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COQUITLAM RESERVOIR KOKANEE/SOCKEYE (*ONCORHYNCHUS NERKA*) HATCHERY REARING, SMOLT RELEASE, AND SMOLT SURVIVAL TESTING, 2016-2017

YEAR 2-3

This project was carried out with the financial support of the BC Hydro Fish and Wildlife Compensation Program

Prepared for:

BC Hydro Fish and Wildlife Compensation Program
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September 2017

LGL LIMITED
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FISH AND WILDLIFE COMPENSATION PROGRAM
A partnership between BC Hydro, the Province of B.C. and Fisheries and Oceans Canada

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EXECUTIVE SUMMARY

In the Coquitlam-Buntzen BC Hydro system, numerous interested parties have a vision of restoring salmon runs that have been obstructed from migrating into the reservoir since 1914. In 2007, the first adult Sockeye Salmon returned to the Coquitlam Reservoir dam following the release of 620 Kokanee/Sockeye smolts in 2005. These fish were transported over the dam and released into the reservoir in a historic ceremony led by the Kwikwetlem First Nation. While the project has been successful in seeing these initial returns of adult Sockeye Salmon to the Coquitlam River, few Sockeye/Kokanee smolts have emigrated from the reservoir in each successive year. The reasons for the continued low numbers in adult returning from the ocean to Coquitlam Reservoir are likely based on low numbers of juvenile smolts leaving the reservoir to the ocean.

To maximize potential for successful establishment of a viable anadromous Sockeye population under existing conditions, progeny from the residential Coquitlam Reservoir Kokanee were used as broodstock for a hatchery intervention. Genetic stock identification of the resident Coquitlam Kokanee concluded the Kokanee to be recent descendants of the anadromous Sockeye population and therefore to be well suited as a locally adapted broodstock.

An egg take project was conducted in Coquitlam Reservoir in the fall of 2015 (Year 1) that resulted in the capture and fertilization of ~10,000 Kokanee eggs, which were transported for incubation and rearing to the smolt stage at the Department of Fisheries and Oceans Canada (DFO)-assigned Rosewall Creek hatchery on Vancouver Island. Disease screening of female Kokanee was performed at the Pathology Lab of the Freshwater Fisheries Society of BC located in Duncan, Vancouver Island.

Following the one and a half year period from egg take in the fall of 2015, Kokanee were held through the initial incubation and later rearing to the fry stage to reach the smolt stage in the April of 2017. At that time, approximately 5,400 smolts were transported from Rosewall Creek Hatchery to the Coquitlam Dam to be released into the Coquitlam River below the dam.

The Kokanee smolts were transported by Rosewall Creek Hatchery staff to Coquitlam Dam on April 12, 2017 and transferred into a 3 m diameter holding tank that was installed by the Port Coquitlam & District Fishing & Hunting Club. Volunteer members of the club also fed the smolts and cleaned the tank on a daily basis throughout the rearing period, from April 12 to April 20 for the majority of the smolts and to May 1 for the remaining 400 smolts.

On April 20, 2017, 5,000 Kokanee smolts were released into the Coquitlam River in a widely publicized ceremony and with First Nations, municipal, and provincial representatives in attendance.

On April 14 and April 24, 2017, a total of 103 Kokanee smolts were surgically implanted with acoustic tags for an acoustic telemetry study in Coquitlam Reservoir. In this study, the approach behaviour of smolts to the dam and its Low Level Outlets (LLOs) was investigated to inform the

planning of a smolt release and surface attraction structure. Detailed results of this study will be reported in an additional report submitted to the Fish and Wildlife Compensation Program (FWCP) in the spring of 2018 and once all study results are analyzed.

As an essential part of the acoustic telemetry study, Kokanee smolts were also used to assess passage survival through the LLOs of Coquitlam Dam in combination with Coho smolt releases. Results from these releases indicated that LLO passage at discharges of 3 m^3/s or less is not harmful to Kokanee or Coho smolts and therefore LLOs can be used as fish surveyors through the dam at typical spring flows. Passage survival at higher flows (8-10 m^3/s) that are likely needed to attract more smolts to the dam and a potential surface smolt release structure was not determined.

In summary, this study was highly successful in raising and releasing Kokanee smolts to return as adult Sockeye and providing smolts for behaviour and survival studies that are essential to the re-establishment of a Coquitlam Sockeye population.

This project represents the first direct intervention to boost the number of Sockeye Salmon returning to Coquitlam Reservoir past the ten fish that returned in 2008 and thus marks a realistic attempt to re-establish Sockeye in Coquitlam Reservoir. To our knowledge, if successful, this would be the first re-establishment of Sockeye based on residual Kokanee in the world and would bolster the hope of the Kwikwetlem First Nation that past wrongs can be made right if a First Nation, a utility company, governments, a municipality, and volunteers work together.

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INTRODUCTION

The restoration of anadromous fish runs, where practical, is a key objective of the BC Hydro Fish and Wildlife Restoration Program (FWRP). This key objective was given the highest possible priority ranking in the "2011 Coquitlam/Buntzen Watershed Salmonid Action Plan" (Fish and Wildlife Compensation Program 2011).

In the Coquitlam-Buntzen BC Hydro system, numerous interested parties including government agencies, the Kwikwetlem First Nation, stewardship groups, environmental Non-Government Organizations (NGOs), and concerned citizens have an interest in restoring anadromous salmon runs in the Coquitlam Reservoir while maintaining Coquitlam Reservoir's important role as a major source of high quality drinking water for the Metro Vancouver Regional District (MVRD).

Re-establishment of anadromous Sockeye salmon is the primary goal for re-introduction because this species was most important to the Kwikwetlem First Nation, as they historically harvested returning Sockeye salmon throughout the Coquitlam Watershed.

Several methods for re-introduction were reviewed in past reports (Plate et al. 2015; Bocking and Gaboury 2003). This included utilizing donor stocks from different Sockeye salmon populations that shared similar genetic makeup (e.g., lower Fraser river stocks) and early migratory run-time, to utilizing the local resident Kokanee stock of Coquitlam reservoir (Plate et al. 2015). Nelson and Wood (2007) investigated the genetic heritage of Kokanee in Coquitlam Reservoir and Kokanee smolts in Coquitlam River. They concluded that Kokanee smolts sampled in Coquitlam River are most likely smolts from Coquitlam Reservoir. Genetic stock identification checks supported the hypothesis that Coquitlam Reservoir Kokanee are recently derived from an anadromous Sockeye. Gill raker number and the length of Coquitlam Kokanee indicates that these fish have similar characteristics to Sockeye-Kokanee hybrids (Bussanich et al. 2006), which supports the interpretation that Coquitlam Reservoir Kokanee are likely recent descendants of Sockeye Salmon. These results suggest that capability for re-anadromization likely exists in Coquitlam Kokanee populations and that it is feasible to pursue this objective. This suggestion was proven in 2007, when two adult Sockeye returned, and in 2008 when ten adult Sockeye returned from a release of 621 Kokanee in 2005.

An average 1% survival from Sockeye smolt to adult has been estimated for returns from brood years 2005−2011 for the Alouette system and a similar survival rate can be expected for Coquitlam Sockeye (Mathews et al. 2012). Using this survival rate, a smolt release of 5,000 fish would produce an escapement of approximately 50 adult fish under current low ocean productivity conditions.

Currently, few Sockeye smolts are emigrating from the reservoir (Table 1) based on monitoring results from Rotary Screw Traps (RSTs) that were first deployed in Coquitlam River in spring of 2000. Prior to 2000, emigration numbers are unknown. In 2007, 2 adult Sockeye Salmon returned to the Coquitlam Reservoir Dam (Figure 1) following the release of 621 Kokanee/Sockeye smolts in 2005 (unpublished data from BC Hydro, James Bruce). These fish

were transported over the dam and released into the reservoir in a historic ceremony led by the Kwikwetlem First Nation. While the project has been successful in seeing these initial returns of Sockeye Salmon to the Coquitlam River, fewer Sockeye/Kokanee smolts have emigrated from the reservoir in each successive year. The reason for the low level of emigration and resulting low numbers of returning adult Sockeye Salmon is unknown. Three potential hypotheses that may explain the low number of emigrating Sockeye Salmon smolts are:

1. Sockeye Salmon/Kokanee smolts are unable to find the Low Level Outlets (LLOs) of the reservoir to migrate to sea or perish when passing through the LLOs before reaching the downstream trap;
2. The standing crop of Sockeye Salmon/Kokanee in the reservoir is too small to enable a significant outmigration of smolts; and,
3. There are few Sockeye Salmon/Kokanee that have the genetic predisposition to migrate to sea.

