207115		56	Mar-13-01		212.0	80.0
	1F3B207115		52.3	Jun-24-05		218.0	75.0
	1F3B207115		52.3	Jun-16-06		215.5	73.0
	1F3B207115		56	Jun-07-07		216.0	90.0
	1F3B207115		52.3	Jun-02-09		218.0	78.0
	1F3B207115		56	Jun-09-09		219.0	70.0
F	985120022620878	Waneta	56.6	Jun-22-12	F4	182.0	50.0
	985120022620878		75.2	Jun-16-06		159.0	34.0
F	7F7D4D174C	Waneta	56	Jun-22-12	F4	208.5	77.0
	7F7D4D174C		2.2	Jul-05-93		133.0	16.8
	7F7D4D174C		2.2	Aug-14-94		139.0	N/A
	7F7D4D174C		2.2	Sept-24-96		138.0	20.5
	7F7D4D174C		2	Jul-04-02		166.5	79.0
F	426505161B	Koot Eddy	10.1	Jun-25-12	F4	191.0	69.0
	426505161B	HLK	0.1	Ma-06-03		177.5	53.6
	426506011B	HLK	0.1	Apr-25-01		152.5	34.1

Table 3: Broodstock capture history for adult male White Sturgeon caught in the lower Columbia River in 2012 and spawned at the KTH. Koot Eddy: Kootenay eddy; Mat. Code: an established maturation code used to describe the maturation state of the gonad; RKM: river kilometre measured from the HLK dam; Rialto: Rialto Creek; DOCK: Private dock at RKM 3.5;

Sex	PIT TAG #	Station Name	RKM	Capture Date	Mat. Code	Length (cm)	Weight (kg)
M	42381E6117	Koot Eddy	10.5	June-06-12		191.0	60.0
M	4204067834	Koot Eddy	10.5	June-06-12		175.0	40.0
M	7F7D1C4C51	DOCK		June-06-12	M2	197.0	62.0
	7F7D1C4C51		4.5	November-08-92		138.5	17.7
	7F7D1C4C51	HLK	0.1	June-18-03		188.0	50.9
M	4239441550	Waneta	56.6	June-11-12		175.0	45.0
M	985120022605171	Waneta	56.6	June-11-12	M2	186.0	56.0
	985120022605171			June-01-05		167.0	37.2
M	985121005952954	HLK	0.1	June-18-12	M2	177.0	43.0
	985121005952954			June-10-10		172.0	39.0
M	7F7D4F6A1F	Fort Sheppard	52.3	June-21-12	M2	181.0	49.0
	7F7D4F6A1F		52.3	March-23-94		195.0	50.0
	7F7D4F6A1F		52.3	November-11-94		188.0	81.6
	7F7D4F6A1F		52.3	March-13-01		176.0	41.4
	7F7D4F6A1F		52.3	June-07-04		N/A	N/A
	7F7D4F6A1F		52.3	June-14-05		179.0	44.0
	7F7D4F6A1F		52.3	June-09-08		182.0	50.0
	7F7D4F6A1F		52.3	June-13-08		182.0	50.0
	7F7D4F6A1F		52.3	June-01-09		180.5	41.0
	7F7D4F6A1F			June-01-09		180.5	41.0
M	42616E411F	Waneta	56	June-22-12	M2	178.0	43.0
	42616E411F		0.1	April-27-01		146.0	28.2
M	7F7D4F723C	Rialto		June-26-12	M2	184.0	56.0
	7F7D4F723C		52.3	March-30-95		113.0	10.9
	7F7D4F723C		52.3	March-13-01		125.0	14.1
	7F7D4F723C		52.3	April-30-02		127.5	15.4
M	4239436C4E	Waneta	56	June-28-12		212.0	90.0

4.0 TRANSPORT

When an adult was captured and determined to be mature or maturing, it was loaded into the sturgeon transport tank using a stretcher or a tube net at the nearest access point to the river. The transport tank was filled with ambient river water using a fire pump. To minimize stress during transport and to facilitate healing abrasions that may have occurred during capture and handling, salt (heavy metal free sodium chloride) was added to the tank water to 1 % (w/v). Oxygen was supplied to saturation to the tank through aeration stones that were recessed into the tank floor and was not monitored during transport. Twice during each transport, staff checked the fish for duress and none was noted. All fish transported from the site of capture to the KSH had no discernable negative effects of transport apparent on arrival or after placement into tanks at the KSH. Transport times were approximately four hours.

On arrival, hatchery culture tank temperatures were matched with transport tank temperatures. The fish were transferred from the transport tank to the culture tank using a tube net and physical labour. Fish were monitored hourly following arrival at the hatchery until deemed necessary. No adverse effects (e.g. stress; abrasion or reddening of the ventral surfaces or fin margins) of transport were noted.

An alternate route had to be used on June 6th 2012 when transporting four adults back to the hatchery due to road closures. Total transport time the adults were exposed to (6 hours) was longer compared to normal (4.5 hours) transport routes and procedures that typically occur. Fortunately, a detour via Nelson and over the Kootenay Lake ferry was successfully in getting the adults to their culture tanks at the hatchery with no discernable negative effects (e.g. stress). This event demonstrates the species capability to endure transport stress for longer periods then recorded for this population. We would like to acknowledge BC Ferries for their assistance in transporting the tanks and trucks across Kootenay Lake on short notice.

5.0 ADULT HOLDING CONDITIONS

For male fish, water temperatures were held at ambient river temperatures (about 11°C) and increased to 15°C after LHRHa injections were given. Female fish were held on heated water (15°C) throughout captivity. Water temperatures were increased from ambient river temperatures to 15°C in 2°C increments.

