



**Interactions of Wild and Hatchery Pink Salmon in Prince  
William Sound**

**Final Report for 2017**

**For Alaska Department of Fish and Game  
Contract CT 160001756**

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## ABSTRACT

This is the sixth in a series of annual progress reports on data collection and analysis for studies of hatchery-wild interactions of Pink Salmon in Prince William Sound (PWS), Alaska. The Prince William Sound Science Center (PWSSC), under contract to Alaska Department of Fish & Game (ADF&G), continued data collection for an investigation of the relative survival (fitness) of hatchery-origin and wild-origin offspring following natural spawning. Research in 2017 was focused on PWS Pink Salmon fitness studies. Similar studies of Chum Salmon in SEAK were managed by Sitka Sound Science Center and are therefore reported elsewhere. During the 2017 field season, 58,688 individual Pink Salmon were sampled during repeated visits to the spawning grounds of five PWS streams that had been previously sampled for this research. Tissue samples were collected for DNA extraction from each fish for the eventual analysis of relative hatchery-wild survival. Otoliths were collected from the same specimens for identification of possible hatchery origin (hatchery Pink Salmon in Alaska have thermally marked otoliths that were used to determine hatchery- or wild-origin). Every other tray of otolith samples collected from Stockdale and Hogan Creeks, and a subset of eight trays of otoliths collected from the other three fitness streams, were read for inclusion in statistical analyses, to produce estimates of hatchery fractions for each stream. The following hatchery fractions were determined: Paddy Creek (35%), Erb Creek (26%), Hogan Bay (69%), Gilmour Creek (25%), and Stockdale Creek (10%). These values are similar to those calculated for 2015, which was also a high run year for Pink Salmon. Results of the relative survival of PWS Pink Salmon hatchery-origin and wild-origin offspring will be available following final field sampling in 2018 and subsequent pedigree analyses completed in 2019.

## INTRODUCTION

Prince William Sound Science Center (PWSSC) has continued scientific data collection and analysis services requested this year under the State of Alaska contract CT 160001756 "... to conduct a research program to address interactions of Wild and Hatchery Pink and Chum Salmon in Prince William Sound and Southeast Alaska". This is the sixth annual report, focusing on results from 2017 data collection, as well as summarizing results from 2013 through 2017.

The overall plans and intentions of this contracted research are guided by two documents: 1) the ADF&G RFP 2013-1100-1020, dated May 7, 2012 entitled "Interactions of Wild and Hatchery Pink and Chum Salmon in Prince William Sound and Southeast Alaska and 2) the PWSSC proposal for the project, dated June 29, 2012. The overarching purposes of the multi-year research are to:

- Estimate the proportion of the annual runs of Pink Salmon and Chum Salmon in Prince William Sound (PWS) comprised of first-generation offspring of hatchery salmon.
- Determine the extent and annual variability in straying into natural streams of hatchery Pink Salmon in PWS and Chum Salmon in PWS and Southeast Alaska (SEAK), and
- Assess the impact on fitness (productivity) of wild Pink Salmon and Chum Salmon stocks due to straying of hatchery fish into natural streams.

The 2017 field research consisted of only one major activity: adult sampling of Pink Salmon in five PWS streams to collect tissues for DNA extraction and otolith samples for the ongoing fitness studies. The otolith sampling also allowed for estimation of the hatchery fractions of spawning salmon in the five streams. With such limited data, no credible estimates of hatchery fractions in spawning populations by district or in PWS as a whole were calculable for 2017, as they were for 2013 – 2015 (Knudsen et al. 2016).

The methods in this report reflect guidance in the RFP, some refinements made following the 2012 preliminary field season (Buckhorn et al. 2013), and the 2013-2015 full seasons (Knudsen et al. 2015a,b, and Knudsen et al. 2016), as well as changes made as a result of consultation with the Science Panel in November 2012, December 2013, December 2014, and April 2015. The 2017 field sampling protocol is presented in Appendix A. This report includes summaries of sample collection during 2017 for the DNA-based fitness studies. Otolith and DNA samples were shipped to the ADF&G Gene Conservation Lab (GCL) on October 18, 2017. Otolith samples were subsequently sent by ADF&G to the lab in Cordova for processing. Otolith sample results were used to generate hatchery fraction estimates for streams sampled in 2017. In addition, otolith results will be used in Pink Salmon fitness studies that will be reported following the final 2018 sampling season and subsequent pedigree analyses planned for completion in 2019.

# ADULT SAMPLING IN STREAMS

## Background

Based on the original RFP from ADF&G, there were two primary purposes for sampling adult Pink Salmon and/or Chum Salmon in streams through the course of these studies: 1) to further assess the degree and the range of inter-annual variability in hatchery straying rates; and 2) determine the effects of hatchery fish spawning with wild populations on the fitness of wild populations. The former objective was addressed by collecting otoliths from spawned out adults in 64 streams across PWS and SEAK between 2013 and 2015. The otoliths were examined in ADF&G laboratories to determine whether the individuals were of hatchery or wild origin. The now completed results are estimates of the percent of hatchery fish for each stream, district, and regional spawning population over three years (Knudsen et al. 2016). The latter objective is being addressed by collecting tissues for DNA analysis, as well as otoliths, from adults in a subset of the same streams, referred to here as “fitness” streams. The DNA “markers” of these parents can be used to identify either their pre-emergent offspring collected the following spring, or progeny returning to the streams as adults, so that relative reproductive success (fitness) of hatchery- and natural-origin fish can be estimated for both males and females.

## Methods

Tissues for DNA extraction (primarily heart and muscle) were collected along with otoliths from adult Pink Salmon in 2017 in PWS. (Samples taken from Chum Salmon in SEAK were managed by the Sitka Sound Science Center and are therefore reported elsewhere.) This required repeated sampling of the five fitness study streams in PWS (Figure 1). The 2017 methods were identical to the methods used in 2016 (Knudsen et al. 2015a, b, Knudsen et al. 2016, Gorman et al. 2017).

The RFP originally specified that fitness study streams have sampling targets of 500 individuals in high-stray-rate streams and 1,000 individuals in streams with lower stray rates. Subsequent discussions with ADF&G GCL staff and the Science Panel in late 2013, and again in December 2014 and April 2015, indicated the importance of exceeding the original sampling targets from these streams. Therefore, a strategy of maximizing the number of samples from fitness streams was implemented in 2015, and maintained in 2016 and 2017, to increase the statistical power of pedigree analyses through larger sample sizes.

### **Logistical Strategy**

From July 30 through September 20, 2017, three field crews sampled five of the original six fitness streams for adult Pink Salmon otoliths and DNA (Figure 1). The Science Panel dropped Spring Creek from the experimental design in 2016 and 2017 because of the extremely low hatchery fractions, particularly in even, low-run years such as 2016, which would have made fitness comparisons implausible in this stream.

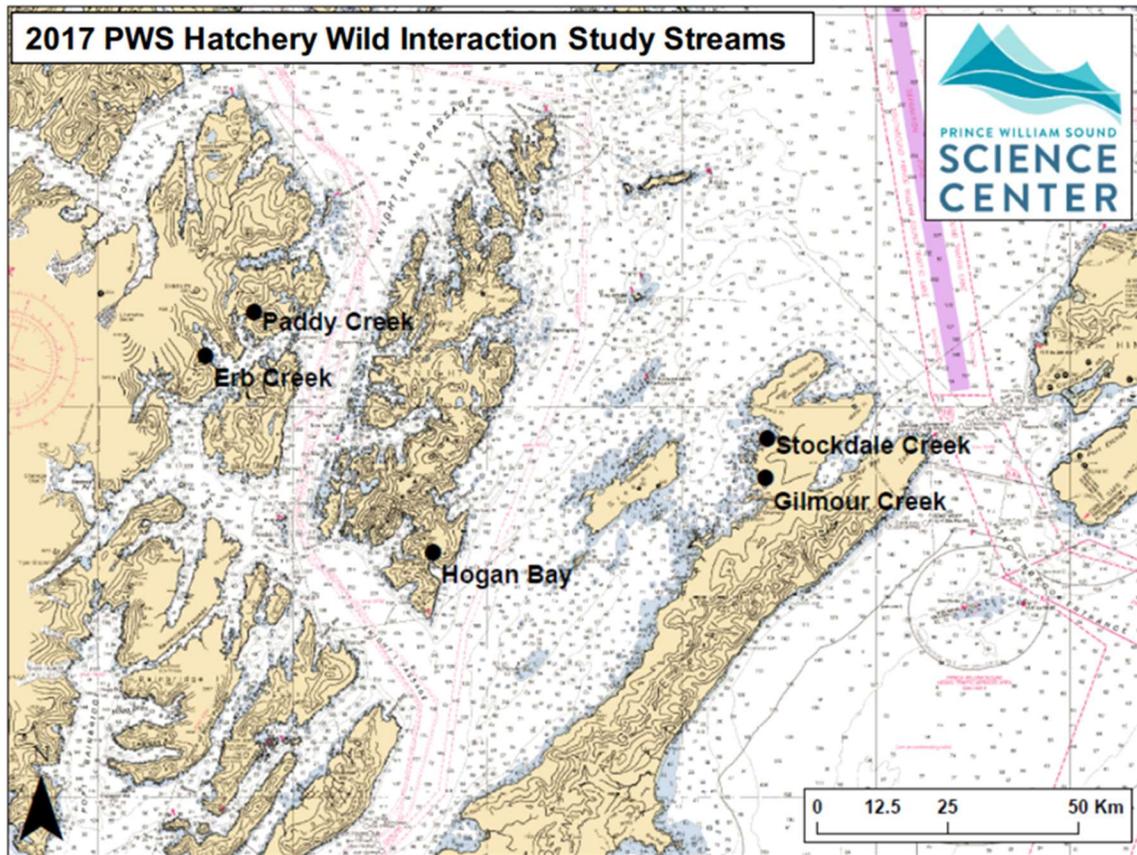


Figure 1. Streams sampled during 2017 for Pink Salmon otoliths and DNA tissues in PWS.

All PWS field crews were directly employed by PWSSC in 2017. Stream sampling is a dynamic process influenced by historic run timing, in-season fish abundance, weather, crew location, and distance between streams. Each field crew primarily worked independently and the 2017 crew leaders were astute in making decisions to maximize efficiency and achieve sampling goals. There were two live-aboard, vessel-based crews, the *M/V Auklet* and *F/V Alexandra*, that sampled Hogan, Gilmour, and Stockdale creeks, and a land-based, camping crew at Paddy Bay that sampled Erb and Paddy creeks. All crews trained in Cordova between July 24 and 28, 2017. Training included boating, bear and firearms safety, CPR and First Aid, protocol review, tablet use, and data entry. The *Auklet* and Paddy Bay field crews deployed the Saturday after training and the *Alexandra* crew deployed August 20, 2017 to sample at Hogan Bay during the peak run. Of note, half of the field crew in 2017 included returning crew leaders and technicians from previous hatchery-wild field seasons conducted in PWS.

For the first three weeks of the season, until the *F/V Alexandra* was deployed, the *M/V Auklet* crew was responsible for sampling Stockdale, Gilmour, and Hogan Bay fitness streams. Distributing sampling effort between streams was at times challenging due to weather, tides, and sample availability. However, the *M/V Auklet* crew made 40 successful stream visits to those three fitness streams before making their only port call

between August 21-23, 2017. While the *M/V Auklet* was in port, the Alexandra crew sampled Stockdale, Gilmour, and Hogan Bay Creeks. When the *M/V Auklet* returned, the *F/V Alexandra* crew remained at Hogan Bay Creek, sampling every day to capture the peak run, while the *M/V Auklet* crew sampled Stockdale and Gilmour every day or every other day for the peak run. The end of sampling was dictated mostly by charter vessel and field crew availability (see Discussion).

The PWSSC camping crew was stationed in Paddy Bay from July 29 to September 15, 2017. The Paddy crew was responsible for sampling at Erb Creek and Paddy Creek and made 74 stream visits to these creeks. To access study creeks, the Paddy crew used a rigid hull inflatable skiff to navigate to and from Erb Creek, and camped and hiked to Paddy Creek from the base camp at Paddy Bay. The Paddy crew sampled Paddy and Erb Creeks every day except during peak abundance when the crew maximized efficiency by intensively sampling each creek on alternate days. The *M/V Auklet* assisted Paddy camp set-up and take-down by deploying camp on July 29th and demobilizing camp on September 15, 2017.

### **Stream Sampling Methods and Execution**

Upon arriving at a study stream, crew leaders would indicate where to begin and how to focus on post-spawner and carcass collection depending on stream size and tide stage. Sampling began in either the upper stream reaches or lower intertidal zone, and crews worked together for speed, or leapfrogged in separate teams for efficiency. Crews were equipped with shotguns, first aid kits, and VHF radios and inReach satellite communication devices for safety. All efforts were made to sample and survey as much of the stream length as possible, accounting for factors such as carcass availability, incoming tide, deep water, strong current, impassable barriers, and bears.

After determining and marking the start location of a survey, all crew members began targeted species collection. Sample collection success at any given processing area depended on carcass abundance and sampling goals. After collecting a sufficient number of carcasses, the latitude and longitude of the processing area was marked on the tablet and the crew began sampling.

Carcasses were aligned in rows of eight by six, mimicking the 48 deep well plates (DWP). The popular cutting technique for accessing both heart DNA tissue and otoliths was to make two cuts. First, a horizontal cut dorsal to the eye was made to expose the brain cavity and otoliths. Second, a ventral cut was made perpendicular to, and slightly posterior of, the isthmus below the gill juncture. This cut exposed tissue of the bulbus arteriosus, a piece of which was removed for genetic analysis. Otoliths and tissue were placed in the DWP trays and stored in high concentration ethanol (See Appendices A and B for specific stream sampling protocols).

The last phase of stream sampling was to perform a fish survey to establish a rough index of fish abundance at the time of the sampling visit. When fish sampling was close to completion, two or more crew members conducted both a live and dead estimate of all

salmon species within the survey bounds. If multiple people were counting the same species, estimates were discussed at the end of the survey and averaged to produce a final count. When the survey was complete, a responsible crew member marked the end location of the survey, checked the count numbers, and made any additional comments.

### **Communication and Data Transmission**

All crews filed float plans with PWSSC and checked in daily with the stream PI on Delorme inReach devices, satellite phone, or personal cell phones. Satellite and cell phones were used when longer conversations were necessary. Each night, crews checked in, reported daily count and sample numbers, and backed up data on their laptop computer and to a secondary external drive. Data was transmitted to the host database daily, or as soon as internet service was available. Between the tablets, laptop computers, external drive backup, and regular data upload to the host database, the likelihood of data being lost was very low and no data was lost in 2017.

After completion of a final quality control review in Cordova at the end of the season, the fitness stream otoliths and tissues were shipped to the Anchorage ADF&G GCL on October 18, 2017, where tissues for DNA extraction were removed from the DWPs and otoliths were shipped back to Cordova ADF&G lab for processing. Upon shipment of DWPs to ADF&G GCL, all field data had been transmitted to the project database. Electronic data delivery to ADF&G followed the quality control review so that otolith and DNA results could be matched to the field observation data.

### **Specific Biological Sampling Methods**

Every effort was made to use consistent field methodologies throughout the data collection within and across years. Detailed methodological protocols were developed to guide the field data collection (Appendices A and B). The protocols were developed primarily from previous practices established within ADF&G, modified as necessary to facilitate the current study, and from experience during 2013 to 2016 field seasons. The protocols included specific methods for biological sampling including techniques for collecting post-spawned adult salmon, extracting otoliths, measuring lengths, determining sex, and collecting tissues for Pink Salmon DNA analysis in PWS fitness streams.

All field data were collected on-site using electronic tablets running an Android application developed specifically for collecting this project's data (developed under a subcontract to Finsight LLC, now Resource Data, Inc.). Guidance for the use of the field tablet application for data collection was integrated into the protocols. A more rigorous process of field and post-field quality control was implemented in 2014 and improved in 2015 and 2016. All otolith and DNA samples were checked for completeness and accuracy at the end of each sample tray row, before leaving a processing area, and at the end of the day. Data errors were immediately corrected in the tablet or on the laptop.

A project SQL database was also established in 2013 and updated for the 2014-2017 seasons by Resource Data, Inc. Field data was backed up nightly on laptop computers and then uploaded to the host database from the laptops whenever the crews had access to the internet. The survey data were imported nightly from the tablets to laptop computers where they were run through a series of quality assurance checks on a custom laptop

application. Corrections were made, if necessary, while the day's activities were easy to recall.

### **Hatchery Fraction Data Analysis**

As in 2013 - 2016, the objectives of the PWS field sampling in 2017 included estimates for the fractions of hatchery fish in each spawning population of Pink Salmon. However, unlike earlier years when hatchery fractions were estimated for over two dozen streams, only five streams were visited in 2017. Each sampled stream was visited repeatedly from late July through late September (Appendices C and D). The number of dead and live salmon of each species was usually counted in the stream during each visit, and otolith samples were taken from dead or moribund salmon during each visit. Otoliths were excised from each sampled salmon, and its origin (hatchery or wild) was determined in the ADF&G Cordova lab later after sampling had finished.

A single sample of  $m_{iv}$  salmon was quasi-randomly selected during each visit ( $v$ ) to a stream ( $i$ ). The estimated hatchery fraction for the  $i^{\text{th}}$  stream in 2017 is the weighted average of the estimates across visits:

$$\bar{q}_i = \sum_{v=1}^{V_i} w_{iv} q_{iv}, \text{ where } w_{iv} = \frac{C_{iv}}{\sum_{v'=1}^{V_i} C_{iv'}}, \text{ and } q_{iv} = \frac{\sum_{j=1}^{m_{iv}} y_{ijv}}{m_{iv}}, \text{ and} \quad (1a, 1b, 1c)$$

where  $v$  denotes a visit,  $V_i$  the number of visits to the  $i^{\text{th}}$  stream,  $C_{iv}$  the number of dead salmon counted during a visit, and  $y_{ijv}$  is the score ("1" if it's a hatchery fish, or a "0" if otherwise) of the  $ijv^{\text{th}}$  sampled fish. An approximation of the variance for the weighted average is:

$$\text{var}(\bar{q}_i) = \sum_{v=1}^{V_i} w_{iv}^2 \text{var}(q_{iv}), \text{ where } \text{var}(q_{iv}) = \frac{q_{iv}(1 - q_{iv})}{m_{iv} - 1} \quad (2a, 2b)$$

The calculations described above were first explicitly framed in Excel and then coded into R statistical software for repetitious analytical runs.

Because of limited funding, the Science Panel decided that every other tray of collected otoliths from Stockdale and Hogan Bay creeks would be read for 2017. For the other three streams (Erb, Paddy, and Gilmour creeks), eight trays of collected otoliths were read for estimating a hatchery fraction for each of those spawning grounds. Systematic sampling (number of trays divided by eight) was used to select the eight full otolith trays from those three streams.

## **RESULTS**

Final results comparing the relative survival of hatchery- and wild-origin offspring will be available after the last PWS Pink Salmon field season in 2018 and subsequent DNA tissue analyses are completed in 2019 (approximately 2024 for the SEAK Chum Salmon relative fitness analysis). The 2017 stream surveys, sample sizes, and previous years'

results for estimated hatchery fractions for the five PWS fitness streams are reported below.

### PWS Pink Salmon Fitness and Hatchery Fraction Sampling

Overall, 2017 sampling was successful at all five selected Pink Salmon PWS fitness study streams where 58,688 DNA tissues and pairs of otoliths were taken (Table 1). Rather than having a numerical sampling goal in 2017, it was decided that sampling would occur every day on Paddy and Erb Creeks and every other day for the other three streams. This strategy allowed the crews to collect the large number of samples necessary for analysis of relative survival (Table 1). The number of samples varied per stream visit (Appendix C). Foot survey-based live and dead counts were made on all stream surveys (Appendix C) and the dead counts were later used to weight the hatchery fraction estimates per visit. (See Appendix D for more details on the sampling of each PWS stream.)

Table 1. A stream-by-stream summary of sampling and hatchery fractions for five PWS Pink Salmon streams in 2017. Hatchery fractions for each stream were weighted by observed dead salmon counts taken during each visit. Note that the five streams sampled in 2017 were sampled because of relatively high fractions of hatchery fish found in previous years. Therefore, the fractions are not representative of the district in which they are located or of PWS as a whole.

| Stream name              | AWC code     | Stream visits 2017 | Samples collected 2017 | Samples used for hatchery fraction 2017 | Hatchery fraction weighted by dead counts per visit |       |       |       |       |
|--------------------------|--------------|--------------------|------------------------|---|---|-------|-------|-------|-------|
|                          |              |                    |                        |   | 2013  | 2014  | 2015  | 2016  | 2017  |
| Paddy C <sup>a</sup>     | 226-20-16010 | 40                 | 8,467                  | 285                                     | 0.154   | 0.595 | 0.328 | 0.206 | 0.352 |
| Erb C <sup>a</sup>       | 226-20-16040 | 34                 | 14,572                 | 366                                     | 0.113   | 0.228 | 0.214 | 0.100 | 0.263 |
| Hogan Bay <sup>a</sup>   | 226-30-16810 | 45                 | 18,836                 | 9,152                                   | 0.640   | 0.915 | 0.583 | 0.205 | 0.687 |
| Gilmour C <sup>a,b</sup> | 227-20-17480 | 23                 | 5,247                  | 352                                     | NA  | 0.557 | 0.225 | 0.116 | 0.250 |
| Stockdale C <sup>a</sup> | 227-20-17520 | 37                 | 11,566                 | 5,585                                   | 0.163   | 0.735 | 0.240 | 0.078 | 0.095 |

<sup>a</sup>Otolith collections from all five creeks were subsampled - see Methods.

<sup>b</sup>Gilmour Creek was not sampled in 2013 to estimate the hatchery fraction of its spawning Pink Salmon population.

## DISCUSSION

In comparison with previous years, sampling in 2017 (58,688) exceeded the total samples collected for these same five streams in previous years:

2013: 2,789 (note Gilmour was not sampled in 2013)

2014: 7,939  
2015: 46,386  
2016: 37,549.

Fewer samples in 2013 and 2014 were primarily due to a reduced sampling effort in those two years.

In general, the 2017 field season was extremely rainy, and this proved challenging for field crews. Despite the less than optimal field conditions, crews were successful in sampling and maintained good attitudes throughout the field season. Charter vessel captains and crew were experienced and worked well with field crews this season. Bears proved to be a greater presence this year than in previous field seasons. In many cases, tissue samples were collected from carcasses that were depredated and missing otoliths. However, these samples are still important as they could represent offspring from previously sampled parents with known hatchery or wild otolith designations.

The intention was to sample the entire spawning run in each of the five streams. We were very successful at sampling the beginning of each run as indicated by the live counts and sample numbers in Appendix C. By adding the *MV Alexandra* crew just prior to the run peak, we were able to increase the sampling intensity at the peak in Hogan, Gilmour and Stockdale creeks. Although the runs were not entirely completed in mid-September, the *Auklet* had to return to Cordova by September 16, thus the field season ended with anywhere from several hundred to over 2,000 (Erb) live fish remaining in the creeks (Appendix C). The *MV Alexandra* crew continued to sample Hogan Creek through September 20, but there were still over 4,000 live fish in Hogan on the last day of sampling. Thus, the end of the season was mainly driven by charter vessel logistics and field crew availability.

Interestingly, hatchery fractions calculated for 2017 were very similar to those calculated during 2015, the previous high run year for Pink Salmon in PWS. Stockdale Creek, however, had a much lower hatchery fraction calculated for 2017 than 2015. Overall, hatchery fractions are generally consistent among high run years for Pink Salmon in these select streams in PWS (2013, 2015, and 2017).

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## APPENDIX A. STREAM SURVEYS AND ADULT SALMON COLLECTION

This protocol will be used to prepare for and conduct surveys of streams for the hatchery-wild interactions study in Prince William Sound (PWS) in 2017. The purpose of the study is to collect salmon otolith and DNA samples, and data on sex, fish length, fish height, and morbidity state, as a basis for estimating the fraction of hatchery-origin salmon and to study the relative survival (fitness) of hatchery- and wild-origin Pink Salmon in five PWS streams. Table A1 designates the streams and species to be sampled and the types of sampling at each stream in 2017. Details about collecting measurements and biological samples from each specimen are described in the next protocol (Appendix B). This protocol is about collecting fish from streams.