Other hypotheses may be developed in the future.

Table 1 Summary of RST data collected on the Lower Coquitlam River from 2005 to 2013. LLO Release refers to the duration that a Low Level Outlet was in use delivering a flow pulse >3 m^3/s. Capture efficiencies were those reported for Coho smolts caught in RST 3. Smolt abundance estimates were based on RST 3 catch data as well. Sockeye smolts were not observed in 2014. Six smolts were counted in 2015 (S. Dowdall, Generation Maintenance, pers. comm.).

Year	LLO Release duration (days)	Catch				Capture Efficiency**	Smolt Abundance	Fork Length (%)	
		RST 4	RST 3	RST 2*	Total			< 100mm	> 100mm
2005	10	192	398	21	621	0.26	1531	85	15
2006	25	55	137	50	242	0.29	472	52	48
2007	0	-	54	72	126	0.28	193	100	-
2008	9	8	4	-	12	0.21	19	100	-
2009	60	71	50	9	130	0.2	250	53	47
2010	60	16	8	2	26	0.2	40	100	-
2011	63	107	21	14	142	0.19	111	99	1
2012	60	18	6	7	21	0.2	30	97	3
2013	60	1	-	-	1	-	-	-	1

* Sum of 2 RST's fished in tandem

** For RST3

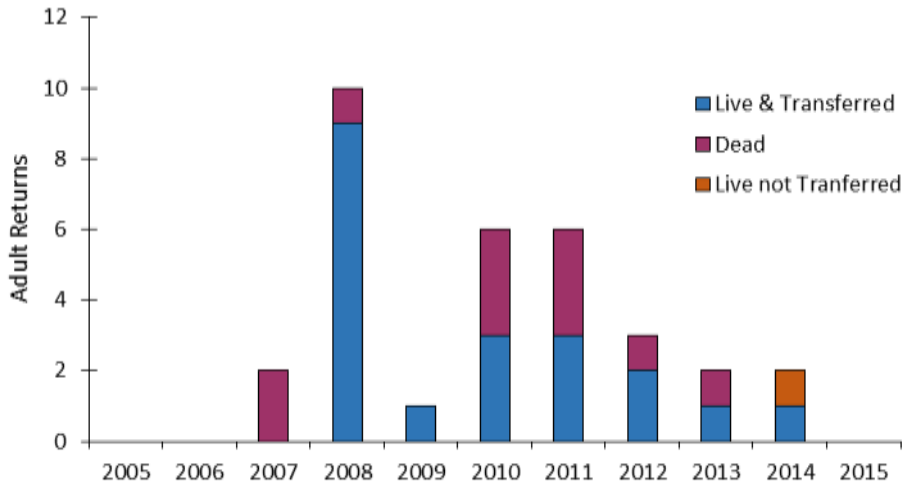


Figure 1 Count of captured adult Sockeye Salmon in the Coquitlam River. Live fish were trucked and released into the Coquitlam Reservoir. The majority of dead fish in the traps were the result of river otter predation. In 2015, one Sockeye was misidentified and not released into the reservoir (B. Wilson, BC Hydro, pers. comm.).

The objectives of this project were to:

1. Rear 5,000 Kokanee fry to the smolt stage for release into the Coquitlam River and to boost the number of adult Sockeye returning as spawners into the Coquitlam River and Reservoir in 2019.

2. Provide 200 Kokanee smolts for a three dimensional and high precision acoustic telemetry study that is aimed at monitoring Kokanee smolt behaviour in the Coquitlam Reservoir forebay and when approaching Coquitlam Dam and the entrance to the LLOs. Results from this study will be informing the assessment of technical feasibility for a planned smolt outlet structure.

3. Provide 400 Kokanee smolts for a smolt survival study during LLO passage. In the past, it has been speculated that Kokanee smolts did not survive LLO passage and therefore a Coquitlam Reservoir smolt outlet structure was not to be connected to the LLOs, but an LLO independent release tunnel or pipe. As for the last objective, results from this study will be informing the assessment of technical feasibility for a planned smolt outlet structure.

This project is the initial step in the “Establishment Phase” in the greater attempt to restore the Coquitlam anadromous Sockeye stock.

GOALS

The primary tasks for this project are to:

1. Rear 6,000 Kokanee fry to the smolt stage in Rosewall Creek hatchery.

2. Install a smolt holding tank including water supply and outflow plumbing below Coquitlam Dam.

3. Transport 6,000 Kokanee smolts from Rosewall Creek Hatchery on Vancouver Island to the holding tank installed below Coquitlam Dam.

4. On a daily basis, feed and maintain the 6,000 smolts from arrival to release into Coquitlam River for approximately 2 weeks to allow for olfactory imprinting to Coquitlam Reservoir water.

5. Implant 100 Kokanee smolts with acoustic tags for release into Coquitlam Reservoir as the basis for an acoustic telemetry study assessing approach behaviour to Coquitlam Dam in three dimensions.

6. Inform the feasibility of a smolt release structure directly connected to the LLOs, through the release of batches of 100 Kokanee smolts into the LLOs at different discharges to evaluate passage survival, physical trauma, and general condition when re-captured below the dam in a Fyke Net and a RST.

MATERIALS AND METHODS

Study Area and Setting

The project was carried out in Coquitlam Reservoir. The reservoir is approximately 4 kilometres north of the city of Coquitlam and 10 kilometres north of the Lougheed Highway. Its southern end is located at (UTM 10) coordinate 5466685 N and extends to the north to coordinate 5478860 N. The most eastern point of the reservoir is near (UTM 10) coordinate 517560 E and reaches to coordinate 513890 E to the west.

Coquitlam Reservoir is a major source of drinking water for Metro Vancouver and covers approximately 1,200 ha. Water is also stored in the Coquitlam Reservoir to generate electricity through the Buntzen System and to maintain fish flows downstream into the Lower Coquitlam River. The reservoir surface is at an elevation of 154 metres above sea level (masl) at full pool, and has a mean and maximum depth of 87 m and 185 m, respectively (Table 2). The reservoir is approximately 12 km long and has an average width of roughly 1 km.

The Coquitlam Reservoir is ultra-oligotrophic and therefore characterized by low nutrient concentrations (Phosphorous limited), low phytoplankton biomass, and good water clarity. Its relatively cool water temperature regime, high dissolved oxygen levels, and favourable water quality conditions make it suitable for resident cold-water fishes. Coquitlam Reservoir supports salmonids including Kokanee Salmon (*Oncorhynchus nerka*), as well as Coastal Cutthroat Trout (*Oncorhynchus clarki clarki*), and coarse fishes including Peamouth Chub (*Mylocheilus caurinus*), Northern Pikeminnow (*Ptychocheilus oregonensis*), Largescale Sucker (*Catostomus macrocheilus*), Prickly Sculpin (*Cottus asper*), and Three-spine Stickleback (*Gasterosteus aculeatus*) (Bocking and Gaboury 2003; Plate et al. 2011; Plate et al. 2012).

Coquitlam Reservoir has low zooplankton stocks (1.2 µg/L) which are typical for an ultra-oligotrophic system. Nevertheless, based on hydroacoustic survey results (Bussanich et al. 2006; Plate et al. 2011; Plate et al. 2012) the standing stock and growth of Kokanee to the smolting stage supports the notion that the reservoir can feed enough juvenile Kokanee to allow for the establishment of a healthy Sockeye smolt population.

Table 2 Morphological characteristics of Coquitlam Reservoir (Nordin and Mazumder 2005; James 2000).