6.0 SPAWNING

In the 2012 spawning season, Polarization Index's (PIs) were calculated for all female fish immediately following capture and ranged from as high as 0.168 to as low as 0.07 (Table 4). The last three females captured during broodstock collection had PI's that were near or below the benchmark of 0.10 needed for induction of spawning and were spawned on July 4th. The first five females captured had PI values above the benchmark and, though they progressed slowly, were ready to spawn on July 18th

Table 4: Adult female White Sturgeon capture information and Polarization Index (PI) calculations for 2012 spawning events. Koot Eddy: Kootenay eddy.

Sex	Pit Tag #	Weight kg	Capture Date	Capture Location	Initial PI	PI Check June 21	PI Check June 28	PI Check July 9	Spawn Date
F	985161000180641	46	Jun-06	Koot Eddy	0.132	0.131		0.092	July 18
F	7F7D381E45	76	Jun-08	Koot Eddy	0.168	0.139		0.113	July 18
F	985161000056632	55	Jun-11	Waneta	0.157		0.158	0.109	July 18
F	7F7D222A69	78	Jun-20	Genelle	0.160			0.111	July 18
F	1F3B207115	105	Jun-21	Waneta	0.153			0.077	July 18
F	985120022620878	50	Jun-22	Waneta	0.070				July 4
F	7F7D4D174C	77	Jun-22	Waneta	0.070				July 4
F	426505161B	69	Jun-25	Koot Eddy	0.100				July 4

6.1 Spawning Induction

As the PI values of the females decreased, three females were induced to spawn on July 4th. Eggs from each female were split into 5 portions and each portion was fertilized with each of the 5 males that were induced on the same day. Milt was checked for motility prior to use for each fertilization process on a visual, arbitrary score of Poor, Good or Excellent, and only milt with Good or better was used to fertilize eggs.

The remaining 5 females were induced to spawn on July 18th. Eggs from each female were once again split into 5 portions and each portion fertilized with each of the 5 remaining males. Males used in the first spawning event were not reused.

Three females were unsuccessful in spawning in 2012. First, Female 985120022620878, caught at Waneta Eddy and spawned on July 4th, was found to contain the parasite *Polypodium hydriforme* within her gametes. Samples were collected and provided to Nathaniel Evans, Department of Ecology and Evolutionary Biology, University of Kansas. After several discussions involving the FFSBC fish health lab, DFO officials and others UCWSRI partners, the decision was made to destroy the eggs from this female and return her to the river. Though identified in other sturgeon species, this is the first observation of this parasite for the Upper Columbia River population. Second, two females (1F3B207115 and 7F7D381E45) spawned on July 18th had 0 % survival to hatch. The reasons for this are unknown. Both females were crossed with all five males available the day of spawning.

6.2 Breeding schedule

Milt and eggs are obtained, handled, and fertilized as per established protocols (see 2007 Annual Report). Fertilized eggs from each of the male/female crosses are incubated in separate MacDonald jars until hatch whereupon they are combined into a single family based on maternal lineage. Thus, all half-sibling crosses of a single female are combined to create a maternal family that is reared separately.

6.3 LHRHa Injection and Gamete Collection:

Adults are induced to spawn using an LHRHa treatment (Table 5). The procedure for the maturation determinations followed methods described in the Broodstock Evaluation section of the Hatchery Manual for the White Sturgeon (Conte et al. 1988; Pub. 3322; U. CA; USFWS). This LHRHa treatment regimen for female sturgeon consists of two doses of LHRHa given 12 hours apart: a loading (10%) and resolving (90%) dose. Total dose is 50 µg/kg which is split with the initial dose being 5 µg/kg and the resolving dose being 45 µg/kg. Female fish begin to ovulate and release eggs approximately 24 hours after the resolving injection of LHRHa.

Once a female has been observed to be releasing eggs as evidenced by the presence of eggs on the tank floor, the water level is dropped in the tank. Staff enter the tank and place the fish ventral side up onto a hooded stretcher with a water hose providing fresh water flowing over the gills. Sufficient egg volumes to provide for the targeted juvenile numbers are collected from the female using manual expression through the urogenital opening or by extraction through an incision using a modified cesarean section method. To meet production and research targets, cesarean sections may be more frequently used as this method can sometimes more fully permit the expression of ovulated eggs.

Male fish are held at 10°C until they are needed to supply milt. Male fish are intramuscularly injected with a single bolus dose (10 µg/kg) of LHRHa in saline 1-3 days prior to intended use. At the time of the injection, the water temperature in the male tanks was increased to 15°C until after spawning events were complete.

Milt was collected several hours before egg fertilization is conducted to minimize workload; milt remains viable for several hours when kept on ice. Once egg collection was complete, the water temperature in the male sturgeon tanks was decreased back down to 10°C. This allows the male to "shut down" and possibly be used again for subsequent spawning events following the methods described above.

Table 5: LHRHa injection dosage volumes for adult White Sturgeon spawned in 2012. See table footnote for LHRHa solution concentrations.

Sex	Pit Tag #	Weight Kg	Capture Date	Capture Location	Initial Dose (ml)	Resolving Dose (ml)
F	985161000180641	46	Jun-06	Koot Eddy	0.46	0.83
F	7F7D381E45	76	Jun-08	Koot Eddy	0.76	1.37
F	985161000056632	55	Jun-11	Waneta	0.55	0.99
F	7F7D222A69	78	Jun-20	Genelle	0.78	1.40
F	1F3B207115	105	Jun-21	Waneta	1.05	1.89
F	985120022620878	50	Jun-22	Waneta	0.50	0.90
F	7F7D4D174C	77	Jun-22	Waneta	0.77	1.39
F	426505161B	69	Jun-25	Koot Eddy	0.69	1.24
M	42381E6117	60	Jun-06	Koot Eddy	0.88	
M	4204067834	40	Jun-06	Koot Eddy	0.59	
M	7F7D1C4C51	62	Jun-06	Dock Set	0.91	
M	4239441550	45	Jun-11	Waneta	0.66	
M	985120022605171	56	Jun-11	Waneta	0.83	
M	985121005923954	43	Jun-18	HLK	0.63	
M	7F7D4F6A1F	49	Jun-21	Ft Shepard	0.72	
M	42616E411F	43	Jun-22	Waneta	0.63	
M	7F7D4F723C	56	Jun-26	Rialto	0.83	
M	4239436C4E	90	Jun-28	Waneta	1.33	

