Columbia River Project Water Use Plan

Columbia White Sturgeon Management Plan

White Sturgeon Conservation Aquaculture

CLBWORKS-25: Mid-Columbia River White Sturgeon Conservation Aquaculture (Year 2)

CLBWORKS-34: Lower Columbia River White Sturgeon Conservation Aquaculture (Year 6)

Works Period: May 2013 – May 2014

Prepared by: Freshwater Fisheries Society of British Columbia

Ron Ek
Mike Keehn
Ken Scheer
Adrian Clarke

Freshwater Fisheries Society of British Columbia
Kootenay Trout Hatchery
Fort Steele, British Columbia, V0B 1N0

May 10, 2014

COLUMBIA WHITE STURGEON CONSERVATION FISH CULTURE PROGRAM

KOOTENAY STURGEON HATCHERY

2013 Annual Report

Ron Ek and Mike Keehn

May 10, 2014

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. 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 2013. Juvenile sturgeon were released into the Mid-Columbia (n=6,013) and Lower Columbia (n=1,800) Rivers, and all targets associated with the various research egg requests were met.

Capture of mature adult white sturgeon for use as hatchery broodstock was conducted over a five week period, starting on June 10th, and ending July 12th, 2013. Effort was dispersed throughout the lower Columbia River from Hugh Keenlyside dam (HLK) in Castlegar, BC, down to the Canadian/USA border below Trail, BC. Eight mature female and eight mature males were captured and transported to the Kootenay Trout Hatchery (KTH) Sturgeon Facility. Two spawning events were conducted in 2013, with the first occurring on the 26th of June and the second on July 17th. Six of the females were successfully spawned (three on the first spawn and three on the second spawn) with three of the males (two on the first spawn and one on the second spawn) reating a total of nine half sibling families. The two unused females were returned to the river pre-spawn and one of the females was implanted with an acoustic transmitter prior to release so movements post release could be monitored (e.g. spawning location).

The two spawning events produced a total of 418,759 green eggs. Fertility, neuralization and hatch rates were variable in 2013, with neuralization ranging from as low as 35.5%, and as high as 75% between family groups in the first spawn. The second spawning event, where only one male was used, had a much higher and consistent neuralization rate, ranging from 87.6% to 93.0% in the family groups.

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. Five projects building on prior studies of white sturgeon larvae.
- Golder and Associates Ltd. used eggs (n=500) on mats with cameras to study predation at Waneta Eddy.
- In addition, blood samples were collected to assess stress cortisol levels in white sturgeon for Jon Wong, UBC

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. 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, assessed the ploidy of the white sturgeon families being reared at KTH. Two families were identified as having a proportion of 12n individuals: Family 1 was composed of 3% 12n sturgeon while Family 4 was composed of 6 % 12n individuals. Subsequently, we assessed the ploidy of each individual to be released from Family 4 (n=200) to ensure only 8n white sturgeon were released into the lower Columbia River.

DNA samples were collected by taking tissue samples from the dorsal fin of all adults and from a subset of juveniles from each half-sib family. These samples were then preserved in ethanol. The DNA tissue was shipped to UBC and BC Hydro for analysis. 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 2014 on May 1st and 5th in the lower Columbia River and on May 7th in the Mid-Columbia River. A total of 1,800 juvenile sturgeon were released into

the Lower Columbia River between Hugh Keenlyside dam (HLK) near Castlegar BC, and the USA border. This release number was lower than the target of 4,000 due to a lower number of contributing males compared to previous years. Releases in the lower Columbia River occurred at HLK (n=900), Kootenay Eddy (n=300), Genelle (n=300) and Beaver Creek (n=300). Average weight of these fish was 100 grams. An additional 6,013 juvenile sturgeon were released into the Mid-Columbia River in the Revelstoke area. All of these were released at Shelter Bay Provincial Park and averaged 202 grams in weight. There were school and public release events associated with the sturgeon releases at HLK in Castlegar and Shelter Bay near Revelstoke with over 850 school kids participating. Due to lower juvenile release numbers, the public event which is normally held at HLK had to be cancelled. Instead, a public event was held at the Castlegar and District Community Complex and the Waneta Plaza on May 5th. The public was invited to come to the site to see and touch the juveniles before their release into the Columbia, and to have any questions answered and concerns addressed.

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.

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 caught using setlines and transported to the Kootenay Trout Hatchery (KTH) sturgeon facility from June 10th through to July 12th, 2013. In all, eight female fish with near mature eggs and eight male fish were retained and transported to the KTH. 2013 proved to be a difficult year for finding producing males with flowing milt or even mature gonads. The capture program duration was extended for longer (5 weeks) then previous years (3-4 weeks) to try to collect more mature males.

All fish were returned to the Columbia River as near to capture location as possible. Prior to their release, all fish had ovarian fluid or milt collected, and a fin clip taken, in duplicate, for DNA analysis. Tissue samples were stored in ethanol and stored at KTH before shipping to UBC and BC Hydro for permanent storage. To assist with a UBC study on stress cortisol levels on captured sturgeon we collected blood samples according to study directions on all sturgeon adults prior to spawning, at spawning and prior to release back into the river. Table 1 and Table 2 show 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. Capture locations, size, and timing of spawning of adult White Sturgeon broodstock in 2013.