Attribute	Measure
Lake Volume (m^3)	1,044,000,000
Mean depth (m)	87
Surface area (km^2), (ha)	12 (1200)
Watershed area (km^2)	212
Watershed area contributing to reservoir (km^2)	191
Watershed to Reservoir area ratio [a]	15.9:1
Normal operating elevation (m)	137.48 - 154.86
Normal operating elevation range (m)	17.4
Average annual precipitation (rain) (mm)	3576.8
Average annual precipitation (snow) (mm)	158.2
Inflow (m^3/yr)	725,000
Mean inflow (m^3/s)	23
Water Residence time (yr)	1.44
Sedimentation rates - 1967-1997 (g/m^2/yr), (t/km^2/yr)	192 (1.92x10^2)
Sedimentation rates - 1990-2002 (g/m^2/yr)	267
Sedimentation rates - 1905-2002 (mm/year) [b]	1.8mm /year

[a] to the mouth of lower Coquitlam

[b] over the period

1. Methods: Kokanee fry rearing to the smolt stage in Rosewall Creek hatchery and transport to Coquitlam Dam

DFO approved space at their Rosewall Creek Hatchery facility on Vancouver Island for rearing of the Kokanee fry to the smolt stage from April 2016 to April 2017. Fry were fed daily with an amount of 3% to 1% of body weight while increasing in size. Feed crumble size was adjusted to fish size and weight from very fine to smolt crumble between April 2016 and April 2017. Fish were reared in 3 m diameter rearing tanks at low densities of <10 kg/m^3. Once typical signs of initial smolting, such as silvering, scale loss, and restlessness, had been detected at the end of March 2017, feeding was reduced and preparations were made for transport to Coquitlam Dam on April 12, 2017. Through the period from April 2016 to March 2017, Rosewall Creek Hatchery and LGL Ltd. staff communicated about fish condition and release plans. In early April of 2017, fish transport to Coquitlam Dam was planned between hatchery and LGL Ltd. staff and the Port Coquitlam & District Fishing & Hunting Club (PCDFHC). Club members prepared tanks and

started flow before the fish arrived at Coquitlam Dam on April 12, 2017 and were present for fish delivery.

2. Methods: Installation of a smolt holding tank including water supply and outflow plumbing below Coquitlam Dam

The PCDFHC also installed a 3 m diameter x 1.2 m fiberglass flow-through tank, a 4.5 m length x 0.8 width and 0.5 m depth aluminum raceway, and a 2 m x 0.6 m rectangular tank at the base of Coquitlam Dam. In addition, water supply and plumbing for all tanks was installed by the PCDFHC in advance of the smolt arrival. The 3 m tank was installed for the rearing of 5,000 smolts during the 23 day olfactory imprinting period to Coquitlam Reservoir water. The rectangular tank was installed to hold the 100 acoustically tagged fish separate from non-tagged fish before release into Coquitlam Reservoir and the raceway facilitated release of the fish from the main tank into the river. Communication was ongoing between volunteers for the PCDFHC and LGL Ltd. staff during planning, set-up, and smolt delivery.

3. Methods: Daily feeding and maintenance in holding tank at Coquitlam Dam

Based on input from DFO hatchery staff facilitated by LGL Ltd., PCDFHC volunteers cleaned tanks and fed fish daily through the 23 day imprinting period until the ceremonial and widely publicized smolt release into Coquitlam River on May 4, 2017. Fish were handfed daily with an approximate amount of 1% body weight as a maintenance diet and in preparation for release.

4. Methods: Planning and execution of acoustic tag implant surgery and subsequent smolt release into Coquitlam Reservoir

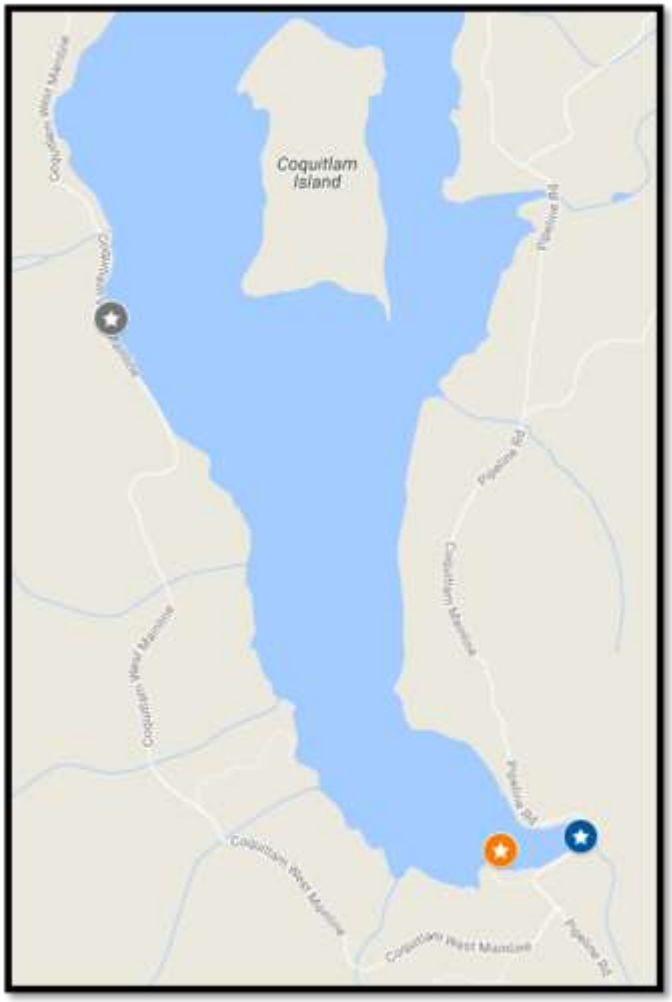
Acoustic tag implant surgery was carried out on the first 50 smolts on April 14, 2017 and on an additional 63 smolts on April 24, 2017. All details regarding the Standard Operating Procedures (SOPs) for the surgery can be found in Appendix A: Fish Tag Implant Surgery SOP. In general, PCDFHC volunteers and LGL Ltd. staff worked hand in hand to facilitate the following:

- set up of a shelter and all surgery and supporting equipment;
- the capture of smolts in the holding tank;
- transport of smolts from holding tank to pre-surgery tank;
- fish sedation;
- surgery and respiration;
- placement into the recovery tank; and,
- final placement into the holding tank for tagged smolts.

While 20% or 10 smolts died following the first surgery on April 14 (see discussion), not a single of the 63 fish died following the second surgery on April 24. This resulted in a total release of 103 tagged fish into Coquitlam Reservoir, as summarized in Table 3.

Table 3 Summary of release locations, numbers, dates, and discharge through LLOs of tagged Coquitlam Kokanee smolts in 2017 and map showing colour-coded release locations.

Release Group	Discharge through LLOs	Total	2017 Date
Dam 1	3 cms	15	April 18
Dam 2	8 cms	10	April 23
Dam 3	3 cms	10	April 28
Dam 4	8 cms	13	May 3
Boom 1	3 cms	10	April 18
Boom 2	8 cms	5	April 23
Boom 3	3 cms	10	April 28
Boom 4	8 cms	10	May 3
Buntzen 3	3 cms	10	April 28
Buntzen 4	8 cms	10	May 3
Total		103	



5. Methods: Smolt releases into the LLOs to evaluate passage survival, physical trauma, and general condition

To assess survival and condition following LLO passage, it was planned to release four groups of 100 Kokanee smolts into the LLOs at two discharges (3 m^3/s and 8 m^3/s) and starting from two release locations (trash racks and directly into LLOs). Unfortunately, only the first release was accomplished with Kokanee smolts and the remaining smolts kept for the survival study died following a lightning storm that tripped the breaker on the water pump, cutting off the water supply to the tank and leading to oxygen depletion in the tank.

Therefore, the other three releases of 100 fish each into the LLOs were conducted using Coho Salmon smolts of the same size as the Kokanee smolts (average 11 g). The Coho smolts were provided by the PCDFHC hatchery. Kokanee and Sockeye smolts were released into a bucket mounted onto a 17 m (6 cm diameter) PVC pipe while water pumped from the reservoir into

the bucket was creating pressure to push the fish through the pipe into the trash racks or the LLOs of the Coquitlam Dam sluice tower as shown in Figure 2.

