Columbia River Project Water Use Plan

Columbia River White Sturgeon Management Plan

White Sturgeon Conservation Aquaculture – 2018 Annual Report and Comprehensive Program Report

CLBWORKS-24: Mid-Columbia River White Sturgeon Experimental Aquaculture
CLBWORKS-25: Mid-Columbia River White Sturgeon Conservation Aquaculture **(Implementation Year 7)**
CLBWORKS-34: Lower Columbia River White Sturgeon Conservation Aquaculture **(Implementation Year 11)**

WUP Works Period: May 2007 – May 2019
Program Period: 2001-2019

Prepared by: Freshwater Fisheries Society of British Columbia

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

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Mid and Lower Columbia River White Sturgeon Conservation Aquaculture Comprehensive Report: Study Period 2001 to 2019

Prepared for: BC Hydro

Prepared by: Freshwater Fisheries Society of British Columbia

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1. Background

The population of White Sturgeon in the Canadian portion of the Columbia River has been undergoing recruitment failure for several decades (Hildebrand and Parsley 2014). 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; http://uppercolumbiasturgeon.org) 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 support the population until such time as stock abundance/age structure and habitat conditions can support a self-sustaining population.

The white sturgeon conservation aquaculture program was initiated in 2001 with a focus on the lower Columbia River, which encompasses 57.0 km of the Columbia River from Hugh L. Keenleyside (HLK) Dam near Castlegar British Columbia to the international border. A parallel program was initiated in the US in 2003, to produce juvenile sturgeon for release into Lake Roosevelt. Finally, in 2007, the program was extended to the Middle Columbia River Canada, which is a 235 km section of the Columbia from HLK to Revelstoke Dam and includes the Arrow Lakes Reservoir. Annual releases of sub-yearlings have occurred into all locations since their inception. The key overarching objectives of the aquaculture program were originally to prevent extirpation and retain the genetic diversity of the wild population. In order to achieve these objectives, the program followed an adaptive management approach that continually incorporated new methods and key results into the decision-making process. The adaptive management of the program was conducted in partnership with UCWSRI partners from both Canada and the US that included agencies, first nations, industry, and other stakeholders.

The main objective of this report is to provide a comprehensive summary of the Canadian conservation aquaculture methods and results since inception (2001) until present (spring 2019). While all major aspects of the program are described, further details about methods and results can be found within annual reports (details in FFSBC 2008-2018).Notably, the program experienced a significant shift in methods where the use of wild adult broodstock to produce juveniles for release was transitioned to a program focused on collecting wild-origin embryos and larvae to rear both streamside on the Columbia River and in the traditional hatchery environment. Accordingly, the report is structured for each of those periods (Section 2.1 and 2.2).

2. Methods

There have been a number of adaptive changes to release targets and production methodologies over the course of the aquaculture program concurrent with improved understanding of Columbia River Sturgeon ecology and rearing, however, one key decision was the shift in approach and methods to how progeny were produced for rearing and subsequent release. While the early years of the program focused on direct spawning of wild adults (broodstock), results from post-release survival monitoring found that genetic diversity of the hatchery-origin population at large in the Columbia River was reduced compared to the time of release from the hatchery(McLellan and Crossman unpublished data). In order to address deficiencies in the objective pertaining to retaining genetic diversity of the wild population, the program piloted and then fully adopted an approach focused on collections of embryos and larvae that were produced from wild spawning events. This approach was shown to improve genetic diversity compared to direct spawning of adults for both Upper Columbia white sturgeon (Jay et al. 2014) and other sturgeon species (e.g. Crossman et al. 2011). Accordingly, each approach is described separately in this report and Figure 1 shows the general design that was implemented.

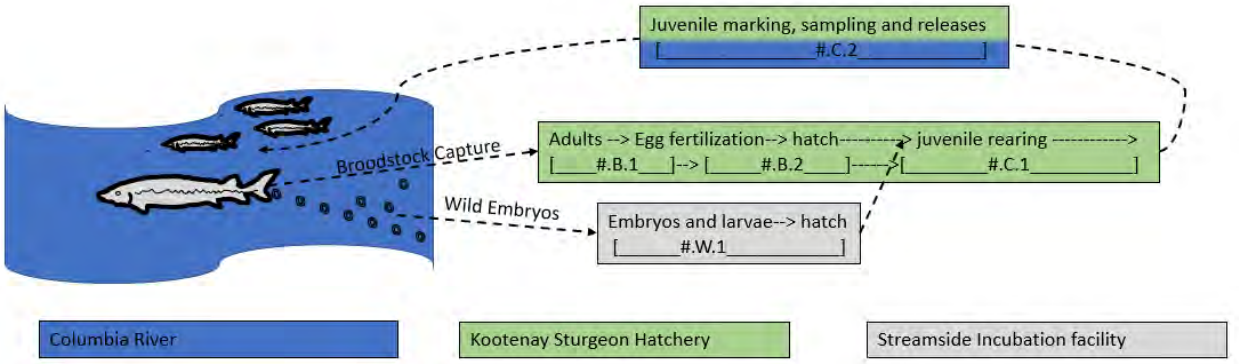

Figure 1. Schematic of the two different approaches used to produce progeny for the conservation aquaculture program for Columbia River White Sturgeon including broodstock (2001-2014) and collections of wild progeny (2014-2019). (B = broodstock, W = Wild embryos or larvae, C = combined sources).

2.B Broodstock Approach

At the initiation of the conservation aquaculture program, hatchery production of a year class was initiated using the approach of capturing mature wild adults (broodstock) from the Columbia River, transporting them to the hatchery for holding and direct spawning through gamete extraction. This approach was a more traditional aquaculture method allowing spawning matrices to be developed in a controlled setting. Broodstock capture was initiated in 2001 and continued for over a decade, and significant learning and improvements were continuously made along the way. The incorporation of new information and specific techniques is apparent in the increasing level of detail within the annual reports over the years (e.g. FFSBC 2008-2015). This comprehensive report will focus on clearly summarizing the key steps that were consistently needed to run the broodstock program over all brood years (2001 to 2014).

During the period of broodstock capture and direct spawning, the conservation aquaculture program was tasked with several different objectives (e.g. FFSBC 2008-2013), including the following:

- The capture, transportation, care, breeding, and ultimate release of mature adult sturgeon to provide for an annual target number of genetically distinct families or secondarily subfamilies (The target numbers for adult capture varied over years and increased to a maximum of 20: e.g. 2003 quoted a target of 6 per sex, and 2013 quoted 10 per sex).
- The successful incubation, rearing and subsequent release of up to 12, 000 sub-yearling sturgeon (or alternate number as set by the TWG). The source of the released juveniles should be approximately evenly distributed among the genetically distinct brood families.
- The annual marking and tagging of all sturgeon according to established protocols. Marking of sturgeon included scute removal to designate either brood year or origin (hatchery produced vs wild collection), Passive Integrated Transponder (PIT) tags to track capture data individuals, and acoustic tagging of a subset of individuals to track movement and/or survival as the TWG directs.
- 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 as the TWG directs.

2.B.1 Adult Methods

The adult methods employed by the conservation aquaculture program were previously detailed in the Upper Columbia River Adult White Sturgeon Capture, Transport and Handling Manual (2006). The 52-page report provides helpful diagrams and photographs to visualize the equipment setup as well as datasheet templates and other information for readers needing more detailed methodological information. Specific details can be found in each of the annual

reports found at:
https://www.bchydro.com/toolbar/about/sustainability/conservation/water_use_planning/southern_interior/columbia_river/columbia-sturgeon.html

Adult Capture

SET LINES

Set lines were the primary capture method used to obtain mature adult broodstock. Set lines are highly effective for white sturgeon, less size-selective than other methods, have little by-catch of other species, and have demonstrated high survival rates of captured fish (Elliot and Beamsderfer 1990, Thomas ad Haas 1999)

Line configuration and bait

Set lines were initially configured as either “long lines” or “medium lines” when the broodstock program began. Long lines measured up to 183 m in length with 30 circle halibut hooks (sizes 14/0 and 16/0) attached at 4.6 m intervals. A medium line configuration (40 m mainline; 8 hooks) was used in more confined target areas (i.e. small eddies). In both cases the configuration consisted of 64 mm diameter nylon mainline with 0.7 m ganglion lines that were clipped (64mm stainless steel swivel clip) onto the mainline and tipped with a hooks. Note that the smallest hook size (12/0) stated in the Upper Columbia capture manual was excluded in these set line configurations, as only large mature individuals were targeted for broodstock. Some changes were made to the original configuration over time (J. Crossman Pers comm): 1) Starting in 2008, only the shorter “medium” lines were used for broodstock and “longlines” were dropped; and 2) From 2010 onward only 20/0 hook size was used to reduce the number of smaller or immature fish captured.

The baits used on set lines were varied over the years dependent on availability. Fresh or freshly frozen Kokanee (available from local spawning channels and eggs stations each fall),

Eastern Brook Char (sometimes pickled) and Rainbow Trout (Available from Kootenay Trout hatchery) as well as commercially available pickled squid have all been used successfully.

Deployment and Retrieval

The set lines were deployed by attaching one end of the mainline to a large steel or concrete anchor (approximately 5kg) and float line. Once the anchor was deployed the boat would slowly reverse as a crewmember on the bow played out the mainline and clipped on (swivel clips) the shorter ganglion lines with baited hooks at the specified regular intervals. Adjustments to the direction, anchoring system and float line were made according to the water depth and flow conditions. In areas of higher flow, additional anchors and/or a shoreline were used to secure the set line in place and on the bottom. The preferred direction of deployment was perpendicular to shore as this provides the maximum dispersal of bait scent to potentially attract fish, however, in higher flow conditions the direction must be altered to the direction of flow (parallel the shoreline).

The retrieval procedure began by lifting the downstream float line and then idling the boat along the mainline so that the float line, anchor, and mainline could be fed into a plastic tub, while the hooks were removed into a separate pale. Slightly different systems of open pulleys or winches were used to retrieve the mainline over the years and depending on the velocity conditions of a given line set.

When a fish was suspected to be on a set line hook, the set line would then be pulled up off the bottom more slowly to enable white sturgeon to de-gas or ‘burp’. When fish were captured, they were removed from the mainline and tethered to on the side of the boat, so that all fish could be processed after the set line was completely cleared. Fish tethered using the swivel clip and ganglion remained docile and secured to the boat safely. When lines were to be reset for another night, all hooks would be checked for integrity, and old bait replaced with fresh bait.

ANGLING

A supplementary capture method for adult broodstock was angling. Angling is also species and size selective and has the added benefits of (1) reducing stress and injury due to a shorter timeframe between hookup and removal, (2) being able to access areas/habitats that were not practical for set line deployment.

Angling gear consisted of a sturdy single piece rod and an in-line reel spooled with 27 to 58 kg test nylon braided or monofilament line. Terminal tackle was attached using a three-way swivel; the mainline from the rod was secured to one eye of the swivel, a weight (up to 700 g) was tied to another eye of the swivel for anchoring on the river bottom, and a (~1m) leader tipped with a stainless steel hook (same sizes as set lines) was tied to the third eye of the swivel. This swivel system was found to be the most tangle-free fishing method in the river currents.

Preferred bait for angling included eastern brook trout (obtained from Kootenay Trout Hatchery) soaked overnight in garlic, rainbow trout, eulachon, dew worms, pickled squid, roe, and lamprey.

Adult Biological and Stage of Maturity Assessment

Following capture, biological sampling begins following the protocols in WSG manual. The most important biological metrics for broodstock selection were: previous capture history, sex and stage of maturity of the individual. Initially each sturgeon was scanned for presence of a PIT tag, and if found, the capture history was reviewed to ensure that the fish was a potential brood candidate (i.e. not be used for brood previously and obtain sex information). If a PIT tag was not detected, or the fish was deemed a good candidate from PIT information, then sex and maturation sampling began. For males, fish were examined for flowing milt (confirmation of a mature male) by applying suction with a Tygon tube (3.2mm inside diam.) and 50 ml syringe to the urogenital opening. If no milt was found, then a surgery was performed to confirm the sex

and maturation stage. Surgery was performed on all adults greater than 150cm as per following steps (protocol is also outlined in the WSG manual):

1. All surgical and other support equipment were laid out in an accessible and sterile location before fish were handled for processing. All instruments were sterilized in a 10% Germaphene solution, and all crewmembers handling the fish wore sterile latex gloves. Care was taken to maintain all surfaces and instruments that may contact the incision sterile throughout the surgery process.
2. The fish was then positioned on its back in a stretcher, and fresh water was continuously supplied over its gills via a garden hose and pump system. A damp cloth was used to cover the bulk of the fish's exposed underside to keep the fish cool and the skin moist. The ventral surface of the fish around the incision area was swabbed with Betadine or an approved disinfectant and then covered with a sterile surgical drape.
3. The abdominal area near the incision location (three to four ventral scutes anterior to the urogenital opening), was disinfected by swabbing with Betadine. A 1.5 to 2.0 cm long incision was made with a sterile scalpel through the body wall just off the mid-line, taking care not to cut any internal organs.
4. If the gonad was not visible through the incision, an otoscope equipped with a veterinary head and speculum would be inserted into the incision to help examine the gonads. Maturity stages were assessed according to the qualitative histological classifications described by Conte et al. 1988 (Table 1) the newer classification table set out by Bruch, et al. 2001 (Table 2), and further updated in Webb et al. 2018. The sex and maturation stage code were recorded and a short description of the left and right gonad (where possible) was written in the comments. Samples of ovarian fluid and milt collected from all adults potentially contributing gametes were screened for viruses (IPNV, WSHV1, WSHV2 and WSIV) using standard tissue culture methods as described in Procedures for the Detection of Viruses as listed in the Canadian Fish Health Protection Regulations Manual of Compliance.

5. After examination and removal of a sample of the gonad, the incision was closed using a half circle, reverse cutting-edge needle, wedged to a 0 (4.0 metric) Polydioxanone suture (PDS). When the body wall was thick (greater than 0.5 cm), a vertical mattress stitch was used (for thinner body walls, a simple stitch).
6. Sutures were spaced approximately 1 cm apart and slack (approximately 2.0 to 4.0 mm) provided in the sutures helped to prevent tissue damage caused by swelling during the healing process. The sutured incision was swabbed with Betadine to disinfect the area.
7. After the surgery, the fish deemed not suitable for broodstock were lowered overboard in the stretcher and released once normal orientation, swimming movements, and gill activity were observed. Those that were selected as potential broodstock were transferred to a holding sleeve in the river (when appropriate) and then to a holding transport trailer tank (Transport process outlined below).

Table 1. Sexual maturity codes for white sturgeon (from Conte et al. 1988).

