



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

Progress Report for 2016

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

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ABSTRACT

This is the fifth 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). 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. The research in 2016 was focused only on PWS Pink Salmon fitness studies because the experimental design allowed a hiatus of fitness data collection on Chum Salmon in SEAK. In 2016 field sampling, 37,549 individual Pink Salmon were sampled during repeated visits to the spawning grounds of five PWS streams that had been previously sampled for this research. DNA tissue samples were collected from each fish for the eventual analysis of relative survival. Otoliths were collected from the same specimens for identification of possible hatchery origin (hatchery Pink Salmon in Alaska have thermally marked otoliths that were used to determine hatchery or natural origin). All otolith samples collected from Stockdale and Hogan Creeks were analyzed, and collections from the other three pedigree streams were subsampled. Fractions of hatchery Pink Salmon were estimated to range from 7.8 percent to 20.6 percent for the five PWS spawning populations. These values are lower than in previous years. While several factors could explain this pattern, these results likely reflect higher survival of natural Pink Salmon relative to hatchery Pink Salmon, as compared to the 2014 adult run, based on harvest records reported by ADF&G. Results of the relative survival of PWS Pink Salmon hatchery-origin and wild-origin offspring will be forthcoming after final field sampling in 2018 and the subsequent DNA tissue analyses are completed in 2019.

INTRODUCTION

Prince William Sound Science Center (PWSSC) is engaged in continuing 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 fifth annual report, focusing on the results of 2016 data collection and analysis, as well as summarizing some results from 2013 through 2016.

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 2016 field research consisted of only one major activity: adult sampling of Pink Salmon in five PWS streams to collect DNA and otolith samples for the ongoing fitness studies. The otolith sampling also allowed for estimation of the hatchery fractions of spawning salmon in only 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 2016, 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 because of consultation with the Science Panel in November 2012, December 2013, December 2014, and April 2015. A complete, revised 2016 field sampling protocol is presented in Appendix A. This report includes summaries of sample collection during 2016 for the DNA-based fitness studies. DNA samples were delivered to the ADF&G Gene Conservation Lab. Pink Salmon fitness analysis will be reported after the 2018 final sampling and the subsequent DNA tissue analyses are completed in 2019. Similarly, otoliths collected during 2016 were submitted to the ADF&G lab in Cordova for processing. Otolith results will be used for the fitness analysis as well. This report includes analysis of hatchery fractions for the five PWS streams.

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 interannual 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. The otoliths were examined in ADF&G laboratories to determine whether the individuals are of hatchery or wild origin. The now completed results are estimates of the percent and sometimes numbers of hatchery fish for each stream, district, and regional spawning population over three years (Knudsen et al. 2016). The latter objective is being addressed by collecting tissues for DNA analysis, as well as otoliths, from adults in a subset of the same streams, referred to here as “fitness” streams. The DNA “markers” of these parents can be used to identify either their pre-emergent offspring collected the following spring, or progeny returning to the streams as adults, so that relative reproductive success (fitness) of hatchery- and natural-origin fish can be estimated for both males and females. Field sampling in 2016 focused only on PWS Pink Salmon because the experimental design of the study allowed for a hiatus in sampling the very few 3-year-old adult Chum Salmon of potential known parentage that were expected to return in SEAK.

Definitions:

Hatchery salmon – Originated in a hatchery.

Wild salmon – Originated in a stream from wild x wild spawning. i.e., no hatchery-origin heritage.

Natural salmon – Originated in a stream from parents that could have been of wild, natural, or hatchery-origin, and can include purely wild or unknown origins.

Methods

DNA tissues were collected along with otoliths from adult Pink Salmon in 2016 in PWS only. This required repeated sampling of only the five fitness study streams in PWS (Figure 1). The 2016 methods were only slightly varied for improvement of the methods used in 2013-2015 (Knudsen et al. 2015a, b, Knudsen et al. 2016).

The RFP originally specified that fitness study streams have sampling targets of 500 individuals in expected high-stray-rate streams and 1,000 individuals in streams with expected lower stray rates. Subsequent discussions with ADF&G Gene Conservation Laboratory 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 increased in 2015 and maintained in 2016 to make every effort to sample the low-fraction fitness streams every day and the high-fraction streams every other day.

Logistical Strategy

From July 31 through September 17, 2016, two - 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 experiment in 2016 because the extremely low hatchery fraction, particularly in even, low run years such as 2016, would not yield meaningful results in the relative fitness study.)

All field crews were directly employed by the PWSSC in 2016. Stream sampling is a dynamic process influenced by current fish abundance, historic run timing, weather, crew location, and distance between streams. Each field crew was unique and independent and the 2016 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*, which sampled Hogan Creek, Gilmour Creek and Stockdale Creek, and a land-based, camping crew stationed in Paddy Bay that sampled Erb Creek and Paddy Creek. The Auklet, Alexandra, and Paddy crews trained July 25-29, 2016. Training included boating, bear and firearms safety, CPR and First Aid, protocol review, tablet use, and data entry. The Auklet and Paddy Bay field crews were deployed the Saturday after training and the Alexandra crew was deployed August 21, 2016 to sample at Hogan Bay during the peak run.

In 2016, the Auklet crew completed a total of 85 stream visits and the Alexandra crew completed 27 stream visits. For the first three weeks of the season, until the *F/V Alexandra* was deployed, the *M/V Auklet* crew was responsible for sampling three fitness streams; Stockdale, Gilmour, and Hogan Bay creeks. Distributing sampling effort between streams was at times challenging due to weather, tides, and sample availability, however, the *M/V Auklet* crew made 57 successful stream visits to those three fitness streams before making their first and only port call (August 23-26). While the *M/V Auklet* was in port, the Alexandra crew sampled Stockdale, Gilmour, and Hogan Bay creeks. When the *M/V Auklet* returned, the *F/V Alexandra* crew remained stationed 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 2016 season ended, with 0 – 50 live fish in all creeks, between September 11 and 17, 2016.

The PWSSC camping crew in 2016 was stationed in Paddy Bay from July 30 to September 14, 2016. The Paddy crew was responsible for sampling at Erb Creek and Paddy Creek and made a total of 37 stream visits to both Erb and Paddy creeks. The Paddy crew used a rigid hull inflatable skiff to navigate between Erb Creek and camp and hiked to Paddy Creek from camp. As stated in the field sampling protocol (Appendix A), the goal was to sample Paddy and Erb creeks daily. This pattern was followed during periods of low sample abundance but, during peak abundance, the crew determined they could maximize their efficiency by sampling the two creeks intensively on alternate days. The *M/V Auklet* assisted Paddy camp set-up and take-down by deploying camp on July 31st and demobilizing camp on September 14, 2016.

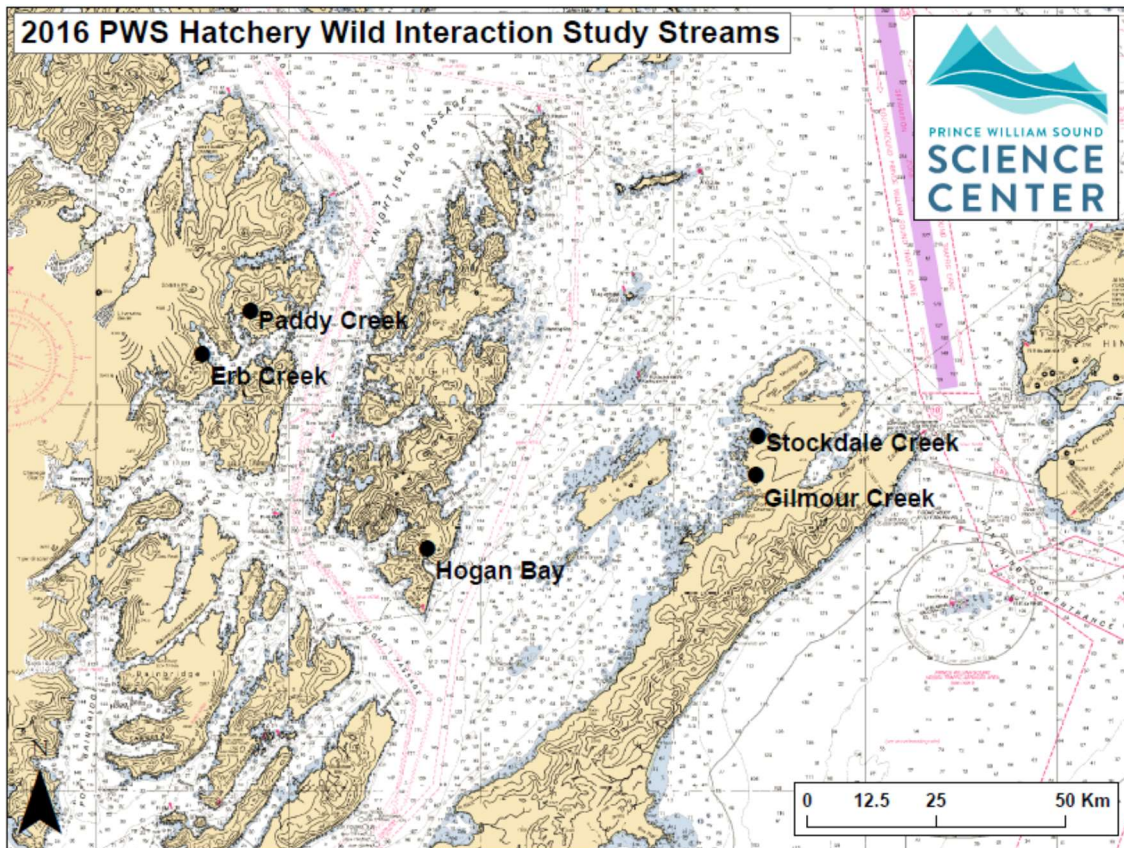


Figure 1. PWS streams sampled for Pink Salmon otoliths and DNA tissues in 2016.

Stream Sampling Methods and Execution

Upon arriving at a study stream, the crew leader 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. When crews were sampling streams daily, they sometimes sampled about half of the creek on one day and the other half the following day.

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

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

The last phase of stream sampling was to perform a fish survey to establish a rough index of fish abundance at the time of the sampling visit. When fish sampling was close to completion, two or more crew members conducted both a live and dead estimate of all salmon species within the survey bounds. If multiple people were counting the same species, estimates were discussed at the end of the survey and averaged to produce a final count. When the survey was complete, a responsible crew member marked the end location of the survey, checked the count numbers, and made any additional comments.

Communication and Data Transmission

All crews had float plans and checked in daily with the PWSSC 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 2016.

After completion of a final quality control review in Cordova at the end of the season, the fitness stream otoliths and tissues were delivered to the Anchorage ADF&G GCL on October 6, 2016, where otoliths were extracted and shipped back to the Cordova ADF&G office for processing. 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 2016 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 2015 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, 2015, and 2016 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, 2014, and 2015, the objectives of the PWS field sampling in 2016 included estimates for the fractions of hatchery fish in each spawning population of Pink Salmon. However, unlike earlier years when hatchery fractions were estimated for over two dozen streams, only five streams were visited in 2016. 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 2016 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, the Science Panel decided that all otoliths from only Stockdale and Hogan Bay creeks would be read for 2016. For the other three streams (Erb, Paddy, and Gilmour creeks), approximately 380 otoliths were read for estimating hatchery fraction on each of those spawning grounds. A stratified design was used to

select the 380 individuals by processing eight full otolith trays from each of the three streams. Based on the number of trays collected, every 23rd tray was read for Erb, every 12th for Paddy, and every 14th for Gilmour. Numbers of samples for calculating hatchery fraction are shown in Table 1.

RESULTS

Final results comparing the relative survival of hatchery- and natural-origin offspring will be forthcoming after the last PWS Pink Salmon field sampling in 2018 and the subsequent DNA tissue analyses are completed in 2019 (approximately 2024 for the SEAK Chum Salmon relative fitness analysis). The 2016 and previous years' results of the hatchery fractions estimated for the five PWS fitness streams are reported below.

PWS Pink Salmon Fitness and Hatchery Fraction Sampling

Overall, 2016 sampling was successful at all five selected Pink Salmon PWS fitness study streams where 37,549 DNA tissues and pairs of otoliths were taken. Rather than having a numerical sampling goal in 2016, 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 thought by the Science Panel to be necessary for analysis of relative survival (Table 1). The number of samples varied per stream visit (Appendix C). Foot survey-based live and dead counts were made on all stream surveys (Appendix C) and the dead counts were later used to weight the hatchery fraction estimates per visit. (See Appendix D for more details on the sampling of each PWS stream.)

Hatchery fractions of the five spawning Pink Salmon populations ranged from 7.8 percent at Stockdale Creek to 20.6 percent in Paddy Creek in 2016 (Table 1).

Table 1. A stream-by-stream summary of sampling and hatchery fractions for five PWS Pink Salmon streams in 2016. Average seasonal hatchery fractions for each stream were weighted by observed dead salmon counts taken during each visit. Note that the five streams sampled in 2016 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 2016	Samples collected 2016	Samples used for hatchery fraction 2016 ^a	Average hatchery fraction weighted by dead counts per visit			
					2013	2014	2015	2016
Paddy C	226-20-16010	37	3,273	328	0.154	0.595	0.328	0.206
Erb C	226-20-16040	37	8,091	383	0.113	0.228	0.214	0.100
Hogan Bay	226-30-16810	34	13,007	12,830	0.640	0.915	0.583	0.205
Gilmour C ^a	227-20-17480	20	4,178	378	NA	0.557	0.225	0.116
Stockdale C	227-20-17520	31	9,000	8,709	0.163	0.735	0.240	0.078

^aOtolith collections from Paddy, Erb, and Gilmour creeks were subsampled - see Methods.

^bGilmour Creek was not sampled in 2013 to estimate the hatchery fraction of its spawning Pink Salmon population.

DISCUSSION

The 2016 hatchery fractions in the five spawning streams were notably less than in previous years, especially compared to 2014, the previous even-year run (Table 1). Reduced estimated hatchery fractions could possibly have been influenced by two types of factors: natural variations and/or sampling effects.

Population-level factors that could have contributed to lower fraction of hatchery Pink Salmon in the spawning streams include 1) greater survival or abundance of naturally produced fish relative to hatchery fish or 2) more efficient commercial harvest of available hatchery fish than of natural fish. Evidence from the commercial catch over the past few years suggests some combination of lower relative survival of hatchery fish and/or higher survival of natural fish in 2016 and corroborates the findings of reduced stream hatchery fractions. Comparing 2016 catches to the previous even-year run in 2014, wild catch increased from 1.8 million to 2.2 million while hatchery catch decreased from 36.6 million to 6.4 million, as shown below.

	Millions of Pink Salmon harvested ^a			Percent hatchery
	Hatchery	Wild	Total	
2013	69.2	17.4	86.7	80
2014	36.6	1.8	38.4	95
2015	64.5	25.5	90.1	72
2016	6.4	2.2	8.6	74

^aData from Stormy Haught ADF&G, Cordova

Much less likely sampling-related explanations for the lower hatchery fraction in 2016 include 1) lack of otolith marks on hatchery fish, 2) misreading of otolith marks from hatchery fish and/or 3) biased or scant sampling of fish on spawning grounds. On the last point, it is also possible, although unlikely, that the subsampling of otolith trays from Paddy, Erb, and Gilmour creeks could have biased the hatchery-natural fraction but the pattern of low hatchery fraction was consistent across all five streams. We doubt that any of these factors contributed to the relatively low fraction of hatchery spawners observed in the streams because all PWS hatchery Pink Salmon have their otoliths thermally marked prior to release, the otolith reading techniques at the ADF&G Cordova lab have been consistent and include quality controls, and a very high number of samples were collected from the streams, representing all portions of the run (Table 1).