Spawn Year	Sex	Pit Tag#	Weigh t (kg)	Length (cm)	Capture Date	Capture River Km	Capture Location	Spawn Date	Release Date	Release River Km	Release Location
2013	F	985121001732553	45	177	6/12/13	10.5	Kootenay Eddy	6/26/13	7/04/13	7.0	Robson Eddy
2013	F	4239084A26	42	176	6/12/13	6.5	Sturgeon Island	6/26/13	7/02/13	7.0	Robson Eddy
2013	F	985120022626419	75	205	6/17/13	56.0	Waneta Eddy	6/26/13	7/02/13	7.0	Robson Eddy
2013	F	4158445F7C	47	180	6/19/13	56.0	Waneta Eddy	7/17/13	7/22/13	49.0	Beaver Creek
2013	F	1F4B4B7952	79	201	6/20/13	56.0	Waneta Eddy	n/a	7/19/13	49.0	Beaver Creek
2013	F	7F7D190073	96	208	6/28/13	54.9	American Eddy	7/17/13	7/23/13	49.0	Beaver Creek
2013	F	7F7D185C29	84	225	7/09/13	56.0	Waneta Eddy	7/17/13	7/22/13	49.0	Beaver Creek
2013	F	7F7D4F6C19	88	225	6/20/13	52.3	Fort Shepherd Eddy	n/a	6/24/13	49.0	Beaver Creek
2013	M	4238456407	42	180	6/11/13	13.5	Kinnard Bridge	6/26/13	7/04/13	7.0	Robson Eddy
2013	M	420B272429	50	190	6/13/13	15.0	Fishy Beach	n/a	7/10/13	49.0	Beaver Creek
2013	M	41616A4B08	66	202	6/18/13	49.0	Beaver Creek	n/a	7/08/13	49.0	Beaver Creek
2013	M	7F7D1C3835	36	165	6/18/13	52.3	Fort Shepherd Eddy	n/a	7/08/13	49.0	Beaver Creek
2013	M	141221610A	41	163	6/21/13	52.3	Fort Shepherd Eddy	n/a	7/10/13	49.0	Beaver Creek
2013	M	7F7E6A3845	56	0	6/25/13	10.5	Kootenay Eddy	6/25/13	7/18/13	7.00	Robson Eddy
2013	M	7F7D381619	36	165	7/03/13	0.1	HLK Eddy	n/a	7/18/13	7.00	Robson Eddy
2013	M	985161000066843	72	208	7/10/13	56.0	Waneta Eddy	7/17/13	7/19/13	49.0	Beaver Creek

2.1 Brood Capture History

Table 2. Broodstock capture history for white sturgeon captured during the 2013 brood acquisition and successfully spawned at the Kootenay Sturgeon Hatchery. Please see Appendix 1 for a description of sexual maturity codes. Yellow highlight show fish captured in 2013.

Tag Number	Recap	Date	Water Body	River Km	Station Name	Length (cm)	Weight (kg)	Mat Code	Fate
985121001732553	New	06/12/13	COL	10.5	Kootenay Eddy	177.0	45.0	4	H
4239084A26	New	06/12/13	COL	10.5	Kootenay Eddy	176.0	42.0	4	H
985120022626419	1	06/17/13	COL	56	Waneta Eddy	205.0	75.0	4	H
985120022626419	0	05/20/05	FDR	114	100	183.0	59.9	13	A
4158445F7C	1	06/19/13	COL	56	Waneta Eddy	180.0	47.0	4	H
4158445F7C	0	04/15/98	COL	0.1	SSL 0.1M	136.0	20.0	97	
1F4B4B7952	1	06/20/13	COL	56	Waneta Eddy	201.0	79.0	4	H
1F4B4B7952	0	06/22/05	COL	52.3	SSL 52.3	197.0	68.0	15	H
7F7D190073	1	06/28/13	COL	56.5	American Eddy	208.0	96.0	4	H
7F7D190073	1	04/27/94	COL	52.3	AB 52.3	174.5	47.7	13	
7F7D190073	1	04/26/94	COL	52.3	AB 52.3	174.0	45.0	97	
7F7D190073	1	03/28/93	COL	56	SSL55.8R	181.0	49.5	97	
7F7D190073	1	07/12/92	COL	52.3	SSL 52.3	177.0	59.1	97	
7F7D185C29	1	07/09/13	COL	56	Waneta Eddy	225.0	84.0	4	H
7F7D185C29	1	04/24/01	COL	0.1	SSL 0.1L	194.5	54.5	17	A
7F7D185C29	1	06/11/94	COL	0.1	SSL 0.1L	177.0	49.1	97	
7F7D185C29	1	11/27/92	COL	0.1	SSL 0.1L	168.0		1000	
7F7D185C29	1	07/12/92	COL	56	SSL 55.8R	175.0	41.8	12	
7F7D4F6C19	1	06/20/13	COL	52.3	Fort Shepherd Eddy	225.0	88.0	4	H
7F7D4F6C19	1	06/14/11	COL	52.3	Fort Shepherd Eddy	219.0	76.0	Adult	A
7F7D4F6C19	1	03/15/01	COL	52.3	SSL 52.3	209.5	74.3	12	A
7F7D4F6C19	1	03/16/94	COL	56	SSL 55.8R	206.0	72.7	13	
4238456407	New	11/06/13	COL	13.5	Kinnaird	180.0	42.0	2	H
420B272429	New	13/06/13	COL	15	Fishy Beach	190.0	50.0	2	H
41616A4B08	New	18/06/13	COL	49	Beaver Creek	202.5	66.0	2	H
7F7D1C3835	1	18/06/13	COL	52.3	Fort Shepherd Eddy	165.0	36.0	2	H
7F7D1C3835	1	06/20/11	COL	52.3	Fort Shepherd Eddy	154.0	28.0	Adult	A
7F7D1C3835	1	06/15/09	COL	52.3	52.3 SSL	147.0	20.9	97	A
7F7D1C3835	1	06/01/09	COL	52.3	52.3 SSL	149.0	24.0	97	A
7F7D1C3835	1	06/09/08	COL	52.3	AB 52.3	145.0	62.0	97	A
7F7D1C3835	1	06/19/06	COL	52.3	AB 52.3	139.0	20.0	97	A
7F7D1C3835	1	06/15/06	COL	52.3	AB 52.3	137.0	25.0	97	A
7F7D1C3835	1	06/07/04	COL	52.3	AB 52.3L				
7F7D1C3835	1	05/10/04	COL	52	AB 52.0L	134.5	15.9	97	
7F7D1C3835	1	05/14/03	COL	52.3	SSL 52.3	128.5	13.6	98	A
7F7D1C3835	1	05/01/02	COL	52.3	SSL 52.3	121.0	13.6	98	A
7F7D1C3835	1	08/08/94	COL	52.3	AB 52.3		11.3	1000	
7F7D1C3835	1	07/23/94	COL	52.3	AB 52.3	111.8	11.4	1000	
7F7D1C3835	1	11/11/92	COL	52.3	SSL 52.3	110.5	7.7	98	
141221610A	1	06/21/13	COL	52.3	Fort Shepherd Eddy	163.0	41.0	2	H
141221610A	1	06/01/09	COL	52.3	52.3SSL	152.0	26.0	97	A
141221610A	1	06/15/05	COL	52.3	SSL 52.3	140.5	18.0	97	A
7F7E6A3845	1	06/25/13	COL	10.5	Kootenay Eddy	123.0	56.0	2	H
7F7E6A3845	0	05/04/04	COL	0.1	SSL 0.1M	183.0	0.2	1	
7F7D381619	1	07/03/13	COL	0.1	HLK Eddy	165.0	36.0	2	H
7F7D381619	0	04/21/98	COL	1.2	SSL 1.2R	153.5	27.3	97	
985161000066843	New	10/07/13	COL	56	Waneta Eddy	208.0	72.0	2	H