### Targeted Number of Fish to Sample

The analytical goal for otolith and DNA tissue samples from each fitness stream is **the highest proportion of population (maximum number of samples) possible**. Two of the fitness study streams will be sampled every day (Paddy, Erb). The three other streams may be sampled every other day (Table A1) although every day is preferable (Gilmour, Stockdale and Hogan Bay Creeks). These latter streams will be sampled every day for 2 weeks during the peak runs in late August. Sampling will thus be maximized and distributed evenly to best represent the run.

The samples collected at each stream will be representative of all individuals within that population. Therefore, the samples should be spread as evenly as possible throughout the stream length, at least within a few-day period. It is acceptable to work on one section of a stream on one day if the other sections of the stream are covered on subsequent days, keeping sampling effort proportional to the relative carcass and spawn-out abundance along the stream course.

**Table A1. Streams and species to be sampled in PWS in 2017  
(see also the “Tote Board” on Google Drive).**

| Stream name | Stream code  | Target species | Fitness stream | Sampling events per season |
|-------------|--------------|----------------|----------------|----------------------------|
| Erb C       | 226-20-16040 | DNA pink       | Yes            | Daily                      |
| Paddy C     | 226-20-16010 | DNA pink       | Yes            | Daily                      |
| Hogan Bay   | 226-30-16810 | DNA pink       | Yes            | Every other day            |
| Gilmour C   | 227-20-17480 | DNA pink       | Yes            | Every other day            |
| Stockdale C | 227-20-17520 | DNA pink       | Yes            | Every other day            |

### Tote Board

Individual sampling goals for each visit to a stream, and a method for tracking progress toward the goals, is established in a spreadsheet in Google Drive. Crew leaders with access to the Google Drive should update the tote board each day. If there is no access to

the internet, crew leaders should text their final sample numbers daily to the coordinator who will update the tote board.

## **Preparations for Surveys**

Preliminary preparations for your survey (usually the night before) at base camp or on live-aboard boats are as follows.

### *√ Charge all tablets and scanners fully*

The tablets are key to data collection and organization. One tablet may not last all day for sampling, so having and keeping a full charge is essential to success. Charge tablets preferably on 110 ac – they will charge quicker. (See separate instructions for scanner set-up as needed.)

- \* To conserve tablet battery life,
  - Turn wi-fi off in the field, put into airplane mode, and turn sound off.
  - Turn power all the way off when not using for more than a few minutes.
  - Set the display time-out to 1 minute or less.
  - Change the display to automatic rather than keeping it on high all the time.
  - Do not use tablet after charging until in the field (or recharge).
  - Do not leave the tablet connected to a computer or battery pack when the source unit is off – the tablet may discharge.

### *√ Clean and inspect guns and other safety gear*

Be sure shotguns are unloaded and clean guns according to standard methods. Unless in the field, guns should be kept unloaded. Be sure all safety gear is ready and operable.

### *√ Dry and patch waders*

### *√ Prepare for the next sample visit*

Crew leader will consult tide graph to determine the sampling survey strategy. Crew leader assigns tasks to personnel accordingly (see stream surveys below). Make every effort to visit each stream during a time that encompasses one low tide and plan your survey so that the low intertidal is included in sampling.

### *√ Prepare sampling kits*

Select the trays and labels that will be needed for the next survey and be sure they are ready for field deployment (take extras). Be sure you have all the necessary tools, back-up data sheets, and safety gear ready (see next protocol).

## **Stream Surveys**

A stream survey is defined as a set of Processing Areas on one stream and one date, with a beginning and ending point along the stream. A survey includes information about a day's activity on a stream such as demarcating the beginning and end locations of the

survey. A careful count of the total number of observed alive and dead target species in the survey area also will be made for each survey, with a few exceptions noted below. The survey can be conducted as the crew moves either upstream or downstream.

### *Arriving at the Study Stream*

Work with the skipper of your landing craft to deploy as close as possible to the starting location of your day's work. Know your estimated pick-up time and location if the landing craft is not staying on shore. Establish radio contact. Ascertain bear safety. Know who is carrying your first aid kit and sampling permit(s). You may need to hike to the start of the stream survey.

Depending on the situation, the Crew Leader designates tasks among personnel according to real-time needs, which will vary by reach and stream. This is to expedite sampling while ensuring safety.

### *Starting a Survey*

The starting point of the survey will depend on the tide stage and will be determined by the Crew Leader. It is important to include the lowest point of the stream at the day's low tide because carcasses often accumulate there.

If the tide is low and will be incoming while at the stream, start at the lowest point and work upstream. Designate one or two team members to count salmon, live and dead, as you progress upstream. Conduct specimen sampling as you go (see Specimen protocol below).

If the tide is high or medium upon arrival but dropping, then start the survey upstream and begin your survey heading downstream. Count live/dead fish as you progress upstream, but sample specimens as you move back downstream.

If the stream is forked or braided, or if there are two study streams in very close proximity, you may break into two teams but only if there are at least two people on each team and both teams have a gun. At no time should anyone work alone.

Treat a tributary or separate stream as a separate survey. Create a new survey in the app and select the appropriate stream name. Then log beginning and ending survey points for the tributary or separate stream.

The survey Begin Location and End Location entered into the tablet are meant to describe the linear extent of your daily survey on each stream. The Begin Location or End Location can be oriented either downstream to upstream or vice versa, as long as, between the two, they described the entire survey extent.

Depending on the number of fish that can be sampled, decide whether to split into two teams or all work together. You may use one or two tablets. **(If you use two tablets, add**

**the live/dead counts to only one tablet, unless you are working on separate tributaries under separate surveys.)**

Designate one tablet as the primary survey tablet. If that tablet is lost, malfunctions, or the battery is close to dying, continue with another tablet. Re-establish the survey in the second tablet. There is no need to enter the same data previously entered on the previously used tablet (except the live/dead counts – see below).

**A special case occurs when two separate surveys are independently conducted on the same day and single stream (not separate tributary).** An example is when one team starts surveying at the upper end, the other from the lower end, and they meet in the middle. Using two tablets, establish separate surveys each with their own locations, processing areas, specimen data, etc. However, before uploading both surveys into the laptop app be sure 1) to number the processing areas differently between the two surveys, 2) combine the live/dead counts from both surveys into the final totals, either in one tablet, or when you are editing the surveys in the laptop app, and 3) make sure that the overall begin/end points for the total survey (uppermost and lowermost point surveyed on the stream that day) are either in one tablet, or entered when you are editing the surveys in the laptop app.

#### Data Entry

At the beginning point of the survey,

- Turn on the tablet and the HW App
- Hit the Stream Survey tab in the HW app
- Select ADD SURVEY
- Tap the bar under Stream Code and select the stream from the drop-down list.
- Tap Survey Date bar to set the date. A calendar will pop up with today's date highlighted. Be sure it is correct. You can change it if necessary by touching the calendar. Finish by selecting "Set".
- Add comments – anything about the survey such as weather conditions, stream water levels and flows, or especially anything unusual about the survey. You should come back to this screen anytime you note something that should be reported for this survey.
- Tap Survey Samplers. Tap and enter First Name and Last Name (consistently from tablet to tablet) and then tap Add Person for anyone not on the list. Check the boxes of all the people who are conducting this survey and hit Save Samplers. Close with the tablet back button.
- When you are at the physical beginning point of the survey, select Survey Locations. Wait for the GPS accuracy number to go down and the number of satellites to go up, then tap in the Begin Location box and tap Capture Location. Let the App average lat/long for about a minute and then hit Finish (The lat/long can also be manually entered from a different GPS unit if necessary).

## Counting Salmon

Either before, or while progressing through a survey, usually moving upstream, count the number of live and dead salmon. In PWS, count Pink, Chum, Chinook, Coho, and Sockeye salmon separately. It will be preferable to have one person count all live and another person count dead of each species. To avoid over estimating dead counts, count whole carcasses and heads, not tails or bodies without heads. Allow technicians counting to do so before the fish are disturbed due to fish collection for sampling. In many lighting conditions, it will help to wear polarized glasses. **Include all dead fish from previous sampling in your dead count if the carcasses are still visible on the survey.**

Use hand tally-whackers while surveying to keep count of fish, although at times you may be counting by tens, hundreds, or even thousands, so you need to be aware of integrating the counting units. Use the counting accumulator on the tablet to enter counts at natural stopping points, or if you stop to process fish, to get a grand tally of the counts. (If the primary tablet is expected to quit due to low battery, try to transfer the live/dead count into the replacement tablet.) The live/dead counts will be used as a component of the statistical analysis for this research. Therefore, every effort should be made that these counts are good quality. If conditions do not allow for a complete count, do the best you can. Then enter the codes for survey quality into the live/dead count page in the tablet.

The count pertains to the stretch of stream between the Begin Survey and End Survey locations, so don't be too concerned about getting counts from exactly the same stretch of stream every time – they will be adjusted for the stream length surveyed.

On fitness streams that are visited every day, try to count fish every day but, if you are unable to sample all the available fish for otoliths and DNA, you may skip counting live/dead salmon for one or two days, but do a complete count of the entire stream at least every third day. If you are visiting a fitness stream every-other-day and the fish are too abundant to sample all, you may skip live/dead counts every other visit (i.e., do a complete count every fourth day). This is because getting more otolith and DNA samples is a higher priority.

### Data Entry

To accumulate salmon counts:

- From the Survey page, hit the Live/Dead Counts bar
- Select Species to Count for your target species and hit Add
- In the species box, enter the number in each category and hit Save on the top bar
- To add counts to the accumulator, select Add Live/Dead Counts from the drop-down menu. Select the species you want to add counts for and enter the additional numbers to the species dialogue box and hit Save (notice that the accumulator adds these numbers to the tally).
- To add more species to the list, select Add Species to Count from the drop down menu.
- Check the “No Alive Count” or “No Dead Count” checkboxes ONLY if you did not count fish. If the count was zero, enter 0 in the count spaces.

### *Processing Areas*

Processing Areas are defined as locations on a stream during a survey where a set of specimens are gathered, measured, and sampled. Details on Processing Areas and associated data entry are found in Appendix B.

### *Fish Collection*

Fish to be sampled can be collected by any practical means. **Only collect spawned out, dying, or dead adults.** Use gaffs, spears, snagging gear (rod and reel with treble hooks), dip nets, baskets, or hands to gather fish. Use a beach seine if practical (only if a school is observed to contain spawn-outs) but select only the spawn-outs and release the rest. Spawn-outs will usually have flatter, flaccid abdomens than pre-spawning or spawning fish. If eggs or milt flow very freely from a gently squeezed individual, it should be released to continue spawning. Some spawned out females will have a few remaining eggs spurt out when squeezed – if many eggs come out of a live fish, release the fish. Spawning males have pure white milt - if squeezing a male produces watery or no milt, sample it, otherwise release it. Try to avoid catching and squeezing the same individuals repeatedly. Eventually they will appear to be post spawned even though they aren't. Sample all dead fish, regardless of their remaining egg or milt content.

If a fish's gonads have been removed by predators, sample the fish. Gonad-predated individuals will have a small hole in the abdomen and the gonads will be entirely missing. Check the "Preyed Upon" box on the tablet as appropriate. Also in fitness streams, if the fish is sampled (dead or alive) with more than a little eggs or milt, check the "Partial Spawner" box on the tablet. If the eggs and milt do not easily release and carcass has completely full gonads check the "Pre-Spawner" box.

### *Completing the Survey*

There are two steps to completing a survey. One occurs at the physical end point of a survey. Take the "End Location" GPS data. Please also always make general comments about the survey such as weather conditions, stream water levels and flow, or especially anything unusual about the survey.

The other step occurs after all samples have been collected (but before leaving the stream). Review all the data in the tablet for that survey, including all the Processing Area and Specimen data for every sampling tray, to make sure it all makes sense and is complete (more detail on this in the next protocol).

### Data Entry

To finish a survey:

- Hit “Finish Survey” on the top menu bar or go to the Surveys page and re-select the current survey.
- Select Survey locations
- Tap on the End Location box, wait for the GPS accuracy number to go down and the number of satellites to go up, then hit Capture Location, wait for a minute, and hit Finish.
- Add final, detailed comments about the survey.

### **Post-Survey Data Management**

Immediately upon return to your base camp or live-aboard boat, take the following steps to process data while the entire crew remembers the events of the day. It is critically important to complete the following list immediately to ensure that the data are of highest quality and any errors that occurred during the day are addressed immediately.

√ *Enter data if necessary*

Enter any data that was collected on paper that day into a tablet for the appropriate survey (or you may enter it in the QA procedure on the laptop, as described below).

√ *Back up all data collected to date onto laptop*

- 1) Every evening after every survey, regardless of whether you have connection to the internet, connect the tablet to the laptop. Start the tablet and open the tablet HW app. Tap on Export Surveys to create a file ready to move the file to the laptop from the tablet.
- 2) Using Windows Explorer on the computer, go to the tablet’s directory and find the folder called “Exports”. Copy that text file to the subdirectory “C:\HW\Exports from tablets”.
- 3) Rename each day’s text file by adding the date to the end of the name, such as “exportedSurveys 7-21-15”.
- 4) Then insert a thumb drive and copy the text file on the laptop directory to back it up on the thumb drive.
- 5) Open the HW application on the laptop. Use the “Import Surveys” to import data from the text file to the SQL database on the laptop. This step is to again back up the data into the SQL database on the laptop and preserve the data from loss.

### √ *QA Procedure*

1) Review the data on the tabs called Stream Surveys, Processing Areas, and Stream Specimens, to be sure it is correct. This is your chance to edit the data if there are any errors. Expand on the Stream Survey Comments, the Processing Area descriptions, or the comments for individual fish as much as necessary.

2) On the Stream Surveys tab, open and run the QA process tray-by-tray, for all trays from each survey. Carefully open each tray, and look in all the cells to be sure the samples match the data. Enter the information requested in each row of the QA dialogue box, and be sure the first and last cells in the tray match the data, and that all missing otoliths and DNA samples match between the tray and the data. Continue with that process until all the errors and warnings are resolved.

IMPORTANT -- If you discover errors in the DNA tray cells that cannot be easily resolved, please keep the samples but flag the data from the first questionable cell forward by adding the comment “UNCERTAIN PAIRING” plus any other potentially helpful details in each data row for potentially erroneous data. If you find more than two otoliths in a cell, do not guess which one is in the wrong cell – make comments as described above for every uncertain cell (data row).

3) After inspecting DNA trays, fill every cell with ethanol to 3/4 cell volume, replace the lids, and wrap the tray tightly with plastic wrap. It is critical that all DNA tray wells be filled  $\frac{3}{4}$  with ethanol and that the entire tray is wrapped tightly with saran wrap. The ethanol WILL EVAPORATE if this is not done properly, which will compromise the integrity of the sample for DNA extraction. Store the DNA trays in a secure location for safe transport.

4) Once a QA is successfully finished, note that it is locked. If you later discover that edits are needed, use the Unlock Survey button, then make the changes and redo the QA process.

### √ *Upload data to database*

As soon as the internet is available, use the “Transmit Stream Surveys” tab in HW laptop app to upload the most recent survey data from the laptop to the host database. Enter the names of all the people who participated in a survey if/when prompted. Check the boxes of the surveys you want to transmit and hit the Transmit Surveys button. There is usually no harm in retransmitting previous surveys, but only retransmit if you have modified a previous survey for some reason (which should only be done if you realize there is a mistake in a previous survey or its associated data). Usually it will be preferable to make your corrections in the HW Laptop App.

√ *Update the tote board*

Also whenever internet is available, access the tote board on Google Drive, update it with the date of the stream visit, number of samples obtained, and the number of live and dead of the target species observed during the day's surveys. If there is no internet available, call, text, or in-reach your project coordinator to report the daily survey numbers. Then use the tote board to help plan the next day's activities and sampling goals.

## **APPENDIX B. PROTOCOLS FOR COLLECTING, PROCESSING, AND SHIPPING BIOLOGICAL SAMPLES FROM INDIVIDUAL SALMON**

The purpose of this protocol is to describe the background and methods for sexing and measuring fish and for collecting, processing, and shipping otolith and DNA tissue samples from streams in the Hatchery-Wild Interactions Study. See the Appendix A for protocols on which streams and how to collect fish.

### **Background**

Otoliths and DNA tissues will be collected from “fitness” streams to reconnect offspring, subsequently collected as fry or as returning adults, to their parents so that relative reproductive success of hatchery- and natural-origin fish can be estimated for both males and females.

### **Otoliths**

Thermal marking is one of the methods being utilized to identify and manage hatchery-released Pink Salmon. When the embryonic fish are incubating in hatcheries, the water temperature is raised and lowered according to a pre-determined schedule that results in a predictable sequence of visibly enhanced growth increments or “thermal rings” on the fishes’ otolith bones. We will collect the sagittal otoliths and send them first to ADF&G’s Gene Conservation Lab (GCL) in Anchorage where heart tissue and otolith samples will be further processed. Otoliths will be sent by ADF&G’s GCL to the Cordova ADF&G lab for processing. In the lab, the left sagittal otolith is glued to a glass slide and then ground down on fine grit sandpaper. When the center of the otolith is reached, it is examined under a microscope for the presence of a thermal mark. Fish with no thermal mark are then designated as wild, while fish having a thermal mark can be identified as to their hatchery release group.

### ***DNA Tissues***

The ADF&G GCL will use bulbus arteriosus tissue samples (described below) that we collect from dead or moribund fish for genotyping. These genotypes will allow for the reconstruction of individual pedigrees (family trees) that, when paired together with identification as hatchery or wild-origin fish from otoliths, will provide the basis for comparing the relative survivals of stream-spawned natural, hatchery, or natural x hatchery crosses.

### **Preparing for Specimen Sampling**

#### ***Tray Preparation***

Prepare the sampling trays in advance of field work. Adhere labels to the trays and always try to use tray numbers in sequence. In “fitness” streams, use 48 deep well plates

(dwp) or trays. When using 48dwp trays, always be sure to add the otoliths and the DNA tissue from each fish to same tray well (the otoliths will be separated out of the 48dwp trays into new trays at the GCL lab).

*Preparing DNA/Otolith 48dwp Trays*

Trays for DNA sampling in fitness streams (Figure B1) hold only 48 samples and they will be used to collect and store both the otoliths and the DNA tissue from the same fish in each well.



**Figure B1.** An empty 48dwp tray for DNA tissue and otoliths (cover off).

Pre-printed barcode labels for each 48dwp have been provided by GCL. Keep label sheets packed in a ZipLoc bag to keep them dry prior to application. Labeling 48dwp trays should be done beforehand in a dry area to insure the label adheres. Apply label on notched side (picture below) of each plate. Barcode label should be facing the sampler as you take samples. After adhering labels, cover the barcode in clear packing tape to protect it from wear.



Before processing samples on a given day, it will be important for genetic tray bar code labels to be covered with clear packing tape before leaving the boat or camp. Blank fields on the labels should be filled out using pencil (No. 2 soft) as you use the trays. Record the

following information: Stream Identifier: \_\_\_\_\_ (Stream Name and AWC Number),  
Date: \_mm/dd/yy (see photo of label below).

Each plate will hold 48 samples. By the end of each day, add clear packing tape to the written portion of the label to insure the label information is retained even if ethanol leaks out. Further, during very rainy conditions, having tape on the label will help preserve the label information (water or ethanol will gradually dissolve the label). Please avoid pre-labeling and taping trays that will NOT be used in a given day as to avoid incorrect information being used the following day.



### Field Equipment for Sampling Otoliths and DNA

Pack the following items for field sampling otoliths and DNA:

|   |                               |
|---|-------------------------------|
| 1) Butcher knives with deep 6-8" blade        | 10) Pencils - No.2 soft       |
| 2) Forceps (fine point)                       | 11) Cutting boards            |
| 3) Cotton and vinyl gloves                    | 12) Knee pads                 |
| 4) 48dwp trays with labels; barcode taped     | 13) Bright yellow glass beads |
| 5) 48dwp tray cover mats                      | 14) Tray holders              |
| 6) Carrying case for trays - spill prevention | 16) Neoprene cuffs            |
| 7) Calipers and Measuring tape                | 17) Tablets                   |
| 8) EtOH in large nalgene bottles              |                               |
| 9) Nalgene squirt bottles for EtOH            |                               |

### Processing Specimens

#### *Processing Areas*

Collect spawn-outs or carcasses from the study stream (as described in Appendix A) into an area where you can work on cutting the heads and extracting otoliths and DNA.

Processing Areas are defined as locations on a stream during a survey where a set of specimens are gathered, measured, and sampled. Every time you stop to process fish, create a new Processing Area. Limit the length of a given Processing Area stream reach to approximately 200 meters, or preferably less, and try to process your samples approximately in the middle of the <200m stream reach (this is because we want to track approximately which portion of the surveyed stream the adults were collected from). Each Processing Area will be uniquely identified within a survey.

### Data Entry

To establish a Processing Area:

- On the main menu, tap Processing Areas
- Select “Add Processing Area” from the top menu
- Enter a sequential number for the Processing Area ID, starting with 1 (the PA ID numbers do not need to be spatially sequential on the stream)
- Add a written description of the Processing Area
- Tap on the Processing Area Location box. Wait for the GPS accuracy number to go down and the number of satellites to go up, then hit “Capture Location”

### *Processing Samples*

Line fish up in rows of 8 for collecting otoliths and DNA, it helps to leave extra space between rows that are the end row and beginning row of two trays. The line-up helps to keep track of which fish has been sampled for each required attribute or specimen sample and parallels the filling order of the 48dwp trays.

At each Processing Area, **work through collecting specimen information in the following order: sex, length, height, morbidity condition, DNA tissues, and otoliths.** You can enter data into the tablet for more than one row at a time prior to processing these fish, but you must double check each row after the otoliths/DNA are collected, before moving on to the next row. **After you have collected the required samples from a fish, leave the fish in place within its row until done with the processing area.** This allows for a final physical comparison of the data in the tablet with the actual fish. There are several types of errors that can be resolved if the fish are still lying in place on the gravel bar, in the exact order that they were in when the data was entered into the tablet. Once you have finished working up all your fish for the Processing Area, spread the fish carcasses into the stream, move to next Processing Area and create a new Processing Area in HW App.

All data recorded for specimens is associated with the tray where the specimens are deposited. Before you can start recording data about specimens, you must set up a tray in the HW tablet application. Use a portable barcode scanner to enter the full tray number into the tablet. If for some reason your scanner is inoperable, you may type the full number into the HW app. Have one person read the number, while another types, and then read it back to verify. (See separate instructions for scanner set-up if necessary.)

### Data Entry

To establish a specimen tray:

- From the Processing Area page, Tap the Sampling Trays bar
- On the Sample Trays page, hit ADD SAMPLE TRAY in the upper right corner
- Scan the tray barcode ID or type the tray ID number and
- If necessary, select the species and/or the tray type and hit DONE

### ***Sex, Length, and Height Measurements***

Collect sex, height, length, and morbidity state before dissecting otoliths and DNA.

Male Pink Salmon are usually identifiable by their kype (a secondary characteristic of extension of jaw and enlargement of teeth) and they usually have a noticeable hump in their dorsal outline. Sometimes the sex is not obvious, if in doubt; squeeze the area just anterior of the vent to see evidence of milt or eggs. If still in doubt about a fish's sex, cut the abdomen to look for evidence of eggs or testes.

Measure the mid-eye to hypural (MEH) length of each fish (in mm) using calipers. For this measurement, place one end of caliper at the middle of the eye and take the measurement at the end of the hypural bone. If uncertain where the hypural bone is, use your thumb to flex the caudal fin laterally – it will flex at the end of the hypural bone. Once you learn where the hypural bone is, you will be able to feel it with your thumb. When measuring with a tape, stretch the tape taut. If a carcass is emaciated or mostly just skeleton, align the carcass in its normal form before measuring. If any portion of the skeleton between the head and tail is missing, do not measure length or height (but still sample for otoliths and DNA).

