



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

Final Report for 2018

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

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ABSTRACT

This is the seventh 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 2018 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 2018 field season, 36,550 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 reproductive success (RRS). 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 natural-origin). An effort is still underway to read all otoliths from Stockdale and Hogan Bay creeks in 2017 and 2018. In addition, a subset of eight trays of otoliths collected from the other three fitness streams (Paddy, Erb, and Gilmour Creeks) were read. For this report we estimated hatchery fractions weighted by abundance (dead counts) for each stream. The following hatchery fractions were determined: Paddy Creek (7.6%), Erb Creek (6.7%), Hogan Bay (42.3%), Gilmour Creek (6.3%), and Stockdale Creek (5.7%). These values are low compared to previous year's estimates. A decision by the Science Panel was made in late 2018 to continue field work in three pedigree streams in 2019: Hogan Bay, Gilmour, and Stockdale Creeks. Results of the relative reproductive success of PWS Pink Salmon hatchery-origin and natural-origin spawners will be available following the final field sampling 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 seventh annual report, focusing on results from 2018 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 2018 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 2018, 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 2018 field sampling protocol was unchanged from 2017 and is presented in Appendix A. This report includes summaries of sample collection during 2018 for the DNA-based fitness studies. Given the interest of the ADF&G Gene Conservation Lab in early sample processing, we arranged two separate sample shipments to Anchorage in 2018. The first transfer was 58 sample trays to ADF&G on 22 August 2018 during a Cordova port call (consisting of samples from Stockdale, Gilmour, and Hogan Bay), followed by a final delivery of 903 sample trays by van to Anchorage on 22 September 2018. 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 2018. At the time of this report, not all the otoliths had been read for the 2018 field sampling, so please consider our estimates of hatchery fractions as preliminary.

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 natural origin. The now-completed results are estimates of the percent of hatchery-origin fish for each stream, district, and regional spawning population over three years (Knudsen et al. 2016, Knudsen et al., manuscript in prep). 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 (RRS) of hatchery- and natural-origin fish can be estimated for both males and females. This analysis is also referred to as pedigree analysis in this report.

Methods

Tissues for DNA extraction (primarily heart, but sometimes other tissues) were collected along with otoliths from adult Pink Salmon in 2018 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 2018 methods were identical to the methods used in 2017 and previous years (Knudsen et al. 2015a, b, Knudsen et al. 2016, Gorman et al. 2017, Gorman et al. 2018).

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, 2017 and 2018, to increase the statistical power of pedigree analyses through larger sample sizes.

Logistical Strategy

From August 5 through September 15, 2018, 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 2016-2018 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.

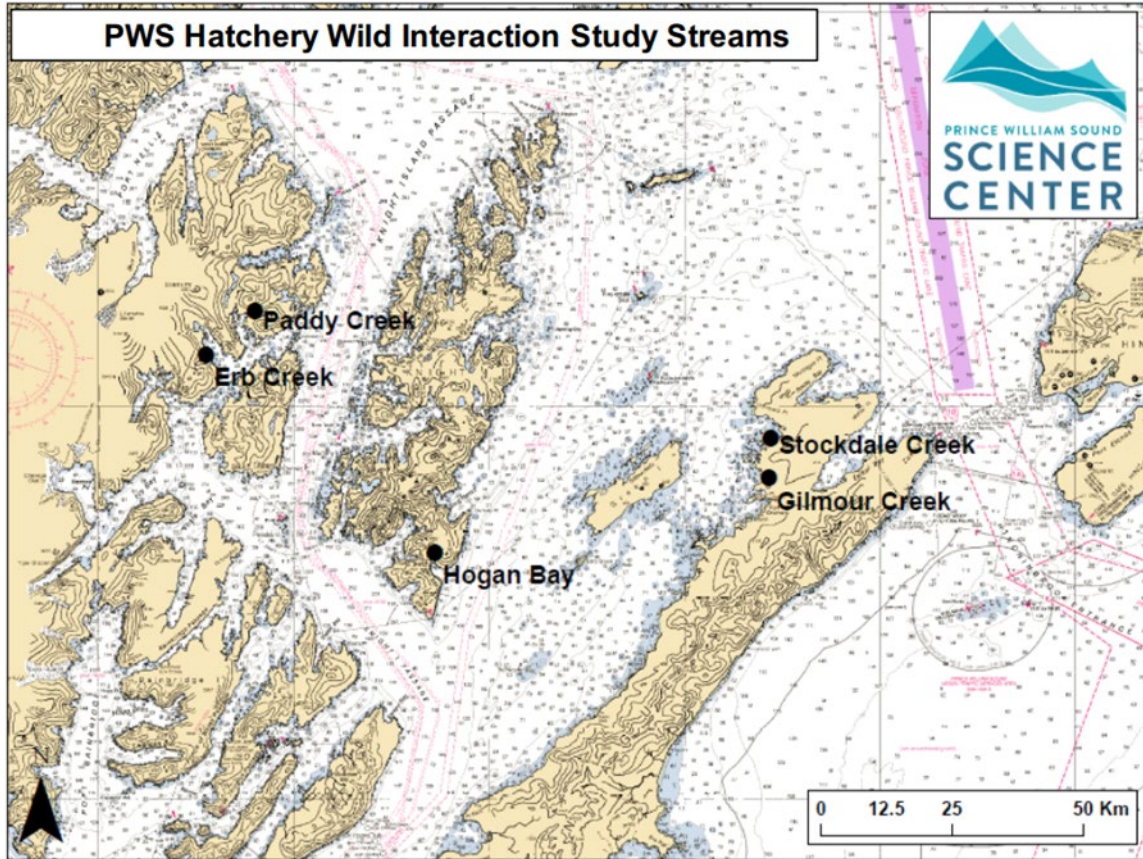


Figure 1. Streams sampled during 2018 for Pink Salmon otoliths and DNA tissues in PWS.

All PWS field crews were directly employed by PWSSC in 2018. 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 2018 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 30 to August 3, 2018. Training included boating, bear and firearms safety, CPR and First Aid, protocol review, tablet use, and data entry. The *M/V Auklet* and Paddy Bay field crews left port at 0700 on August 4, 2018, Paddy Camp was deployed later that day, and sampling began on August 5, 2018. An additional crew deployed to Hogan Bay on August 18, 2017 to sample at Hogan Bay during the peak run. Of note, there was only one returning member from previous field seasons, so the early training period was critical to get new recruits prepared for the field season. Julia McMahon, a UAF graduate student and previous crew leader, assisted in training

the field crew during the Cordova training week to assure the protocol was followed as in previous years.

For the first two weeks of the season (August 5-18, 2018), 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 sampled for 14 continuous days to those three fitness streams before making their only port call between August 21-22, 2018. While the *M/V Auklet* was in port, the *F/V 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 total number of stream visits in 2018 on Hogan Bay, Gilmour, and Stockdale Creeks were 35, 19, and 22, respectively (Table 1). The end of sampling was dictated by a dramatic drop in numbers of fish entering the streams (see Discussion).

The PWSSC camping crew was stationed in Paddy Bay from August 4 to September 12, 2018. The Paddy crew was responsible for sampling at Paddy Creek and Erb Creek and made 30 and 36 stream visits, respectively, to these creeks. To access study creeks, the Paddy crew used a rigid hull inflatable skiff to navigate to and from Erb Creek, 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 crew was assisted at times by a crew of graduate students from UAF working on a stream life study. The UAF crew captured, tagged, and released 1000 adult Pink Salmon in each stream. The UAF crew was responsible for sampling recovered, tagged fish in each stream and followed AHRP sampling protocol. The samples were saved in separate trays and were included in the sample delivery to ADF&G at the end of the season. The *M/V Auklet* assisted Paddy camp set-up and take-down by deploying camp on August 4, 2018 and demobilizing camp on September 12, 2018.

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 with GPS, 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 GPS 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 2018.

After completing a sample inventory, samples were delivered to ADF&G in two separate shipments during 2018. The first 58 trays were delivered to ADF&G during a Cordova port call on August 22, 2018, and the final shipment, consisting of a total of 903 trays, were delivered by van to Anchorage on September 22, 2018. In Anchorage, 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 2017 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 2014-2018 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 - 2017, the objectives of the PWS field sampling in 2018 included estimates for the fractions of hatchery fish in each spawning population of Pink Salmon. However, unlike 2013-2015 when hatchery fractions were estimated for over two dozen streams, only five streams were visited in 2018. 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^{th} stream in 2018 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^{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^{th} sampled fish. An approximation of the variance for the weighted average is:

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

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 in the past, the Science Panel decided that otoliths reading would be limited to every other tray of collected otoliths from Stockdale and Hogan Bay creeks in 2017. Since that decision, more funding was obtained to read the other half of the otoliths sampled in 2017 (K. Shedd, ADF&G, pers. comm.). We pulled data from the database on May 31, 2019 to conduct the final analyses for 2018 reported here. The otolith readings on every other tray of 2018 collections from Hogan Bay Creek and Stockdale Creek are on-going, so our results only reflect the otolith reads as of the end of May, 2019. As in previous years, eight trays of collected otoliths in 2018 were read for the other three streams (Erb, Paddy, and Gilmour creeks), for estimating hatchery fractions. Systematic sampling (number of trays divided by eight) was used to select the eight otolith trays from those three streams (although some of those selected trays were less than full). As in the analysis for 2017, additional otolith readings from fish that were tagged and recovered in 2018 in a stream life project in Erb and Paddy Creeks led by UAF were included in the computation of hatchery fractions that appear in Table 1 (number of tagged fish recovered from Erb Creek: 336, Paddy Creek: 118).

RESULTS

The 2018 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, 2018 sampling was successful at all five selected Pink Salmon PWS fitness study streams where 36,550 DNA tissues and pairs of otoliths were taken (Table 1). Rather than having a numerical sampling goal in 2018, 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 RRS (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 2018. Hatchery fractions for each stream were weighted by observed dead salmon counts taken during each visit. Note that the five streams sampled in 2018 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 2018	Samples collected 2018	Number of otoliths read 2018 ^a	Sampling Year					
					2013	2014	2015	2016	2017	2018
Paddy C	226-20-16010	30	4,511	301	0.154	0.595	0.328	0.206	0.352	0.076
Erb C	226-20-16040	36	9,655	670	0.113	0.228	0.214	0.100	0.263	0.067
Hogan Bay	226-30-16810	35	14,292	10,376	0.640	0.915	0.583	0.205	0.687	0.423
Gilmour C ^b	227-20-17480	19	2,901	366	NA	0.557	0.225	0.116	0.250	0.063
Stockdale C	227-20-17520	22	5,191	4,903	0.163	0.735	0.240	0.078	0.095	0.057

^aOtolith collections from all five creeks were subsampled - see Methods.

^bGilmour 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 2018 (36,550) was lower than that sampled in 2017 owing largely to a smaller run typical of an even year. The sample size was comparable to the most recent even year sampling effort (2016):

2013: 2,789 (note Gilmour was not sampled in 2013)
2014: 7,939
2015: 46,386
2016: 37,549
2017: 58,688

In general, the 2018 field season was marked by a lack of rain and low flows in the latter half of the field season due to a persistent high-pressure weather system in the region. Crews were very successful in surveying and sampling all streams during the field season. Training the new field recruits was very successful with the help of Julia McMahon, and the field crew benefited from the experience of the charter vessel captains and one returning crew member from 2017. Black bears were commonly observed (as in previous years) at Hogan Bay, Paddy, and Erb Creeks. Only two observations of brown bears were made on Stockdale and Gilmour Creeks during the 2018 field season (see more detailed accounts in annual stream summaries in Appendix D). In many cases, tissue samples were collected from carcasses that were depredated and missing otoliths. The low flows of 2018 appeared to make Pink Salmon even more vulnerable to predation compared to previous years. However, these depredated samples are still important as they could represent offspring from previously sampled parents with known hatchery- or natural-origin otolith designations. It should be noted that the target of 384 (8 full DWP trays) was not achieved in Paddy and Gilmour Creeks (Table 1) as a result of depredated carcasses where the crew were unable to recover otoliths.

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 *F/V 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. While we were initially planning on sampling through September 20, live counts on all the study streams dropped markedly in early September. Sampling ended on September 9, 2018 on Stockdale and Gilmour Creeks (live counts on the final day of sampling were less than 100 and 10, respectively) and on September 11, 2018 on Paddy and Erb Creeks (live counts less than 200 and 50 respectively, Appendix C). The *F/V Alexandra* crew continued to sample Hogan Creek through September 15. On that final day of sampling at Hogan Bay, no live fish were observed in the upstream, freshwater habitat, and only 100 live fish were counted in the intertidal. Thus, the end of the season in 2018 was dictated by the dramatic drop in live fish numbers in all the study creeks.

The results here indicate lower hatchery fraction values compared to previous years (Table 1). There was only one case where values of hatchery fraction in the spawning

population were lower than that estimated in 2018: 2016 in Hogan Bay (21.5%, compared to 42.3% in 2018, Table 1). One possible explanation is poor survival of hatchery releases in recent years, although changes in rates of donor straying (i.e. the % of the hatchery run that strays to wild streams) or the strength of the run of natural-origin Pink Salmon in PWS could also explain these results.

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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 2018. 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 2018. 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 2018
(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 or Alternate Days
Paddy C	226-20-16010	DNA pink	Yes	Daily or Alternate Days
Hogan Bay	226-30-16810	DNA pink	Yes	Daily or Alternate Days
Gilmour C	227-20-17480	DNA pink	Yes	Daily or Alternate Days
Stockdale C	227-20-17520	DNA pink	Yes	Daily or Alternate Days

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).

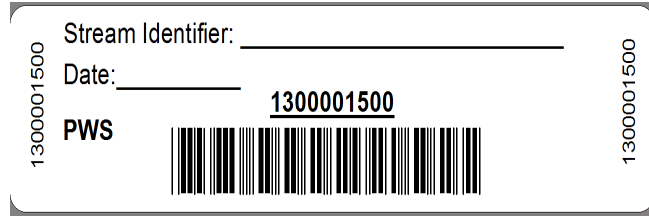
Pre-printed barcode labels for each 48dwp have been provided by GCL. Keep label sheets packed in a ZipLoc bag to keep them dry prior to application. Labeling 48dwp trays should be done beforehand in a dry area to insure the label adheres. Apply label on notched side (picture below) of each plate. Barcode 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.

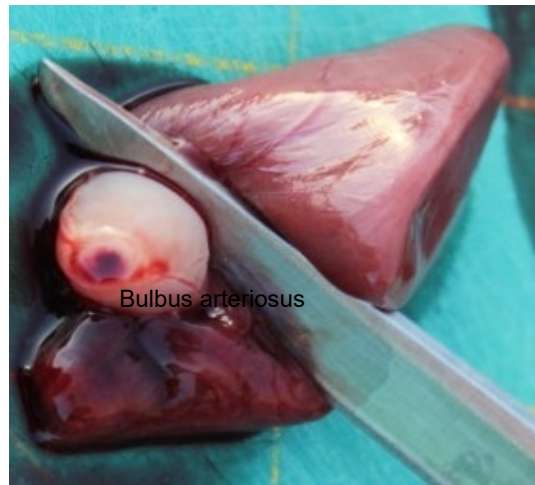


Figure B3. Bulbus arteriosus attached to heart.

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).

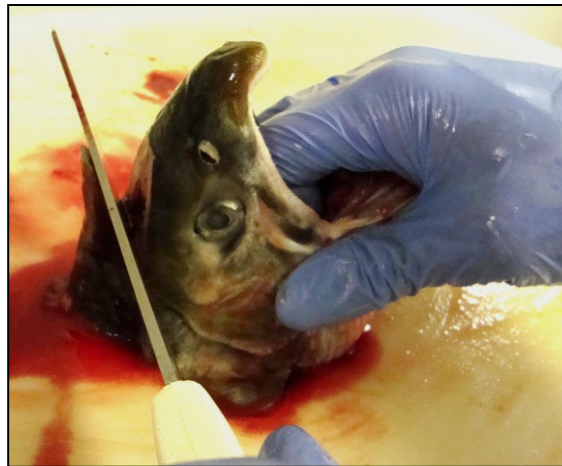


Figure B4. Slice the top of head off.



Figure B5. Remove brain tissue.