Code	Developmental State Description
Male	
01	Non-reproductive, testes appear as thin strips with no pigmentation
02	Maturing; small testes; some folding may be apparent; translucent, smoky pigmentation
03	Early reproductive; large testes, folds beginning to form lobes; some pigmentation still present; testes more white than cream coloured
04	Late reproductive; testes large, often filling posterior of body cavity; white with little or no pigmentation
05	Ripe; milt flowing; large white lobular testes; no pigmentation
06	Spent; testes pinkish-white, flaccid, and strongly lobed
00	General unknown maturity
Female	
10	General unknown maturity
11	Non-reproductive; ovaries small, folded with no visible oocytes; tissue colour white to yellowish
12	Pre-vitellogenic; moderate size ovary with small eggs present (0.2 to 0.5 mm diameter); may have "salt and pepper" appearance
13	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	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 appearance
15	Ripe; eggs fully pigmented and easily detached from ovarian tissue; eggs 3.0 to 3.4 mm in diameter
16	Spent; ovaries are flaccid with some residual fully developed eggs
17	Pre-vitellogenic with atretic oocytes; small eggs (<0.5 mm diameter) present; dark pigmented tissue present that may be reabsorbed eggs
Unknown	
97	Adult based on size, no surgical examination
98	Juvenile/sub-adult based on size, no surgical examination
99	Gonad undifferentiated or not visible during surgical examination

Table 2. Sexual maturation state description for White Sturgeon (from Bruch et al. 2001)

Code	Developmental State Description
Male	
M0	Male based on previous capture; general unknown maturity.
M1	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	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	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.
Female	
F1	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	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	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	"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 diameter.
F5	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	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	Virgin female juvenile; small feathery looking, beige ovarian tissue attached to a thin strip of adipose fat tissue.

Adult Transport

Adult sturgeon that were selected as candidates for broodstock must be transported to Kootenay Trout Hatchery facility. The selected adults were loaded (using a stretcher) into a large (1.5m deep tank mounted on a 4.8m flat deck trailer), insulated tank filled with ambient river water and oxygenated to 90-100% with standard oxygen tank and diffuser equipment. Rock salt (heavy metal free) was added to the water (5% solution) to prevent osmotic stress, sterilize and aid with any bacterial and/or fungal infections, and to treat any minor injuries (i.e. hook wounds and surficial rope abrasions) sustained during capture. Staff regularly check fish for duress during the travel period which was approximately 4 hours from river to hatchery.

Adult Holding

Once broodstock have arrived at the hatchery they were placed in large (3.7m diameter), circular holding tanks and segregated by sex (maximum of four males or two females in a single tank). All tanks were equipped with covers preventing natural light from entering, but lights were set to simulate the natural photo period. The tank water was initially held at a temperature to match ambient river temperatures, and salt treatments continued as in the transport tank. Fish were closely monitored at hourly intervals immediately after arrival.

All adult sturgeon were continually monitored for health and reproductive status while being held in the hatchery, and slightly different husbandry protocols were used for males and females. The water temperature in female holding tanks was slowly increased in 1-2C increments per day until it reaches 16C and then remains elevated. Female fish were not fed, and if a female was thought to be close to spawning, she may be placed in a separate tank. Males were kept at the initial receiving temperature (usually 10-11C), and then increased to 15C after being induced to spawn (described below). In the initial years of program (2011 to 2005), males were fed Eastern Brook trout fingerlings (5-10g individual size) starting with 40 fish on arrival and additional fish were added (ad libitum) depending on the rate of consumption. Feeding of males ceased after 2005 and females were not fed at any time during

the program. Fish used as feed come from stocks designated as disease free. All tanks were covered but were exposed to a simulated natural photoperiod.

Adult Maturation Stage and Health Monitoring

As outlined above, adults undergo an initial maturation check when captured to determine the approximate state of maturation and select the most appropriate individuals. However, most individuals require significant additional maturation time while holding in the hatchery. Male spawn timing can be effectively controlled by hormone injections, but females must first develop their gonads to an appropriate state (requiring elevated water temperatures) before hormone injections can be used to induce spawning. Therefore, it was necessary to continually monitor the females to predict the timing of breeding potential. The "ripeness" of eggs was tracked using egg stage polarization index (PI) of the germinal vesicle (GV) and germinal vesicle breakdown (GVBD)(Conte et al. 1988). Depending on a subjective evaluation of the PI conducted immediately after capture, the condition of the fish and a visual appraisal of the biopsy eggs, a second biopsy will be conducted within 3-4 weeks after exposure to elevated water temperatures (15-16°C) in the hatchery.

The monitoring protocol requires a small sample of eggs (~20) to be removed through a small surgical incision in the abdominal wall. These eggs were placed into a 150ml beaker of Ringers' solution, placed on a hot plate and boiled for five to eight minutes. This process hardens the yolk and fixes the position of the nucleus (germinal vesicle, GV). The sample was cooled by placing it on ice and when cool, drained and placed in a 10% formalin solution overnight. To determine GV position relative to the center of the egg and the periphery, the egg was bisected and examined under a dissecting microscope. This procedure was accomplished by grasping the egg with a pair of tissue forceps and making a cut along the animal-vegetal axis using a sharp single-edged razor blade. The position of the GV was then observed and recorded as a proportion of 1.0. For example, a PI of 0.15 would mean a GV migration of 85% of the distance between the center of the egg and the periphery. Previous work (Conte et al. 1988) has shown that PI levels less than 0.10 are optimal to begin a spawning induction treatment with the

peptide human luteinizing hormone releasing hormone analogue (LHRHa). When a female has been determined to be ready for spawning induction she may be placed into a separate tank and injected with LHRHa.

Males did not need to be monitored for maturation status, as maturation timing can largely be controlled by LHRHa injection (see spawning induction section below).

Spawning Induction

Both male and female were induced to spawn using LHRHa. The target dosage of LHRHa for females was 50 µg/kg, which was administered in two doses given 12 hours apart: a loading (10%) and resolving (90%) dose. Female fish begin to ovulate and release eggs approximately 24 hours after the resolving injection of LHRHa. Once a female has been observed to be releasing eggs as evidenced by the presence of eggs on the tank floor, the water level was 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. Target egg volumes were collected from the female using manual expression through the urogenital opening or by extraction through an incision using a modified caesarean section method. To meet production and research targets, caesarean sections may be more frequently used as this method can sometimes more fully permit the expression of ovulated eggs.

Unlike females, male fish were held at 10°C until the LHRHa injections were administered for spawning induction. At that time the males tank temperature will be increased to 15C until breeding was complete. Male fish were intramuscularly injected with a single bolus dose (10 µg/kg) of LHRHa in saline 1-3 days prior to intended use. Milt was collected several hours before egg fertilization to create an efficient work process, since milt remains viable for several hours when kept on ice. Once egg collection was complete, the water temperature in the male sturgeon tanks was decreased back down to 10°C. This allows the male to “shut down” and possibly be used again for subsequent spawning events following the methods described above.

Breeding design for spawn events

The breeding design varied from the start to end of the broodstock program, starting with a monogamous mating design (1 female to 1 male) in initial years (2001 to 2007), and switching to a full factorial mating design from 2008 until termination in 2014. The factorial mating design implemented used approximately equal numbers of males and females (5 to 8 of each sex per year) and each female was crossed with each available male. This method maximizes the effective population size (Ne; e.g. Bartron et al. 2018) and is a common approach for conservation work with a limited breeding population.

The number of eggs allocated for each family was pre-determined by dividing the breeding design into overall release targets. Variation from the target breeding design and/or family specific egg targets could occur if a contributing male or female could not be induced to spawn or had poor quality gametes that reduced fertilization success.

Broodstock release

After spawning events, broodstock fish were held for about three additional days before being 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 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 (some years acoustic transmitters were added to the fish for studies). 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.

2.B.2 Early life stage methods

Early life stage in this report refers to all developmental stages after gametes collection from fertilization to hatch. Aquaculture of White Sturgeon is marked by several key milestones that are briefly summarized in Table **3** and described in more detail in the following sections.

Table 3. A simplified table of the key stages and actions for early rearing of White Sturgeon.

Stage	**Day**	**ATUS**	**Action**
Eggs and sperm	0	0	Fertilization, deadhesion and disinfection process
Fertilized eggs	1 (8-12hrs)	6-8	Fertilization check
Neuralized eggs	3 (72hrs)	44	Neurulation check and estimate fertilization rate
Hatch	8-10	112-140	Remove shells and enumerate egg losses
Larvae	20	290	First feeding

Egg fertilization process

Eggs to be fertilized were separated into stainless steel bowls (one bowl per maternal family). Sperm from a single male was added one bowl from each different female (prior to 2008 there was only one bowl per female, but in later years, female eggs were divided into separate bowls to match the number of males). The sperm and eggs were gently mixed while adding water and then after one minute the fertilized eggs were rinsed and a silt compound (a commercially manufactured compound called Fullers Earth) was added to the bowl to de-adhere the eggs. Gentle mixing continued until the eggs were no longer sticky which took up to and over one hour depending on the eggs. A constant water temperature was maintained throughout this process by setting the stainless-steel mixing bowls within a trough containing 14.5C running water and continually adding fresh silt-water to the individual bowls. After deadhesion, the eggs were disinfected using the standard practice of submerging eggs in an Ovadine (100ppm) and water solution for 10 minutes.

Incubation and fertilization checks

After egg fertilization, deadhesion, and disinfection was complete each individual family bowl of eggs was measured in volume to ml and transferred to an individual upwelling jar (industry standard McDonald jar type). The upwelling jars were plumbed to trough inflow so that the water flowed through the jar and spilled out running down the full length of the trough to the outflow. Trough water temperatures remained fixed at 14.5C at the source and flow was initially set at 5-10L/min with regular checks to ensure sufficient movement to prevent suffocation by fungus. An initial fertilization check would be performed after 8-12 hours of incubation by removing a small sample of eggs and individually examining the eggs in a Petri dish under a compound microscope. Eggs were examined to confirm cell division (2 to 4 cell divisions are expected in the 8-12 hour range), and therefore that fertilization had occurred. A more quantitative estimate of fertilization rate was obtained at 72 hours by removing a sample of 100 eggs. These later stage eggs were examined individually in a Petri dish for a visible notochord. Healthy eggs observed to have a notochord had completed neurulation, and the total number of neuralized eggs out of 100 was recorded as the fertilization rate for a given jar of eggs. This percentage could then be multiplied by the original volume estimated egg number in the jar to achieve a prediction of the number of progeny expected to eventually flow from the jar to the trough. Immediately after the neuralization rate had been confirmed the flow rate in the jars was increased to 20L/min as the eggs has passes the sensitive stage and additional flow increases egg movement aids in prevention of fungus. Water temperature remained constant from fertilization to hatch at 14.5C. Each upwelling jar was covered with a black cloth to prevent direct light exposure and labelled with the parental Male and Female PIT codes.

From Jar to Trough

The incubating eggs start to hatch out on or about day 8 (112 ATU), and normally by day 10 (140 ATU) all larvae have hatched out and have swum or spilled out into the receiving trough. Rearing troughs measured 3m long by .5m wide and were equipped with silicone sealed screens at inflow and outflow ends to ensure no escapement. The flow rate in troughs was set at 10-12 L/min and meant to exchange water, while taking care not to disturb larvae or force them to

expend energy swimming. During the initial hatching period it was important to ensure that egg shells were continually cleaned from screens. In the early years (2001 – 2007) a temporary angled screen was added mid trough to trap egg casings and facilitate removal before plugging of outflow screen, however, this was abandoned in later years as egg abundance targets decreased and experience increased. In addition to cleaning the egg shells all dead eggs were recorded as losses from the group, and this provides an updated abundance estimate for the free embryo stage.

2.W Wild Origin Approach (2014 – Ongoing)

Background and Objectives

The conservation sturgeon aquaculture program successfully reared and released nearly 200,000 sturgeon between 2001 and 2014. However, survival of hatchery-origin juveniles in the upper Columbia River population has been higher than originally predicted, with more than 20,000 individuals estimated to be at large in the population (BC Hydro 2018). Within the hatchery population, certain year classes are estimated to be in higher abundance than the existing wild population (~3,000 mature individuals), as a result of higher survival for year classes released at larger body size. Of further concern, within year class genetic diversity has been estimated to be reduced relative to the time of release from the hatchery (McLellan and Crossman unpublished data) as a result of disproportionate survival among maternal family groups. This reduction in genetic diversity compared to at the time of release from the hatchery during the broodstock approach led to a largescale adaptive management change, by initiating a shift in egg source away from broodstock capture to capture of wild progeny. This had been developed for lake sturgeon conservation aquaculture (Crossman et al. 2011) to improve genetic diversity, and field work in the Columbia demonstrated it was feasible to capture large numbers of wild larvae and eggs downstream of known spawning locations (primarily Waneta). Work by Jay et al. (2014) demonstrated an improvement in genetic diversity and, given the objectives of the overall program to rebuild abundance and maintain

genetic diversity, the use of wild progeny was deemed as the optimal source for conservation aquaculture program going forward. Therefore, in 2014 a mobile streamside rearing facility was piloted as an incubation method for wild eggs in conjunction with the traditional broodstock capture. Following 2014 the broodstock program was discontinued and the wild-origin approach has continued to date.

2.W.1 Early life stage methods

Capture of wild origin sturgeon

Sturgeon are broadcast spawners which allows for gametes to be sampled with passive sampling gear (Egg mats and Drift nets [AKA D-rings]). The effectiveness of egg mats and D-rings depends on the placing gear in the proper location and time relative to discrete spawning events, as well as the river conditions that influence the ability to maintain gear in certain areas of the river. Therefore, timing and location of capture effort was an important consideration.

Capture Equipment

Egg mats

Egg mats were comprised of a steel frame (0.77 X 0.92m) that enclose a latex covered animal hair filter material. The filter material was present on both sides of the frame, so when deployed, one side of the filter material was always sitting upward where drifting eggs and free embryos could lodge and attach. The egg mat system was secured into place on the river bottom with two 30 kg claw anchors attached to one another with an approximately 6 m galvanized steel chain and a float line which attached to the front anchor; a 10 m rope attached the rear anchor to the egg mat with a second float line (15 to 45 m depending on depth and flow) coming off the rear of the egg mat for retrieval. Near shore egg mat sample locations were tied to shore instead of having a second float line.

In order to retrieve the egg mats the appropriate float line was pulled up by the field crew, wrapped around the winch of the boat and retrieved. The egg mat was detached from the rope

system and replaced with a fresh egg mat so that the system could be immediately re-deployed. This allowed the field crew to re-locate to a secure location along the shoreline to carefully search through the trapped the egg mat debris for eggs and embryos.

Data collected at each egg mat included: station name; UTM; channel; set and pull date and time; water temperature; set depth; number of eggs; number of free embryos; number of eggs/free embryos preserved; other species/observations; and, any relevant comments.

D-rings

The D-ring (drift net) capture method has been successfully used to collect White Sturgeon eggs/free embryos in the middle and lower Columbia rivers in Canada as well as throughout the Columbia River in the USA. Drift nets consist of a D-shaped metal frame (0.8 m wide by 0.6 m high) opening into tapered plankton net with collection cup at the end (3.6 m long, 0.16 cm knotless mesh, 11.4 cm diameter collection bottle). In addition, a flow meter (e.g., General Oceanics) was fixed to the D-ring frame at the top of the opening to measure the volume of water sampled. Drift nets were set on the same anchor systems and/or rope used for egg mat sampling (Section above).

Retrieval of the drift net was similar to the egg mat in that the float line was retrieved and the winch used to pull the drift net into the boat. Upon retrieval of the drift nets, the contents of the bottle and drift net were emptied into a white bucket. The contents of the bucket were further diluted with river water into small white wash-basins and field crew members sorted through the debris to find and collect any White Sturgeon eggs/free embryos using tweezers.

Data recorded for drift net sampling included: station name; river km and channel location; set and pull date and time; water temperature; set depth; start/end readings of the flow meter for calculation of water volume sampled; number of eggs/ free embryos; number preserved; other species observed; and, relevant comments.

