PSC Northern Fund 2016 Annual Project Report

Project Title: Monitoring occurrence and prevalence of *Ichthyophthirius multifiliis* (Ich), *Loma salmonae* (Loma), and infectious hematopoietic necrosis virus (IHNV) in Skeena River sockeye salmon stocks.

Introduction: The 2016 sample period represents the 2nd year of PSC funding towards monitoring of Skeena River sockeye salmon stocks for viral and parasitic infections. Similar to 2015, sampling and diagnostic efforts focused on the detection of three pathogens, *Ichthyophthirius multifiliis* (Ich), *Loma salmonae* (Loma), and infectious hematopoietic necrosis virus (IHNV), which are known to be present in the Skeena watershed and have attributed to devastating losses in sockeye salmon. Additionally, as was performed on the 2015 samples, histology was conducted in 2016 to continue to compile a pathogen/parasite inventory list at various life stages of Skeena River sockeye. Further, comparator sockeye stocks from the Fraser River were again sampled in 2016 in order to compare pathogen presence with that observed in Skeena sockeye. These comparator samples are reported herein as they provide necessary epidemiological context to our understanding of fish health in Skeena sockeye salmon. The 2016 sample collection details, methods and diagnostic results are presented in the following Methods and Results sections.

Methods:

Fry Sampling

Fry collections began in April 2016 at each of the three spawning channels (Pinkut Creek, Fulton River, and Nadina River) during spring enumeration activities. Fry collections occurred over the entire outmigration period with a sample of greater than 25 fry taken every third or fourth day from each location until the migration ceased. For virological analysis, all fry sampled from one location at one timepoint were placed into a whirl-pak bag and immediately frozen. For histological analysis, an additional 20 fry were collected at three separate timepoints to represent the beginning, middle and end of the outmigration period. Fry collected for histology were placed immediately into 10% neutral buffered formalin. All samples were labeled with the date of sampling and the location. At the conclusion of the fry collection period, samples were shipped overnight to the Pacific Biological Station via Air Canada.

Spawning Adult Sampling and Ich & Loma Monitoring

Retuning spawning adults were sampled for virological analysis during the peak spawning period (mid-September) at each of the three spawning channels. The virological sample consisted of ovarian fluid or milt samples collected from newly dead or moribund post spawned fish. The numbers of virological samples collected are summarized in Table 1. To monitor for Ich and Loma, a minimum of 50 fish were sampled at three time points at each of Fulton, Pinkut, and Nadina Spawning Channels. At time of collection, fish were visibly inspected for the presence of the Ich by identifying the parasitic trophont stage often viewed around the top of the head and operculum. Subsequently a gill arch was removed from the fish and microscopically inspected and scored for presence of Ich by the observation of the infective motile stage of the parasite (known as theronts). For identification of Loma, spores of the parasite were detected in a wet mount preparation from the gill. Lastly, from each of Fulton, Pinkut, and Nadina spawning channels, 10 adult sockeye salmon, coinciding with peak spawning periods, were preserved into 10% neutral buffered formalin for histological analysis.

Smolt Sampling

Babine River sockeye smolts were collected at the Babine River counting fence during the outmigration from Babine Lake. Sampling for virological analysis began on May 2, 2016 and continued through to June 9 representing 22 independent collection periods to amass a total of 110 samples (5 fish per collection). Smolts collected for viral analysis were pooled by day into whirl-pak bags and immediately frozen. Bags were labeled with the date of sampling and the location. Additionally ten smolts were collected near the beginning, middle,

and end of the migration period and placed into 10% neutral buffered formalin. Prior to fixation, the smolts were quickly euthanized and opened from the anal vent to the isthmus with precaution to not damage the internal organs. One operculum was removed and the entire fish was placed into fixative with multiple fish sampled on the same day placed into one bottle.

Virology and Histology

Reproductive samples from adult sockeye salmon and tissue samples from fry/smolts life stages were assayed for IHNV using cell culture methodologies. Tissues were homogenized in Hank's balanced salt solution (HBSS) to prepare a final inoculum of 2%-4% (w/v) solution. The homogenate was centrifuged at 2500 x g for 15 minutes. Ovarian fluid is diluted 1:2 and 1:10 with HBSS. Dilutions prepared from samples were inoculated (0.1ml) into wells containing monolayers of EPC cells prepared 24 hours prior to inoculation. Cells were incubated at 15 C and observed 2-3 times per week for a period of 3 weeks for the presence of cytopathic effects (CPE). Samples showing plaque formation or CPE typical of IHN virus were passed to new monolayers of EPC cells and representative positive virus isolates were confirmed as IHN virus by reverse transcription polymerase chain reaction (RT-PCR). All IHN virus isolates will be archived at -80° C at the Pacific Biological Station for future investigation. For histological analysis, tissues preserved in in 10% (v/v) buffered formalin were processed by routine methods, sectioned at 5μm and stained with hematoxylin and eosin (H&E).

Table 1. Summary of 2016 sockeye salmon samples collected for virological and histological examination

Watershed	Spawning Channel	# of samples collected		
		Adult	Fry	Smolt
Skeena	Fulton River	Viral = 414* Histo = 10	Viral = 398* Histo = 121**	Viral = 55 Histo = 50
	Pinkut Creek	Viral = 101 Histo = 10	Viral = 175 Histo = 150***	
Fraser	Nadina River (comparator stock)	Viral =100 Histo = 10	Viral = 75 Histo = 62	Not applicable

*Adult samples taken for viral analysis were collected from Fulton River (n=100), Channel #1 (n=100), Channel #2 (n=201) and before the counting fence (n=10). Fulton fry samples were collected from Fulton River + Channel #1 (n=199) and Channel #2 (n=199). Pinkut Creek fry samples were collected from the channel (n=100) and the creek (n=75).