REFERENCES

- Buckhorn, M., T. Kline, and V. O’Connell. 2013. Interactions of Wild and Hatchery Pink Salmon and Chum Salmon in Prince William Sound and Southeast Alaska. Annual Report for 2012, For Alaska Department of Fish and Game Contract IHP-13-013, Cordova, Prince William Sound Science Center, Alaska.
- Knudsen, E., M. Buckhorn, K. Gorman, D. Crowther, K. Froning, M. Roberts, L. Marcello, B. Adams, V. O’Connell, D. Bernard. 2015a. Interactions of Wild and Hatchery Pink Salmon and Chum Salmon in Prince William Sound and Southeast Alaska. Final Progress Report for 2013, For Alaska Department of Fish and Game Contract IHP-13-013, Cordova, Prince William Sound Science Center, Alaska.
- Knudsen, E., M. Buckhorn, K. Gorman, P. Rand, M. Roberts, B. Adams, V. O’Connell, D. Bernard. 2015b. Interactions of Wild and Hatchery Pink Salmon and Chum Salmon in Prince William Sound and Southeast Alaska. Final Progress Report for 2014, For Alaska Department of Fish and Game Contract IHP-13-013, Cordova, Prince William Sound Science Center, Alaska.
- Knudsen, E., P. Rand, K. Gorman, J. McMahon, B. Adams, V. O’Connell, and D. Bernard. 2016. Interactions of Wild and Hatchery Pink Salmon and Chum Salmon in Prince William Sound and Southeast Alaska. Final Progress Report for 2015, For Alaska Department of Fish and Game Contract IHP-13-013, Cordova, Prince William Sound Science Center, Alaska.

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 2016. 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 2016. 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 2016 (see also the “Tote Board” on Google Drive).

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

Tote Board

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

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

Preparations for Surveys

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

√ Charge all tablets and scanners fully

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

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

√ Clean and inspect guns and other safety gear

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

√ Dry and patch waders

√ Prepare for the next sample visit

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

√ Prepare sampling kits

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

Stream Surveys

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

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

Arriving at the Study Stream

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

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

Starting a Survey

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

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

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

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

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

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

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

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

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

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

Data Entry

At the beginning point of the survey,

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

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, but not all, 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, as a way 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 of good quality. If the 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 do that 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).

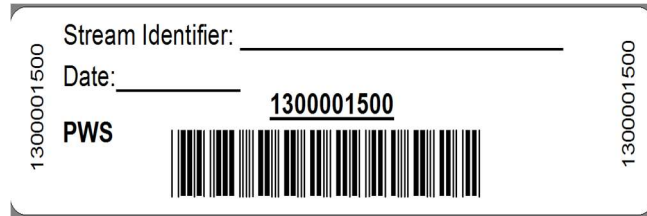
Pre-printed barcode labels for each 48dwp have been provided by GCL. Keep label sheets packed in a ZipLoc bag to keep them dry prior to application. Labeling 48dwp trays should be done beforehand in a dry area to insure the label adheres. Apply label on notched side (picture below) of each plate. Barcode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

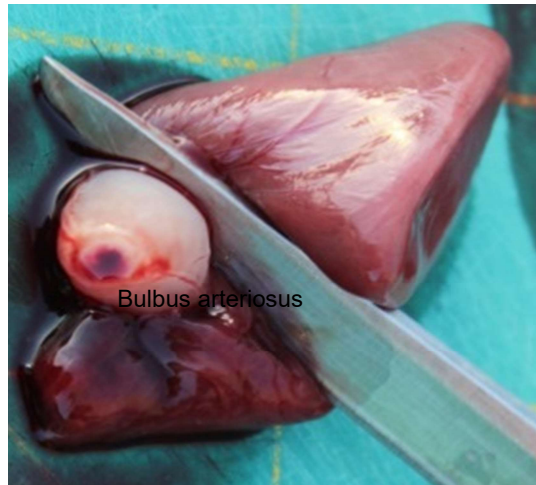


Figure B3. Bulbus arteriosus attached to heart.

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.

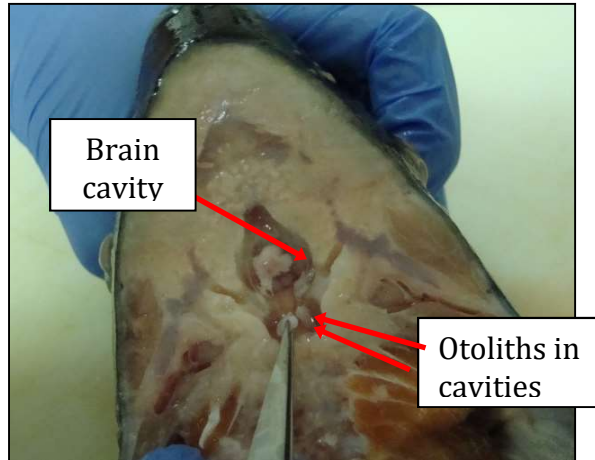


Figure B6. Otoliths in two lateral wells posterior to brain cavity.

Using 48dwp Trays for DNA Tissue and Otoliths

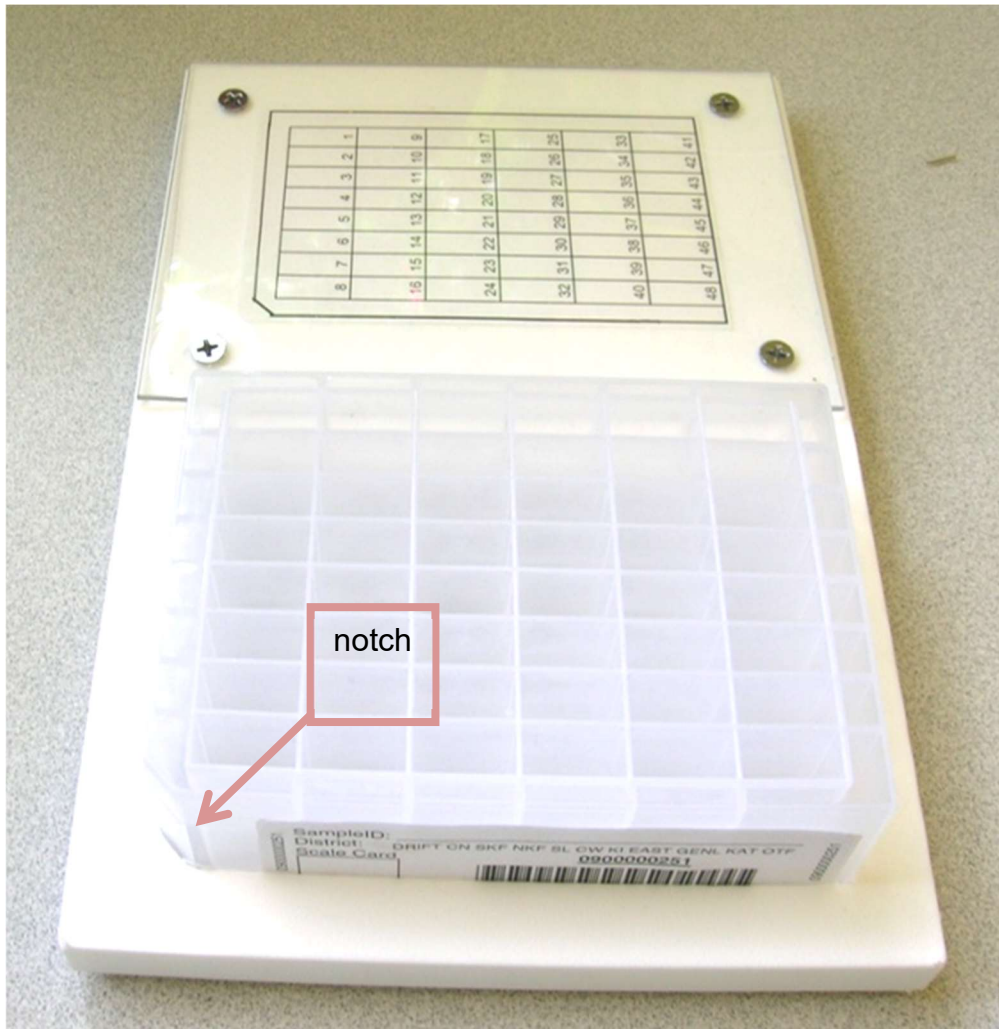
It is important to try to collect the best **quality DNA tissue samples to ensure reliable results**. Tissue quality degrades after death, so moribund and recently dead fish will yield best results. Frequent visits to streams will provide better opportunity to sample fresher spawn-outs. However, sample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

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.

Delivery Slip for HWI Samples to Cordova Otolith

Page ____ of ____

(An Excel sheet like this can be created starting with the download of a Tray Inventory report from the Keta.Finsight web site.)

Sample Date	Sample Location (AStream #)	Species	# Otoliths Sampled	Tray #	Sample Type	Comments
<i>e.g.:</i> 7/4/2013	<i>Erb Creek</i> (226-20-16040)	<i>Pink</i>	48	1300001757	<i>Pedigree</i>	<i>One oto in cells A5, D2</i> <i>Samplers: J. Doe, B. Smith</i>

Date sent to Cordova Lab:

Date received by Cordova Lab:

Person responsible for delivery:

Recipient:

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

Stream Name	Adfg Stream Code	Survey Date	Number of Specimens	Pink Alive Count	Pink Dead Count	Chum Alive Count	Chum Dead Count
Erb C	226-20-16040	7/31/2016	0	183	0	2	0
Erb C	226-20-16040	8/1/2016	4	183	4	3	0
Erb C	226-20-16040	8/2/2016	2	975	2	3	0
Erb C	226-20-16040	8/3/2016	3	603	3	4	0
Erb C	226-20-16040	8/4/2016	13	607	14	6	0
Erb C	226-20-16040	8/5/2016	7	944	13	4	0
Erb C	226-20-16040	8/6/2016	12	1007	23	5	0
Erb C	226-20-16040	8/7/2016	12	1087	25	2	1
Erb C	226-20-16040	8/8/2016	25	1965	44	7	2
Erb C	226-20-16040	8/9/2016	21	1699	54	7	3
Erb C	226-20-16040	8/10/2016	48	1771	83	14	2
Erb C	226-20-16040	8/11/2016	64	1709	130	16	6
Erb C	226-20-16040	8/12/2016	26	1380	90	6	4
Erb C	226-20-16040	8/13/2016	104	2125	193	4	1
Erb C	226-20-16040	8/14/2016	35	1875	112	2	4
Erb C	226-20-16040	8/15/2016	129	2526	283	4	7
Erb C	226-20-16040	8/16/2016	194	3071	533	1	23
Erb C	226-20-16040	8/17/2016	107	2500	438	3	10
Erb C	226-20-16040	8/18/2016	265	3135	934	2	17
Erb C	226-20-16040	8/19/2016	213	1861	403	30	2
Erb C	226-20-16040	8/20/2016	370	3126	1072	1	25
Erb C	226-20-16040	8/21/2016	462	3347	1383	3	15
Erb C	226-20-16040	8/22/2016	232	2360	1202	2	9
Erb C	226-20-16040	8/24/2016	602	1450	1426	2	10
Erb C	226-20-16040	8/26/2016	858	2782	1455	0	0
Erb C	226-20-16040	8/28/2016	864	2572	2058	1	4
Erb C	226-20-16040	8/30/2016	768	2515	2246	0	9
Erb C	226-20-16040	9/1/2016	864	1369	2729	0	1
Erb C	226-20-16040	9/3/2016	624	915	2706	0	0
Erb C	226-20-16040	9/5/2016	787	465	2978	0	0
Erb C	226-20-16040	9/6/2016	148	180	2265	0	1
Erb C	226-20-16040	9/8/2016	143	54	2884	0	2
Erb C	226-20-16040	9/9/2016	27	16	1613	0	0
Erb C	226-20-16040	9/10/2016	22	2	2205	0	3
Erb C	226-20-16040	9/11/2016	34	0	2284	0	0
Erb C	226-20-16040	9/12/2016	2	0	1370	0	0

Erb C	226-20-16040	9/13/2016	0	0	1261	0	0
Gilmour C	227-20-17480	8/1/2016	0	12	0	115	34
Gilmour C	227-20-17480	8/3/2016	2	47	2	143	45
Gilmour C	227-20-17480	8/5/2016	0	3	0	17	113
Gilmour C	227-20-17480	8/7/2016	0	19	1	22	63
Gilmour C	227-20-17480	8/9/2016	2	116	2	27	66
Gilmour C	227-20-17480	8/11/2016	3	251	5	33	70
Gilmour C	227-20-17480	8/13/2016	0	342	3	7	18
Gilmour C	227-20-17480	8/15/2016	2	1402	3	8	24
Gilmour C	227-20-17480	8/17/2016	23	1655	28	8	52
Gilmour C	227-20-17480	8/18/2016	20	2283	18	7	15
Gilmour C	227-20-17480	8/21/2016	144	753	276	1	31
Gilmour C	227-20-17480	8/25/2016	286	3375	758	0	0
Gilmour C	227-20-17480	8/28/2016	432	607	742	0	0
Gilmour C	227-20-17480	8/30/2016	672	753	2445	0	0
Gilmour C	227-20-17480	9/1/2016	480	437	1880	0	0
Gilmour C	227-20-17480	9/3/2016	624	224	2832	0	0
Gilmour C	227-20-17480	9/5/2016	384	186	700	0	0
Gilmour C	227-20-17480	9/7/2016	250	276	1593	0	0
Gilmour C	227-20-17480	9/9/2016	64	95	1265	0	0
Gilmour C	227-20-17480	9/12/2016	1	1	62	0	0
Hogan Bay	226-30-16810	7/31/2016	0	0	0	0	0
Hogan Bay	226-30-16810	8/2/2016	0	0	0	0	0
Hogan Bay	226-30-16810	8/6/2016	0	673	1	19	0
Hogan Bay	226-30-16810	8/10/2016	17	427	25	2	1
Hogan Bay	226-30-16810	8/12/2016	10	774	13	1	0
Hogan Bay	226-30-16810	8/16/2016	89	3330	116	6	1
Hogan Bay	226-30-16810	8/20/2016	603	3230	859	6	31
Hogan Bay	226-30-16810	8/27/2016	336	4072	2278	0	0
Hogan Bay	226-30-16810	8/29/2016	576	3137	2550	0	0
Hogan Bay	226-30-16810	8/31/2016	576	2773	2670	0	0
Hogan Bay	226-30-16810	9/3/2016	576	2030	5464	0	0
Hogan Bay	226-30-16810	9/5/2016	480	3426	4813	0	0
Hogan Bay	226-30-16810	9/6/2016	576	2558	3623	0	0
Hogan Bay	226-30-16810	9/8/2016	624	-	-	-	-
Hogan Bay	226-30-16810	9/10/2016	576	2013	1542	0	0
Hogan Bay	226-30-16810	9/12/2016	720	640	3795	0	0
Hogan Bay	226-30-16810	9/14/2016	384	815	1795	0	0
Hogan Bay	226-30-16810	9/16/2016	288	396	476	0	0
Paddy C	226-20-16010	7/31/2016	0	0	0	0	0
Paddy C	226-20-16010	8/1/2016	0	0	0	0	0