Egg and Larvae handling

The captured contents of egg mats were obtained by carefully inspecting the matt surface and picking off embryos and larvae. D-ring mesh and capsule contents were rinsed into a white

bucket, and then transferred in small diluted aliquots to several white plastic inspection trays. The trays allowed for contrast and dispersion of debris when searching for the tiny (often sediment covered) embryos.

The developmental stage of all sturgeon embryos was be determined in the field using a hand lens following the stages assigned by Dettlaff et al. (1993) and the manual developed by Jay et al. (2016). Embryos were grouped into similar developmental categories prior to transfer to the streamside hatchery trailer where each group was reared in a segregated jar and trough section (see below). Development categories are as per Table 4 below.

Table 4. Developmental categories used in the field to segregate rearing groups.

Developmental Category	Stage
Cleavage - Gastrulation	1 - 14
Yolk Plug	15 - 18
Neurulation - Heart formation - Pre-Hatch	19 - 35

Streamside incubation trailer

A streamside rearing trailer was created from a 14' by 8' cargo trailer to allow eggs and embryos to incubate at natural stream temperatures. The cargo trailer was set up with an electrical and plumbing system to enable pumping of river water through a series of MacDonald (upweller) jars, that spilled into a single trough with dividers to capture and segregate progeny from separate spawning events (Figure 2).

Figure 2. Streamside Incubation Facility (outfitted cargo trailer) used for rearing wild origin White Sturgeon progeny on the Columbia River.

The captured live embryos and larvae were immediately transferred to the streamside incubation facility. Embryos were placed into hatching jars whereas larvae were placed directly into the trough. In both cases they would be segregated based on their estimated spawning event group created from developmental stage (Table 4) and capture location.

Five incubation jars (MacDonald Type; J30, Dynamic Aqua-Supply Ltd., Surrey, BC) were available to group embryos by their unique spawning date and location. Water was flow through pumped from the Columbia River and maintained at approximately 5 L/min to ensure adequate embryo separation and oxygenation. Upon hatch, yolk-sac larvae were flushed from the hatching jars directly into a segregated section of the rearing trough associated with each hatching jar (larvae would be placed directly into a section of the trough for their spawning event group). The troughs were supplied with artificial substrate (1" diameter sinking Bio-Spheres; Dynamic Aqua-Supply Ltd. Surrey, BC) which allowed yolk-sac larvae to burrow into interstitial spaces mimicking behaviour documented in the wild (McAdam 2011). To reduce sediment in the incubation jars and tanks, water was filtered (254 micron; Spin-Down Separator, Denton, TX) and tanks were cleaned twice a week by purging to remove sediment and waste. All yolk-sac larvae were transported to the Kootenay Sturgeon Hatchery within 7

days of hatch in bags of ambient river water filled with oxygen. Temperature loggers inside the facility recorded air, river water, and facility tank water temperatures.

Transport of Larvae

Larvae need to be in the hatchery for rearing before 10 days post hatch to ensure success for first feeding. FFSBC staff made regular trips to the facility to retrieve larvae and transport them back to KTH to meet the 10 day deadline.

The larvae transport process was as follows: (1) Place two plastic bags (heavy duty clear) in a 10L pail and fill with 4-6 liters of ambient river water; (2) Carefully count and move a single spawn event group of larvae from their trough compartment into the bag; (3) Expel residual air from inner bag, then insert tygon hose from oxygen tank to fill the inner bag with oxygen, then twist and seal with electrical tape, then pull up the outer bag, twist and seal as the inner bag; (4) Places the larvae bag inside a chest cooler containing at least four bubble wrap covered ice packs, close the cooler lid, and place in the truck; (5) Transport to Kootenay hatchery with extra bags and oxygen on hand; (6) Once arriving at the hatchery, float each larvae bag within the destined container to allow the water temperature in the bag to slowly equilibrate with the container water, then finally cut open the bag and release the fish into their container. As in the streamside incubation facility, each spawning group was placed into a separate container.

2.C Juvenile life stage methods

2.C.1 Juvenile rearing

Larval transition to feeding

The two different approaches for acquiring sturgeon to bring into the hatchery, merge together once fish reach the larval stage. Therefore, the methods for the entire 2001 to present period are integrated for this life stage and beyond.

The sturgeon larvae remain largely sedentary over the first 10-15 days post hatch while they consume their yok reserves and continue to develop body parts and organs. The transition to

first feeding is a critical life stage for many fish species in both aquaculture and natural settings that can largely determine the relative success of a cohort. White Sturgeon require special attention at this stage.

The troughed larvae start to change behaviour at about day 18 (260 ATUs) where they begin to congregate into discrete groups within the available area of the trough (as opposed to an evenly distributed spread of individuals). Food can be introduced soon after this behaviour is observed and typically occurs on day 20 (290 ATU). The initial feeding was done by hand with the following process: drain level of pond/trough slightly to expose a damp side, place food onto the wet area on side of trough, allow water level to rise back to previous level so that fish can feed off sides of the trough. In addition to the hand feedings a 24-hour belt feeder was operated to continually dispense a target amount of food as close to the edge of the circular as possible. Each time the pond was drained down for hand feeding (placing feed on the pond walls), the old feed was gently swept out of the pond drain.

The type of food used for Sturgeon rearing has varied over the years for a variety of reasons. At program initiation BioDiet (a commercial feed from Skretting) was found to work very effectively, but at that time it contained bovine additive. However, in 2004 mad cow disease occurred in North America, and this prevented the import of products containing cow blood (which was a part of the BioDiet formula). When the alternate bovine free version of the commercial feed was used, the Sturgeon did not appear to respond well at first feeding and staff scrambled to try a variety of alternatives. A special concoction was developed at the Kootenay Trout Hatchery to encourage sturgeon larvae to feed. The mixture used the new BioDiet commercial trout food base (Skretting/Bio Oregon) that was mixed with a series of additives: krill powder, Cyclop-eeze (a biologically engineered organism of the Copepod family to increase concentrations of essential omega-3 fatty acids, biological pigments and other nutrients), freeze-dried blood worms, and anchovy oil. The additives were added in the highest proportion at first feeding (1/3 of total mass), and then gradually reduced as fish progressed until fish reached a size of 1g after which only the commercial food was used. In 2013

Cyclopeeze ceased to be manufactured and another special concoction was created using Golden Pearl (manufactured by Artemia) and krill (Table 5). At time of writing this report it is understood that Golden Pearl is also being phased out, and the tentative plan is to switch to Biodiet and screened, freeze-dried krill by 2020.

Table 5. Diet mixes used to feed different stages of Sturgeon in Kootenay Trout Hatchery from 2013 to present.

Fish weight (g)			Biodiet (pellet size mm)	Krill	Golden Pear
0.2	to	1.0	27% (0.3)	53%	20%
1.0	to	1.7	30% (0.3), 20% (0.5)	40%	10%
1.7	to	6.6	20% (0.3), 70% (0.5)	10%	0%
6.7	to	20.0	40% (0.5), 40% (0.7), 20% (1.0)	0	0
20.0	to	50.0	19% (0.5), 38% (0.7), 38% (1.0), 5% (1.2)	0	0
50.0	to	greater	40% (0.7), 40% (1.0), 20% (1.2)	0	0

Feeding rates were to excess (50% of body weight per day) for first two weeks, and continuous cleaning of the trough was necessary. After the first two weeks, the feed amount was based on temperature and body weight as per Table **6**, and the feed sizes also increased to match fish size (starting at 0.3mm and up to 1.2 mm by about day 45). The relationship between body weight and feed rate in Table 6 is a simple power function with a slope of -0.355, and a temperature dependent intercept that corresponds to the row for 1g mean body weight. The early larvae were reared in troughs for about 45 days, and then moved to self-cleaning circular tanks.

Table 6. White Sturgeon feeding rate guidelines as a function of average size and ambient temperature (units are percent of body mass per day).

Mean Body	Temperature (˚C)				
Weight (g)	9	11	13	15	17
0.05	21.6	2.7	5.9	4.6	5.2
0.10	16.9	2.9	5.7	4.7	5.2
0.15	14.6	3.1	5.6	4.7	5.1
0.20	13.2	3.2	5.5	4.7	5.1
0.25	12.2	3.3	5.4	4.7	5.1
0.50	9.5	3.6	5.3	4.8	5.1
0.75	8.2	3.8	5.2	4.8	5.1
1.0	7.4	3.9	5.1	4.8	5.1
2.5	5.4	4.4	4.9	4.9	5.1
5.0	4.2	4.8	4.8	5.0	5.0
7.5	3.6	5.0	4.7	5.0	5.0
10.0	3.3	5.2	4.6	5.0	5.0
25.0	2.4	5.9	4.4	5.1	5.0
50.0	1.9	6.4	4.3	5.1	5.0
75.0	1.6	6.7	4.2	5.2	5.0
100.0	1.5	7.0	4.2	5.2	5.0
250.0	1.0	7.8	4.0	5.3	4.9
500	0.8	8.5	3.9	5.3	4.9

Feed studies

The importance of diet was recognized early in the broodstock program, and feed comparison studies were formally implemented in collaboration with academic institutions and/or FFSBC science staff in 2008 and 2009 as many products were available. No final reports were located for these studies; however, the 2009 study archived the raw data and study description in FFSBC files (which was sufficient for analyses within this current report). In 2009, 12 troughs of individual family groups were stocked with equal amounts of hatched sturgeon (5300 fish per

trough). There were four feed treatments applied to the 12 troughs giving 3 replicates per treatment as shown in Table **7**. The feed treatments involved comparing two widely available commercial pellet feeds, Biodiet from Skretting and EWOS, with and without a special concoction of additives meant to illicit feeding behaviour. The feed mixture for additive treatments on file states: 1kg commercial feed + 30g of dried kill + 30g of Cyclopeeze + 15g of bloodworms, therefore the additives were a very small proportion of the overall feed mix (7%) in the experiment. Fish were troughed on July 30th, and then sampled at 10-11 day intervals until the last check on September 21st, which totals to a data set of 72 observations.

Table 7. Experimental design for 2009 diet study. Each treatment is applied to a single family group in a separate trough, and the initial number (5300) and weight (0.4g) was constant for all treatments.

Feed base	Additive	Family	Sample Dates
Biodiet	No	5	6
Biodiet	No	9	6
Biodiet	No	12	6
Biodiet	Yes	1	6
Biodiet	Yes	8	6
Biodiet	Yes	10	6
EWOS	No	3	6
EWOS	No	6	6
EWOS	No	7	6
EWOS	Yes	2	6
EWOS	Yes	4	6
EWOS	Yes	11	6
Grand Total			72

For this report, the data was summarized qualitatively to show the full growth and survival trajectories, and then quantitatively at two discrete time intervals. The first interval examined was 21 days post troughing, as this time step represented the highest growth and mortality period and is likely closely tied to the first feeding event. The second time step analyzed, was the final number and size of fish at day 52, as this represents the number and size of fish

resulting from trough rearing that would be moved to circulars. These discrete points were chosen as opposed to assuming a specific growth or survival function and fitting the time series, which is non-linear.

Juvenile grading, culling and rearing

As juveniles grow, they were regularly sub-sampled (volumetrically) to estimate overall abundance and biomass, as well as assessed individually for size, disease or deformities. Subsamples of 5-30 juveniles from all family groups were screened for viruses at 30 days and 60 days post hatch. These 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.

Once mortality rates appear to have stabilized and increasing tank biomass begins to reach capacity (individuals were approximately 0.2g in size), the fish will be graded. Typically, large individual variation in size was observed, and fish were graded in order to improve overall growth and survival. Fish were individually sorted into separate tanks for large or small categories, and this represents the first complete population census during the rearing process (only estimates from egg estimates and losses were recorded prior to this). The grading of individuals by size into two tanks reduces overall population densities and allows smaller individuals to more effectively compete for a fair ration of available food. This allows the smaller fish to achieve increased growth rates, so that they catch up to the larger individuals, and the overall group can more uniformly achieve the target release size. Regardless of size grade, all families or spawn groups were maintained separate, until after the fish were individually marked (see marking and releases below).

After fish have been graded and censused, a decision on whether culling was needed to reach release targets was made. Culling was conducted from 2001-2014 (the entire broodstock period) to meet release targets. Numbers of fish culled in each year varied and was conducted in early winter prior to the application of tags. To minimize artificial selection culled fish were randomly selected in equal amounts from each of the size category tanks. Individual, randomly

selected fish were counted out into a vessel containing 500mg/l TMS (tricaine methanesulphonate) according to FFSBC Standard Operation Procedure: Euthanasia.

2.C.2 Juvenile sampling, marking and releases

The conservation and research basis of the sturgeon conservation aquaculture program required that the fish be uniquely identifiable when recaptured in the wild. Therefore, prior to release (once the fish reached a size of approximately 50g) each juvenile sturgeon underwent up to three marking procedures: (1) Each individual was implanted with 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. Upon recapture in the wild, PIT tags allow individual level tracking of brood family as well as the ability to test the influence of factors such as release size, location, year, month, etc. on survival. (2) All hatchery fish also have a set number/location of scutes removed so that they are quickly visually identifiable in the field. The side of the body from which scutes were removed was changed to match the source/stage that were first reared in the hatchery from broodstock spawn events (left side) to wild origin larvae (right side). (3) In some years (most notably 2010), fish were also implanted with an acoustic tag, which allowed for tracking of individual level movements post release. All mark locations are depicted in Figure 3. DNA and blood smears were also taken during the marking process (see Polyploidy testing section below).

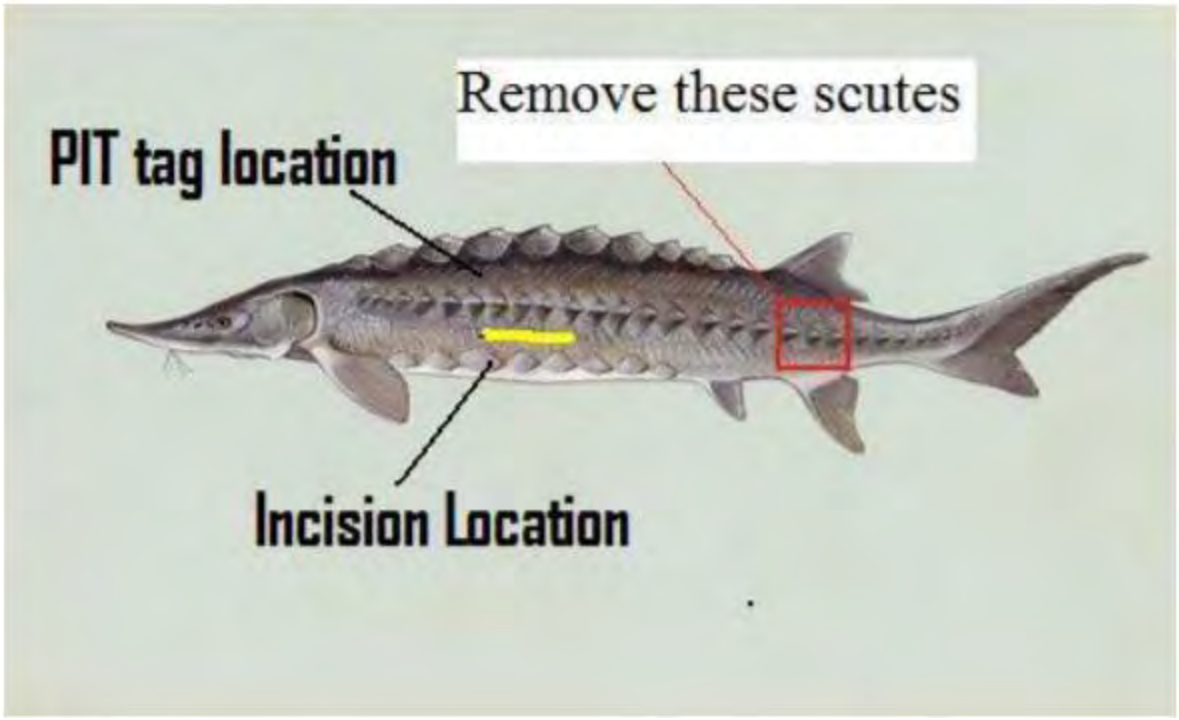

Figure 3. Diagram of physical locations of marks applied to hatchery reared juvenile sturgeon. Incision location refers to the location of acoustic tag insertion in years where acoustic tags were released (image extracted from BC Hydro CLB WORKS-34 2013 report).