3.0 Transport

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 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 make a salt solution of 1%. Oxygen was supplied to saturation to the tank through aeration stones that were recessed into the tank floor. Twice during each transport, staff checked the fish for levels of duress and none was noted. 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 and physical labour. 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 5.3) 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

This year the adults were not fed during their time at the hatchery.

5.0 Spawning

In the 2013 spawning season, all female fish captured had initial PI levels calculated immediately following capture. The three females used in the first spawn had PI levels that were near or below the benchmark of 0.10mm needed for induction of spawning (0.103mm, 0.096mm and 0.047mm), and were spawned on June 26th. Two of the three females used in the second spawn had higher PI values (0.111mm and 0.121mm), but they moved down into acceptable parameters. The third female in the second spawn was captured on June 28th, and

her PI level was 0.080. Two of the eight females were deemed unusable due to being under mature (Table 3). As the PI (Polarization Index) values of the females decreased, three females were induced to spawn on June 26th. Eggs from each female were split into portions and each portion was fertilized with the two males that were induced the same day. The remaining five females were checked again, and three were induced to spawn on July 17th. 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 3. Female Information and PI Calculations for Spring 2013. Water temperatures were 12.5°C on June 18, 14.0°C on June 22, and 15.5°C on June 26.

Pit Tag #	Weight Kg	Capture Date	Capture Location	Initial PI	PI June 14	PI June 19	Spawn Date
985120011732553	45	06/12/14	Kootenay Eddy	0.103	0.103		June 26
4239094A26	42	06/12/14	Sturgeon Island	0.096	0.096		June26
985120022626419	75	06/17/14	Waneta Eddy	0.047	0.047		June 26
4158445F7C	47	06/20/14	Waneta Eddy	0.111	0.111	0.083	July 17
1F4B4B7952	79	06/20/14	Waneta Eddy	0.121	0.121	0.106	N/A
7F7D190073	96	06/28/14	American Eddy	0.080			July 17
7F7D185C29	84	07/09/14	Waneta Eddy	0.095	0.095		July 17
7F7D4F6C19	88	06/25/14	Fort Shepherd Eddy	0.350	0.270		N/A

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 ug/kg which is split with the initial dose being 5 ug/kg and the resolving dose being 45 ug/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 4). 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 then be used again for subsequent spawning at later dates by once again going through the LHRHa injection and water temperature increase.

Table 4. LHRHa injection dosage volumes for adult White Sturgeon spawned in 2013. See table footnote for LHRHa solution concentrations.

Sex	Pit Tag #	Weight kg	Capture Date	Capture Location	Initial ml	Resolved ml
F	985121001732553	45	6/12/13	Kootenay Eddy	0.23	2.03
F	4239084A26	42	6/12/13	Sturgeon Island	0.21	1.89
F	985120022626419	75	6/17/13	Waneta Eddy	0.38	3.38
M	4238456407	42	6/11/13	Kinnard Bridge	0.42	
M	420B272429	50	6/13/13	Fishy Beach	0.50	
M	41616A4B08	66	6/18/13	Beaver Creek	0.66	
M	7F7D1C3835	36	6/18/13	Fort Shephard Eddy	0.36	
F	4158445F7C	47	6/19/13	Waneta Eddy	0.24	2.12
F	1F4B4B7952	79	6/20/13	Waneta Eddy	0.40	3.56
F	7F7D190073	96	6/28/13	American Eddy	0.48	4.32
F	7F7D185C29	84	7/09/13	Waneta Eddy	0.42	3.78
M	141221610A	41	6/21/13	Fort Shephard Eddy	0.41	
M	7F7E6A3845	56	6/25/13	Kootenay Eddy	0.56	
M	7F7D381619	36	7/03/13	HLK Eddy	0.36	
F	7F7D4F6C19	88	6/20/13	Fort Shephard Eddy	0.44	3.96
M	985161000066843	72	7/10/13	Waneta Eddy	0.72	

* 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

5.2 Spawning Summary

The target breeding design is a full factorial mating design where each female is crossed with all available males. This method maximizes the effective population size, and is a common approach for conservation work within a limited breeding population. A modification was made to the matrix this year due to the shortage of males, and not all males could be used for each female (Table 5). The results of the two spawning events and the crosses used in the 2013 breeding season are presented in Table 5. Families were 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.