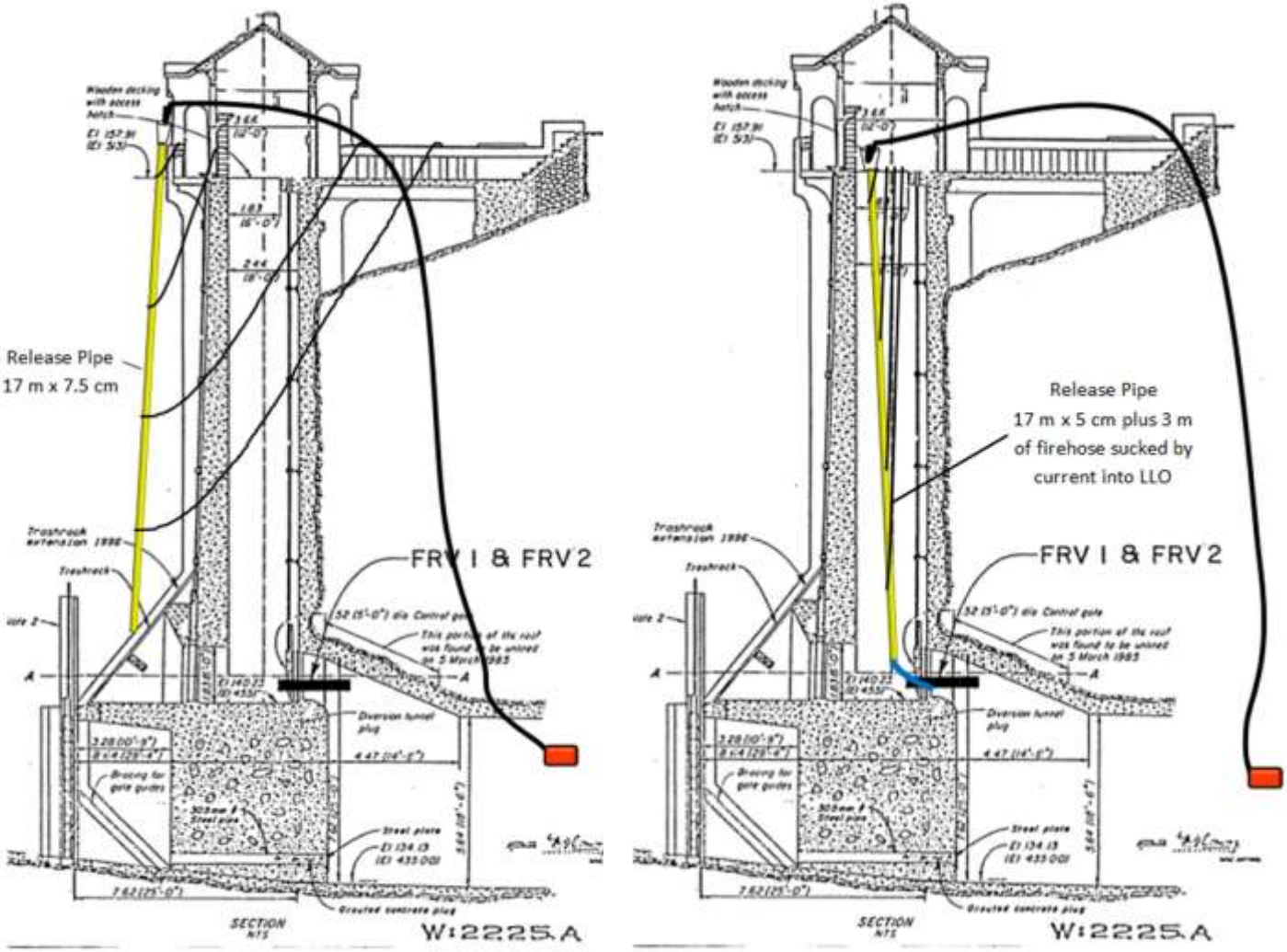


Figure 2 Cross section of the Coquitlam Dam sluice tower and position of the release pipe and bucket (yellow), the water supply hose (black) connected to the water pump (red) for releasing smolts into the sluice tower trash racks (left panel) or the LLOs (right panel). When releasing smolts directly into the LLOs, a 2 m piece of fire hose (blue) was attached to the end of the release pipe to ensure that fish could not escape the current into the chamber upstream of the LLOs.

Table 4 summarizes all release dates, release numbers, discharges, release locations, fish re-capture locations downstream of the dam, and condition of smolts at re-capture.

In addition to the pipe releases into the trash racks and LLOs, dead smolts were also released into the tunnel below the downstream end of the LLOs to determine where fish that would not

survive passage would settle and whether they would be caught in the downstream Fyke Net (2 m x 2 m with lead nets installed 50 below dam) or RST (2.4 m diameter installed 400 m below dam) (Figure 3).

Table 4 Summary of all releases of Kokanee and Coho smolts into the LLOs of Coquitlam Dam:

Date (mm/dd/yy)	05/02/17	05/03/17	05/04/17	Summary	05/31/17	05/31/17	06/01/17	Summary
Time (12 h hh:mm)	14:30	18:00	12:30		22:10	22:30	12:00	
Discharge (m^3/s)	8	3	3		3	3	3	
Number Coho released (N)	106	97	50	253	100	100	100	300
Number Sockeye released (N)	0	0	50	50	0	0	0	
Dead or Alive (D or A)	A	A	A		D	A	D	
Release Location (Trash, LLO or LT=Lower Tunnel)	Trash	Trash	Trash		LLO	LLO	LT	
Clip (A=adipose; UC=upper caudal; LC=lower caudal	Adi	Adi	Adi		Adi + UC	Adi	Adi + LC	
Fyke Net (or Visual) Number Recovered Alive (N) Coho	1	3	1	5	0	29	0	29
Average Condition of Captured Smolts (1 - 5; 1 = no trauma visible)	1		2		1	1	1	
Fyke Net (or Visual) Number Recovered Dead (N)	0	0	0	0	47	3	25	75
Total RST Number Recovered Alive (N)	11	11	12	34	0	55	0	55
Total RST Number Recovered Dead (N)	0	0	0		0	0	0	
Total %Recovered Alive from Alive (%)	11.32%	14.43%	13.00%	12.87%	NA	84.00%	NA	84.00%
Total %Recovered Dead from Dead (%)	NA	NA	NA	0.00%	47.00%		25.00%	36.00%
Total %Recovered Dead from Alive (%)	0.00%	0.00%	0.00%		NA	3.00%	NA	3.00%
Comments		3 Coho smolts observed holding in bay					55 dead found at tunnel outlet in deep water	


Figure 3 Fyke Net (left panel) and Rotary Screw Trap (RST; right panel) used to recover Kokanee and Coho smolts in the Coquitlam River below the dam.

RESULTS AND DICUSSSION

1. **Results and Discussion: Kokanee fry rearing to the smolt stage in Rosewall Creek hatchery and transport to Coquitlam Dam**

Of the 12,236 eggs collected, fertilized, and incubated, a total survival of 49.5% (~6,000 eggs) reached the ponded stage (fry stage) in Rosewall Creek Hatchery. The relatively low survival rate was likely a reflection of a combination of contributing factors during the broodstock capture and egg take such as: 1.) variable degree of egg maturity since fish could not be assessed for ripeness before capture in a depth of 20 m; 2.) physical shock during transport of fertilized eggs since the fertilized eggs were flown via float plane from the mainland to Vancouver Island; 3.) water entry through vent, subsequent egg hardening and micropyle closing following longer times of females under water in the gill nets. Of the 6,000 fry ponded in March of 2016, approximately 5,400 fish survived to the smolt stage and were transported in an oxygenated transport tank on a trailer from Rosewall Creek Hatchery to Coquitlam Dam on April 12, 2017. Very little mortality was observed during transport and smolts were transferred with dip nets into the Coquitlam Dam holding tank. Of the 5,400 smolts in the holding tank, approximately 5,000 were released into the Coquitlam River on April 20, 2017 to migrate to the ocean. The remainder were left in the tank for tagging and LLO passage survival studies. At a typically assumed 1% survival rate applied from smolt to adult stage, an escapement of 50 adult Sockeye can be expected for the fall of 2019 based on the release of 5,000 smolts. Assuming an approximate sex ratio of 1:1, 25 anadromous Sockeye females could produce between 45,000 – 180,000 eggs (1,500 – 6,000 eggs per female; Burgner 1991) and a larger escapement could easily be produced based on these higher numbers past 2019. Selecting the returning anadromous Sockeye broodstock for future hatchery enhancement may also produce smolts with a higher propensity for an anadromous life cycle than offspring from land-locked Kokanee.

If smolt to adult survival is found to be in line with, or higher than, other Lower Fraser Sockeye stocks (>1%) (Mathews et al. 2012) consideration should be given to the construction of a surface smolt collection system with a dam passage option. Once a larger number of Sockeye Salmon is regularly transported above the dam, their offspring should have a higher propensity to leave the reservoir and the need for further hatchery enhancement is hoped to be eliminated. To facilitate large numbers of smolts leaving the reservoir and to accommodate larger (>1,000) Sockeye Salmon escapements, a complete fish ladder will likely need to be added to the Coquitlam Dam structure in the future.