Immediately prior to release (within 1 week) all fish were measured for length and weight in order to accurately reflect their size-at-release. A sub-sample of fish were run through an additional disease screening for the same metrics as the 30 and 60 day tests. The juveniles were transported to the release site using FFSBC transport vehicles according to UCWSRI TWG transport protocols. Over the years, sturgeon were strategically released (as directed by the technical working group) at a variety of sizes, locations and times (see Results) to assess the potential impacts of each of these covariates. In addition, a portion of the release events were used an opportunity to public engagement and education. The public release events allowed school children and general public to have the opportunity to release individual fish into the river at park type release locations (e.g. Beaver Creek, Shelter Bay).

Polyploidy testing

The ploidy (number of sets of chromosomes) of White Sturgeon is typically 8N in Columbia and Kootenay River populations (Schreier et al. 2011, Schreier et al. 2013, Gille et al. 2015). However, 12N White Sturgeon were found to occur (at a rate of up to 50%) at the Kootenay Tribe of Idaho's (KTOI) Sturgeon Hatchery in family groups that were derived from their wild broodstock program. The mechanism of this ploidy shift is unknown, but it is hypothesized to occur at the fertilization stage. Given the findings in the Kootenay River White Sturgeon, the Columbia River Technical Working Group directed the assessment of ploidy for all Columbia River White Sturgeon families reared at the Kootenay Sturgeon Hatchery. The standard protocol set be the Technical Working Group is that individuals found to be 12N will not be released in to the wild (i.e. must be culled).

The original blood smear method for assessing ploidy on White Sturgeon was developed from methods used by FFSBC to assess ploidy in salmonid stocks. In salmonid stocks, there is a visually distinguishable size difference in cell size between diploid and triploid fish when viewing slides of blood smears under a microscope. This same approach was used for White Sturgeon in 2012 using reference slides of known ploidy fish from the Kootenay River population. The slides were imaged under a Leitz Labourlux tri-ocular compound microscope mounted with a Canon Rebel TSi and using a 25X objective. The resulting images were run through an "Image J" program producing a spreadsheet of cell sizes. Staff were able to set up an Excel formula that categorized distinctly different sized cells associated with reference slides. After methods were established and applied in routine monitoring, all suspect 12N slides were re-imaged and re-analyzed for confirmation.

Starting in 2018 a new more efficient and automated method was created using a Coulter Counter (Beckman Coulter Z2). Coulter counters operate on the same basic premise of blood smears using the size of cell nuclei to assign ploidy based on established benchmarks. The equipment manual provides all technical details for operation, but the key setting of note is

that the upper size Tu be set at 9 microns and the lower size Ti be set at 3 microns. The Coulter counter allows many thousands of cells to be measured and is much less labour intensive.

All available data from 12N testing was analyzed using a generalized linear model in R (lme4 package) to assess whether there was an apparent difference in the occurrence of 12N fish between wild origin juveniles and hatchery spawned juveniles. The mixed effects modelling approach was selected to account for the random effect of brood year on 12N rates.

3. Results

3.B Broodstock Approach

3.B.1 Adult Results

Numbers, Timing and Location of Capture

A total of 220 adult sturgeon were captured and transported to the Kootenay Sturgeon Hatchery during the broodstock program from 2001 to 2014. The adults were captured in a variety of locations between Hugh Keenleyside dam and the USA Border, but the dominant site (>50% of total catch) was Waneta Eddy, with significant catch also obtained from immediately below HLK dam, the confluence with the Kootenay River and 3km upstream of Waneta at a location recorded as Fort Shepard (Figure **4**). The broadscale timing and locations of captured broodstock are summarized in Table **8** using Columbia River zones (Five equal 11.2 km reaches starting at Hugh Keenleyside dam down to the USA border) as a location category and month as the timing category. The summarized data shows that capture effort was initially spread across the entire spring and early summer period but narrowed down to the month of June by the end of the program. Furthermore, while 15 unique capture locations were recorded, they can almost exclusively be categorized into Zone 1 or Zone 5 (except for 6 individuals). All of the captured individuals selected for transport were very large at 199cm and 179cm average sizes for females and males respectively (Figure **5**), and all available individual level data is summarized in the appendix (Table A 1). In all the years of brood capture, there was 0 hooking

mortality observed (M Keehn per. Comm.), however, in 2002 one adult died during holding in the hatchery.

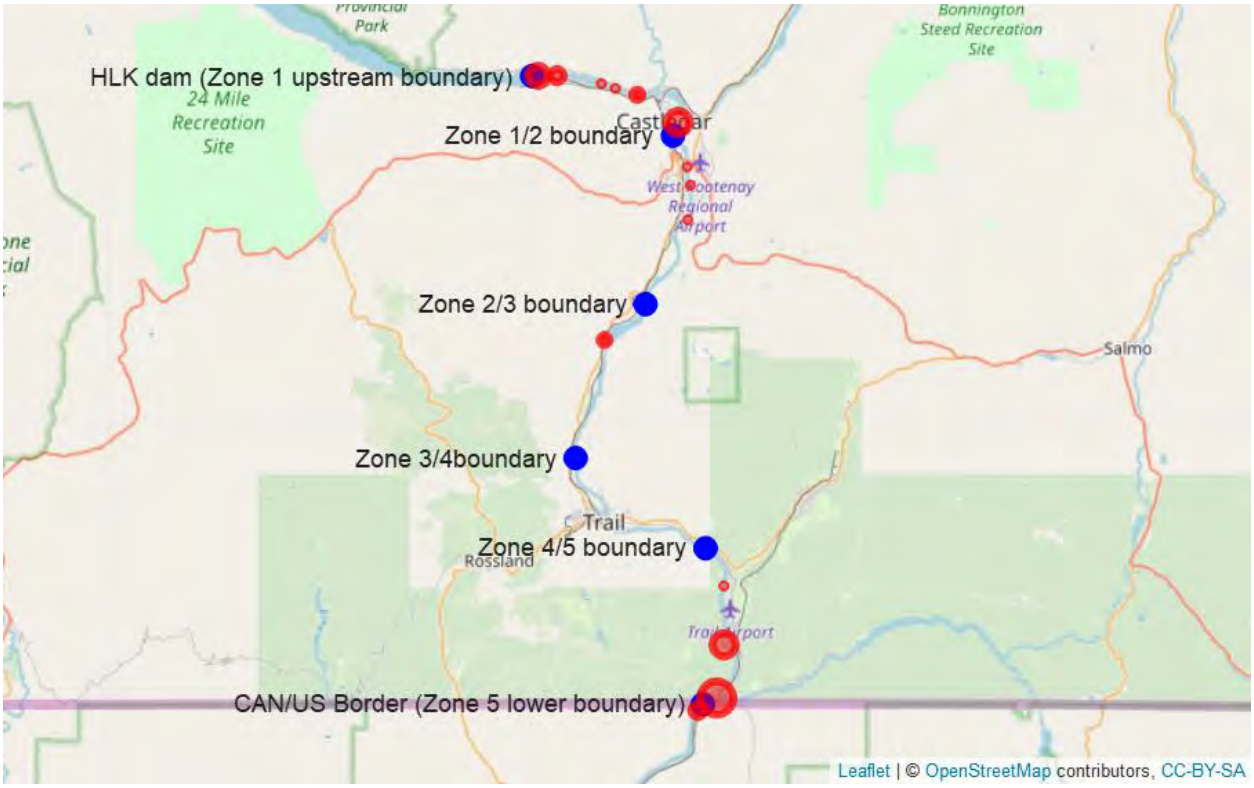

Figure 4. A map of broodstock capture locations (red circles) and zone boundaries (blue circles). The red circles are proportional to the log of the number captured in that location.

Table 8. Summary table of timing and location of adult sturgeon that were captured and transported to Kootenay Trout Hatchery.

Capture timing		Capture Zone					
Year	**Month**	**1**	**2**	**3**	**4**	**5**	**Total**
2001	3					3	3
2001	4	5					5
2001	6					2	2
2001	7					1	1
2002	4	5					5
2002	5					3	3
2002	6					4	4
2003	5	4				7	11
2003	6					2	2
2004	4	2					2
2004	5	3				1	4
2004	6	2				9	11
2005	6	1				19	20
2006	6					17	17
2007	6	4				10	14
2008	6					15	15
2009	6	5				12	17
2010	6	8		3		8	19
2011	6	10	1			10	21
2012	6	7		1		10	18
2013	6	4	1			8	13
2013	7	1				2	3
2014	6	1				6	7
2014	7					3	3
Total		62	2	4	0	152	220

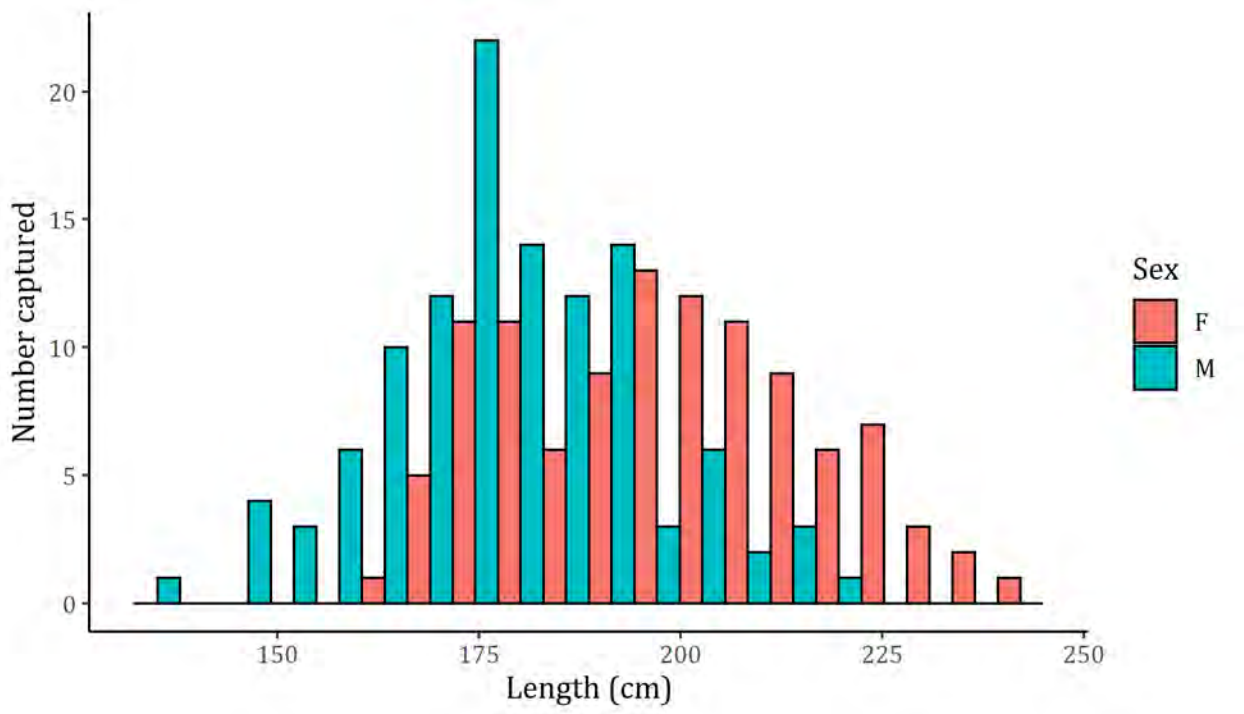

Figure 5. Length frequency distribution of broodstock captured during the 14-year program (N = 220).

Family crosses and spawn events

Out of the 220 adult sturgeon that were captured and transported to the Kootenay Hatchery, 173 were successfully spawned. Several spawning events were performed in each year depending on the timing and capture and maturation, with late June being the most common time period for spawn events (Figure 6).

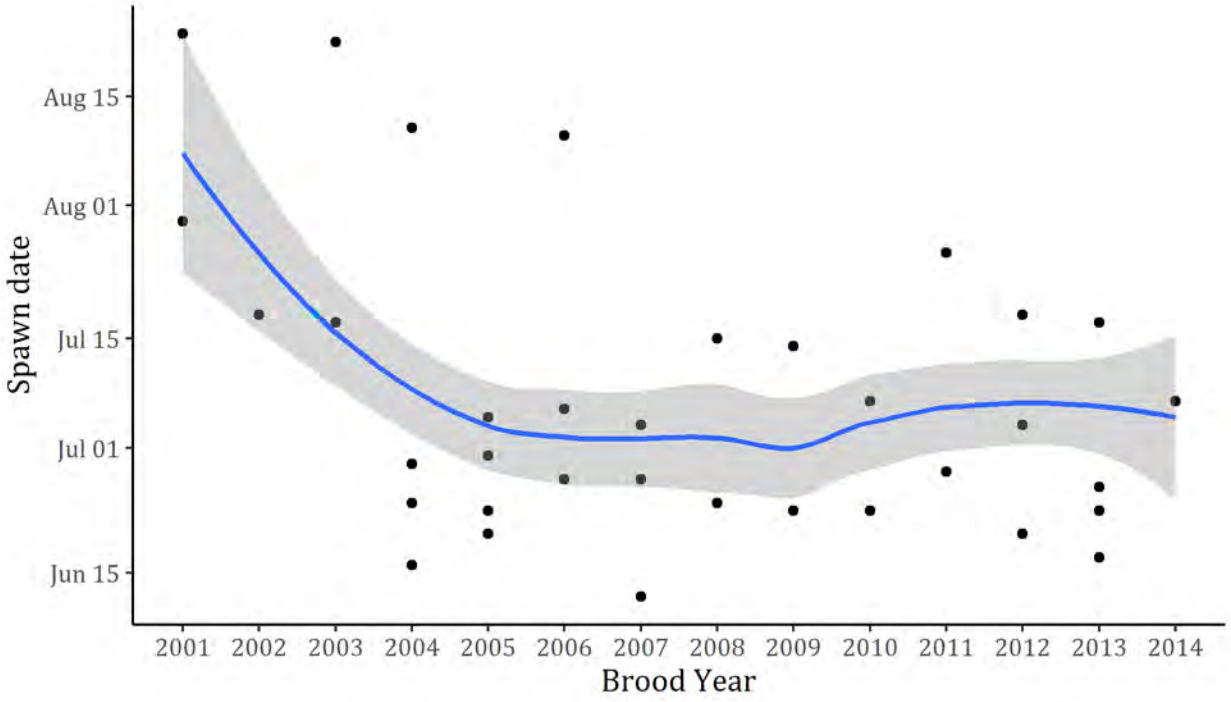

Figure 6. In hatchery spawn timing over the full broodstock capture period from 2001 to 2014. Points are individual spawn events and solid line is a Loess smoothing function to visualize the overall trend.