Male injection-Single dose-10ug/kg - 7 ml Ringer's solution contains 5mg LHRHa.
Female Initial Dose- 5ug/kg (10%) - 10 ml Ringer’s solution contains 10mg LHRHa.
Female Resolving Dose- 45ug/kg - (90%) - 2 ml Ringer's solution contains 5mg LHRHa

6.4 Spawning Summary

The breeding design used is a full factorial mating design. Briefly, an equal number of male and female are used in a matrix where each female is crossed with each available male. This method maximizes the effective population size, and is a common approach for conservation work within a limited breeding population. The results of the two spawning events and the crosses used in the 2012 breeding season are presented in Table 6. Families are reared in separate MacDonald jars until hatch whereupon they are combined into a single family based on maternal lineage. Thus, all half-sibling crosses of a single female are combined to create a maternal family that is reared separately for as long as possible through to release. The five maternal families represented a total of 25 different paternal crosses. As an example, Female 426505161B was crossed with 5 males and at neurulation an equal number of fertilized eggs were combined to hatch out to create Family # 1. Family numbers were equalized prior to being combined later in the rearing cycle (at approximately 4-5 months). The surplus fish were later euthanized.

Table 6: Family structure following adult White Sturgeon spawning events in 2012.

Family	Female PIT Tag #	Male PIT Tag #	Spawn Date
1	426505161B	42381E6117	Jul-04
		4204067834	
		7F7D4F6A1F	
		42616E411F	
		4239436C4E	
2	7F7D4D174C	42381E6117	Jul-04
		4204067834	
		7F7D4F6A1F	
		42616E411F	
		4239436C4E	

Discarded Parasite	985120022620878	42381E6117 4204067834 7F7D4F6A1F 42616E411F 4239436C4E	Jul-04
3	985161000180641	7F7D1C4C51 4239441550 985120022605171 985121005923954 7F7D4F723C	Jul-18
Discarded 0% Fert	7F7D381E45	7F7D1C4C51 4239441550 985120022605171 985121005923954 7F7D4F723C	Jul-18
4	7F7D222A69	7F7D1C4C51 4239441550 985120022605171 985121005923954 7F7D4F723C	Jul-18
5	985161000056632	7F7D1C4C51 4239441550 985120022605171 985121005923954 7F7D4F723C	Jul-18
Discarded	1F3B207115	7F7D1C4C51	Jul-18

0% Fert	4239441550
	985120022605171
	985121005923954
	7F7D4F723C

All eight females were successfully induced to spawn with LHRHa injections as described above. Two of the females did not progress and even though they did release eggs after being injected with LHRHa, the fertilization rates (across all 5 males used) were extremely poor and subsequent larval hatch was almost zero. We attribute this result to lower river temperatures at the time of capture compared to previous years possibly slowing down the maturation rate and influencing our ability to induce them successfully in the hatchery environment. Total egg volume and number was recorded for individual females for the 2012 spawning season and all ovulated eggs that were easily available were taken. The spawning events of July 4th and July 18th produced a total of 612,865 green eggs.

The average neurulation percentage was 58.5% which produced a total of 271,336 viable eggs and larvae (Table 7). These numbers do not include the discarded eggs from Female 985120022620878 that had the parasite.

The remaining eggs and larvae that did not go towards production of juveniles for stocking efforts were used for following:

- Research work with Dr. Steve McAdam and Dan Baker at UBC on substrate effects on larvae quality and swimming performance.
- Kinbasket Reservoir Sturgeon Recolonization Risk Assessment – CLBMON #26 egg survival experiment.
- M. Boucher (FFSBC) Substrate Experiment – To Identify if the use of artificial substrate at a production scale during larval yolk sac phase offers improved growth and survival.

- BC Hydro/FFSBC – (Katy Jay, Masters Student) Cataloguing larval development at varying rearing temperatures. This to be used when determining age/stage of larvae when captures in Columbia River.
- Canada Cryogenetics Services – M. Ritter. Eggs were used to evaluate cryopreservants and fertilization rates.

Table 7: Identification of production families, spawning date, green and neurulated (Neur.) egg numbers and percentage for 2012 egg takes.

Family	Female PIT Tag #	Male PIT Tag #	Spawn Date	Green Eggs	Neur. %	Neur. #
1	426505161B	42381E6117	Jul-04	22,000	88.9	19,558
		4204067834	Jul-04	19,000	36.9	7,011
		7F7D4F6A1F	Jul-04	16,000	82.0	13,120
		42616E411F	Jul-04	16,000	40.1	6,416
		4239436C4E	Jul-04	60,000	71.0	42,800
		Subtotals		133,000	66.8	88,905
2	7F7D4D174C	42381E6117	Jul-04	16,400	94.1	15,432
		4204067834	Jul-04	14,350	93.6	13,432
		7F7D4F6A1F	Jul-04	16,400	94.6	15,514
		42616E411F	Jul-04	14,350	89.2	12,800
		4239436C4E	Jul-04	20,500	4.1	830
		Subtotals		82,000	85.0	58,008
3	985161000180641	7F7D1C4C51	Jul-18	9,925	71.0	7,046
		4239441550	Jul-18	11,910	80.5	9,587
		985120022605171	Jul-18	9,925	75.0	7,443
		985121005923954	Jul-18	9,925	31.0	3,076
		7F7D4F723C	Jul-18	9,925	30.0	2,977
		Subtotals		51,610	87.0	30,129
4	7F7D222A69	7F7D1C4C51	Jul-18	10,320	78.0	8,049

