**PSC Northern Fund 2015 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:** March 31, 2015 marks the completion of the first year of PSC funding towards monitoring of Skeena River sockeye salmon stocks for viral and parasitic infections. In particular sampling and diagnostic efforts over the past year 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. In addition to the specific diagnostics, exploratory diagnostics and sampling were conducted to establish a pathogen/parasite inventory list at various life stages of Skeena River sockeye. In order to compare the IHNV findings of Skeena sockeye with other sockeye populations in different watersheds, similar virological collections were performed on a Fraser River sockeye salmon stock. The sample collection details, methods and outcome of the diagnostics are presented in the following Methods and Results sections.

**Methods:**

*Fry Sampling*

Fry collections began in April 2015 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 4, 2015 and continued through to June representing 32 independent collection periods to amass a total of 160 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 Earle's balanced salt solution (EBSS) to prepare a 2% w/v solution. The homogenate was centrifuged at 3000 x g for 10 minutes and an additional 10 fold dilution was prepared to produce a 0.2% solution. Ovarian fluid is diluted 1:2 and 1:20 with EBSS. Dilutions prepared from samples were inoculated (0.1ml) into wells containing monolayers of EPC and CHSE-214 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 2015 sockeye salmon samples collected for virological and histological examination

| Watershed | Spawning Channel | # of samples collected | | |
|---|---|---|---|---|
| | | Adult | Fry | Smolt |
| Skeena | Fulton River | Viral = 431*<br>Histo = 10 | Viral = 349*<br>Histo = 120** | Viral = 160<br>Histo = 30 |
| | Pinkut Creek | Viral = 115<br>Histo = 10 | Viral = 148<br>Histo = 60 | |
| Fraser | Nadina River | Viral = 108<br>Histo = 10 | Viral = 150<br>Histo = 60 | Not applicable |

*Adult samples taken for viral analysis were collected from Fulton River (n=105), Channel #1 (n=108), and Channel #2 (n=208). Fry samples were collected from Fulton River (n=150) and Channel #2 (n=199).

** Histological specimens were collected from Fulton River (n=60) and Channel #2 (n=60).

**Results:**

*Ich & Loma monitoring in adult sockeye*

Regional biologist summary is pending.

Fulton: Gill arch samples survey results for samples taken Sept 1, 16+17, and Sept 28:

- Ich prevalence progressed over the sampling periods (4%, 80% and 87% positive, respectively); with increasing severity seen overtime as well.
- Loma progressed over the sampling periods (8%, 10% and 13% positive, respectively), but remained relatively low prevalence. Increasing severity was seen overtime as well, with more than half the final sample being scored 'too numerous to count'.

Nadina: Gill arch samples survey results for a single sample taken Sept 3:

- 100% prevalence of Ich (n=14) with counts ranging from 17 – 120.
- No Loma was seen.

Pinkut: Gill arch samples survey results for samples taken Sept 2 and 15$^{th}$:

- Ich prevalence progressed over the sampling periods (4% and 16%, respectively); but counts remained fairly low (<30).
- Loma progressed over the sampling periods (8% and 20% positive, respectively), but remained relatively low prevalence. Increasing severity was fairly constant at < 30 per gill arch, except for two gills from the second sample that were scored as TNTC.

*Virological analysis – IHNV detection*

Returning spawning adults sampled at Pinkut Creek and Fulton River Spawning Channels were positive for IHNV. The prevalence of IHNV in the Skeena sockeye adults ranged from 1.4 % to 9.5 %. The numbers of virus positive adult fish from the Skeena River system are summarized in Table 2. All 108 Sockeye adults collected from Nadina River Spawning Channel tested negative for IHNV. Additionally, all sockeye fry (n= 647) and smolts (n=160) sampled in 2015 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 | 115 | 10 | 8.7 |
| Fulton River Spawning Channel #1 | 108 | 9 | 8.3 |
| Fulton River Spawning Channel #2 | 208 | 3 | 1.4 |
| Fulton River | 95 | 9 | 9.5 |
| Fulton River below the main fence | 15 | 0 | 0 |

Nucleotide sequence analysis of a subset of the IHNV positive samples revealed all isolates were identical to one another over the 303 bp central region of the glycoprotein gene that was sequenced. This virus sequence type has been observed previously in stocks of Sockeye salmon in British Columbia and Washington. This Sequence types denoted as mG265U is one of many IHNV variants that collectively are referred to the U genogroup, which to date are the only IHNV types identified in British Columbia and Alaska. The details of the number of specimens and sample numbers that were sequenced are detailed in Table 3.