Of the 173 spawned adults, 160 successfully contributed to viable progeny that were released into the Columbia River. The reduced number was a function of sterile males where fertilization did not occur or complete mortality of family groups during early rearing. A list of each specific family cross for each brood year is in the Appendix (Table A 2), and a summary of the family crosses in (Table 9). Table 9 shows the basic calculation of effective population size for each brood year as $Ne = 4NmNf/(Nm + Nf)$ (Wright 1931).

Table 9. Summary of contributing adults, total crosses (families) and effective population size (Ne) for each brood year.

Brood Year	Unique Females	Unique Males	Total crosses	Ne
2001	2	4	5	5.3
2002	2	5	5	5.7
2003	2	5	6	5.7
2004	5	4	9	8.9
2005	8	8	9	16.0
2006	6	7	7	12.9
2007	4	5	6	8.9
2008	5	6	16	10.9
2009	7	8	33	14.9
2010	6	10	17	15.0
2011	8	9	32	16.9
2012	5	10	25	13.3
2013	6	3	9	8.0
2014	5	5	25	10.0
Grand Total	71	89	204	158.0

3.B.2 Early Life Results

Fertilization to Hatch

The number of eggs collected from a female was based on back calculating from the target release number based on expected mortality rates. One of the initial major sources of loss that must be accounted for is unfertilized or non-viable eggs, and this was tested by counting the proportion of neurolated eggs out of a sample of 100 eggs from an upweller. A summary of data obtained for most years of the program indicated that % neurulation averages approximately 60% but was highly variable between groups with a standard deviation of approximately 33% (Table 10). Examination of raw data shows neurulation estimates from individual upwellers range from 0 to 99%, and this large variation can be found even within spawning dates and sibling groups. It was typically assumed that neurolated eggs will hatch with minor losses, so this measure multiplied by the number of green eggs in a group was

expected to be equivalent to the hatched larvae. Green egg estimates were derived from multiplying the volume of eggs obtained by the estimated number of eggs per liter (calculated from sub samples or using an average from previous sampling). The eggs per liter values were not routinely recorded into a long-term database but ranged from 23,000 to 44,500 and averaged 36,400 in raw data files from 2008 to 2012.

Table 10. Percent neuralization over years where data were available. Green eggs refer to eggs estimated from volume. The mean and standard deviation were based on the number of different upwellers checked in a given year (reported as N). Each upweller sample represents approximately 100 eggs that were individually counted for neurulation.

Brood Year	Green eggs estimate	Mean % Neurolated	St. Dev. of % Neurolated	N
2004	329,400	50.0	42.4	18
2006	353,600	30.5	24.6	17
2008	180,215	76.3	22.8	16
2010	1,345,750	66.0	35.1	22
2011	468,214	68.6	25.1	26
2012	418,830	57.2	31.6	25
2013	418,038	71.2	23.2	6
2014	520,978	53.0	22.6	5
Average		59.1	28.4	

3.W Wild Origin Approach

3.W.1 Captured embryos and larvae

Wild embryos and larvae have been consistently captured during the last decade, although capture rates can be variable (Table 11). In the time period since 2014 when wild embryos and larvae have been used in the conservation aquaculture program the total catch has varied from under 400 to over 6000. Waneta has been the most consistent capture location by a large margin, however, significant numbers of embryos and larvae have been captured just below Hugh Keenleyside dam in a few years. The use of wild origin embryos and larvae does present several challenges for aquaculture (e.g. sediments stuck to eggs, transport between facilities,

etc.). Thus the number of embryos and larvae captured does not reflect the starting number of live larvae arriving at the hatchery (see bottom row Table 11)

Table 11. Sampling effort and capture numbers for White Sturgeon embryos and larvae by sampling location. Larvae categorized as "Hatchery" refers to the total number of resulting larvae received at Kootenay Sturgeon Hatchery in each year.

	Effort			Embryos			Larvae			
Year	Waneta	Kinnaird[b]	ALH	Waneta	Kinnaird	ALH	Waneta	Kinnaird	ALH	Hatchery
2008	19,500	16,657	-	3,950	0	-	220	1	-	-
2009	22,054	976	-	1,792	0	-	41	5	-	-
2010	18,317	2,104	5,692	4,891	1	42	105	8	115	-
2011	19,932	2,547	6,152	2,552	2	185	25	32	308	-
2012	16,675	197	2,929[b]	360	0	6	17	0	0	-
2013	14,739[a]	517	680[b]	410	0	0	0	5	0	-
2014	19,405	2,538	2,665	5,762	6	0	67	13	0	1,951
2015	22,291	3,036	1,373[b]	253	0	0	56	8	1	174
2016	14,796	2,633	1,006[b]	6,473	0	0	959	17	0	2,245
2017	11,290	1,289	2,146[b]	2,475	1	511	584	14	159	1,452
2018	7714	2,758	2,290[b]	9,970	0	3	587	4	14	1,940

[a] No drift net sampling effort, [b] No egg mat sampling effort

3.C Juvenile Life Stage

3.C.1 Juvenile growth, survival and diet studies

Sturgeon larvae develop and grow at rapid rates, which makes first feeding a concentrated and sensitive time period. Typically, the fish were minimally handled at this stage, and data on growth and survival rates were not compiled from the first feeding period over all the years. The diet study data from 2009 was very valuable as individual family groups were carefully monitored over small time intervals and early growth and survival patterns were well documented (Figure **7**). The raw data indicate an exponential gain in weight over the first 50 days and a non-linear mortality function. Mortality patterns show substantial mortality occurs in in the first 21 days (highest mortality rate was between 11 and 21 days), and then stabilizes at a much lower rate after that point.

Raw data patterns in Figure **7** suggest that commercial base used in diet has an influence on mortality patterns, but the effects on growth and use of additives are not visually apparent. The raw data also highlights the potential random effect of family that can influence results, but unfortunately, the same family was never used for multiple treatments. Modelling the final weight and final number within a generalized linear model found that the commercial feed base had a significant effect on both growth and survival, but the special additives did not have a significant effect (although results were in a positive direction when using additives). These results suggest that additives likely have a minor effect if at all, but the effect might be masked by using a relatively low proportion of additives, and the unknow effect of family relative to the treatments.

Regardless of whether specific diet concoctions had significant rearing impacts, the Biodiet plus additives treatment was valuable data as it closely resembled the actual diet and husbandry practices implemented during the sturgeon aquaculture program at Kootenay Sturgeon Hatchery. The treatment resulted in an average (52 day) survival rate of 65% for the three families exposed whereas the average survival over all groups was 54%. Fish weight was more similar among groups, with the Biodiet plus additives having an average final size of 1.62g compared to an overall average of 1.47g. Given that it was not standard practice to continuously record growth and survival at 10-day intervals these data are helpful in understanding the overall growth and survival trajectories expected during initial juvenile rearing.

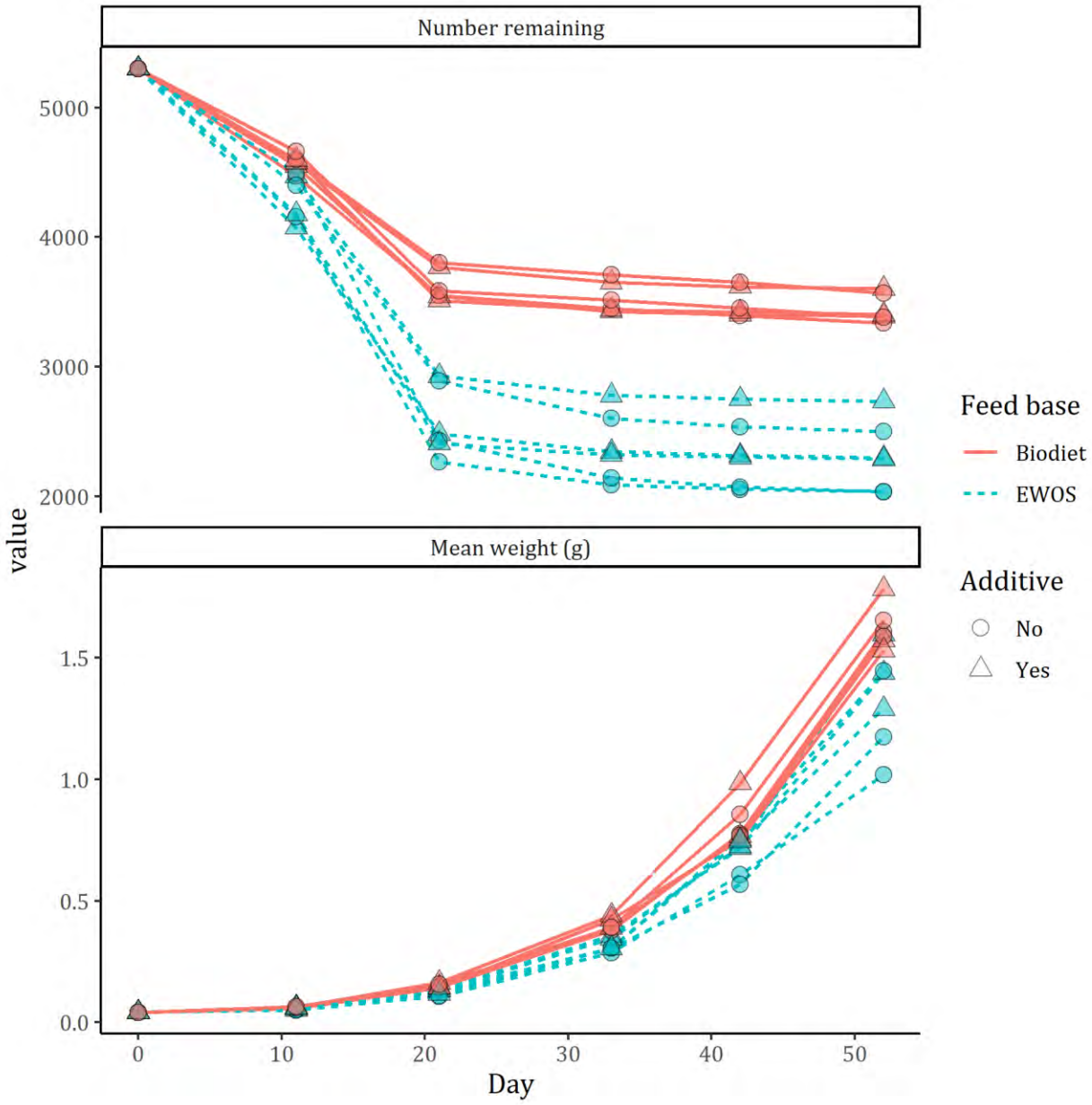

Figure 7. Survival (upper) and growth (lower) of juvenile white sturgeon reared with different commercial food bases (Biodiet/Skretting and EWOS) and with and without additives (indicated as Yes/No in graph).

There were not continuous data available to follow growth and survival in later stages of juvenile rearing after the period covered by the diet studies. During the broodstock period, the standard practices required substantial grading and culling at these stages, which makes

interpretation of survival and growth rates extremely difficult. However, in the more recent years of wild origin progeny there was not intentional culling of fish, and so it was possible to observe the overall survival and growth rates from received larvae to yearlings (or older) released. Table 12 documents numbers of each brood year class from larvae to release. The survival rate was quite consistent and averages 52% overall.

Table 12. Numbers of wild larvae received at Kootenay Sturgeon Hatchery for each brood year and the resulting number of juveniles (yearlings or older) released.

Brood Year	Starting larvae	Released	Survival rate
2014	1,951	1,095	56%
2015	174	76	44%
2016	2,245	1,224	55%
2017	1,452	607	42%
2018	1,940	741	55%*
Totals	7,762	3,918	52%*

*Some 2018 brood year fish (N = 323) still held in hatchery at time of writing and were summed with released to calculate 2018 cohort and overall survival.

3.C.2 Juvenile releases

The 2001 to 2014 broodstock based conservation aquaculture program has released a total of 1,532,226 sturgeon into the Columbia River. However, most of these releases (1,363,588) were released as larvae which would be expected to have very low if any survival. The 168,714 sturgeon released as yearlings or older are the more relevant quantity of released fish. Even at the older age, survival has been found to be size dependent (BC Hydro CLBMON-29 2016), and a trend towards larger release size is notable in the stocking records (Figure 8). The timing of release was varied somewhat over the timeseries, but primarily is focused on the month of May.

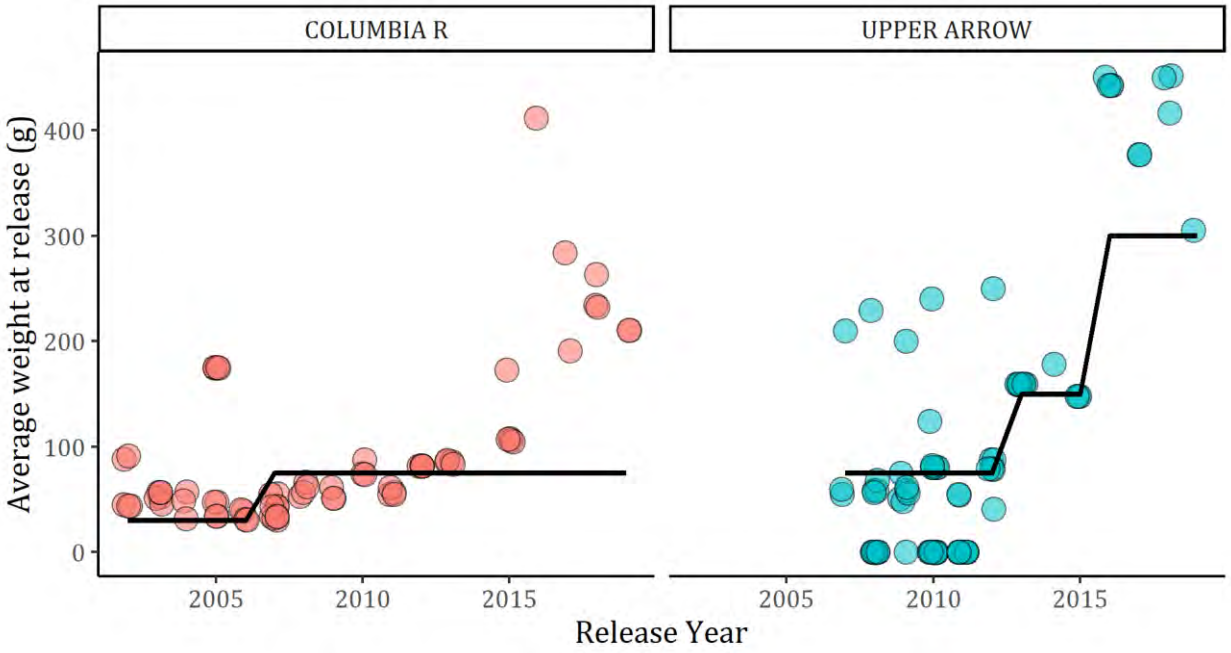

Figure 8. Trends in release size (g) over the full span of the aquaculture program to 2019. Each point is a unique release group. Panels are labelled by release location: Columbia R = All release sites below HLK, and Upper Arrow = All releases size above HLK. Groups appearing as 0g, were larvae experimentally released into Upper Arrow. The solid back lines depict the minimum size-at-release targets, which changed for both areas over time. Points are jittered for visual discrimination.

