Columbia River Project Water Use Plan

Columbia White Sturgeon Management Plan

White Sturgeon Conservation Aquaculture – 2014 Annual Report (Year 7)

CLBWORKS-25: Mid-Columbia River White Sturgeon Conservation Aquaculture
CLBWORKS-34: Lower Columbia River White Sturgeon Conservation Aquaculture

Works Period: May 2014 – May 2015

BC Hydro and Power Authority

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May 10, 2015

COLUMBIA WHITE STURGEON CONSERVATION FISH CULTURE PROGRAM

KOOTENAY STURGEON HATCHERY

2014 Annual Report

Chad Fritz

May 10, 2015

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 (UCWSRI), with hatchery reared juveniles being released over the last decade into both the lower (since 2001) and Mid-Columbia (since 2007) Rivers in Canada. Conservation aquaculture and release of juvenile White Sturgeon into the mid and lower Columbia Rivers as part of the Columbia Water Use Plan was successful in 2014. Juvenile sturgeon from wild adult broodstock captured and transferred to and spawned at the Kootenay Trout Hatchery (KTH) were released into the Mid-Columbia and Lower Columbia Rivers, and all targets associated with the various research egg requests were met.

At the April 2013 UCWSRI Technical Working Group (TWG) meeting members identified and ranked the primary goals for a sturgeon conservation aquaculture program focused on rearing wild caught eggs and larvae in a streamside white sturgeon conservation aquaculture facility (the Facility). The primary goal of the Facility is to augment the genetic diversity of supplemental progeny and the Facility was piloted in the summer of 2014. The Facility is located on the Canadian section of the transboundary reach of the Columbia River near Waneta and supports the incubation and hatch of wild caught eggs. Wild caught eggs (n = 5,567) were incubated to hatch and all resulting larvae (n = 1,951) were transported to the KTH to be reared and released as juveniles in spring 2015.

Capture of mature adult white sturgeon for use as hatchery broodstock was conducted over a five week period, starting on June 9th, and ending July 4th, 2014. Effort was dispersed throughout the lower Columbia River from Hugh Keenlyside dam (HLK) in Castlegar, BC, to the Canada/USA border below Trail, BC. Five mature females and six mature males were captured and transported to the KTH Sturgeon Facility. One spawning event took place on July 9, 2014, with all five females successfully spawned with five males. The sixth male was previously captured in 2006 and contributed towards juveniles released in the fall of 2006 and spring of 2007. In 2014, this male was brought to KTH to serve as backup in the eventuality that one or more of the other five males did not spawn but was not needed. A total of five maternal

families were created using a full factorial mating design where each female is crossed with each males. All adult sturgeon were released back into the Columbia River by July 18, 2014.

A total of 530,978 green eggs were produced from this single spawning event. Fertility, neuralization and hatch rates were variable, with neuralization ranging from as low as 0%, and as high as 98% between family groups, depending on the male to female cross.

Eggs were first allotted to satisfy production targets for release of juveniles into the Mid and lower Columbia Rivers. Surplus eggs and larvae produced from the two spawning events were used for the following research projects.

- University of British Columbia – Drs. Steve McAdam, Colin Brauner, David Close and Sang Yung. Projects building on prior studies of white sturgeon larvae.
- Golder and Associates Ltd., on behalf of Columbia Power Corporation. Eggs (n=500) were adhered to mat material and cameras were used to study predation, predator densities and species composition at Waneta Eddy.
- University of Saskatchewan. Dr. Markus Hecker. Ongoing investigations of the sensitivity of white sturgeon to different environmental pollutants.
- Colville Confederated Tribes. Jason McLellan and Charlee Capaul. 40,000 first-feeding larvae, transported to Kootenay Tribal Hatchery, Bonners Ferry, Washington State.

The ploidy of wild white sturgeon has previously been determined to be octaploid (8N) in Columbia and Kootenay River populations. Concern was raised when a large number of 12N white sturgeon were discovered in juvenile family groups which were offspring of wild adult Kootenay River White Sturgeon spawned at the Kootenay Tribe of Idaho (KTOI) Sturgeon Hatchery in 2013. The mechanism of this ploidy shift is unknown but it is thought to occur at the fertilization stage. Given that there are potential implications to the UCWSRI program, the Freshwater Fisheries Society, under the direction of the TWG, again assessed the ploidy of the white sturgeon families being reared at KTH. One family (family 1) was identified as having 12% 12n individuals (n=100). Subsequently, the ploidy of each individual to be released from Family 1 (n=1693) was assessed to ensure only 8n white sturgeon were released into the lower Columbia River.

Samples from all Columbia adults, as well as the juveniles, were also sent to the FFSBC Fish Health lab for screening. The FFSBC Fish Health Lab tested sturgeon samples for several viruses (IPNV, WSHV1, WSHV2, and WSIV). This testing was done on adults and juveniles from each family as defined by federal and provincial fish transplant permits. Final results from these tests were negative.

Juvenile releases occurred in the spring of 2015 on May 5th and 8th in the lower Columbia River and on May 7th in the mid-Columbia River. A total of 2,800 production and 1,095 wild progeny juvenile sturgeon were released into the lower Columbia River between Hugh Keenlyside Dam (HLK) near Castlegar BC, and the USA border below Trail BC. Releases of production juveniles in the lower Columbia River occurred at Millennium Park in Castlegar (n=1,000), Robson upper boat launch (n=600), Genelle (n=600) and Gyro Park in Trail (n=600). Average weight of these fish was 107 grams. A total of 1,095 wild progeny juveniles were released at Beaver Creek, at an average of 172.5 grams. An additional 3,288 juvenile sturgeon were released into the mid-Columbia River near Revelstoke BC. All of these were released at Shelter Bay Provincial Park and averaged 147.8 grams in weight. There were school and public release events associated with the sturgeon releases at Millennium Park in Castlegar, Gyro Park in Trail, Beaver Creek near Trail and Shelter Bay near Revelstoke.

Table of Contents

List of Tables

Acknowledgements

This Project is funded by the BC Hydro Water Use Plan for the Columbia River and the Fish and Wildlife Compensation Program on behalf of its program partners BC Hydro, the Province of B.C., First Nations and the public, who work together to conserve and enhance fish and wildlife impacted by the construction of BC Hydro dams.

We also acknowledge the Upper Columbia White Sturgeon Recovery Initiative Technical Working for their contribution to this program.

1.0 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. 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; Hildebrand and Parsley 2014). 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 Columbia White Sturgeon 2012 Annual Report) 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.

CLBWORKS#24, Mid-Columbia White Sturgeon Experimental Aquaculture, was implemented during the first four years of the program (2008-2011). During this period, the focus was 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 initiated in 2012 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.
In discussions at the UCWSRI TWG meetings in 2013, the alternative for a sturgeon conservation aquaculture program focused on rearing wild caught eggs and larvae both instream as well as in a streamside white sturgeon conservation aquaculture facility (the Facility) was brought up. Such programs have been found to result in improved genetic diversity, have more natural rearing conditions, and have allowed for the development of collection and rearing methods that incorporate more aspects of the species reproductive ecology compared to more traditional rearing practices for both white sturgeon and other sturgeon species. This alternative consisted of both standard aquaculture practices (broodstock collection) and wild source eggs and larvae that would be reared to stocking size at the KTH and specifics were:
Wild progeny (eggs/larvae) to be imprinted at their respective spawning areas within streamside rearing containers and the surviving progeny transferred to and reared at KTH until a minimum of 9 months of age.
Broodstock crossed at 1 spawning event (5 females | 5 males) with progeny reared at KTH.
This report specifically describes the conservation fish culture activities undertaken by the Freshwater Fisheries Society of BC at their Kootenay white sturgeon conservation facility to meet the objectives defined by CLBWORKS#25 and CLBWORKS#34.