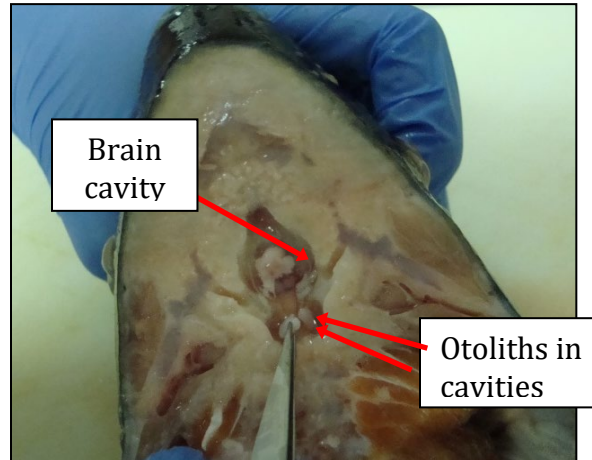


Figure B6. Otoliths in two lateral wells posterior to brain cavity.

Using 48dwp Trays for DNA Tissue and Otoliths

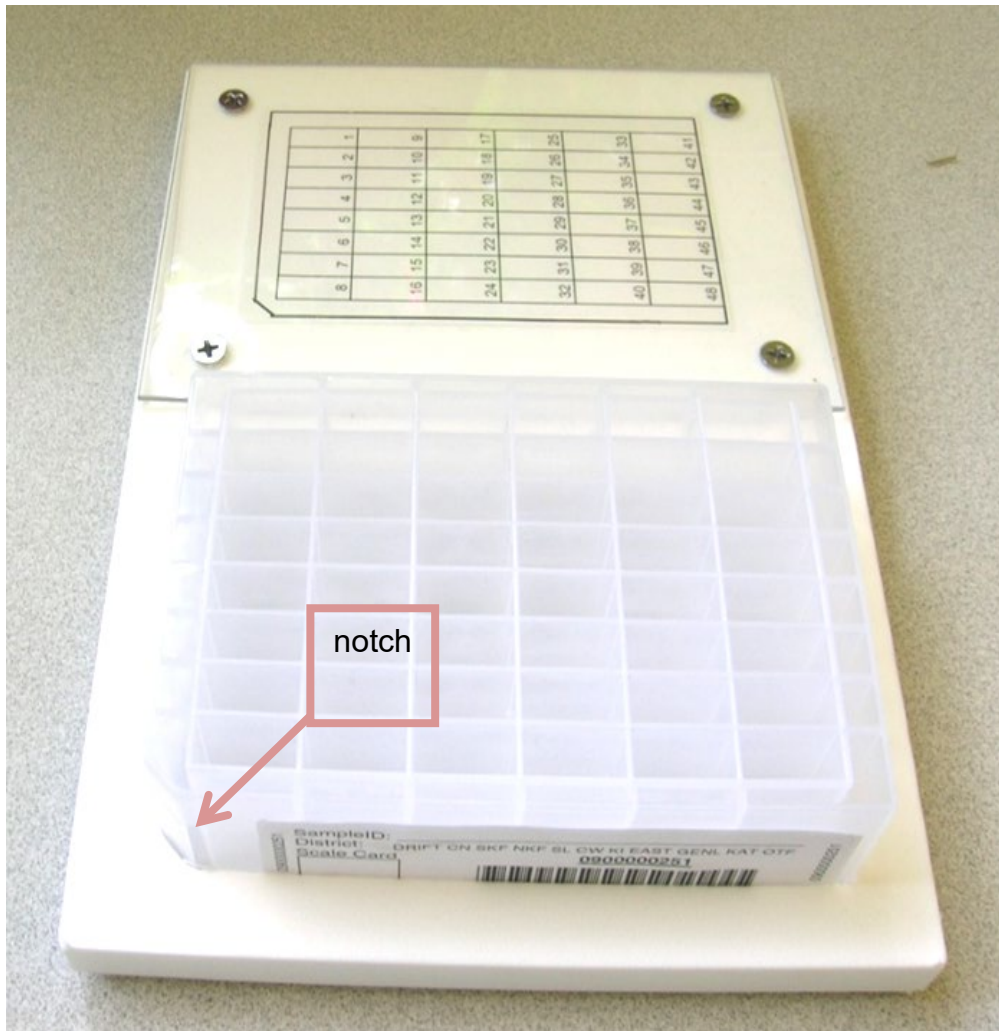
It is important to try to collect the best **quality DNA tissue samples to ensure reliable results**. Tissue quality degrades after death, so moribund and recently dead fish will yield best results. Frequent visits to streams will provide better opportunity to sample fresher spawn-outs. However, sample 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.

Person responsible for delivery:

Recipient:

Date sent to Cordova Lab:

Date received by Cordova Lab:

APPENDIX C. PWS PINK SALMON SURVEY SUMMARY

Stream Name	AWC	Date	Specimens	Live Count	Dead Count
Erb C	226-20-16040	8/6/18	2	616	2
Erb C	226-20-16040	8/7/18	12	1554	39
Erb C	226-20-16040	8/8/18	80	1578	240
Erb C	226-20-16040	8/9/18	54	3567	300
Erb C	226-20-16040	8/10/18	138	1587	438
Erb C	226-20-16040	8/11/18	180	4182	1284
Erb C	226-20-16040	8/12/18	120	4509	666
Erb C	226-20-16040	8/13/18	110	3615	558
Erb C	226-20-16040	8/14/18	271	7548	885
Erb C	226-20-16040	8/15/18	149	10650	1500
Erb C	226-20-16040	8/16/18	339	12174	2034
Erb C	226-20-16040	8/17/18	1	-	-
Erb C	226-20-16040	8/18/18	614	7200	3339
Erb C	226-20-16040	8/20/18	279	3900	1118
Erb C	226-20-16040	8/22/18	608	6300	2688
Erb C	226-20-16040	8/23/18	8	-	-
Erb C	226-20-16040	8/24/18	469	6720	1116
Erb C	226-20-16040	8/25/18	32	-	-
Erb C	226-20-16040	8/26/18	881	4200	3792
Erb C	226-20-16040	8/27/18	17	-	-
Erb C	226-20-16040	8/28/18	576	9000	4272
Erb C	226-20-16040	8/29/18	16	-	-
Erb C	226-20-16040	8/30/18	604	12717	3456
Erb C	226-20-16040	8/31/18	31	-	-
Erb C	226-20-16040	9/1/18	697	5244	2200
Erb C	226-20-16040	9/2/18	35	-	-
Erb C	226-20-16040	9/3/18	735	4246	6650
Erb C	226-20-16040	9/4/18	15	-	-
Erb C	226-20-16040	9/5/18	835	1736	5100
Erb C	226-20-16040	9/6/18	298	1638	6186
Erb C	226-20-16040	9/7/18	690	822	6488
Erb C	226-20-16040	9/8/18	348	524	6260
Erb C	226-20-16040	9/9/18	576	639	9540
Erb C	226-20-16040	9/10/18	137	314	5262
Erb C	226-20-16040	9/11/18	181	351	8013
Erb C	226-20-16040	9/12/18	1	-	-

Stream Name	AWC	Date	Specimens	Live Count	Dead Count
Paddy C	226-20-16010	8/10/18	22	46	58
Paddy C	226-20-16010	8/11/18	6	256	20
Paddy C	226-20-16010	8/12/18	25	1797	108
Paddy C	226-20-16010	8/13/18	34	1062	174
Paddy C	226-20-16010	8/14/18	36	264	123
Paddy C	226-20-16010	8/15/18	153	993	495
Paddy C	226-20-16010	8/17/18	311	3816	1044
Paddy C	226-20-16010	8/18/18	2	-	-
Paddy C	226-20-16010	8/19/18	329	2271	1458
Paddy C	226-20-16010	8/20/18	2	-	-
Paddy C	226-20-16010	8/21/18	124	3716	601
Paddy C	226-20-16010	8/22/18	5	-	-
Paddy C	226-20-16010	8/23/18	572	6954	2922
Paddy C	226-20-16010	8/24/18	10	-	-
Paddy C	226-20-16010	8/25/18	604	2832	2718
Paddy C	226-20-16010	8/26/18	12	-	-
Paddy C	226-20-16010	8/27/18	597	3837	2676
Paddy C	226-20-16010	8/28/18	2	-	-
Paddy C	226-20-16010	8/29/18	540	1515	2524
Paddy C	226-20-16010	8/30/18	31	-	-
Paddy C	226-20-16010	8/31/18	482	2433	3029
Paddy C	226-20-16010	9/1/18	12	-	-
Paddy C	226-20-16010	9/2/18	413	645	1650
Paddy C	226-20-16010	9/3/18	13	-	-
Paddy C	226-20-16010	9/4/18	148	141	2193
Paddy C	226-20-16010	9/5/18	4	-	-
Paddy C	226-20-16010	9/6/18	124	15	2094
Paddy C	226-20-16010	9/7/18	17	-	-
Paddy C	226-20-16010	9/8/18	64	81	1545
Paddy C	226-20-16010	9/10/18	30	126	510
Hogan Bay	226-30-16810	8/5/18	1	1312	1
Hogan Bay	226-30-16810	8/6/18	14	5007	42
Hogan Bay	226-30-16810	8/8/18	24	2124	72
Hogan Bay	226-30-16810	8/10/18	89	1155	288
Hogan Bay	226-30-16810	8/12/18	62	2625	294
Hogan Bay	226-30-16810	8/14/18	96	2847	414
Hogan Bay	226-30-16810	8/16/18	194	2535	750
Hogan Bay	226-30-16810	8/18/18	183	4230	1524

Stream Name	AWC	Date	Specimens	Live Count	Dead Count
Hogan Bay	226-30-16810	8/19/18	303	8205	1821
Hogan Bay	226-30-16810	8/20/18	128	13110	1317
Hogan Bay	226-30-16810	8/21/18	118	8295	1071
Hogan Bay	226-30-16810	8/23/18	528	9990	4392
Hogan Bay	226-30-16810	8/24/18	432	12510	5712
Hogan Bay	226-30-16810	8/25/18	624	13476	7353
Hogan Bay	226-30-16810	8/26/18	576	13482	14082
Hogan Bay	226-30-16810	8/27/18	480	8410	6798
Hogan Bay	226-30-16810	8/28/18	720	15870	10128
Hogan Bay	226-30-16810	8/29/18	480	11925	13026
Hogan Bay	226-30-16810	8/30/18	480	5800	9240
Hogan Bay	226-30-16810	8/31/18	720	-	-
Hogan Bay	226-30-16810	9/1/18	480	4820	11610
Hogan Bay	226-30-16810	9/2/18	672	6600	18585
Hogan Bay	226-30-16810	9/3/18	624	3324	13986
Hogan Bay	226-30-16810	9/4/18	1008	-	-
Hogan Bay	226-30-16810	9/5/18	480	420	12050
Hogan Bay	226-30-16810	9/6/18	576	648	20160
Hogan Bay	226-30-16810	9/7/18	720	1126	19548
Hogan Bay	226-30-16810	9/8/18	720	-	-
Hogan Bay	226-30-16810	9/9/18	768	82	18880
Hogan Bay	226-30-16810	9/10/18	1056	-	-
Hogan Bay	226-30-16810	9/11/18	649	1005	26640
Hogan Bay	226-30-16810	9/12/18	288	813	21540
Hogan Bay	226-30-16810	9/13/18	364	807	23760
Hogan Bay	226-30-16810	9/14/18	130	363	24930
Hogan Bay	226-30-16810	9/15/18	44	228	20820
Gilmour C	227-20-17480	8/8/18	6	1826	12
Gilmour C	227-20-17480	8/10/18	129	5230	264
Gilmour C	227-20-17480	8/12/18	67	1623	378
Gilmour C	227-20-17480	8/14/18	38	3873	303
Gilmour C	227-20-17480	8/16/18	13	7151	166
Gilmour C	227-20-17480	8/18/18	171	2115	669
Gilmour C	227-20-17480	8/19/18	36	3210	465
Gilmour C	227-20-17480	8/20/18	12	3000	336
Gilmour C	227-20-17480	8/21/18	30	3380	85
Gilmour C	227-20-17480	8/23/18	331	4125	1077
Gilmour C	227-20-17480	8/25/18	477	7440	2940
Gilmour C	227-20-17480	8/27/18	384	6720	4245

Stream Name	AWC	Date	Specimens	Live Count	Dead Count
Gilmour C	227-20-17480	8/29/18	240	2400	3260
Gilmour C	227-20-17480	8/31/18	265	1862	4400
Gilmour C	227-20-17480	9/2/18	288	869	7270
Gilmour C	227-20-17480	9/4/18	240	295	6900
Gilmour C	227-20-17480	9/6/18	119	312	8800
Gilmour C	227-20-17480	9/8/18	50	96	4600
Gilmour C	227-20-17480	9/9/18	6	34	2980
Stockdale C	227-20-17520	8/7/18	20	16290	54
Stockdale C	227-20-17520	8/9/18	88	29985	273
Stockdale C	227-20-17520	8/11/18	17	26430	159
Stockdale C	227-20-17520	8/13/18	83	26400	336
Stockdale C	227-20-17520	8/15/18	58	36330	378
Stockdale C	227-20-17520	8/17/18	146	36870	663
Stockdale C	227-20-17520	8/19/18	288	11550	282
Stockdale C	227-20-17520	8/20/18	81	-	-
Stockdale C	227-20-17520	8/22/18	486	36714	3480
Stockdale C	227-20-17520	8/24/18	576	10500	4065
Stockdale C	227-20-17520	8/26/18	384	25500	17100
Stockdale C	227-20-17520	8/28/18	312	18700	15840
Stockdale C	227-20-17520	8/29/18	240	-	-
Stockdale C	227-20-17520	8/30/18	426	8400	21900
Stockdale C	227-20-17520	8/31/18	273	-	-
Stockdale C	227-20-17520	9/1/18	303	4878	24720
Stockdale C	227-20-17520	9/2/18	288	-	-
Stockdale C	227-20-17520	9/3/18	496	2100	18000
Stockdale C	227-20-17520	9/4/18	313	-	-
Stockdale C	227-20-17520	9/6/18	177	314	7600
Stockdale C	227-20-17520	9/7/18	98	460	11800
Stockdale C	227-20-17520	9/8/18	26	12	2100

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

2018 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). The UAF tagging crew conducted daily searches for tagged fish on each creek, thus some sampling was completed every day of the season (see Appendix C). As a result the tagging crew contributed some samples to the AHRP study. 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.