Measure and record body height unless there is damage or advanced deterioration that would compromise the measurement. Measure a specimen body height with calipers from the anterior insertion of the dorsal fin to the anterior insertion of the pelvic fin.

If for some reason you do not have calipers for measuring length and height, please use a measuring tape, but be sure to note in the survey or individual fish comments that you used a measuring tape.

Examine the fish or its gills to determine “morbidity” state and record in the tablet Card View: The morbidity categories are alive (at the time of capture), pink gills, grey gills, or rotten gills.

### Data Entry

To record sex, length, and body height:

- On the Sample Trays page, tap the new tray. This will bring you to the tray list view (notice the cell numbers are pre-entered)
- Tap on the first cell to open the card view for the first specimen.
- Be sure that the Species, Processing Area ID, Tray #, and Cell # are correct
- Select the sex of the first fish – if unknown, leave “Unknown” checked
- Enter the MEH length in mm for the first fish – if unable to measure full length, check “No Length”
- Enter the body height in mm for the first fish
- Enter the morbidity state
- To enter data for the next fish, swipe to the left, and continue for the first 8 or 12 fish (depending on the tray type)
- Add comments about missing otoliths and/or DNA issues in the Card View Comments
- You can return to the SPECIMEN LIST (upper right) at any time to be sure you are tracking the correct fish in the row and view all the data you have collected thus far.

### *Cutting Heads for Sampling DNA and/or Otoliths*

There are several possible methods for cutting salmon heads to retrieve otoliths. The method described below works well for sampling both DNA tissue and otoliths.

Place the fish on a cutting board so that you can safely cut the head completely off just posterior to the posterior edge of the operculum (Figure B2). This will expose the heart with the bulbus arteriosus attached (Figure B3). See description below for further instructions on sampling DNA tissue.



**Figure B2. Cut head off.**



**Figure B3. Bulbus arteriosus attached to heart.**

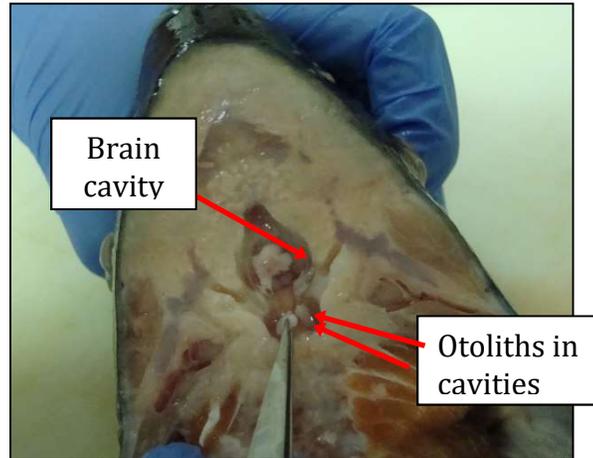
Place the cut head on the cutting board and slice the top off (Figure B4). This should reveal the brain cavity. Remove the brain tissue (Figure B5). The otoliths will be in two wells just posterior of the brain cavity (Figure B6).



**Figure B4. Slice the top of head off.**



**Figure B5. Remove brain tissue.**



**Figure B6. Otoliths in two lateral wells posterior to brain cavity.**

### ***Using 48dwp Trays for DNA Tissue and Otoliths***

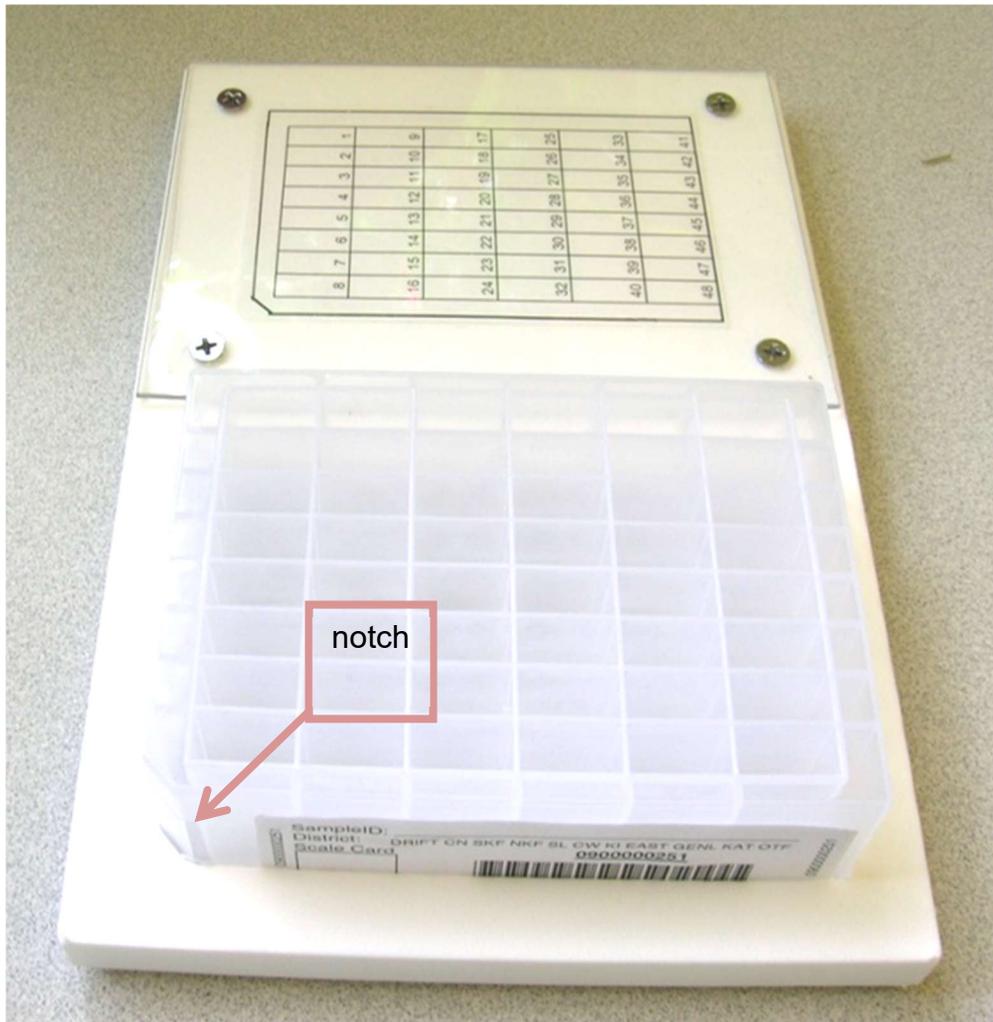
It is important to try to collect the best **quality DNA tissue samples to ensure reliable results**. Tissue quality degrades after death, so moribund and recently dead fish will yield best results. Frequent visits to streams will provide better opportunity to sample fresher spawn-outs. However, sample any previously un-sampled fish you can find, regardless of state. Be sure to record the morbidity state in the tablet. Tissue samples must be immersed in ethanol (EtOH) immediately upon sampling. At a processing area, fill wells  $\frac{1}{4}$  to  $\frac{1}{2}$  full of EtOH from the squirt bottle before you deposit the tissue and otoliths into the tray cells. Please be sure to fill each well at least  $\frac{3}{4}$  full of ethanol during QA once you get back to base camp or live-aboard vessels.

Use one (or more) 48dwp trays per stream, date, and species for both the DNA tissue and the otoliths (Figure B1). **Never place otoliths and DNA tissue from more than one**

**stream or sampling date into the same 48dwp!** Even if you have a 48dwp containing only ONE otolith/DNA tissue sample for a stream visit, send it in that way.

Steps for sampling fitness stream otoliths and DNA tissues into **48dwp** are:

a. Set the pre-labeled 48dwp into the tray holder so that the notched edge of the tray aligns with the holder. This will guide you to the correct numbering of the individual wells and the order of sample placement. Be sure the label is filled out and facing the sampler as you sample (Figure B7). Fill wells  $\frac{1}{4}$  to  $\frac{1}{2}$  full of EtOH, this helps transfer tissues and otoliths from forceps to the well.



**Set-up for sampling:**

1. Place 48dwp in sampling guide; barcode label should be facing you as you sample. Use numbered well sample grid for referencing well position and for sampling direction. **DO NOT DEVIATE** from individual well filling pattern.

**Figure B7. Proper set-up of 48dwp in sampling tray holder.**

b. Deposit DNA tissue and otoliths samples into 48dwp one column (vertical) at a time, top to bottom, starting with the left-most column. After each column is filled, move right and start at the top of the next column (see numbered sample grid below). Use rubber mat to cover sampled and un-sampled (empty) columns to protect the samples and to guide yourself to the correct column.

|   |    |    |    |    |    |
|---|----|----|----|----|----|
| 1 | 9  | 17 | 25 | 33 | 41 |
| 2 | 10 | 18 | 26 | 34 | 42 |
| 3 | 11 | 19 | 27 | 35 | 43 |
| 4 | 12 | 20 | 28 | 36 | 44 |
| 5 | 13 | 21 | 29 | 37 | 45 |
| 6 | 14 | 22 | 30 | 38 | 46 |
| 7 | 15 | 23 | 31 | 39 | 47 |
| 8 | 16 | 24 | 32 | 40 | 48 |

c. For each fish, cut about ¼-inch piece of the bulbus arteriosus from a fish (see picture) and place it in the appropriate well within the 48dwp (Figure B3).

d. If the bulbus arteriosus is lost or unusable, use other heart tissue of approximately the same size for the DNA sample. If the heart is missing or lost, sample other tissue of approximately the same size in the following order: other heart tissue, muscle, liver, or fin (avoid fungus). Check “Alternate DNA tissue” in the HW App. If there is no DNA tissue sample, skip putting tissue in that well and Check DNA tissue missing. Make other comments about the fish accordingly.

e. Be sure to record the “morbidity” state in the tablet: alive, pink gills, grey gills, rotten.

f. Wipe or rinse knife blade and/or tweezers between fish to reduce cross contamination among DNA samples.

g. **FROM SAME FISH**, excise both otoliths and deposit the otoliths into the well that corresponds to the fish number. Put **two** otoliths in **same** well as bulbus arteriosus. Note that otoliths can be collected from even the most deteriorated fish – the carcasses don’t have to be fresh.

With tweezers, pluck the left otolith from its well (Figure B6). The otoliths are about ¼ inch long in Pink Salmon. Place the otolith onto the back of your hand or neoprene cuff and then recover the right otolith from the right well. It may take some probing, especially if the cut is not just right, or the carcass is old.

The otoliths are encased in a tissue bag surrounded by fluid. Gently rub or tweeze the tissue off the otoliths (Figure B8), clean off any blood and place both of them into the next well of the tray.



**Figure B8. Cleaning an otolith on a neoprene glove or sleeve.**

Place the pair of otoliths from one fish into the tray wells following the top-to-bottom, left-to-right, scheme described above.

If an otolith is accidentally cut in half, place both halves in the appropriate tray well. If one of the two otoliths is lost during the process, place a colored bead into the well with the other otolith. If both otoliths are lost, place two colored beads in the cell for that fish.

h. After each column is complete, check all the cells to be sure none were skipped and that every cell has one tissue sample and two otoliths (or beads). If you find discrepancies, go back to the last known accurate sample, and start over with new fish. Discard confused samples. Do not try to guess which tissues or otoliths in the confused cells go with which fish.

i. After tissue samples are taken, make sure **all tissues are covered with EtOH** before sealing plates with rubber impermeamats. Use the squirt bottle to fill wells  $\frac{3}{4}$  full, if needed. EtOH/tissue ratio should be **at least 3:1** to preserve tissues.

j. Cover finished trays with a rubber mat lid pushed tightly down into cells and secure with two perpendicular rubber bands. Place the tray(s) upright into a used plastic tray bag for transport in a pack from the field. Keep the trays upright in the pack to reduce leakage and prevent otoliths from sticking to the lid and getting lost when opened.

k. The squirt bottle should be emptied after **each sampling session; it will leak** if not emptied.

## **Cleaning, Storing, and Shipping Samples**

### ***Inspecting DNA and Otolith Samples***

Soon after returning to the live-aboard boat or other lodging, while performing data QA on the HW laptop, carefully inspect 48dwp trays for any damage or mis-location of the samples. If samples do not match data, carefully adjust data if appropriate. If there are any questions about the alignment of data with samples in their cells, keep the samples, but flag the data from the first questionable cell forward by adding the comment “UNCERTAIN PAIRING” plus any other potentially helpful details, in each data row for potentially erroneous data. If you find more than two otoliths in a cell, do not guess which one is in the wrong cell – make comments as described above for every uncertain cell (data row). If this occurs for a large number of samples, it may be necessary to return to the stream, to collect additional samples.

If for any reason there is confusion, be sure to make notes in the laptop data accordingly at the survey and specimen level as appropriate.

After inspection of DNA trays, replace the rubber mat tightly into the cells. Then use the plastic wrap to tightly seal the tray by wrapping several times in both directions. Store the finished DNA trays in a safe place until delivery to the PWS Science Center.

It is critically important for all DNA trays to be delivered to the PWS Science Center during each port call, or as often as possible throughout the season. This will ensure that samples are stored as safely as possible and also that DNA trays get delivered to ADF&G’s Gene Conservation Lab as quickly as possible to avoid any possible issues with ethanol evaporating from the tray wells.

### ***Pre-Shipment QA Process***

When the trays are returned to the Science Center, it is important that each tray is documented as received at the Center and that it be visually inspected to ensure that the number of samples matches the number of records in the database. This will be accomplished for every batch of trays arriving to the Center by:

### Data Entry

- Download the most recent version of the Tray Inventory report from the keta web site and save it as an Excel spreadsheet.
- Sort the tray inventory so it's convenient to compare to the batches of trays.
- Create two new columns in the spreadsheet: "Tray received" and "Samples verified".
- Scan the first tray code into the first "Tray received" cell.
- Count/observe the number of samples (filled cells) in the tray to verify that it contains the same number of samples as indicated in the spreadsheet.
- If the sample numbers match, scan the tray code into the "Samples verified" cell.
- If the sample numbers do not match, follow the procedures on the previous page under *Inspecting DNA and Otolith Samples* to rectify the data and the samples.

## ***SHIPPING***

All transfer of trays from PWSSC to ADF&G must be done according to the protocols established below. All shipments must be accompanied by an inventory list showing the stream names and AWC numbers and the number of trays collected, by date, as well as the inspection verification described above.

### ***48dwp Trays***

All 48-dwp trays will be shipped to the ADF&G GCL lab in Anchorage. These are hazardous materials shipments because of the EtOH, so haz-mat packing and shipping procedures must be followed.

Ship to: Alaska Department of Fish and Game  
Gene Conservation Laboratory  
333 Raspberry Road  
Anchorage, AK 99518

Questions on shipping the 48dwps to the GCL should be directed to Judy Berger 907-267-2175.



|  |  |  |  |  |  |  |
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|  |  |  |  |  |  |  |

**Person responsible for delivery:**

**Recipient:**

**Date sent to Cordova Lab:**

**Date received by Cordova Lab:**

**APPENDIX C. PWS PINK SALMON SURVEY SUMMARY  
BY STREAM AND DATE**

| <b>Stream Name</b> | <b>AWC</b>   | <b>Survey Date</b> | <b>Specimens</b> | <b>Live Count</b> | <b>Dead Count</b> |
|--------------------|--------------|--------------------|------------------|-------------------|-------------------|
| Paddy C            | 226-20-16010 | 7/31/2017          | 0                | 0                 | 0                 |
| Paddy C            | 226-20-16010 | 8/1/2017           | 0                | 0                 | 0                 |
| Paddy C            | 226-20-16010 | 8/2/2017           | 0                | 0                 | 0                 |
| Paddy C            | 226-20-16010 | 8/3/2017           | 0                | 2                 | 0                 |
| Paddy C            | 226-20-16010 | 8/4/2017           | 0                | 0                 | 0                 |
| Paddy C            | 226-20-16010 | 8/5/2017           | 0                | 0                 | 0                 |
| Paddy C            | 226-20-16010 | 8/6/2017           | 0                | 0                 | 0                 |
| Paddy C            | 226-20-16010 | 8/7/2017           | 0                | 0                 | 0                 |
| Paddy C            | 226-20-16010 | 8/8/2017           | 0                | 0                 | 0                 |
| Paddy C            | 226-20-16010 | 8/9/2017           | 4                | 20                | 4                 |
| Paddy C            | 226-20-16010 | 8/10/2017          | 63               | 756               | 63                |
| Paddy C            | 226-20-16010 | 8/11/2017          | 165              | 535               | 208               |
| Paddy C            | 226-20-16010 | 8/12/2017          | 45               | 719               | 156               |
| Paddy C            | 226-20-16010 | 8/13/2017          | 118              | 800               | 172               |
| Paddy C            | 226-20-16010 | 8/14/2017          | 238              | 993               | 338               |
| Paddy C            | 226-20-16010 | 8/15/2017          | 238              | 667               | 430               |
| Paddy C            | 226-20-16010 | 8/16/2017          | 245              | 609               | 617               |
| Paddy C            | 226-20-16010 | 8/17/2017          | 157              | 508               | 693               |
| Paddy C            | 226-20-16010 | 8/18/2017          | 185              | 671               | 701               |
| Paddy C            | 226-20-16010 | 8/19/2017          | 2                | -                 | -                 |
| Paddy C            | 226-20-16010 | 8/20/2017          | 392              | 525               | 526               |
| Paddy C            | 226-20-16010 | 8/21/2017          | 74               | 104               | 213               |
| Paddy C            | 226-20-16010 | 8/22/2017          | 284              | 1171              | 631               |
| Paddy C            | 226-20-16010 | 8/23/2017          | 3                | -                 | -                 |
| Paddy C            | 226-20-16010 | 8/24/2017          | 306              | 849               | 836               |
| Paddy C            | 226-20-16010 | 8/26/2017          | 232              | 1983              | 725               |
| Paddy C            | 226-20-16010 | 8/27/2017          | 5                | -                 | -                 |
| Paddy C            | 226-20-16010 | 8/28/2017          | 714              | 1310              | 1363              |
| Paddy C            | 226-20-16010 | 8/30/2017          | 692              | 1608              | 1200              |
| Paddy C            | 226-20-16010 | 9/1/2017           | 709              | 2113              | 1230              |
| Paddy C            | 226-20-16010 | 9/2/2017           | 11               | -                 | -                 |
| Paddy C            | 226-20-16010 | 9/3/2017           | 483              | 873               | 3000              |
| Paddy C            | 226-20-16010 | 9/4/2017           | 3                | -                 | -                 |
| Paddy C            | 226-20-16010 | 9/5/2017           | 775              | 452               | 1487              |
| Paddy C            | 226-20-16010 | 9/6/2017           | 3                | -                 | -                 |
| Paddy C            | 226-20-16010 | 9/7/2017           | 764              | 1907              | 2849              |
| Paddy C            | 226-20-16010 | 9/8/2017           | 1                | -                 | -                 |

| Stream Name | AWC          | Survey Date | Specimens | Live Count | Dead Count |
|-------------|--------------|-------------|-----------|------------|------------|
| Paddy C     | 226-20-16010 | 9/9/2017    | 435       | 967        | 1296       |
| Paddy C     | 226-20-16010 | 9/11/2017   | 533       | 886        | 2692       |
| Paddy C     | 226-20-16010 | 9/13/2017   | 588       | 502        | 2509       |
| Paddy C     | 226-20-16010 | 9/15/2017   | 0         | 167        | 1012       |
| Erb C       | 226-20-16040 | 7/31/2017   | 0         | 210        | 0          |
| Erb C       | 226-20-16040 | 8/2/2017    | 11        | 325        | 11         |
| Erb C       | 226-20-16040 | 8/3/2017    | 35        | 1075       | 42         |
| Erb C       | 226-20-16040 | 8/4/2017    | 138       | 1198       | 167        |
| Erb C       | 226-20-16040 | 8/5/2017    | 115       | 1102       | 217        |
| Erb C       | 226-20-16040 | 8/6/2017    | 189       | 880        | 333        |
| Erb C       | 226-20-16040 | 8/7/2017    | 217       | 825        | 615        |
| Erb C       | 226-20-16040 | 8/8/2017    | 133       | 909        | 721        |
| Erb C       | 226-20-16040 | 8/9/2017    | 144       | 726        | 786        |
| Erb C       | 226-20-16040 | 8/10/2017   | 169       | 2671       | 717        |
| Erb C       | 226-20-16040 | 8/11/2017   | 112       | 1384       | 816        |
| Erb C       | 226-20-16040 | 8/12/2017   | 86        | 3852       | 410        |
| Erb C       | 226-20-16040 | 8/13/2017   | 155       | 2398       | 706        |
| Erb C       | 226-20-16040 | 8/14/2017   | 293       | 3319       | 749        |
| Erb C       | 226-20-16040 | 8/15/2017   | 365       | 3252       | 893        |
| Erb C       | 226-20-16040 | 8/16/2017   | 390       | 3371       | 939        |
| Erb C       | 226-20-16040 | 8/17/2017   | 457       | 3548       | 2287       |
| Erb C       | 226-20-16040 | 8/18/2017   | 384       | 6017       | 1416       |
| Erb C       | 226-20-16040 | 8/19/2017   | 637       | 5727       | 1530       |
| Erb C       | 226-20-16040 | 8/21/2017   | 550       | 2855       | 1275       |
| Erb C       | 226-20-16040 | 8/23/2017   | 888       | 3750       | 2675       |
| Erb C       | 226-20-16040 | 8/25/2017   | 735       | 3880       | 1799       |
| Erb C       | 226-20-16040 | 8/27/2017   | 768       | 10040      | 2006       |
| Erb C       | 226-20-16040 | 8/29/2017   | 688       | 4064       | 1236       |
| Erb C       | 226-20-16040 | 8/31/2017   | 576       | 900        | 1236       |
| Erb C       | 226-20-16040 | 9/2/2017    | 960       | 3000       | 2017       |
| Erb C       | 226-20-16040 | 9/3/2017    | 672       | -          | -          |
| Erb C       | 226-20-16040 | 9/4/2017    | 1440      | 3200       | 4000       |
| Erb C       | 226-20-16040 | 9/6/2017    | 865       | 3600       | 6600       |
| Erb C       | 226-20-16040 | 9/8/2017    | 864       | 3325       | 5430       |
| Erb C       | 226-20-16040 | 9/10/2017   | 576       | 1732       | 6154       |
| Erb C       | 226-20-16040 | 9/12/2017   | 672       | 2700       | 6235       |
| Erb C       | 226-20-16040 | 9/14/2017   | 288       | -          | -          |
| Erb C       | 226-20-16040 | 9/15/2017   | 0         | 510        | 4346       |
| Hogan Bay   | 226-30-16810 | 7/30/2017   | 0         | 12         | 0          |
| Hogan Bay   | 226-30-16810 | 8/1/2017    | 1         | 28         | 1          |