		4239441550	Jul-18	17,200	12.5	2,150
		985120022605171	Jul-18	17,200	20.5	3,526
		985121005923954	Jul-18	10,320	62.0	6,398
		7F7D4F723C	Jul-18	12,040	0.0	0
		Subtotals		67,080	29.0	20,123
5	985161000056632	7F7D1C4C51	Jul-18	23,760	75.5	17,938
		4239441550	Jul-18	15,840	73.0	11,653
		985120022605171	Jul-18	13,860	65.0	9,009
		985121005923954	Jul-18	15,840	58.5	9,266
		7F7D4F723C	Jul-18	15,840	0	0
		Subtotals		85,140	79.0	47,866
		Total	Jul-04	215,000	69%	146,913
		Total	Jul-18	203,830	48%	98,118
		Grand Total		418,830	58.5%	24,5031

7.0 BROODSTOCK RELEASE

After hatchery spawning events, fish are held for about three additional days before being returned to the Columbia River. This additional time in captivity is to assure the staff that the fish are recovered fully from the spawning event and that there are no fish health issues that should be addressed prior to release. Before fish are returned to the river a DNA sample (fin clip) is collected. This sample is placed in ethanol, labelled and stored on site in a secure dry area at the KTH. Fish are also re-checked for the presence of a PIT tag to ensure future identification. The same holding and transport equipment used to transport fish from the river to the hatchery is employed to carry them back to the river, where they are released as near as possible to the capture area. In 2012, all adult releases were completed without incident and all fish appeared well at time of release.

8.0 ACOUSTIC TAGGING ADULT BROODSTOCK

All adults taken to the hatchery were implanted with acoustic transmitters (Vemco model V16-6H-R64K, Table 8) prior to release back into the lower Columbia River. These transmitters allow for fish movements to be tracked when they pass by

stationary receivers (Vemco model VR2W) that are situated in various locations in the river. The transmitters used have an estimated life of 3,000 days or nearly 10 years. This will allow movement data to be collected on these adults to determine if or when they return to spawn and if there are additional spawning areas being used in the river.

Table 8: Vemco tag and PIT tag number for adults. Koot: Kootenay eddy; Dock set: Private dock at River Km 3.5; Rialto: Rialto creek.

Sex	Pit Tag #	Vemco Tag No.	Vemco Tag Code	Capture Date	Capture Location	Release Date
F	985161000180641	1106214	44397	Jun-06	Koot Eddy	July 20
F	7F7D381E45	1106215	44398	Jun-08	Koot Eddy	July 20
F	985161000056632	1137768	34190	Jun-11	Waneta	July 23
F	7F7D222A69	1137769	34191	Jun-20	Genelle	July 24
F	1F3B207115	1137770	34192	Jun-21	Waneta	July 23
F	985120022620878	1137771	34193	Jun-22	Waneta	July 6
F	7F7D4D174C	1137772	34194	Jun-22	Waneta	July 6
F	426505161B	1137773	34195	Jun-25	Koot Eddy	July 10
M	42381E6117	1137774	34196	Jun-06	Koot Eddy	July 19
M	4204067834	1137775	34197	Jun-06	Koot Eddy	July 19
M	7F7D1C4C51	1137776	34198	Jun-06	Dock Set	July 25
M	4239441550	1137777	34199	Jun-11	Waneta	July 26
M	985120022605171	1137778	34200	Jun-11	Waneta	July 26
M	985121005923954	1137779	34201	Jun-18	HLK	July 25
M	7F7D4F6A1F	1137780	34202	Jun-21	Ft Shepard	July 10
M	42616E411F	1137781	34203	Jun-22	Waneta	July 11
M	7F7D4F723C	1137782	34204	Jun-26	Rialto	July 24
M	4239436C4E	1137783	34205	Jun-28	Waneta	July 11

9.0 EGG INCUBATION AND LARVAL DEVELOPMENT

Fertilized eggs were placed in MacDonald Jars for incubation with water outflow from the jars directed into stainless troughs. Jars were positioned over individual troughs that were labelled and segregated by family. FFSBC staff ensured adequate flow (8 – 12 litre per minute) to maintain egg separation and oxygenation, while guarding against egg loss from jars as they become more buoyant during development. Dead eggs were removed at intervals throughout the day to control the development of fungal infestations. Egg condition and number were monitored to ensure juvenile and larval release goals were met.

Hatch out for eggs was between 8-10 days post fertilization at 15 °C for all families of the 2012 brood year. Free embryos emerged from the MacDonald jars and were flushed into stainless troughs containing 10-12 lpm flowing water at a depth of 10 cm. Water levels were controlled by a standpipe and larvae were protected from the exit flows by a stainless mesh screen. Water flows are set to exchange water, but not unduly disturb larvae and cause them to swim. Overhead partitions on the troughs provide cover for the larvae.

Approximately one-half of Family 1 was involved in an artificial substrate study (see Appendix 1) where larvae were provided with substrate enabling them to hide. After 100 days of incubation the study was ended and the experimental fry were combined with control fry and the family reared as one group.

After about 10 days feed was introduced into the larval tanks. A custom formulation was produced at the hatchery that contained standard Bio-Oregon semi moist starter feed with added dried krill powder and the commercial product Cyclop-eeze (Argent Chemicals). Proportions of the ingredients varied with the progress of the larvae, but in general, the additives represented one-third of the feed mass at the beginning of feeding and progressed to straight Bio-Oregon feed by 1 g in size.

Feed was presented to feeding larvae by hand in two methods. At first, feed was continuously (24h) applied to the water surface and pressed to the trough wall below the waterline. Young sturgeon rise to feed on the vertical surfaces after their primary introduction to feed on the bottom of the trough. Feeding is done on an *ad libitum* basis as directed by the fish culturist. As fish develop, feed is pressed on to the tank wall in addition to being delivered the water surface by a belt feeder, both in excess of need.