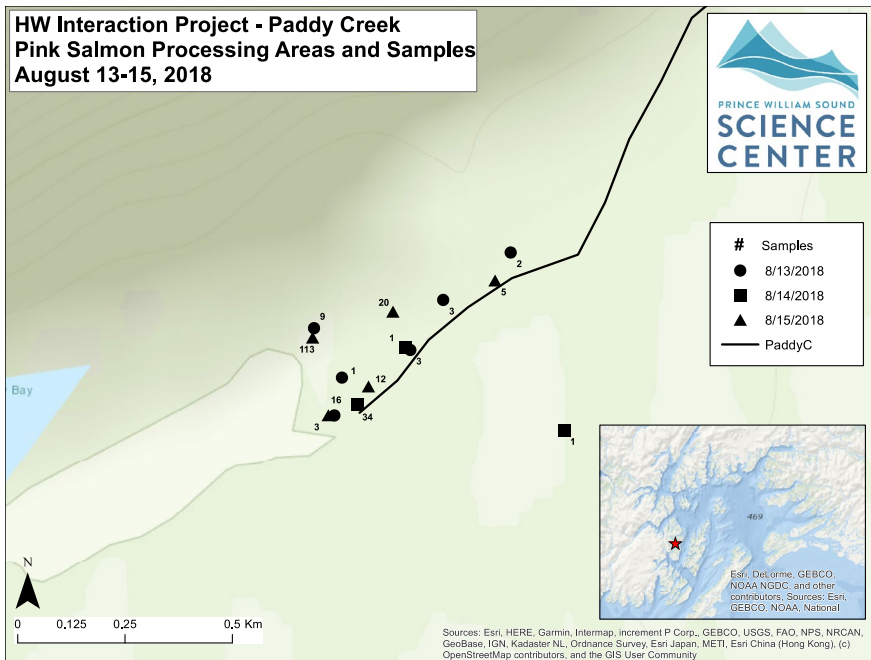
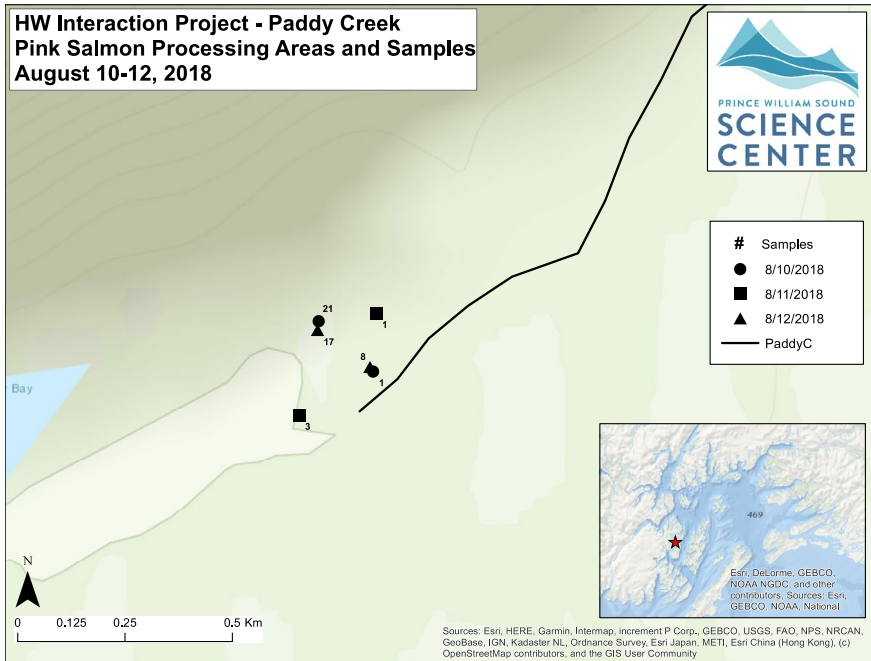
Paddy and the tagging crews sampled at Paddy Creek 30 times between 08/10/2018 and 09/10/2018 and collected 4,724 Pink Salmon otolith and tissue samples. Fish were present on the first and last day of sampling, but live counts were low at the start of the season (live count = 46, dead count = 58) and live count at the end of the season was low (126), so we are confident that the crew was present during the majority of the run. As in previous years, fish numbers peaked in mid-August, slowly tapering off through September.

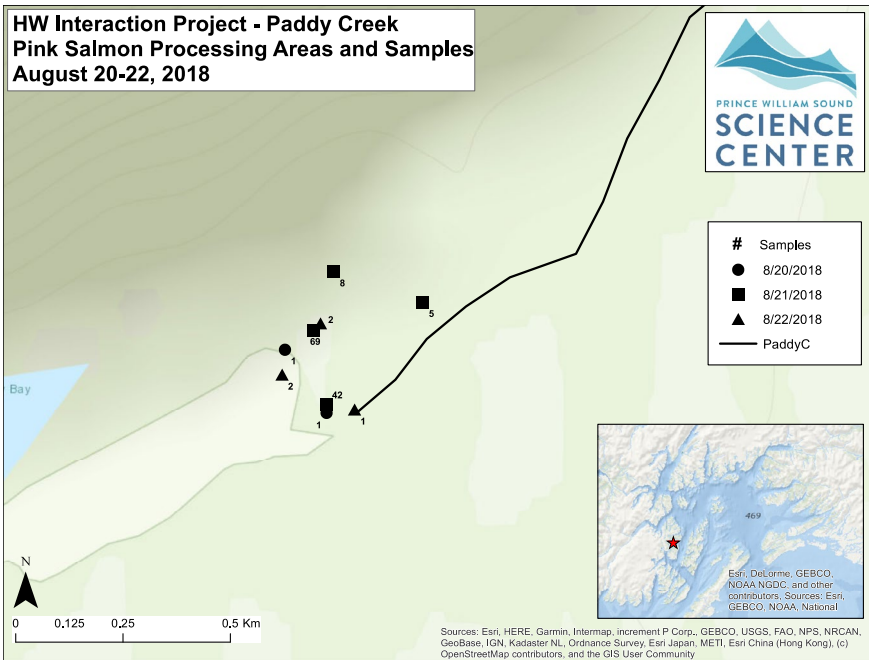
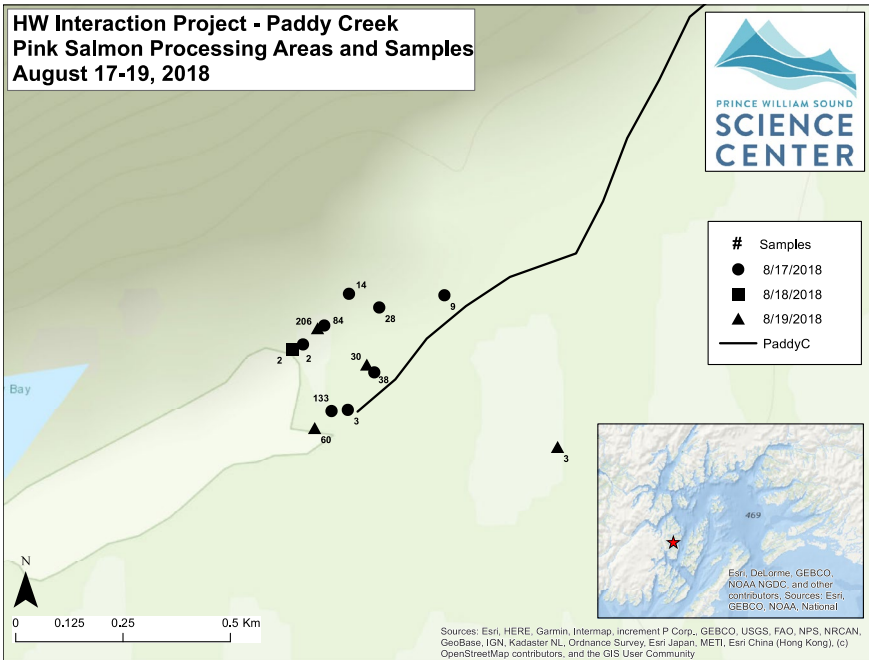
Extent of stream sampled: There are two creeks that flow into the head of Paddy Bay, one from the northeast and one from the east. Looking upstream, Paddy Creek is the stream on the left side (NE) and an unnamed creek flows from the right (E), for our sampling purposes we call this unnamed creek Paddy 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.

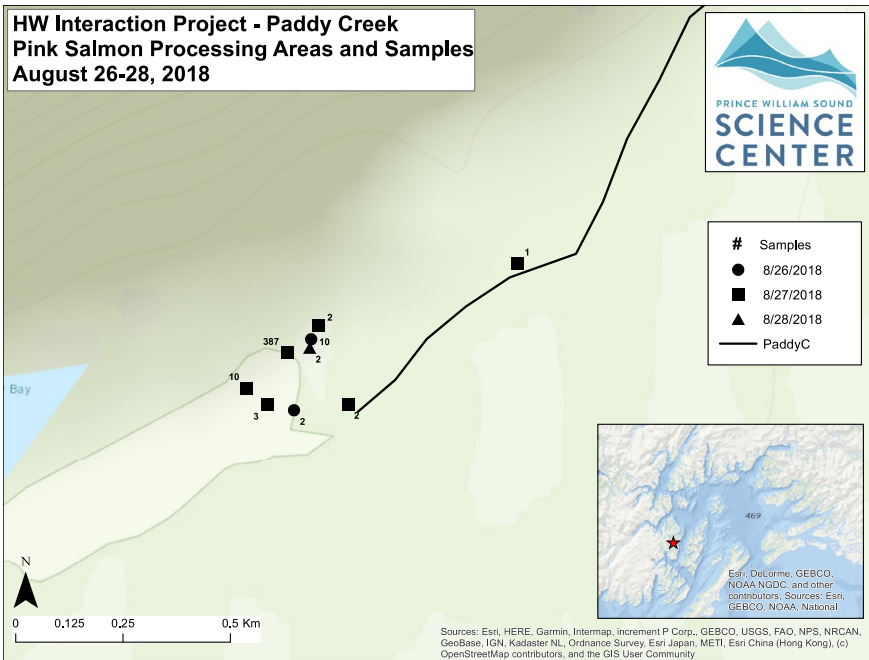
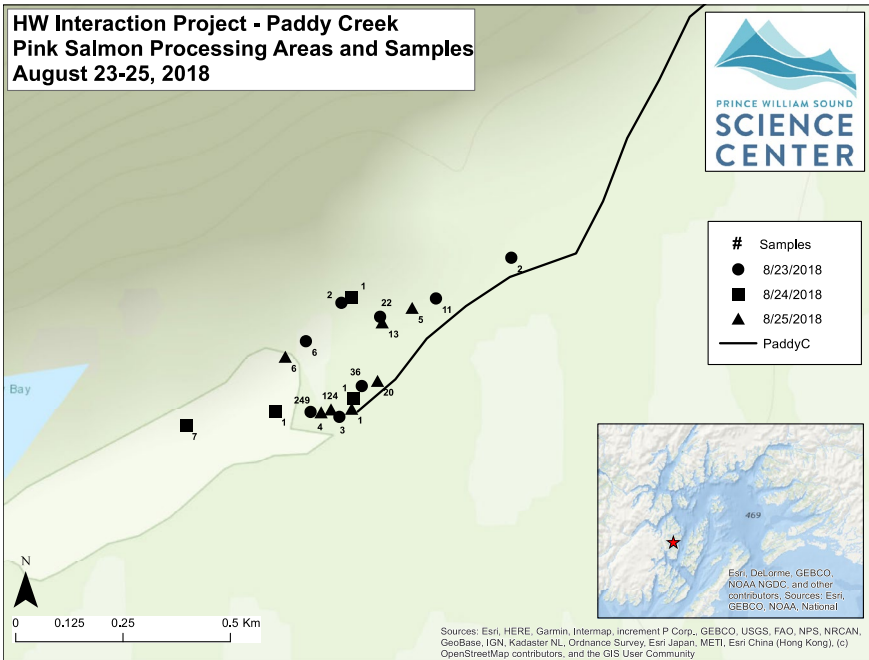
Unusual events: The season was marked by extremely low flows due to lack of precipitation during the latter half of the season. Pink salmon appeared to be more vulnerable to predation compared to previous years due to the low water conditions.

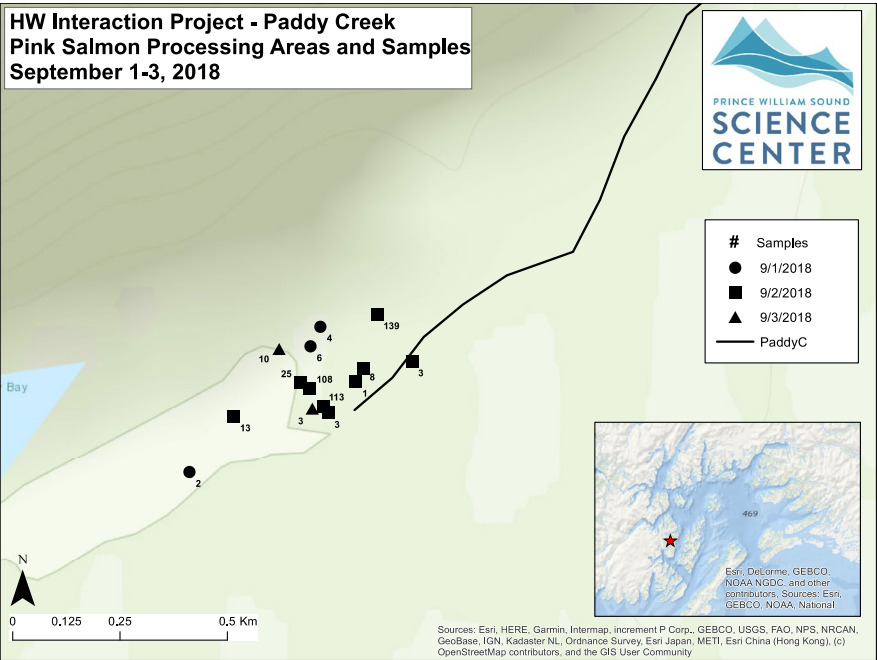
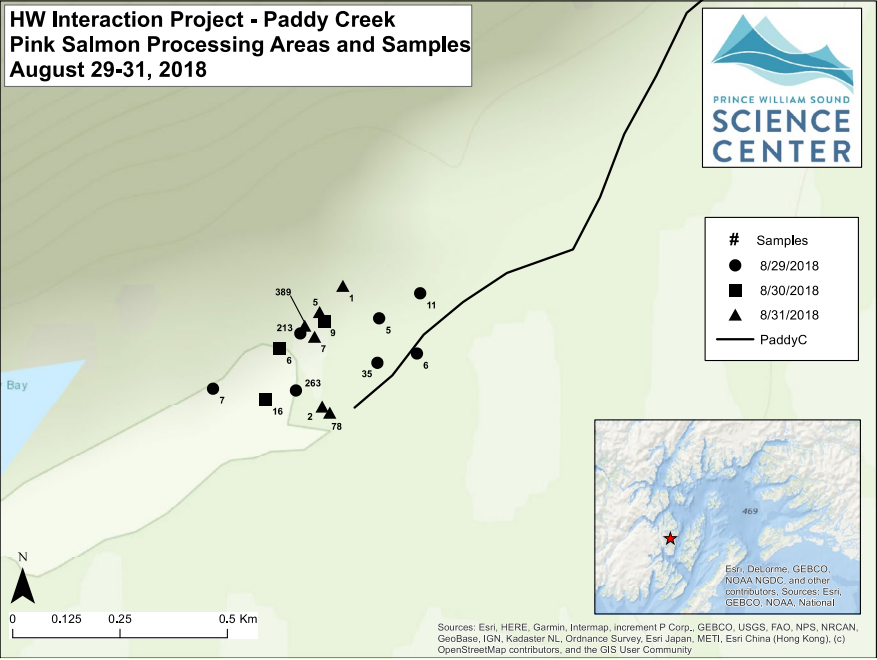
Access, safety, or logistics issues: Paddy Creek is approximately 1 km from camp - access is easy and dependable except in extreme flooding events. We routinely observed black bears (as many as eight at one time) that have become habituated to the presence of humans. No close

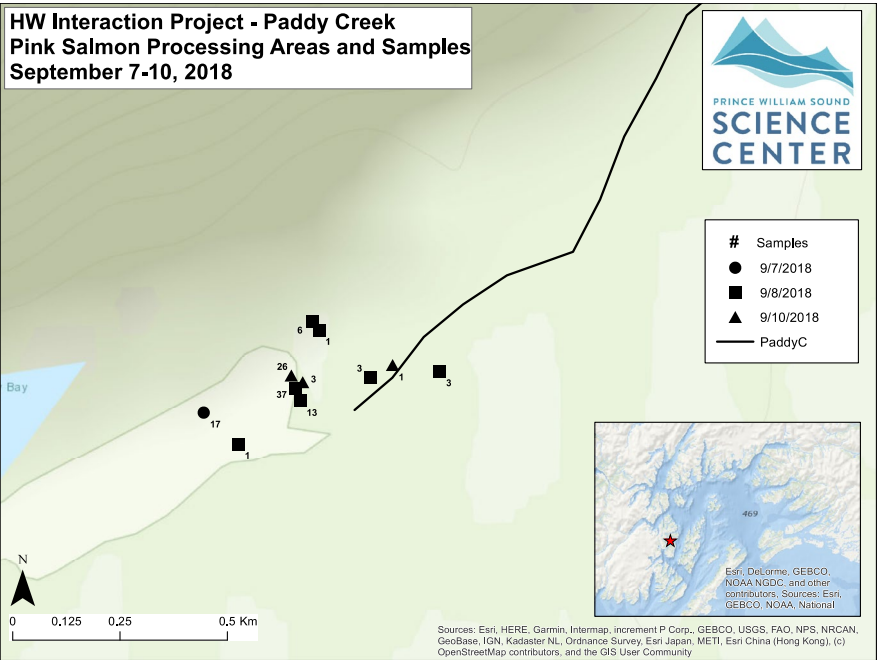
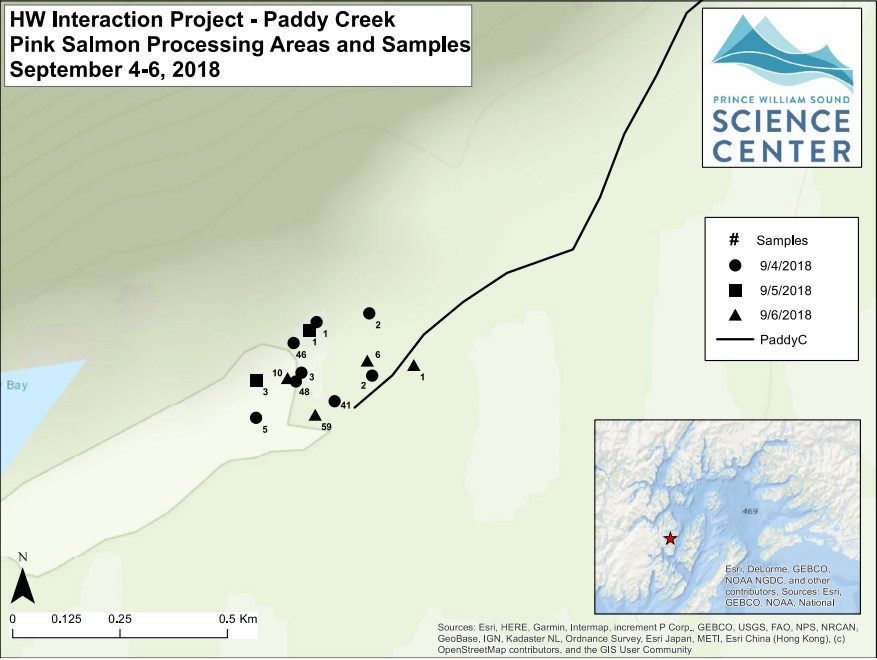
encounters occurred during the 2018 field season.