2. **Results and Discussion: Installation of a smolt holding tank including water supply and outflow plumbing below Coquitlam Dam**

The smolt holding tanks installed by the PCDFHC kept fish in good health from April 12 to April 20, 2017, with mortality rates of 30 fish per day for the first three days after transport and very few mortalities (< 5 smolts per day) after that for a total of 2% over a 12 day period. Of the remaining 400 fish, 100 were used for a LLO passage survival study and the remaining fish died due to a power failure based on a lightning strike, as reported in the Methods section. For future holding of juvenile fish at the dam, it is recommended to install a water flow back-up system connected to an independent power source or a low flow warning system with notification capabilities.

3. **Results and Discussion: Daily feeding and maintenance in holding tank at Coquitlam Dam**

Smolts were fed daily and grew visibly. Growth was not quantified to avoid handling stress, since the main focus of this project was the release of as many smolts as possible into the Coquitlam River for migration into the ocean. The PCDFHC volunteers also maintained the holding tank in excellent condition as part of their daily feeding and cleaning routine.

4. **Results and Discussion: Planning and execution of acoustic tag implant surgery and subsequent smolt release into Coquitlam Reservoir**

The acoustic implant surgery on April 24, 2017 was highly successful without any mortalities for the second batch of 63 fish. Implant surgery on the first batch of 50 smolts on April 14 nevertheless resulted in the loss of 20% of the fish or a total of 10 mortalities. The first batch of surgeries was carried out with the same care and attention to detail as the second batch of surgeries, but was also carried out two days after the transport stress, when mortalities without the surgery handling also spiked in the holding tank. We therefore strongly believe that the two day period between transport and surgery was too short and should be longer in future studies. Alternatively, surgery in Rosewall Creek a week before the transport would have likely been the best alternative but was rejected by the DFO Ethics Committee following discussions and submission of applications by LGL Ltd.

5. **Results and Discussion: Smolt releases into the LLOs to evaluate passage survival, physical trauma, and general condition**

Based on anecdotal information about Sockeye smolt mortalities in Coquitlam River RSTs it was assumed that passage through the LLOs was harmful and could lead to injury and mortality in

the majority of smolts passing. The results obtained in this study paint a different picture, at least for the average spring discharge of 3 m^3/s through one of the LLOs. The results summarized in Table 4 show that only 13% of the 303 Kokanee and Coho smolts released into the sluice tower trash rack at a discharge of 8 m^3/s on May 2 and of 3 m^3/s on May 3 and 4 were recovered in the combined catch of the Fyke Net and RST in the Coquitlam River below the dam. Nevertheless, all of recovered fish were alive and in good condition. This low recovery rate could have been based on either very low survival through the LLOs or high survival through the LLOs but low rate of entrainment into the LLO. Since no dead fish were observed or recovered in the Fyke Net or RST, the latter explanation appeared more plausible and additional release tests were planned accordingly. A cabled underwater camera with a surface monitor was deployed into the chamber between the trash rack and the LLO when flow was stopped and it became obvious that smolts that were sucked through the trash rack and into the chamber could have easily escaped the current leading into the LLO by swimming up or to the side and into back eddies with little current. The water-filled part of the chamber had an approximate width of 2 m, a length of 3.5 m, and at the observed reservoir elevations above 150 m a height of approximately 10 m, while the LLO opening is about 1 m in diameter when fully opened and less at a discharge of 3 m^3/s (when the majority of the fish where released). Therefore, the majority of the chamber had little current and fish could easily remain in the chamber for an extended period of time. Once discharge was switched from one LLO to another, flow in the chamber with the smolts stopped all together and smolts could have easily swam back into the reservoir through the trash racks without encountering any current. Therefore, we designed the second smolt release contraption without the possibility for fish to escape the current into the LLOs and installed the release pipe inside the chamber downstream of the trash racks inside the sluice tower with a fire hose attached to the end that was directly sucked into the LLO. Based on the releases using this modified set-up, combined Fyke Net and RST recovery rates under consideration of their respective catch efficiency increased to 84% for fish released alive and 36% for fish released dead during the May 31 and June 1 releases at 3m^3/s (Table 4). All of the fish recovered alive were in good condition without any physical trauma and were released. Only 3% of the fish released alive were recovered dead and upon visual examination and indicators for time of death such as "*rigor mortis*" and skin colour, those fish all died in the Fyke Net shortly before recovery and not during passage through the LLOs the night before. We can therefore assume that at least for discharges around 3 m^3/s or lower passage mortality through the LLOs is close to 0%. The LLOs may therefore be suitable as a conveyor of smolts from the reservoir into the river and could therefore be connected directly to a smolt surface collector that will likely be needed to create surface attraction flows for smolts in the forebay of Coquitlam Reservoir.

RECOMMENDATIONS

Based on the results of the work carried out in this project, the following recommendations are made:

1. While passage of Kokanee and Coho smolts through the LLOs at a 3 m^3/s discharge appears to not injure or kill Kokanee or Coho smolts, the same has to be proven for

discharges of 8 m^3/s or higher, which are likely needed to attract smolts to a surface smolt collector system that may be installed in the Coquitlam Reservoir forebay.

2. Incubation and rearing of Kokanee caught in 20 m depth in a gillnet can be accomplished successfully, but slightly lower rates of initial survival from fertilization to hatching have to be expected. For future egg takes (if available), returning Sockeye spawners that can be visually assessed or held to maturity in a hatchery may be the option with higher egg to smolt survival.

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APPENDIX A: FISH TAG IMPLANT SURGERY SOP

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Field Standard Operating Procedures

Surgical Tagging Implantation Procedures Used in GCPUD 2016 Acoustic Telemetry Studies

Purpose: To provide guidelines and standard protocols for surgical tagging of juvenile salmonids for the Grant County Public Utility District (GCPUD) 2016 (Year 3 of 3) survival analysis. Year 1 and Year 2 results can be found in Hatch et al. (2015) and Hatch et al. (2016) respectively.

Area of Applicability: For all LGL staff involved in surgical tagging of juvenile salmonids for the GCPUD survival analysis. However, the Standard Operating Procedures (SOP) can be applied to most other projects in a lab environment, and is an excellent resource for anyone wanting to learn about fish tagging.

SURGICAL EQUIPMENT AND MATERIALS NEEDED:

- Dissolved oxygen (DO) meters which include temperature readings
- pH meter
- Total dissolved gas (TDG) meter
- Autoclave (M11 Ultraclave, Midmark) (available at Wanapum Dam)
- Tags, acoustic and PIT, PIT tag reader
- Tricaine methanesulfonate (MS-222; 53 g/L stock solution)
- Baking soda – sodium bicarbonate (buffering solution), same concentration as MS-222
- Stress Coat – original concentration and 25% solution 250 ml/L
- 20 L buckets marked at 10 L and clearly labeled “HEAVY” (referring to anesthetic buckets)
- 20 L numbered recovery buckets with lids
- Pair of 20 L buckets (gravity feed) marked at 10 L connected by rubber tubing with in-line shut-off valves – one labeled “MS-222” and one labeled “fresh water”
- Syringes for measuring anesthetic (60 cc)
- Oxygen delivery system
- Small and large dip nets – some modified as sanctuary nets
- Nitrile gloves
- Waterproof scale measuring to the nearest 0.5 g

- Plastic weigh boats
- Surgery table – plexi-glass box with a built in v-shaped trough (trough is covered with plastic cupboard lining to avoid mucus and scale loss). Plastic hoses are also connected to the surgery table for outtake and intake of water. The water from outtake hose is emptied into a 20 L bucket.
- Trays for holding solutions used to clean surgical tools
- Needle drivers
- Forceps
- Disposable scalpels (size #15)
- Germiphene (bactericidal, fungicidal, and virucidal; diluted 16 ml of Germiphene to 1 L of water)
- Chlorhexidine solution (Nolvasan; 30 ml/L) prepared with distilled water
- Sutures, size 5-0 (VICRYL coated with tapered RB-1 needle – antibacterial type)
- Distilled water as a rinse solution for disinfectant (Chlorhexidine)
- 2 L bottles to make up stock solutions for Stress Coat, Germiphene, and MS-222
- Spray bottles for Stress Coat and disinfectant
- Shop towels
- Sharps container and Ziploc Bags
- Data sheets, writing tools, computer

Procedures:

1) Collection and Pre-Tag Holding

A. Verify that proper collection and transport permits have been obtained and are in possession at the time of collection and tagging. All staff involved in the tagging procedures must be aware of permit restrictions. Copies of the permits are to be visible at the collection facility.