2.0 Brood Capture

The procedures for brood-stock acquisition follow those reported in earlier Annual reports (FFSBC 2012; FFSBC 2013). For a copy of the procedures manual, please contact FFSBC at the Kootenay Trout Hatchery in Wardner BC or BC Hydro in Castlegar BC. This report includes

data from retained and transported adult fish only. Information on all fish caught and released can be obtained from BC Hydro.

Adult sturgeon were captured using setlines and transported to the Kootenay Trout Hatchery (KTH) sturgeon facility from June 9th through to July 4th, 2014. In all, five female fish with near mature eggs and six flowing male fish were retained and transported to the KTH. Brood capture proved somewhat difficult with respect to capturing mature males, and an extra week of effort was required (July 1 – 4) to capture enough males (see Table 1 for numbers of contributing adults).

Table 1. Number of Broodstock spawned at KTH from 2001 – 2014.

Year	Females	Males
2001	4	5
2002	2	5
2003	2	5
2004	6	7
2005	6	5
2006	6	6
2007	4	5
2008	5	6
2009	7	9
2010	8	10
2011	8	9
2012	6	10
2013	6	3
2014	5	5
	75	90

All fish were returned to the Columbia River as near to their capture location as possible. Prior to their release, all fish had ovarian fluid or milt collected to be sent to the FFSBC's Fish Health lab for disease testing. Table 2 and Table 3 show the capture and life history information on all adults taken to the hatchery including sex, PIT Tag number, capture location, capture date, length, weight, spawn date and date of release.

Table 2. Capture details for adult White Sturgeon broodstock retained and transported to KTH, 2014.

Spawn Year	Sex	Pit Tag #	Weight (kg)	Length (cm)	Capture Date	Capture - river km	Capture Location	Spawn Date	Release Date
2014	F	985161000179510	60	189	06/18/2014	6.5	Sturgeon Island	07/09/2014	07/16/2014
2014	F	985120022601339	145	244	06/23/2014	56.5	American Eddy	07/09/2014	07/18/2014
2014	F	985120030505824	89	211	06/23/2014	56.0	Waneta Eddy	07/09/2014	07/16/2014
2014	F	115619232A	80	205	06/25/2014	52.3	Fort Shepherd Eddy	07/09/2014	07/14/2014
2014	F	985120020810967	105	231	06/27/2014	56.5	American Eddy	07/09/2014	07/14/2014
2014	M	900254000111534	25	136.5	06/26/2014	56.0	Waneta Eddy	07/09/2014	07/10/2014
2014	M	900254000130209	28	144.5	06/26/2014	56.0	Waneta Eddy	07/09/2014	07/11/2014
2014	M	985120019170580	57	176	07/01/2014	56.0	Waneta Eddy	07/09/2014	07/10/2014
2014	M	7F7D4E730C	52	188	07/01/2014	56.5	American Eddy	not spawned	07/10/2014
2014	M	7F7D4F162A	80	202	07/02/2014	56.5	American Eddy	07/09/2014	07/11/2014
2014	M	7F7D1C6D5C	62	193	07/02/2014	49.0	Beaver Creek	07/09/2014	07/11/2014

2.1 Brood Capture History

Table 3. Broodstock capture history for white sturgeon captured during the 2014 brood acquisition and successfully spawned at the Kootenay Sturgeon Hatchery (KTH). Please see Appendix 1 for a description of sexual maturity codes. Rows highlighted in yellow indicate adults captured and transported to KTH in 2014.

PIT Tag Number	Date	Water Body	River Km	Station Name	Length (cm)	Weight (kg)	Mat Code
985120022601339	22/06/06	FDR Lake	75.2	SSL75.3	231.00	121.00	15
985120022601339	23/06/14	Columbia	56.5	American Eddy	244.00	145.00	F4
985120030505824	23/06/14	Columbia	56	Waneta Eddy	211.00	89.00	F4
115619232A	25/06/14	Columbia	52.3	Fort Shepard Eddy	205.00	80.00	F4
115619232A	18/08/98	FDR Lake	122.1	1002	141.00	23.00	
900254000111534	26/06/14	Columbia	56	Waneta Eddy	136.50	25.00	M2
985120020810767	27/06/14	Columbia	56.5	American Eddy DS	231.00	105.00	F4
7F7D4E730C	01/07/14	Columbia	56.5	American Eddy US	188.00	52.00	M2
7F7D4E730C	09/06/93	Columbia	56	SSL55.8R	150.50	25.40	97
7F7D4E730C	30/04/02	Columbia	52.3	SSL52.3	166.00	34.00	97
7F7D4E730C	23/06/05	Columbia	52.3	AB52.3	173.00	43.00	3
7F7D4E730C	06/06/06	Columbia	56	SSL56.0R	175.00	42.00	M2
7F7D4F162A	02/07/14	Columbia	56.5	American Eddy US	202.00	80.00	M2
7F7D4F162A	18/03/94	Columbia	2.2	SSL2.2R	136.00	23.20	98
7F7D4F162A	17/10/94	Columbia	2.2	SSL2.2R	144.00	27.30	97
7F7D4F162A	13/06/07	Columbia	1.2	SSL1.2R	182.50	56.00	97
985120019170580	01/07/14	Columbia	56	Waneta DS	176.00	57.00	M2
7F7D1C6D5C	13/06/11	Columbia	10.5	Koot Eddy	191.0	52.0	M1
7F7D1C6D5C	02/07/14	Columbia	10.5	Kootenay Eddy DS	193.00	62.00	M2
7F7D1C6D5C	16/08/91	Columbia	6.4	SSL6.4R	166.80	36.40	1000
7F7D1C6D5C	09/07/92	Columbia	0.1	SSL0.1L-K	163.00	36.40	2
7F7D1C6D5C	08/07/93	Columbia	0.1	SSL0.1L-K	177.00	39.10	3
900254000130209	26/06/14	Columbia	56	Waneta US	144.50	28.00	M2
985161000179510	18/06/14	Columbia	5.5	Sturgeon Island	189.00	60.00	F4

3.0Transport

When an adult sturgeon was captured and it was determined to be at a suitable maturation stage, it was loaded into the sturgeon transport tank using a stretcher or a tube net. This was done streamside at the nearest access point to the river. The transport tank was filled on site with ambient temperature river water, using a gas water 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 make a salt solution of 1%. Oxygen was supplied to saturation to the tank through aeration stones recessed into the tank floor. Twice during each transport, staff checked the tank water Oxygen level using an Oxygaurd Handy O_2 meter and visually observed the fish for levels of duress. All fish travelled well during transport from the site of capture to KTH with no negative effects of transport apparent. Transport times were approximately four hours.

On arrival, hatchery tank temperatures were matched with transport tank temperatures. The fish were transferred from the transport tank to the culture tank using a tube net. Fish were monitored hourly following arrival at the hatchery until the staff was off duty, and again the following morning.