Paddy C	226-20-16010	8/2/2016	1	3	1	0	0
Paddy C	226-20-16010	8/3/2016	1	2	1	1	0
Paddy C	226-20-16010	8/4/2016	0	1	0	0	0
Paddy C	226-20-16010	8/5/2016	0	5	0	0	0
Paddy C	226-20-16010	8/6/2016	0	23	0	1	0
Paddy C	226-20-16010	8/7/2016	0	40	0	0	0
Paddy C	226-20-16010	8/8/2016	0	93	0	0	0
Paddy C	226-20-16010	8/9/2016	3	85	3	0	0
Paddy C	226-20-16010	8/10/2016	3	64	4	0	0
Paddy C	226-20-16010	8/11/2016	1	57	2	0	0
Paddy C	226-20-16010	8/12/2016	0	104	1	0	0
Paddy C	226-20-16010	8/13/2016	2	202	3	0	0
Paddy C	226-20-16010	8/14/2016	7	350	7	0	0
Paddy C	226-20-16010	8/15/2016	9	278	10	0	0
Paddy C	226-20-16010	8/16/2016	14	984	22	0	4
Paddy C	226-20-16010	8/17/2016	69	877	88	0	3
Paddy C	226-20-16010	8/18/2016	35	915	91	0	5
Paddy C	226-20-16010	8/19/2016	67	1023	140	2	4
Paddy C	226-20-16010	8/20/2016	99	740	177	0	4
Paddy C	226-20-16010	8/21/2016	125	623	287	0	1
Paddy C	226-20-16010	8/22/2016	15	82	63	0	1
Paddy C	226-20-16010	8/23/2016	109	1081	256	0	3
Paddy C	226-20-16010	8/25/2016	234	1261	399	0	0
Paddy C	226-20-16010	8/27/2016	517	931	883	0	0
Paddy C	226-20-16010	8/29/2016	426	514	1043	0	0
Paddy C	226-20-16010	8/31/2016	437	530	1272	0	1
Paddy C	226-20-16010	9/2/2016	347	353	1129	0	0
Paddy C	226-20-16010	9/4/2016	356	288	1375	0	0
Paddy C	226-20-16010	9/6/2016	119	222	1166	0	0
Paddy C	226-20-16010	9/7/2016	94	192	1283	0	0
Paddy C	226-20-16010	9/9/2016	107	101	1232	0	0
Paddy C	226-20-16010	9/10/2016	50	51	1229	0	0
Paddy C	226-20-16010	9/11/2016	22	19	1113	0	0
Paddy C	226-20-16010	9/12/2016	2	11	168	0	0
Paddy C	226-20-16010	9/13/2016	5	99	104	0	0
Stockdale C	227-20-17520	8/1/2016	2	1205	2	22	3
Stockdale C	227-20-17520	8/3/2016	2	1810	4	20	7
Stockdale C	227-20-17520	8/5/2016	3	1419	4	28	7
Stockdale C	227-20-17520	8/6/2016	31	2162	29	33	15
Stockdale C	227-20-17520	8/7/2016	0	338	5	2	3
Stockdale C	227-20-17520	8/8/2016	15	4436	24	73	20

Stockdale C	227-20-17520	8/9/2016	13	6335	18	11	28
Stockdale C	227-20-17520	8/10/2016	12	4772	19	43	17
Stockdale C	227-20-17520	8/11/2016	79	3189	111	33	32
Stockdale C	227-20-17520	8/12/2016	41	1583	57	17	24
Stockdale C	227-20-17520	8/13/2016	22	5743	27	12	7
Stockdale C	227-20-17520	8/14/2016	8	1003	17	-	3
Stockdale C	227-20-17520	8/15/2016	55	4898	65	22	30
Stockdale C	227-20-17520	8/16/2016	80	4885	147	10	42
Stockdale C	227-20-17520	8/17/2016	113	10078	372	22	51
Stockdale C	227-20-17520	8/19/2016	293	12728	645	4	86
Stockdale C	227-20-17520	8/21/2016	176	800	650	0	0
Stockdale C	227-20-17520	8/22/2016	467	300	700	-	5
Stockdale C	227-20-17520	8/23/2016	576	4089	1069	0	0
Stockdale C	227-20-17520	8/26/2016	551	9810	3454	0	1
Stockdale C	227-20-17520	8/27/2016	480	5583	3920	1	1
Stockdale C	227-20-17520	8/29/2016	720	2465	3280	0	0
Stockdale C	227-20-17520	8/31/2016	816	338	3360	0	0
Stockdale C	227-20-17520	9/2/2016	672	960	4100	0	0
Stockdale C	227-20-17520	9/4/2016	624	3113	6108	0	1
Stockdale C	227-20-17520	9/6/2016	624	381	5600	0	0
Stockdale C	227-20-17520	9/7/2016	288	200	3000	0	0
Stockdale C	227-20-17520	9/8/2016	768	90	2350	0	0
Stockdale C	227-20-17520	9/9/2016	480	113	3569	0	0
Stockdale C	227-20-17520	9/10/2016	648	68	2875	0	0
Stockdale C	227-20-17520	9/11/2016	341	41	4374	0	1

Dash (-) indicates live/dead estimates were not made

APPENDIX D. PWS STREAM SUMMARIES

Paddy Creek
226-20-16010
Pink Salmon Fitness

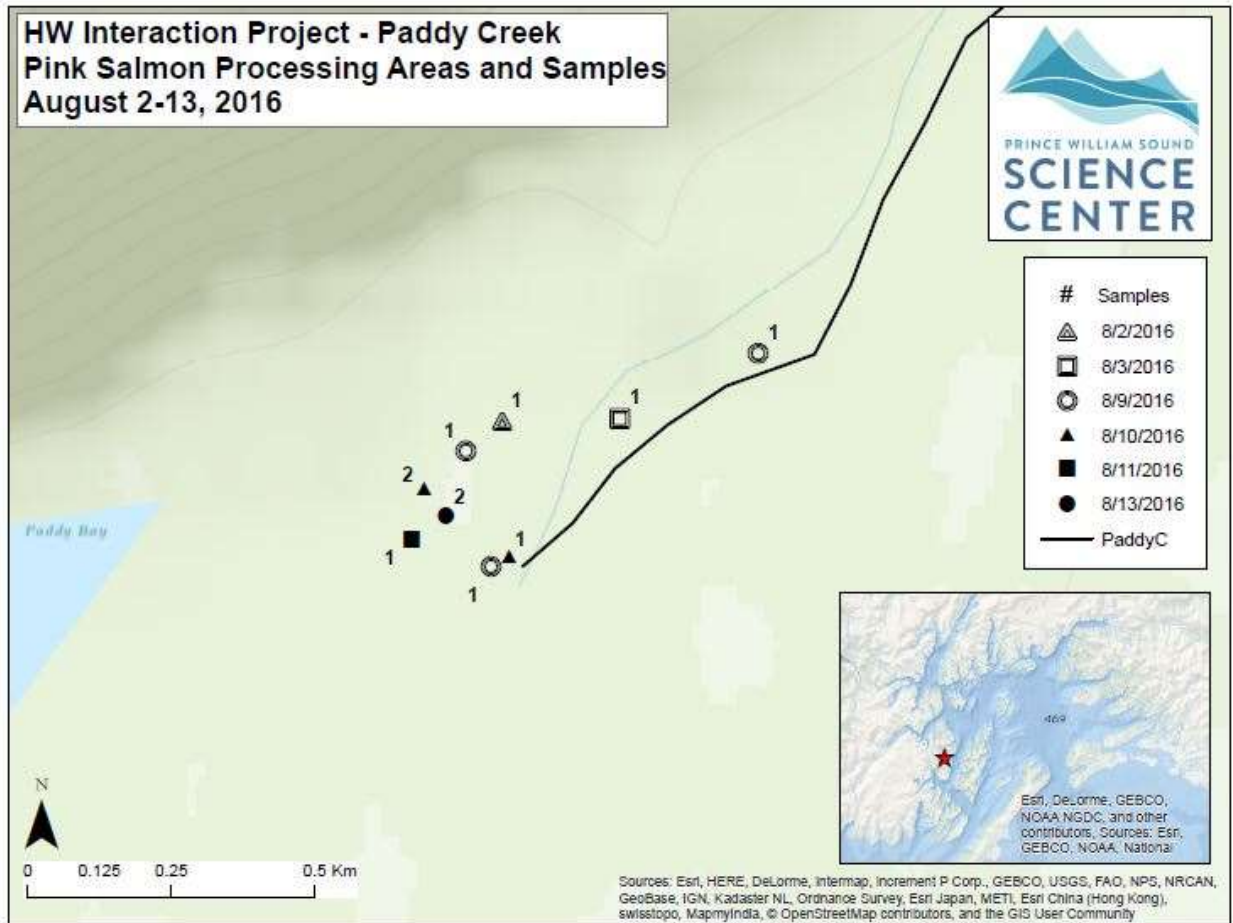
2016 samples collected and run timing: Sampling duties for Paddy and Erb Creeks were the responsibility of the PWSSC camping crew (Paddy crew). Paddy crew surveyed at Paddy Creek every day except during peak season, when they surveyed Paddy and Erb Creeks every other day to maximize the number of samples collected at both creeks (by reducing total transit time to Erb Creek from every day to every other day). Paddy Creek is a small muskeg creek with three branches, and for our sampling purposes we called them Paddy Creek, Paddy Right Tributary and Paddy Left Tributary (description in the next section). The following summary is comprehensive for all the Paddy tributaries. Paddy crew sampled at Paddy Creek 37 times between 07/31/2016 and 09/13/2016 and collected 3,273 Pink Salmon otolith and tissue samples. The run started very slowly with 23 fish observed on 08/6/2016 and by 08/15/2016 there were still less than 300 live fish observed. The next day, 08/16/2016, live fish numbers peaked for 12 days with an average of 940 and a high of 1,261 Pink Salmon observed until 08/27/2016. On 08/29/2016, the run dropped below 600 fish and quickly tapered to 2 live fish by 09/13/2016; the last day sampled. Unlike last season, creek levels stayed high, Pink Salmon easily accessed spawning grounds all season long, and there were very few stranded, pre-spawned samples. The spawning run was a total of 43 days in 2016.

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. In Paddy Creek, live Pink Salmon were not seen in 2016 past a medium cascade that was not a barrier in 2015. 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 either 2015 or 2016. The extent of Paddy Left Tributary is a 1.5-m log-jam sieve where water drains through a buildup of loose cobble before trickling through the log-jam. 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: In contrast to the dry, sunny weather of 2015, there was an incredible amount of rain during the summer of 2016. The water was so high one day that we were unable to cross Paddy Creek; a creek that doesn't wet your feet on most days.

Access, safety, or logistics issues: Paddy Creek is approximately 1 km from camp - access is easy and dependable except for in extreme flooding events.

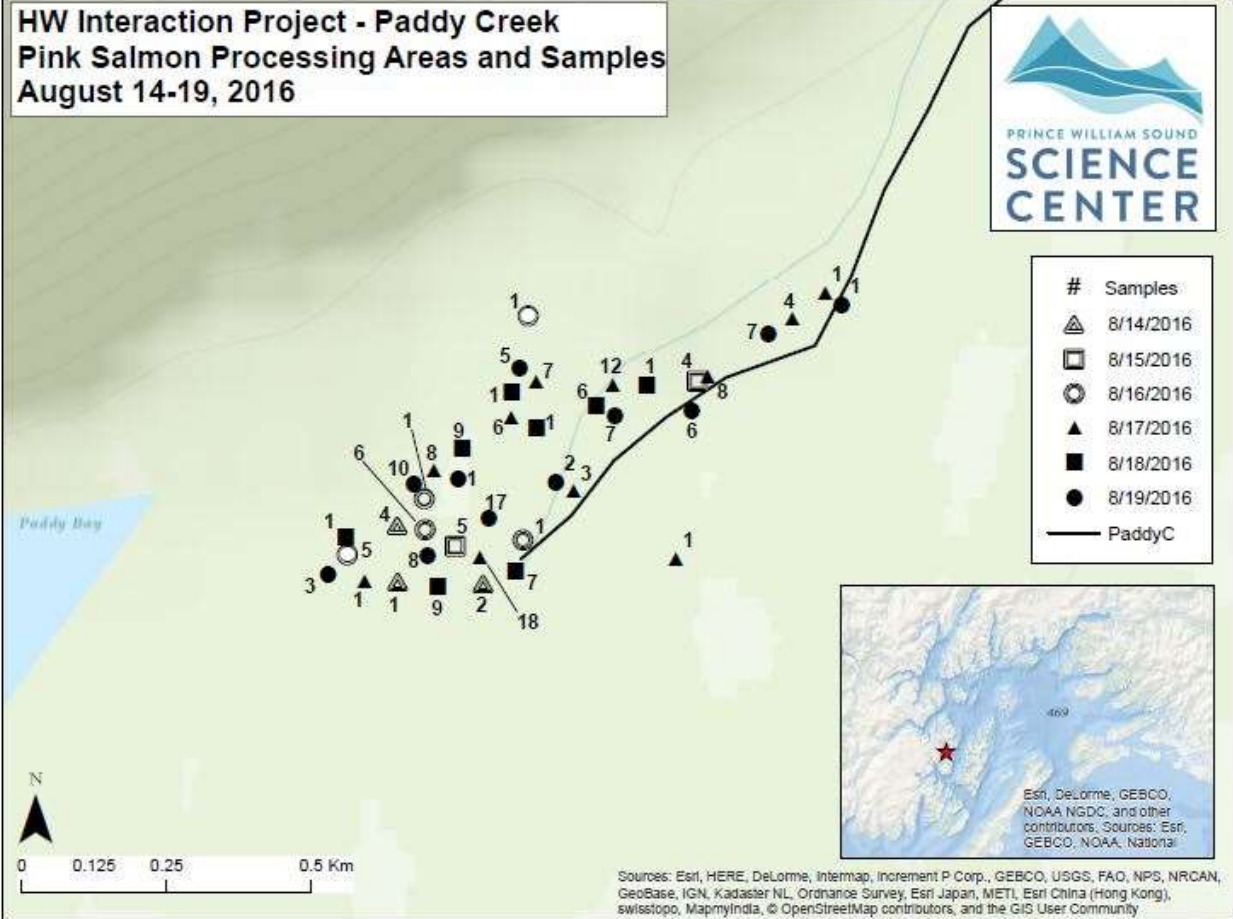
Recommendations, changes, and other notes: Paddy Crew recommends that the Left Tributary be abandoned in 2017. The effort needed to sample the Left Tributary was very high for the amount of samples collected. Paddy crew also recommends that all the tablets get SD cards to back up the tablet information. Paddy crew had a tablet malfunction- the charging port no longer accepted a charge and we had to quickly extract the data before the device ran out of power. SD cards would also provide backup in case a device was immersed in water, dropped or broke the touch screen.



**HW Interaction Project - Paddy Creek
Pink Salmon Processing Areas and Samples
August 14-19, 2016**



#	Samples
△	8/14/2016
□	8/15/2016
○	8/16/2016
▲	8/17/2016
■	8/18/2016
●	8/19/2016
—	PaddyC

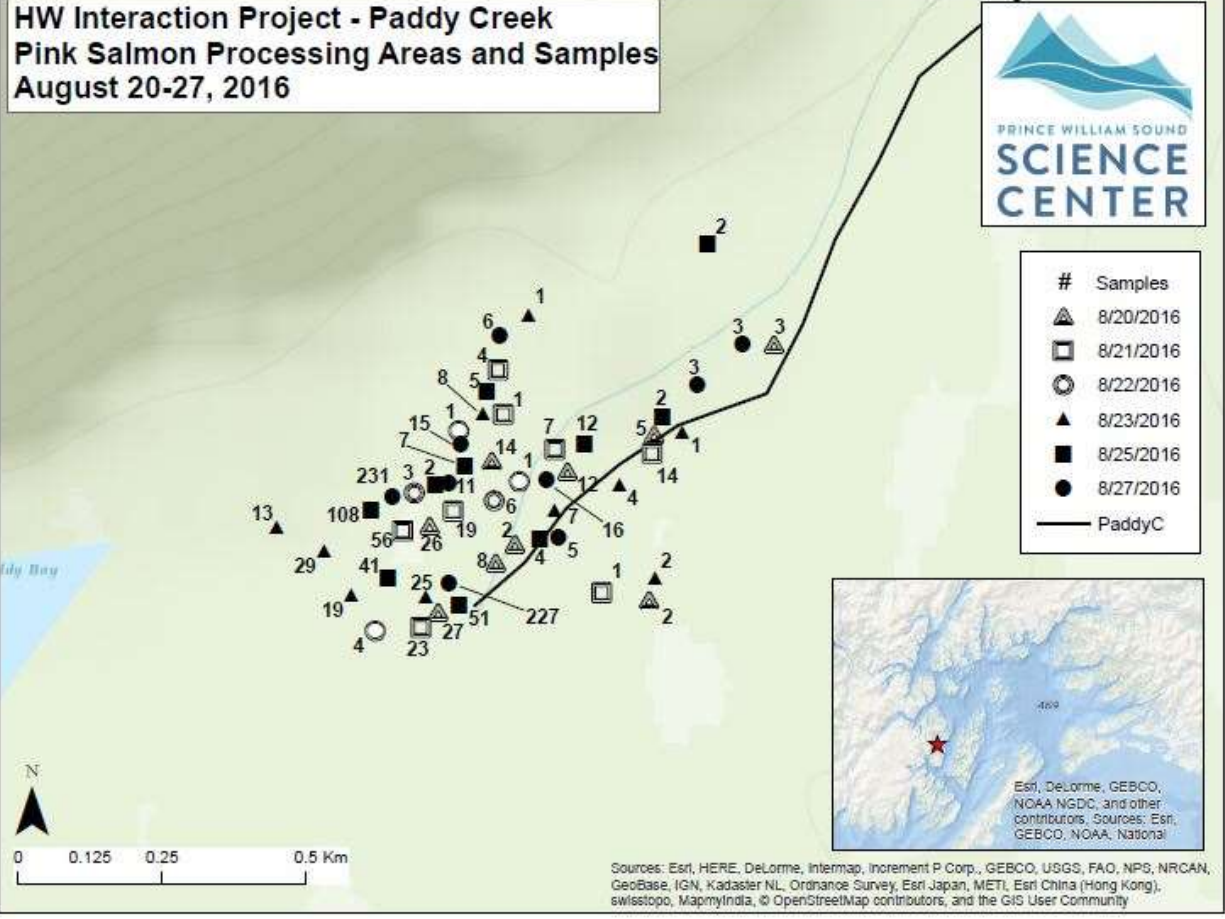


Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Paddy Creek
Pink Salmon Processing Areas and Samples
August 20-27, 2016**