B. After being gatewell dipped, fish are delivered in a tank on the back of a truck. The truck tank releases the fish into the pesculator holding tank; the fish are then pescalated up from the tank through a long tube and into the sorting trough. At the sorting trough the taggable fish (correct species, suitable size, and in acceptable condition) are placed into holding tanks and the rest of the fish are released. 150 ml of MS-222 stock solution is added to the trough to create a light sedation that reduces the handling stress on the fish. Given the water level in the trough will drop as the fish are netted out, it is helpful to keep a bucket of extra MS-222 which can then be used to restore the water level in the trough. To make a bucket of the same MS-222 concentration as the trough, add 7.3 ml of MS-222 to a 20 L bucket of river water. Whenever MS-222 is used, it is buffered with the same quantity of sodium bicarbonate. See Section xii.

C. All pre-tag holding tanks are scrubbed, flushed, and re-filled with water at the beginning of the season. All pre-tag holding tanks should have no light access (i.e., windows) as darkness is thought to reduce Frustrated Smolt Syndrome (FSS).

D. Pre-tag holding density is approximately 30 Steelhead smolts per tank, which is about half the density recommended as maximum by aquaculturists (50 g of fish per L of water). Holding tanks must have flowing, untreated river water supplied at all times when in use.

E. The pre-tag holding period begins once fish are placed in holding tanks. Pre-tag holding times for study fish should be between 15 to 24 hours and not to exceed 36 hours. For example, if fish are delivered at noon, and tagging the following day runs from 8 AM until 4 PM, then pre-tag holding times will range from 20-28 hours, and any fish tagged after 12 PM would be over the recommended holding time. All fish (including gatewell dipped fish) should be delivered by 5 PM to allow pre-tag holding times to range from 15-23 hours.

F. Each species collected is held in a separate holding tank to reduce stress (only one species collected and tagged in 2016). Record the species and collection date on each pre-tag holding container using erasable wax pencils.

G. A random sample of pre-tag holding containers are monitored for DO and temperature twice daily while housing fish.

2. Fish Size Criteria

A. The maximum tag weight to body weight ratio, or tag burden, must be known to calculate minimum fish size. The tag burden for the 2016 GCPUD smolt studies is 3%. To determine tag weight, 10% of all tags were weighed and the maximum weight of 0.36 g was used. Given PIT tags are also inserted into the fish, the final tag weight must include the addition of 0.1 g for the PIT tag, resulting in a maximum tag weight of 0.46 g. Hence if adhering to a 3% tag burden the smallest fish allowable for tagging is 15.3 g. Given the weigh scales are precise to 0.5 g the minimum fish size was rounded up to the 0.5 g, resulting in a minimum fish size of 15.5 g.

B. A maximum fish size limit is also followed to avoid tagging very large fish that may potentially residualize. Weight is the preferable measure for both minimum and maximum limits as tag burden is calculated based on weight. Also, as an aside, during the tagging procedure weights are measured first hence handling time is reduced if a fish is culled immediately after being weighed. The 2016 maximum size limit of Steelhead is 89 g; this maximum weight for Steelhead was

determined in years past based on size distributions and a weight / length regression.

3. Pre-Tag Preparations

A. Environmental conditions – All staff must be trained on all water quality measuring equipment.

i. Dissolved oxygen (DO): will be measured in mg/L in a random sample of pre- and post-tag holding tanks/buckets while housing fish.

ii. Measurements will be taken using a DO meter.

iii. DO concentrations in pre- and post-tag holding tanks/buckets should be between 8 mg/L and 13 mg/L. If readings are outside of this range, inform your field manager and check for limited water flow to the tank which may cause low oxygen levels. Add supplemental oxygen or increase flow if necessary.

iv. Water temperature: will be measured in °C in a random sample of pre- and post-tag holding tanks/buckets while housing fish. Temperature may be taken with a DO meter. Temperature of the pre- and post-tag holding tanks/buckets should be within 2° C of the ambient water temperature. If readings are outside this range inform your field manager and change the water.

v. Air temperature: within the surgical area should be controlled to ensure that the fish on the surgical table are not too hot or too cold. Typically, the tagging trailer and post-op recovery trailer are equipped with swamp coolers.

vi. Total dissolved gas (TDG): will be measured as percent saturation twice a day in the head-box and in a recovery bucket. Measure once at the start of the day and again at the end of tagging for the day.

vii. Measurements will be taken using a TDG meter (various models)

viii. Gas super saturation (TDG > 110%) may lead to gas bubble disease and must be avoided. Contact your field manager if TDG approaches 110%. Check that water inflows are being off-gassed before use.

4. Setup of Equipment

i. Tags should be activated, tested, and prepared for implantation (procedure depends on tag type).

ii. Disinfect all tags in diluted Chlorhexidine solution (i.e., Nolvasan) for a minimum of 10 minutes. The tags are then transferred to a distilled water rinse bath then actively rinsed under running distilled water. The tag will be placed, using forceps, in a vessel of distilled water just prior to implantation. Note that some chemicals in certain disinfectants may adversely affect the coating on the transmitters.

iii. Prepare surgical table and equipment for use.

iv. Setup measuring board and scale. All staff must be trained on the use of all weight and length measuring equipment.

v. Ensure the scale is functioning properly. Scales should be calibrated at the start of the season and whenever they are moved, and should be recalibrated as necessary.

vi. Spray approximately 1-2 ml of undiluted Stress Coat on the surgical table.

vii. Recovery buckets must be filled with untreated river water and supplied with oxygen if necessary (See Section A.

viii. Administration of anesthetic: The effectiveness of MS-222, as an anesthetic, varies with factors such as temperature, size of fish, species, exposure, and fish density. Adjustment of the anesthesia concentration should be based on the amount of time it takes for a fish to lose equilibrium. However, the anesthesiologist should target between 60-80 mg/L MS-222 concentration level for salmonids. Communication among the tagging crew is very important to ensure that MS-222 is only administered to the anesthetic buckets and gravity feed buckets once. Never administer MS-222 into a bucket until you confirm that no one else has.

ix. Fill the “HEAVY” anesthetic bucket with 10 L of untreated river water. Start with adding approximately 10 ml of MS-222 stock solution to yield a concentration of 53 mg/L. Adjust the amount of anesthesia concentration accordingly based on the amount of time it takes for a fish to lose equilibrium and the amount of water in the bucket.

x. Fill both gravity feed buckets with 10 L of untreated river water. Add 3 ml of MS-222 stock solution to the bucket marked “MS-222” for a light dose for Steelhead. Do NOT add MS-222 to the bucket marked “fresh water”.

xi. Stress Coat helps maintain the slime coat on the fish, which helps prevent infection and reduce stress. Add the Stress Coat stock solution (Stress Coat solution is 25% Stress Coat, 75% river water) to all buckets (anesthetic, anesthetic gravity feed, and recovery buckets). For every 10 L of water, add

10 ml Stress Coat stock solution. Undiluted Stress Coat is lightly sprayed to the weigh boat and surgery table.

xii. MS-222 is acidic and changes the pH level of water, therefore it is desirable to buffer the water by adding sodium bicarbonate. Add the same quantity of buffer as the MS-222. The easiest way to achieve this is by making the sodium bicarbonate stock solution at the same concentration as the MS-222 solution so that an identical amount of milliliters of each solution is added to the buckets. For example, if 3 ml of MS-222 stock solution is added to a bucket, then 3 ml of sodium bicarbonate stock solution must also be added. The MS-222 stock solution has been prepared by adding 1 kg of MS-222 powder to 5 gallons of water, yielding a stock solution of 52.63 mg/ml, or 53 g/1 L. When preparing the buffer stock solution weigh out 53 g of sodium bicarbonate and add it to 1 L of river water.

xiii. Water in all buckets (anesthetic and gravity feed) should be changed and monitored periodically to minimize dilution of anesthetic water and temperature changes (such as a temperature change of more than 2° C above or below ambient water temperature) and to ensure that there is sufficient water in your gravity feed buckets to last the complete duration of a surgical tag insertion. Typically, water in the anesthetic and gravity feed buckets is discarded and refilled after approximately twenty fish are tagged. During warm days water may need to be changed more frequently due to rapid increases in water temperature.

xiv. All gravity-feed “MS-222” and “fresh water” buckets will be monitored for dissolved oxygen. Oxygen levels should be maintained near saturation. It is the surgeon’s or anesthesiologist’s call to add oxygen to the necessary buckets.

xv. Anesthetic and fresh water buckets should be filled and prepared just prior to tagging.

xvi. It is recommended that sanctuary nets be used for the transfer of fish between pre-tag holding tanks and “HEAVY” anesthetic buckets.