4.0 Adult Holding Conditions

For male fish, the water temperature was increased from ambient (about 10°C) to 15°C after LHRHa injections (see section 5.1) were applied. Female fish were held on heated water (15°C) throughout captivity. Water temperatures in tanks holding adults were increased from ambient river temperatures to 15°C in 2°C increments.

4.1 Adult Feeding

As a matter of practice adults are not fed during their time at the hatchery.

5.0 Spawning

In 2014, all female fish captured and retained to be transported to the hatchery had initial PI levels calculated immediately following capture. All five females had initial Polarizing Index (PI) levels below the benchmark of 0.10 mm needed for induction of spawning (see Table 4). Eggs from each female were once again split into portions and each portion fertilized with one male. Milt was checked for motility prior to use for each fertilization process and only milt with 'good' or better scores was used.

Table 4. Female Information and Initial PI Estimates, Spring 2014.

Pit Tag#	Weight (kg)	Length (cm)	Capture Date	Capture Location	Initial PI	Release Date
985161000179510	60	189	06/18/2014	Sturgeon Island	0.098	07/16/2014
985120022601339	145	244	06/23/2014	American Eddy	0.074	07/18/2014
985120030505824	89	211	06/23/2014	Waneta Eddy	0.082	07/16/2014
115619232A	80	205	06/25/2014	Fort Shepherd Eddy	0.082	07/14/2014
985120020810967	105	231	06/27/2014	American Eddy	0.077	07/14/2014

5.1 LHRHa Hormone Injection and Gamete Collection

The LHRHa treatment regimen for female sturgeon consists of two doses of LHRHa given 12 hours apart: a loading dose (10%) and a resolving dose (90%). 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 will begin to ovulate and release eggs 24 hours after the resolving injection of LHRHa (Table 4). Once a female has been observed 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. Male fish are held at 10°C until they are needed to supply milt. When a female has been assessed as mature and is ready for induced spawning, male fish are intramuscularly injected 1-3 days prior with a single bolus dose (10 µg/kg) of LHRHa in saline (Table 5). At the time of the injection, the water temperature in the tanks is increased to 15°C for the time remaining until after spawning. Once spawning is over, the water temperature in the male tank is decreased back down to 10°C. This allows the

male to “shut down” and if necessary be used again for subsequent spawning at later dates by using the same methods.

Table 5. LHRHa injection dosage volumes for adult White Sturgeon spawned in 2014.

Sex	Pit Tag#	Weight (kg)	Capture Date	Capture - river km	Capture Location	Initial Dose (ml)	Resolving Dose (ml)
F	985161000179510	60	06/18/2014	6.5	Sturgeon Island	0.60	1.08
F	985120022601339	145	06/23/2014	56.5	American Eddy	1.45	2.61
F	985120030505824	89	06/23/2014	56.0	Waneta Eddy	0.90	1.40
F	115619232A	80	06/25/2014	52.3	Fort Shepherd Eddy	0.80	1.44
F	985120020810967	105	06/27/2014	56.5	American Eddy	1.05	1.89
M	900254000111534	25	06/26/2014	56.0	Waneta Eddy	0.37	
M	900254000130209	28	06/26/2014	56.0	Waneta Eddy	0.41	
M	985120019170580	57	07/01/2014	56.0	Waneta Eddy	0.84	
M	7F7D4E730C	52	07/01/2014	56.5	American Eddy	not spawned	
M	7F7D4F162A	80	07/02/2014	56.5	American Eddy	1.18	
M	7F7D1C6D5C	62	07/02/2014	49.0	Beaver Creek	0.91	

5.2 Spawning Summary

The target breeding design is a full factorial mating design where each female is crossed with all available males (see Table 6). This method maximizes the effective population size, and is a common approach for conservation work within a limited breeding population. Results from the July 9 spawning event are presented in Table 7. Families were reared in separate MacDonald jars until hatch whereupon they were 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.

Table 6. Breeding design for adult White Sturgeon spawned in 2014.

Family 1	Female PIT #	Male PIT #
1	115619232A	900254000111534
		900254000130209
		985120019170580
		7F7D4F162A
		7F7D1C6D5C
2	985120022601339	900254000111534
		900254000130209
		985120019170580
		7F7D4F162A
		7F7D1C6D5C
3	985120020810967	900254000111534
		900254000130209
		985120019170580
		7F7D4F162A
		7F7D1C6D5C
4	985120030505824	900254000111534
		900254000130209
		985120019170580
		7F7D4F162A
		7F7D1C6D5C
5	985161000179510	900254000111534
		900254000130209
		985120019170580
		7F7D4F162A
		7F7D1C6D5C

Total egg volume and number was recorded for individual females for the 2014 spawning season and all ovulated eggs that were easily available were taken from spawning female fish. The spawning event on July 9th produced 530,978 green eggs and a total of 256,656 larvae. Neurulation rates were very variable across the different male to female crosses, ranging from 0% to 98%. One male (PIT # 985120019170580) was identified as having poor milt motility upon visual inspection prior to spawning, and as a result produced very poor results: of the five one to one crosses with this male, three produced no neurulation, one produced 17% neurulation and one produced 7.4%. All other males showed good to excellent milt motility and as such produced much better neurulation rates.

Eggs were first used to satisfy juvenile production targets for release into the mid and lower Columbia River's. The release targets for 2014 were 4,000 (as many from wild caught eggs as possible) for the section from below the Hugh L Keenlyside dam to the USA border raised to

80+ grams in size and as many 100 + gram juveniles for release in the Mid-Columbia River as possible.

Surplus eggs and larvae were used for following:

- University of British Columbia – Drs. Steve McAdam, Colin Brauner, David Close and Sang Yung. Projects building on prior studies of white sturgeon larvae.
- Golder and Associates Ltd., on behalf of Columbia Power Corporation. Eggs (n=500) were adhered to mat material and cameras were used to study predation, predator densities and species composition at Waneta Eddy.
- University of Saskatchewan. Dr. Markus Hecker. Ongoing investigations of the sensitivity of white sturgeon to different environmental pollutants.
- Colville Confederated Tribes. Jason McLellan and Charlee Capaul. 40,000 first-feeding larvae, transported to Kootenay Tribal Hatchery, Bonners Ferry, Washington State.

6.0 Broodstock Release

After hatchery spawning events fish were held for three additional days and then returned to the Columbia River. This additional time in captivity was to assure the staff that the fish were recovered fully from the spawning event and that there were no fish health issues that should be addressed prior to release. Before being returned to the river all adults were 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 was employed to transport back to the river, where they were released as near as possible to the capture area. All adult releases were completed without incident and all fish appeared well at time of release.

7.0 Acoustic Tagging of Adult Broodstock

No acoustic tags were implanted into hatchery broodstock this year.

8.0 Incubation and Larval Development

Fertilized eggs were placed in MacDonald Jars set at 5 liters per minute (lpm) flow 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 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 microbial infestations. Egg condition and number were monitored to ensure juvenile and larval release goals were met (Table 7).

Table 7. Number of green eggs, neurolation and hatch rates for all contributing females, spring 2014.