The database of all released individuals with uniquely identifiable PIT tags that originated from the broodstock program sums to 172,043 in Canada as well as 8258 that were released in the USA (Table 13). The location of release has initially coincided with easily accessible sites proximate to known spawning locations: (1) below HLK and (2) Beaver Creek which is near Waneta spawning site. However, in 2010 small numbers of fish were released in all zones as part of a movement study, which found that fish released near Waneta had a high probability of emigrating to the USA (James Crossman, BC Hydro, Personal Communication). Therefore, after that year the fish destined to be released in zone 5 were split between zone 5 and zone 3 (Gennelle boat launch), and the zone 5 site was moved upstream to the Trail airport. In addition, the zone 1 release location was changed to include Kootenay eddy instead of releasing at HLK only. Releases above Hugh Keenleyside Dam began in 2008 and generally

focused at Revelstoke, but Shelter bay (River km 178) has been used consistently since 2011. Also of note, stocking time was varied between groups 12am and 12pm in Revelstoke releases of May 2007.

Table 13. All releases of brood and wild origin progeny reared at Kootenay Sturgeon Hatchery and released into the Columbia River. Columbia River zones refer to five 11.2 km sections between Hugh Keenleyside Dam and the USA border.

Source	Release Year	Columbia Zone of Release					Arrow	USA	Total Releases
		1	2	3	4	5			
Brood Spawn	2002	4,407				4,264			8,671
	2003	5,724				6,079			11,803
	2004	4,828				4,867		1,882	11,577
	2005	8,996				8,881		3,755	21,632
	2006	7,267				7,603			14,870
	2007	6,237				5,915	4,206		16,358
	2008	3,216				3,233	6,534		12,983
	2009	3,738				400	8,118		12,256
	2010	3,458	15	14	15	515	9,575		13,592
	2011	2,500		1,000		510	8,078	2,621	14,709
	2012	3,189		1,000			6,567		10,756
	2013	2,293		875		869	5,944		9,981
	2014	1,200		300		301	6,017		7,818
	2015	1,600		600		600	3,288		6,088
Wild Capture	2015					1,095			1,095
	2016					76	1,324		1,400
	2017					800	1,589		2,389
	2018	378				229	975		1,582
	2019	100				100	541		741
	Total	59,131	15	3,789	15	46,337	62,756	8,258	180,301

*2019 releases up to July 1, 2019.

Autopolyploidy

Results from screening of hatchery reared fish (since brood year 2013) have demonstrated the 12N occurrence to be very low (Table 14), but fish originating from broodstock have significantly higher rates of occurrence than fish originating from wild captured embryos (GLM effect of origin $p < 0.0001$). Fitting the data to a generalized linear mixed model (glm function

Origin + Brood Year + Method, binomial family, in R lme4 package) indicates that broodstock origin fish are approximately 40X more likely to be 12N than wild origin progeny. The higher occurrence in juveniles derived from in-hatchery broodstock spawning does support the hypothesis that autopolyploidy is influenced (unintentionally) during the very early developmental stages at, or immediately after, fertilization. However, the consistent (but extremely rare) occurrence of 12N wild origin fish confirms that there is low level natural occurrence of 12N fish.

The recent advancement in 12N assessment technology to the use of a Coulter counter has made ploidy assessment much more efficient and presumably more accurate. In 2018 a set of 112 individuals were analyzed with the two methods for comparison. The blood smear method and Coulter counter both detected 5 of the individuals as being 12N, but the Coulter method also detected two more individuals as 12N suggesting that historical data may underestimate the true prevalence of 12N. The bias could be proportionally substantial (i.e. 2/7 = 29%), but since 12N rates are so low this would still only represent a tiny proportion of released individuals (i.e. less than 1% of the fish released as 8N could have been 12N since ploidy monitoring began).

Table 14. The proportion of hatchery reared fish testing positive for 12N ploidy**.**

Origin	Brood Year	Method	Sample	12N	Proportion 12N
Hatchery	2013	ImageJ	180	9	0.050
Hatchery	2014	ImageJ	1,693	124	0.073
Wild	2014	ImageJ	1,098	2	0.002
Wild	2015*	ImageJ	63	1	0.016
Wild	2016	ImageJ	1,209	1	0.001
Wild	2017	ImageJ	737	3	0.004
Wild	2018	Coulter	1,063	4	0.004
Wild	2018*	ImageJ	112	5	0.045
Wild	2018*	Coulter	112	7	0.063

* 2018 Paired re-sampling of same 112 individuals to compared methods.
* In 2015, an additional 2,154 wild origin sturgeon larvae captured in the USA were transferred to Kootenay Sturgeon hatchery and all 2,154 were confirmed as 8N using Image J method.

4. Discussion

The Upper Columbia White Sturgeon Recovery Initiative has accomplished a vast amount of work towards numerous aspects of recovery of this population (e.g. see Upper Columbia White Sturgeon Recovery Initiative Operational Plan updated annually by the technical working group). The conservation aquaculture program has been a vital component of the initiative and the successful establishment of hatchery juveniles in the Columbia River has prevented extirpation of the population. The present abundance of sturgeon in the upper Columbia is estimated to be 5 to 10 times greater than without intervention for Canada and USA respectively. Moreover, one of the key recovery plan objectives of 2,500 reproductive adults in Canada has been found to be easily achievable with the current stocking program given current estimates of survival rates.

This report documents all the available data associated with works at the Kootenay Sturgeon Hatchery since 2001. The data had not previously been presented in a comprehensive format and the full data set allows for better understanding of key parameters, performance measures and their uncertainty. Starting from the earliest point of production, fertilization and neurulation, were quite low and highly variable in Columbia White Sturgeon. The neurulation rates and variability did not improve with experience; thus, these results suggest that others planning to implement White Sturgeon broodstock programs should account for high variability at this stage and set green egg collection targets accordingly. Moving to the next life stage, first feeding, the diet study data indicated that outcomes were more consistent among families, and that the rearing methods used (e.g. diet type) can significantly (and consistently) alter the loss rate. The loss rate at this stage is significant (average survival of 54%) but was significantly altered by rearing methods (i.e. Biodiet feed base improved average survival by 21% over EWOS). Therefore, our data suggests that careful planning and monitoring of diet and feeding practices during the first feeding stage is likely to yield the most benefits in White Sturgeon aquaculture, which matches findings for other sturgeon species (e.g. Gisbert and Williot 1997,

Valentine et al. 2017). The diet study monitoring was very intensive (census every 10 days); however, the significant loss period was typically complete by day 21 (although slightly elevated mortality observed between day 21 and 33 for EWOS base), and therefore a starting inventory at troughing and an ending inventory at approximately one month should provide all necessary information for understanding effects of first feeding practices. The mortality rates from day 21 to day 52 in the diet study data were very low (5 and 10% for Biodiet and EWOS respectively) and although data collection did not continue past day 52, the summary of survival from larvae to release (Table 12) shows that mortality typically continues to be minimal until release. Nonetheless, the program may benefit from setting up more frequent reporting intervals for survival trends in the long-term database, so that the effects of any changes to aquaculture practices or available feed types can be assessed. At present the number of fish being reared is very low (release target is currently 200 Sturgeon to Canadian Columbia River below HLK) and start from a later life stage, so this should facilitate data recording and storage.

There has been routine monitoring for spontaneous autopolyploidy in the Upper Columbia aquaculture program since 2014 following evidence of potential hatchery induced autopolyploidy in other systems (Drauch Shreir et al. 2011, Schrier et al. 2013). Sampling now includes 6 brood years in hatchery, in addition to numerous age classes of hatchery and wild origin fish from stock assessments (BC Hydro CLBMON-28 2017). The autopolyploidy monitoring data from Upper Columbia White Sturgeon is quite valuable in that it includes various age classes and spans the transition in sourcing hatchery progeny from broodstock to wild capture. The data in Table 14 suggests that hatchery practices do somehow (unintentionally) increase the rate of 12N progeny as broodstock source progeny did have higher 12N rates than wild captured embryos and larvae (despite a less efficient detection method). However, the stock assessment data in BC Hydro CLBMON-28 2017, provides the strongest evidence of the effect of hatchery influence as sub-adult hatchery fish always had between 1 to 4% 12N occurrence over four years of monitoring (N = 1678), whereas wild adult occurrence was only one 1 out of 490 (0.2%). Still, the monitoring of wild origin progeny used in the conservation hatchery program is conclusive that 12N spontaneous autopolyploidy does

occur naturally at a very low rate, since the processes expected to influence autopolyploidy would occur at the time, or immediately after (within minutes), of fertilization (Gille at al. 2015), which is before there is any chance of handling or hatchery influence. The observed rates of 12N in wild adults captured during stock assessment is very low at 0.2%, but this is within the range observed in the wild origin hatchery data. As more stock assessment and hatchery data is collected, it may be possible to ascertain if there is any survival disadvantage of 12N fish in the wild (i.e. a lower rate in wild stock assessment adults than wild captured embryos).

At the start of the aquaculture program there were many unknowns, and this was the Freshwater Fisheries Society first attempt at rearing Sturgeon. The Kootenay Sturgeon Hatchery has been significantly refined over the years in many aspects that were not captured in datasets, such as hatchery infrastructure and staff experience. Moreover, the aquaculture program as guided by the UCSRI technical working group has been a continual learning process and always benefitted from an adaptive management approach. The program has evolved in terms of methodological changes documented in this report, but also overall targets have changed as more information on population statistics and life history parameters have come available. A list of the key milestones and adaptive changes are presented in Table 15.

The most fundamental adaptive change in the program has been the switch from spawning captured broodstock to capture of wild embryos and larvae. The change has significantly increased the genetic diversity of the released juveniles which is a key objective of the program. While significant field effort was needed to capture the embryos and larvae, capture of adult broodstock was also labour intensive and involved handling and transporting large and extremely valuable remnant wild individuals. By the end of the broodstock program, catch rates of wild origin brood decreased significantly due to the increasing by-catch of hatchery fish (i.e. the ratio of hatchery to wild fish caught in the final year of broodstock capture was 4:1, J. Crossman pers. comm.). The capture rates of wild origin embryos and larvae cannot be predicted with certainty; however, the program has also dramatically decreased the target

release numbers over time, as evidence for high survival rates of released fish has accumulated. At this point, there is a large number of age classes and juvenile sturgeon in the system, so that the risk to overall recovery targets associated with inconsistent numbers of wild progeny is very low. Furthermore, as hatchery fish gradually increase in density and recruit to the spawning population, the densities (and therefore CPUE) of embryos and larvae in the system should begin to increase and a decision will need to be made of how to incorporate these progeny into the program if they reflect genetics of fish previously released in larger numbers from the hatchery.

The UCWSRI continues to work towards a better understanding of the mechanisms of recruitment failure and any potential restorative actions, however, at the time of writing this report there is still no clear path forward to address recruitment failure outside of aquaculture. Thus, there must be a sustained effort to continually review and improve the aquaculture program in order to decrease artificial selection as much as possible. For example, there could be fine scale local adaptation within spawning locations of the Canadian Columbia River, and using only locally sourced embryos for releases (currently not a strictly enforced objective) could be a requirement in the future. Also, the technical working group has discussed the potential negative effects of holding fish for extended periods in the hatchery before release, and whether accepting a lower initial survival rate may be preferential to setting size-targets that require long term (sometimes 2 to 3 years) rearing (which may have longer term behavioural or artificial selection effects).

Overall, this report is part of the adaptive management process to periodically review historical data and trends to ensure the most appropriate path is selected going forward. At this point in time, some of the pressure to prevent population extinction has been relieved from the large number of juveniles released to date, and confirmation of their survival from ongoing stock assessment work (e.g. BC Hydro CLBMON-28 2018). Moreover, the switch to wild origin sources for hatchery rearing has reduced concerns about potential negative impacts of hatchery selection. Still, ongoing adaptive management changes to the aquaculture program are

foreseeable in the context of issues such as: high transboundary survival rates and system carrying capacity, recreational fisheries in the US, and low upper Columbia survival rates and potential influence of size at release. Notable the technical work group members in Canada have recently agreed to a substantial decrease in stocking rates based on population forecasts using all available survival data. The US technical working group members are also setting targets based on the updated forecasts, but their stocking rates may increase slightly to allow for recreational harvest opportunity.

Table 15. Summary of adaptive management changes made during the last 18 years of the White Sturgeon conservation aquaculture program in transboundary and Canadian reaches of Columbia River.

					Targets							
					Adults		Releases					
Area	**Milestone**	**Adaptive change**	**Period applied**	**Approach**	**Canada**	**US**	**Canada**	**US**	**Total**	**Min. size (g)**	**Families**	**Breeding**
Below HLK Dam	Program Initiation	-	2002-2003	Broodstock	2,500	2,500	12,000	0	12,000	30	>= 5	Partial Factorial
	Releases begin on US side of border	Release numbers	2004-2006	Broodstock	2,500	2,500	12,000	4,000	16,000	30	>= 7	Partial Factorial
	Program review in 2006. Survival higher than expected and diversity less than expected.	Release numbers and size	2007-2010	Broodstock	3,000	3,000	4,000	4,000	8,000	75	>= 7	Full Factorial
	Pilot years for wild progeny	Gamete source	2010-2015	Both	3,000	5,000	4,000	4,000	8,000	75	>= 7	Full Factorial
	2012 recovery plan revision	Release numbers and size, and source potentially	2012	Both	2,000	5,000	2,500	2,500	5,000	75	>= 7	Full Factorial
	Wild progeny adopted	No more broodstock origin released	2015-on	Wild origin	2,000	5,000	2,500	2,500	5,000	75	-	-
	High juvenile survival and US fishery	Stocking rate changes, exploitation targets	2019 - on	Wild origin	2,000	5,000	200	*3,000*	*3,200*	200	-	-
Above HLK Dam	Program Initiation	-	2007-2012	Broodstock	NA	-	≤12,000	-	≤12,000	75	>= 7	Full Factorial
	Apparent low survival	Experimental changes to SAR	2013-2015	Both	NA	-	≤12,000	-	≤12,000	150	-	-
	Apparent low survival	Experimental changes to SAR	2016-on	Wild origin	NA	-	≤12,000	-	≤12,000	300	-	-

*2019 stocking rate targets yet to be confirmed in US

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6. Appendix

Table A 1. Complete summary of adult captures as part of the Canadian Columbia River White Sturgeon broodstock program.