Erb Creek
226-20-16040
Pink Salmon Fitness

2018 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. A tagging crew from University of Alaska Fairbanks was present during the entire season and contributed some samples to the AHRP study (as described in the Paddy Creek summary, above).

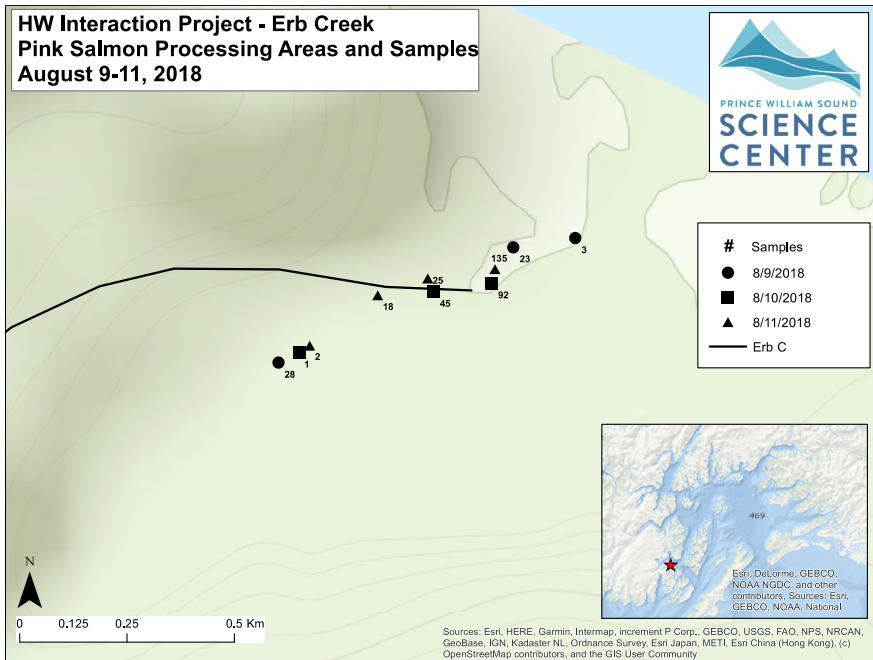
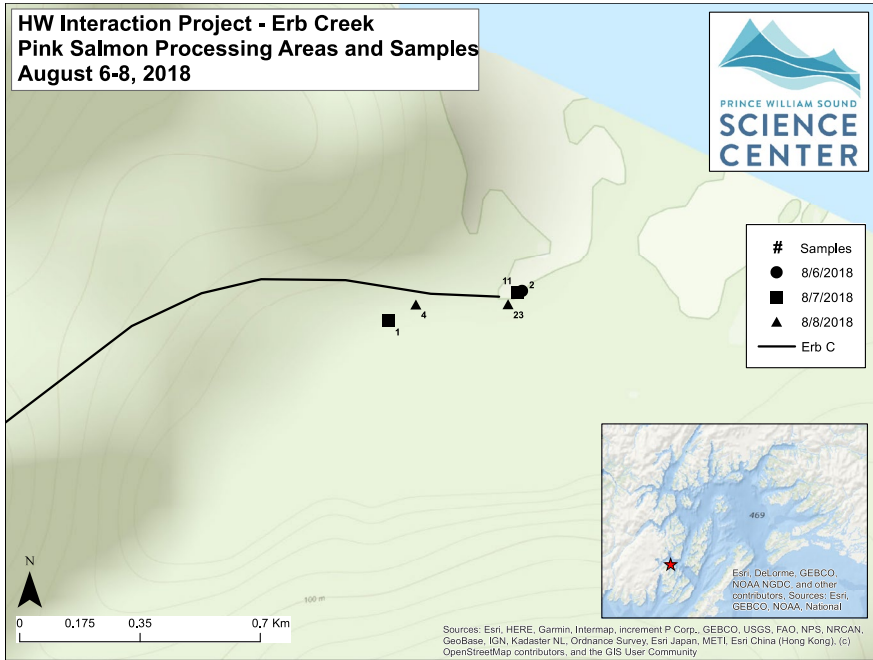
Paddy and tagging crews visited Erb Creek 36 times between 8/06/2018 and 9/12/2018, collecting a total of 10,139 Pink Salmon otolith and tissue samples. The run peaked in mid-August and remained high for the rest of the month, and then declined rapidly in early September. Live Pink Salmon numbered 351 on the final day of sampling.

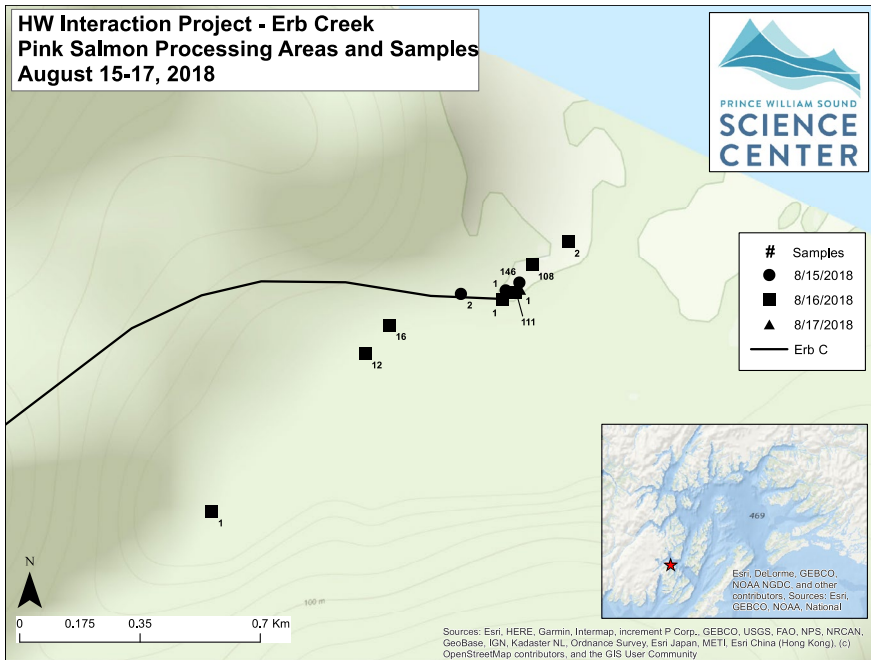
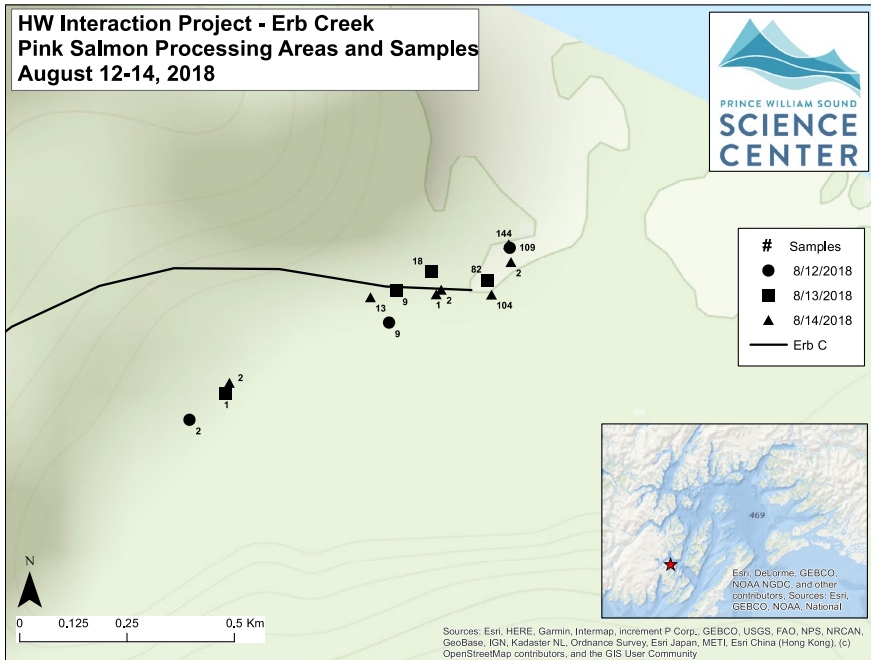
Extent of stream sampled: From 8/06/2018 through 9/12/2018, sampling efforts were spread over the entire length of spawning habitat.

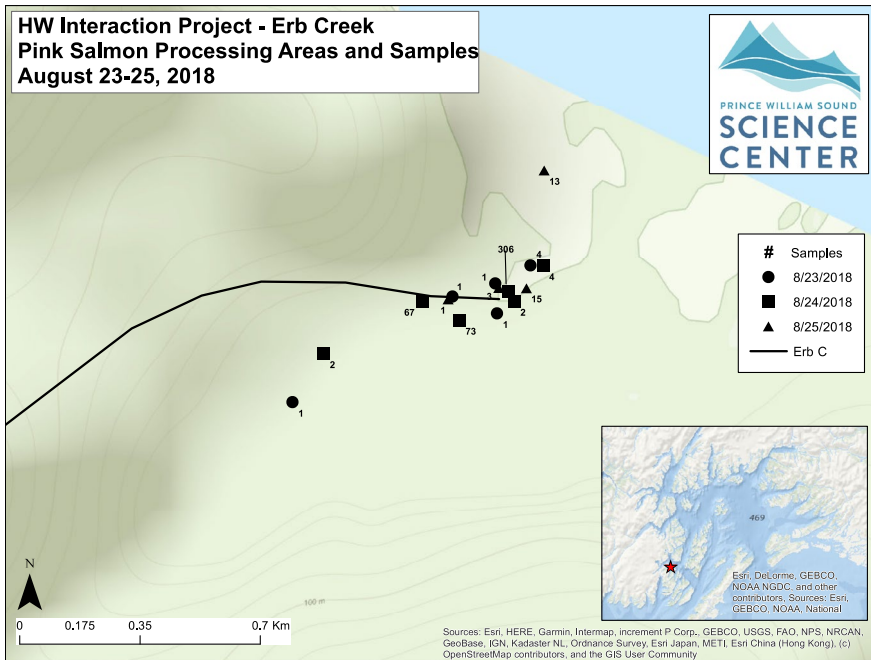
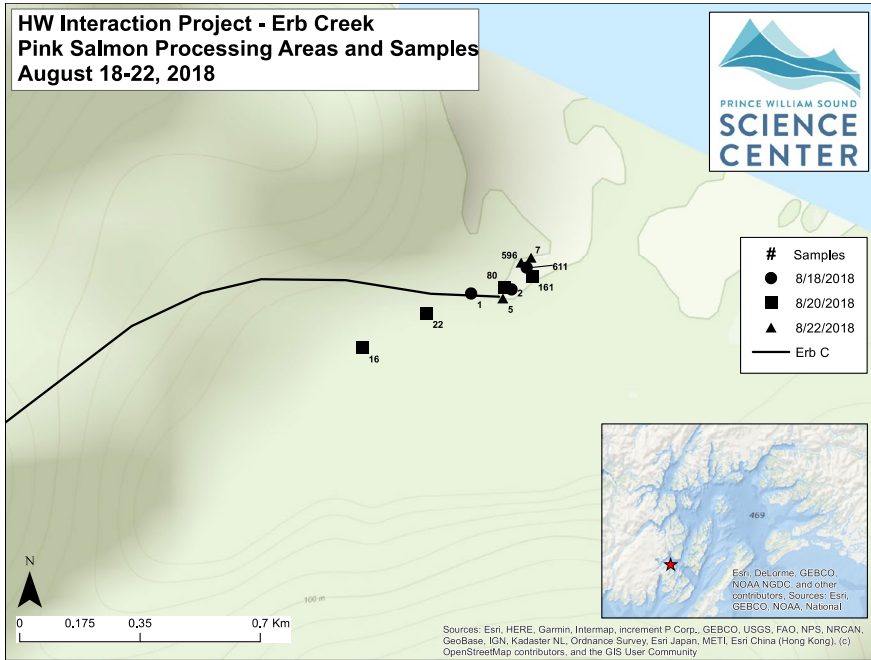
Unusual events: The season was marked by extremely low flows due to lack of precipitation. Pink salmon appeared to be more vulnerable to predation compared to previous years due to the low water conditions.

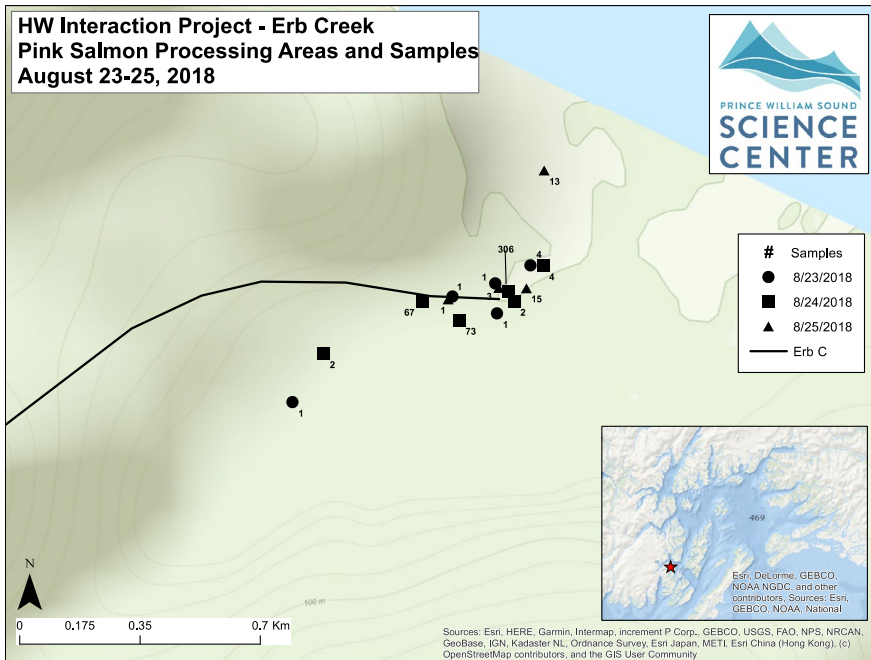
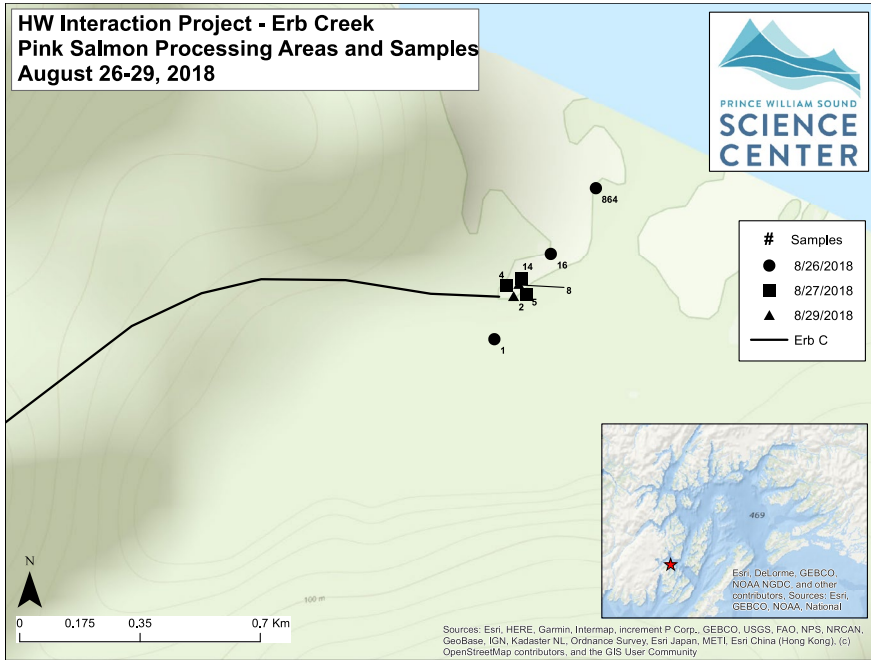
Access, safety, or logistics issues: As described in the above summary for Paddy Creek, bears on this stream have become habituated to the presence of the sampling crews. One crew member encountered a younger, small black bear at close range (approximately 1 m). Once the bear detected the presence of the crew member, it quickly darted off. There was a larger black bear that appeared to be more brazen than the others, often approaching in close proximity to the crew. In all these cases, the crew made loud noises and rang a bell, and eventually the bear would move on.