Family	Female	Spawn Date	# Green Eggs	% Neurulation	# Hatched
1	115619232A	07/09/14	112,310	72.9%	76,623
2	985120022601339	07/09/14	116,910	55.3%	55,328
3	985120020810967	07/09/14	107,483	67.5%	65,980
4	985120030505824	07/09/14	105,735	55.3%	47,415
5	7F7D185C29	07/09/14	78,540	14.8%	11,310

Time to hatch is dependent on water temperature and ranged from 8-10 days post fertilization at 15°C for all families. At the initiation of hatch, flows in the MacDonald Jars were increased to 10-12 lpm, and as larvae emerged they were flushed into stainless troughs being supplied with flow from the upweller, at about 10cm depth. Water level was controlled by a standpipe and larvae were protected from the exit flows by a stainless mess screen (1 mm^2). Water flows were set to exchange water, but not unduly disturb larvae and cause them to swim. Overhead partitions on the troughs provided cover for the larvae from light. All of the larvae were provided Bioballs as an artificial substrate to allow them to hide and conserve resources. The Bioballs provide artificial habitat similar to what the fish would experience in natural conditions.

After about 10 days, introduction of feed was started into the larval tanks. A custom formulation was produced at the hatchery that contains standard Skretting Nutra XP starter feed with added dried krill powder and the commercial product Cyclopeze (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 Skretting commercial feed by about 1g 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 was done on an *ad libitum* basis as directed by the fish culturist.

As fish developed, feed was delivered to the tank wall and water interface by a belt feeder in excess of need.

Common fish culture practices include cleaning tanks and screens daily on an ‘as needed’ basis. The monitoring of feeding activity was likewise observed daily. In this fashion, the care and culture of sturgeon, especially for the younger, more vulnerable life stages was continuous throughout the working day.

8.1 Juvenile Rearing

During the juvenile rearing process, fish are graded based on size to improve growth and survival. At grading, fish were hand-picked into either large or small categories and placed into separate tanks. This was the first time during the rearing process when a complete inventory of fish was established. Numbers for all prior milestones of development were then back-calculated from this point. The splitting of fish between tanks decreases densities and reduces tank effects on growth. Secondly, non-competitive access to feed is important to the smaller, downgraded fish. These fish will recover from any feeding competition and quickly establish a higher growth rate. As post-release survival is estimated to be positively influenced by size at release, it is important that annual size at release targets are met. As a standard fish culture practice, feed rations are set based on fish biomass (percent feed per day). As biomass increases so does the size and amount of feed provided to ensure consistent growth.

During grading, smaller fish remained in troughs or smaller circular tanks until they caught 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 occurred equally from all tanks to ensure that artificial genetic selection was minimized to the extent possible. Briefly, fish were randomly selected from rearing containers using small nets and counted out into a vessel containing 500mg/l TMS (tricaine methanesulphonate) according to FFSBC Standard Operation Procedure: Euthanasia. Culling continued until the desired numbers of fish remain in the culture container.

8.2 Wild Progeny

In the Spring of 2014 a streamside rearing facility was piloted on the banks of the Columbia River near Waneta. The facility was constructed within a 14 by 8 foot cargo trailer for transportability. The trailer was parked at a streamside location just upstream of Waneta eddy, supplied with power and plumbed to allow river water to be pumped through a series of MacDonald Jar upwellers set up over small rearing troughs. Wild source eggs were collected in river and taken directly to the container to be placed into flowing MacDonald Jars. There they were incubated to hatch and the larvae retained in a compartmentalized collection trough (equipped with Bioballs for cover and hiding) for a maximum of 7 days post-hatch. Larvae need to be in the hatchery for rearing before 10 days of age to ensure they are successfully weaned onto feed, so FFSBC staff made regular trips to the facility to retrieve larvae and transport them back to KTH. At the KTH, wild larvae were reared in quarantine using the same juvenile rearing techniques and protocols described in section 8.1, and the same marking and tagging protocols described in section 10.2, with the exception of the lateral scute removal, which was done on the right side of the wild progeny fish. No culling of wild progeny took place as it was determined that all available fish would be released. Fish were kept in separate groups based on the date of collection in the river, and all mortalities were preserved in ethyl alcohol for DNA samples, until all fish were large enough to have tissue samples taken and preserved. At this time, the three groups were mixed and treated as one for the duration of the rearing time.

9.0 Autopolyploidy Assessment

The ploidy of wild white sturgeon has previously been determined to be 8N in Columbia and Kootenay River populations. Concern was raised when a large number of 12N white sturgeon were discovered in progeny family groups which were derived from wild Kootenay River white sturgeon brood and were being cultured in the artificial rearing environment at the Kootenay Tribe of Idaho's (KTOI) Sturgeon Hatchery. The mechanism of this ploidy shift is unknown but it is thought to occur at the fertilization stage. In partnership with a commercial sturgeon farm and with the assistance of University of California at Davis, blood samples were collected from family groups at KTOI and sent to UC Davis for Flow/Cytometry analysis. Through this process it was determined that some of the family groups at KTOI were composed of up to 50% 12N sturgeon. Given that there are potential implications to the Upper Columbia River recovery program, the Freshwater Fisheries Society, under the direction of the TWG, assessed the

ploidy of the white sturgeon families being reared at KTH. Two families were composed of some level of 12n individuals: Based on an initial analysis Family 1 was composed of approximately 13% 12N, and a subsequent re-submission (n=51) was found to be 11.8% 12N the remaining five families were found to be 100% 8N. As in 2013 it was determined that there were benefits associated with maximizing genetic contribution by releasing as many 8N individuals from family 1 as possible. For this reason samples were taken from each individual in family 1 (n=1693) and sent to the FFSBC's Fish Health Lab for analysis. A total of 124 fish were confirmed 12n from this sample (7.3%) and were removed from the group (see Table 8).

Table 8. Percent of 12n white sturgeon individuals among family groups reared at the Kootenay Trout Hatchery in 2014.

Family	# Samples	# Confirmed 12N	% Confirmed 12N
1	100	13	13
1 (re-sub)	51	6	11.8
2	100	0	0
3	100	0	0
4	100	0	0
5	100	0	0

10.0 Releases

10.1 Larval Releases

There were no larval releases of 2013 year class sturgeon.

10.2 Juvenile Releases

This year the stocking number for the Lower Columbia section was to include as many wild progeny sturgeon as possible, with the addition of juveniles produced from broodstock crosses to make up the release target of 4,000. A total of 1,951 larvae were transported to the KTH, from three separate spawning "events" that were estimated to occur at Waneta Eddy. Survival was good, approximately 62% from larvae to release age (see Table 9). It was determined that wild progeny would be released as close to place of origin as possible, so Beaver Creek below Trail was chosen as the release site. This meant that a total of 2,800 hatchery spawned juveniles were released into the Lower Columbia (see Table 11). As in previous years, as many juveniles as possible (up to 7,000) were to be released into the Mid-Columbia section, at

a greater (75g+) size, but this year it was determined that a portion of each family was to be held for an extra year, to be released as two year olds at a larger size (>300g).. As a result, a total of 3,288 juveniles were released into the Mid-Columbia reach, at Shelter Bay near Revelstoke BC.

Table 9. Numbers of wild origin White Sturgeon collected from the lower Columbia River and reared at KTH from July 2014 to May of 2015.