| Stream Name | AWC          | Survey Date | Specimens | Live Count | Dead Count |
|-------------|--------------|-------------|-----------|------------|------------|
| Hogan Bay   | 226-30-16810 | 8/3/2017    | 48        | 577        | 48         |
| Hogan Bay   | 226-30-16810 | 8/5/2017    | 103       | 427        | 131        |
| Hogan Bay   | 226-30-16810 | 8/7/2017    | 50        | 152        | 125        |
| Hogan Bay   | 226-30-16810 | 8/9/2017    | 83        | 174        | 178        |
| Hogan Bay   | 226-30-16810 | 8/11/2017   | 126       | 738        | 260        |
| Hogan Bay   | 226-30-16810 | 8/13/2017   | 48        | 1494       | 165        |
| Hogan Bay   | 226-30-16810 | 8/15/2017   | 242       | 898        | 488        |
| Hogan Bay   | 226-30-16810 | 8/16/2017   | 119       | 1802       | 337        |
| Hogan Bay   | 226-30-16810 | 8/17/2017   | 243       | 2840       | 610        |
| Hogan Bay   | 226-30-16810 | 8/18/2017   | 90        | 2689       | 694        |
| Hogan Bay   | 226-30-16810 | 8/19/2017   | 345       | 3450       | 840        |
| Hogan Bay   | 226-30-16810 | 8/20/2017   | 88        | 3900       | 248        |
| Hogan Bay   | 226-30-16810 | 8/21/2017   | 187       | 2970       | 575        |
| Hogan Bay   | 226-30-16810 | 8/22/2017   | 528       | 4858       | 948        |
| Hogan Bay   | 226-30-16810 | 8/23/2017   | 192       | 6150       | 1364       |
| Hogan Bay   | 226-30-16810 | 8/24/2017   | 288       | 8540       | 1845       |
| Hogan Bay   | 226-30-16810 | 8/25/2017   | 234       | 10150      | 640        |
| Hogan Bay   | 226-30-16810 | 8/26/2017   | 384       | 11480      | 561        |
| Hogan Bay   | 226-30-16810 | 8/27/2017   | 528       | 10270      | 789        |
| Hogan Bay   | 226-30-16810 | 8/28/2017   | 624       | 6700       | 1954       |
| Hogan Bay   | 226-30-16810 | 8/29/2017   | 162       | -          | -          |
| Hogan Bay   | 226-30-16810 | 8/30/2017   | 480       | 11730      | 1807       |
| Hogan Bay   | 226-30-16810 | 8/31/2017   | 719       | 6400       | 1739       |
| Hogan Bay   | 226-30-16810 | 9/1/2017    | 672       | 9700       | 2966       |
| Hogan Bay   | 226-30-16810 | 9/2/2017    | 528       | 20630      | 4001       |
| Hogan Bay   | 226-30-16810 | 9/3/2017    | 576       | 8100       | 2448       |
| Hogan Bay   | 226-30-16810 | 9/4/2017    | 624       | 10500      | 5270       |
| Hogan Bay   | 226-30-16810 | 9/5/2017    | 192       | 9700       | 7420       |
| Hogan Bay   | 226-30-16810 | 9/6/2017    | 480       | 17290      | 7174       |
| Hogan Bay   | 226-30-16810 | 9/7/2017    | 768       | 9200       | 12320      |
| Hogan Bay   | 226-30-16810 | 9/8/2017    | 768       | 9900       | 15710      |
| Hogan Bay   | 226-30-16810 | 9/9/2017    | 528       | 17200      | 16440      |
| Hogan Bay   | 226-30-16810 | 9/10/2017   | 768       | 8500       | 21280      |
| Hogan Bay   | 226-30-16810 | 9/11/2017   | 768       | 8200       | 13970      |
| Hogan Bay   | 226-30-16810 | 9/12/2017   | 624       | 4300       | 4170       |
| Hogan Bay   | 226-30-16810 | 9/13/2017   | 480       | 5100       | 13660      |
| Hogan Bay   | 226-30-16810 | 9/14/2017   | 1500      | 8300       | 21750      |
| Hogan Bay   | 226-30-16810 | 9/15/2017   | 528       | 4300       | 9500       |
| Hogan Bay   | 226-30-16810 | 9/16/2017   | 624       | 3650       | 8630       |
| Hogan Bay   | 226-30-16810 | 9/17/2017   | 576       | 1960       | 1800       |

| Stream Name | AWC          | Survey Date | Specimens | Live Count | Dead Count |
|-------------|--------------|-------------|-----------|------------|------------|
| Hogan Bay   | 226-30-16810 | 9/18/2017   | 672       | 3800       | 11200      |
| Hogan Bay   | 226-30-16810 | 9/19/2017   | 624       | 3160       | 11800      |
| Hogan Bay   | 226-30-16810 | 9/20/2017   | 624       | 4390       | 20050      |
| Gilmour C   | 227-20-17480 | 7/31/2017   | 0         | 2          | 0          |
| Gilmour C   | 227-20-17480 | 8/2/2017    | 0         | 2          | 0          |
| Gilmour C   | 227-20-17480 | 8/4/2017    | 0         | 38         | 0          |
| Gilmour C   | 227-20-17480 | 8/6/2017    | 0         | 12         | 0          |
| Gilmour C   | 227-20-17480 | 8/8/2017    | 1         | 6          | 1          |
| Gilmour C   | 227-20-17480 | 8/10/2017   | 1         | 147        | 1          |
| Gilmour C   | 227-20-17480 | 8/12/2017   | 7         | 63         | 7          |
| Gilmour C   | 227-20-17480 | 8/14/2017   | 3         | 211        | 7          |
| Gilmour C   | 227-20-17480 | 8/16/2017   | 5         | 166        | 10         |
| Gilmour C   | 227-20-17480 | 8/18/2017   | 3         | 530        | 14         |
| Gilmour C   | 227-20-17480 | 8/20/2017   | 10        | 430        | 13         |
| Gilmour C   | 227-20-17480 | 8/23/2017   | 31        | 887        | 48         |
| Gilmour C   | 227-20-17480 | 8/24/2017   | 40        | 862        | 72         |
| Gilmour C   | 227-20-17480 | 8/25/2017   | 26        | 1056       | 53         |
| Gilmour C   | 227-20-17480 | 8/27/2017   | 95        | 2178       | 131        |
| Gilmour C   | 227-20-17480 | 8/29/2017   | 44        | 605        | 50         |
| Gilmour C   | 227-20-17480 | 8/31/2017   | 119       | 992        | 131        |
| Gilmour C   | 227-20-17480 | 9/2/2017    | 605       | 2761       | 770        |
| Gilmour C   | 227-20-17480 | 9/4/2017    | 731       | 1728       | 1366       |
| Gilmour C   | 227-20-17480 | 9/6/2017    | 688       | 1720       | 1026       |
| Gilmour C   | 227-20-17480 | 9/8/2017    | 1118      | 2486       | 2232       |
| Gilmour C   | 227-20-17480 | 9/10/2017   | 821       | 1332       | 1721       |
| Gilmour C   | 227-20-17480 | 9/12/2017   | 899       | 1102       | 2904       |
| Stockdale C | 227-20-17520 | 7/31/2017   | 19        | 218        | 19         |
| Stockdale C | 227-20-17520 | 8/2/2017    | 6         | 508        | 6          |
| Stockdale C | 227-20-17520 | 8/3/2017    | 5         | 779        | 24         |
| Stockdale C | 227-20-17520 | 8/4/2017    | 7         | 622        | 13         |
| Stockdale C | 227-20-17520 | 8/6/2017    | 29        | 1043       | 41         |
| Stockdale C | 227-20-17520 | 8/7/2017    | 17        | 1482       | 51         |
| Stockdale C | 227-20-17520 | 8/8/2017    | 19        | 852        | 53         |
| Stockdale C | 227-20-17520 | 8/9/2017    | 16        | 2012       | 91         |
| Stockdale C | 227-20-17520 | 8/10/2017   | 19        | 1330       | 80         |
| Stockdale C | 227-20-17520 | 8/11/2017   | 1         | -          | -          |
| Stockdale C | 227-20-17520 | 8/12/2017   | 10        | 3933       | 63         |
| Stockdale C | 227-20-17520 | 8/13/2017   | 12        | 3260       | 80         |
| Stockdale C | 227-20-17520 | 8/14/2017   | 26        | 4642       | 48         |
| Stockdale C | 227-20-17520 | 8/16/2017   | 97        | 6159       | 140        |

| Stream Name | AWC          | Survey Date | Specimens | Live Count | Dead Count |
|-------------|--------------|-------------|-----------|------------|------------|
| Stockdale C | 227-20-17520 | 8/17/2017   | 37        | 6035       | 151        |
| Stockdale C | 227-20-17520 | 8/18/2017   | 51        | 6145       | 128        |
| Stockdale C | 227-20-17520 | 8/19/2017   | 49        | 4680       | 193        |
| Stockdale C | 227-20-17520 | 8/21/2017   | 76        | -          | 90         |
| Stockdale C | 227-20-17520 | 8/23/2017   | 469       | 7646       | 655        |
| Stockdale C | 227-20-17520 | 8/24/2017   | 50        | 500        | 75         |
| Stockdale C | 227-20-17520 | 8/25/2017   | 389       | 9369       | 475        |
| Stockdale C | 227-20-17520 | 8/26/2017   | 729       | 9747       | 1065       |
| Stockdale C | 227-20-17520 | 8/27/2017   | 436       | 8452       | 893        |
| Stockdale C | 227-20-17520 | 8/28/2017   | 771       | 8505       | 1683       |
| Stockdale C | 227-20-17520 | 8/29/2017   | 122       | -          | 177        |
| Stockdale C | 227-20-17520 | 8/30/2017   | 808       | 7426       | 1110       |
| Stockdale C | 227-20-17520 | 8/31/2017   | 505       | 2825       | 880        |
| Stockdale C | 227-20-17520 | 9/1/2017    | 1070      | 7065       | 2112       |
| Stockdale C | 227-20-17520 | 9/2/2017    | 192       | 1531       | 429        |
| Stockdale C | 227-20-17520 | 9/3/2017    | 816       | 7273       | 5834       |
| Stockdale C | 227-20-17520 | 9/4/2017    | 288       | 980        | 1980       |
| Stockdale C | 227-20-17520 | 9/5/2017    | 368       | 4705       | 3087       |
| Stockdale C | 227-20-17520 | 9/6/2017    | 528       | -          | -          |
| Stockdale C | 227-20-17520 | 9/7/2017    | 1200      | 1370       | 3257       |
| Stockdale C | 227-20-17520 | 9/9/2017    | 303       | 4880       | 13347      |
| Stockdale C | 227-20-17520 | 9/11/2017   | 1104      | 535        | 2473       |
| Stockdale C | 227-20-17520 | 9/13/2017   | 912       | 1566       | 8963       |

Dash (-) indicates live/dead estimates were not made. Number of samples, live counts and dead counts include contributions from tributaries where applicable.

## APPENDIX D. PWS STREAM SUMMARIES

**Paddy Creek**  
226-20-16010  
Pink Salmon Fitness

**2017 samples collected and run timing:** Sampling duties for Paddy and Erb Creeks were the responsibility of the PWSSC camping crew (Paddy crew). Paddy crew surveyed at Paddy Creek every day except during peak season, when they surveyed Paddy and Erb Creeks every other day to maximize the number of samples collected at both creeks (by reducing total transit time to Erb Creek from every day to every other day). On the days that Paddy crew did not survey Paddy Creek, the tagging crew conducted a tag search. This was documented with a HW survey because tagged samples were included as part of the HW effort. Paddy Creek is a small muskeg creek with three branches and, for our sampling purposes, we called them Paddy Creek, Paddy Right Tributary and Paddy Left Tributary (description in the next section). The following summary is comprehensive for all the Paddy tributaries.

Paddy crew sampled at Paddy Creek 41 times between 07/31/2017 and 09/13/2017. 8,467 pink salmon otolith and tissue samples were collected. Fish were present on the first and last day of sampling, so the full run length was not observed. Fish numbers peaked in mid-August, slowly tapering off through September.

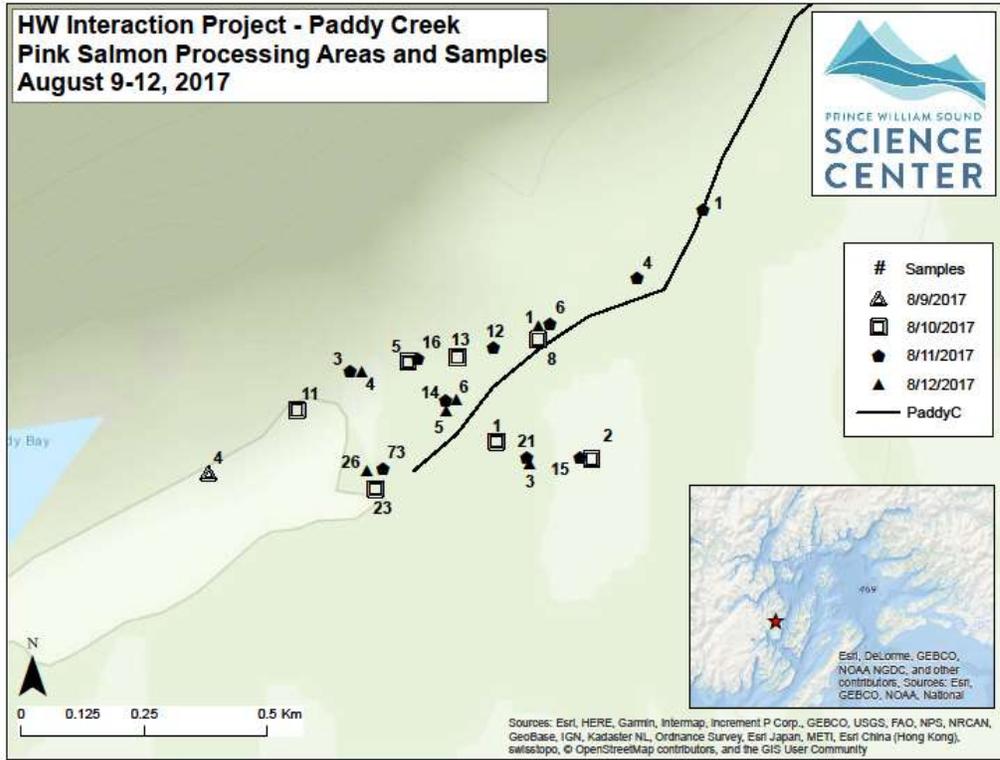
**Extent of stream sampled:** There are two creeks that flow into the head of Paddy Bay, one from the northeast and one from the east. Looking upstream, Paddy Creek is the stream on the left side (NE) and an unnamed creek flows from the right (E), for our sampling purposes we call this unnamed creek Paddy Right Tributary. Additionally, Paddy Creek has a small tributary roughly 100 m from the average high tide. Looking upstream, it flows from the left, and is called Paddy Left Tributary. The extent of all three creeks was determined in 2015. The extent of Paddy Creek is a small muskeg lake about 2.5 km from the mouth. Every year in Paddy Creek, live Pink Salmon are seen as high up as the muskeg lake. The extent of Paddy Right Tributary is a 2.5 m bedrock slide. We never saw fish above that point, live or dead, even during the highest stream flow in 2015, 2016 or 2017. The extent of Paddy Left Tributary is a 1.5-m logjam sieve where water drains through a buildup of loose cobble before trickling through the logjam. Very few fish were observed in this tributary. The majority of spawning activity occurred in the lowest 200m of both Paddy Creek and the Right Tributary.

**Unusual events:** A technician was charged by a bear.

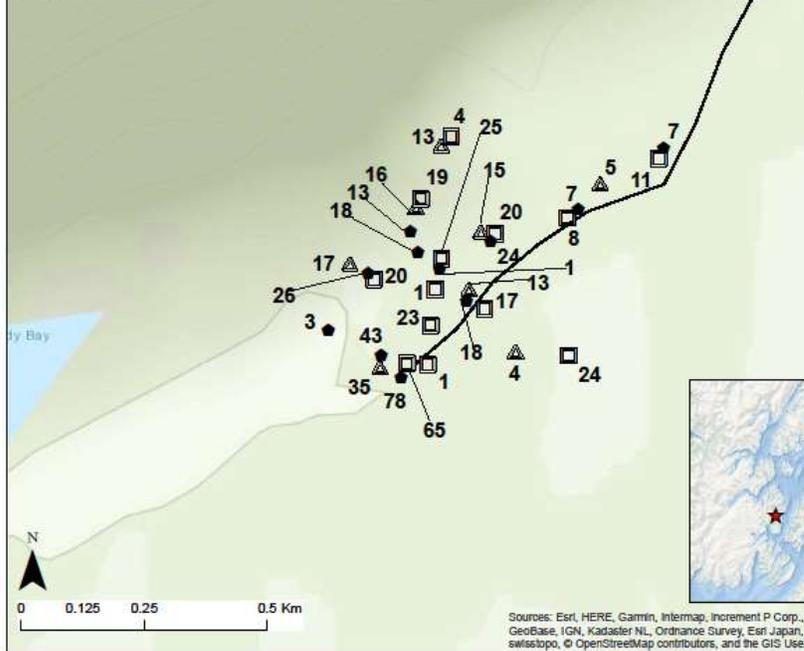
**Access, safety, or logistics issues:** Paddy Creek is approximately 1 km from camp - access is easy and dependable except in extreme flooding events. Paddy Crew recommends, as they did in 2016, that the Left Tributary not be sampled in 2018. The effort needed to sample the Left Tributary was very high for the amount of samples collected. We also had many run-ins with black bears that are totally fearless. After 4 years of seeing technicians on the streams daily, coupled with close-proximity camping, this generation of bears is bold around humans. We had several dangerous encounters with bears there. Bears have not entered the camp but it is only a

matter of time before they do. Paddy Camp should be moved next season, possibly to the island close to the mouth of Erb Creek to avoid possible bear encounters.

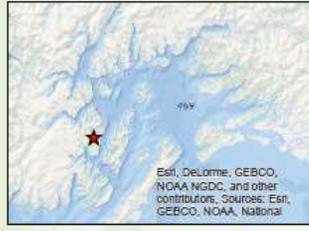
Distribution of Paddy Creek samples by date:



**HW Interaction Project - Paddy Creek  
Pink Salmon Processing Areas and Samples  
August 13-15, 2017**

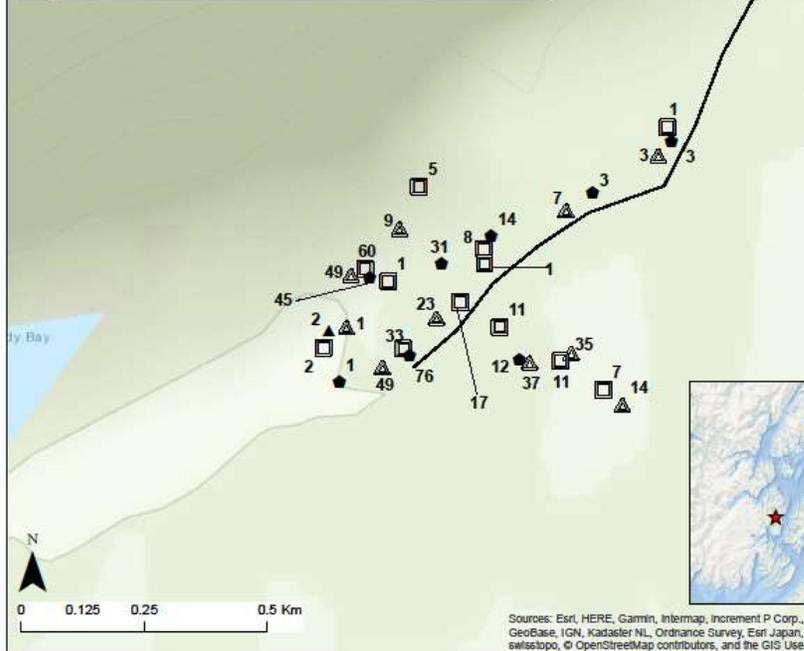


| # Samples | Date      |
|-----------|-----------|
| ▲         | 8/13/2017 |
| □         | 8/14/2017 |
| ●         | 8/15/2017 |
| —         | PaddyC    |



Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Paddy Creek  
Pink Salmon Processing Areas and Samples  
August 16-19, 2017**

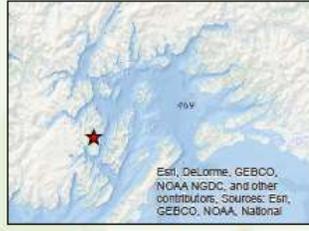
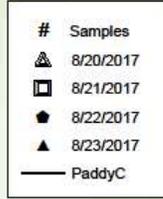
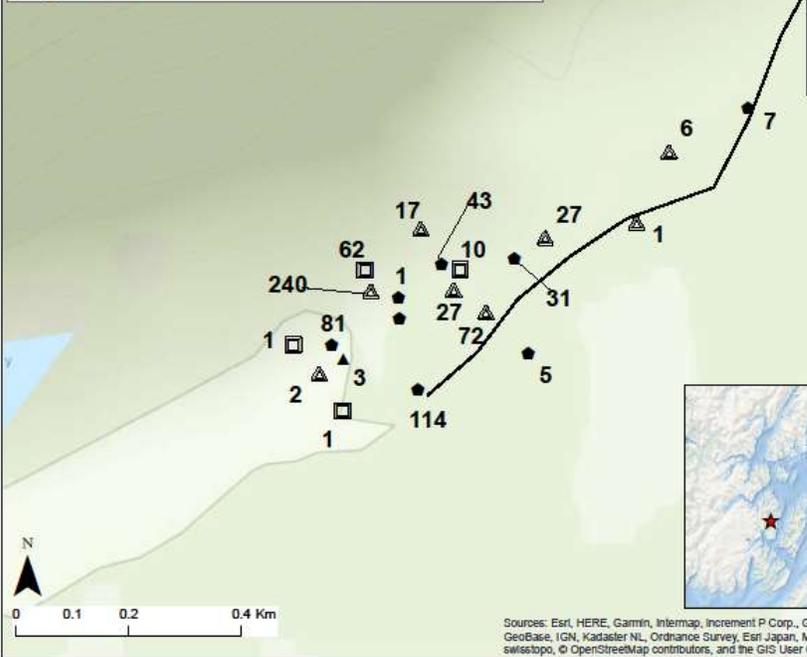


| # Samples | Date      |
|-----------|-----------|
| ▲         | 8/16/2017 |
| □         | 8/17/2017 |
| ●         | 8/18/2017 |
| ▼         | 8/19/2017 |
| —         | PaddyC    |

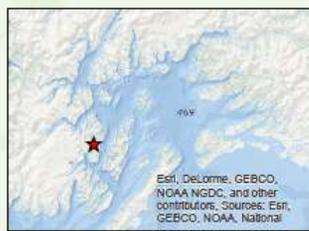
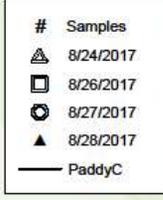
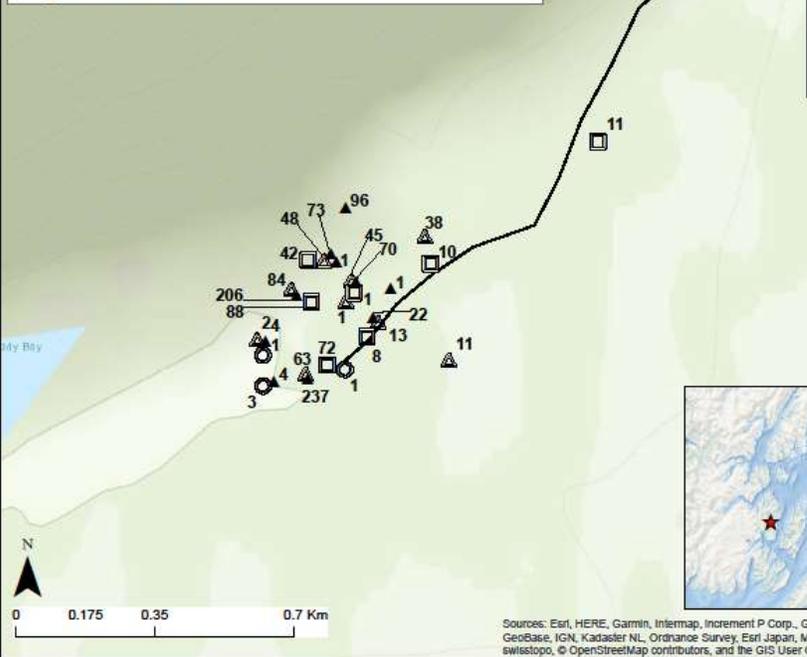


Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Paddy Creek  
Pink Salmon Processing Areas and Samples  
August 20-23, 2017**