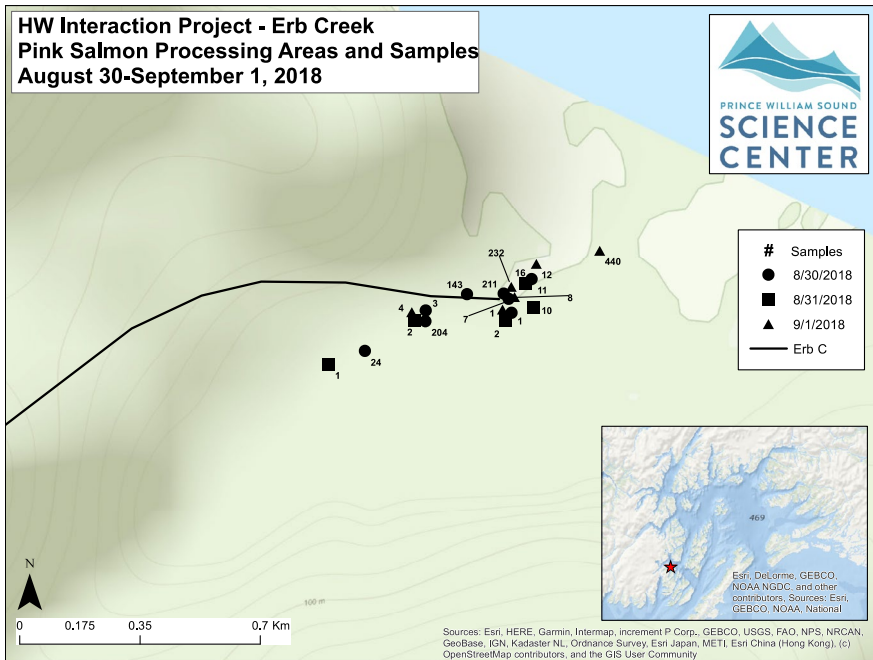
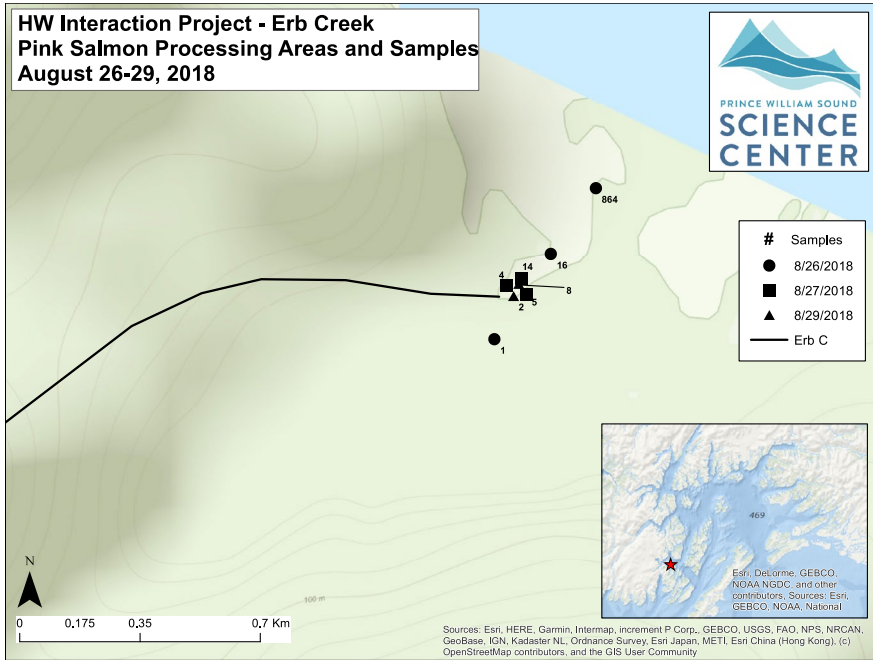
Recommendations, changes, and other notes: The crew did not have any recommendations or additional summary notes of the 2018 season.

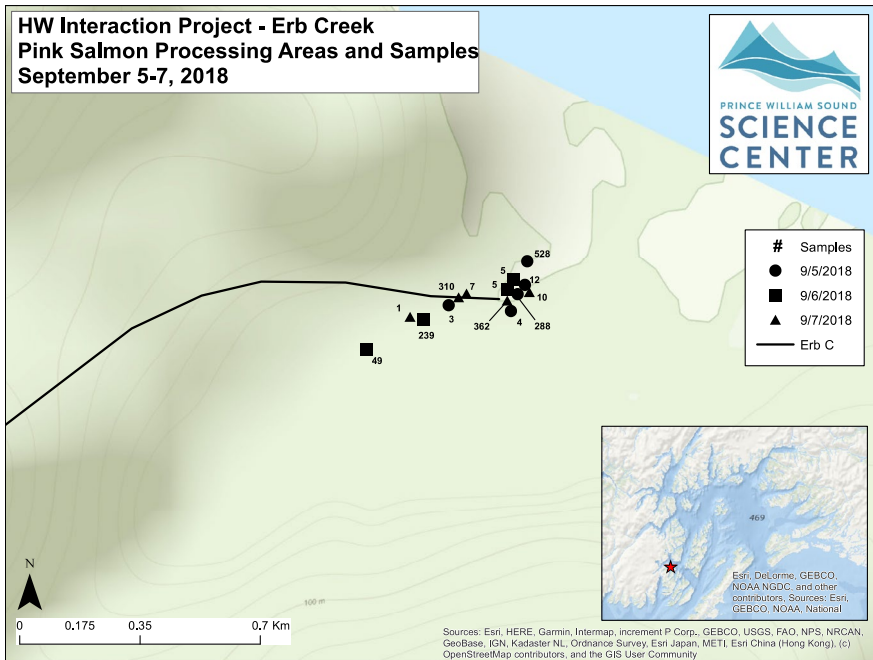
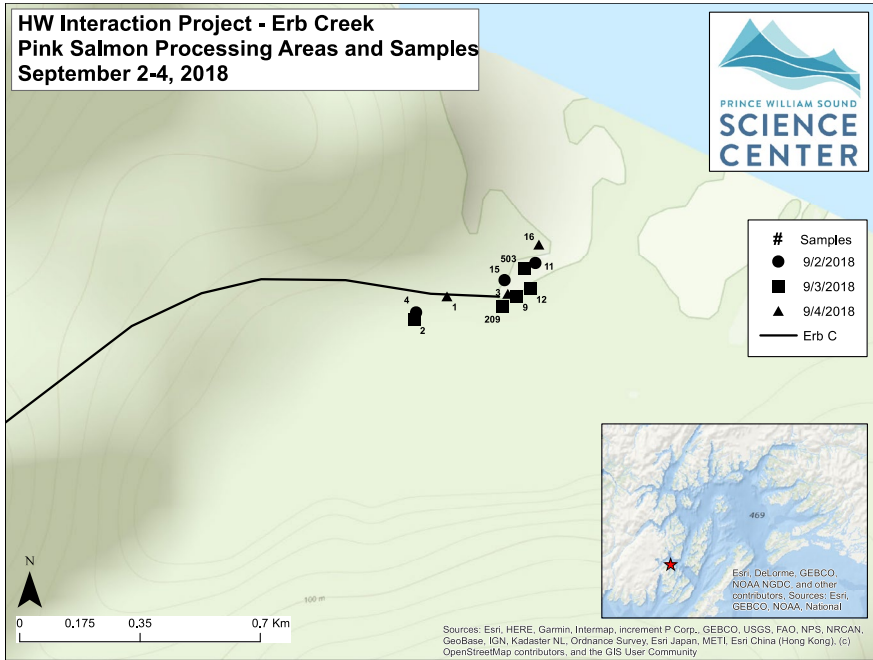












Alexandra was planned to demobilize on the 20th of September, but deployment was shortened due to the drought and shorter run period. Fewer than 100 live fish were left milling in the intertidal when the crew departed on September 16th and none in the creek itself.

Extent of Stream Sampled: The stream was surveyed to the maximum extent the Pink Salmon are capable of accessing at the beginning of the season. 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 used 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 two out of every three days. This was where the carcasses were most concentrated and could be sampled with maximum efficiency. Every third day the upper portion of the stream was sampled. Almost every day a live and dead count of the entire stream was done.

Unusual Events, Access, Safety and Logistics Issues:

One significant rain event occurred on August 20th to 21st which limited sampling due to high flow and inaccessibility in the upper creek. No rain was observed after that – totaling at least 25 days with no rain causing the creek to run completely dry. This also caused frequent, pre-spawner die offs. The crew believed that very few salmon successfully spawned during this period. Any eggs that were deposited became desiccated or were eaten by bears.

Black bear presence at the stream appeared to be similar to that of last year based on information provided in the 2017 crew log. Very few sightings through the first part of August but observations picked up by August 25th. The crew typically saw 5 to 10 bears a day, with a maximum of 12 unique individuals seen in one day. Most encounters resulted in either the bear(s) walking away or the bear and humans both carrying on while respecting each other's space. We rarely had to alter our sampling or locations based on bears. Only one encounter involved defensive behavior, and we successfully deescalated the situation before any attack occurred or bear spray was needed. A few crew members felt unsafe with the guns in the field. We had one unknown illness of a crew member (symptoms included fever and loss of appetite) which was overcome in a matter of days.

Recommendations and Other Notes:

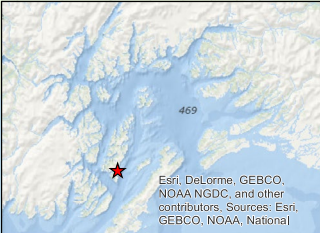
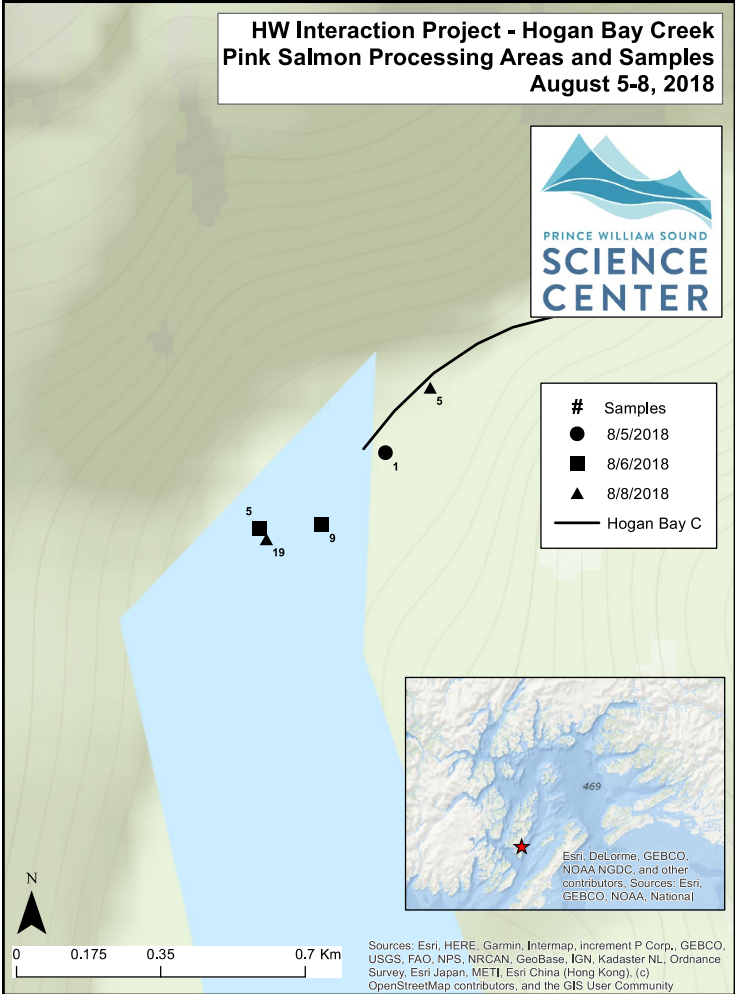
The three-person crew was a suitable size for the assignment. In a few instances two crews worked together at Hogan – when working with another crew on the same creek the Hogan crew found it most efficient and controlled to stay separate instead of trying to work things up as a big group.

All members took precautions to avoid encounters with bears, including never working alone, and making plenty of noise to alert bears of their presence. Each crew member carried bear spray (easily accessible in a holster) at all times while conducting the field work. The crew lead on this team recommended more time be spent on gun safety and gun handling during the training week (or just prior to deployment for crew members that join the field operations later).