#	Samples
▲	8/20/2016
◻	8/21/2016
◯	8/22/2016
▲	8/23/2016
■	8/25/2016
●	8/27/2016
—	PaddyC

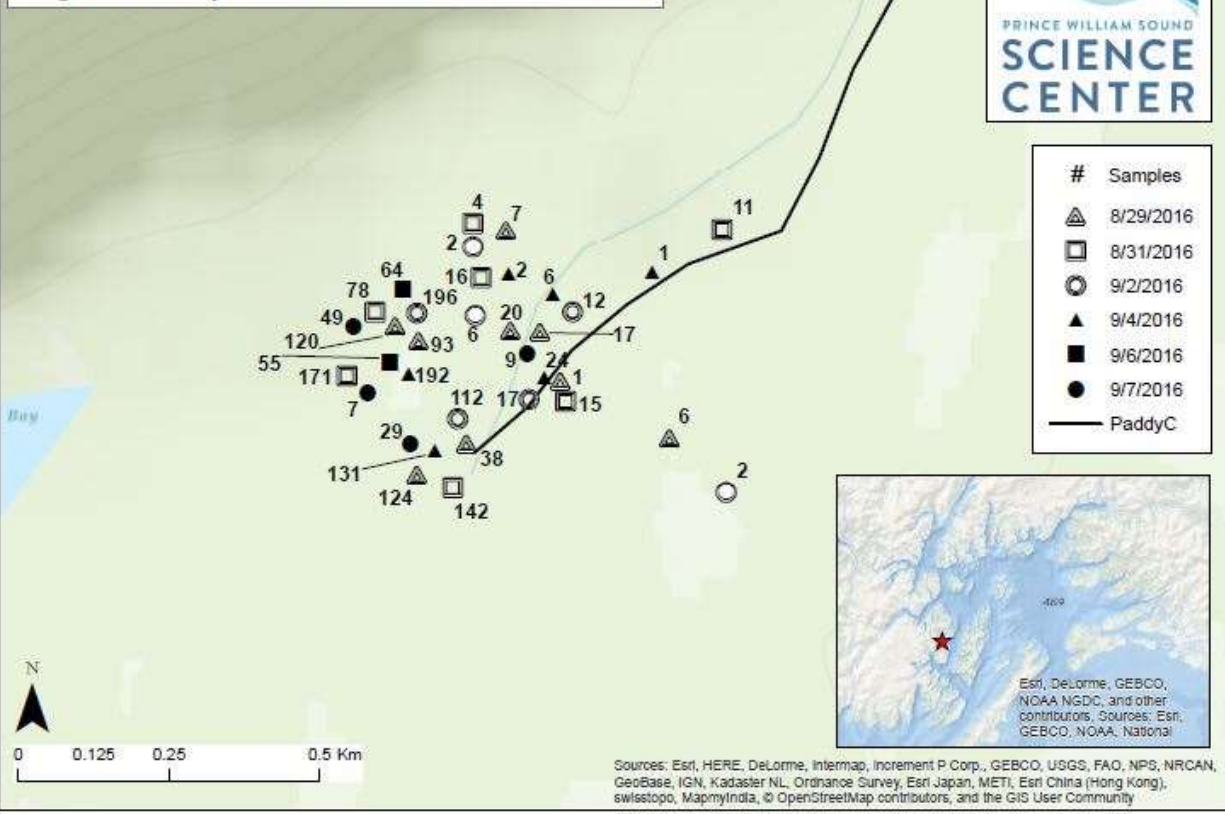


Sources: Esri, HERE, DeLorme, Intermap, Incent P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Paddy Creek
Pink Salmon Processing Areas and Samples
August 29-September 7, 2016**



#	Samples
△	8/29/2016
□	8/31/2016
○	9/2/2016
▲	9/4/2016
■	9/6/2016
●	9/7/2016
—	PaddyC

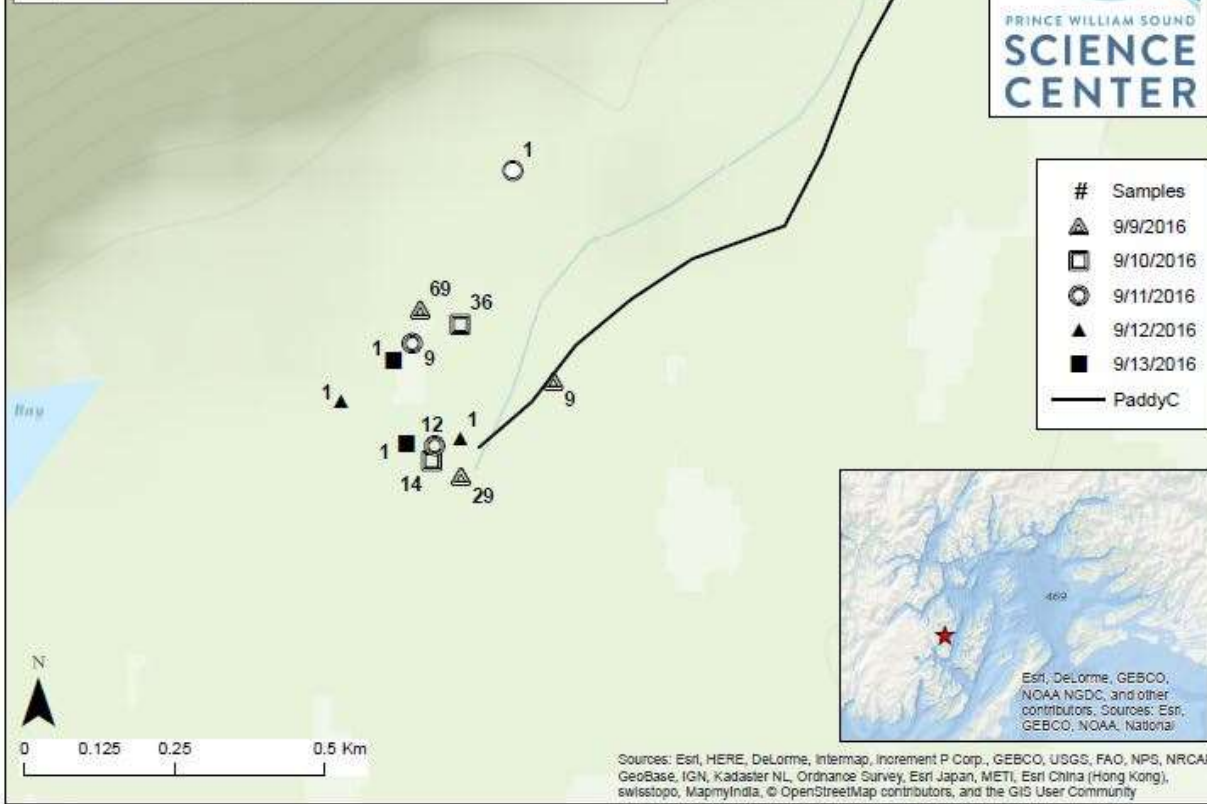


Sources: Esri, HERE, DeLorme, Intermap, Incent P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Paddy Creek
Pink Salmon Processing Areas and Samples
September 9-13, 2016**



#	Samples
▲	9/9/2016
◻	9/10/2016
◯	9/11/2016
▲	9/12/2016
■	9/13/2016
—	PaddyC



Erb Creek
226-20-16040
Pink Salmon Fitness

2016 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 Paddy 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 two small mountain valleys with no tributaries. Paddy crew visited Erb Creek 37 times and collected 8,091 Pink Salmon otolith and tissue samples between 07/31/2016 and 09/13/2016. The run gradually picked up from 183 live fish observed on the first day, 7/31/2016. From 08/08/2016 to 09/01/2016 there was an average of 2,300 and a high of 3,347 live Pink Salmon in Erb Creek. After 09/03/2016 the run sharply decreased from 900 live to 0 live fish on 9/10/2016. Live Pink Salmon were observed in the creek each visit, except for the last 3 days surveyed. Unlike last season, creek levels stayed high, Pink Salmon could access spawning grounds all season long and there were very few stranded, pre-spawned samples. The spawning run was a total of 42 days in 2016.

Extent of stream sampled: From August 20, 2016 through September 5, 2016, un-sampled carcass numbers were too high to sample in one day. During this time, Paddy crew primarily focused on sampling as many carcasses as possible, and secondarily focused on distributing sampling effort over the entire stream. Most spawning activity was in the first 1 km, but spawning was observed from the lower intertidal to the marked survey extent below a long cascade of boulders.

Unusual events: Paddy crew had three sightings of a golden eagle in late August and early September.

Access, safety, or logistics issues: There is a tidal flat in front of Erb Creek would be a very long dry wait if you boated up at high tide and got stranded. The best place to keep the skiff is a tiny, but deep, bight to the northwest of the creek mouth. To keep the skiff even safer and not have to push it to water every low tide, we made a simple pulley system across this bight. After implementing the pulley system we could sample uninterrupted.

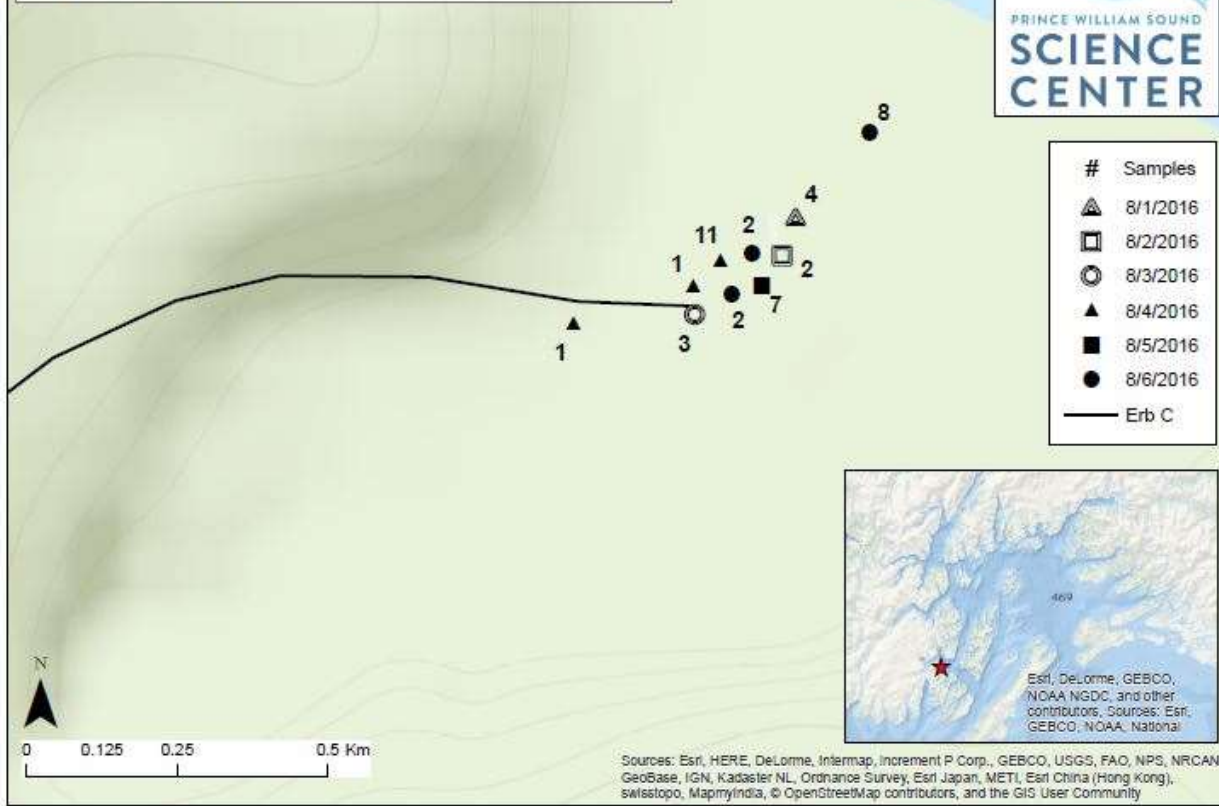
Paddy crew always carried a sealed emergency bucket in the skiff with a portable stove, stove fuel, food and a shelter tarp. We also always carried the GPS in case of fog, it fits nicely in the med kit and already has a track-line between camp and Erb Creek.

Recommendations, changes, and other notes: Paddy crew was three people strong this year, and if the run had been bigger we would not have been able to sample all of the carcasses. The Pink Salmon run in 2017 is forecast to be larger than 2016 and we strongly recommend having a fourth crew member for the summer 2017 field season. Paddy crew recommends SD cards for the tablets. See details in Paddy Creek stream summary.