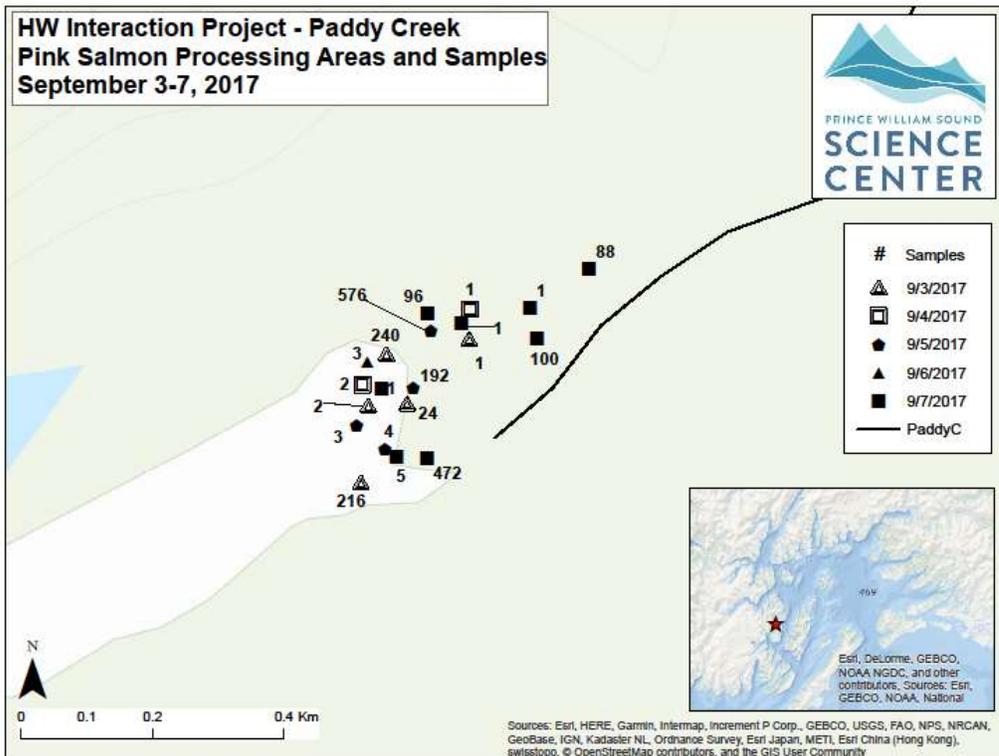
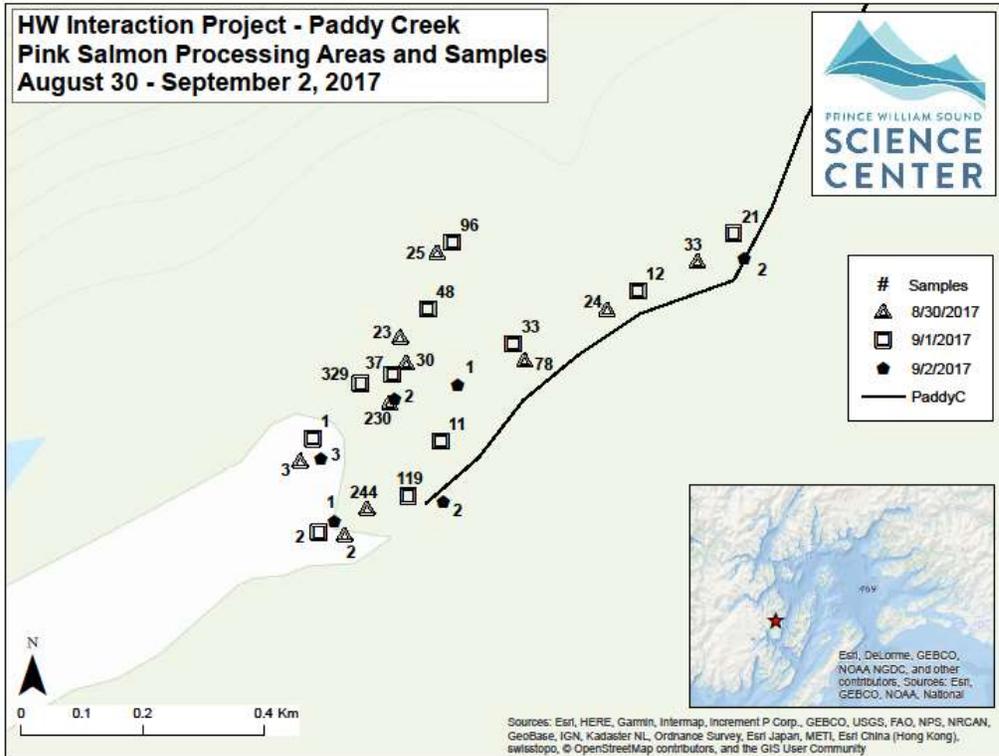


**HW Interaction Project - Paddy Creek  
Pink Salmon Processing Areas and Samples  
August 24-28, 2017**



Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community





**Erb Creek**  
226-20-16040  
Pink Salmon Fitness

**2017 samples collected and run timing:** Sampling duties for Erb and Paddy Creeks were the responsibility of the PWSSC camping crew (Paddy crew). Paddy crew sampled at Erb Creek every day, except during peak season when the crew surveyed Erb Creek every other day in order to maximize the number of samples collected at both creeks. Erb Creek is about twice the size of Paddy Creek, and drains a small mountain valley with no tributaries.

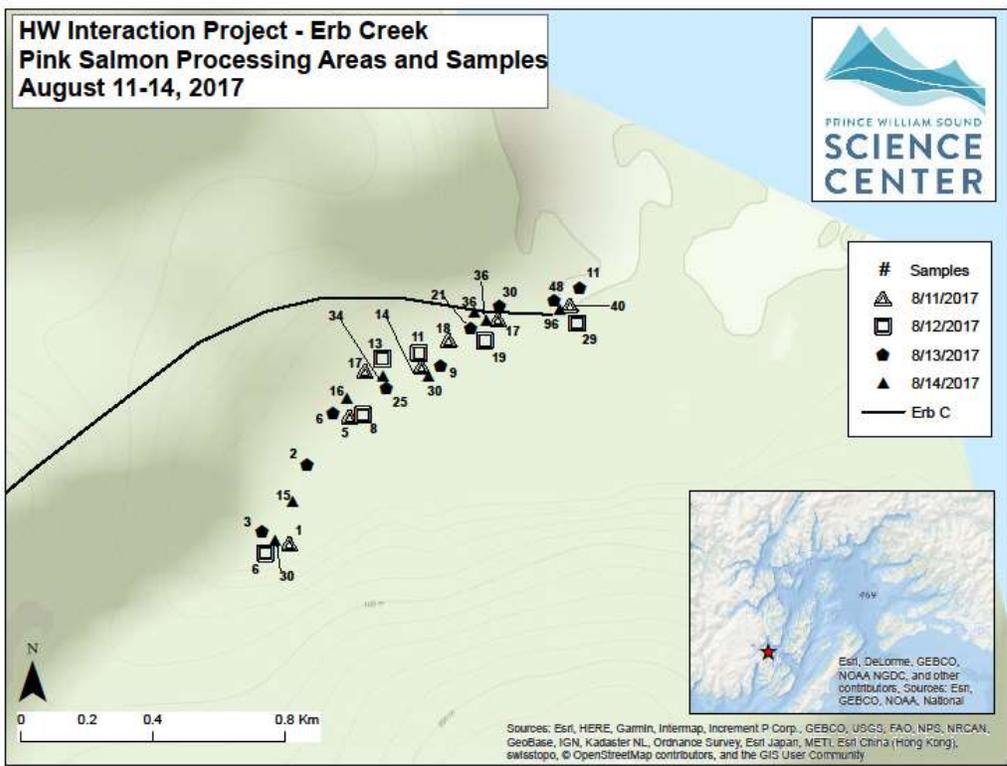
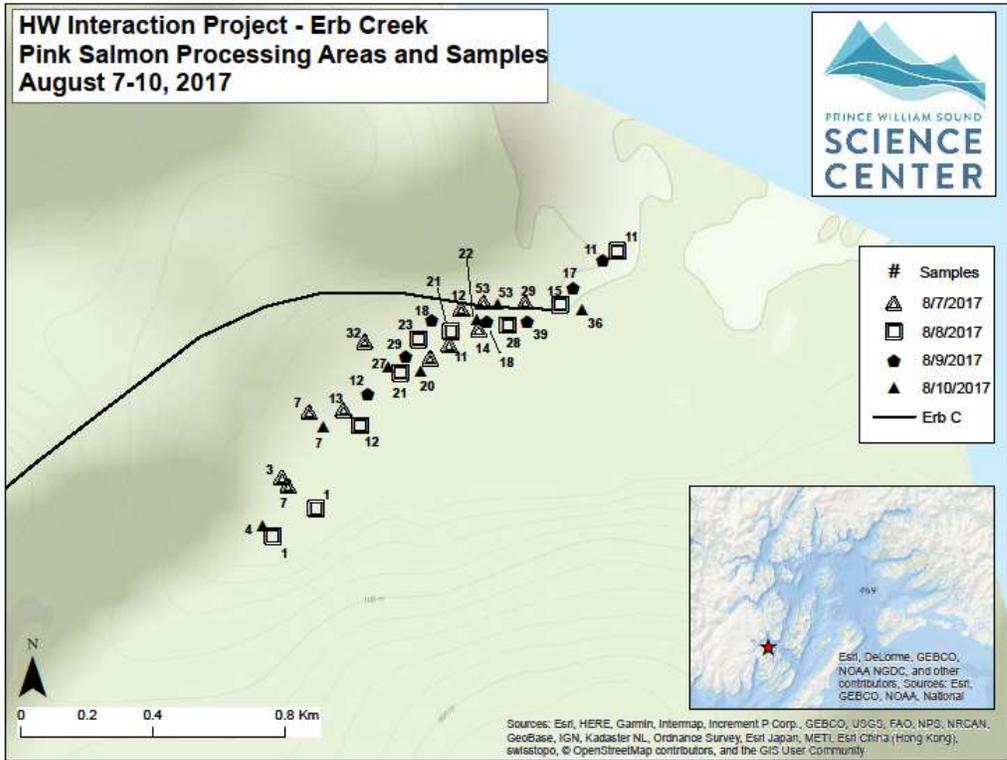
Paddy crew visited Erb Creek 34 times between 7/31/2017 and 9/14/2017, collecting a total of 14,572 pink salmon otolith and tissue samples. The run picked up gradually, peaking in the first days of September. Spawners were present our first sample day, and fresh fish were still arriving to spawn at Erb on 9/14/2017, the last day samples were taken. For this reason, full run length was not observed.

**Extent of stream sampled:** From 7/31/2017 through 8/16/2017, sampling efforts were spread over the entire length of spawning habitat. By mid-August however, mass die-offs greatly increased the amount of samples we had access to. So Paddy crew switched gears and primarily focused on sampling as many carcasses as possible, and secondarily focused on distributing sampling effort over the entire stream. Most spawning activity was in the first 1 km, but spawning was observed from the lower intertidal to the marked survey extent below a long cascade of boulders.

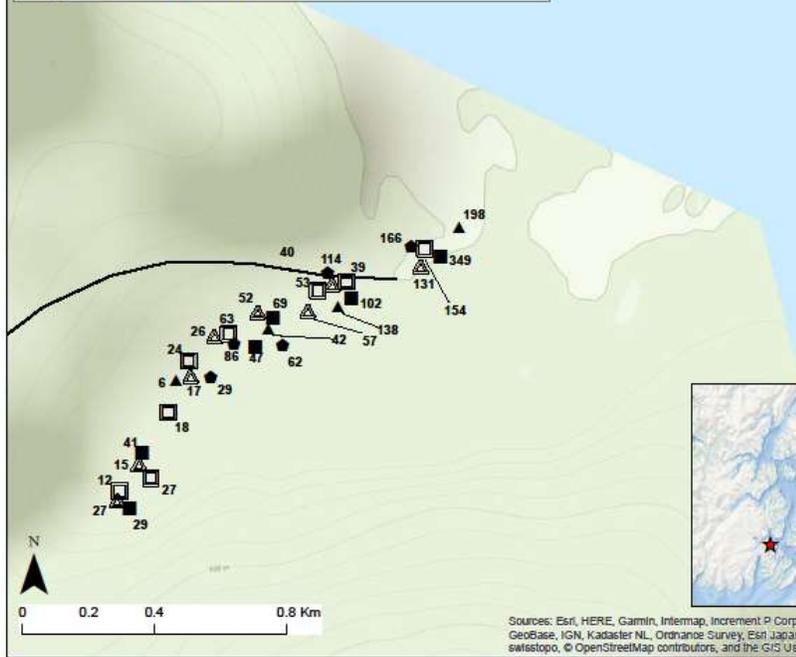
**Unusual events:** A number of high water events this season.

**Access, safety, or logistics issues:** There is a tidal flat in front of Erb Creek that could create a very long, dry wait if you boated up at high tide and got stranded. The best place to keep the skiff is a tiny, but deep, bight to the northwest of the creek mouth. To keep the skiff even safer and not have to push it to water every low tide, we made a simple pulley system across this bight. After implementing the pulley system we could sample uninterrupted. Paddy crew always carried a sealed emergency bucket in the skiff with a portable stove, stove fuel, food and a shelter tarp. We also always carried the GPS in case of fog. It fits nicely in the med kit and has a track-line between camp and Erb Creek.

**Recommendations, changes, and other notes:** Having a 5-person crew allowed for record numbers of samples to be collected. It is encouraged to have as many people as possible sampling.



**HW Interaction Project - Erb Creek  
Pink Salmon Processing Areas and Samples  
August 15-19, 2017**

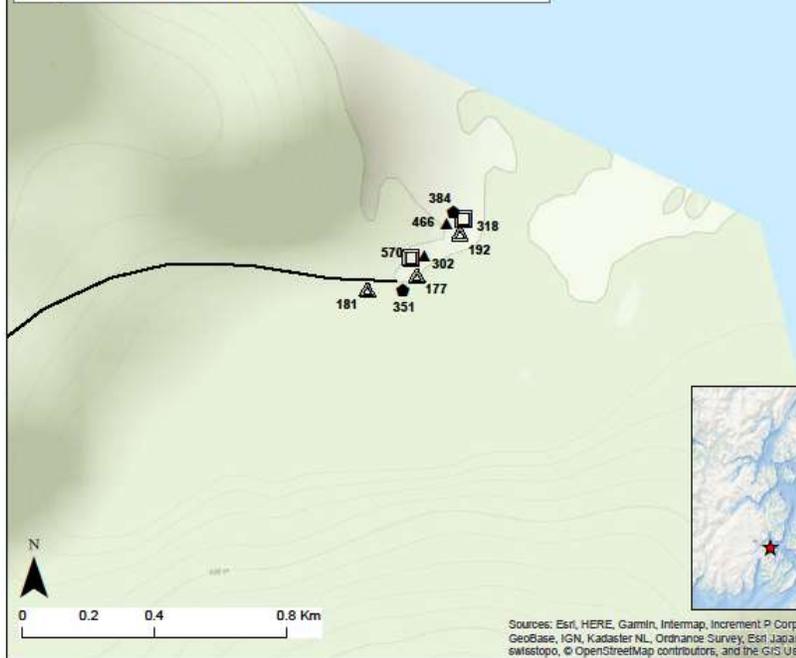


| # | Samples   |
|---|-----------|
| △ | 8/15/2017 |
| □ | 8/16/2017 |
| ● | 8/17/2017 |
| ◆ | 8/18/2017 |
| ■ | 8/19/2017 |
| — | Erb C     |

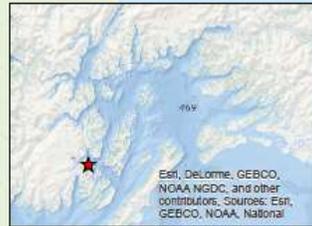


Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community.

**HW Interaction Project - Erb Creek  
Pink Salmon Processing Areas and Samples  
August 21-27, 2017**

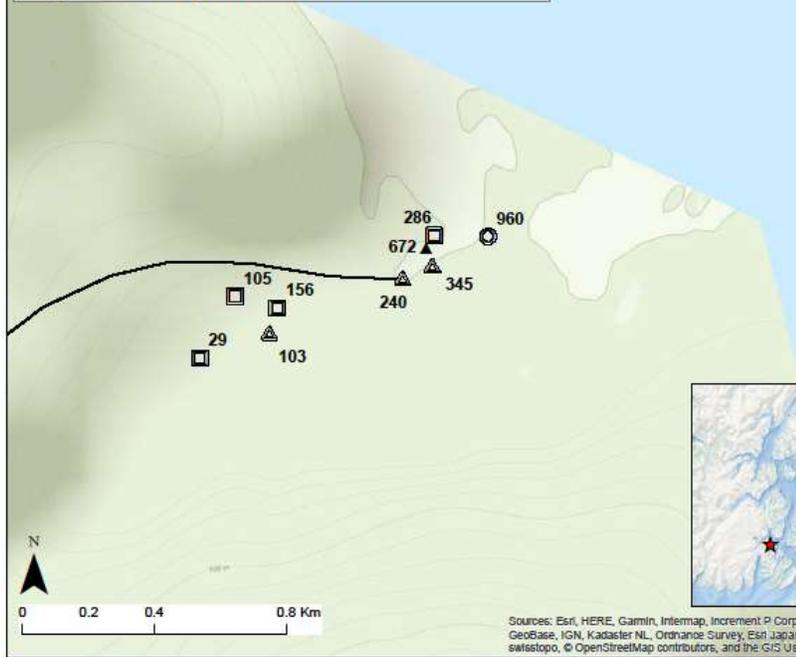


| # | Samples   |
|---|-----------|
| △ | 8/21/2017 |
| □ | 8/23/2017 |
| ● | 8/25/2017 |
| ◆ | 8/27/2017 |
| — | Erb C     |



Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community.

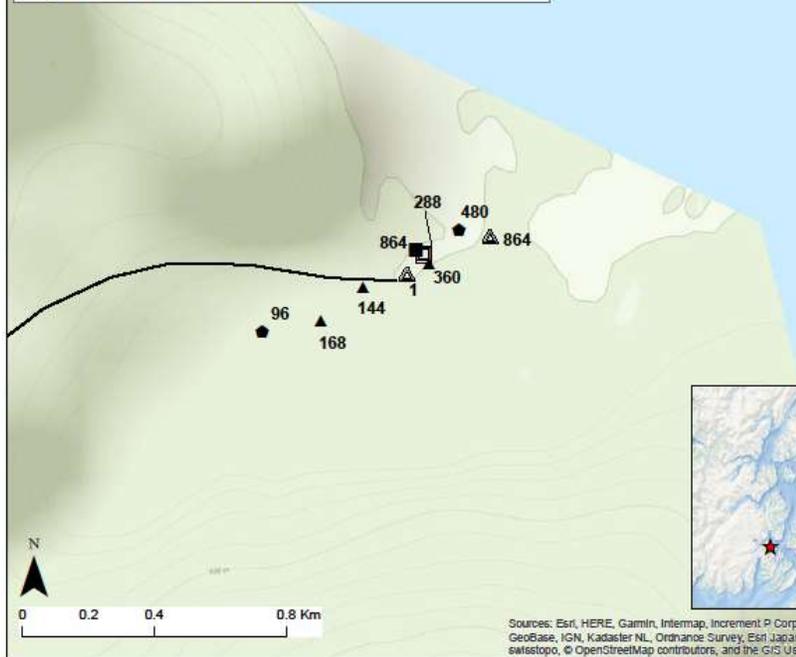
**HW Interaction Project - Erb Creek  
Pink Salmon Processing Areas and Samples  
August 29 - September 4, 2017**



| # | Samples   |
|---|-----------|
| ▲ | 8/29/2017 |
| ◻ | 8/31/2017 |
| ◯ | 9/2/2017  |
| ▲ | 9/3/2017  |
| ◻ | 9/4/2017  |
| — | Erb C     |

Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Erb Creek  
Pink Salmon Processing Areas and Samples  
September 6-14, 2017**



| # | Samples   |
|---|-----------|
| ▲ | 9/6/2017  |
| ◻ | 9/8/2017  |
| ◯ | 9/10/2017 |
| ▲ | 9/12/2017 |
| ◻ | 9/14/2017 |
| — | Erb C     |

Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

**Hogan Bay Creek**  
226-30-16810  
Pink Salmon Fitness

**2017 Samples Collected and Run Timing:** Pink salmon sampling for fitness and hatchery fraction at Hogan Creek from July 30<sup>st</sup> to September 20<sup>th</sup> yielded 18,836 samples over 45 visits. Sampling efforts at Hogan Creek exceeded all previous years. Peak fish counts also exceeded all previous years by a large margin. The run peaked on September 2<sup>nd</sup> when 20,600 live pink salmon were counted in the stream. After that date the rate of spawning deaths increased rapidly, however, fresh fish continued to enter the bay and the stream. The crew saw salmon jumping and actively feeding in the bay and at the mouth of the bay up to the date of departure. Regular rainfall this season maintained the water level in the creek and allowed unrestricted fish passage all season long. Due to the large run size, the rate of fish deaths quickly exceeded the crew's rate of sampling and large numbers of carcasses went un-sampled and eventually washed out of the stream into large piles at the stream mouth. An estimated 4,100 live fish were still present in the stream when the crew demobilized at the end of the season. Some Chum, Sockeye, and Coho Salmon were observed in the stream but never in large numbers.

The stream was initially sampled by the 4-person crew on the Auklet, every other day. On August 23<sup>rd</sup> a 3-person crew working from the vessel Alexandra arrived and sampled the stream every day. Initially the Alexandra was planned to demobilize on the 10<sup>th</sup> of September, but deployment was extended due to the large run. The 3-person crew worked until the 14<sup>th</sup> and then a 2-person crew finished the season on September 20<sup>th</sup>.

Estimating the total run size is complicated by the difficulty of tracking the number of carcasses that have washed out to sea or have decomposed completely in the large piles that formed at the stream mouth. The maximum number of alive and dead fish that was counted was 38,990. A rough estimation of decomposed and washed out fish brings the total run size to 45,000 to 55,000 fish. If this is accurate we were able to sample between 34% and 42% of the total run.

**Extent of Stream Sampled:** In the early season, when the number of dead fish was manageable, the stream was surveyed to the maximum extent the pink salmon are capable of accessing. The accessible length is limited by a series of large cascades too high for the fish to jump. As the death rate climbed a sampling strategy was developed that emphasized collecting as many samples as possible each day, but also ensuring adequate samples were collected along the entire stream length on a regular basis. This strategy involved sampling fish in just the lower, intertidal part of the stream 2 out of every 3 days. This is where the carcasses are most concentrated and can be sampled with maximum efficiency. Every 3<sup>rd</sup> day the upper portion of the stream was counted and sampled.

**Unusual Events:** Large numbers of stranded fish were recorded largely due to a regular cycle of heavy rain, which would flood side channels, followed by a day or two of little rain causing the channels to go dry with fish in them. Twice there were exceptionally intense rain storms that caused the creek to rise to very high levels and led to nearly all the carcasses in the upper stream to wash out beyond the stream mouth, where they were no longer accessible for sampling. There was also two intense wind events during the season. The first storm we stayed in Hogan Bay, but

the live-aboard vessel drug anchor through the night. For the second storm the crew moved to a neighboring bay (Snug Harbor) with better protection and weathered the storm fine that night. The move to shelter required two days be cut short on the stream.