Brood Year	Capture Date	Capture Location	Spawn Date	Release Date	Sex	PIT	FL (mm)	Wt (g)
2001	15-Mar-01	Fort Shepard	23-Aug-01	7-Sep-01	F	4264713044	227	81
2001	19-Apr-01	Bob's Hole	30-Jul-01	20-Sep-01	F	42656F031A	178	41
2001	20-Mar-01	Fort Shepard	Not Spawned	19-Sep-02	M	7F7D502515	170	35
2001	17-Apr-01	Kootenay Eddy	23-Aug-01	20-Sep-01	M	4264797C36	200	51
2001	26-Jun-01	Waneta	30-Jul-01	7-Sep-01	M	7F7D4F211B	216	59
2001	25-Apr-01	HLK	Mort	died 28 Aug 02	F	426506011B	174	34
2001	27-Apr-01	HLK	Not Spawned	20-Sep-01	F	7F7D224271	202	56
2001	6-Jul-01	Waneta	Not Spawned	18-Sep-01	F	4264712614	235	81
2001	13-Mar-01	Fort Shepard	30-Jul-01	18-Sep-01	M	7F7D4D1329	220	59
2001	17-Apr-01	Kootenay Eddy	30-Jul-01	20-Sep-01	M	7F7D491874	164	29
2001	20-Jun-01	Waneta	30-Jul, 23-Aug, 01	18-Sep-01	M	42647D7B37	184	33
2002	23-Apr-02	HLK	18-Jul-02	1-Oct-02	M	7F7D220955		52
2002	23-Apr-02	HLK	18-Jul-02	1-Oct-02	M	7F7D1D1231		61
2002	17-Apr-02	Kootenay Eddy	18-Jul-02	2-Oct-02	M	464581E7B		53
2002	19-Apr-02	HLK	Not Spawned	2-Oct-02	M	7F7D1C206F		50
2002	25-Jun-02	Waneta	18-Jul-02	19-Sep-02	M	4264551446		44
2002	26-Jun-02	Waneta	18-Jul-02	19-Sep-02	M	42634C245B		30
2002	1-May-02	Fort Shepard	Not Spawned	9-Aug-02	F	426508183C		69
2002	27-Jun-02	Waneta	18-Jul-02	18-Sep-02	F	7F7D1D0B11		83
2002	25-Jun-02	Waneta	18-Jul-02	9-Aug-02	F	7F7D1C5C60		64
2002	17-Apr-02	Kootenay Eddy	Not Spawned	20-Aug-02	F	4264602042		63
2002	2-May-02	Fort Shepard	Not Spawned	17-Oct-02	F	4255471903		39
2002	2-May-02	Fort Shepard	Not Spawned	18-Sep-02	F	115552157A		78
2003	21-May-03	Waneta	17-Jul, 25-Aug, 4 Sep	14-Sep-03	M	42657B3C4C	189	50
2003	21-May-03	Waneta	17-Jul, 25-Aug, 4 Sep	28-Aug-03	M	7F7D187B26	174	50
2003	21-May-03	Waneta	17-Jul, 25-Aug, 4 Sep	14-Sep-03	M	42647D276C	172	45
2003	23-May-03	Waneta	Not Spawned	26-Aug-03	M	4265591832	163	31
2003	10-Jun-03	Waneta	28-Jul, 22-Aug, 03	26-Aug-03	M	4269693465	150	26
2003	10-Jun-03	Waneta	28-Jul, 22-Aug, 03	28-Aug-03	M	42657D0125	171	43
2003	6-May-03	HLK	Not Spawned	14-Sep-03	F	4269515607	184	48
2003	7-May-03	HLK	17-Jul-03	23-Jul-03	F	7F7D497658	172	50
2003	9-May-03	HLK	Not Spawned	23-Jul-03	F	4264617C0D	189	68
2003	14-May-03	Fort Shepard	22-Aug-03	28-Aug-03	F	42657C7B47	191	59
2003	21-May-03	Waneta	Not Spawned	26-Aug-03	F	426168094B	207	81
2003	23-May-03	Waneta	Not Spawned	25-Jun-03	F	4265517BLF	197	72

2003	6-May-03	HLK	Not Spawned	12-May-03	F	7F7D211362	199	68
2004	30-Apr-04	HLK	Not Spawned	24-Jun-04	M	131473731A		48
2004	4-May-04	HLK	16-Jun-04	24-Jun-04	M	7F7D42061F		40
2004	4-May-04	HLK	16-Jun-04	13-18 Aug 04	M	7F7D49475D		34
2004	4-May-04	HLK	Not Spawned	24-Jun-04	M	7F7E6A391D		41
2004	11-Jun-04	Kootenay Eddy	11-Aug-04	6-Jul-04	F	7F7D311727		95
2004	16-Jun-04	Waneta	24-Jun-04	25-Jun-04	F	7F7D500107		45
2004	18-Jun-04	Waneta	24-Jun-04	25-Jun-04	F	7F7D494B31		39
2004	18-Jun-04	Waneta	24-Jun-04	25-Jun-04	F	7F7D31165E		59
2004	22-Jun-04	Waneta	29-Jun-04	6-Jul-04	F	7F7E6A3C3D		100
2004	17-Jun-04	HLK	Not Spawned	13-18 Aug 04	M	7F7E6A4A11		23
2004	18-Jun-04	Waneta	24,29 Jun 04	13-18 Aug 04	M	7F7E687116		30
2004	12-Jun-04	Waneta	24-Jun-04	13-18 Aug 04	M	7F7D4E5E33		47
2004	22-Jun-04	Fort Shepard	Not Spawned	13-18 Aug 04	M	426465351D		41
2004	23-Jun-04	Waneta	24,29 Jun 04	13-18 Aug 04	M	7F7D37534A		45
2004	29-Apr-04	HLK	Not Spawned	21-Jul-04	F	42647F5002		43
2004	11-May-04	Waneta	Not Spawned	15-Jul-04	F	7F7D3F700C		107
2004	9-Jun-04	Waneta	16-Jun-04	17-Jun-04	F	7F7D31051E		45
2005	7-Jun-05	Waneta	23-Jun-05	27-Jun-05	F	422D357649	212	75
2005	7-Jun-05	Waneta	Not Spawned	6-Jul-05	F	7F7D4E5518	235	114
2005	7-Jun-05	Waneta	20-Jun-05	22-Jun-05	F	422D39032F	205	60
2005	7-Jun-05	Waneta	20,23,30 Jun 05	2-Jul-05	M	42386A7E34	162	38
2005	8-Jun-05	Fort Shepard	20,30 Jun 05	2-Jul-05	M	42617E5C72	185	48
2005	14-Jun-05	Waneta	Not Spawned	6-Jul-05	F	985120019235248	178	45
2005	16-Jun-05	Waneta	23-Jun-05	27-Jun-05	F	141216392A	218	102
2005	16-Jun-05	Waneta	20,23 Jun 05	28-Jun-05	M	7F7D314476	176	37
2005	17-Jun-05	Waneta	23-Jun-05	12-Jul-05	M	141263327A	166	40
2005	17-Jun-05	Waneta	23-Jun-05	29-Jun-05	M	222374420B	191	57
2005	17-Jun-05	Waneta	23-Jun-05	27-Jun-05	F	7F7D35204D	180	50
2005	21-Jun-05	Waneta	23-Jun-05	12-Jul-05	M	141234451A	152	31
2005	21-Jun-05	Waneta	23-Jun-05	12-Jul-05	M	7F7D215849	154	30
2005	22-Jun-05	Fort Shepard	30-Jun-05	5-Jul-05	F	1F4B4B7952	197	68
2005	22-Jun-05	Waneta	30-Jun-05	5-Jul-05	F	141255321A	185	54
2005	22-Jun-05	Waneta	30-Jun-05	7-Jul-05	M	7F7D1C7510	172	48
2005	22-Jun-05	Waneta	6-Jul 05 cryo	7-Jul-05	M	141217395A	149	24
2005	29-Jun-05	Kootenay Eddy	6-Jul 05 cryo	7-Jul-05	M	42386D5D5F	170	44
2005	28-Jun-05	Waneta	5-Jul-05	13-Jul-05	F	4238646404	170	44
2005	28-Jun-05	Waneta	5-Jul-05	13-Jul-05	F	242385A5B75	213	84
2006	20-Jun-06	Fort Shepard	10-Aug-06	11-Aug-06	F	7F7D4F2157	190	68
2006	21-Jun-06	Waneta	6-Jul-06	7-Jul-06	F	7F7D50264A	215	85
2006	21-Jun-06	Waneta	27-Jun-06	29-Jun-06	F	7F7D4F457D	171	45
2006	06-Jun-06	Waneta	6-Jul-06	7-Jul-06	F	985161000179261	216	90
2006	06-Jun-06	Waneta	Not Spawned	19-Jul-06	F	7F7D217872	196	85

2006	07-Jun-06	Waneta	27-Jun-06	29-Jun-06	F	7F7D22067B	181	52
2006	13-Jun-06	Waneta	27-Jun-06	29-Jun-06	F	985161000072510	206	80
2006	05-Jun-06	Waneta	10-Aug-06	11-Aug-06	F	985161000072575	202	85
2006	05-Jun-06	Waneta	6-Jun-06	7-Jun-06	M	7F7D4F211B	193	55
2006	06-Jun-06	Waneta	6-Jul-06	7-Jul-06	M	7F7D4E730C	175	42
2006	08-Jun-06	Waneta	27-Jun-06	19-Jul-06	M	985161000184384	156	32
2006	15-Jun-06	Waneta	27-Jun-06	19-Jul-06	M	985161000061823	194	64
2006	06-Jun-06	Waneta	10-Aug-06	11-Aug-06	M	985161000178903	169	41
2006	19-Jun-06	Waneta	27-Jun-06	29-Jun-06	M	7F7D221610	148	25
2006	06-Jun-06	Waneta	27-Jun-06	29-Jun-06	M	985161000184236	184	54
2006	08-Jun-06	Waneta	27-Jun-06	19-Jul-06	M	985161000183619	156	31
2006	07-Jun-06	Waneta	7-Jun-06	7-Jul-06	M	42656B1546	178	45
2007	05-Jun-07	Waneta	Not Spawned	7-Jul	F	42657C7B47	210	68
2007	06-Jun-07	Waneta	12-Jun	25-Jun	F	1F6C77126C	207	80
2007	22-Jun-07	Kootenay Eddy	27-Jun	28-Jul	F	7F7D186E66	182	62
2007	22-Jun-07	Waneta	27-Jun	5-Jul	F	4158696408	226	120
2007	19-Jun-07	Kootenay Eddy	4-Jul	6-Jul	F	985161000057769	205	73
2007	08-Jun-07	Waneta	Jun 12 / 27	7-Jul	M	7F7D4F2F69	170	40
2007	15-Jun-07	HLK	Not Spawned	28-Jun	M	985120019232551	0	37
2007	15-Jun-07	Waneta	4-Jul	8-Jul	M	985161000065272	180	51
2007	26-Jun-07	Waneta	27-Jun	29-Jun	M	4265646344	166	39
2007	15-Jun-07	Kootenay Eddy	Not Spawned	28-Jun	M	985120019237290	157	33
2007	07-Jun-07	Fort Shepard	Not Spawned	5-Jul	M	426465351D	187	52
2007	16-Jun-07	American Eddy	27-Jun	29-Jun	M	7F7D21605B	0	45
2007	07-Jun-07	Waneta	4-Jul	6-Jul	M	985120025166158	166	35
2007	08-Jun-07	Fort Shepard	4-Jul	6-Jul	M	7F7D490B02	168	37
2008	04-Jun-08	Fort Shepard	24-Jun	25-Jun	F	4262057965	217	93
2008	04-Jun-08	Waneta	Not Spawned	16-Jul	F	985161000056033	0	92
2008	10-Jun-08	Waneta	24-Jun	25-Jun	F	985161000063939	227	98
2008	03-Jun-08	Waneta	15-Jul	17-Jul	F	7F7D216159	216	85
2008	04-Jun-08	Fort Shepard	15-Jul	17-Jul	F	4264615E3A	186	60
2008	10-Jun-08	Waneta	15-Jul	16-Jul	F	115552157A	214	92
2008	11-Jun-08	Waneta	Not Spawned	27-Jul	F	4238164254	0	83
2008	11-Jun-08	Fort Shepard	Not Spawned	11-Jul	F	42617D343D	0	104
2008	06-Jun-08	Waneta	Jun 24 / Jul 15	18-Jul	M	426465351D	191	53
2008	03-Jun-08	Waneta	15-Jul	29-Jul	M	985161000066162	207	73
2008	06-Jun-08	Waneta	Jun 24 / Jul 15	27-Jul	M	985161000067865	197	62
2008	13-Jun-08	Waneta	15-Jul	18-Jul	M	4238433152	173	34
2008	12-Jun-08	Waneta	Jun 24 / Jul 15	18-Jul	M	4266037619	200	58

2008	03-Jun-08	Waneta	15-Jul	29-Jul	M	985161000060917	173	42
2008	03-Jun-08	Waneta	15-Jul	29-Jul	M	7F7D1C7C1A	173	52
2009	16-Jun-09	Waneta	23-Jun	24-Jun	F	7F7D216541	193	63
2009	4-Jun-09	Waneta	23-Jun	26-Jun	F	7F7D211362	207	73
2009	16-Jun-09	Waneta	23-Jun	24-Jun	F	415B562501	196	67
2009	4-Jun-09	Kootenay Eddy	23-Jun	24-Jun	F	7F7D1C1D5D	180	45
2009	1-Jun-09	Waneta	14-Jul	20-Jul	F	7F7D49161D	58	58
2009	5-Jun-09	Kootenay Eddy	14-Jul	15-Jul	F	7F7B030F1D	168	37
2009	4-Jun-09	Sturgeon Island	14-Jul	15-Jul	F	42385F3D39	176	43
2009	11-Jun-09	Fort Shepard	Not Spawned	2-Jul	F	42647D1632	200	68
2009	16-Jun-09	Fort Shepard	Not Spawned	2-Jul	F	7F7D186622	181	62
2009	9-Jun-09	Waneta	Jun 23 / Jul 14	20-Jul	M	7F7D224474	186	61
2009	4-Jun-09	Kootenay Eddy	23-Jun	25-Jun	M	4110E451E	160	34
2009	12-Jun-09	Kootenay Eddy	Jun 23 / Jul 14	15-Jul	M	985161000182601	172	46
2009	2-Jun-09	Waneta	Not Spawned	21-Jul	M	7F7D1C6A60	186	54
2009	11-Jun-09	Waneta	23-Jun	26-Jun	M	7F7D1C676B	176	48
2009	3-Jun-09	Waneta	Jun 23 / Jul 14	21-Jul	M	985161000071544	169	44
2009	16-Jun-09	Waneta	14-Jul	21-Jul	M	985161000056222	179	45
2009	5-Jun-09	Waneta	23-Jun	20-Jul	M	985161000067588	186	49
2010	01-Jun-10	Kootenay Eddy	23-Jun	24-Jun	F	7F7D22402D	198	67
2010	02-Jun-10	Genelle	23-Jun	28-Jun	F	7F7D1D0B11	198	95
2010	08-Jun-10	Sturgeon Island	Not Spawned	14-Jun	F	985121006007569	185	61
2010	15-Jun-10	Waneta	23-Jun	25-Jun	F	985121005953125	233	130
2010	01-Jun-10	Waneta	23-Jun	25-Jun	F	115533135A	220	96
2010	10-Jun-10	Kootenay Eddy	23-Jun	24-Jun	F	985121006000498	200	68
2010	10-Jun-10	Waneta	Not Spawned	12-Jul	F	7F7D4F7224	215	85
2010	02-Jun-10	Kootenay Eddy	7-Jul	8-Jul	F	985161000129029	198	51
2010	10-Jun-10	Kootenay Eddy	7-Jul	8-Jul	F	115934096A	216	87
2010	15-Jun-10	Waneta	7-Jul	9-Jul	F	985121006205 45	218	100
2010	12-Jun-10	Waneta	23-Jun	9-Jul	M	985121005952316	193	47
2010	12-Jun-10	Waneta	23-Jun	9-Jul	M	985121005951809	181	44
2010	16-Jun-10	Waneta	23-Jun	9-Jul	M	985121005665450	213	66
2010	11-Jun-10	Rialto	Jun 23 / Jul 14	29-Jun	M	985121005664010	167	39
2010	10-Jun-10	Rialto	23-Jun	29-Jun	M	985121005952005	173	41
2010	09-Jun-10	Genelle	7-Jul	13-Jul	M	985121005950118	189	57
2010	10-Jun-10	5.1KM	7-Jul	13-Jul	M	985121005962077	170	35
2010	03-Jun-10	Waneta	7-Jul	12-Jul	M	41586E5529	176	46
2010	08-Jun-10	Genelle	23-Jun	29-Jun	M	985121005620411	191	51