**HW Interaction Project - Hogan Bay Creek
Pink Salmon Processing Areas and Samples
August 5-8, 2018**



- # Samples**
- 8/5/2018
 - 8/6/2018
 - ▲ 8/8/2018
 - 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), (c) OpenStreetMap contributors, and the GIS User Community

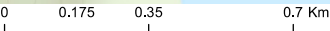
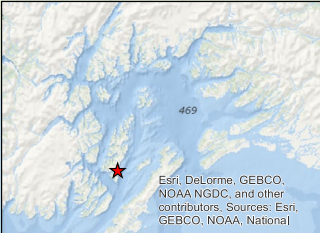
**HW Interaction Project - Hogan Bay Creek
Pink Salmon Processing Areas and Samples
August 10-14, 2018**



- # Samples**
- 8/10/2018
 - 8/12/2018
 - ▲ 8/14/2018
 - Hogan Bay C

- 18 ●
- 11 ●
- 33 ●
- 15 ▲
- 20 ▲
- 65 ●

- 4 ■
- 9 ■
- 12 ■
- 12 ▲
- 14 ▲

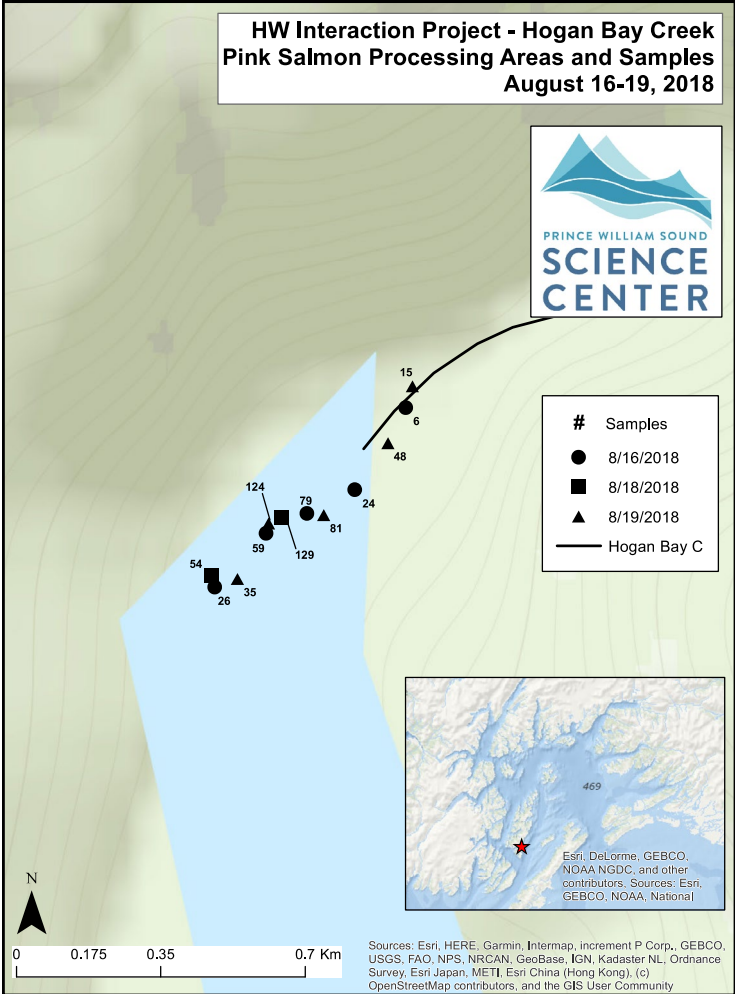


Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Hogan Bay Creek
Pink Salmon Processing Areas and Samples
August 16-19, 2018**



#	Samples
●	8/16/2018
■	8/18/2018
▲	8/19/2018
—	Hogan Bay C

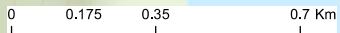
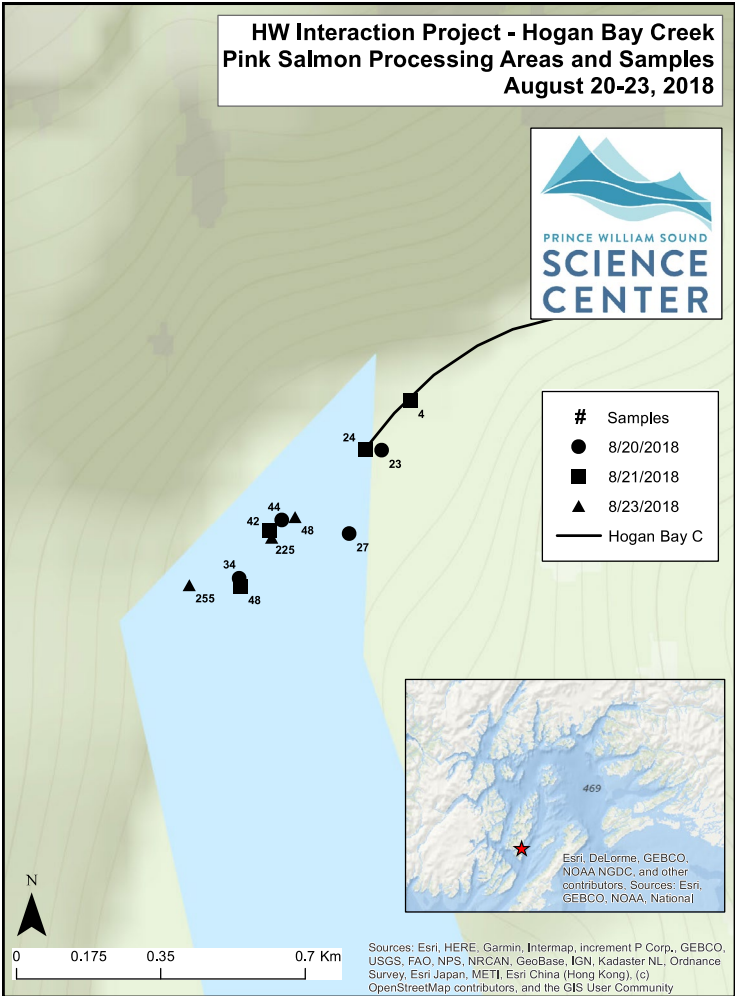


Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Hogan Bay Creek
Pink Salmon Processing Areas and Samples
August 20-23, 2018**



#	Samples
●	8/20/2018
■	8/21/2018
▲	8/23/2018
—	Hogan Bay C

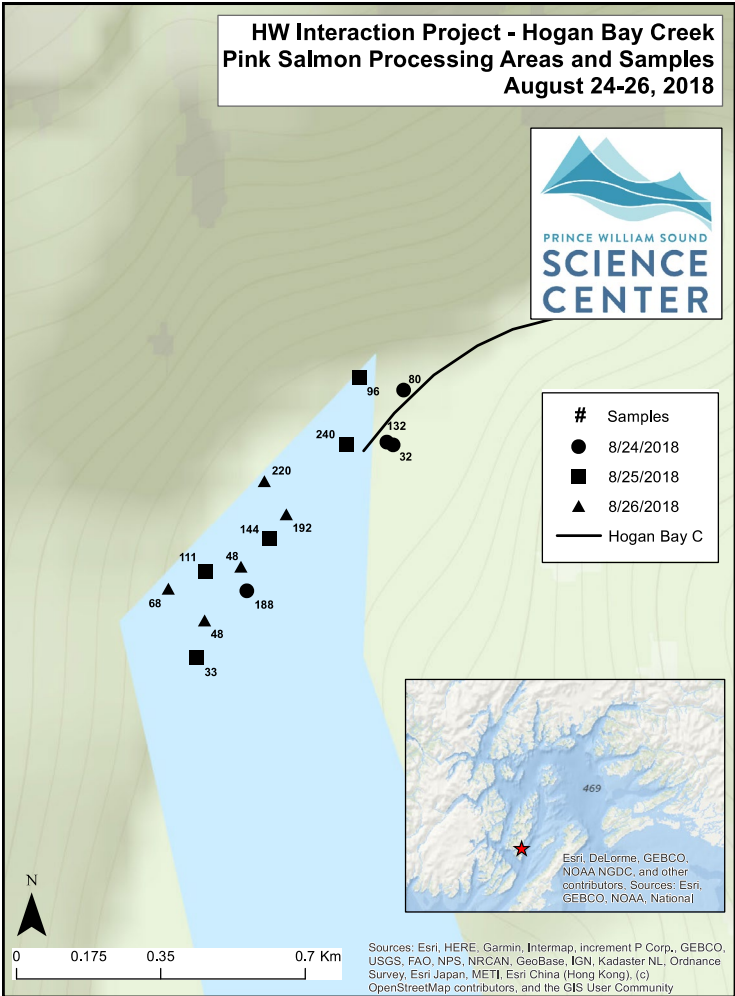


Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Hogan Bay Creek
Pink Salmon Processing Areas and Samples
August 24-26, 2018**



#	Samples
●	8/24/2018
■	8/25/2018
▲	8/26/2018
—	Hogan Bay C



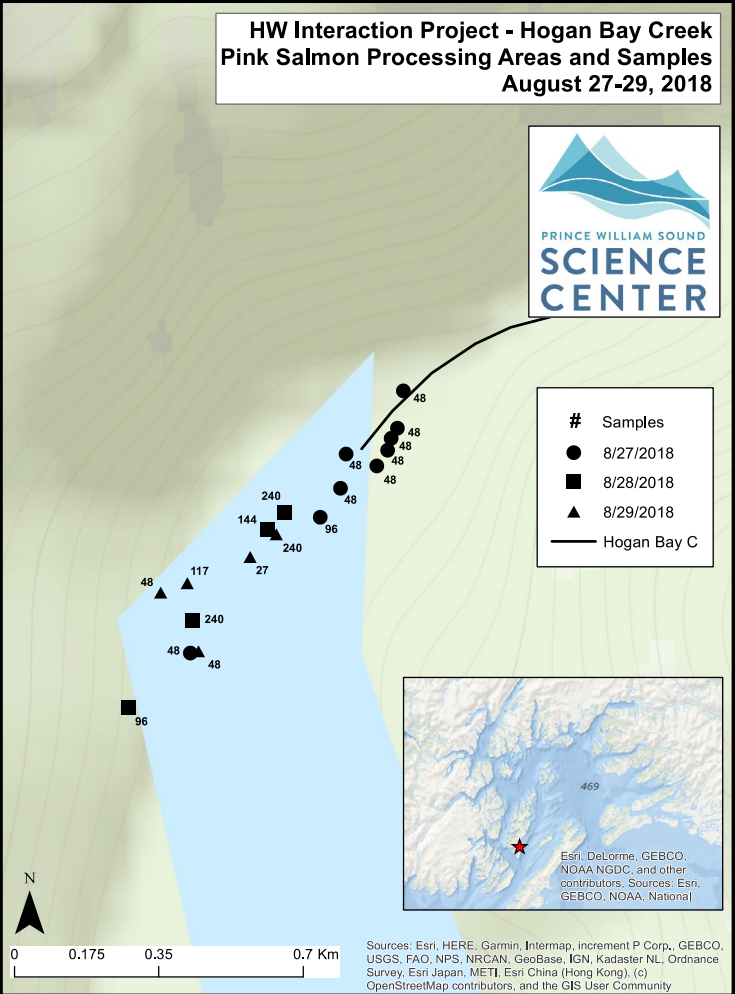
Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Hogan Bay Creek
Pink Salmon Processing Areas and Samples
August 27-29, 2018**



Samples

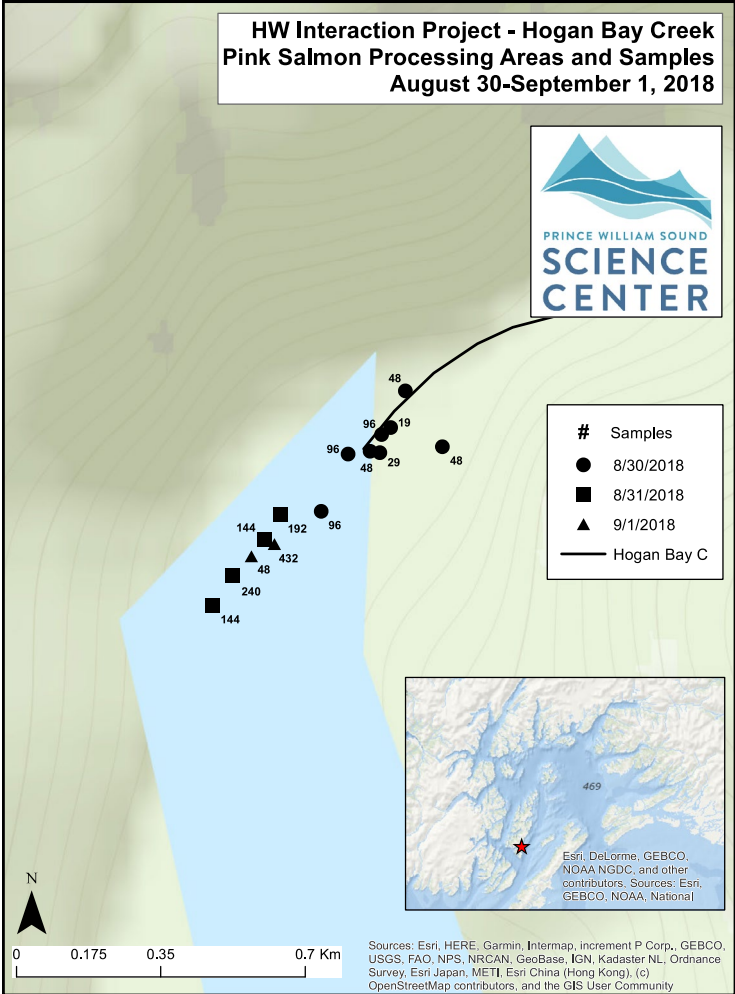
- 8/27/2018
- 8/28/2018
- ▲ 8/29/2018
- Hogan Bay C



**HW Interaction Project - Hogan Bay Creek
Pink Salmon Processing Areas and Samples
August 30-September 1, 2018**



- # Samples**
- 8/30/2018
 - 8/31/2018
 - ▲ 9/1/2018
 - Hogan Bay C

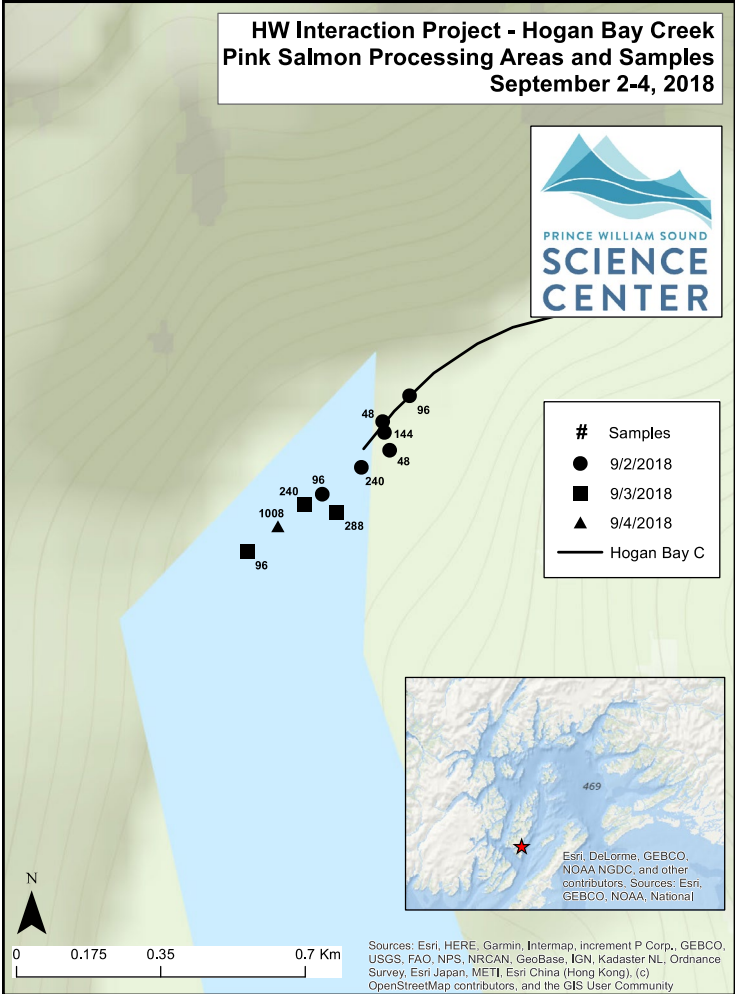


Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Hogan Bay Creek
Pink Salmon Processing Areas and Samples
September 2-4, 2018**



- # Samples**
- 9/2/2018
 - 9/3/2018
 - ▲ 9/4/2018
 - Hogan Bay C



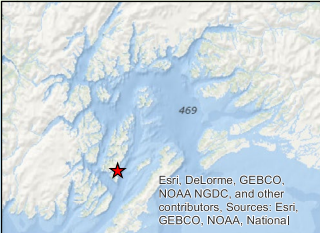
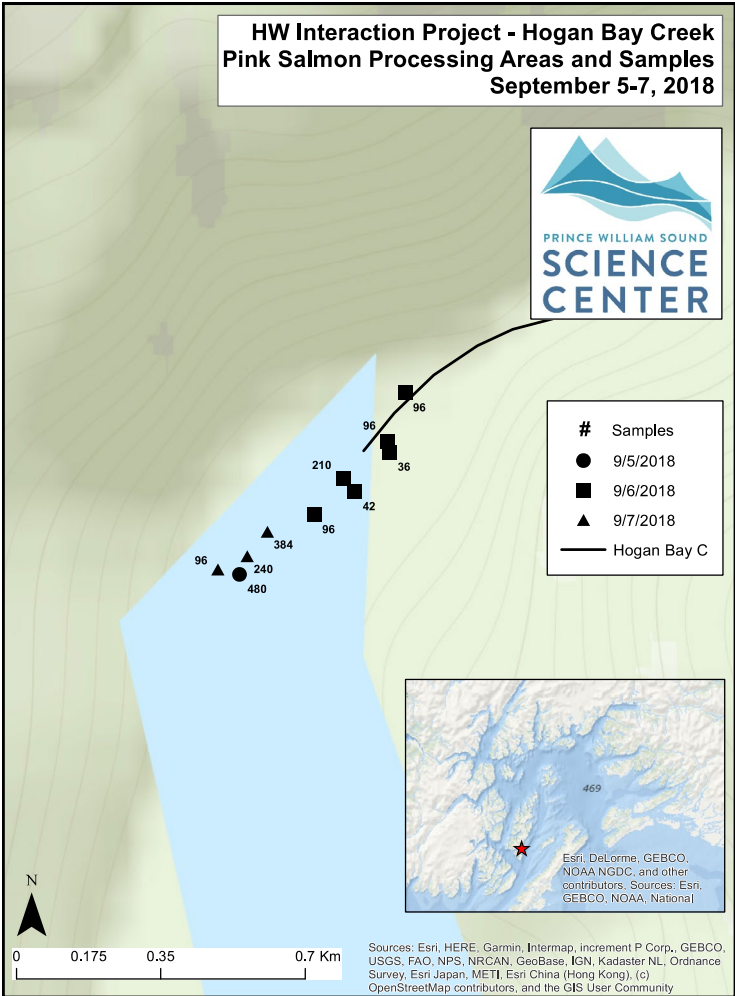
0 0.175 0.35 0.7 Km