**HW Interaction Project - Erb Creek
Pink Salmon Processing Areas and Samples
August 1-6, 2016**



#	Samples
▲	8/1/2016
◻	8/2/2016
○	8/3/2016
▲	8/4/2016
■	8/5/2016
●	8/6/2016
—	Erb C

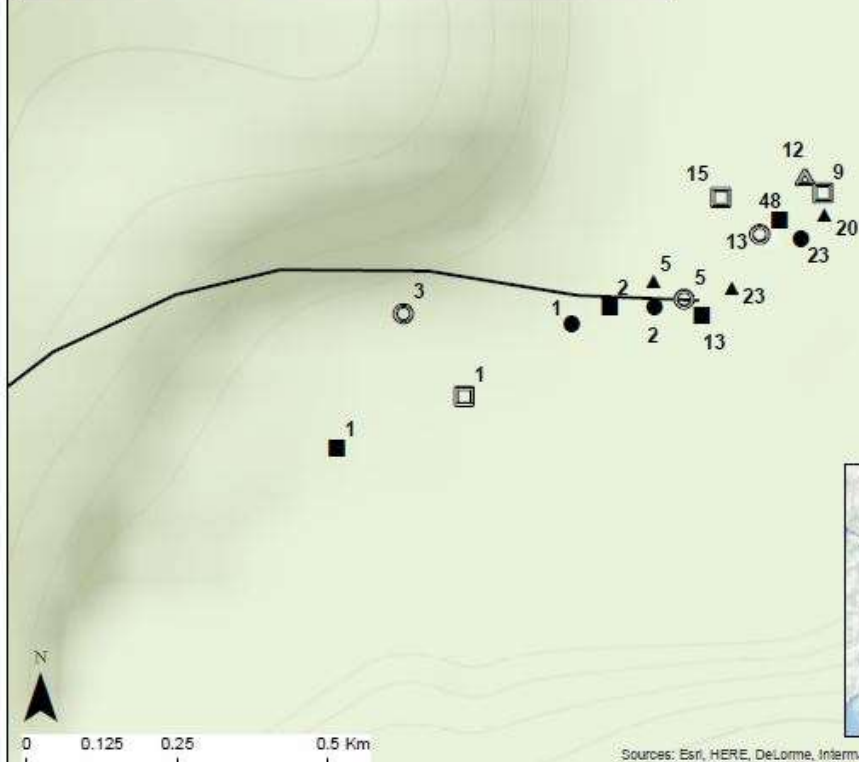


Sources: Esri, HERE, DeLorme, Intermap, Incent P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Erb Creek
Pink Salmon Processing Areas and Samples
August 7-12, 2016**



#	Samples
▲	8/7/2016
◻	8/8/2016
⊙	8/9/2016
▲	8/10/2016
■	8/11/2016
●	8/12/2016
—	Erb C

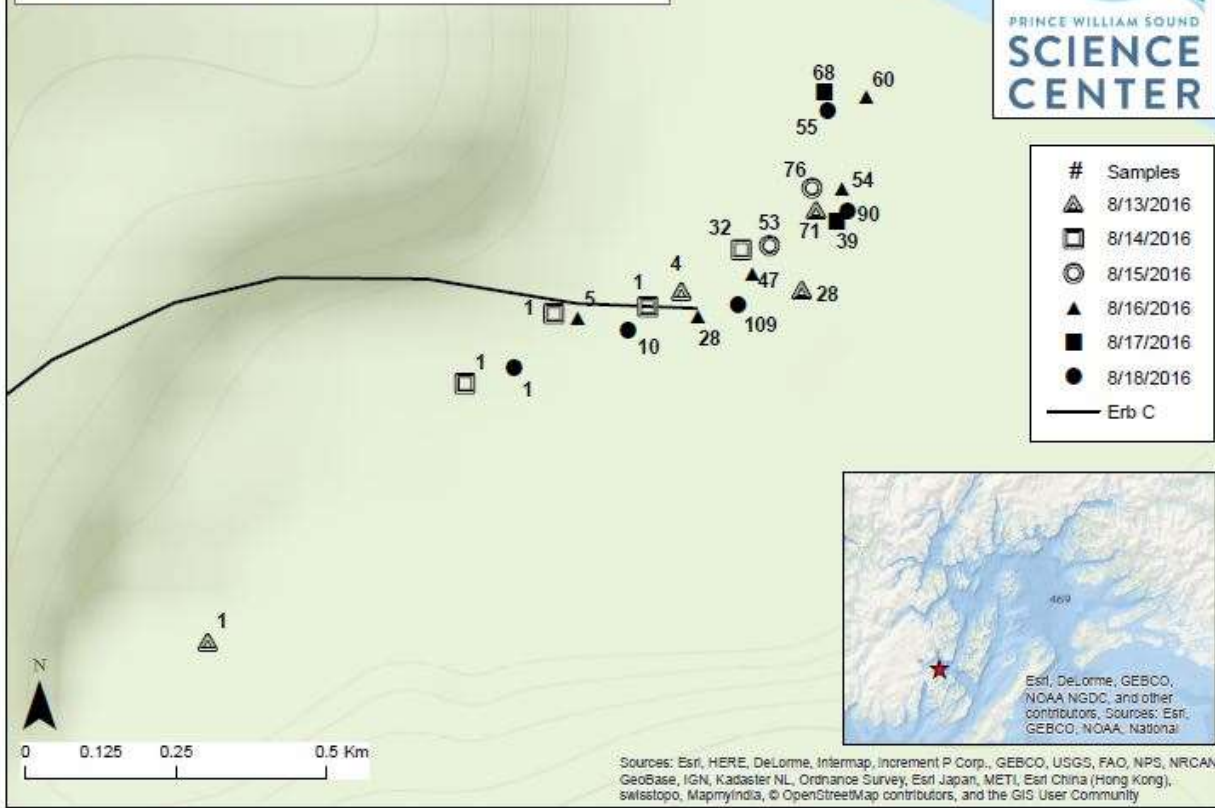


Sources: Esri, HERE, DeLorme, Intermap, Incent P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Erb Creek
Pink Salmon Processing Areas and Samples
August 13-18, 2016**



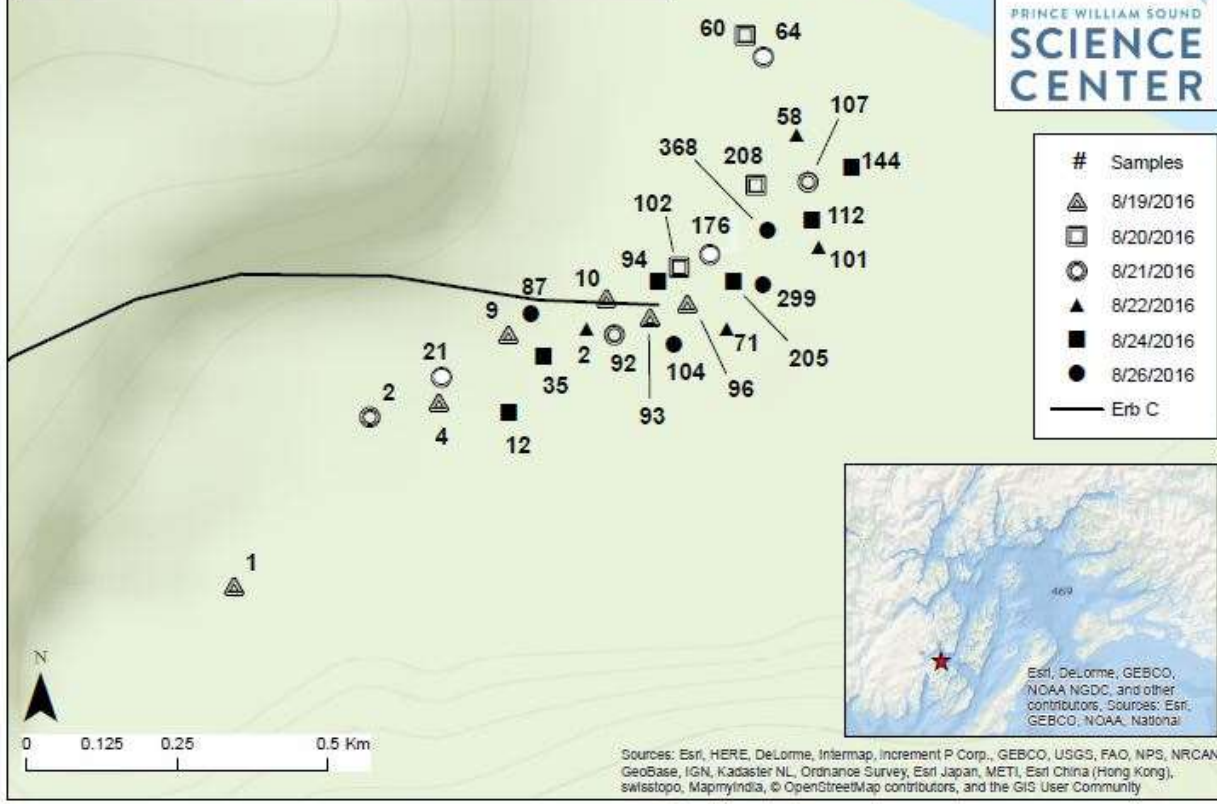
#	Samples
▲	8/13/2016
◻	8/14/2016
◯	8/15/2016
▲	8/16/2016
■	8/17/2016
●	8/18/2016
—	Erb C



**HW Interaction Project - Erb Creek
Pink Salmon Processing Areas and Samples
August 19-26, 2016**

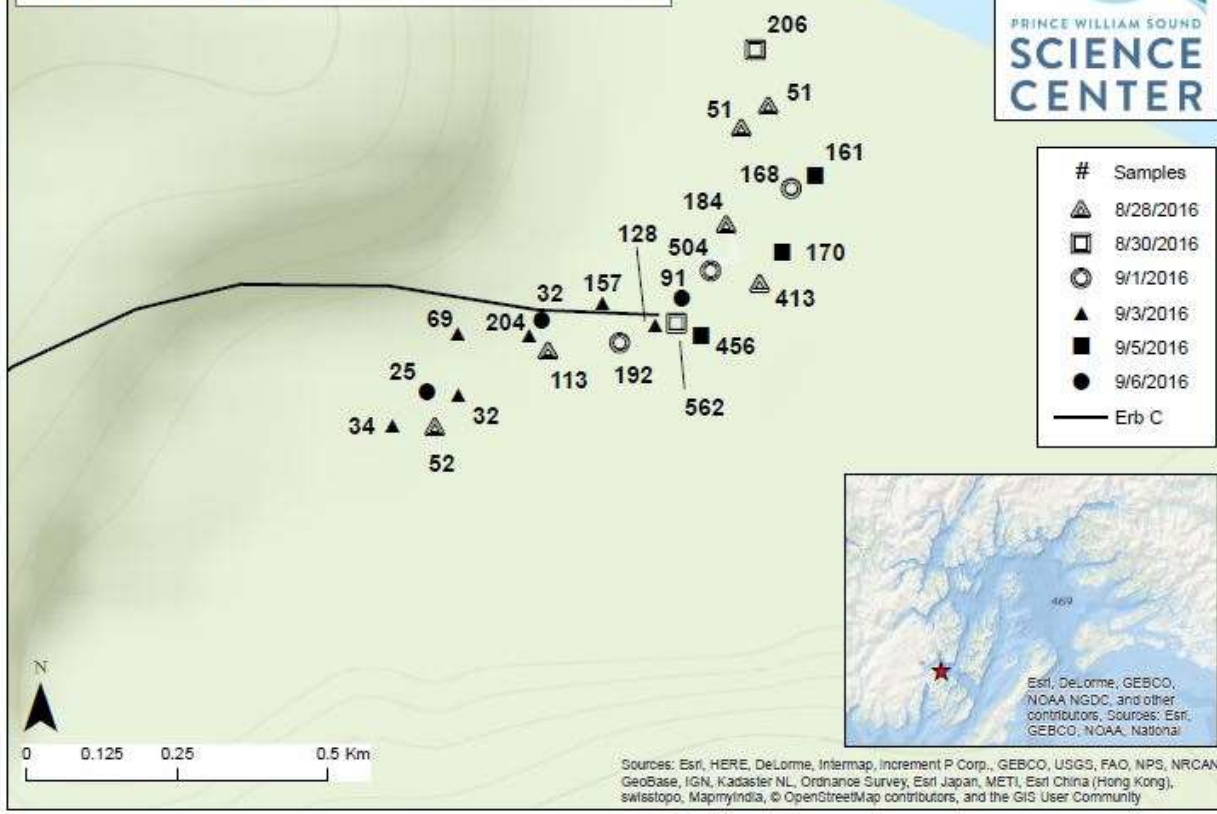


#	Samples
△	8/19/2016
□	8/20/2016
○	8/21/2016
▲	8/22/2016
■	8/24/2016
●	8/26/2016
—	Erb C



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

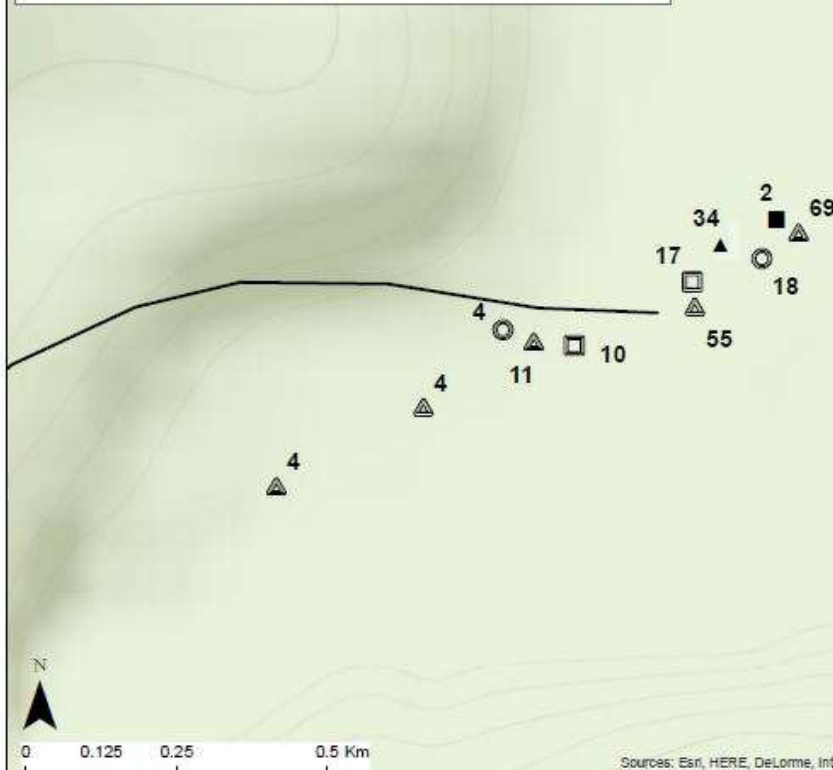
**HW Interaction Project - Erb Creek
Pink Salmon Processing Areas and Samples
August 28-September 6, 2016**



**HW Interaction Project - Erb Creek
Pink Salmon Processing Areas and Samples
September 8-12, 2016**



#	Samples
▲	9/8/2016
◻	9/9/2016
○	9/10/2016
▲	9/11/2016
■	9/12/2016
—	Erb C



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

Hogan Bay Creek
226-30-16810
Pink Salmon Fitness

2016 samples collected and run timing: Sampling for otoliths and heart tissue at Hogan Creek was successful with 34 visits during the 2016 season (7/31/16 - 9/17/16). The sampling visits resulted in 13,009 samples collected. The first fish observed in the stream were seen on the third visit (8/4/16). There were two distinct peaks in the run due to weather. The first peak being on visit 12 (8/24/16) though this was observed with a new crew not used to the stream so another possible peak was observed by the original crew just a few days later on visit 14 (8/28/16). From here the live numbers dipped due to a long period of warm dry weather lasting from the peak until visit 20 (9/3/16), which brought rain back to our part of the sound. During the period of dry weather the stream level dipped so low that the lower part of the stream went completely dry and forced the salmon in the salt to wait until rain returned. The second peak was observed on visit 22 (9/5/2016), after the creek level rose from rain, the fish in the salt water entered the system and quickly disappeared from the bay. The number of live fish then trailed off until our last sampling day (9/17/16). Some Chum, Sockeye, and Coho Salmon were observed in the stream but never in large numbers.

Extent of Stream Sampled: The crew was able to successfully sample the entire stream for almost every visit unless hindered by high waters which occurred only a few times or by a massive number of samples forcing them to stay in one area all day. The ability to sample much of the stream was helped due to the short length of Hogan Creek. The creek ends at the falls, which appeared to be an impassable barrier to all species of salmon. Fish were observed only up to the third large pool in the falls system.

Unusual Events: Once during the season the water levels dipped very low due to continued sunny weather (8/24/16 – 9/3/16). This caused parts of the stream to go dry during low tide and forced many fish to stay in the salt water for an extended period. Fish who attempted to go upstream during this time often were trapped in ever shrinking pools, often resulting in prespawn death. There were also an unusual number of black bear sightings for this season. We sighted at least one black bear almost every day and often saw more than that. There were several family groups as well as several lone bears.

Access, Safety, or logistics issues: Access to the entire stream was almost never hindered. Even on days after or during extreme rain the stream was almost always passable, the short length of the creek system assisted this. The downside of the short creek length was that the system is heavily influenced by the tides. Approximately one third of the system with salmon present was in the intertidal. This made sampling on days with extreme tides or mid day high tides difficult. On days of spring tides the seawater could reach into the grass at the base of the woods. This severely limited the processing areas that were usable in the lower parts of the stream.

There were two main collection sites for salmon on the stream. The first was the intertidal, which had two deep pools that collected many of the dead as they were being flushed from the system; this also included the lower intertidal flats. The second was a gravel bar in the high intertidal, which, as the stream flowed across it, caught many of the salmon carcasses due to shallow water and many rocks.