Common fish culture practice of sturgeon mimics that of trout culture in that tanks and screens are cleaned throughout the day on an 'as needed' basis. The monitoring of fish health and feeding activity is likewise observed during daily routine. In this fashion, the care and culture of sturgeon, especially for the younger, more vulnerable life stages is continuous throughout the working day.

9.1 Juvenile Rearing

Once sturgeon fry are on feed and attrition through a lack of start-feeding has abated routine fish culture of cleaning, feeding and monitoring water quality ensues for the duration of juvenile rearing. Fish below a gram average in weight are fed *ad libitum* to about 15-30% body weight per day. As fish pass their first month of tank culture post initiation of feeding, the transition from starter diets to larger commercial trout or salmon diets (e.g.: Bio Oregon; Nutra-XP) ensues and the ration drops to 2-4% body weight per day. Feed is offered by clockwork belt feeders 24h per day and the feeders are refreshed daily. Size differences appear soon after fish are actively feeding, usually by August. Water flows to the tanks are in the order of 1 litre per kilogram of biomass and may be increased to ensure the current maintains self cleaning in the tank. Water quality (e.g.: O_2) is measured daily.

Juvenile White Sturgeon are graded in order to decrease densities and reduce tank effects on growth like competition for food. This allows juveniles to recover from any feeding competition and quickly establish a higher growth rate. As post-release survival is assumed to be positively influenced by size at release, it is

commonly practiced to grow the fish as large as possible to time of release. Additionally, the larger graded fish need an appropriate feed size to continue a positive growth pattern. Grading begins in September as sizes of the fish diverge and continues through the rearing process on a three to four week schedule. At grading, fish are hand-picked into either large or small categories and placed into separate tanks. This is the first time during the rearing process when a complete inventory of fish is established.

During grading, smaller fish remain in troughs or smaller circular tanks until they catch up on growth. Further grading and culling may occur, but care is taken to ensure that smaller fish are not excluded so that they will contribute to the final release numbers. Culls for population density control are from all tanks in equal measure to ensure that artificial selection is minimized. Briefly, fish are randomly selected from rearing containers using small nets and counted out into a vessel containing 500 mg/l TMS (tricaine methanesulphonate) according to FFSBC Standard Operation Procedure: Euthanasia. Culling continues until the desired numbers of fish remain in the culture container.

10.0 RELEASES 2012

10.1 Juvenile Releases

In February, juvenile White Sturgeon are individually marked with a PIT tag that is inserted into the dorsal musculature at the midpoint between the dorsal and lateral scute line inferior to the anterior margin of the dorsal crest scute line (Figure 1). Each fish is also individually marked externally by the removal of two left side scutes according to a prescribed coding formula corresponding to year class. In early April, the PIT tag number, length (cm) and weight (g) data are recorded for each fish prior to release. By uniquely marking each individual fish, recaptured fish can subsequently be identified to their specific release location and date of release in addition to family record. Juveniles are transported in FFSBC fish transport vehicles according to UCWSRI TWG transport protocols.

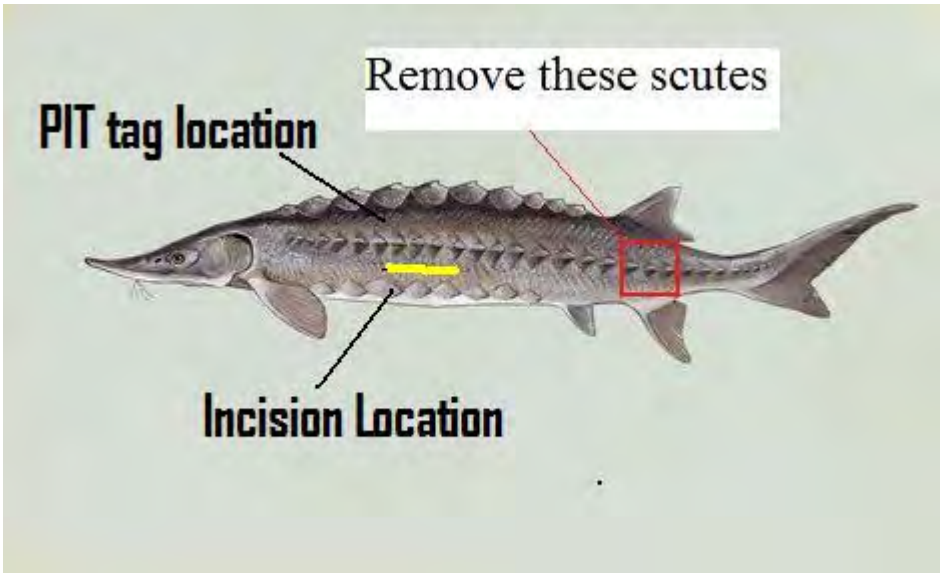

Figure 1: Scute removal and PIT tag locations.

Juvenile releases into the lower Columbia River occurred on May 1st and 2nd, 2013. Average weight of released fish was 86 g (Table 9). A total of 4,037 juvenile sturgeon were released into the Canadian Lower Columbia River at the HLK near Castlegar (n = 1,400), Kootenay Eddy (n = 893), Genelle (n = 875) and Beaver Creek (n = 869).

A further 5,944 juvenile sturgeon were released April 30th and May 9th into the Canadian Mid-Columbia River downstream of Revelstoke. All of these individuals were released at Shelter Bay Provincial Park. These juveniles were much larger than have been released in the past, and averaged 170 grams in weight each (Table 9).

Table 9: Numbers, average length and weight, and release locations for hatchery reared juvenile White Sturgeon released into the lower and mid-Columbia Rivers in 2013.