Table 5. Breeding design for adult White Sturgeon spawned in 2013.

Family	Female #	Male #1	Male #2
1	985121001732553	7F7E6A3845	4238456407
2	4239084A26	7F7E6A3845	4238456407
3	985120022626419	7F7E6A3845	4238456407
4	7F7D190073	985161000066843	
5	7F7D185C29	985161000066843	
6	4158445F7C	985161000066843	

Total egg volume and number was recorded for individual females for the 2013 spawning season and all ovulated eggs that were easily available were taken from spawning female fish. The spawning events of June 26th, and July 17th produced 418,038 green eggs. The average neurolation percentage was 44% which produced a total of 274,759 available eggs and larvae.

Eggs were first used to satisfy juvenile production targets for release into the Mid and lower Columbia River's. The release targets for 2013 were 4,000 for the section from below the Hugh L Keenlyside dam to the USA border raised to @ 80+ grams in size and as many 175 + gram juveniles for release in the Mid-Columbia River as possible. Initially the target that was estimated was 4,000 175 gram juveniles but 6,000 averaging 202 grams were available at the time of release. The average size for the lower Columbia River ended up being 100 grams each.

Surplus eggs and larvae were used for following:

- University of British Columbia – S. McAdam, Colin Brauner, David Close and Sang Yung. 5 Projects building on prior studies of white sturgeon larvae.
- Golder and Associates used eggs (500) on mats with cameras to study predation on eggs at Waneta Eddy.
- Collecting blood samples for stress cortisol levels in white sturgeon for Jon Wong, UBC

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 fish were returned to the river in 2013, a DNA sample (fin clip) was collected. This sample was placed in ethanol, labelled and stored on site in a secure dry area at the KTH. Fish were 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 was employed to carry them 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

Only one adult taken to the hatchery was implanted with an acoustic transmitter (Vemco modelV16-6H-R64K, Table 6). This was a female that did not spawn successfully and the assumption was that by implanting an acoustic transmitters, any post release movemenets related to spawning in the wild could be monitored. This implanted tag allows for the fish movement to be tracked if it passes 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.

8.0 Incubation and Larval Development

Fertilized eggs were placed in MacDonald Jars for incubation with water outflow from the jars directed into stainless, free-embryo 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 6).

Table 6. Identification of production families, spawning date, green and neurulated (Neur.) egg numbers and percentage for 2013 egg takes.

Family	Female	Spawn Date	# Green Eggs	Neurulation %	Hatched
1	985121001732553	6/26/13	40400	75.0%	30300
2	4239084A26	6/26/13	77140	49.5%	38184
3	985120022626419	6/26/13	109395	35.5%	39425
4	7F7D190073	7/17/13	95450	82.2%	78913
5	7F7D185C29	7/17/13	64468	91.2%	58970
6	4158445F7C	7/17/13	31185	93.0%	28967

Time to hatch Ranged from 8-10 days post fertilization at 15°C for all families of the 2013 brood year. Free embryos emerged from the MacDonald jars and were flushed into stainless troughs that contained 10-12 lpm flowing water of about 10cm depth. Water level was controlled by a standpipe and larvae were protected from the exit flows by a stainless mess screen. 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. All of the larvae were provided bioballs as an artificial substrate to allow them to hide and conserve energy. The bioballs provide artificial habitat similar to what the fish would experience in natural conditions.

After about 10 days the feed was introduced into the larval tanks. A custom formulation was produced at the hatchery that contains standard Skrettings 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 Bio-Oregon feed by about the 1g stage.

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 practice of sturgeon mimics that of trout culture in that tanks and screens were cleaned throughout the day on an ‘as needed’ basis. The monitoring of fish health and feeding activity was likewise observed during daily routine. 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

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 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, as fish size 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.

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 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 UC 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 3% 12N sturgeon while Family 4 was composed of 27% 12N sturgeon (Table 7).

Table 7. Percent of 12n white sturgeon individuals among family groups reared at the Kootenay Trout Hatchery in 2013. This is based on initial analysis of 30 samples per family**.**

	Total #	**Total # sampled**	**8N**	**12N**	**% 8N**	**% 12N**
Family 1	1596	30	29	1	97%	3 %
Female						
9.85121E+14						
Male						
7F7E6A3845						
4238456407						
Family 2	1594	30	30	0	100%	---
Female						
4239084A26						
Male						
7F7E6A3845						
4238456407						
Family 3	1592	30	30	0	100%	---
Female						
9.8512E+14						
Male						
7F7E6A3845						
4238456407						
Family 4	1378	30	22	8	73%	27%
Female						
7F7D190073						
Male						
9.85161E+14						
Family 5	1364	30	30	0	100%	---
Female						
7F7D185C29						
Male						
9.85161E+14						
Family 6	1394	30	30	0	100%	---
Female						
4158445F7C						
Male						
9.85161E+14						

After the TWG meeting on April 2nd it was determined that there were benefits associated with maximizing genetic contribution by releasing a portion of Family 4 in Spring 2014. For that reason the FFSBC Fish Health lab analysed 300 individuals from Family 4 for ploidy levels to come up with a release number of 200 known 8N individuals. After that analysis of a much larger sample was complete it was determined that Family 4 was composed of 6 % 12N individuals, which was lower than the original 27%. We assume that the difference in the result was that fish selected for the first analysis (n=30) were mainly smaller individuals (downgrades) while the later analysis of the larger sample (n=300) was random for size. If true, this may suggest that 12N sturgeon are smaller than their 8n counterparts which may relate to performance differences between 8N and 12N fish.