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), (c) OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Hogan Bay Creek
Pink Salmon Processing Areas and Samples
September 5-7, 2018**



- # Samples**
- 9/5/2018
 - 9/6/2018
 - ▲ 9/7/2018
 - Hogan Bay C

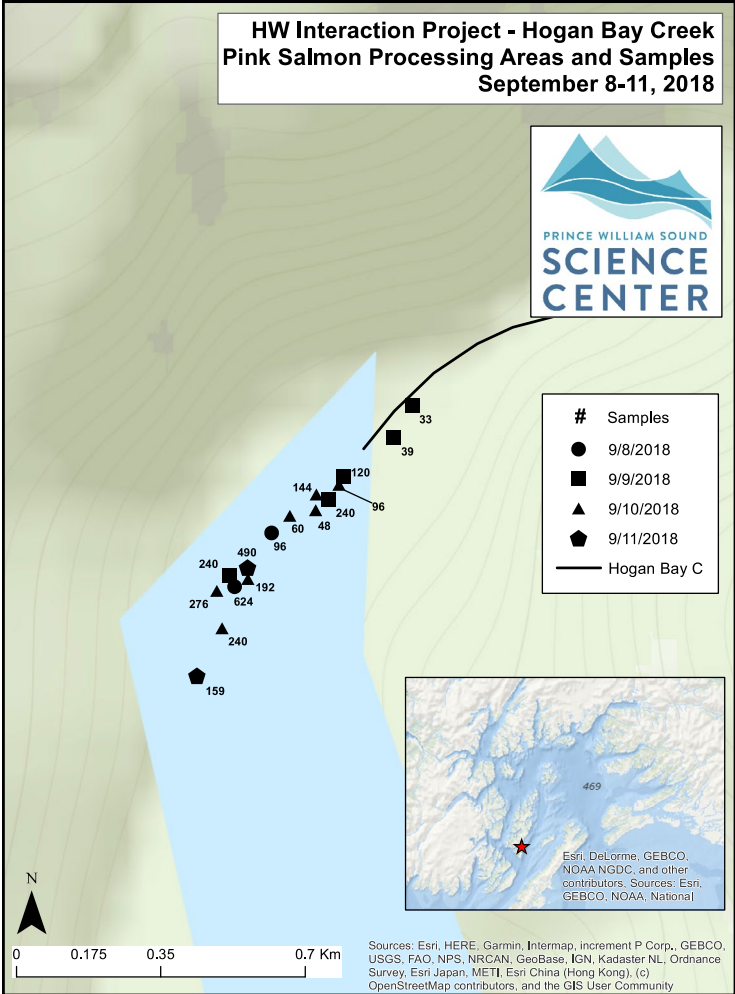


Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

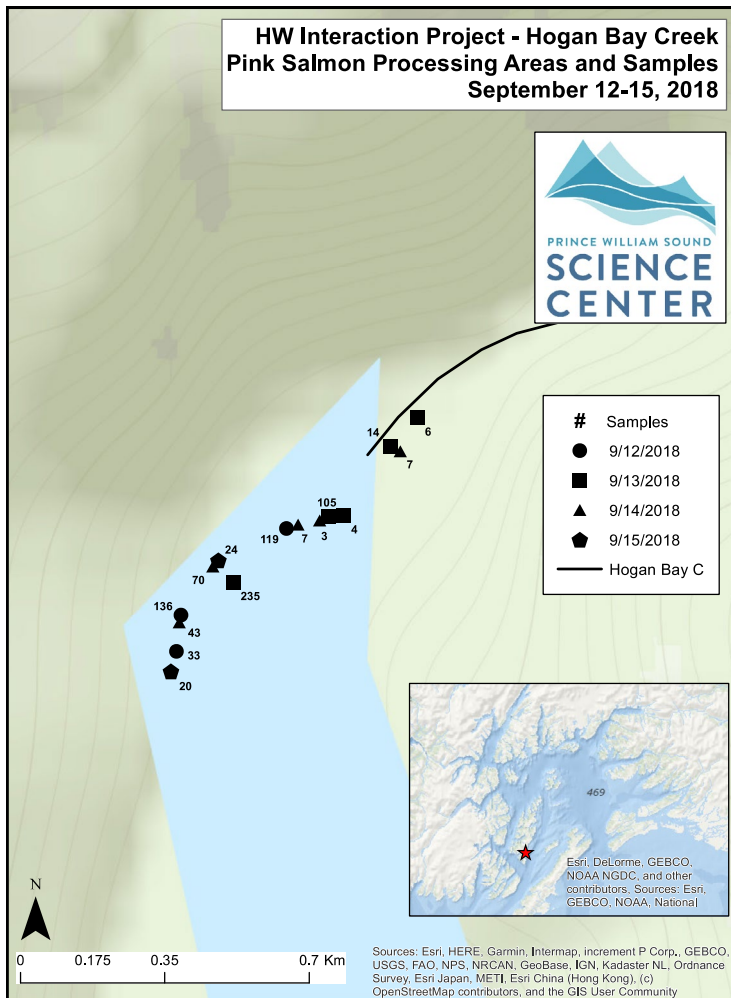
**HW Interaction Project - Hogan Bay Creek
Pink Salmon Processing Areas and Samples
September 8-11, 2018**



#	Samples
●	9/8/2018
■	9/9/2018
▲	9/10/2018
⬠	9/11/2018
—	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), (c) OpenStreetMap contributors, and the GIS User Community



Stockdale Creek
227-20-17520
Pink Salmon Fitness

2018 Samples Collected and Run Timing: Pink salmon sampling for fitness and hatchery fraction at Stockdale Creek from August 7th to September 8th yielded 5,179 samples over 22 visits. On the first visit of the season (8/7/2018), 16,290 live Pink Salmon were counted, but very few dead fish were present (<100).

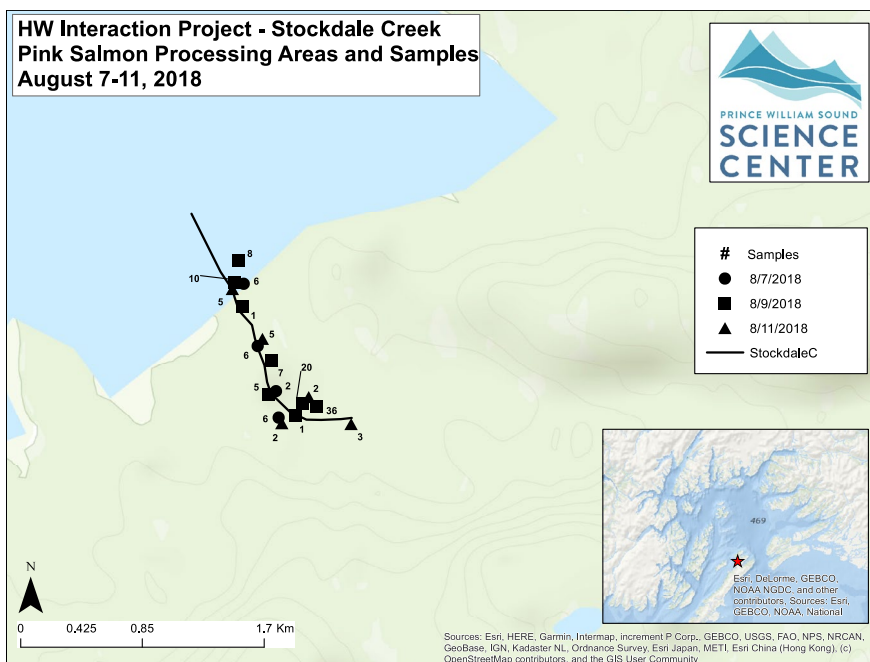
In-stream live counts increased steadily until 8/17/18 when 36,870 live and 663 dead pinks were counted. Heavy rains on and around 8/20/18 hampered our sampling efforts and likely flushed many dead fish into the intertidal. The crew returned to Cordova on 8/21/18 for a ~30hr resupply and personnel change, before resuming sampling Stockdale on 8/24/18. After the rains, a relative

drought ensued, and water levels in Stockdale subsided steadily until the last sampling day on 9/8/18. Many redds appeared exposed as the waters subsided, and many fish died pre-spawning as they were stranded in gravel banks. Dead fish rotted quickly, and toward the end of our sampling season, outpaced our ability to sample before they decomposed entirely.

Extent of Stream Sampled: In concurrence with 2018 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, sampling dead fish in the lower reaches was prioritized, with live/dead counts conducted along the entire reach every other day.

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.

Unusual Events: Two bear encounters occurred while sampling Stockdale. The first, on 8/9/18, occurred while all four crew members waded at chest height through a cut between logjams below the fork. Grunting and shuffling was heard from the heavily vegetated riverbank above head level. The crew slowly backed away with bear spray readied, and detoured over the opposite bank. The bear was not sighted. The second event occurred on 8/17/18 at a safe distance. A young brown bear was sighted in the lower reaches of the stream, fishing in the shallow bar mid-stream. The crew observed the bear for a few minutes, before our noise and slow approach encouraged the bear to leave.

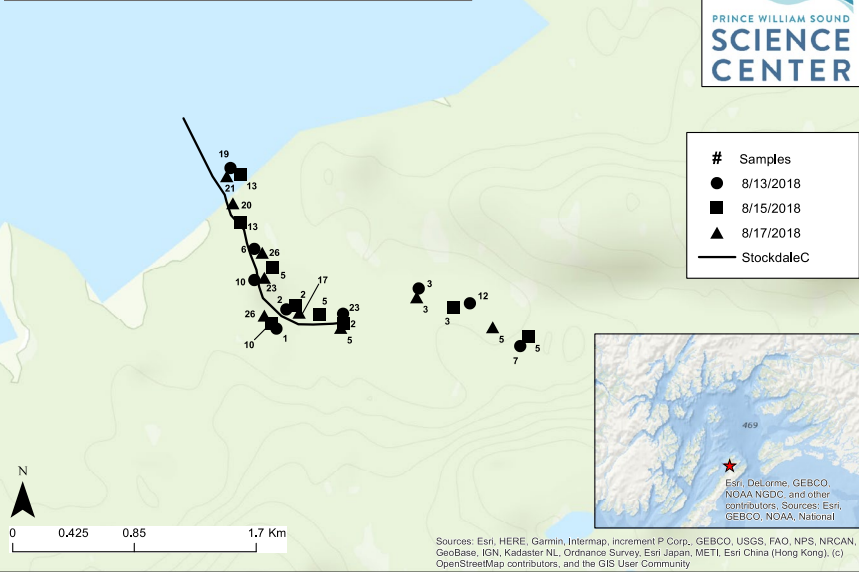


**HW Interaction Project - Stockdale Creek
Pink Salmon Processing Areas and Samples
August 13-17, 2018**



Samples

- 8/13/2018
- 8/15/2018
- ▲ 8/17/2018
- StockdaleC

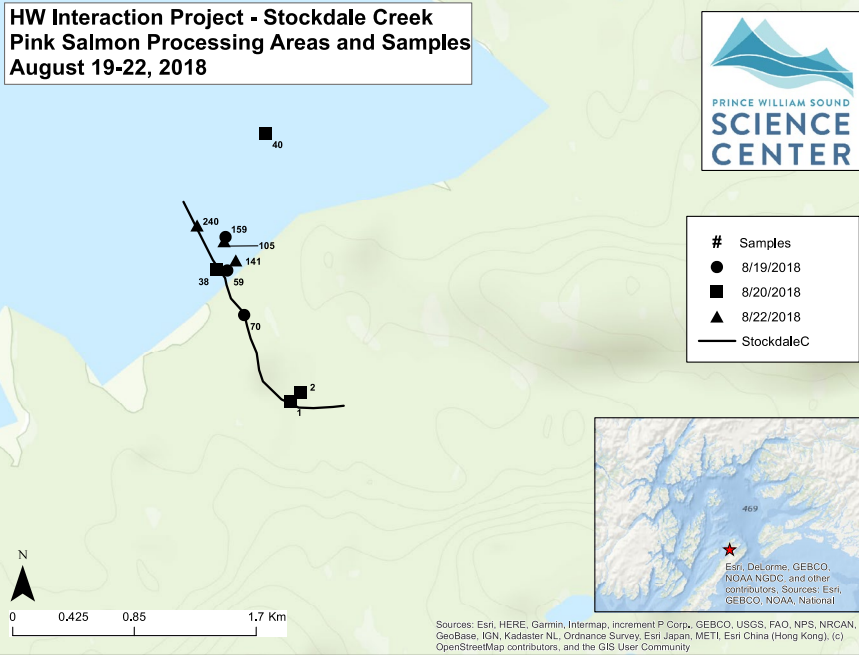


**HW Interaction Project - Stockdale Creek
Pink Salmon Processing Areas and Samples
August 19-22, 2018**



Samples

- 8/19/2018
- 8/20/2018
- ▲ 8/22/2018
- StockdaleC

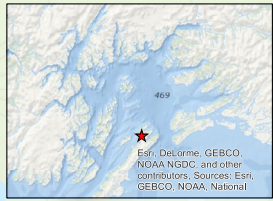
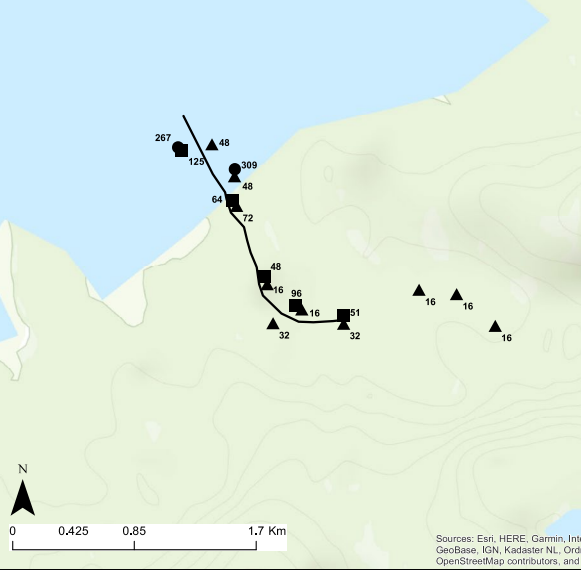


**HW Interaction Project - Stockdale Creek
Pink Salmon Processing Areas and Samples
August 24-28, 2018**



Samples

- 8/24/2018
- 8/26/2018
- ▲ 8/28/2018
- StockdaleC



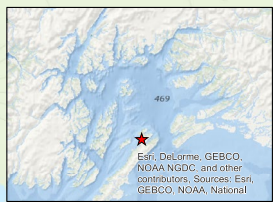
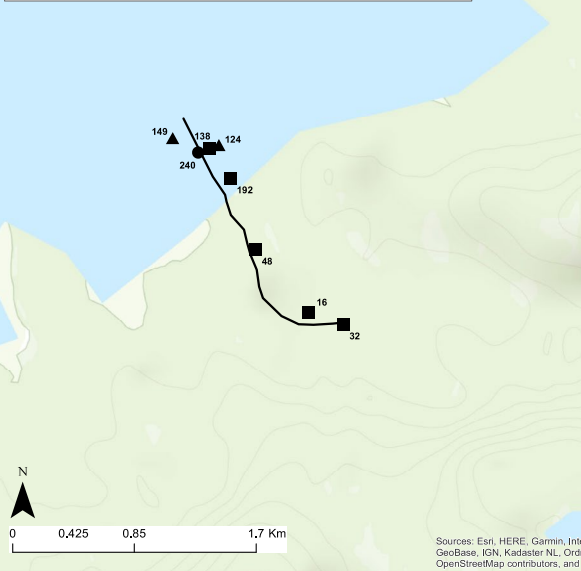
Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Stockdale Creek
Pink Salmon Processing Areas and Samples
August 29-31, 2018**