Location	Release Date	River Km	Family	Number	Average FL cm	Average Wt g
HLK	02-May-13	0.1	1	280	22.0	82.7
			2	280	22.5	86.6
			3	280	22.8	89.8
			4	280	22.2	91.8
			5	280	22.3	84.8
		Total / Average		1,400	22.3	87.1
Kootenay Eddy	01-May-13	10.5	1	174	22.0	82.7
			2	175	22.5	86.6
			3	175	22.8	89.8
			4	184	22.2	91.8
			5	185	22.3	84.8
		Total / Average		893	22.0	83.5
Genelle	01-May-13	24	1	175	22.0	82.7
			2	175	22.5	86.6
			3	175	22.8	89.8
			4	175	22.2	91.8
			5	175	22.3	84.8
		Total / Average		875	22.3	86.1
Beaver Creek	01-May-13		1	177	22.0	82.7
			2	176	22.5	86.6
			3	176	22.8	89.8
			4	170	22.2	91.8
			5	170	22.3	84.8
		Total / Average		869	22.3	86.1
Revelstoke	08-May-13	178	1	923	27.2	159.4
			2	940	28.7	180.3

	2	592	28.0	161.7
	2	386	23.2	92.4
	3	1,080	28.0	172.0
	4	891	28.0	166.0
	5	1,132	29.0	183.0
Total / Average		5,944	27.4	159.3
Year Class Totals		9,981	22.0	81.5

11.0 RELEASE EVENTS

Each year school and public release events are planned and organized by Angus Glass of the Fish & Wildlife Compensation Program (FWCP) and FFSBC staff along with volunteers from many other agencies. The FWCP works on behalf of its partners, BC Hydro, the B.C. Ministry of Environment and Fisheries and Oceans Canada, to conserve and enhance fish and wildlife populations impacted by the construction of BC Hydro dams in the Columbia Basin.

The FWCP and BC Hydro are the primary funders for the Columbia River white sturgeon aquaculture program and is an active partner in the UCWSRI. It also recognises that the sturgeon recovery work will take many years and will only be successful if the community and the younger generations become more connected with the fish. With that goal in mind FWCP organizes juvenile sturgeon release events for the public in Creston and, on behalf of the UCWSRI, juvenile sturgeon release events for school children and public in Trail, Castlegar and Revelstoke.

A public release event took place at Beaver Creek on May 1st with 300 school students and public assiting in the release of the juvenile fish. One very large public release events took place on May 2nd, 2013 with over 900 school students, each getting to release their own sturgeon below the HLK dam. After the school children released fish, another 250 general public came to help release the rest of the required numbers.

Another public release event took place in Revelstoke on May 9th with another 150 school children and public at the Shelter Bay Provincial Park boat launch site.

12.0 FISH HEALTH TESTING SUMMARY

12.1 Virus Screening for Broodstock

Samples of ovarian fluid and milt collected from spawners contributing gametes were screened for viruses (IPNV, WSHV1, WSHV2 and WSIV) using standard tissue culture methods as described in Section 10: Procedures for the Detection of Viruses as listed in the Canadian Fish Health Protection Regulations Manual of Compliance. Fluids were collected from egg or milt samples and frozen at -20 prior to testing. All results for ovarian fluid and milt samples were negative for viral or bacterial contamination (Appendix 2).

12.2 Juvenile Screening History

Subsamples of 5-30 juveniles from all family groups were screened for viruses at 30 days post hatch. Fish were processed according to Section 10: 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. Viral culture cell lines used for Broodstock testing were also used for juvenile testing.

12.3 Deformities

In 2012 year class fish, there were a few deformities in a couple of families. No changes were made in the way of early rearing practices that could account for the low deformity rate. As there were so few deformities seen, monthly deformity checks were discontinued and deformities were then noted when fish were handled during the marking process. No particular deformities were seen with the few that did occur being classed as spinal or fin deformities.

13.0 PERMITS

A SARA permit was obtained for all the Columbia sturgeon culture activities including adult transport, holding, spawning, rearing, research and releases.

As with other years permits were obtained from the Introductions and Transfer Committee for adults, as well as for spring release of juveniles and larval releases.

14.0 PRESENTATIONS AND MEETINGS

1. Spawning events July 4th and July 18th at KTH.
2. Nov 13/14/15– Ron Ek and Dr. Jim Powell attended UCWSRI TWG meeting in Castlegar.
3. February 19 to 22, Marcus Boucher, *Substrate trials update*, FFSBC Annual General Meeting, Kelowna B.C.
4. April 58/9/10 Mike Keehn and Dr. Jim Powell attended UCWSRI TWG meeting in Nelson and presented updated plan for spring release events and dates.
5. Monthly TWG conference calls Ron Ek and Dr. Jim Powell.

APPENDIX 1.0 RESEARCH PROJECTS

1.1 Larval quality indicators study: Background and Research proposal

Principal investigator(s) (name, affiliation, contact info): Colin Brauner, Professor, UBC Zoology

Purpose/study title: This study will be a continuation of last year's research into larval quality indicators. The project is being conducted by Colin Brauner's lab as part of a TWG project and has close links to recovery needs. The project goal is to understand factors that affect larval survival and to investigate indicators of larval quality that can be used to evaluate field samples.

Location(s) where study will occur: UBC, Vancouver.

Short description of study (describing objectives, rationale for this specific request, expected outcome, communication plan for results): The study objects are to rear larvae under 2 or 3 combinations of substrate and flow. Samples would be taken at regular intervals (e.g. 5, 10, 15, and 30 days) and archived for later analysis. We wish to investigate whether a stronger growth response can be obtained as compared to methods used in 2010 (i.e. if we use separate aquaria vs. recirculation). If a strong growth response is achieved results from 2011 would be available for analysis to strengthen results obtained in 2010.

Please provide details of how the results will be used: Results will be used to support ongoing research programs in white sturgeon. Since a TWG member is directly involved in conducting this research (Steve McAdam), this helps guide the work so that it continues to address issues that are relevant to the TWG and sturgeon recovery. It also ensures that results are communicated to the TWG in a timely manner. Any reports and publications produced as a result of this work will be provided to the TWG.