10.0 Releases

10.1 Larval Releases

There were no larval releases of 2013 year class sturgeon.

10.2 Juvenile Releases

The number of juvenile sturgeon being released into the Canadian section of the lower Columbia River from the 2013 year class (spring 2014) was adjusted this year due to the low number of males (3 total) contributing to the progeny to be released through discussions with Dr. James Crossman and Ron Ek. This year the overall adult contribution was low compared to the previous few years as shown in Table 8.

Table 8. The number of adults contributing to the juvenile sturgeon releases in the Canadian section of the Columbia River from 2009 to 2013**.**

Year	Total Adults Used
2013	9
2012	15
2011	17
2010	18
2009	16

As recommended by the TWG, the mating designs from 2009-present were used to inform the number of juveniles contributed by each half-sib family group at the time of stocking. Since maternal family groups are pooled during the rearing process and survival is high, we assumed equal paternal contributions from the time of pooling to the time of release. We took the stocking number (4000) and divided by the number of half-sib family groups created in that year (across both spawning events). This approach provided us with an estimate of the number of

juveniles per family at the time of stocking. We then averaged that number across the 2009 through 2012 years and the result was 189 individuals per family (Table 9). We then applied that average to the 2013 half-sib family groups and it resulted in **1702** juveniles available for stocking for all families combined. The break down was 1135 from the first spawning event of 3 females and 2 males, and 567 from the second spawning event of 3 females and 1 male. This number of juveniles ensured that the public release event would not be impacted and fish were released at multiple locations in the river as has been the practice for the past several years.

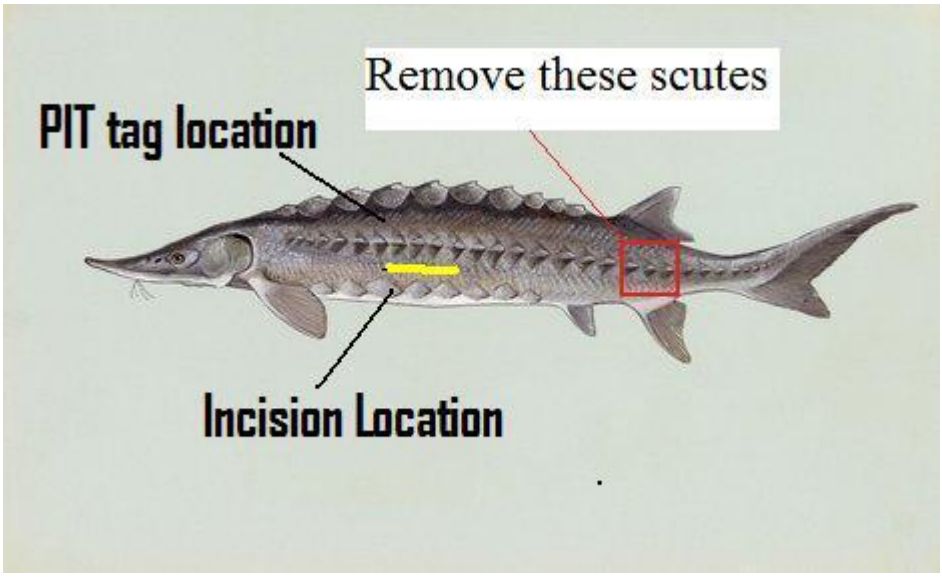
Table 9. The number of individuals per family at the time of stocking in the Canadian section of the Columbia River from 2009-2012.

Year	Average/family
2012	160
2011	111
2010	235
2009	250
Average	**189**

As a comparison, if the breeding plan was followed for the target number of adults (10 females and 10 males) with two different spawning events it would have resulted in 80 juveniles per half-sib family for a total of 720 juveniles for stocking. We felt that the upper level for spawning each year is a target that has not been achieved since the program has been initiated despite increased brood stock sampling. It was determined that weighting the number of fish released by incorporating the mating design from the past few years is a defensible approach. Using that methodology it was determined that 1702 juvenile sturgeon were to be released into the Canadian section of the Columbia River in spring 2014.

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. Then in early April the PIT tag number, length and weight data were recorded for each fish prior to releases. 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 occurred in the spring of 2014. In the Lower Columbia on May 1st and 5th fish were released at an average size of 100 grams. A total of 1,702 juvenile sturgeon were released into the Canadian Lower Columbia River at Keenlyside Dam near Castlegar (n=900), Kootenay Eddy (n=300), Genelle (n=300) and Beaver Creek (300). As well 6013 juvenile sturgeon were released May 7th into the Canadian Mid-Columbia River in the Revelstoke area. All of these were released at Shelter Bay Provincial Park. These sturgeon were much larger than have been released in the past as they averaged 202 grams each (Table 10).

Table 10. Numbers, average length and weight, and release locations for juvenile white sturgeon released into the lower Columbia (LCR) and Mid-Columbia (MCR) Rivers in the spring of 2014**.**