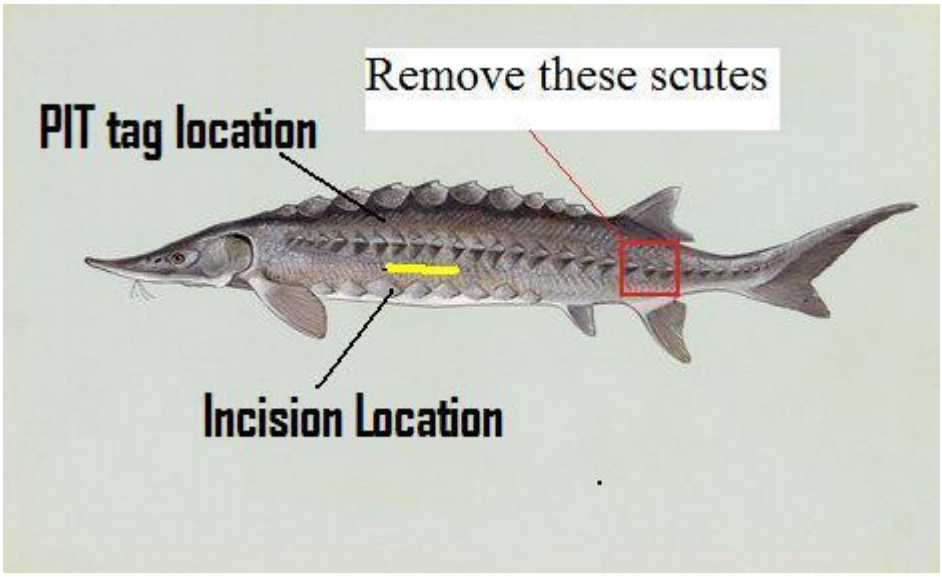
	Arrival at KTH	Number Larvae	Number Dec 1	Number Released Spring '15	Number Held to Fall '15
Event 1	July 1-3	130	80		
Event 2	July 10-15	1737	1173		
Event 3	July 18-22	84	48	1095	139

Table 10. Total number of hatchery reared juvenile White Sturgeon released annually into the Columbia River in Canada and the United States from 2002 – 2015.

	Canada		USA		
Release Year	Fall	Spring	Spring	Fall	Total
2002		8,671			8,671
2003		11,803			11,803
2004		9,695	1,881		11,576
2005		12,748	3,755		16,503
2005	5,039				5,039
2006		10,828	4,351		15,179
2006	4,042				4,042
2007		8,123	3,422		11,545
2007	4,029				4,029
2008		6,448	3,821		10,269
2009		4,141	3,537		7,678
2010		3,947	3,873		7,820
2010				522	522
2011		4,010	3,869		7,879
2011				3,590	3,590
2012		4,000			4,000
2012				302	302
2013		4,037			4,037
2014		1,801			1,801
2015		7,183			7,183
	13,110	97,435	28,509	4,414	143,468

In February the juvenile fish were individually handled to insert a PIT tag into the dorsal musculature at the midpoint between the dorsal and lateral scute line inferior to the anterior margin of the dorsal fin (see Diagram 1). PIT tag, length and weight data was recorded for each individual fish starting in mid-April, to be as close to release time as possible. This was done to ensure no additional growth occured in-hatchery that may be attributed to post-release growth. Each individual fish can subsequently be identified to its 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.

Diagram 1: Scute removal and PIT tag locations.



Juvenile releases took place in the spring of 2015 over a period of three days, May 5th, 7th, and 8th. Fish from hatchery crosses of broodstock were released in the Lower Columbia Reach (LCR) at Millennium Park in Castlegar (n = 1,000), Gyro Park in Trail (n = 600), Genelle (n = 600) and Robson (n = 600). Wild origin progeny were released at Beaver Creek near Trail (n = 1,095), which is the closest release site to the streamside facility where they were hatched. Releases took place in the Mid-Columbia Reach (MCR) at Shelter Bay near Revelstoke (n = 3,288) (see Table 11).

Table 11. Columbia juvenile release summary, Spring 2015

Columbia Sturgeon Releases 2015	Release Date	River (km)	Family	Number	Average FL (cm)	Average weight (g)	Weight (kg)
LCR - Millennium Park	05/05/2015	10.5	1	200	25.3	126.0	25.1
			2	200	23.8	96.0	19.2
			3	200	24.2	100.0	19.9
			4	200	24.2	99.0	19.7
			5	200	25.0	119.0	23.8
			Totals	**1000**			**107.7**
LCR – Gyro Park, Trail	05/05/2015	39.5	1	120	25.2	124.0	14.8
			2	120	24.0	101.0	12.1
			3	120	24.1	98.0	11.7
			4	120	24.1	97.0	11.6
			5	120	24.5	104.0	12.5
			Totals	**600**			**62.7**
LCR - Genelle	08/05/2015	24.0	1	120	25.0	118.0	14.1
			2	120	23.5	95.0	11.4
			3	120	24.0	97.0	11.6
			4	120	25.0	96.0	11.5
			5	120	25.1	120.0	14.4
			Totals	**600**			**63.0**
LCR – Robson upper b/l	08/05/2015	4.0	1	120	24.7	120.0	14.4
			2	120	24.1	99.0	11.9
			3	120	24.5	104.0	12.5
			4	120	24.0	92.0	11.1
			5	120	25.2	120.0	14.4
			Totals	**600**			**64.3**
MCR - Revelstoke	07/05/2015		1	503	27.5	152.0	76.4
			2	751	27.2	146.0	109.0
			3	586	26.8	140.0	82.0
			4	678	26.7	125.0	84.8
			5	765	27.8	158.0	120.8
			Totals	**3283**			**473.0**
Beaver Creek (Wild Progeny)	05/05/2015	49.0		1095	29.0	172.5	189.0
	Production	**LCR**		**2800**			**290.7**
		MCR		**3283**			**473.0**
	Wild Progeny	**LCR**		**1095**			**189.0**

10.3 Release Events

Each year large school and public release events are planned and organized by Angus Glass of the Fish & Wildlife Compensation Program (FWCP) and FFSBC staff, along with the assistance of 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 FWCP is an active partner in the Upper Columbia White Sturgeon Recovery Initiative (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 organized 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.

This year's releases included school events at Millennium Park in Castlegar, with approximately 700 students attending, Beaver Creek near Trail, with approximately 300 students, and at Shelter Bay near Revelstoke, with approximately 200 students involved. Public events were held at Gyro Park in Trail and at Shelter Bay, and as in years past were very well attended and received.

11.0 Fish Health Testing Summary

11.1 Virus Screening for Broodstock and Juveniles

Samples for virus screening were collected from all mature adults (ovarian fluid and milt) and a subsample of juveniles was collected from all family groups (5-30 per family). Fluids were collected from egg or milt samples and frozen at -20 prior to testing. All samples were screened for viruses (IPNV, WSHV1, WSHV2 and WSIV) 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 (Fisheries and Oceans 2011)[1]. All results were negative for viral or bacterial contamination and are included in Appendix 1.

11.2 Deformities

Very few deformities were observed in the 2014 year class. 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 pattern of deformities was observed, the majority noted were minor fin deformities.

12.0 Permits

In June of 2013 a five year SARA permit was obtained for all the Columbia sturgeon culture activities including adult transport, holding, spawning, rearing, research and releases. This permit is valid to June 2018. All necessary Introductions and Transfer Committee (ITC) permits for adult and juvenile transfers were obtained..