1.2 DNA Sampling

Thirty DNA samples total of sturgeon fins were taken from members of each family group and preserved in labelled jars containing ethanol.

DNA samples also removed from the adults.

Samples were shipped to BC Hydro and UBC for storage and analysis.

1.3 Vemco Tagging of Adult Brood

All 20 of the mature adults taken to the hatchery were implanted with V16-6H-R64K-Coded Vemco tags. These implanted tags allow for these adults movements to be tracked when they pass by stationary VR2 receivers that are situated in various locations in the river. The Vemco tags have a life of 3000 days or nearly 10 years. This tagging should allow adults to be tracked to see if and when they return to spawning areas and if there are additional spawning areas being used in the river.

1.4 Kinbasket Reservoir Sturgeon Recolonization Risk Assessment - CLBMON#26

Location(s) where study will occur:

Upper Columbia River – Near Golden BC

Short description of study (describing objectives, rationale for this specific request, expected outcome, communication plan for results):

This study is designed to continue the process of assessing the potential for white sturgeon colonization of Kinbasket Reservoir and upper Columbia River. Management questions derived from the description and rationale statements, and objectives described by the WUP include:

- Are there suitable spawning habitats, free embryo hiding habitats, larval habitats and under-yearling and older juvenile foraging shelter-sites available in relatively contiguous circumstances within the study area?
- What is the most applicable conservation aquaculture approach to establishing a sturgeon stock in the study area?

Ecological risks have been partially assessed through previous. However, after several additional years of data collection in the rest of the basin including free embryo and larval life stage habitat needs, genetic assessment of stock differences in the Columbia system, aquaculture (pathogen testing, better breeding procedures, food tests), and the three years of concerted efforts to locate and sample sturgeon in Kinbasket Resevoir, suggest we should be able to take a more comprehensive look at ecological risks associated with release of cultured fish into the upper basin. Determining whether such habitats are present will involve sampling habitat parameters in likely candidate areas, and comparing these to already described habitat units from healthy sturgeon populations. If feasible, enclosures may be used to assist in determining the suitability of different sites for egg survival and development, and potentially larval survival.

The enclosure experiment will be designed and replicated within and among different potential spawning locations within the Upper Columbia River above Kinbasket Reservoir. Fertilized eggs will be placed on filter material, placed within enclosures that will prevent larval escapment, and deployed near the river bottom. Enclosures will be removed every few days and the surviving eggs will be counted, imaged for developmental stage, and the enclosure re-deployed. At hatch, all larvae will be preserved for later morphological measurements (e.g. total length, yolk-sac volume). Other measurements recorded from the enclosures will include daily water temperature, water velocity, and dissolved oxygen. Eggs will be placed in the river following the second spawning event in July.

We acknowledge the logistical difficulties of completing this type of an experiment in this region of the Columbia River. This year (2011) will be more of a pilot year and if successful, the experiment will be expanded for 2012.

1.5 Production-level trials using artificial substrate to rear larval white sturgeon.

Marcus Boucher, Junior Research and Development Biologist, FFSBC

Purpose/Objective:

To identify if the use of artificial substrate (1" Bio-Spheres) at a production scale during the larval yolk sac phase offers improved growth and survival. Growth and survival will be monitored beyond the larval stage to assess if differences in treatment groups persist. The overall goal will be to assess if using this substrate is an effective addition to current larval sturgeon rearing methods.

Background Information:

Gravel substrates have long been known to be integral to the life histories of many riverine fish species, particularly during the early life stages. Natural and artificial rearing substrates have often been used in hatcheries in the culture of salmonid alevins, as this generally produces larger fry than rearing methods without substrate (Bams 1969; Fuss and Johnson 1982; 1988; Hansen and Møller 1985; Murray and Beacham 1986; Peterson and Martin-Robichaud 1995). Similarly, rearing larval white sturgeon in substrate during their yolk sac phase has been shown to provide significantly greater growth, as well as greater survival, and improved physiological condition (Boucher, unpublished).

A previous experiment showed that growth of larval white sturgeon reared in either gravel or artificial substrate (1" Bio-Sphere) had similar growth rates. This suggests that Bio-Spheres may be a suitable substrate to use in a culture facility (Boucher, unpublished). So far, substrate rearing experiments have been carried out within a lab setting with relatively low densities and have not yet been implemented at a production scale. The objective of the proposed study is to identify if the use of artificial substrate at a production scale is a beneficial addition to current larval sturgeon rearing methods. Growth, survival, and physiological metrics will be monitored beyond the larval stage to assess if differences in treatment groups persist.

1.6 Pedigree analyses provide estimates of the effective number of breeding adults and number of spawning events for White Sturgeon in the Upper Columbia River, Canada.

Kathleen Jay, James A. Crossman and Kim T. Scribner

Molecular techniques allow for the examination of levels of recruitment to the egg or larval stage and/or reproductive success of adult fish. We estimated the number of spawning adults (N_b) and effective breeding number (N_e) from collected eggs and larvae in each of two years using a pedigree analysis based on genotypic data from 12 microsatellite loci. We also produced a timetable of white sturgeon larval development that incorporated variation due to family and temperature to improve estimates of fertilization dates for wild caught larvae. The total annual number (mean ± SD) of contributing adults and breeding adult effective size among spawning areas (n=2) was 109.0 ± 8.5 (N_b) and 71.5 ± 12.0 (N_e), respectively. Genetically derived estimates of numbers of spawning adults were concordant with empirical estimates of the number of available breeders annually based on sex ratios and maturation stages of adults captured during broodstock programs. Further, genetic data determined egg mats (vs. drift nets) captured offspring that better represented the total spawning population with the majority of N_b represented by progeny at the egg stage. However, these results were influenced by lower sampling effort due to the hydrology of the spawning area. We also used inferred adult contributions to pedigreed larvae produced within and among events to validate the inferred timing of different spawning events based on developmentally staged eggs which is commonly used as a surrogate measure of adult spawning events. Low estimates of the number of spawning adults and inferred reproductive skew based on pedigree analysis revealed that effective breeding numbers are considerably lower than the number of breeders (N_e:N_b = 0.64 ± 0.17; mean ± SD) suggesting that the genetic diversity of progeny during this early life stage is low and effective population size is likewise small. Results from this work help to describe the reproductive ecology of this population and can be used to revise ongoing recovery strategies (e.g. conservation aquaculture programs).