Columbia Sturgeon Juvenile Release 2014	Release Date	River (km)	Family	Number	Average FL (cm)	Average weight (g)	Weight (kg)
LCR (HLK)	5/1/14	0.1	1	200	22	97	19.4
			2	200	23	98	19.6
			3	200	24	125	25
			4	100	25	152	15.2
			5	100	24	106	10.6
			6	100	23	103	10.3
			Totals	900	23	100	100.1
LCR (Kootenay Eddy)	5/1/14	10.5	1	67	22	97	6.5
			2	67	23	98	6.5
			3	67	24	125	8.4
			4	33	25	152	5
			5	33	24	106	3.5
			6	33	23	103	3.5
			Totals	300	23	100	33.4
LCR (Genelle)	5/1/14	24	1	67	22	97	6.5
			2	66	23	98	6.5
			3	67	24	125	8.4
			4	33	25	152	5
			5	33	24	106	3.5
			6	34	23	103	3.5
			Totals	300	23	100	33.4
LCR (Beaver Creek)	5/1/14	49	1	67	22	97	6.5
			2	67	23	98	6.5
			3	66	24	125	8.4
			4	33	25	152	5
			5	34	24	106	3.5
			6	33	23	103	3.5
			Totals	300	23	100	33.4
MCR (Revelstoke)	5/8/14	178 SB	1	1173	28	201	237.8
			2	1328	28	189	247.5
			3	1192	28	219	256.0
			4	83	28	117	9.7
			5	1134	28	205	227.0
			6	1103	28	198	216.0
			Totals	6013	28	202	1194.0
	Total	Revelstoke		6013	28	202	2388.0
	Total	Lower		1800	23	100	351.6
Year Class Totals				**7813**			**1394.0**

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 volunteers from many other agencies assist in these very busy and successful events. 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.

One very large public release event took place with over 850 school students, each getting to release their own sturgeon below the Keenlyside dam on May 1st, 2014. There usually is a public release event later that day, but because there were fewer fish to release this year this public event did not take place. Instead the public was invited to come out to view the sturgeon juveniles and ask questions on May 5th in the Waneta Plaza parking lot and the Castlegar Recreation facility lot. In addition, a public release event took place at Beaver Creek on May 5th with 250 school students and public attending. Finally, a release event took place in Revelstoke on May 7th with another 150 school children and public at the Shelter Bay Provincial Park boat launch site. There was considerable press in attendance at the release events with some examples of newspaper articles in Appendix 3.

11.0 Fish Health Testing Summary

11.1 Virus Screening for Broodstock

Samples of ovarian fluid and milt were collected from spawners. Contributing gametes 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. 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; see Appendix 1.

11.2 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 X: Procedures for the Detection of Viruses as listed in the Canadian Fish Health Protection Regulations Manual of Compliance. All examined juveniles tested negative for the above listed viruses. Viral culture cell lines used for Broodstock testing were also used for juvenile testing.

11.3 Deformities

In 2013 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.

12.0 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 year's permits, these were obtained from the Introductions and Transfer Committee for adults, as well as for spring release of juveniles and larval releases.

13.0 Presentations and Meetings

- Large Spawning events June 26th and July 17th at KTH.
- Nov 19, 20, 21– Ron Ek, Adrian Clarke, and Mike Keehn attended UCWSRI TWG meeting in Spokane.
- February 18 to 20, FFSBC Annual General Meeting, Kelowna B.C.
- April 1 & 2, 2014 Ron Ek, Adrian Clarke, and Mike Keehn attended UCWSRI TWG meeting in Nelson and presented updated plan for spring release events and dates and autopolyploidy findings.
- Monthly TWG conference calls Adrian, Ron & Mike.

Appendix 1. 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 coloured.
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

Number Requested (total and by parent if needed):

Fertilized eggs – 8,000 split equally between 3 full sib families
Additional requirements – our preference is the following:

> **Parents** - eggs would be an equal split of 3 full sibling families
> **Timing** – our preference would be the first spawning, but either would work
> **Transport** – I you could airfreight them to Vancouver as in the past that would be appreciated. We can cover air freight costs if needed. Eggs will be picked up by Steve McAdam (contact details are: Steven McAdam, BC Ministry of Environment 2202 Main Mall UBC, V6T 1Z4, cell phone # 604-908-3474, e-mail steve.mcadam@gov.bc.ca)

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 Bruner'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.

Disposition of fish/embryos after study concludes: All fish will be either sampled or euthanized at the end of this study.

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.

Appendix 2.2 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.

Appendix 2.3 Egg Predation Study, Golder and Associates Ltd.

To demonstrate that post-Project flow changes when the White Sturgeon Flow Augmentation Program – Post Project Enhancement (WSFAP-PPE) is in effect [will] or will 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, will be used to document evidence of egg predation by recording egg loss and egg predators in recorded images. A DIDSON camera will also be used to record egg predator density in the vicinity of the MatCam. DIDSON cameras and seeded MatCams will be deployed concurrently at two locations to monitor egg predator densities, determine species composition, and detect egg predation.

Appendix 3: Fish Health Testing

The 2013 Columbia sturgeon year class reared well. There were three diagnostic cases submitted from this year class. There were 6 families reared and tested in 2013. Results from the Broodstock testing, juvenile Family testing, diagnostic submissions 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.

Please note spawning dates were not provided with submitted samples.

Table 1: Broodstock Testing for 2013

Case Number:	2012-1039						
Sample Tissue		Virology Cell lines				Bacteriology	
Reproductive fluid	PIT Number	CHSE-214	EPC	WSSK	WSS-2	TSA	HS
Ovarian fluid	2553	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Ovarian fluid	4A26	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Ovarian fluid	6419	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Ovarian fluid	D073	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Ovarian fluid	5C29	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Ovarian fluid	5F7C	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Milt	3845	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Milt	6407	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected
Milt	6843	NEG	NEG	NEG	NEG	No pathogenic bacteria detected	No pathogenic bacteria detected

Table 2: 45-60 day Juvenile Family testing results 2013:

Case Number	Family ref #	# fish submitted	Virology				Bacteriology	
			CHSE-214	EPC	WSSK	WSS-2	TSA	HS
2013-1080 Submission date: AUG 21, 2013	Fam 1	60 Total 30 Virology 30 Bacteriology	NEG	NEG	NEG	NEG	30/30 No pathogenic bacteria detected	30/30 No pathogenic bacteria detected
2013-1081 Submission date AUG 21, 2013	Fam 2	60 Total 30 Virology 30 Bacteriology	NEG	NEG	NEG	NEG	30/30 No pathogenic bacteria detected	30/30 No pathogenic bacteria detected
2013-1082 Submission date: AUG 21, 2013	Fam 3	60 Total 30 Virology 30 Bacteriology	NEG	NEG	NEG	NEG	30/30 No pathogenic bacteria detected	30/30 No pathogenic bacteria detected
2013-1083 Submission date: SEPT 5, 2013	Fam 4	60 Total 30 Virology 30 Bacteriology	NEG	NEG	NEG	NEG	30/30 No pathogenic bacteria detected	30/30 No pathogenic bacteria detected
2013-1084 Submission date: SEPT 5, 2013	Fam 5	60 Total 30 Virology 30 Bacteriology	NEG	NEG	NEG	NEG	30/30 No pathogenic bacteria detected	1/30 Yellow pigmented colonies. Positive for *Flavobacterium psychrophilum.*
2013-1085 Submission Date: SEPT 5, 2013	Fam 6	60 Total 30 Virology 30 Bacteriology	NEG	NEG	NEG	NEG	30/30 No pathogenic bacteria detected	30/30 No pathogenic bacteria detected

Diagnostic submissions Columbia White Sturgeon 2013 year class

Table 3: Submission #1

Case Number	Family ref #	# fish submitted	Virology Salmonid		Virology Sturgeon		Bacteriology	
			CHSE-214	EPC	WSSK	WSS-2	TSA	HS
2013-1102 Submission date: Oct 2, 2013	Fam 2 Container # C-8	20 Affected sample	Cell line not used	Negative No viral or filterable replicating agents detected.	Negative No viral or filterable replicating agents detected.	Negative No viral or filterable replicating agents detected.	20/20 No pathogenic bacteria detected	20/20 No pathogenic bacteria detected

Comments: A few fish had been showing signs of reddening around the base of the snout, in the mouth and gill area and belly which was causing mortalities. This was occurring in some of the other families and containers as well but this container was showing the highest number. Samples were shipped in Oct 2nd for diagnostic processing. In conjunction with the virus work and the kidney inoculation onto TSA and HS other work included: Skin wet mounts, and Gram stains on Gill and skin smears.

Results: Gram negative rods were found in the Gills and on the Skin of a few fish in low to moderately high numbers resulting in an external bacterial infection. It has been observed previously that Sturgeon tend to express reddened areas in certain areas of the skin when stressed. The reddening observed may have been a combination of the bacterial infection and the stress of the infection.

Treatment: It was recommended to hatchery staff to try a Chloramine T treatment of September 28th at 10 ppm static bath for 1 hour. This was followed up with a 0.5% Salt static bath administered for 30 minutes for 2 consecutive days October 5th and 6th.

Table 4: Submission #2

Case Number	Family ref #	# fish submitted	Virology Salmonid		Virology Sturgeon		Bacteriology	
			CHSE-214	EPC	WSSK	WSS-2	TSA	HS
2013-1105 Submission date: Oct 31, 2013	Fam 2 Container # C-8	20 Affected sample	Virology not done	Virology not done	Virology not done	Virology not done	1/15 Presumptive for *Aeromonas spp (likely hydrophila)*	15/15 kidney tissue-no pathogenic bacteria detected 15/15 spleen tissue –no pathogenic bacteria detected

Comments: Staff was experiencing ongoing chronic losses in Family 4 located in circular #4 and the sturgeon were developing fungus on some of the gills and on the ventral surface where the operculum's meet. Morts were showing signs of some reddening around the mouth and on the ventral surface. The hatchery submitted 15 samples for diagnostic work. Tests performed included: Gram stain on Gill and skin smears. Kidney tissue inoculated onto Tryptic Soy Agar (TSA) and Sheih's (HS) media as well as spleen tissue inoculated onto Sheih's (HS) media. In addition spleen tissue was taken for PCR testing for *Flavobacterium psychrophilum.*

Results: Gram negative rods were found in a few of the gill and skin smears. These rods were classified as being longer and fatter and were found in low to moderately high numbers. No pathogenic bacteria was isolated from the kidney or spleen tissue on the bacterial media's however 5/15 spleens tested positive for *Flavobacterium psychrophilum* based on PCR results. The reddening observed may have been a combination of the bacterial infection and the stress of the infection. The fungus development was a concern and was thought to be possibly caused by the higher rearing temperature in conjunction with an external bacteria infection.

Treatment: It was recommended to the hatchery staff to try using a Chloramine T treatment when needed at a dosage rate of 10 ppm for 45 minutes for 2 consecutive days alternating with a salt treatment. The hatchery staff increased the salt bath to 1% for 30 minutes on alternate days and decreased the temperature. Approval was requested and received from the Vet to do an Aquaflor treatment at a dosage rate of 15 mg/Kg BW/day for 10 days however this option was not used as the alternating Chloramine T and salt treatment appeared to be effective.

Table 5: Submission #3

Case Number	Family ref #	# fish submitted	Virology Salmonid		Virology Sturgeon		Bacteriology	
			CHSE-214	EPC	WSSK	WSS-2	TSA	HS
2013-1105 Submission date: Oct 31, 2013	Fam 3 Container # C-15	16 Affected sample	Cell line not used	Negative No viral or filterable replicating agents detected.	Negative No viral or filterable replicating agents detected.	Negative No viral or filterable replicating agents detected.	5/16 presumptive for *Aeromonas spp (likely hydrophila)*	16/16 kidney tissue –no pathogenic bacteria 16/16 spleen tissue –no pathogenic bacteria

Comments: Staff was experiencing ongoing chronic losses in Family 4 located in circular #4 and the sturgeon were developing fungus on some of the gills and on the ventral surface where the operculum meets. Morts were showing signs of some reddening around the mouth and on the ventral surface. The hatchery submitted 15 samples for diagnostic work. Tests performed included: Gram stain on Gill and skin smears. Kidney tissue inoculated onto Tryptic Soy Agar (TSA) and Sheih's (HS) media as well as spleen tissue inoculated onto Sheih's (HS) media. In addition spleen tissue was taken for PCR testing for *Flavobacterium psychrophilum.*

Results: A large percentage of the gills and skin smears had long thin gram negative rods in heavy numbers. No pathogenic bacteria was isolated from the kidney or spleen tissue on the bacterial media's however 10/16 spleens tested positive for *Flavobacterium psychrophilum* based on PCR results. The reddening observed may have been a combination of the bacterial infection and the stress of the infection. The fungus development was a concern and was thought to be possibly caused by the higher rearing temperature in conjunction with an external bacteria infection.