** Histological specimens were collected from Fulton River (n=62) and Channel #2 (n=59).

***Histological specimens were collected from Pinkut Channel (n=70) and Pinkut Creek (n=70)

Results:

Ich & Loma monitoring in adult sockeye

Fulton: Gill arch samples survey results for samples taken Aug 30, Sept 13 and 27:

- Ich prevalence and severity progressed over the sampling periods (12%, 54% and 65% positive, respectively).
- Loma remained at a relatively low prevalence but did show a slight progression over the sampling periods (2%, 3% and 10% positive, respectively), but remained relatively low prevalence. Increasing severity was seen overtime as well, with the final sample having several fish that had too high a parasites load to enable accurate counting.

Pinkut: Gill arch samples survey results for samples taken Sept 1 and 14th:

- Ich prevalence progressed over the sampling periods (22% and 34% positive, respectively); however the severity of infection decreased. In the initial sample on Sept 1, over 45% of the positive fish had to high a parasite load to allow counting while in the second sample none of the positive fish had a count higher than 5.
- Loma prevalence remained at 14% for each of the two sampling times yet severity decreased slightly.

Nadina: Gill arch samples survey results for samples taken Aug 31 and Sept 15:

- Increased prevalence over time with the initial sample yielding 88% and the subsequent sampling resulting in 100% prevalence of Ich (n=15). The severity of infection was also greatly increased in the second sample period with nearly half of the fish having counts greater than 200.
- Loma prevalence and severity increased over the sample period, with prevalence starting at 6% and reaching 53% at the second sampling time point. One positive fish at the second sample timepoint had a count of 125.

Virological analysis – IHNV detection

Returning spawning adults sampled at Pinkut Creek and Fulton River Spawning Channels were positive for IHNV. Pre-fence Fulton river samples were negative for IHNV. The prevalence of IHNV in the Skeena sockeye adults in spawning areas ranged from 21 % to 71.269 %. The numbers of virus positive adult fish from the Skeena River system are summarized in Table 2. All 100 Sockeye adults collected from Nadina River Spawning Channel tested negative for IHNV. Additionally, all sockeye fry (n= 648) and smolts (n=55) sampled in 2016 were negative for IHNV.

Table 2. Summary of IHNV positive detections in returning spawning adult Sockeye salmon populations

Sample location	Total samples collected	# of IHNV positive samples detected	Prevalence (%)
Pinkut Creek Spawning Channel	101	72	71.29
Fulton River Spawning Channel #1	100	49	49.00
Fulton River Spawning Channel #2	204	72	35.29
Fulton River	100	21	21.00
Fulton River – below the main fence	10	0	0
Nadina (comparator stock)	100	0	0

Necropsy Findings

10 fish were collected for necropsy and pathogen screening to assess individual and site overall health. Gill and blood cytology, bacteriology, virology, serology for *Renibacterium salmoninarum* and histopathology are being run on an individual fish level for all fish from Pinkut, Fulton, and Nadina. The complete analyses of these fish will be completed by the beginning of April.

- Bacteria Kidney disease

Sample location	Fish #	Elisa reading	Result
Fulton River Spawning Channel (Main Fence)	1	0.0750	Negative
	2	0.0837	Low level detection
	3	0.0920	Low level detection
	4	0.1037	Low positive

	5	0.1233	Low positive
	6	0.0850	Low level detection
	7	0.0883	Low level detection
	8	0.0797	Negative
	9	0.0853	Low level detection
	10	0.0930	Low level detection
Pinkut Creek Spawning Channel	2	0.088	Low level detection
	3	3.575	High Positive
	4	0.118	Low Positive
	43	0.082	Low level detection
	44	0.142	Low Positive
	62	0.116	Low Positive
	64	0.094	Low level detection
	84	0.134	Low Positive
	98	0.084	Low level detection
	102	0.102	Low Positive
Nadina River Spawning Channel	31	0.095	Low level detection
	33	0.104	Low Positive
	34	0.162	Low Positive
	24	0.170	Low Positive
	43	0.144	Low Positive
	49	0.177	Low Positive
	55	0.134	Low Positive
	56	0.113	Low Positive
	57	0.260	Moderate Positive
	71	0.084	Low level detection

Conclusion:

The deliverables as proposed in the second year of PSC Funding towards monitoring Skeena Sockeye for virus and parasites were successfully meet in that over 1,000 fish were analyzed. In addition to the originally proposed deliverables, sampling was undertaken at Nadina River spawning channel, a Fraser River tributary, to provide a comparator stock. This additional sampling resulted in an increase of over 200 samples beyond what was originally proposed to be analyzed. It is also worth noting that in addition to increased samples sizes, the number of laboratory tests as originally proposed was also expanded to include a histopathology evaluation of fish from each stock.

These additions to the monitoring program did significantly increase laboratory processing however are proving beneficial to our foundation of knowledge concerning the health of these stocks. With the 2nd year analyses nearly complete, we will begin to explore the variability in virus and parasite occurrence between the two years and begin to determine what factors contribute to the variation observed within and between Skeena and Fraser River Sockeye stocks.