5. Implantation of Tags

A. Anesthetizing Fish

i. A fish is dip-netted (using a sanctuary net) from the large holding container and placed in the “HEAVY” anesthetic bucket. Secure the lid of the anesthetic bucket as soon as the fish is in the bucket. After one minute the fish will have slowed down enough to quickly check for abnormalities (visual check only while the fish

remains unhandled in the sedative). If the fish is rejected after one minute then it is transferred to a fresh water recovery tank and released into the river.

ii. If the fish is not rejected after one minute it remains in the "HEAVY" anesthetic bucket. The fish should take an additional 1-3 minutes to lose equilibrium and then it is ready for tagging. If after sedating a few fish, you notice that the time required for fish to lose equilibrium is more or less than normal, then adjust the concentration of the anesthetic up or down. If loss of equilibrium takes less than one minute or greater than 5 minutes, reject the fish. The data recorder will document the time the fish entered "HEAVY" to monitor the duration that each fish is in "HEAVY" anesthetic.

iii. Once the fish loses equilibrium, the surgeon will visually screen the fish for tags, fin clips, fungus, disease, descaling, bloated belly, or any obvious abnormalities. Relay any necessary information to the data recorder. See LGL's culling SOP for the type of fish that are acceptable for tagging.

iv. Rejects – If the fish is unacceptable for tagging, transfer the fish to a fresh water tank and release it into the river once it has regained equilibrium.

B. Recording fish length and weight

i. Transfer (using sanctuary net) the anesthetized fish to the scale and weigh the fish to the nearest 0.5 g. The data recorder will stop the "HEAVY" sedation timing once the fish is removed from the "HEAVY" bucket. The data recorder will also document the start time of surgery at this point.

ii. Transfer the fish to the measuring board (on the surgery table) and measure the fork length to the nearest millimeter. Data must be vocally relayed to the data recorder to avoid data errors. The data recorder should then record this information and repeat numbers back to avoid any miscommunication.

iii. Any fish dropped on the floor or ground before tagging must be rejected. A fish that flops from the tagging trough into the surgical table during tagging can still be tagged; exception, if MS-222 water enters the incision, reject the fish. If a fish is dropped on the floor after it is tagged, remove the tag, and reject the fish.

C. Surgery

iv. Place the fish on the surgery table ventral side up. Anesthetic should be administered through the gravity feed tubing as soon as the fish is on the surgery table. The tubing must be placed just inside the mouth or as close as possible so the water flows across the gills. The flow of water should be just enough to cover the gills. If the flow is too low, the fish will flare its gills and become agitated. Adjust to a flow that keeps the respiration of the fish normal throughout the

surgery. Use the in-line valve to control the flow of anesthetic, fresh water, or a mixture of both. Start with a constant flow of anesthetic and monitor the condition of the fish.

v. Using a scalpel, make an incision, approximately 8 mm in length (dependent on tag size), about 3 mm away from and parallel to the mid-ventral line. Start your incision a few millimeters anterior to the pelvic girdle (Figure 4). The incision should be just deep enough to penetrate the peritoneum (the thin membrane separating the gut cavity from the musculature), avoiding the internal organs. The spleen is close to the incision position, so pay close attention to the depth of the incision.

vi. There is no exact specification for what size scalpel blade to use for each fish. For most smolts, we use a veterinary purchased #15 disposable scalpel. You may prefer to use a micro-scalpel on small fish. You can decide which scalpel you prefer in the pre-season training session.

vii. One scalpel blade can be used on about 7-10 fish before it becomes dull. If the blade is pulling roughly or making jagged incisions, it needs to be changed immediately.

viii. If you believe you cut an internal organ, do not implant the tag, stitch the incision, and reject that fish. Excessive bleeding should be noted on the datasheet.

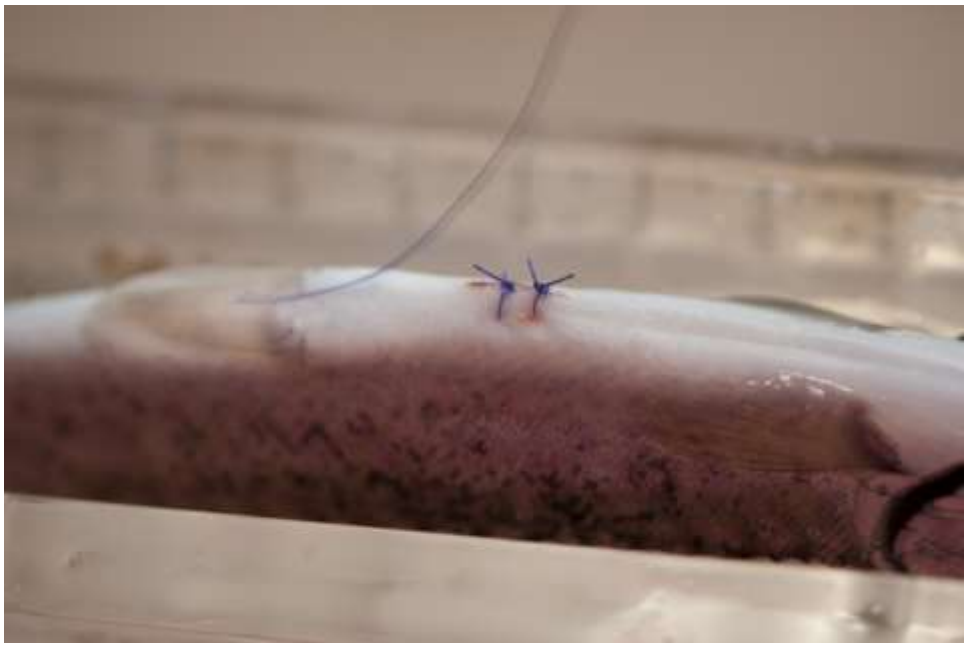

Figure 4. Ventral view of a juvenile salmonid showing the location and proper placement of incision.

ix. If a PIT tag is to be inserted with an acoustic tag do this first by gently pushing the PIT tag into the body cavity. The acoustic tag can then be gently inserted. The transmitter end is inserted first through the incision and should be pointing to the head of the fish; the battery end of the tag should then be pointing to the caudal (Figure 5). The tag should lie directly under the incision and will aid in keeping the organs from protruding through the incision. If either tag is dropped prior to implanting into the fish it will be necessary to soak the tag in a Chlorhexidine solution for a minimum of 10 minutes, then rinsed thoroughly with distilled water before being put into another fish.

Figure 5. Ventral view of a juvenile salmonid showing the insertion of an acoustic tag into the body cavity.

x. Begin suturing the incision. Two interrupted stitches are usually used to close the incision, depending on the size of the tag and incision.

xi. To make a stitch, lock the needle (at the end of the suture) in the needle drivers so the needle point faces you. Enter the outside edge of the incision on the side farthest from you and exit through the other edge of the incision, pulling the suture perpendicular through the two edges. The needle should enter and exit the skin as close to the edge of the incision as possible without tearing the skin (~ 2 mm from edge of incision). Pull the needle and suture through the skin to leave a tag end of about 2-3 cm of suture material protruding from the needle entrance location, then release the needle from the needle drivers. With your non-dominant hand, grasp the long end of the suture material (usually with thumb and forefinger) at or below the needle, and make two forward wraps (i.e.,

away from your body) around the tip of the needle driver, which should be held in your dominant hand. With the two wraps still around the needle driver, grasp the short end of suture material with the needle driver and tighten the stitch by pulling the wraps off the needle driver and pulling both ends of suture material perpendicular to the incision. On the first knot, the dominant hand holding the needle driver should pull toward your body and the non-dominant hand should pull away from your body. Tighten the suture lightly, just so the edges of the incision meet, but do not overlap, pucker, or bulge the edges of the incision. The second knot is in reverse order from the first knot and there is only one wrap with the suture material around the needle holder. The second knot is looser than the first, again taking care not to overlap, pucker, or bulge the edges of the incision. This allows room for tissue swelling. On the third knot, grasp the long end of suture material with your non-dominant hand, make one reverse wrap (i.e., towards your body) around the end of the needle driver, grasp the short end of suture with the needle driver, and tighten the stitch. This time, the knot should be tightened by pulling your dominant hand (holding the needle drivers) away from you and your non-dominant hand toward you. This completes one stitch. Cut the suture with the needle drivers, leaving ends approximately 3 mm in length. See "Resource Documents" list for further discussion of suture materials and knot types.