Treatment: It was recommended to the hatchery staff to try using a Chloramine T treatment when needed at a dosage rate of 10 ppm for 45 minutes for 2 consecutive days alternating with a salt treatment. The hatchery staff increased the salt bath to 1% for 30 minutes on alternate days and decreased the temperature. Approval was requested and received from the Vet to do an Aquaflor treatment at a dosage rate of 15 mg/Kg BW/day for 10 days however this option was not used as the alternating Chloramine T and salt treatment appeared to be effective.

Table 6: Pre-release sample test for brood year 2013 submitted Feb 13th, 2014.

Case Number	Family ref #	# fish submitted	Virology Salmonid		Virology Sturgeon		Bacteriology	
			CHSE-214	EPC	WSSK	WSS-2	TSA	HS
2014-1012 Submission date: Feb 13th, 2014	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 2013 Columbia sturgeon families reared well during larval rearing but there were a few diagnostic issues during the Fall where various containers developed reddening and around the mouth and on the ventral surface near the junction of the base of the operculum. In some isolated cases this also resulted in fungus development in the gills and some increased mortality events. The events were not restricted to any one container or family group but appeared to move through various groups over a period of a few weeks. Although approval to administer an Aquaflor treatment was approved by the Vet, the hatchery effectively mitigated the losses and reduced the incidence of reddening and fungus by introducing an alternating regime of Chloramine T (using a dosage rate of 10 ppm for 30-45 minutes) and salt static baths (dosage rate 1.0% for 30 minutes).

The pre-release sample results indicate that the 2013 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.

Summary prepared by: **Date prepared:** April 29th, 2014

Sherry Mead
Fish Health Unit Manager
Freshwater Fisheries Society of BC
1080 Wharncliffe Rd
Duncan BC V9L 2K7
P: 250-737-1444
E: sherry.mead@gofishbc.com

Appendix 4: Newspaper Articles

Juvenile Sturgeon Release for the Public
at Shelter Bay

April 25, 2013
ARROW LAKES It's that Sturgeon time of year once more! The annual Sturgeon release event, hosted by the Upper Columbia White Sturgeon Recovery Initiative (UCWSRI), will occur between 11.30 a.m. and 1.30 p.m., on Wednesday May 7, at Shelter Bay Provincial Park, and the public are invited.

"During the session, we host elementary school students and members of the public, and the mix works well," says Gerry Nellestijn, chair of the Community Working Group of the UCWSRI. "For young and old alike, it is a great opportunity to help an endangered species. The fish look - and feel - like creatures from prehistoric times; in fact they have remained largely unchanged for 175 million years. By getting the community, particularly the younger generations, involved, and increasing awareness, we feel there are much better chances for the survival of this population."

The release event is organized by the Fish and Wildlife Compensation Program in the Columbia Region, (a partnership between BC Hydro, the Province of B.C., First Nations and the public), with support from BC Hydro, Revelstoke Rod and Gun Club, Columbia Power Corporation, Fortis BC, and the Freshwater Fisheries Society of B.C. (FFSBC).
Approximately 5,500 ten month-old juvenile White Sturgeon, raised by FFSBC at the Bull River hatchery in the East Kootenay through a program funded by BC Hydro, will be released; they are produced from wild adults, caught last June.

The average weight of the fish to be released at Shelter Bay is about 200 grams; this is twice the average weight of fish recently released in the Columbia River below Hugh Keenleyside Dam. Juveniles are being released at a larger body size to help researchers determine if it improves survival of the sturgeon through the first winter following the release.

Though releases of hatchery raised sturgeon into the Arrow Lakes have been occurring since 2007, very few individuals have been recaptured as a part of ongoing monitoring programs. It will take time to be able to tell whether recovery efforts in Arrow Lakes Reservoir are making a difference. In an area as large as Arrow Lakes Reservoir, finding and capturing small white sturgeon is extremely difficult. Further monitoring is planned for the next four years.

"The population of sturgeon in Arrow Lakes Reservoir is estimated at roughly 50 adults and, though they are known to spawn near Revelstoke, there is no evidence that any of the hatched young survive," says James Crossman, white sturgeon biologist for BC Hydro. "Juvenile white sturgeon stocked from the hatchery provide us with an important learning tool to assess survival, growth, and determine important habitats for juvenile sturgeon in Arrow Lakes Reservoir."

The Sturgeon Recovery Initiative is a partnership of more than 20 stakeholders from government, First Nations, industry, community and environmental organizations. Sturgeon recovery includes research to determine the causes of decline, release of hatchery-reared

juveniles from wild stock adults, restoration of habitat, and monitoring and management of water flows.

For more information about the sturgeon release event call the BC Hydro office at 250-365-4550, and to find out more about the UCWSRI visit www.uppercolumbiasturgeon.org.
30

For more info about the above, call Angus with the Fish & Wildlife Compensation Program, 250-352-1300, or email angus.glass@bchydro.com