APPENDIX 2: FISH HEALTH TESTING
Columbia Sturgeon

Fish Health

Summary Report

2012

The 2012 Columbia sturgeon year class reared well. There were no diagnostic cases submitted from this year class. There were 5 families reared and tested in 2012. Results from the Broodstock testing, juvenile Family testing, and Pre-release submissions are summarized in table format below. Columbia juvenile sturgeon were tested and found to be negative for the following viruses:

White Sturgeon Iridio Virus	(WSIV)
White Sturgeon Herpesvirus I & II	(WSHV I & II)
Infectious Hematopoiestic Necrosis Virus	(IHNV)
Infectious Pancreatic Necrosis Virus	(IPNV)
Viral Hemmoragic Septicemia Virus	(VHSV)

Broodstock testing, and Family group sampling were run on the following cell lines: Sturgeon cell lines have been obtained and propagated from UC Davis lines.

White Sturgeon Skin	(WSSK)
White Sturgeon Spleen	(WSS-2)
Epithelioma papulosum cyperinid	(EPC)
Chinook Salmon Embryo-214	(CHSE-214)

The pre-release assay was run on the following cell lines:

White Sturgeon Skin	(WSSK)
White Sturgeon Spleen	(WSS-2)
Epithelioma papulosum cyperinid	(EPC)
Chinook Salmon Embryo-214	(CHSE-214)

All assays were conducted based on the OIE standards for international fish health monitoring and trade.

2.1 Broodstock Testing for 2012:

Please note spawning dates were not provided with submitted samples

Case Number:	2012-1049						
Sample Tissue		Virology Cell lines				Bacteriology	
Reproductive fluid	PIT Number	CHSE-214	EPC	WSSK	WSS-2	TSA	HS
Ovarian fluid	985161000180641	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Ovarian fluid	7F7D381E45	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Ovarian fluid	985161000056632	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Ovarian fluid	7F7D222A69	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Ovarian fluid	1F3B207115	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Ovarian fluid	985120022620878	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Ovarian fluid	7F7D4D174C	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Ovarian fluid	426505161B	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Milt	42381E6117	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Milt	4204067834	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Milt	7F7D1C4C51	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected

Milt	4239441550	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Milt	985120022605171	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Milt	985121005923954	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Milt	7F7D4F6A1F	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Milt	42616E411F	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Milt	7F7D4F723C	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Milt	4239436C4E	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected

2.2 45-60 day Juvenile Family testing results 2012

Case Number	Family ref #	# fish submitted	Virology				Bacteriology	
			CHSE-214	EPC	WSSK	WSS-2	TSA	HS
2012-1095 Submission date: SEPT 6, 2012	Fam 1	60 Total 30 Virology 30 Bacteriology	NEG	NEG	NEG	NEG	18/30 Presumptive for non-pigmenting Aeromonas spp (hydrophila) via tube tests. Considered non-pathogenic.	30/30 No pathogenic bacteria detected
2012-1096 Submission date SEPT 6, 2012	Fam 2	60 Total 30 Virology 30 Bacteriology	NEG	NEG	NEG	NEG	30/30 No pathogenic bacteria detected Considered non-pathogenic.	30/30 No pathogenic bacteria detected
2012-1097 Submission date: SEPT 25, 2012	Fam 3	60 Total 30 Virology 30 Bacteriology	NEG	NEG	NEG	NEG	23/30 Presumptive for vibrio related spp. via tube tests and sensitivity tests Considered non-pathogenic.	30/30 No pathogenic bacteria detected
2012-1098 Submission date: SEPT 25, 2012	Fam 4	60 Total 30 Virology 30 Bacteriology	NEG	NEG	NEG	NEG	8/30 Presumptive for vibrio related spp. via tube tests and sensitivity tests Considered	30/30 No pathogenic bacteria detected

							non-pathogenic.	
2012-1099 Submission date: SEPT 25, 2012	Fam 5	60 Total 30 Virology 30 Bacteriology	NEG	NEG	NEG	NEG	30/30 No pathogenic bacteria detected	1/30 Yellow pigmented colonies. Negative for *Flavobacterium psychrophilum*. No pathogenic bacteria detected

2.3 Pre-release sample test for brood year 2012 submitted Feb 14th, 2013.

Case Number	Family ref #	# fish submitted	Virology Salmonid		Virology Sturgeon		Bacteriology	
			CHSE-214	EPC	WSSK	WSS-2	TSA	HS
2013-1017 Submission date: Feb 14th, 2013	Mixed all families,	60 Random sample	Negative No viral or filterable replicating agents detected.	Negative No viral or filterable replicating agents detected.	Negative No viral or filterable replicating agents detected.	Negative No viral or filterable replicating agents detected.	12/60 Presumptive for vibrio-like spp. Considered non-pathogenic bacteria	60/60 No pathogenic bacteria detected

2.4 Summary Comments; Disease Screening

The 2012 Columbia sturgeon families reared well during larval rearing. The pre-release sample results indicate that the 2012 year class of Columbia White sturgeon appear to be healthy and free of any viral or bacterial pathogens. Hatchery records indicate losses since October have been minimal. The health status of the stock for release is considered to be very good.

May 23rd , 2013

Sherry Mead

Fish Health Unit Manager

Freshwater Fisheries Society of BC