Table 3. Summary of IHNV sequence types isolated from returning adult Skeena River sockeye salmon populations.

| Location | # of samples sequenced | IHNV Glycoprotein Sequence type | Sample #'s |
|---|---|---|---|
| Pinkut Creek Spawning Channel | 7 | mG265U/mG267U | 14, 20, 21, 22, 23, 24, 25 |
| Fulton River Spawning Ch.1 | 8 | mG265U/mG267U | 188, 245, 348, 349, 350, 442, 580, 586 |
| Fulton River Spawning Ch. 2 | 3 | mG265U/mG267U | 136, 261, 314 |

*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 were run on an individual fish level for all fish from Pinkut and Nadina. All tests except bacteriology were run on Fulton samples (bacteriology omitted due to difficulty preserving sample quality

All sites - Blood smears were all negative for *Cryptobia;* no *Loma* were seen on the gill samples.

Fulton main fence sockeye health summary (3 females, 7 males):

1. mild parasitic gill disease
    - 2/10 gills appeared normal
    - *Ichthyophthirius mulitifiliis* was found on 8/10 gills – average count for one gill arch = 8.2 (median = 2.5. mode = 1, min = 0, max = 45, std dev = 13.9)

    - *Trichodina* 1/10, bacteria 1/10, epithelial hyperplasia 1/10 were seen on the gills via histology
2. mild parasitic kidney infestation
    - CKX 7/10
    - *Sphaerospora* 7/10
3. incidental internal parasites (low numbers, limited associated pathology)
    - *Philonema*, *Anisakis*, unidentified nematodes
4. Very mild *Renibacterium salmoninarum* infection 2/10 at a low level of detection by ELISA

Nadina sockeye health summary (5 females, 5 males):

1. moderate-to-severe parasitic gill disease
    - *Ichthyophthirius mulitifiliis* was seen on 10/10 gill arches – average counts = 137.8 (median=150, mode = 150, min = 28, max = too numerous to count, std dev = 38.6)
    - Epithelial hyperplasia and fusion of secondary lamellae 4/10 (moderate compromise) and autolysis 4/10 (severe compromise) indicate that respiratory functions were compromised to varying degrees in most of these fish
2. parasitic kidney infestation
    - *Parvicapsula minibicornis* was seen in the kidney glomeruli of 10/10 fish
        - Heavy in 3/10; medium in 4/10, and light in 3/10
        - Mature spores were seen in the lumen of the kidney tubules in 7/10
3. Incidental internal parasites (low number, limited associated pathology):
    - Unidentified nematodes 7/10 and an unidentified cestode 1/10 in the pyloric caeca
4. Note: Ich was also seen in 5/10 skin sections from this site, suggesting additional osmoregulatory challenge for these fish
5. *Aeromonas salmonicida* (furunculosis) cultured from the kidney of in 1/10 fish
6. 9/10 kidneys cultured *A. hydrophila* or *Vibrio sp.*
7. All samples were negative for *Renibacterium salmoninarum* by ELISA

Pinkut sockeye health summary (6 females, 4 males):

1. Moderate-to-severe bacterial gill disease and mild-to-severe parasitic gill disease
    - Heavy bacteria were seen 7/10 and an amoeba-like organism 2/10, these were associated with focal extensive epithelial hyperplasia and fusion of secondary lamellae 7/10, hypertrophy 4/10, edema 3/10; indicate that respiratory functions were compromised to varying degrees in most of these fish
    - *Ichthyophthirius mulitifiliis* was seen on 4/10 gill arches – average counts = 2.3 (median=2, mode = 0, min = 0, max = 6, std dev = 2.0) – this was considered an incidental finding
    - Other incidental gill findings included: low numbers of *Trichodina* 6/10, fungal hyphae 1/10, *Ichthyophous hoferi* 1/10, *Salmonicola* 1/10
2. Parasitic kidney infestation
    - CKX 10/10
    - *Sphaerospora* in tubule lumens 9/10
3. Incidental internal parasites:

- *Philonema* (pyloric caeca and intestines), *Anasakis*, unidentified worms and cestode in 4/10 intestines, unidentified nematodes 4/10 intestinal wall, unidentified worm-like structure seen within the brain 1/10

4. Very mild *Renibacterium salmoninarum* infection 9/10 at a low level of detection by ELISA
5. *Aeromonas salmonicida* (furunculosis) cultured from the kidney of in 1/10 fish
6. 8/10 kidneys cultured *A. hydrophila* or *Pseudomonas sp*.

**Conclusion:**

The deliverables as proposed in the first 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 time causing delays in our final reporting, but will undoubtedly prove beneficial to our foundation of knowledge concerning the health of these stocks. With subsequent monitoring of these stocks we will begin to explore the variability in virus and parasite occurrence and the drivers behind such variation observed in these Skeena and Fraser River Sockeye stocks.