The number of bear sightings and sign this season was alarming, though there were no aggressive encounters, a family group of five and three were both observed along with several individuals. We surprised a family group on a rainy day, and although they ran off, if the mother had decided to turn on us it could have quickly turned into a dangerous situation. Caution is advised on this stream; the bears became accustomed to our presence and became very comfortable around us, often staying within 100m of us while we worked. Though the bears never seemed to be aggressive and anytime we attempted to scare them off they always left, at least for a time, this stream does present a high encounter rate with these large predators.

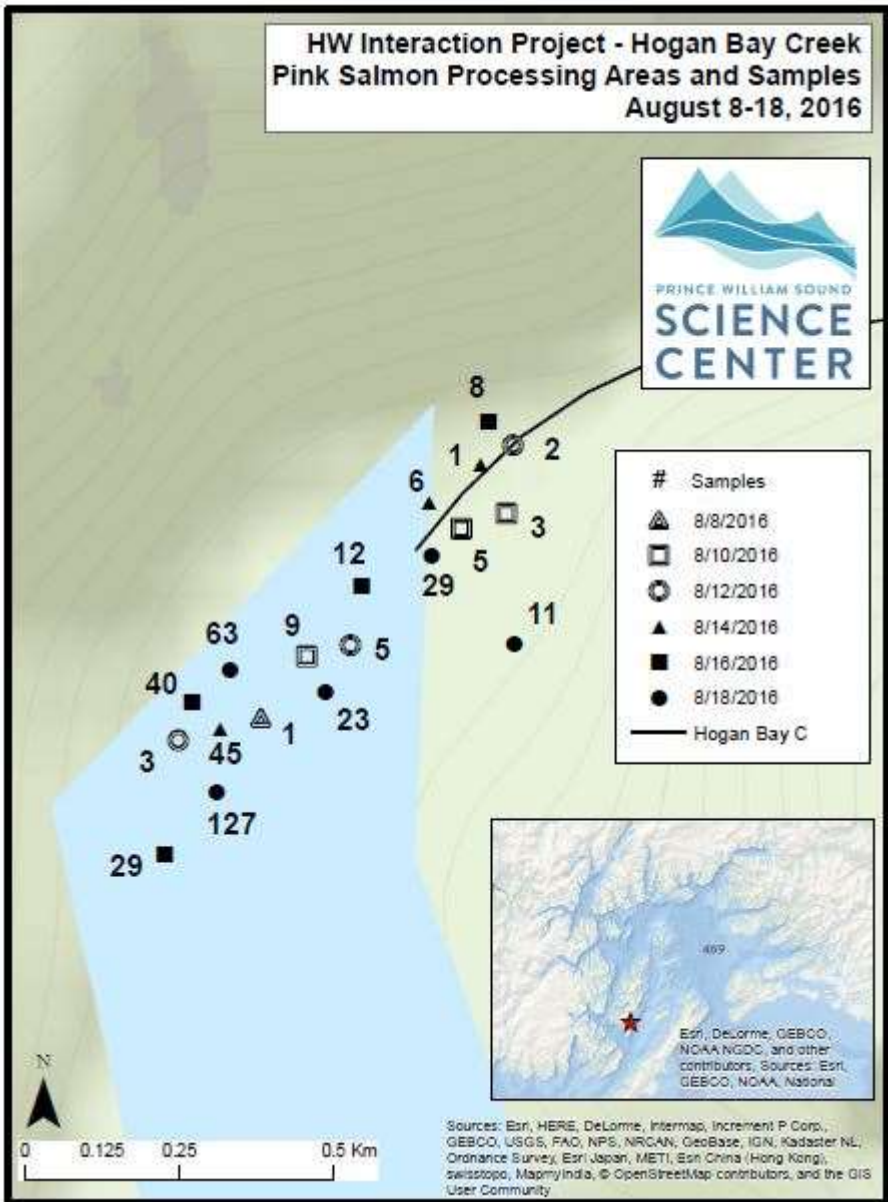
Recommendations, changes, and other notes: we suggest extending the sampling season for this stream just a few more days. This is the same suggestion as last season. Our last day of the season (9/17/16) still had samples to collect and around 50 live fish still in the stream. It would be nice to collect until every reasonable sample had been collected.

The temperature/depth logger deployed at the end of last season was readjusted to sit in the deeper part of the same pool. The unit was buried when deployed at the end of the last season, however, this is not the protocol for the sensors and we decided for this season to follow the same protocol as the other deployed sensors and left it unburied.

**HW Interaction Project - Hogan Bay Creek
Pink Salmon Processing Areas and Samples
August 8-18, 2016**

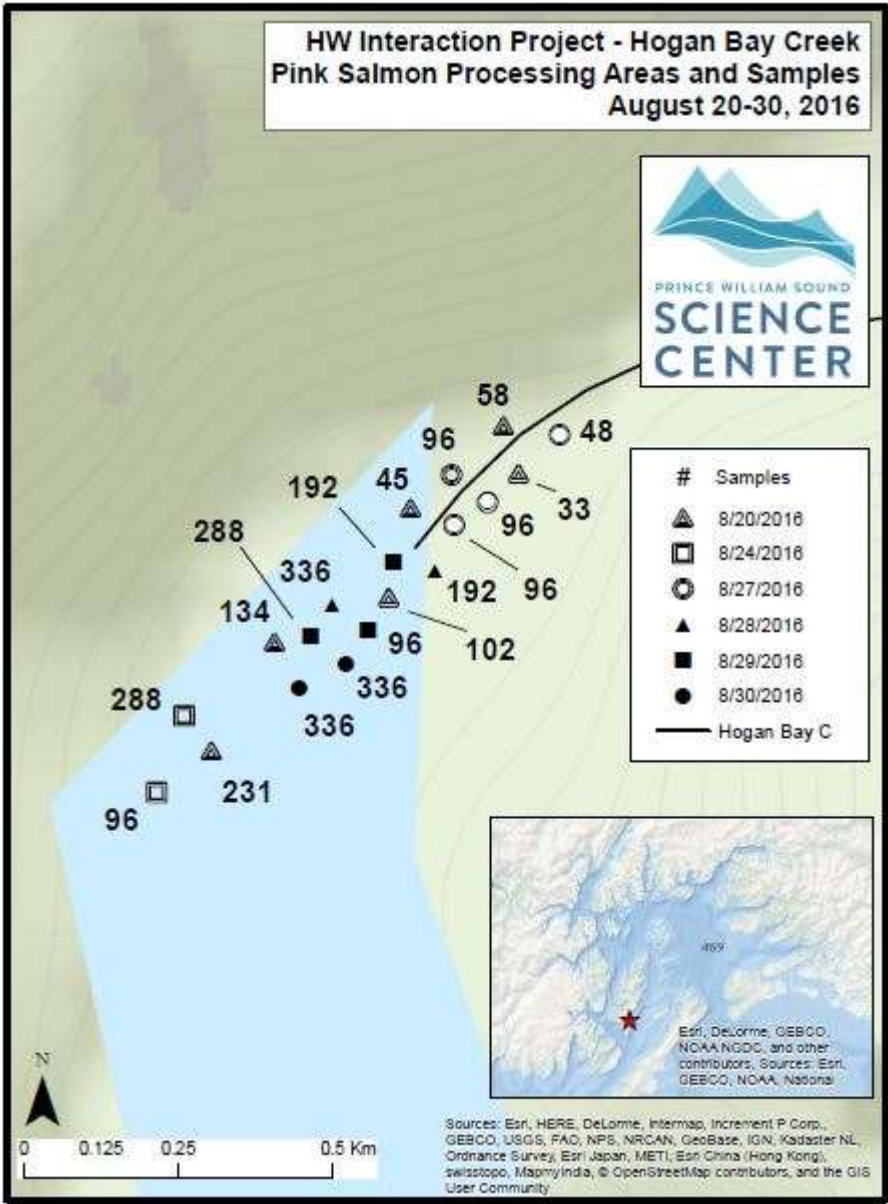


#	Samples
▲	8/8/2016
◻	8/10/2016
⊙	8/12/2016
▲	8/14/2016
■	8/16/2016
●	8/18/2016
—	Hogan Bay C



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

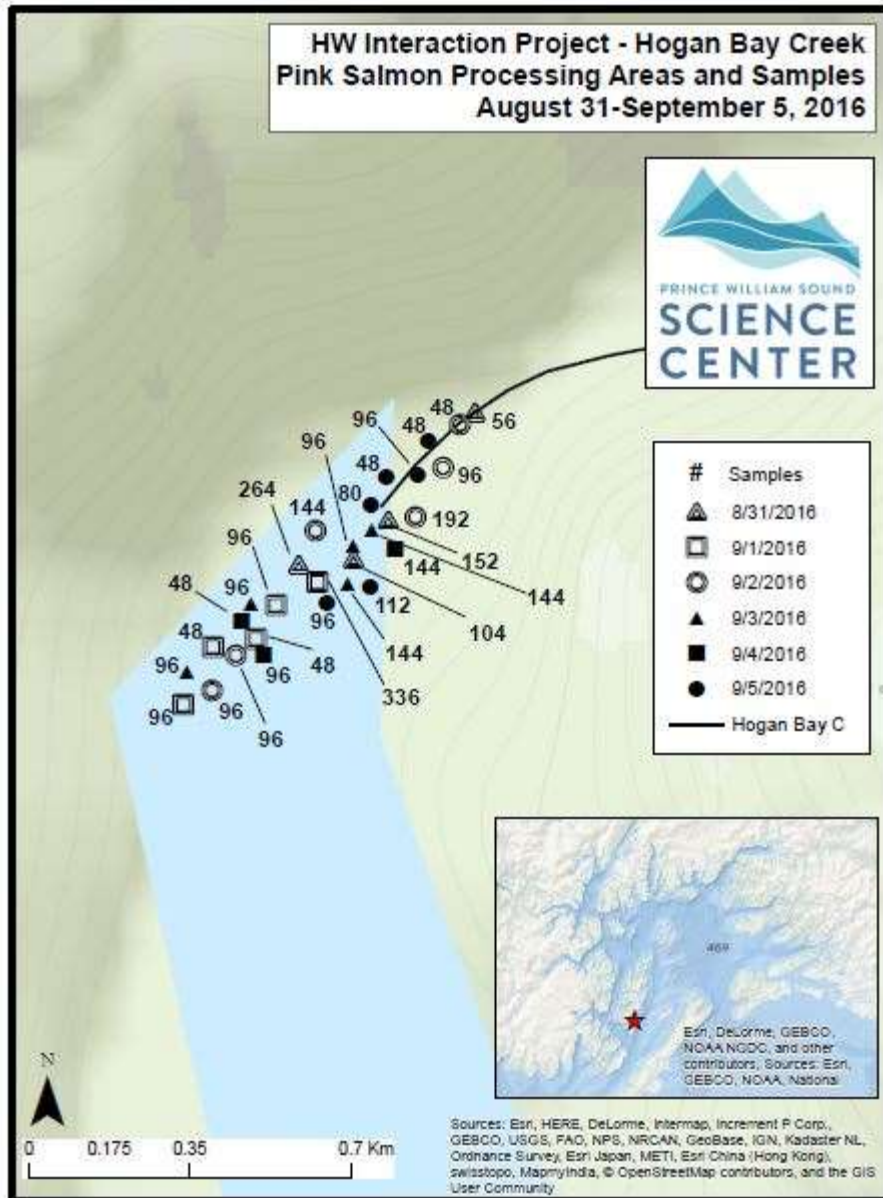
**HW Interaction Project - Hogan Bay Creek
Pink Salmon Processing Areas and Samples
August 20-30, 2016**



**HW Interaction Project - Hogan Bay Creek
Pink Salmon Processing Areas and Samples
August 31-September 5, 2016**



#	Samples
▲	8/31/2016
□	9/1/2016
○	9/2/2016
▲	9/3/2016
■	9/4/2016
●	9/5/2016
—	Hogan Bay C

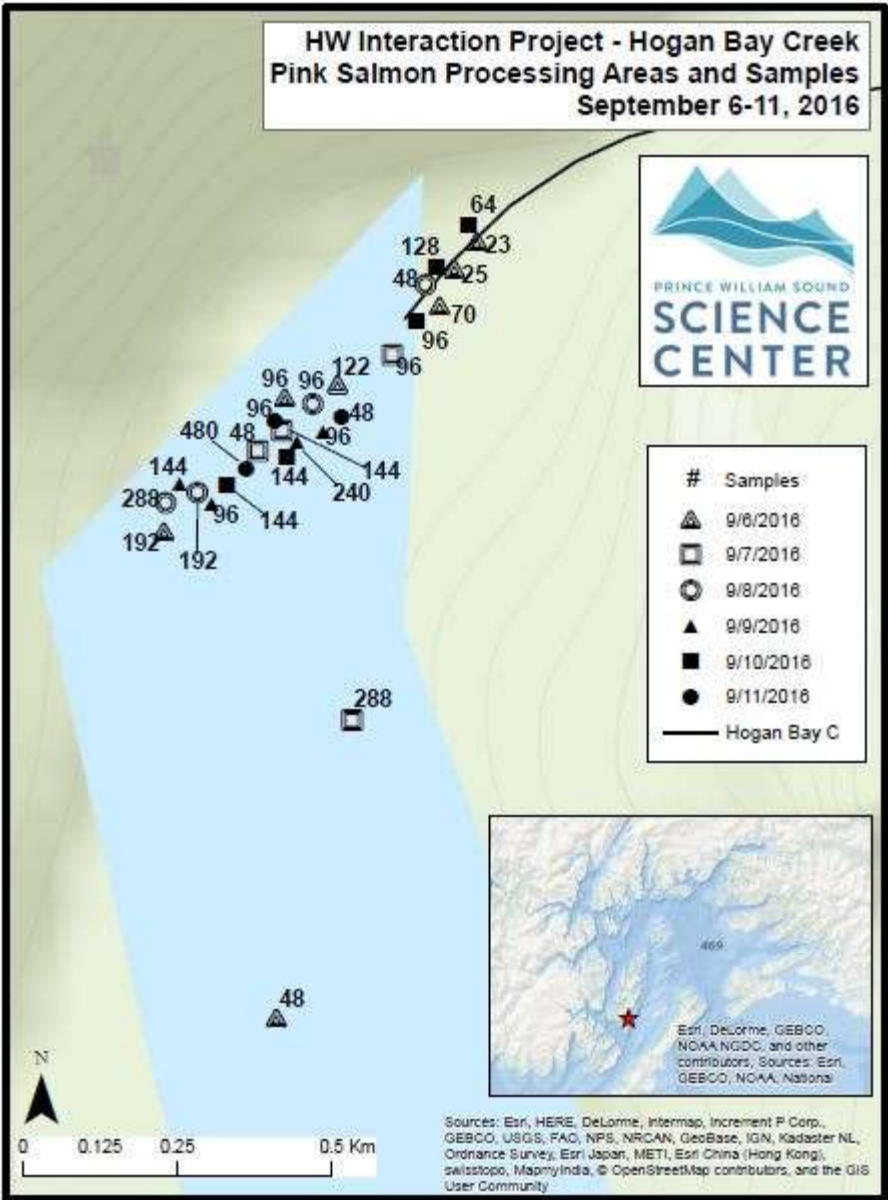


Sources: Esri, HERE, DeLorme, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