xii. When making wraps around the needle driver, it is easiest to make the wraps closer to your hand holding the suture, rather than closer to the fish. After making wraps around the needle driver, be sure to grab (with the needle drivers) the short end of the suture close to the end of the suture material. If you grab the suture material closer to the fish than to the end of the suture, then the tag end will fold onto itself, get tightened into the knot, and leave two strands of suture material in the knot. This is a common mistake.

xiii. When pulling a knot tight, be sure the knot lays flat and does not twist onto itself into a "balled-up" knot. If the knot begins to "ball-up" when pulling tight, it can sometimes be coaxed to lay flat by twisting the suture material between your thumb and forefinger. With experience, each surgeon develops their own "tricks" for making sure the knot lays flat. Although not every knot is perfect, you should strive for perfection on every stitch, rather than settling for an imperfect stitch. An imperfect stitch will be more likely to come untied, possibly resulting in slower wound healing or tag loss, which could ultimately affect the survival of the fish or bias the study results.

xiv. There is no exact specification for what size suture to use. 5-0 sutures can be used for Steelhead smolts. A tapered needle is used on all juvenile salmonids. Since fish come in all sizes, there will be some overlap in these approximate parameters.

xv. Generally, a good time to switch the in-line valve on the gravity feed buckets or hose to untreated river water is just prior to the last stitch. This initiates recovery from anesthesia as early as possible. However, if the fish appears to be inadequately gilling, you should provide a mixture or all fresh water as soon as possible. If the fish is reviving too quickly and the surgery is not yet complete, do not switch to fresh water.

xvi. If the incision is too long, it is acceptable to add a third stitch. Relay this information to the data recorder so that note can be made on the datasheet.

xvii. Because sutures are long, each individual suture (one packet) can be used on 4-6 fish.

xviii. When multiple successive fish are to be tagged, and to keep the tagging process flowing, the anesthesiologist should start sedating the next fish before the first fish's surgical procedure is complete. The surgeon should indicate to the anesthesiologist when the next fish should be sedated. Typically, a good time to start sedation on the next fish is immediately after the previous fish has been taken out of the "HEAVY" bucket.

6. Post-Surgery

A. Transfer the fish from the surgery table directly to a labeled recovery bucket using your gloved hands. The data recorder will document the end time of surgery at this point. The data recorder should also record the label of the bucket as the location for that fish. There should be no more than 2 fish per recovery bucket. Dissolved oxygen levels should be consistent with that of the river as the recovery buckets are filled with water just prior to the fish being placed in the bucket. If there is a delay before a recovery bucket is used (no fish are in the bucket) due to rejecting fish, it is the surgeon's decision whether or not to add oxygen to the recovery bucket. Super saturation of oxygen has been proven to aid in recovery so add supplemental oxygen to the recovery bucket once the first fish is placed in recovery. Remove the supplemental oxygen once the second fish is in recovery and move the bucket to the recovery shed to be placed on flow through river water. The bucket will remain super saturated for a few minutes until all water is flushed, allowing the second fish to also benefit from super saturation during recovery. If several culls occur between the surgeries of the first and second fish, remove the supplemental oxygen from the bucket to avoid a prolonged period of super saturation.

B. Between surgeries, the surgeon should prepare their tools for the next surgery. Disinfect the tools in Chlorhexidine solution and ensure that the scalpel blade and suture are acceptable to use on the next fish. If necessary, replace the scalpel blade and suture. All surgical equipment must be disinfected (by soaking them in Chlorhexidine solution for several minutes – see directions on bottle,

typically at least 10 minutes) between each fish to avoid transmitting disease among individuals. Once disinfected, rinse the tools thoroughly with distilled water. It may be necessary to have multiple sets of surgical instruments which are rotated in order to ensure the minimum recommended soak time between uses (four sets of instruments will be rotated for the 2016 study). Organic debris in the disinfectant bath reduces its effectiveness, so be sure to change the disinfectant baths regularly.

C. Tagged fish are checked at various times throughout the day. Typically tagged fish are checked approximately 30 minutes after surgery. If a tagged fish does not exhibit signs of proper swimming behavior or has not gained equilibrium it is up to the surgeon to decide if the fish should be rejected. Manual ventilation can be attempted by holding the fish in an upright swimming position in the water and moving the fish back and forth to force water flow through the gills. If the fish is rejected then the tag is pulled, soaked in a Chlorhexidine solution for a minimum of 10 minutes, then rinsed thoroughly with distilled water before being put into another fish. The rejected fish is re-sutured and released into the river.

D. Optimal post-op recovery time should be 15-24 hours, and should not exceed 36 hours. Post-op holding time should start when the last fish is tagged. Therefore, the first fish of the day could be held about 8 hours longer than the last fish of the day. Currently, GCPUD study fish are held up to 24 hours after surgery.

7. Cleanup at the End of the Tagging Day

A. Wipe down all counter tops, scales, and measuring boards with the Germiphene solution to disinfect. Germiphene solution should not be made of river water, but rather distilled water.

B. Soak scalpels, forceps, and scissors in Chlorhexidine solution, then rinse with distilled water and thoroughly dry to prevent rusting.

C. Scrub needle drivers with a small brush or scour sponge.

D. All autoclavable surgical equipment should be autoclaved prior to the next day's tagging session.

E. Buckets and nets should be washed with Germiphene solution and then rinsed thoroughly with untreated river water and placed upside down to dry. Store electronics in proper cases.

F. Extra fish (not required for backup) in the pre-tag holding tanks are released back into the river). Empty holding tanks are flushed and re-filled (for use the next day), or left empty.

8. Datasheets

A. The appropriate tagging and water quality data sheets for your project will be filled out before, during and after tagging. At the end of the tagging day, the field manager should review these datasheets to ensure proper collection procedures were followed.

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CULLING GUIDELINES FOR SALMONID SMOLTS

A Criteria for culling fish prior to a tagging session (fish sorters and taggers)

1) Anesthetize all fish with MS-222 prior to handling.

2) Keep fish underwater throughout the examination process, handling fish as quickly and gently as possible.

3) Examine fish individually for external marks before tagging, including:

- **Scale loss: *Normal* (<4%); *Partial* (4-19%); *De-scaled* (>19%);**

Scale loss is calculated per side. For example, 10% scale loss on each side would not be a reject, whereas 20% on one side would. Divide fish into ~ 25% portions as a reference when estimating scale loss. Example of normal (< 4%) scale loss (top) and partial scale loss (4-19%).

- **Bird wounds, head and body injuries, net marks, bleeding, etc.;**

- **Fungus, parasites, leeches, and overall fish health;**

- **Missing or extremely damaged fins (tattered fins are acceptable);**
- **Severe abnormalities (stunted, duplicated fins, etc.);**

- **Signs of Bacterial Kidney Disease (BKD) include hemorrhaging near the eyes and bloating;**

- **Elastomer or visual implant (VI) marks, or other brands;**

- **PIT tag entry scars (between pectoral and ventral fins); and**
- **Cull all fish that have been previously anesthetized, handled, and tagged (except elastomer tagged fish).**

4) Size criteria (exclude):

- Fish weight that will result in a tag burden greater than 3%:
    - Steelhead less than 15.33 g.
- Steelhead greater than 89 g.

- Do not tag any fish that have been dropped or mishandled in any way.

B Criteria for culling fish during a tagging session (criteria for taggers only)

1) Do not tag any fish you feel does not exhibit the same behavior as the group of fish being tagged during each tagging session. For example, if you have a fish that loses equilibrium in substantially less time (compared to the other fish you have been anesthetizing), there is probably a reason. The fish might have been handled recently or is unhealthy.

2) Do not tag a fish that exhibits excess bleeding during tagging.

3) Do not tag a fish that has excess body fluid once incised (sign of BKD).

4) Do not tag a Steelhead with any gill parasites.

C Criteria for culling fish post-tagging (criteria for taggers only)

1) If a tagged fish does not exhibit normal swimming behavior or has not gained equilibrium within 30 minutes of being tagged, it should be rejected. If the fish is rejected then the tag is pulled and put into another fish. The rejected fish should be re-sutured and released into the river.

D Overall

1) Keep an accurate tally of all rejected fish and the reasoning used to reject it. Keep an accurate tally of all surplus fish.

APPENDIX B: FINANCIAL STATEMENT