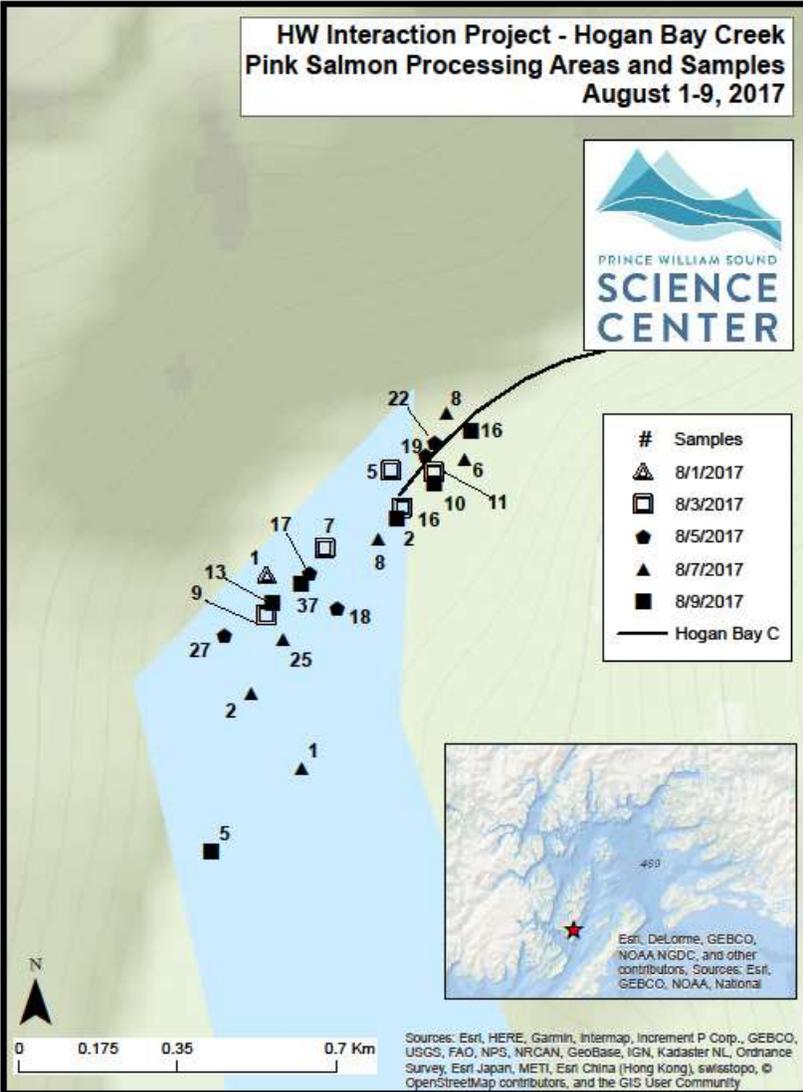
**Access, Safety and Logistics Issues:** Access to the upper stream was limited on one occasion (August 29<sup>th</sup>) by exceptionally high stream flow. Black bear presence at the stream has increased drastically from previous years. The crew typically saw 4 to 10 bears a day, with a maximum of 16 unique individuals seen in one day. An estimated 25-30 bears inhabited the stream valley. Most encounters resulted in either the bear(s) walking away or the bear and humans both carrying on while respecting each other's space. On a handful of occasions the crew was forced to abandon a sample area or change their sampling plan for that day in order to avoid conflict with bears. Only two encounters involved aggressive/defensive behavior and they were successfully deescalated before any attack occurred or bear spray was needed. Further details on safety can be found in the 2017 Hogan Bay Safety Report and details on logistics/tactics can be found in the daily logs.

**Recommendations and Other Notes:** More samples might be collected if the sampling deployment continued one or two weeks later. This year crews were at Hogan Bay before the run began. However, the late-season, 2-person crew would have likely been able to continue their rate of 600 samples a day for at least another week beyond their last day.

**HW Interaction Project - Hogan Bay Creek  
Pink Salmon Processing Areas and Samples  
August 1-9, 2017**



- | # | Samples     |
|---|-------------|
| ▲ | 8/1/2017    |
| □ | 8/3/2017    |
| ● | 8/5/2017    |
| ▲ | 8/7/2017    |
| ■ | 8/9/2017    |
| — | Hogan Bay C |



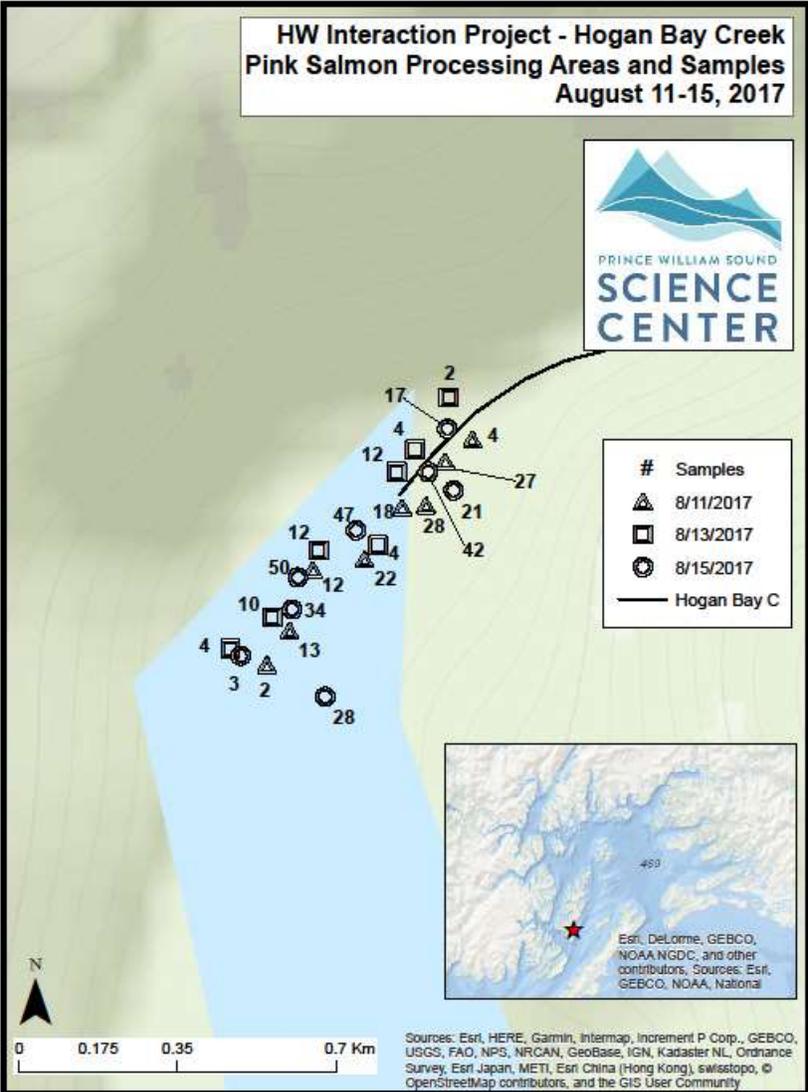
0 0.175 0.35 0.7 Km

Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Hogan Bay Creek  
Pink Salmon Processing Areas and Samples  
August 11-15, 2017**



| # | Samples     |
|---|-------------|
| ▲ | 8/11/2017   |
| ◻ | 8/13/2017   |
| ⊙ | 8/15/2017   |
| — | Hogan Bay C |

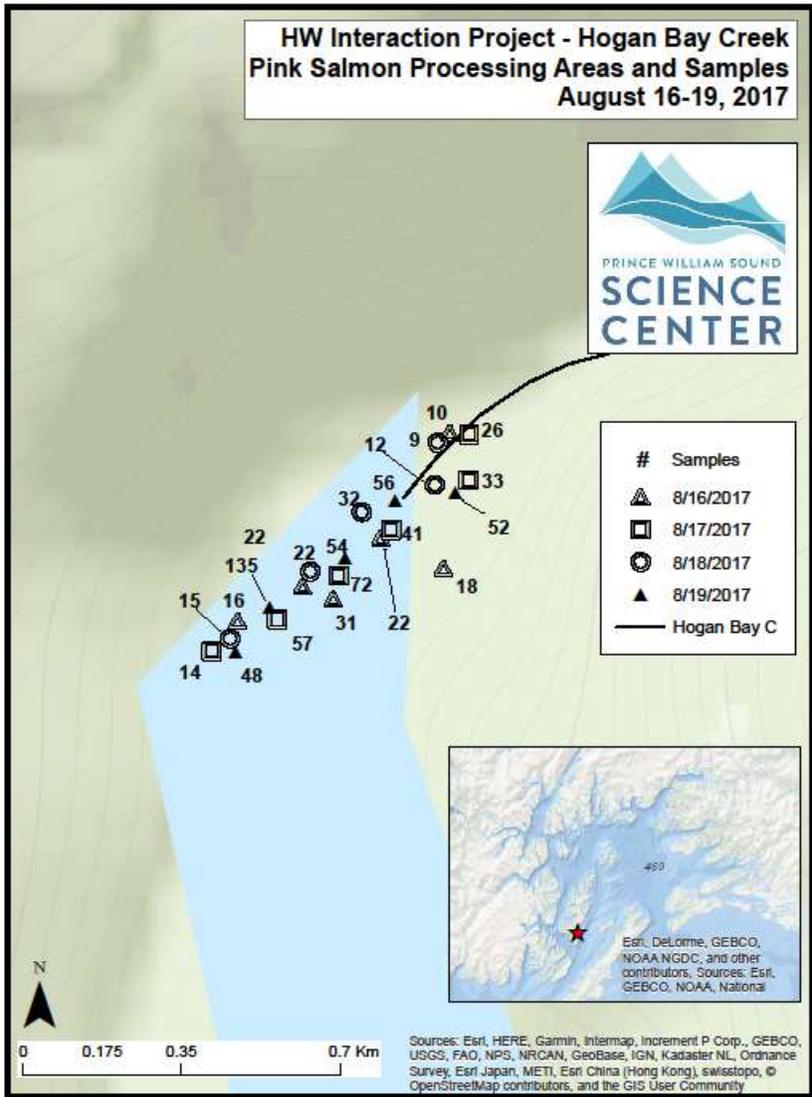


Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Hogan Bay Creek  
Pink Salmon Processing Areas and Samples  
August 16-19, 2017**



| # | Samples     |
|---|-------------|
| ▲ | 8/16/2017   |
| ◻ | 8/17/2017   |
| ⊙ | 8/18/2017   |
| ▲ | 8/19/2017   |
| — | Hogan Bay C |

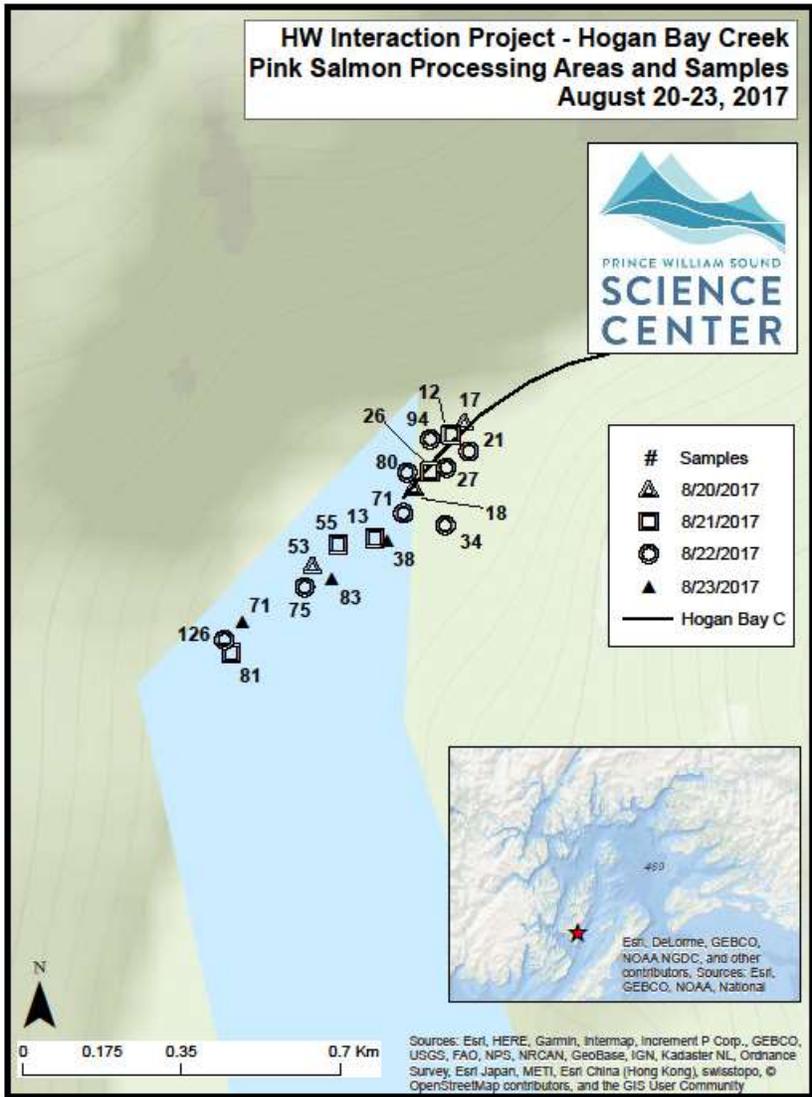


Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Hogan Bay Creek  
Pink Salmon Processing Areas and Samples  
August 20-23, 2017**



| # | Samples     |
|---|-------------|
| ▲ | 8/20/2017   |
| ◻ | 8/21/2017   |
| ⊙ | 8/22/2017   |
| ▲ | 8/23/2017   |
| — | Hogan Bay C |

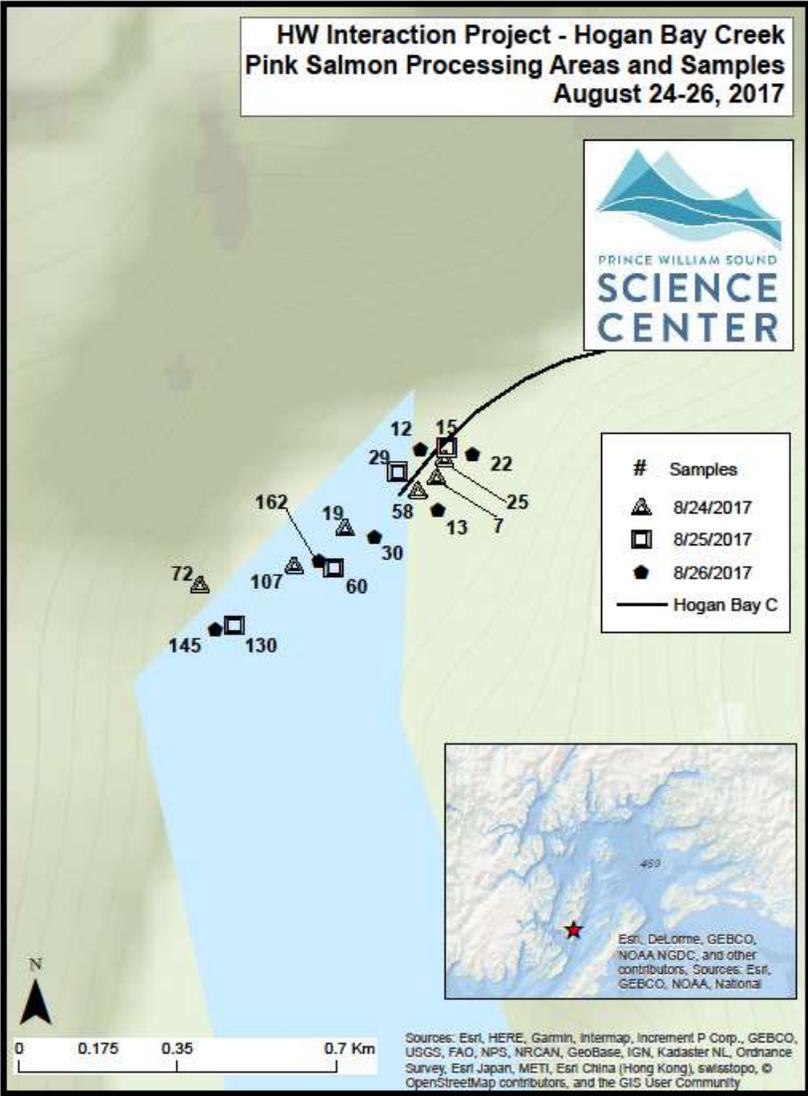


Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Hogan Bay Creek  
Pink Salmon Processing Areas and Samples  
August 24-26, 2017**



| # | Samples     |
|---|-------------|
| ▲ | 8/24/2017   |
| ■ | 8/25/2017   |
| ● | 8/26/2017   |
| — | Hogan Bay C |

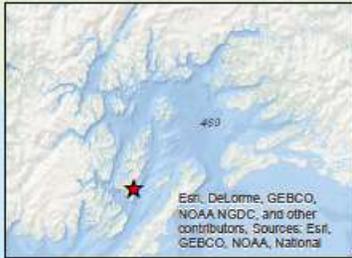
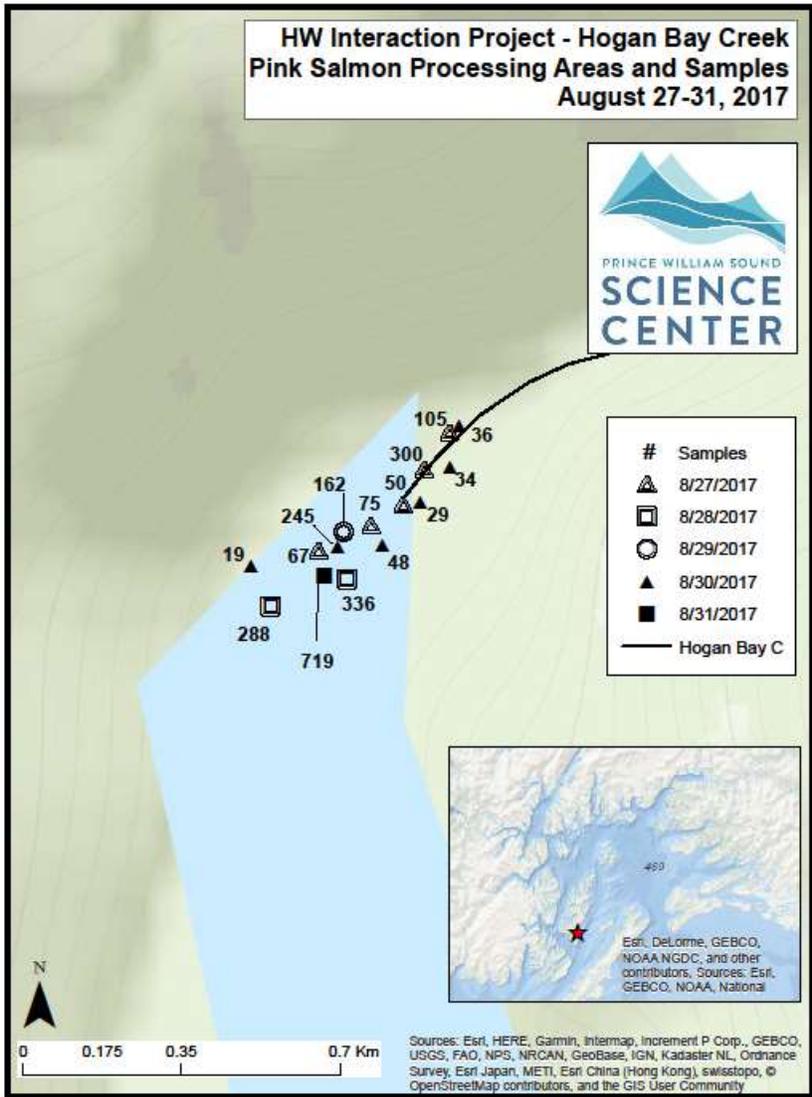


Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Hogan Bay Creek  
Pink Salmon Processing Areas and Samples  
August 27-31, 2017**



| # | Samples     |
|---|-------------|
| ▲ | 8/27/2017   |
| ◻ | 8/28/2017   |
| ◯ | 8/29/2017   |
| ▲ | 8/30/2017   |
| ■ | 8/31/2017   |
| — | Hogan Bay C |

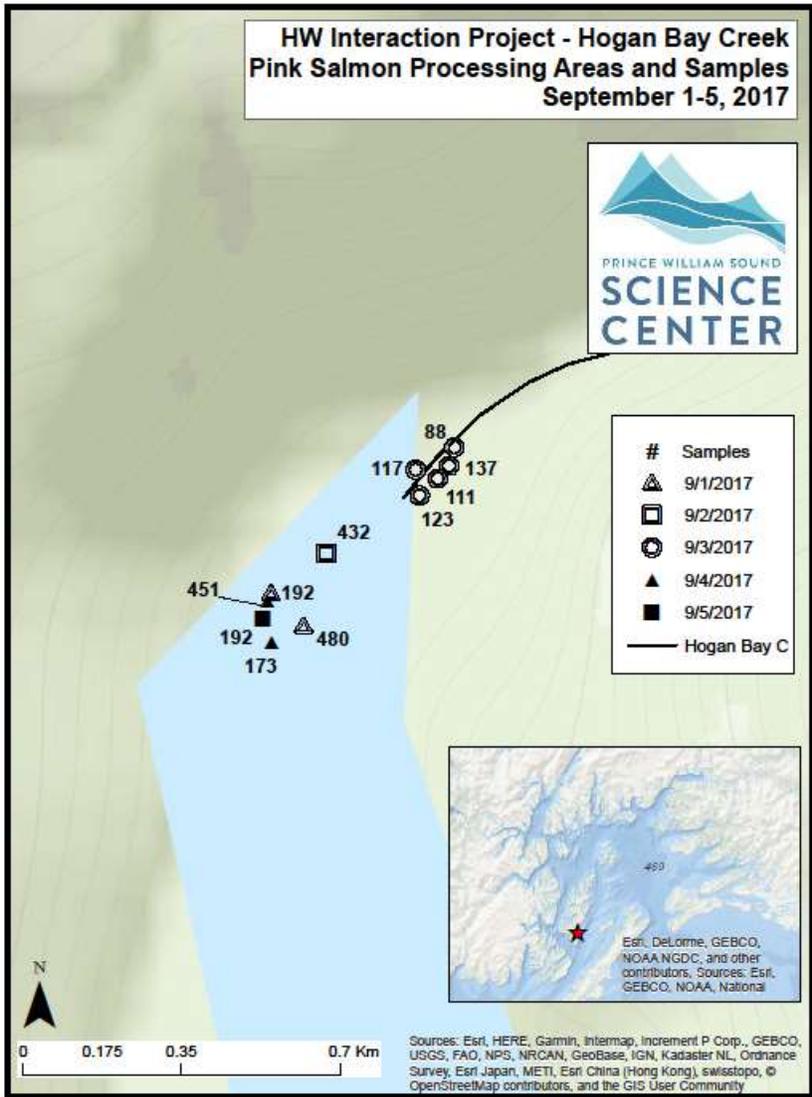


Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Hogan Bay Creek  
Pink Salmon Processing Areas and Samples  
September 1-5, 2017**



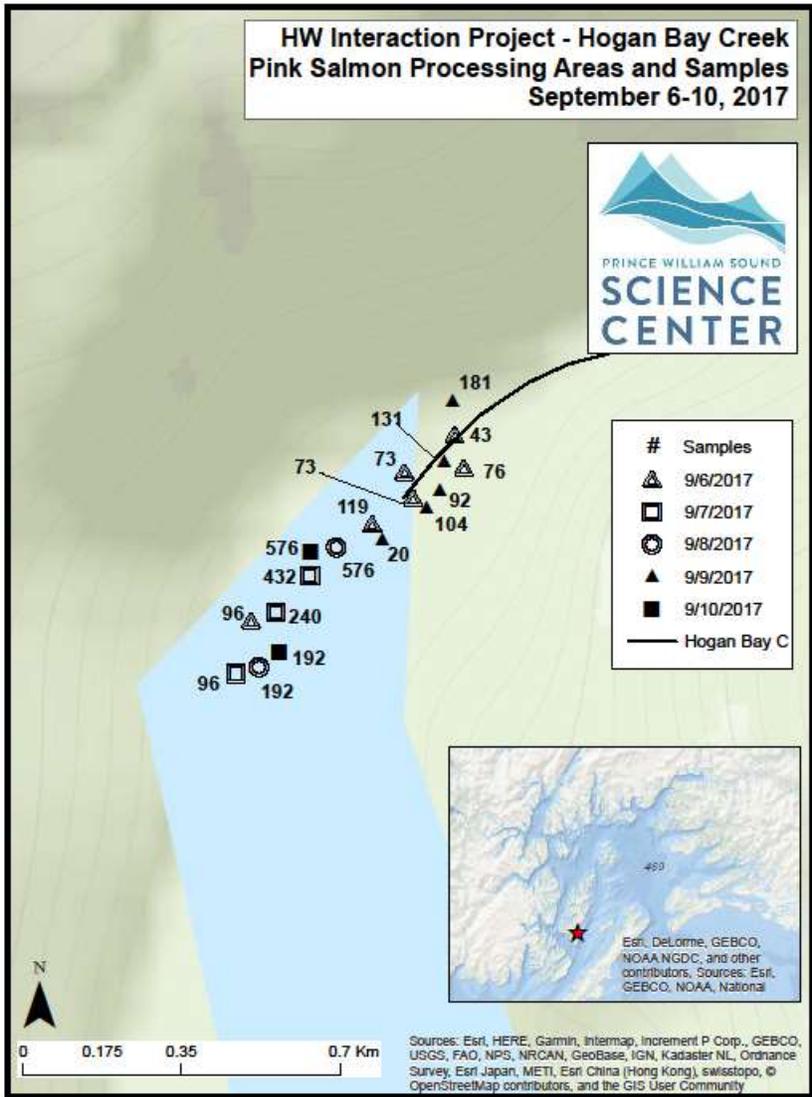
| # | Samples     |
|---|-------------|
| ▲ | 9/1/2017    |
| ◻ | 9/2/2017    |
| ⊙ | 9/3/2017    |
| ▲ | 9/4/2017    |
| ■ | 9/5/2017    |
| — | Hogan Bay C |