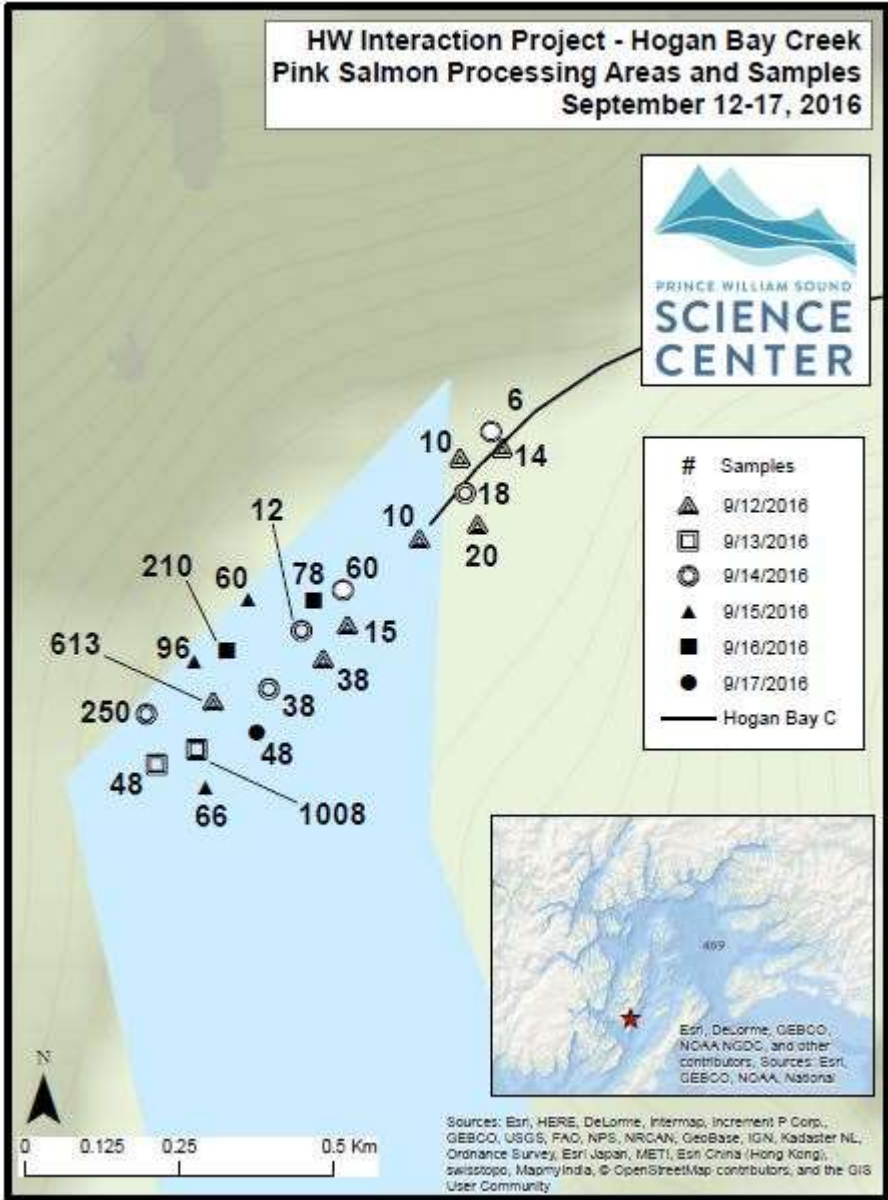
**HW Interaction Project - Hogan Bay Creek
Pink Salmon Processing Areas and Samples
September 6-11, 2016**



#	Samples
▲	9/6/2016
◻	9/7/2016
⊙	9/8/2016
▲	9/9/2016
■	9/10/2016
●	9/11/2016
—	Hogan Bay C



Stockdale Creek
227-20-17520
Pink Salmon Fitness



2016 samples collected and run timing: Pink Salmon fitness sampling at Stockdale Creek successfully collected 9,000 samples from the main branch of Stockdale and one tributary. These samples were collected over the course of 31 visits throughout the entire run. Fish steadily increased by a few hundred each day between visits 1 (8/1/2016) and visit 4 (8/6/2016), then increased dramatically with higher water levels after visit 5 (8/7/2016). Low water events appeared to cause some fish to leave the stream for salt water during spawning, and tidal stranding of pre-spawners was a regular occurrence during the low water levels that were common during the height of spawning. Though high water often made accurate fish counts difficult through the early part of the run, numbers appeared to peak around 8/19/2016, when 12,633 fish were counted in Stockdale main branch. This was the last day a full stream survey was performed until the final day of sampling on visit 31 (9/11/2016). On this last visit, less than 100 live fish were observed. Chum Salmon were also observed in Stockdale early in the season, and Coho Salmon showed up later.

Extent of stream sampled: Mass spawning was observed and mapped from the top of the intertidal zone throughout the extent of the stream. The most heavily utilized spawning reach is in the lower section of Stockdale, an area that was intertidal pre-1964 earthquake, and now holds some of the best pink-spawning habitat at Stockdale. This roughly ¼ mile stretch of stream is perfectly straight with aggraded gravel beds and a wide channel with no bedrock or large wood. This spawning reach is directly above intertidal and pinks can spawn here without expending much energy traveling upstream. A disproportionality high number of Pink Salmon spawned in this area of the stream when compared to other reaches, but the entire stream is utilized by Pink Salmon. The first right fork that branches off the main channel of the stream was included in this year's sample efforts, though it is not well-utilized by pinks and produced an extremely low number of samples. In retrospect this tributary was not worth the crew's time. The main stem of the stream breaks into three separate braids just below this tributary, and these three braids reconvene higher up where the stream first exits the canyon. Fish were observed fairly high up in the canyon this year, an "other" point taken from 8/13/16 shows the highest fish observed by this year's sampling efforts. The "end" survey point from 8/3/16 shows the highest we ever hiked up Stockdale and, while gradient had begun ramping up at this point and spawning gravel had mostly disappeared, it is hard to make an extent call here with any real certainty. True extent on this stream remains undetermined, but there appear to be no definitive barriers and it would be conceivable for pinks to make it all the way up to the muskeg headwaters. Regardless, it seems unlikely that many fish spawn above the floodplain. Spawning reaches within the canyon are very limited, and getting up there in the first place consumes a lot of time and energy. For this reason we followed the lead of crews past and focused little energy on the upper parts of the watershed.

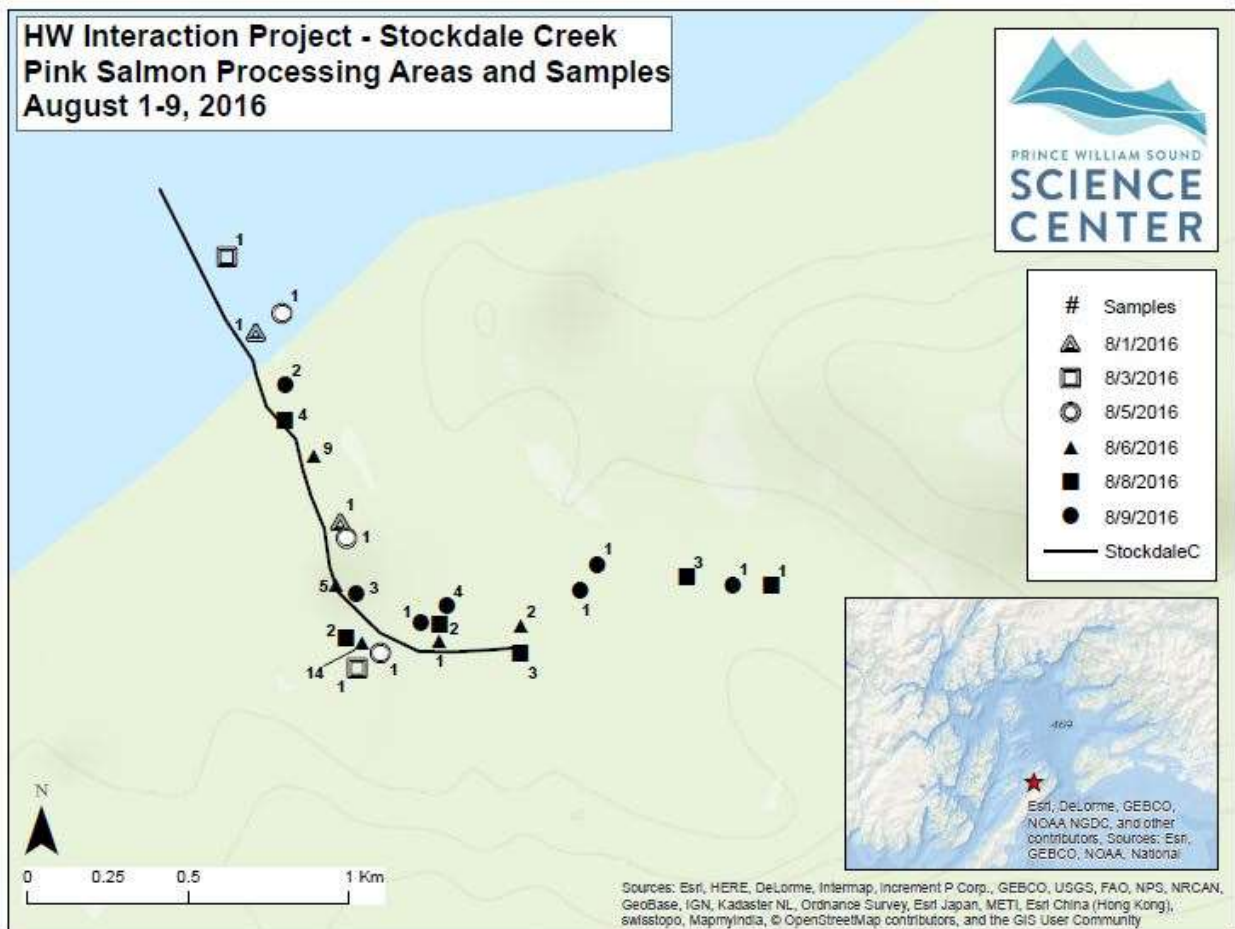
Unusual events: There were a number of times when water levels were low and/or water temperatures were high. These instances resulted in mass die-offs of pre-spawn fish, especially during high tide cycles. The high tide allowed fish up the main channel and when the tide retreated, too many fish concentrated in the small creek pools and died.

Access, safety, or logistics issues: The team experienced a few challenges during visits to this stream. Brown bears are certainly active in the area, and they can appear unexpectedly and in close quarters within the braid system. High water were also a problem. On two separate visits this season Stockdale simply was not safe to cross. These high water events allowed the crew to do some exploration of the muskeg systems and we found a very simple overland route between the

intertidal and floodplain. If you are standing at the mouth of Stockdale looking upstream, the stream makes a 90 degree hard right followed by a 90 degree hard left. Where it makes that first right, go up into the woods on the north (left) side of the stream and follow a small watercourse uphill and then bear right into the muskeg. From this point you can keep the stream to your right and stay up in the muskeg, linking together a series of game trails that will bring you back down to Stockdale between the debris jams. This shortcut saves 30 minutes and is the best way to get upstream when the water is high.

Recommendations, changes, and other notes: Do not include the right fork of Stockdale in next season’s survey attempts. Last year’s crew ignored it completely, we found that fish simply do not use it heavily. Water there is shallow, there is little holding habitat, and substrate seems to be a bit large for good pink redds. Temperature/Pressure Gauge is still installed and was downloaded on 9/11/2016.

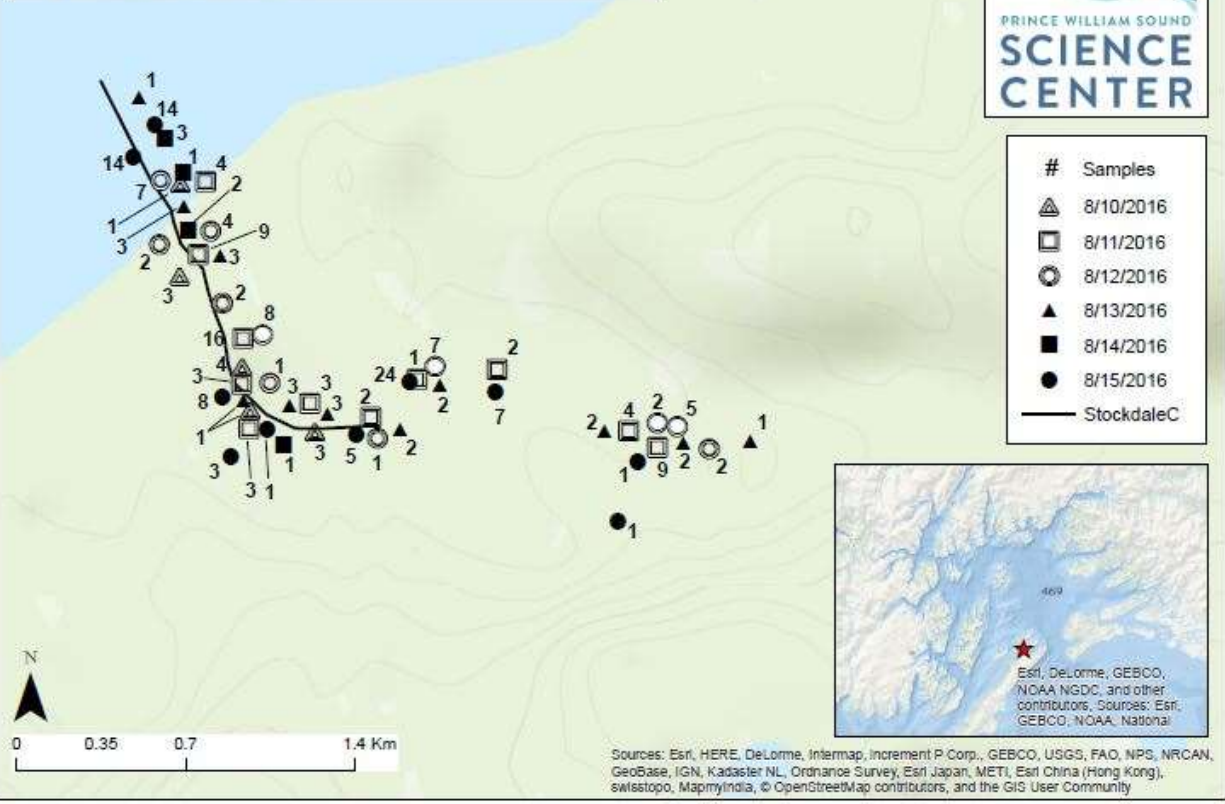
It was an incredible challenge to try and sample all of the carcasses in Stockdale Creek, many carcasses simply disappeared into rot before they could be sampled. The 2017 Pink Salmon run is forecast to be larger than the 2016 run, and as crew lead, I strongly recommend a consistent crew of at least 5 technicians.