2011	22-Jun-11	Waterloo	28-Jun	30-Jun	F	985161000007574	91	49
2011	06-Jun-11	Kootenay Eddy	28-Jun	30-Jun	F	422D2E5218	187	58
2011	11-Jun-11	Waneta	28-Jun	5-Jul	F	985161000056205	198	64
2011	06-Jun-11	Kootenay Eddy	28-Jun	5-Jul	F	42386F274E	180	44
2011	29-Jun-11	Waneta	26-Jul	27-Jul	F	985161000052900	176	52
2011	16-Jun-11	Fort Shepard	26-Jul	28-Jul	F	7F7D4F386F	192	64
2011	09-Jun-11	Rialto	26-Jul	28-Jul	F	7F7D1C477C	202	62
2011	16-Jun-11	Waneta	26-Jul	27-Jul	F	7F7D1C6A69	164	38
2011	06-Jun-11	Rialto	Not Spawned	12-Jul	F	423868474B	191	54
2011	07-Jun-11	Rialto	Not Spawned	12-Jul	F	420B330C0C	174	45
2011	21-Jun-11	Fort Shepard	Not Spawned	25-Jul	M	115612494A	183	45
2011	08-Jun-11	Rialto	28-Jun	25-Jul	M	7F7D220474	200	62
2011	24-Jun-11	Kootenay Eddy	28-Jun	25-Jul	M	7F7D185A73	172	38
2011	06-Jun-11	Sturgeon Island	28-Jun	29-Jun	M	7F7D7D6867	178	39
2011	15-Jun-11	Waneta	Jun 28 / Jul 26	29-Jun	M	4265042C00	183	48
2011	23-Jun-11	Kootenay Eddy	28-Jun	2-Aug	M	985161000181597	170	40
2011	08-Jun-11	Rialto	Jun 28 / Jul 26	2-Aug	M	7F7D191152	178	45
2011	14-Jun-11	Waneta	26-Jul	3-Aug	M	1F4B38134B	176	52
2011	16-Jun-11	Fort Shepard	26-Jul	3-Aug	M	7F7D4E5C3F	193	51
2011	21-Jun-11	Fort Shepard	26-Jul	3-Aug	M	7F7D501A2D	205	75
2011	14-Jun-11	Waneta	Not Spawned	24-Jun	M	42657D0125	195	63
2012	8-Jun-12	Kootenay Eddy	18-Jul	20-Jul	F	7F7D381E45	195	76
2012	21-Jun-12	Waneta	18-Jul	23-Jul	F	1F3B207115	229	105
2012	22-Jun-12	Waneta	4-Jul	6-Jul	F	985120022620878	182	50
2012	25-Jun-12	Kootenay Eddy	4-Jul	10-Jul	F	426505161B	202	69
2012	22-Jun-12	Waneta	4-Jul	6-Jul	F	7F7D4D174C	209	77
2012	6-Jun-12	Kootenay Eddy	18-Jul	20-Jul	F	985161000180641	174	46
2012	20-Jun-12	Genelle	20-Jun	24-Jul	F	7F7D222A69	202	78
2012	11-Jun-12	Waneta	18-Jul	23-Jul	F	985161000056632	188	55
2012	6-Jun-12	Kootenay Eddy	18-Jul	19-Jul	M	42381E6117	191	60
2012	6-Jun-12	Kootenay Eddy	18-Jul	19-Jul	M	4204067834	175	40
2012	21-Jun-12	Fort Shepard	4-Jul	10-Jul	M	7F7D4F6A1F	181	49
2012	22-Jun-12	Waneta	4-Jul	11-Jul	M	42616E411F	178	90
2012	28-Jun-12	Waneta	4-Jul	11-Jul	M	4239436C4E	212	90
2012	6-Jun-12	Dock set	18-Jul	25-Jul	M	7F7D1C4C51	197	62
2012	11-Jun-12	Waneta	18-Jul	26-Jul	M	4239441550	175	45
2012	11-Jun-12	Waneta	18-Jul	26-Jul	M	985120022605171	186	56
2012	11-Jun-12	Waneta	18-Jul	25-Jul	M	985121005923954	186	56
2012	26-Jun-12	Rialto	18-Jul	24-Jul	M	7F7D4F723C	184	56
2013	12-Jun-13	Kootenay Eddy	26-Jun	4-Jul-13	F	985121001732553	177	45
2013	20-Jun-13	Waneta	Not Spawned	19-Jul-13	F	1F4B4B7952	201	79

2013	9-Jul-13	Waneta	17-Jul-13	22-Jul-13	F	7F7D185C29	225	84
2013	12-Jun-13	Sturgeon Island	26-Jun-13	2-Jul-13	F	4239084A26	176	42
2013	17-Jun-13	Waneta	23-Jun-13	2-Jul-13	F	985120022626419	205	75
2013	28-Jun-13	American Eddy	17-Jul-13	23-Jul-13	F	7F7D190073	208	96
2013	20-Jun-13	Fort Shepard	Not Spawned	24-Jun-13	F	7F7D4F6C19	225	88
2013	19-Jun-13	Waneta	17-Jun-13	22-Jul-13	F	4158445F7C	180	47
2013	11-Jun-13	Kinnard Bridge	26-Jun-13	4-Jul-13	M	4238456407	180	42
2013	11-Jun-13	Fishy Beach	Not Spawned	10-Jul-13	M	420B272429	190	50
2013	18-Jun-13	Beaver Creek	Not Spawned	8-Jul-13	M	41616A4B08	202	66
2013	18-Jun-13	Fort Shepard	Not Spawned	8-Jul-13	M	7F7D1C3835	165	36
2013	21-Jun-13	Fort Shepard	Not Spawned	10-Jul-13	M	141221610A	163	41
2013	25-Jun-13	Kootenay Eddy	25-Jun-13	25-Jun-13	M	7F7E6A3845	190	56
2013	3-Jul-13	HLK	Not Spawned	18-Jul-13	M	7F7D381619	165	36
2013	10-Jul-13	Waneta	17-Jul-13	19-Jul-13	M	985161000066843	208	72
2014	25-Jun-14	Fort Shepard	9-Aug-14	14-Jul-14	F	115619232A	205	80
2014	23-Jun-14	American Eddy	9-Aug-14	18-Jul-14	F	985120022601339	244	145
2014	27-Jun-14	American Eddy	9-Aug-14	14-Jul-14	F	985120020810967	231	105
2014	23-Jun-14	Waneta	9-Aug-14	16-Jul-14	F	985120030505824	211	89
2014	18-Jun-14	Sturgeon Island	9-Aug-14	16-Aug-14	F	985161000179510	189	60
2014	26-Jun-14	Waneta	9-Aug-14	10-Jul-14	M	900254000111534	137	25
2014	26-Jun-14	Waneta	9-Aug-14	11-Jul-14	M	900254000130209	145	28
2014	1-Jul-14	Waneta	9-Aug-14	10-Jul-14	M	985120019170580	176	57
2014	2-Jul-14	American Eddy	9-Aug-14	11-Jul-14	M	7F7D4F162A	202	80
2014	2-Jul-14	American Eddy	9-Aug-14	11-Jul-14	M	7F7D1C6D5C	193	62

Table A 2. All family cross for all years of the Canadian Columbia White Sturgeon broodstock program.

Brood Year	Family #	Female PIT		Male PIT
2001	**1**	42656F031A	x	7F7D4D1329
	2	42656F031A	x	7F7D491874
	3	42656F031A	x	7F7D4F211B
	5	4264713044	x	42647D7B37
	6	42656F031A	x	42647D7B37
2002	**1**	7F7D1D0B11	x	7F7D220955
			x	42634C245B
	2	7F7D1D0B11	x	7F7D1D1231
	3	7F7D1C5C60	x	4264551446
	4	7F7D1C5C60	x	464581E7B

2003	**1**	7F7D497658	x	42657B3C4C
	2	7F7D497658	x	42647D276C
	3	7F7D497658	x	7F7D187B26
	4	42657C7B47	x	7F7D187B26
	5	42657C7B47	x	4269693465
	6	42657C7B47	x	42657D0125
2004	**1**	7F7D31051E	x	7F7D49475D
	2	7F7D500107	x	7F7E687116
	3	7F7D494B31	x	7F7D37534A
	4	7F7D31165E	x	7F7D4E5E33
	5	7F7D31165E	x	7F7E687116
	6	7F7E6A3C3D	x	7F7E687116
	7	7F7E6A3C3D	x	7F7D37534A
	8	7F7E6A3C3D	x	7F7D37534A
				7F7E687116
2005	**1**	422D39032F	x	7F7D314476
	2	7F7D35204D	x	7F7D215849
	3	1412166392A	x	222374420B
	4	422D357649	x	141263327A
	5	242385A5B75	x	141234451A
	6	4238646404	x	141234451A
	7*	141255321A	x	42386A7E34
	8*	1F4B4B7592	x	42617E5C72
				7F7D1C7510
2006	**1**	985161000179261	x	7F7D4E730C
	2	7F7D50264A	x	7F7D4D2550
	3	7F7D4F457D	x	985161000183619
	4	7F7D22067B	x	985161000184236
			x	985161000061823
	5	98516100072510	x	985161000184384
	7	985161000072575	x	42656B1546
2007	**1**	1F6C77126C	x	7F7D4F2F69
	2	7F7D186E66	x	7F7D4F2F69
	3	7F7D186E66	x	4265646344
	4	4158696408	x	7F7D21605B
	5	985161000057769	x	985120025166158
	6	985161000057769	x	7F7D490B02
2008	**1**	4262057965	x	426465351D
			x	985161000067865
	2	985161000063939	x	426465351D
			x	985161000067865
	3	7F7D216159	x	4238433152
			x	4266037619
			x	985161000060917
			x	7F7D1C7C1A
	4	4264615E3A	x	4238433152

			x	4266037619
			x	985161000060917
			x	7F7D1C7C1A
	5	115552157A	x	4238433152
			x	4266037619
			x	985161000060917
			x	7F7D1C7C1A
2009	**1**	7F7D216541	x	7F7D224474
			x	985161000071544
			x	4110E451E
			x	985161000182601
			x	7F7D310D2E
			x	7F7D1C676B
	2	7F7D211362	x	7F7D224474
			x	985161000071544
			x	4110E451E
			x	985161000182601
			x	7F7D310D2E
			x	7F7D1C676B
	3	415B562501	x	7F7D224474
			x	985161000071544
			x	4110E451E
			x	985161000182601
			x	7F7D310D2E
			x	7F7D1C676B
	4	7F7D1C1D5D	x	7F7D224474
			x	985161000071544
			x	4110E451E
			x	985161000182601
			x	7F7D310D2E
			x	7F7D1C676B
	5	7F7D49161D	x	985161000056222
			x	985161000067588
			x	7F7D224474
	6	7F7B030F1D	x	985161000056222
			x	985161000067588
			x	7F7D224474
	7	42385F3D39	x	985161000056222
			x	985161000067588
			x	7F7D224474
2010	**1**	985121005953125	x	985121005952316
			x	985121005951809
	2	115533135A	x	985121005665450
			x	985121005664010
	3	985121006000498	x	985121005665450
			x	985121005664010

			x	985121005952316
			x	985121005951809
			x	985121005952005
	4	985161000129029	x	985121005950118
			x	985121005962077
	5	115934096A	x	41586E5529
			x	985121005620411
	6	98512100620545	x	41586E5529
			x	985121005950118
			x	985121005936411
			x	985121005962077
2011	**1**	985161000007574	x	7F7D220474
			x	7F7D185A73
			x	7F7D7D6867
			x	4265042C00
	2	422D2E5218	x	7F7D220474
			x	7F7D185A73
			x	7F7D7D6867
			x	4265042C00
			x	985161000181597
			x	7F7D191152
	3	985161000056205	x	7F7D220474
			x	7F7D185A73
			x	7F7D7D6867
			x	4265042C00
			x	985161000181597
			x	7F7D191152
	4	42386F274E	x	7F7D220474
			x	7F7D185A73
			x	7F7D7D6867
			x	4265042C00
			x	985161000181597
			x	7F7D191152
	5	985161000052900	x	1F4B38134B
			x	7F7D191152
	6	7F7D4F386F	x	1F4B38134B
			x	7F7D191152
			x	7F7D4E5C3F
	7	7F7D1C477C	x	1F4B38134B
			x	7F7D191152
			x	7F7D501A2D
	8	7F7D1C6A69	x	7F7D501A2D
			x	7F7D191152
2012	**1**	426505161B	x	42381E6117
			x	4204067834
			x	7F7D4F6A1F

			x	42616E411F
			x	4239436C4E
	2	7F7D4D174C	x	42381E6117
			x	4204067834
			x	7F7D4F6A1F
			x	42616E411F
			x	4239436C4E
	3	985161000180641	x	7F7D1C4C51
			x	4239441550
			x	985120022605171
			x	985121005923954
			x	7F7D4F723C
	4	7F7D222A69	x	7F7D1C4C51
			x	4239441550
			x	985120022605171
			x	985121005923954
			x	7F7D4F723C
	5	985161000056632	x	7F7D1C4C51
			x	4239441550
			x	985120022605171
			x	985121005923954
			x	7F7D4F723C
2013	**1**	985121001732553	x	7F7E6A3845
			x	4238456407
	2	4239084A26	x	7F7E6A3845
			x	4238456407
	3	985120022626419	x	7F7E6A3845
			x	4238456407
	4	7F7D190073	x	985161000066843
	5	7F7D185C29	x	985161000066843
	6	4158445F7C	x	985161000066843
2014	**1**	115619232A	x	900254000111534
			x	900254000130209
			x	985120019170580
			x	7F7D4F162A
			x	7F7D1C6D5C
	2	985120022601339	x	900254000111534
			x	900254000130209
			x	985120019170580
			x	7F7D4F162A
			x	7F7D1C6D5C
	3	985120020810967	x	900254000111534
			x	900254000130209
			x	985120019170580
			x	7F7D4F162A
			x	7F7D1C6D5C

	4	985120030505824	x	900254000111534
			x	900254000130209
			x	985120019170580
			x	7F7D4F162A
			x	7F7D1C6D5C
	5	985161000179510	x	900254000111534
			x	900254000130209
			x	985120019170580
			x	7F7D4F162A
			x	7F7D1C6D5C

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