**HW Interaction Project - Hogan Bay Creek  
Pink Salmon Processing Areas and Samples  
September 6-10, 2017**



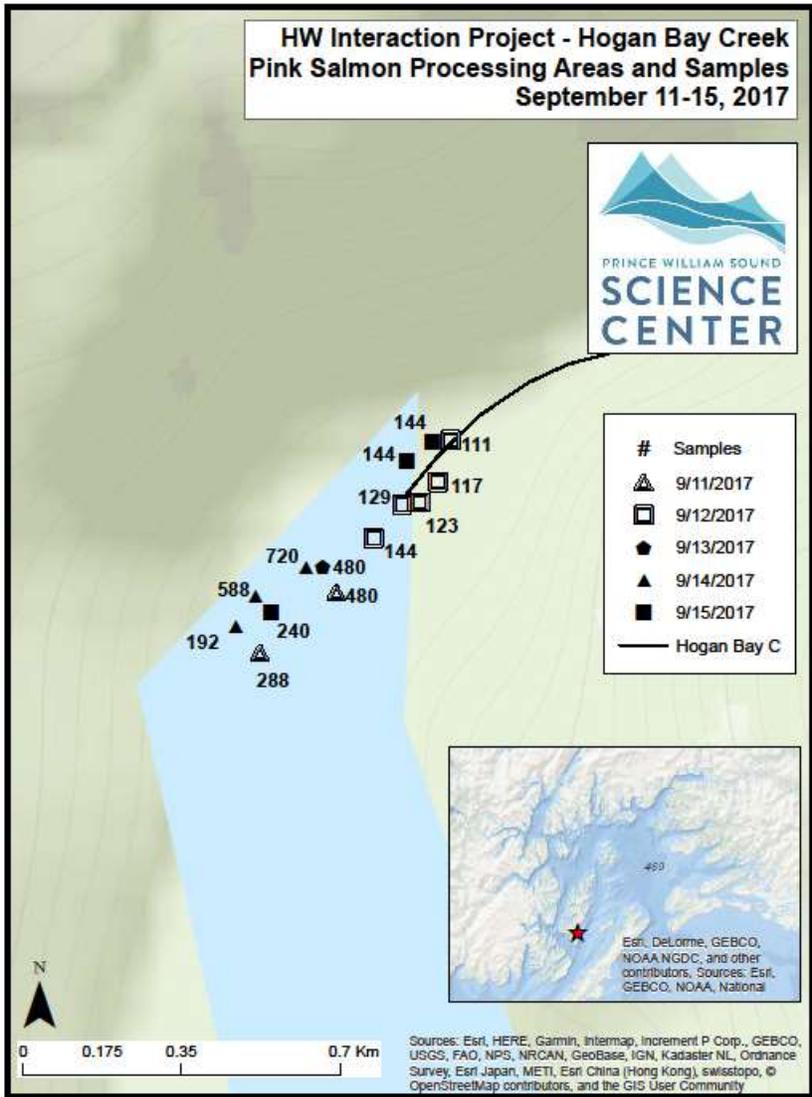
| # | Samples     |
|---|-------------|
| ▲ | 9/6/2017    |
| ◻ | 9/7/2017    |
| ⊙ | 9/8/2017    |
| ▲ | 9/9/2017    |
| ■ | 9/10/2017   |
| — | Hogan Bay C |



**HW Interaction Project - Hogan Bay Creek  
Pink Salmon Processing Areas and Samples  
September 11-15, 2017**



- | # | Samples     |
|---|-------------|
| ▲ | 9/11/2017   |
| □ | 9/12/2017   |
| ● | 9/13/2017   |
| ▲ | 9/14/2017   |
| ■ | 9/15/2017   |
| — | Hogan Bay C |

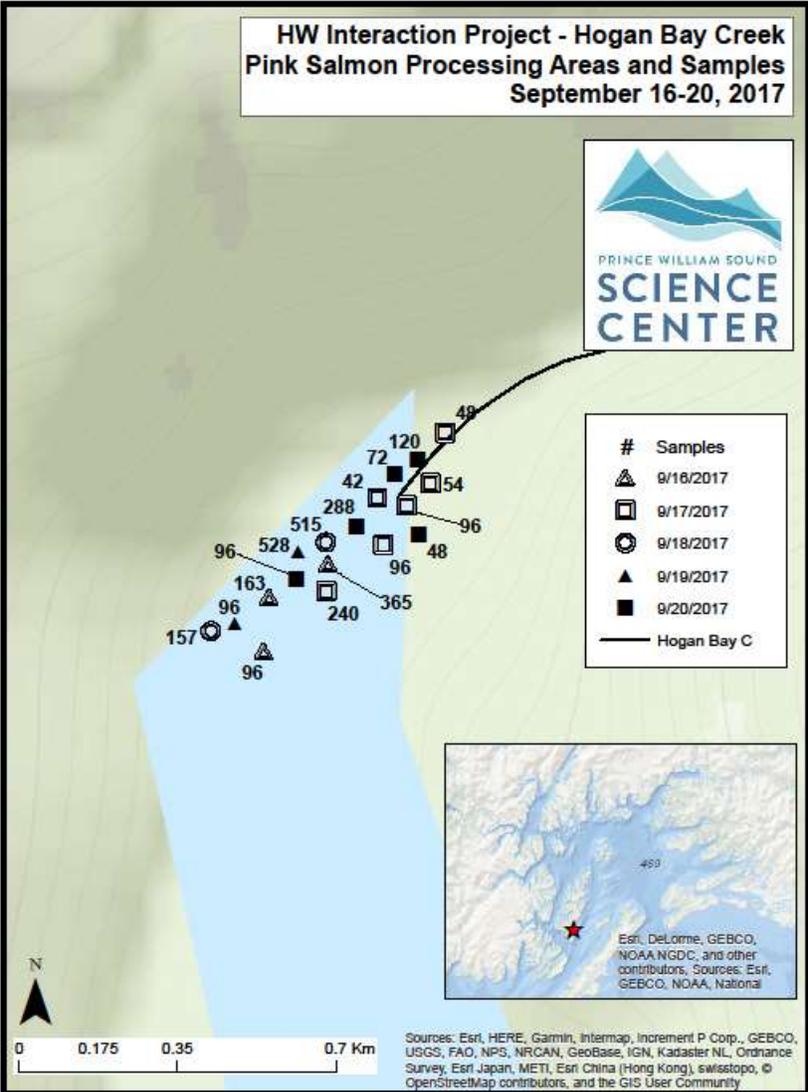


Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Hogan Bay Creek  
Pink Salmon Processing Areas and Samples  
September 16-20, 2017**



| # | Samples     |
|---|-------------|
| ▲ | 9/16/2017   |
| ◻ | 9/17/2017   |
| ⊙ | 9/18/2017   |
| ▲ | 9/19/2017   |
| ■ | 9/20/2017   |
| — | Hogan Bay C |



0 0.175 0.35 0.7 Km

Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

**Stockdale Creek**  
227-20-17520  
Pink Salmon Fitness

**2017 Samples Collected and Run Timing:** Pink salmon sampling for fitness and hatchery fraction at Stockdale Creek from July 31<sup>st</sup> to September 13<sup>th</sup> yielded 11,566 samples over 37 visits. On the first visit of the season (7/31/2017), 217 live Pink Salmon were counted with no live fish observed outside of the intertidal. Spawning activity was first observed on our 9<sup>th</sup> (8/10/2017) visit. Flow remained low in Stockdale Creek until August 11<sup>th</sup>, which may have inhibited fish passage in the early season. Consistent progress upstream was observed until visit 13 (8/14/2017) at which point fish extent matched or exceeded our survey extent. Verifiably post-spawned carcasses were first observed on visit 13 (8/14/2017), with prior dead counts consisting of predated fish only.

In-stream live counts increased steadily until visit 21 (8/25/2017) at which point 9,294 live and 464 dead pinks were counted in the main channel up to the base of the first canyon. Live counts decreased from this point though ocean-bright fish were observed in later visits, suggesting a small influx of fish after the peak. On our final visit on September 9<sup>th</sup>, 1,435 live and 8,183 dead fish were counted in the main channel up to the base of the first canyon.

In 2017, sampling coverage was estimated at 40% of a 30,000 fish run. The whole run size was calculated by first estimating a proportion of observed dead that were sampled over the whole of the season. The known cumulative samples collected were divided by this proportion to give a total dead count for the season. Live fish counted on our final visit were added to this count to provide a total run size.

**Extent of Stream Sampled:** In concurrence with 2017 protocol and prior year's efforts, sampling was spread over the entirety of the system with greatest effort applied to the areas of highest fish concentration. In Stockdale Creek, the first 1.5km from the intertidal up to the divergence of braids below the first canyon held the greatest concentration of live and dead fish. When dead numbers became too great to sample the entire stream in a day, the stream was split in half and alternating visits were applied to the regions above and below the entrance to the meadows.

On visit 35, (9/9/2017), a full count was performed from the lowest reaches of the intertidal up to a one meter logjam with meadows on either side. While passable at very high flow, no carcasses were observed in the logjam despite numerous dead carcasses immediately below. This observation suggests that this feature may have served as an extent to fish passage this season. On visit 35, 18.9% of the dead and 18.8% of the total live were counted in the 2 km surveyed above the base of the canyon. This spatial distribution of live fish concentration was found to vary in response to high flow events, though water flow had remained normal for three days prior to this count.

The left tributary, which begins at the base of the confluence, was surveyed as an independent entity at least every three days as stream flow allowed. Fish extent in the tributary was observed 1 km upstream from its base. At this location, substrate changes from gravel to large cobble and the gradient steepens.

**Unusual Events:** Unlike previous years, no mass-death stranding events were observed at Stockdale Creek in 2017. Large schools of Dolly Varden were observed regularly from visit 3 (8/3/2017) to visit 37 (9/13/2017). Despite frequent signs of predation, particularly early in the season, no bears were encountered.

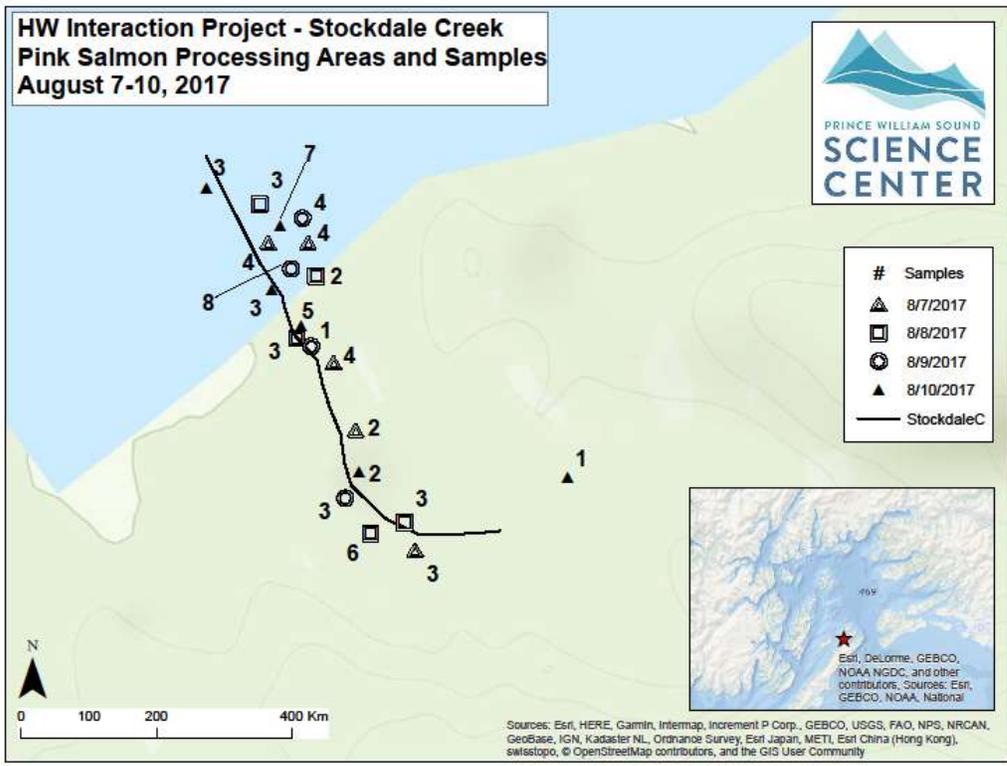
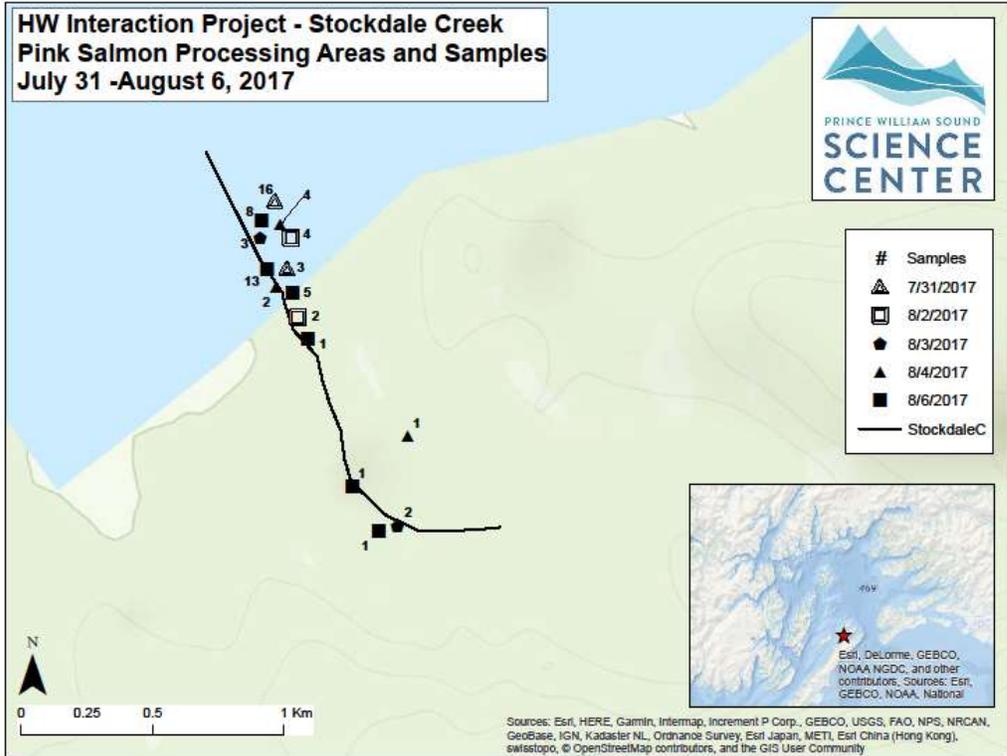
**Access, Safety, or logistical issues:** The muskeg meadow system running stream-right from the upper intertidal to the first logjam was used frequently this season to access the upper portion of the stream and worked well as halfway point at which to divide sampling efforts between days. These meadows also served as an invaluable escape route in high flow conditions. On six visits this season, high water made upstream passage dangerous and inhibited survey efforts. On visit 32 (9/5/2017), after three hours of heavy rain, the stream rose from normal to bank-full and was dangerous to enter at any location. Sampling crews should exercise extreme caution when surveying Stockdale Cr. in high-flow conditions, particularly when traveling around logjams.

**Recommendations, changes, and other notes:** Regular surveys of the left tributary introduce a disproportional sink of the sampling crew's time. Not at all surveying the tributary or reducing efforts to a few visits per season would allow thousands more samples to be collected from the main channel of Stockdale Creek. This would provide a more accurately proportioned sample distribution of the entire stream.

The Pink Salmon run at Stockdale Creek overlaps with late Chum Salmon in early August and a small Coho Salmon run in early July. By late August there are few enough chum present that it is possible, with a four person crew, to assign two counters to both live and dead pinks which allows averaging.

The temperature/depth logger deployed in 2015 was read and redeployed on our final, 9/13/2017 visit. It was readjusted to sit at a depth of 25cm.

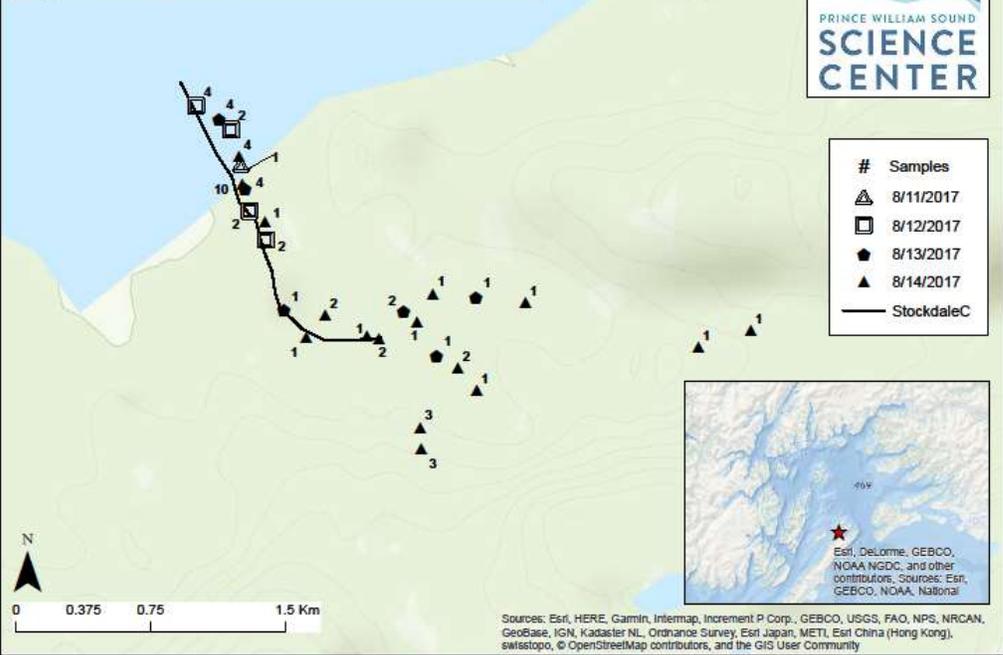
A crew of four technicians was adequate to survey Stockdale Cr. until 8/23/2017, at which point the number of unsampled dead fish exceeded our every-other day sampling capacities. Many carcasses were lost to rot. An additional technician working from the August 24<sup>th</sup> through the end of the season would allow greater coverage during the peak season. Additionally, shifting the entire sampling season back one week, as to begin on September 7<sup>th</sup> and end on September 20<sup>th</sup> would maximize sample collection from the entire run.



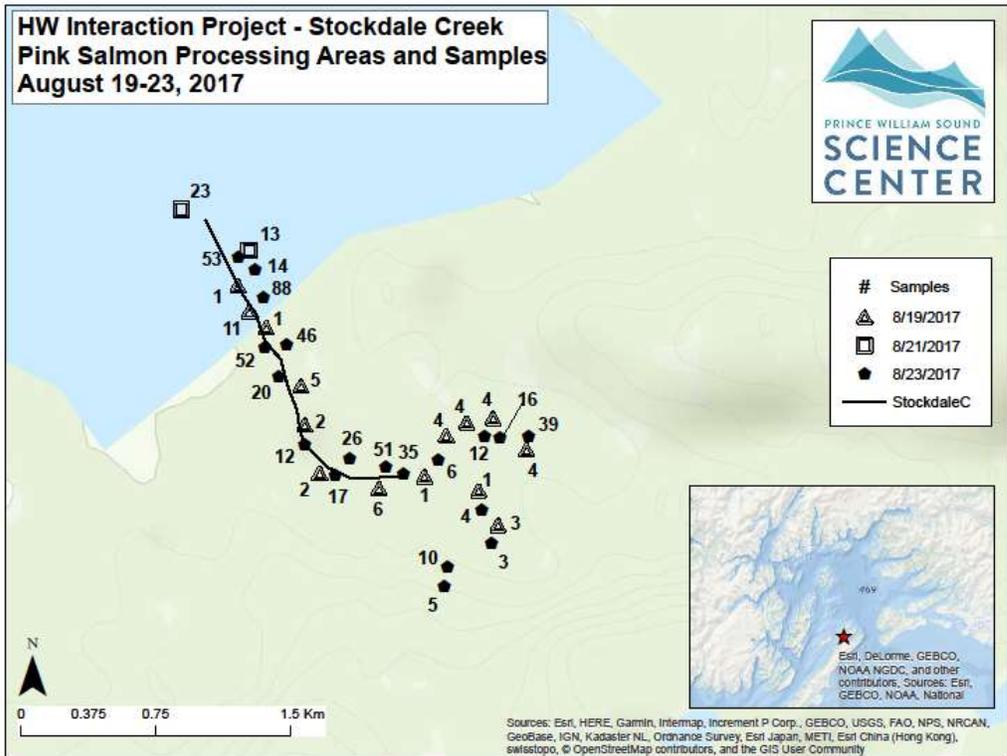
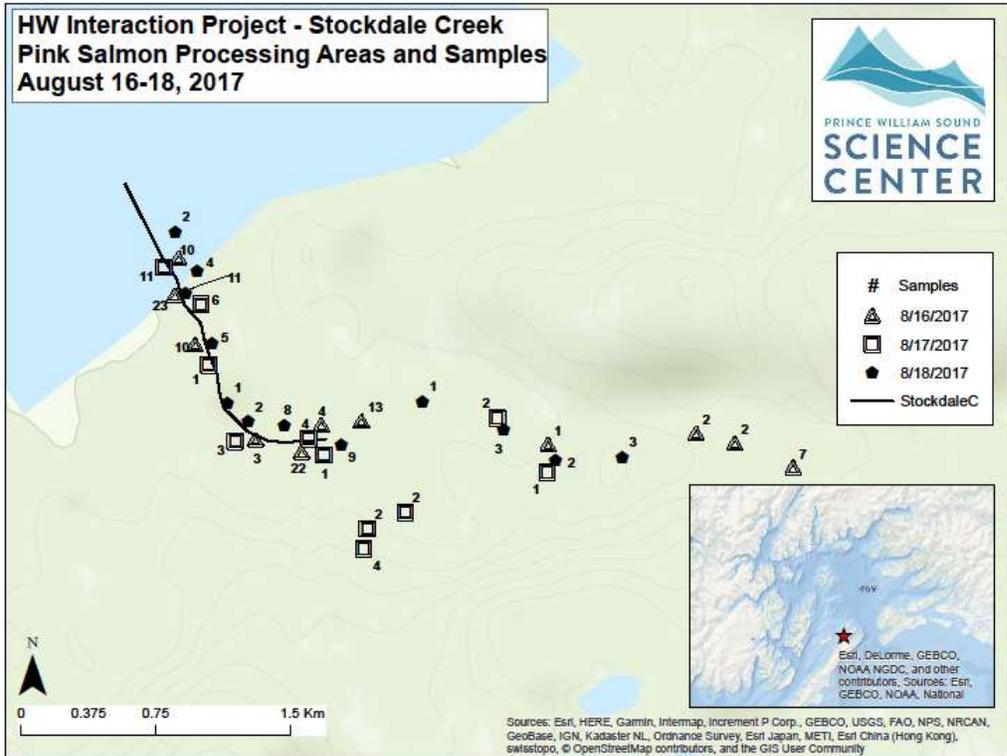
**HW Interaction Project - Stockdale Creek  
Pink Salmon Processing Areas and Samples  
August 11-14, 2017**