**HW Interaction Project - Stockdale Creek
Pink Salmon Processing Areas and Samples
August 10-15, 2016**



#	Samples
▲	8/10/2016
◻	8/11/2016
◯	8/12/2016
▲	8/13/2016
■	8/14/2016
●	8/15/2016
—	StockdaleC

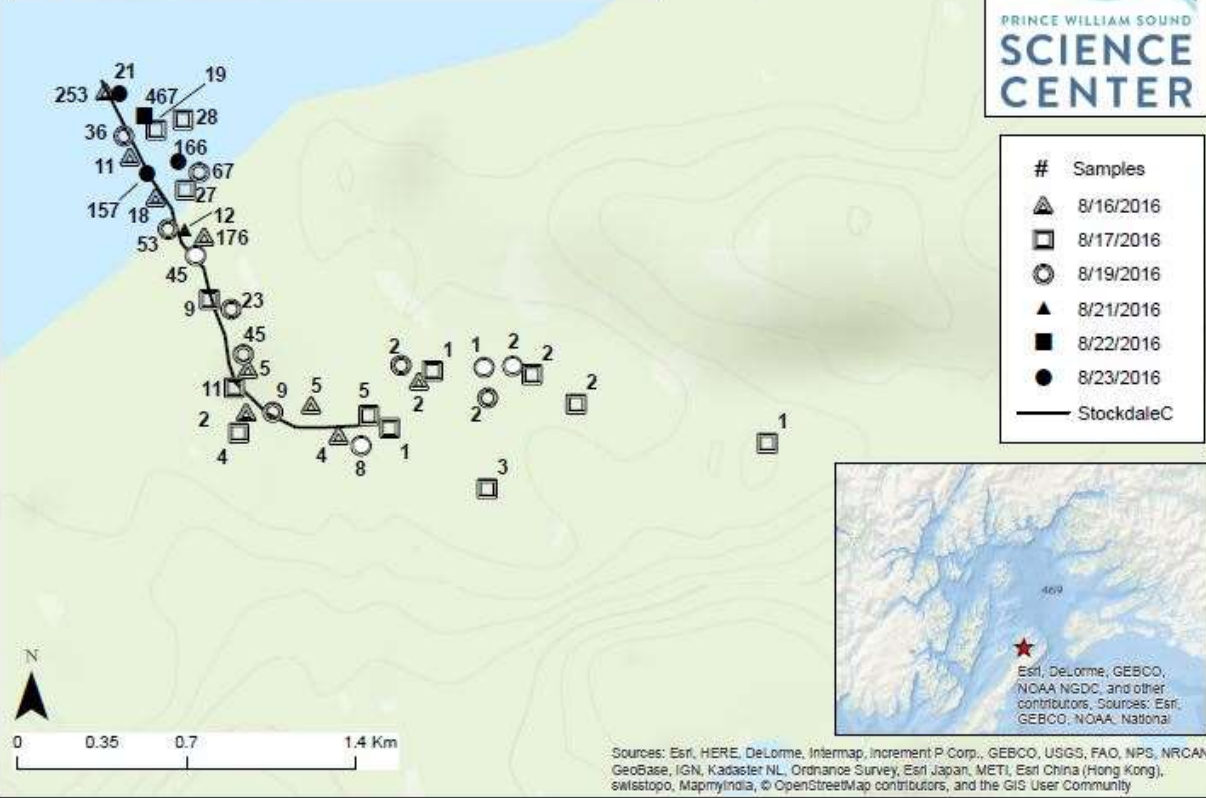


Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Stockdale Creek
Pink Salmon Processing Areas and Samples
August 16-23, 2016**



#	Samples
▲	8/16/2016
□	8/17/2016
○	8/19/2016
▲	8/21/2016
■	8/22/2016
●	8/23/2016
—	StockdaleC

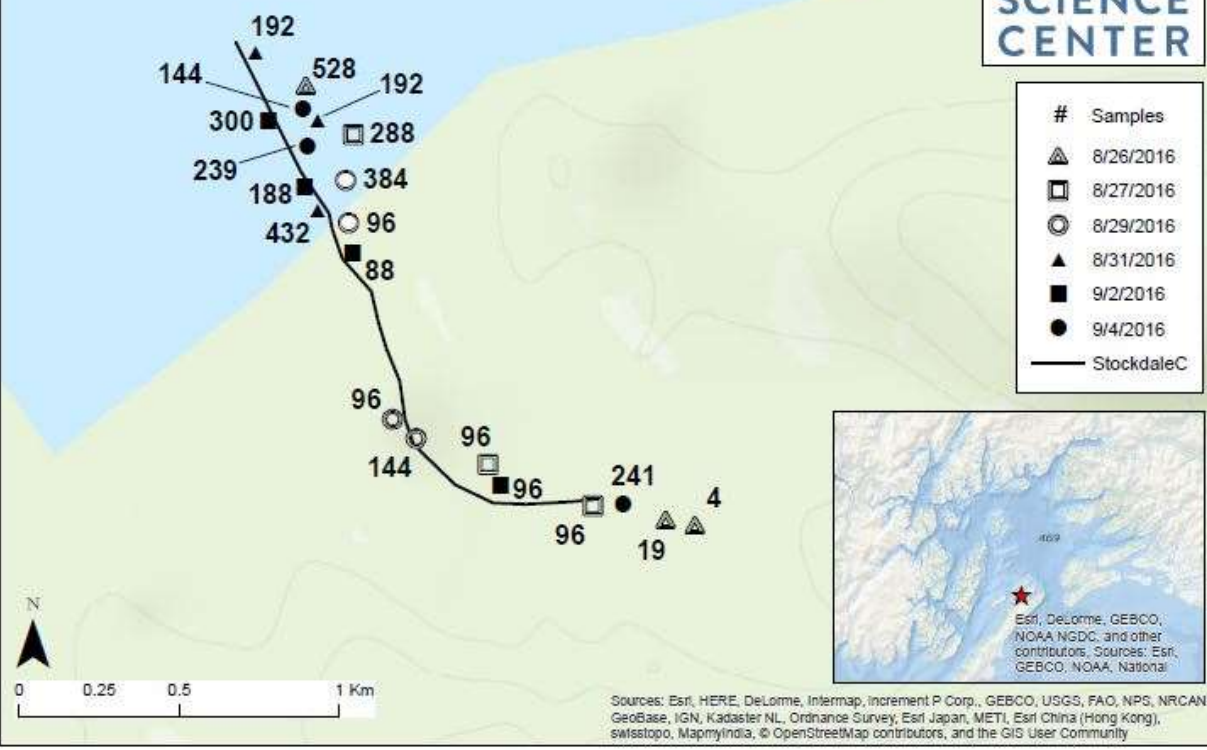


Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

**HW Interaction Project - Stockdale Creek
Pink Salmon Processing Areas and Samples
August 26-September 4, 2016**



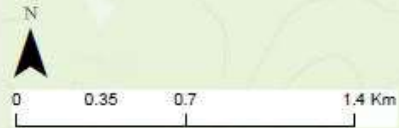
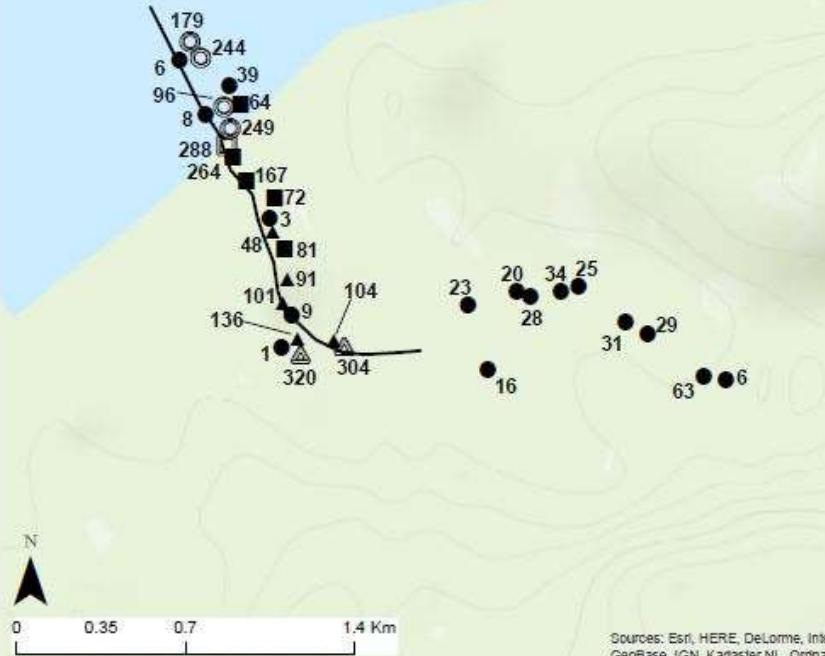
#	Samples
▲	8/26/2016
□	8/27/2016
○	8/29/2016
▲	8/31/2016
■	9/2/2016
●	9/4/2016
—	StockdaleC



**HW Interaction Project - Stockdale Creek
Pink Salmon Processing Areas and Samples
September 6-11, 2016**



#	Samples
△	9/6/2016
□	9/7/2016
○	9/8/2016
▲	9/9/2016
■	9/10/2016
●	9/11/2016
—	StockdaleC



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

Gilmour Creek
227-20-17480
Pink Salmon Fitness

2016 samples collected and run timing: Sampling at Gilmour Creek for Pink Salmon fitness was successful in 2016. Sampling was broken into two daily surveys, one for Gilmour and one for Gilmour Trib. Each survey was performed a total of 20 times through the sampling season, and resulted in 4,282 (2784 Gilmour proper, 1,498 Gilmour trib) total samples collected. The run here was slow to start, the first week of surveys showed just a handful of fish. Numbers were inconsistent over visits and it appears that most of the early fish experienced heavy predation or returned to sea without spawning. On visit 5 (8/9/2016) however, water levels had increased and over 100 fish were recorded. Numbers increased steadily after that, appearing to peak around visit 12 (8/25/2016) with 3,375 fish observed between the two branches. Chum Salmon were present in the early season.

Extent of stream sampled: Mass spawning was observed from the intertidal up both forks of the stream. Survey of the right fork ended at a cascade waterfall that appears to be the Pink extent. A satisfying extent for the main branch was never identified, though no fish made it above the debris jam where most of our surveys ended. Last season Pinks were recorded above this point, but not this year.

Unusual events: Low water was an issue on Gilmour this season. Primary spawning habitat for pinks at Gilmour is within the old intertidal zone that was uplifted during the 64' earthquake. Because of this, trees along the banks of the stream are young and don't supply much wood for maintaining channel complexity. Instead the stream is just aggregated gravel beds, ideal for Pink redds but bad for fish during low water conditions. During the hot days we had at the end of August, low water caused many pre and partial spawners to die.

Access, safety, or logistics issues: The team experienced very few challenges during visits to this stream. There were no bear sightings, though there was certainly evidence of bear predation. Water did get high enough at one point to make crossing the Gilmour main branch too dangerous.

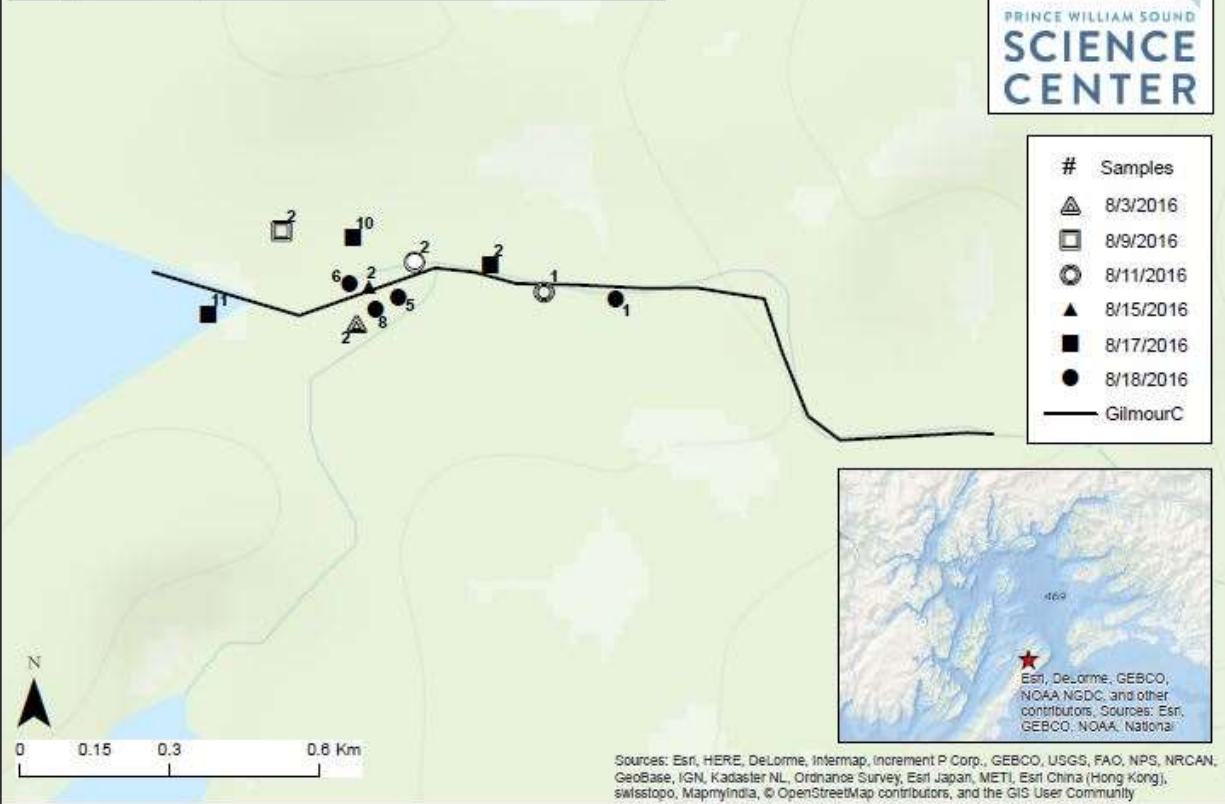
Recommendations, changes, and other notes:

There is a log-jam on the main branch where most of our surveys ended, as fish never made it above that point this season. Last year the crew reported sampling fish from above that point, but not this year. Temperature/Pressure Logger is still installed on this stream and was downloaded on 9/12/2016 before being redeployed. However, upon redeployment it was noticed that the cable anchoring the Gilmour sensor has broken. The cinderblock it is attached to should keep the sensor fairly secure, but it needs to be re-anchored next spring.

**HW Interaction Project - Gilmour Creek
Pink Salmon Processing Areas and Samples
August 3-18, 2016**

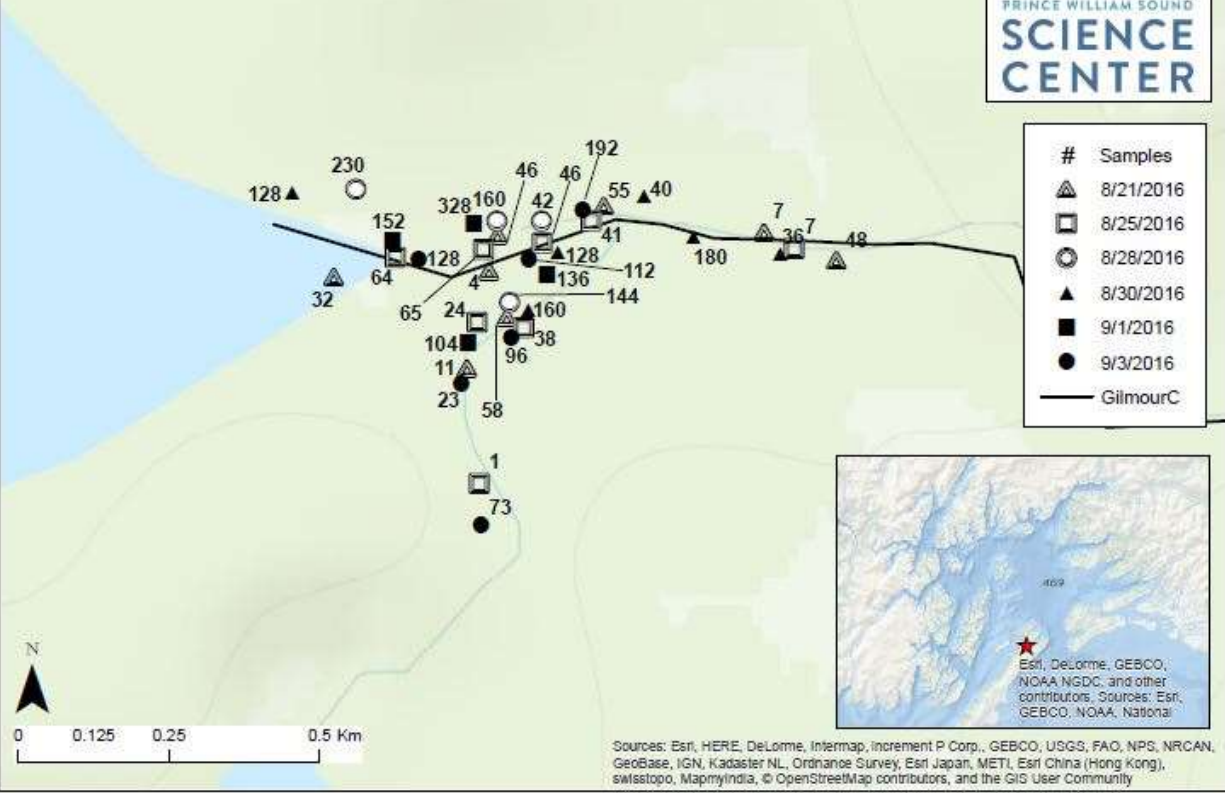


#	Samples
△	8/3/2016
□	8/9/2016
○	8/11/2016
▲	8/15/2016
■	8/17/2016
●	8/18/2016
—	GilmourC



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

**HW Interaction Project - Gilmour Creek
Pink Salmon Processing Areas and Samples
August 21-September 3, 2016**



**HW Interaction Project - Gilmour Creek
Pink Salmon Processing Areas and Samples
September 5-12, 2016**



#	Samples
▲	9/5/2016
□	9/7/2016
○	9/9/2016
▲	9/12/2016
—	GilmourC