[1] Fisheries and Oceans Canada. 1984 (revised 2011). Fish Health Protection Regulations: Manual of Compliance (Fish. Mar. Serv. Misc. Spec. Publ. 31(Revised): iv+50p. Available at: http://www.dfo-mpo.gc.ca/science/environmental-environnement/aah-saa/regulation-reglements-eng.htm#x

Appendix 1.0 White Sturgeon Sexual Maturity Codes

SexMatCode	Se	Development State Description
00	Male	General unknown maturity (male).
01	Male	Non-reproductive, testes appear as thin strips with no pigmentation.
02	Male	Maturing, small testes; some folding may be apparent; translucent, smoky pigmentation.
03	Male	Early reproductive; large testes, folds beginning to form lobes; some pigmentation still present; testes more white than cream colored.
04	Male	Late reproductive; testes large, often filling posterior of body cavity; white with little or no pigmentation.
05	Male	Ripe; milt flowing; large white lobular testes; no pigmentation.
06	Male	Spent; testes pinkish-white, flaccid, and strongly lobed.
10	Female	General unknown maturity (female).
1000	Unknown	Data not available/located in historical database.
11	Female	Non-reproductive; ovaries small, folded with no visible oocytes; tissue colour white to yellowish.
12	Female	Pre-vitellogenic, moderate size ovary with small eggs present (0.2 to 0.5 mm diameter); may have "salt and pepper" appearance.
13	Female	Early vitellogenic; large ovary varying in colour from white to yellowish-cream to light grey; eggs 0.6 to 2.1 mm in diameter.
14	Female	Late vitellogenic; ovaries large with pigmented oocytes still attached to ovarian tissue; eggs 2.2 to 2.9 mm in diameter; sometimes with salt and pepper
15	Female	Ripe; eggs fully pigmented and easily detached from ovarian tissue; eggs 3.0 to 3.4 mm in diameter.
16	Female	Spent; ovaries are flaccid with some residual fully developed eggs.
17	Female	Pre-vitellogenic with attritic oocytes; small eggs (<0.5 mm diameter) present; dark pigmented tissue present that may be reabsorbed
30	Both	Hermaphrodite
97	Unknown	Adult based on size, no surgical examination.
98	Unknown	Juvenile/Sub-adult based on size, no surgical examination.
99	Unknown	Gonad undifferentiated or not visible during surgical examination.
F0	Female	Female based on previous capture; general unknown maturity.

Appendix 1.1 White Sturgeon Sexual Maturity Description

SexMatCode	Sex	Development State Description
F1	Female	Early developing female; pinkish/beige ovarian tissue with brain-like folds and smooth to rough surface, imbedded in heavy strip of fat tissue. Visible whitish eggs are <0.5mm in diameter. Ovarian tissue of previously spawned fish often appears ragged.
F2	Female	Early "yellow egg" female; Yellowish/beige ovarian tissue with deep "brain-like" folds embedded in extensive fat tissue giving it a bright yellow appearance. Eggs, 1 to 2 mm in diameter with no apparent greyish pigmentation.
F3	Female	Late "yellow egg" female; large yellowish ovaries with deep lateral folds and reduced associated fat. Yellow/greenish to grey eggs 2.5 mm in diameter. May indicate next year spawning.
F4	Female	"Black egg" female; Large dark ovaries filling much of the abdominal cavity. Exhibiting a distinct "bulls-eye". Very little fat, Eggs are still tight in the ovary, dark grey to black, shiny and large, >3 mm in
F5	Female	Spawning female; Loose flocculent-like ovarian tissue with eggs free in body cavity shed in layers from deep ovarian folds. Eggs large, from grey to black, similar to F4.
F6	Female	Post spawn female; ovaries immediately after spawning are folded with a mushy pinkish & flaccid appearance, with little/no associated fat. Displays a characteristic abdominal mid-line depression. Large dark degenerated eggs buried amongst small oocytes.
Fv	Female	Virgin female juvenile; small feathery looking, beige ovarian tissue attached to a thin strip of adipose fat tissue.
M0	Male	Male based on previous capture; general unknown maturity.
M1	Male	Developing male; Testes are tubular to lobed, light to dark grey, and embedded in substantial amounts of fat. Testes moderately to deeply lobed have distinct lateral folds.
M2	Male	Fully developed male; Testes large, cream to whitish in colour, deeply lobed and filling most of the abdominal cavity. If captured during active spawning, may release sperm if stroked posteriorly along the abdomen.
M3	Male	Spent/recovering male; Testes size are much reduced, with very distinct lobes and whitish to cream colour.
Mv	Male	Virgin male juvenile; Testes are ribbon-like in appearance with lateral creases or folds, dark grey to cream coloured attached to a strip of adipose fat tissue.

Appendix 2.0 Research Projects

Appendix 2.1 Larval quality indicators study: Background and Research proposal

Fertilized eggs – 10,000 split equally between 4 full sib families.
Unfertilized eggs – 10-20g per female (total 4)
Milt – 10-20 ml milt
Principal investigators: Steve McAdam, BC MOE, Colin Brauner (UBC Zoology), and Sang Yun (UBC Fisheries)
Permit details: 1) UBC Animal care permit: A07-0007; 2) ITC permit for the facilities: #11691; 3) SARA permit: XRSF 20 2014
Purpose/study title: Investigations of the effect of the effects of gravel exposure for yolksac larvae will be continuing. In addition an investigation of ionoregulatory development (i.e. how larvae take up Na, Ca and possibly K from hatch to early feeding larval stage at ~ 25 dph). Additionally, unfertilized eggs and milt are being collected to support some initial genetic sequencing trials. All experiments will be conducted in Colin Brauner's lab at UBC. The tests of substrate effects are closely linked to recovery and this year's will focus on 1) The effect of light regime and 2) the effects of holding yolksac larvae in moving water (which is a closer approximation to riverine conditions).
Location: UBC, Vancouver.
Disposition of fish/embryos: All fish were either sampled or euthanized at the end of this study, as per ITC requirements.
Description of study: Rear larvae under 3 combinations of substrate and flow. Samples taken at 12 dph. Three substrate treatments will be compared based on responses including survival, larval weight, larval appearance, gut development. Previous studies of salmonids and white sturgeon suggest that the effects of substrate condition result from energetic effects caused by decreased movement when substrate is available. Rearing larvae in moving water will directly test this mechanism. Pilot tests in 2013 suggest effects will be clearly detected, but those results need to be confirmed.
The study of ion regulation is not as specifically tied to recovery needs, but is a more basic inquiry into the biology of this species, and in particular how ion uptake is regulated during early development. If variation is seen between substrate treatments it could be informative about the mechanisms and effects of different habitat conditions. In addition the eggs/milt for

genomic analysis will provide information that can assist recovery and management of white sturgeon throughout the province.

Details of how results will be used: Results will be used to support ongoing research programs in white sturgeon. In particular result will assist interpretation of the condition/quality of wild caught larvae. A TWG member is directly involved in conducting this research (Steve McAdam), which helps ensure the work addresses issues relevant to sturgeon recovery. Results will be communicated to the TWG in a timely manner, and reports and publications produced as a result of this work will be provided to the TWG.

Appendix 2.2 DNA SAMPLING

No DNA samples were taken from adults or hatchery spawned juveniles in 2014. DNA samples were taken from all wild progeny fish, either by preserving mortalities or tissue samples of live fish.

Appendix 2.3 Egg Predation Study, Golder and Associates Ltd.