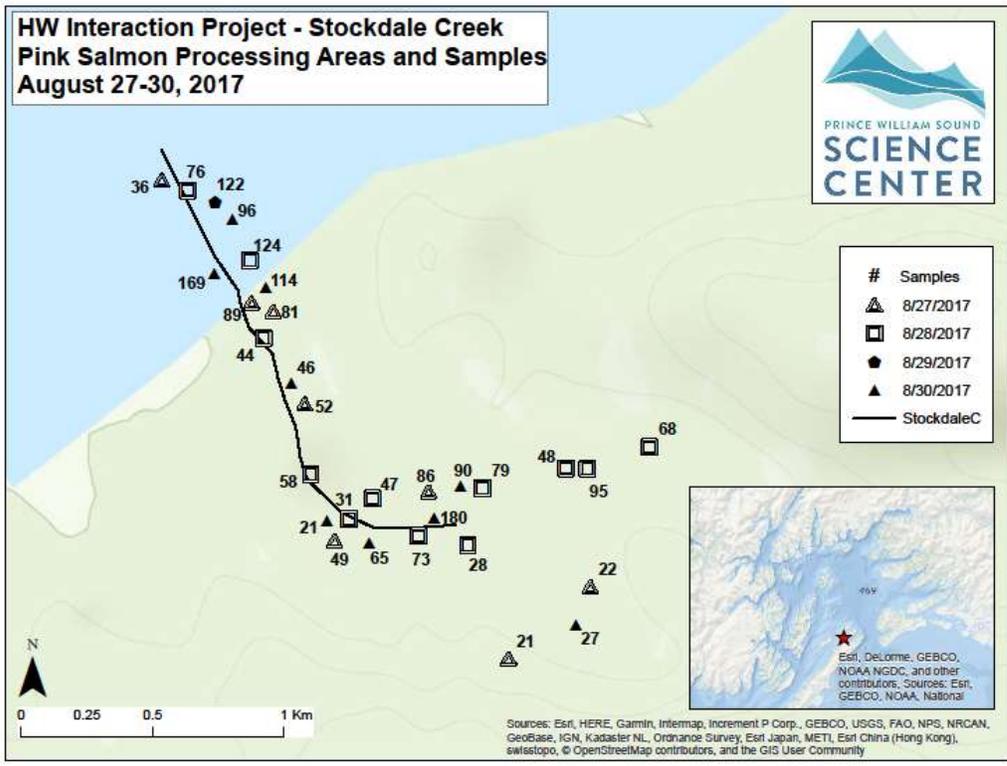
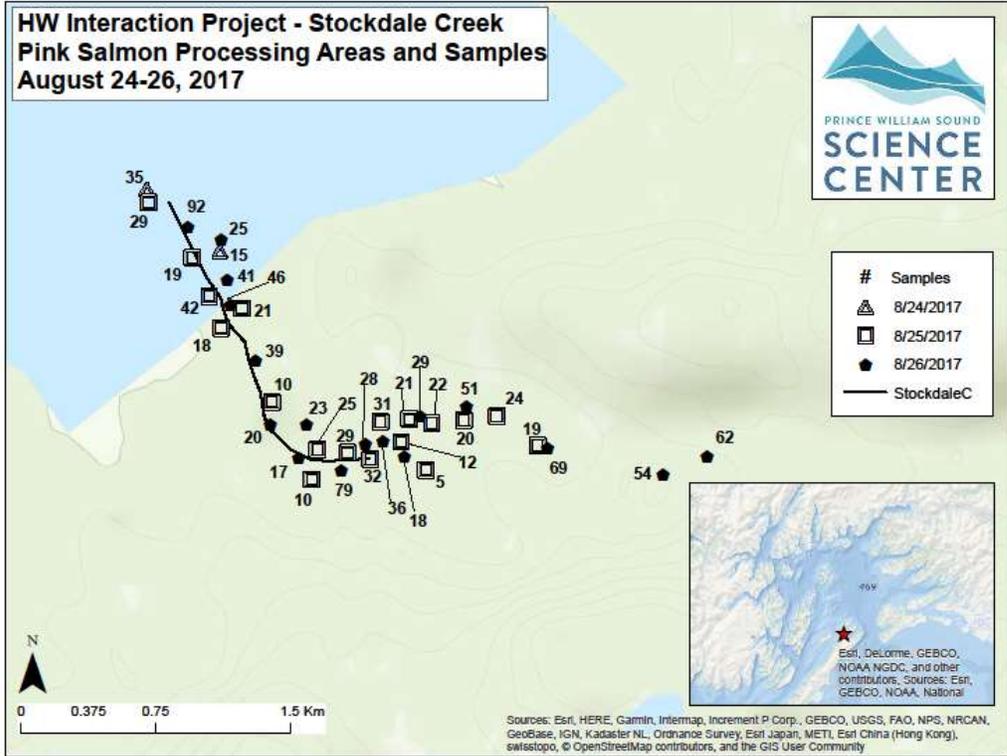


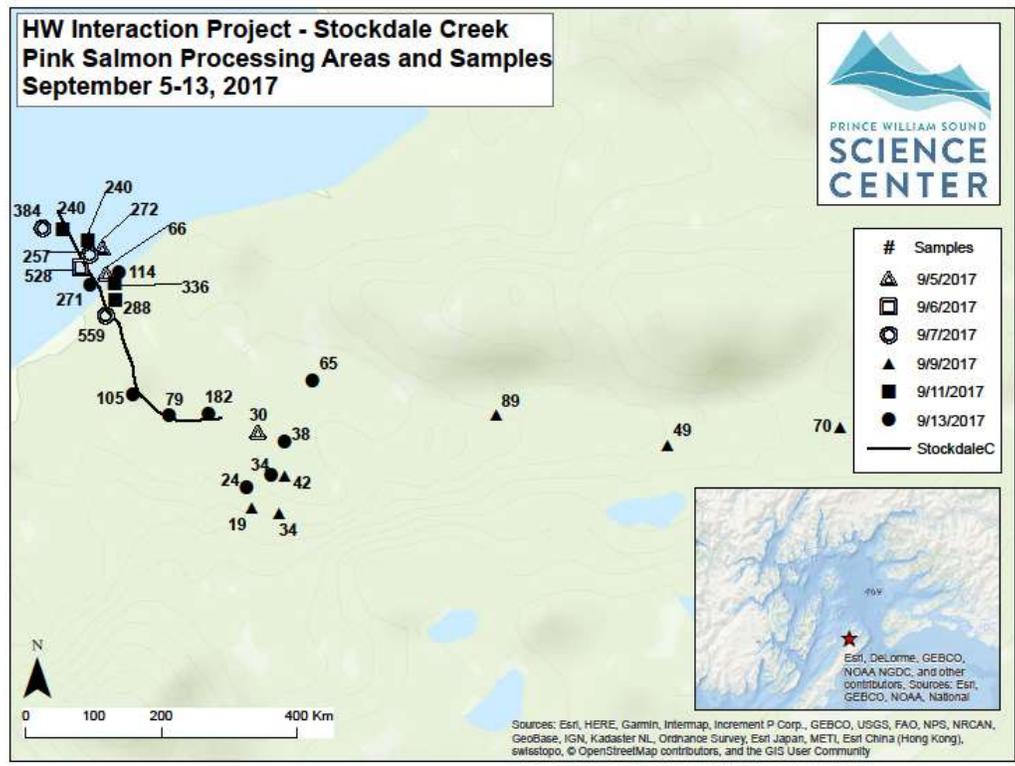
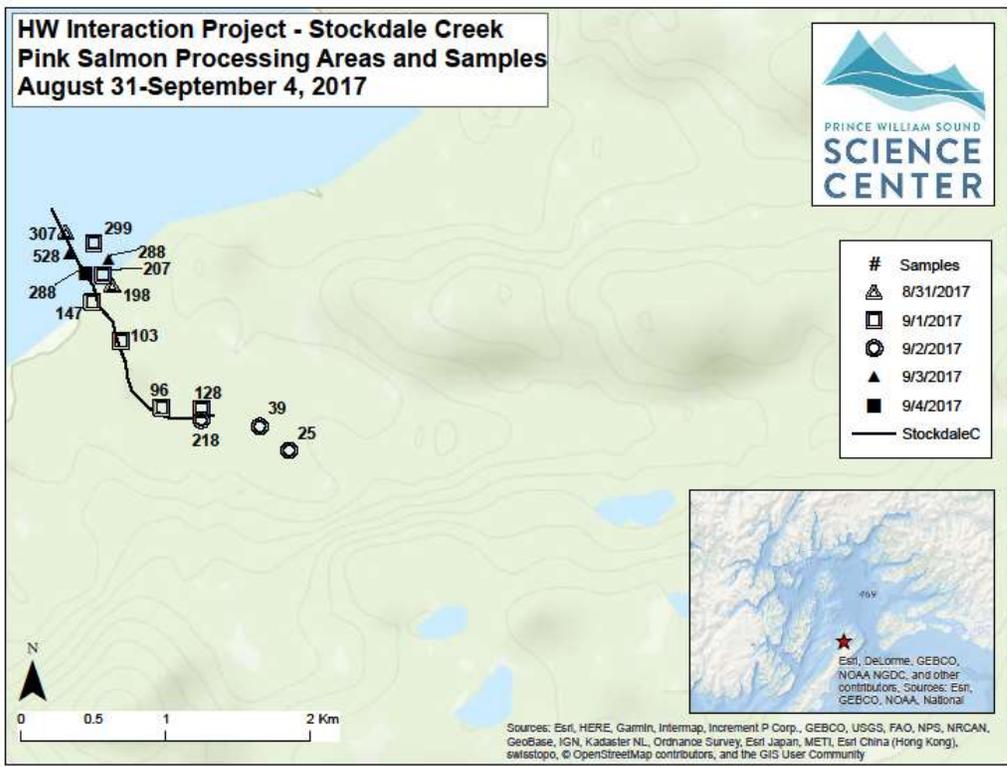
| # | Samples    |
|---|------------|
| ▲ | 8/11/2017  |
| □ | 8/12/2017  |
| ● | 8/13/2017  |
| ▲ | 8/14/2017  |
| — | StockdaleC |



Sources: Esri, HERE, Garmin, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community







**Gilmour Creek**  
227-20-17480  
Pink Salmon Fitness

**2017 Samples Collected and Run Timing:** Pink Salmon sampling for fitness and hatchery fraction at Gilmour Creek from July 31<sup>st</sup> to September 12<sup>th</sup> yielded 5,247 samples over 23 visits. Gilmour Creek's two main branches were surveyed on every visit and their cumulative counts are summarized. On visit one (July 31<sup>st</sup>), two live Pink Salmon were observed. On visit six (8/10/2017), the first live Pink Salmon was observed in the tributary below the lake. Flow remained low at Gilmour Creek until August 10<sup>th</sup> which may have inhibited early season fish passage. In-stream live counts remained low late into the season, despite adequate stream flow. On visit 14 (8/25/2017) 1,056 live and dead Pink Salmon were counted. On visit 15 (8/27/2017) 2,178 live were counted in the same survey area, suggesting a substantial influx of pre-spawn fish. Counts peaked on visit 18 (9/2/2017) at 2,761 live and 770 dead, and then decreased gradually through our last visit on 9/12/2017 at which point 1,102 live and 2,904 dead remained. The total fish count, consisting of live plus dead, increased from visit 18 until the end, suggesting a small influx of prespawn fish after the peak.

In 2017, sampling coverage was estimated at 80% of a 6,500 fish run. The whole run size was calculated by adding our cumulative samples for the season to the number of live and dead fish left unsampled after our final visit. This was possible due to complete sampling coverage (every dead fish observed was sampled) in all but the last visit.

**Extent of Stream Sampled:** Full surveys of both branches were performed on every visit, with exceptions of visits 16 and 17, (8/29/2017, and 8/31/2017) during which stream flow was dangerously high. The left tributary below the lake was regularly surveyed up to a set of double cascades, the upper of which likely serves as a permanent barrier to fish passage. On visit 12 (8/23/2017), after two days of heavy rain, live pink salmon were observed in the main channel above the debris jam. Passage upstream beyond this location was observed in 2015 but not in 2016. A two meter bedrock slide was identified 300m upstream from the debris jam and served as a barrier to fish passage this season. Late in the season, from visit 21 (9/8/2017) on, spawning activity in the tributary below the lake exceeded that in the main channel.

**Unusual Events:** On visit 20, (9/6/2017) large schools of Dolly Varden were observed in the deep pool below the fork and in the upper pools of the tributary below the lake. On this visit, hundreds of Dollies were observed attempting to ascend the cascades below the lake. This phenomenon was not observed on any other visit. Despite frequent signs of predation, no bears were encountered on Gilmour Creek in 2017.

**Access, Safety, or logistical issues:** Two distinct strategies were employed over the course of the season to survey both branches in a single visit. For the first 14 visits (7/31-8/25/2017), the entire crew ascended the main channel to its survey extent and walked West through a series of muskeg meadows to access the top of the tributary below the lake. This route takes 15 minutes and saves significant time that would be spent doubling back on each branch.

For visits 15 through 23 (8/27-9/12/2017), the entire crew of four worked together processing fish in the main channel up to the fork and then split up into two groups of two to survey each of

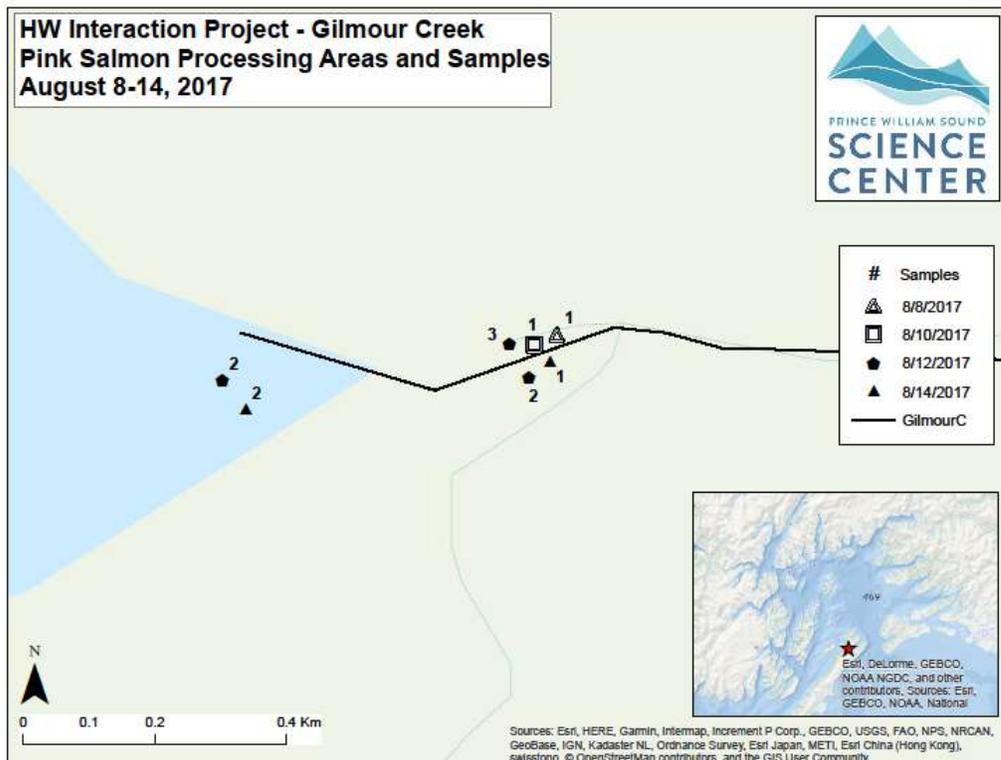
the branches independently. This strategy works well in the sample-sparse upper reaches of Gilmour Cr. Two groups of two can work more efficiently than a single group of four in processing areas with less than a tray of samples.

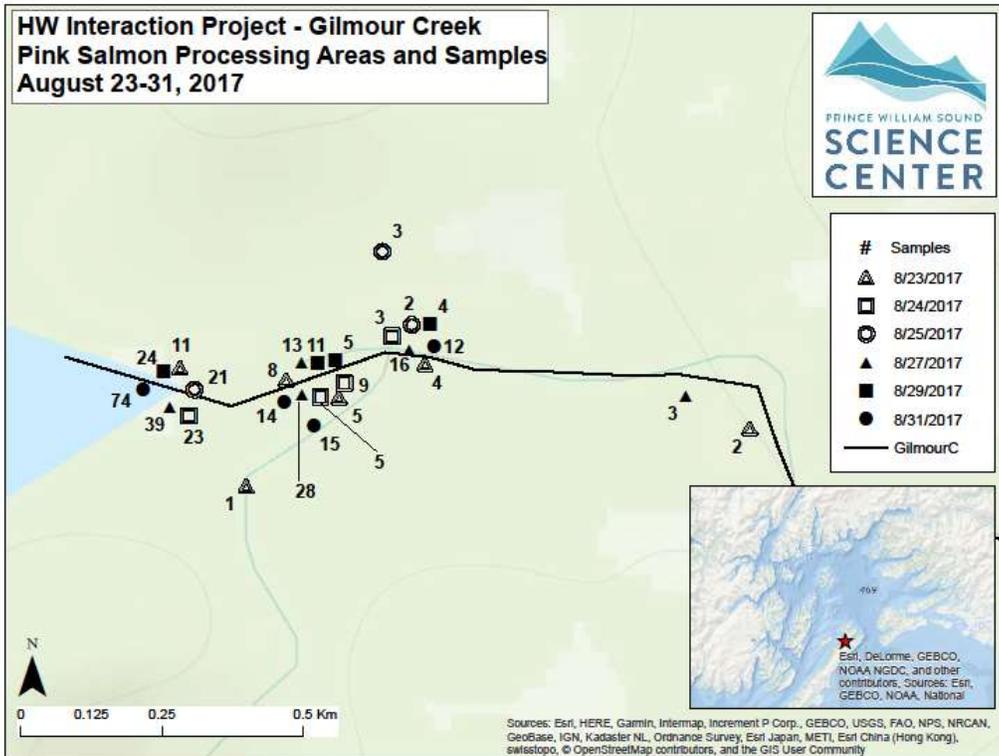
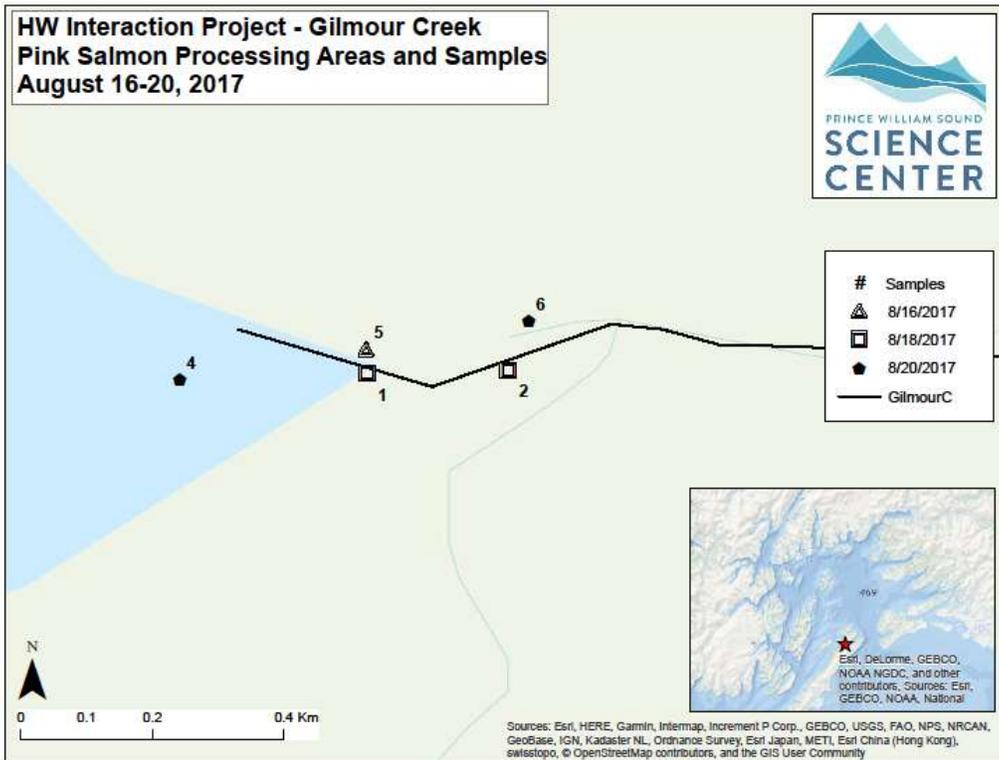
On two visits this season, high water made upstream passage dangerous so that full surveys were not possible. Extreme caution should be taken when descending the cascades below the lake during high flow events.

**Recommendations, changes, and other notes:** The Pink Salmon run at Gilmour Creek overlaps with late Chum Salmon in the early season. By late August there are few enough Chum Salmon present that it is possible, with a four person crew, to assign two counters to both live and dead pinks which allows averaging.

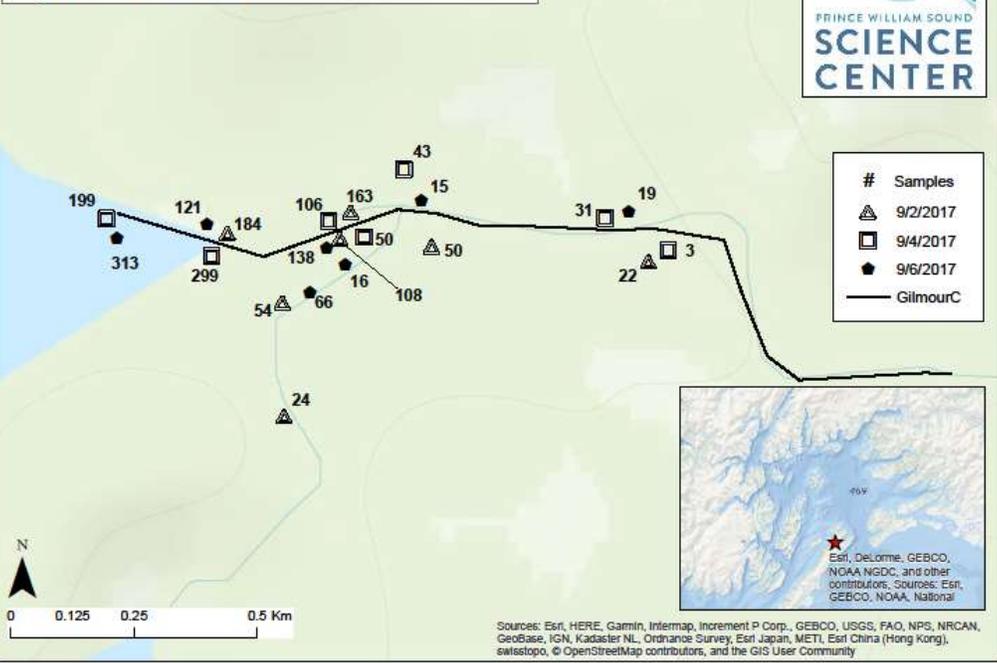
The Gilmour Cr. data logger, deployed in 2015, was found completely dry on our first visit (8/13/2017) and was relocated to its original location, 1 meter upstream. On our final visit on 9/12/2017, it was read and redeployed in the same location.

A crew of four technicians was adequate to fully sample Gilmour Creek every-other day for the entirety of the season. Shifting the entire sampling season back one week as to begin on September 7<sup>th</sup> and end on September 19<sup>th</sup> might maximize sample collection from the entire run.





**HW Interaction Project - Gilmour Creek  
Pink Salmon Processing Areas and Samples  
September 2-6, 2017**



**HW Interaction Project - Gilmour Creek  
Pink Salmon Processing Areas and Samples  
September 8-12, 2017**