Samples

- 8/29/2018
- 8/30/2018
- ▲ 8/31/2018
- StockdaleC



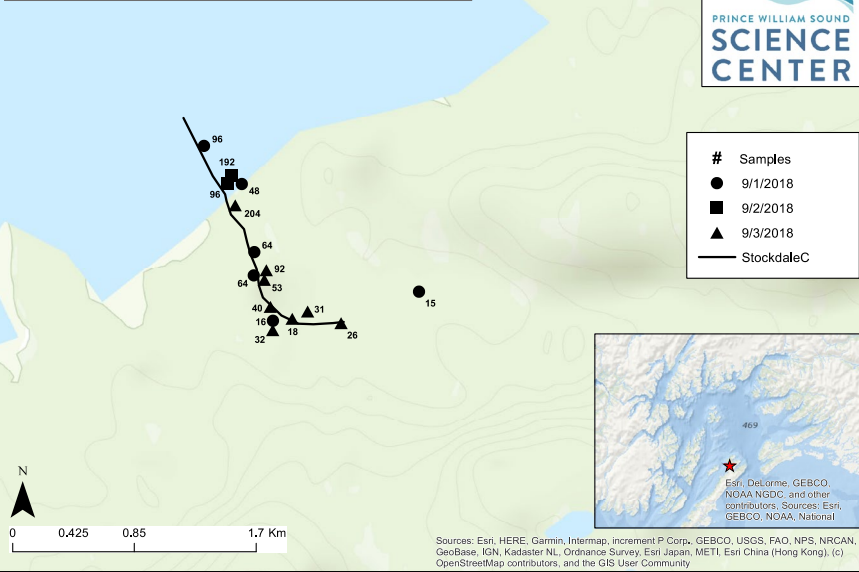
Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Stockdale Creek
Pink Salmon Processing Areas and Samples
September 1-3, 2018**



Samples

- 9/1/2018
- 9/2/2018
- ▲ 9/3/2018
- StockdaleC



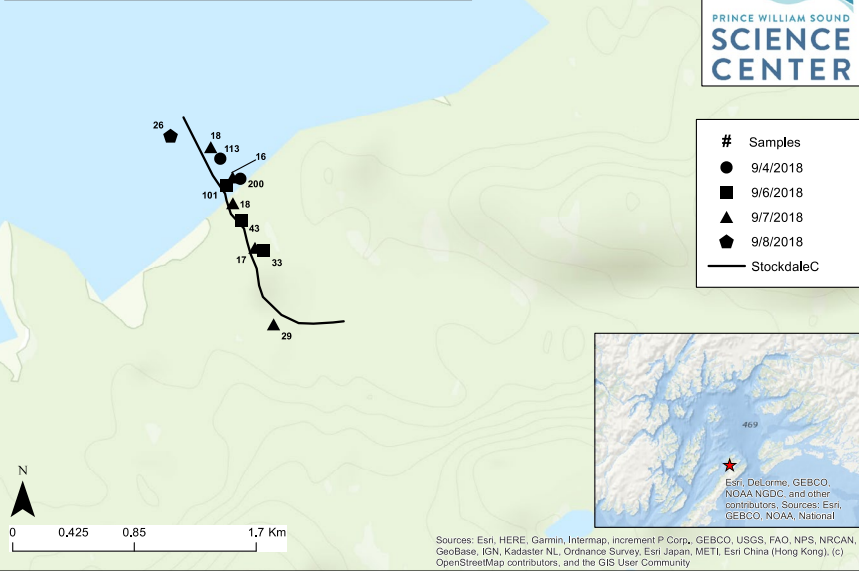
Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Stockdale Creek
Pink Salmon Processing Areas and Samples
September 4-8, 2018**



Samples

- 9/4/2018
- 9/6/2018
- ▲ 9/7/2018
- ◆ 9/8/2018
- 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), (c) OpenStreetMap contributors, and the GIS User Community

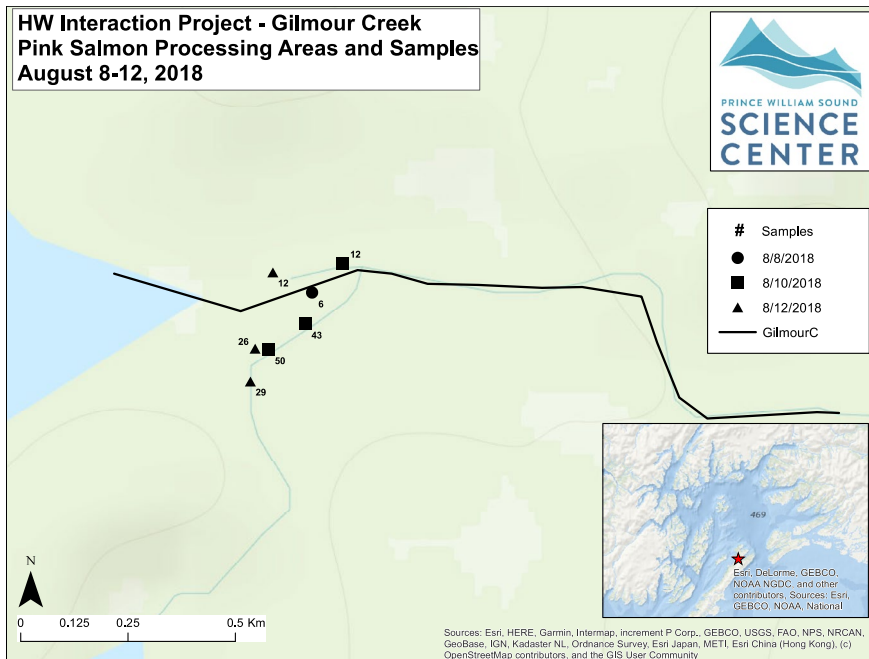
Gilmour Creek
227-20-17480
Pink Salmon Fitness

2018 Samples Collected and Run Timing: Pink Salmon sampling for fitness and hatchery fraction at Gilmour Creek from August 8th to September 9th yielded 2,902 samples over 19 visits. Gilmour Creek’s two main branches were surveyed on every visit and their cumulative counts are summarized. August 16 saw the greatest number of Pink Salmon, with 7,151 live fish counted. Dry conditions after August 21 led to drastic reduction in stream flow, leading to exposure of many redds and stranding of pre-spawn fish on gravel beds.

Extent of Stream Sampled: Full surveys of both branches were performed on every visit. 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. The right fork was easily surveyed until a sharp left turn upstream, where thick vegetation made foot passage more difficult, though possible. Few fish were counted upstream of that bend.

Unusual Events: Despite frequent signs of predation, no bears were encountered on Gilmour Creek in 2018.

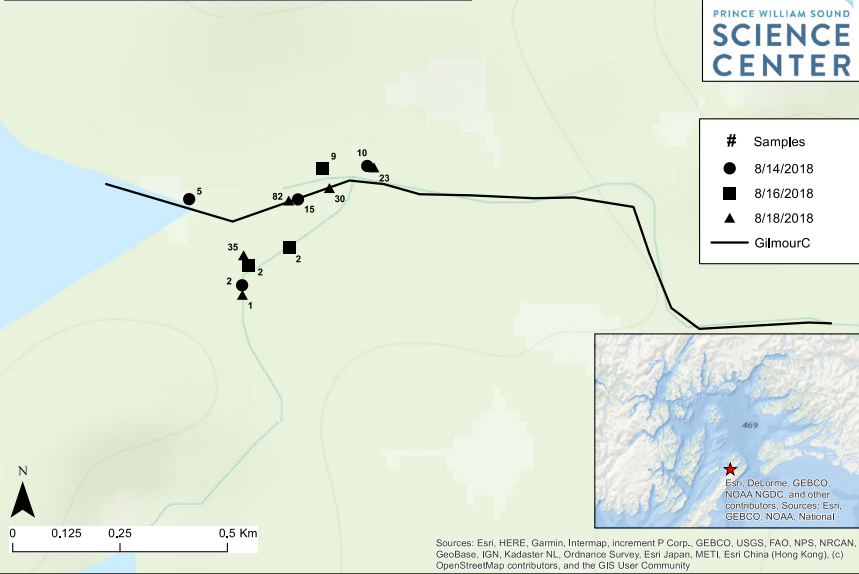
Recommendations, changes, and other notes: During high spring tides, the intertidal extends deep into the lower reaches of the stream and its tributary; it is usually best to begin upstream and work down as the tide recedes, if possible.



**HW Interaction Project - Gilmour Creek
Pink Salmon Processing Areas and Samples
August 14-18, 2018**



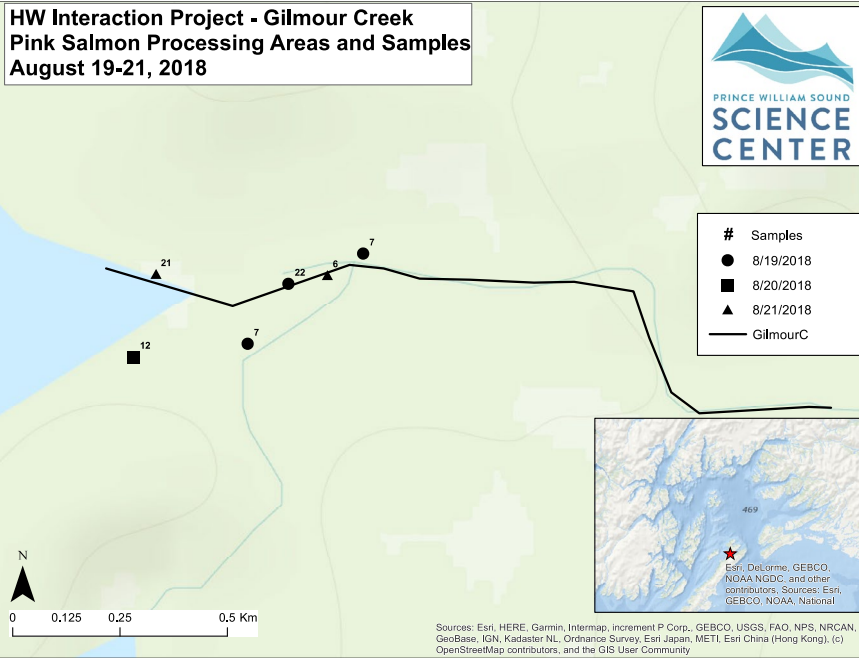
- # Samples**
- 8/14/2018
 - 8/16/2018
 - ▲ 8/18/2018
 - GilmourC



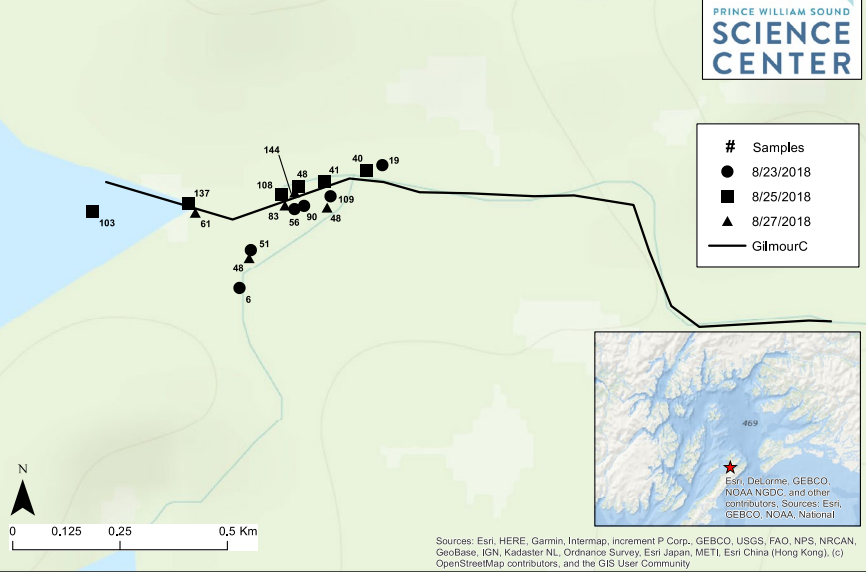
**HW Interaction Project - Gilmour Creek
Pink Salmon Processing Areas and Samples
August 19-21, 2018**



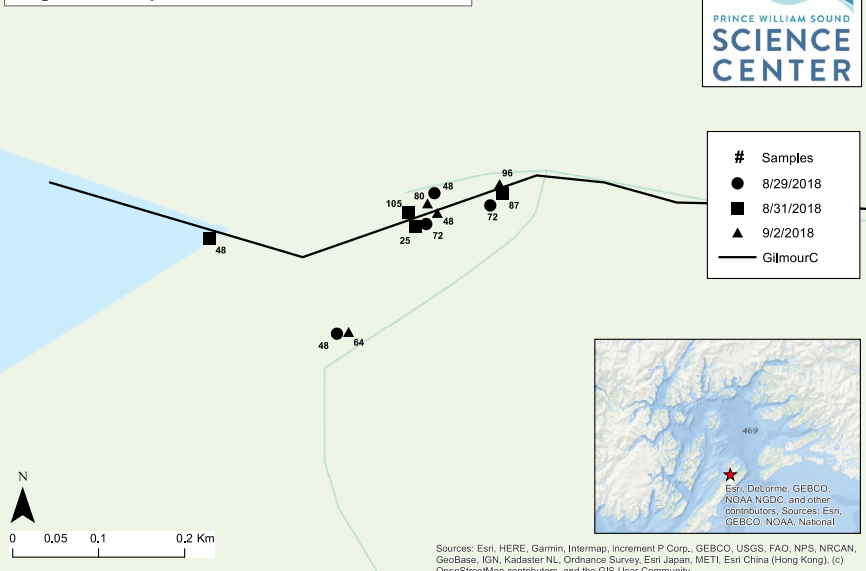
- # Samples**
- 8/19/2018
 - 8/20/2018
 - ▲ 8/21/2018
 - GilmourC



**HW Interaction Project - Gilmour Creek
Pink Salmon Processing Areas and Samples
August 23-27, 2018**



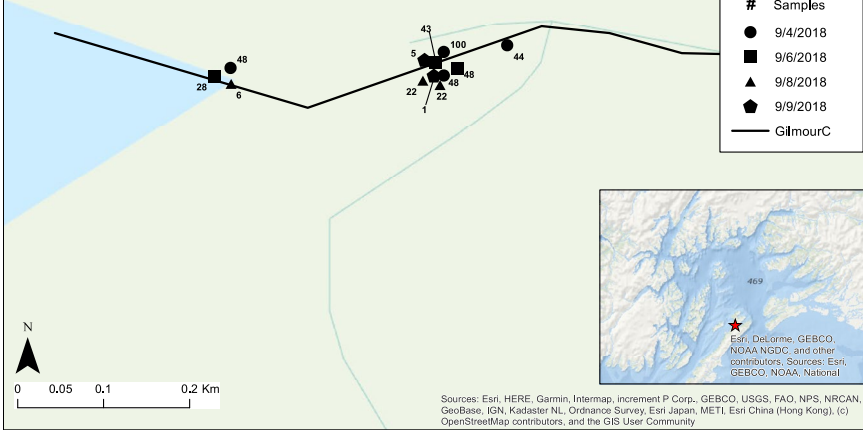
**HW Interaction Project - Gilmour Creek
Pink Salmon Processing Areas and Samples
August 29-September 2, 2018**



**HW Interaction Project - Gilmour Creek
Pink Salmon Processing Areas and Samples
September 4-9, 2018**



- # Samples**
- 9/4/2018
 - 9/6/2018
 - ▲ 9/8/2018
 - ◆ 9/9/2018
 - GilmourC



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), (c) OpenStreetMap contributors, and the GIS User Community