Fertilized eggs – 500

Principal investigator: Golder Associates on behalf of Columbia Power Corporation

Permit details: SARA, ITC, Provincial collection permit

Purpose/study title: Waneta White Sturgeon Egg Predation Monitoring

Location: Below Waneta Dam at the confluence of the Pend d'Oreille and Columbia rivers

Disposition of fish/embryos: Eaten by egg predators or disposed of at the end of each experiment

Description of study: Demonstrate that post-Project flow changes when the White Sturgeon Flow Augmentation Program – Post Project Enhancement (WSFAP-PPE) is in effect do or do not result in increased White Sturgeon egg predation to a degree that will have any detectable effect on recruitment success.

An underwater camera, known as a MatCam, used to document evidence of egg predation by recording egg loss and egg predators in recorded images. A DIDSON camera was also used to record egg predator density in the vicinity of the MatCam. DIDSON cameras and seeded

MatCams were deployed concurrently at two locations to monitor egg predator densities, determine species composition, and detect egg predation.

Report provided to CPC and TWG.

Appendix 2.4. Larval release strategy and hydro-power operations' effect on dispersing larvae.

Larvae – 40,000 pre-feeding larvae.
Principal investigator: Matthew Howell, Fishery Biologist, Colville Confederated Tribes
Permit details:

- A CITES permit will be obtained by the exporter.
- The importer (CCT) will obtain the following permits:
    - Washington Department of Fish and Wildlife Fish Transport permit;
    - Idaho Fish and Game Fish Transport permit;
    - FDA Prior Notice Permit for transport;
    - USFWS AADAP INAD for SE-MARK (INAD #10-987);
    - USFWS Designated Port Exception permit and Declaration for Importation or Exportation of Fish or Wildlife permit;
    - Fisheries and Oceans Canada Introduction or Transfer of Fish or Aquatic Invertebrates (ITC) permit;
    - Species at Risk (SARA) permit.

Purpose/study title: A paired larval release strategy to test the hypothesis that hydro-power operations are the ultimate cause of white sturgeon recruitment failure in the Transboundary Reach of the Columbia River by limiting encounter rates of dispersing larvae with suitable rearing habitat.
Location: The study will occur in the Roosevelt Reach of the Upper Columbia River, WA, USA.
Disposition of fish/embryos: Marked larvae were released into the Roosevelt Reach. As such, the disposition of fish both during and after the study will be dependent upon natural in-river processes. Marked fish recaptured during annual Lake Roosevelt Sturgeon Recovery

Project (LRSRP) and Fall Walleye Indexing (FWIN) fall gill net surveys, will be euthanized, assessed for marks, and either be disposed of appropriately or be fixed for archiving.

Short description of study: This study represents a pilot effort aimed at developing methods to empirically test the hypothesis that persistent recruitment failure in the Transboundary Reach sturgeon population is due to hydro-power operations limiting encounter rates of first-feeding white sturgeon larvae with suitable rearing habitat. Field surveys conducted in the Roosevelt Reach to date suggest that in most years the bulk of first-feeding larvae produced are transported downstream no further than the upper river-reservoir transition zone. LRSRP and FWIN fall gill net surveys in the Roosevelt Reach over the past decade have consistently failed to capture sub-yearling or yearling wild juveniles indicating that suitable early rearing habitat in the upper transition zone is limited. This may be due to some combination of widespread contamination (e.g. deposits of granulated industrial slag), high concentrations of predators (e.g., sculpins and walleye), limited food availability, or some other unknown factor(s).

Details of how the results will be used: The 2014 study results will be used to inform future experimental release strategies. Results will be reported to the Aquatic Animal Drug Approval Partnership (AADAP) and will be communicated to the UCWSRI TWG during the November 2014 and/or April 2015 meetings.

Appendix 2.5. Investigations into the sensitivity of White Sturgeon to contaminants

Fertilized eggs: 10,000

Principal investigator: Markus Hecker, Ph.D.; University of Saskatchewan

Permit details: SARA permit SARA 305**,** BC Transfer of Fish Permit ITC Application #13410

Purpose/study title: Investigation of the sensitivity of white sturgeon to environmental contaminants of concern in Canadian surface waters

Location: University of Saskatchewan

Disposition of fish/embryos: All fish will be euthanized in buffered MS222.

Short description of study: The studies conducted under this permit are part of ongoing investigations of the sensitivity of White Sturgeon (*Acipenser transmontana*) to environmental pollutants such as heavy metals, persistent organic pollutants (POPs), selenium, and other priority contaminants. Earlier studies by my research group have shown that white sturgeon

are highly sensitivity to a range of environmental contaminants of concern including heavy metals, persistent organic pollutants (POPs) and selenium (see Vardy et al. 2011, 2013a,b; Doering et al. 2013, 2014). This unexpected high sensitivity to contaminants is of great concern as it may indicate that current regulations and guidelines aimed to protect aquatic wildlife are not protective of this and potentially other sturgeon species that are currently listed as endangered. Thus, to enable appropriate risk assessment of sturgeon species in Canada, North America and around the world it is critical to understand the sensitivity of these species to environmental contaminants present in surface water and sediments.

Together, these studies will further our understanding regarding the sensitivity of white sturgeon to contaminants of environmental concern in Canada and worldwide.

Details of how the results will be used: The successful completion of the above studies will enable establishing predictive models with the goal to predict sensitivity of white sturgeon and other endangered fish species to environmental contaminants of concern, which ultimately will lead to improved environmental risk assessments and better protection of fish species of concern.

The data generated with these fish will be published in the peer reviewed literature, and all papers will be shared with the hatchery and other stakeholders, and will be publicly available.

Appendix 3: Fish Health Testing

The 2014 Columbia sturgeon year class reared well. There were no diagnostic cases submitted for the 20 year class. There were 5 families reared and tested in 2014. Results from the Broodstock testing (Table 1), juvenile Family testing (Table 2), and Pre-release submissions (Table 3) are summarized in table forma 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 Hematopoietic 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 hav been obtained and propagated from UC Davis lines.

White Sturgeon Skin (WSSK)
White Sturgeon Gonad (WSGo)
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 Gonad (WSGo)
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.

Table 1: Broodstock Testing for 2014:
Please note spawning dates were not provided with submitted samples

Case Number:	2014-1049						
Sample Tissue		Virology Cell lines				Bacteriology	
Reproductive fluid	PIT Number	CHSE-214	EPC	WSSK	WSGo	TSA	HS
Ovarian fluid	985161000179510	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Ovarian fluid	15619232A	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Ovarian fluid	985120030505824	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	Positive for *Flavobacterium psychrophilum* by PCR
Ovarian fluid	985120022601339	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	Positive for *Flavobacterium psychrophilum* by PCR
Ovarian fluid	985120020810967	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Milt	7F7D1C6D5C	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	Positive for *Flavobacterium psychrophilum* by PCR
Milt	7F7D4F162A	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Milt	985120019170580	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Milt	900254000130209	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	Positive for *Flavobacterium psychrophilum* by PCR
Milt	900254000111534	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	Positive for *Flavobacterium psychrophilum* by PCR

Table 2: 45-60 day Juvenile Family testing results 2014:

Case Number	Family ref #	# fish submitted	Virology				Bacteriology	
			CHSE-214	EPC	WSSK	WSGo	TSA	HS
2014-1073 Submission date: SEP 10, 2014	Fam 1	60 Total 30 Virology 30 Bacteriology	NEG	NEG	NEG	NEG	30/30 No pathogenic bacteria detected	4/30 Yellow pigmented colonies. Positive for *Flavobacterium psychrophilum* by PCR
2014-1074 Submission date: SEP 10, 2014	Fam 2	60 Total 30 Virology 30 Bacteriology	NEG	NEG	NEG	NEG	30/30 No pathogenic bacteria detected	30/30 No pathogenic bacteria detected
2014-1075 Submission date: SEP 10, 2014	Fam 3	60 Total 30 Virology 30 Bacteriology	NEG	NEG	NEG	NEG	30/30 No pathogenic bacteria detected	30/30 No pathogenic bacteria detected
2014-1076 Submission date: OCT 2, 2014	Fam 4	60 Total 30 Virology 30 Bacteriology	NEG	NEG	NEG	NEG	30/30 No pathogenic bacteria detected	30/30 No pathogenic bacteria detected
2014-1077 Submission date: OCT 2, 2014	Fam 5	60 Total 30 Virology 30 Bacteriology	NEG	NEG	NEG	NEG	30/30 No pathogenic bacteria detected	30/30 No pathogenic bacteria detected

Table 3: Pre-release sample test for brood year 2014 submitted Feb 19th, 2015.

Case Number	Family ref #	# fish submitted	Virology Salmonid		Virology Sturgeon		Bacteriology	
			CHSE-214	EPC	WSSK	WSGo	TSA	HS
2015-1018 Submission date: Feb 19th, 2015	Mixed all families, 2013 brood year	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.	60/60 No pathogenic bacteria detected	60/60 No pathogenic bacteria detected

Summary Comments:
The 2014 Columbia sturgeon families reared well during larval rearing.

There were no few diagnostic issues during rearing.
There were some yellow pigmented colonies isolated from ovarian fluids, milt samples and from Family 1 during the 45-60 day test period. These were confirmed positive for *Flavobacterium psychrophlium* using the PCR confirmation method. This is just an incidental bacterial finding and not linked to a diagnostic event.
The pre-release sample results indicate that the 2014 year class of Columbia White Sturgeon appear to be healthy and free of any viral or bacterial pathogens. Hatchery records indicate losses during rearing have been minimal. The health status of the stock for release is considered to be very good.

2015 Columbia White sturgeon ploidy results:

Ploidy was again tested this year on all family groups. A sample size of 100 slides was submitted from each family group (Results viewed in Table 4). Only Family 1 was found to have a high percentage of 12N fish. These results were double checked with a second submission of 51 slides (Table 5). Results were still found to be high. A decision was made by the TWG committee to ploidy sample all remaining fish in Family 1 and remove any 12N fish therefore allowing the bulk of the family group to be released into the river population (Table 6). The final results for Family 1 showed 124 of 1693 (7.3%) fish sampled to be of 12N ploidy. These 124 fish were removed from the population prior to release.

Table 7 shows the ploidy results for the Columbia River White Sturgeon wild larvae component. There were 1098 slides processed in this group with all wild sturgeon within target range size being sampled. There is a small group of additional wild larvae that were not sampled at this time as they were too small at time of sampling and are being held over for a Fall release. They will be sampled for ploidy in the summer. These were 2 of 1098 (0.2%) wild larvae identified to be of 12N ploidy.

It remains undetermined what causes the 12N ploidism to occur in the fish population.

Additional ploidy imaging and evaluation was also done on slides collected during brood capture period and during the Fall Juvenile assessment.

Table 4: Initial ploidy results for all 2014 CWS family groups based on sample size of 100 fish per family group:

Family	Date imaged	Confirmed 12N slide#'s	Total # 12N
Family 1	Oct 21	20	80%
Family 2	Oct 29	0	100%
Family 3	Nov 13	0	100%
Family 4	Nov 17	0	100%
Family 5	Nov 19	0	100%

Table 5: Ploidy results for Columbia White Sturgeon Family 1 additional 51 slides submitted:

Family	Date imaged	Confirmed 12N slide#'s	Total # 12N
Family 1	Jan 8	5	90%

Table 6: Ploidy results for Family 1 March 31, 2015 pre-release testing.

Ploidy Results for CWS FAM 1 BY 2014
N= 1693 slides

Group	Slide range	Date imaged	Confirmed 12N slide#'s	Total # 12N
GrpA	1-100	Feb 19	**5, 26, 35, 48, 55, 58, 90**	7
GrpB	101-200	Feb 26	**110, 135, 147, 148, 169**	5
GrpC	201-300	Mar 2	**259, 265**	2
GrpD	301-400	Mar 3	**321, 324, 356, 369, 374**	5
GrpE	401-500	Mar 4	**403, 405, 415, 425, 433, 454, 458, 472, 484, 487, 490, 495, 497, 500**	14
GrpF	501-600	Mar 5	**520, 534, 581, 566**	4
GrpG	601-700	Mar 6	**602, 612, 624, 627, 641, 664, 669**	7
GrpH	701-800	Mar 10	**727,732, 743, 757, 782, 790, 792, 799**	8
GrpI	801-900	Mar 11	**806, 820, 836, 852, 860, 882, 884**	7
GrpJ	901-1000	Mar 16	**906, 928, 929, 951, 958, 960, 962, 970, 972, 967, 983, 984, 986**	13
GrpK	1001-1100	Mar 17	**1037, 1041, 1051, 1052, 1094**	5
GrpL	1101-1200	Mar 18	**1115, 1140, 1144, 1161, 1162, 1190, 1194**	7
GrpM	1201-1300	Mar 20	**1237, 1242, 1249, 1250, 1255, 1256, 1259, 1269, 1270, 1285**	10
GrpN	1301-1400	Mar 23	**1302, 1310, 1323, 1335, 1347, 1367, 1384**	7
GrpO	1401-1500	Mar 24	**1408, 1413, 1423, 1426, 1441, 1463, 1466, 1473, 1479, 1492**	10
GrpP	1501-1600	Mar 25	**1511, 1524, 1525, 1529, 1558, 1578**	6
GrpQ	1601-1693	Mar 27	**1605, 1624, 1653, 1660, 1665, 1673, 1687**	7

Total slides = 1693
Total 12N = 124
% 12N = 7.3%

Table 7: Ploidy for Columbia White Sturgeon wild larvae Brood year 2014

April 30, 2015

Ploidy Results for CWS wild larvae BY 2014
N= 1098 slides

Group	Slide range	Date imaged	Confirmed 12N slide#'s	Total # 12N
GrpA	1-100	Apr 15	--	0
GrpB	101-200	Apr 16	--	0
GrpC	201-300	Apr 17	--	0
GrpD	301-400	Apr 20	--	0
GrpE	401-500	Apr 21	**#454, #409**	2
GrpF	501-600	Apr 22	--	0
GrpG	601-700	Apr 24	--	0
GrpH	701-800	Apr 27	--	0
GrpI	801-900	Apr 28	--	0
GrpJ	901-1000	Apr 29	--	0
GrpK	1001-1100	Apr 29	--	0
GrpL	1101-1200			
GrpM	1201-1300			

Total slides = 1098 to date
Total 12N = 2
% 12N = 0.2%

Summary prepared by: **Date prepared:** May 25th, 2015

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