



Interactions of Wild and Hatchery Pink Salmon in Prince William Sound

Draft Report for 2020

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

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ABSTRACT

This is the ninth in a series of annual progress reports on data collection and analysis for studies of hatchery-wild interactions of Pink Salmon in Prince William Sound (PWS), Alaska. The Prince William Sound Science Center (PWSSC), under contract to Alaska Department of Fish & Game (ADF&G), continued data collection for an investigation of the relative survival (fitness) of hatchery-origin and wild-origin offspring following natural spawning. Research in 2020 was focused on PWS Pink Salmon fitness studies. Similar studies of Chum Salmon in SEAK were managed by Sitka Sound Science Center and are therefore reported elsewhere. During the 2020 field season, 14,121 individual Pink Salmon were sampled during repeated visits to the spawning grounds of two PWS streams that had been previously sampled for this research. Tissue samples were collected for DNA extraction from each fish for the eventual analysis of relative reproductive success (RRS). Otoliths were collected from the same specimens for identification of possible hatchery origin (hatchery Pink Salmon in Alaska have thermally marked otoliths that were used to determine hatchery- or natural-origin). All samples with readable otoliths were analyzed for origin determination from Paddy Creek and Erb Creek. For this report we estimated hatchery fractions weighted by abundance (dead counts) for each stream. The following hatchery fractions were determined: Paddy Creek (15.2%), and Erb Creek (11.5%).

INTRODUCTION

Prince William Sound Science Center (PWSSC) has continued scientific data collection and analysis services requested this year under the State of Alaska contract CT 160001756 “... to conduct a research program to address interactions of Wild and Hatchery Pink and Chum Salmon in Prince William Sound and Southeast Alaska”. This is the ninth annual report, focusing on results from 2020 data collection, as well as summarizing results from 2013 through 2019.

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 2020 field research consisted of only one major activity: adult sampling of Pink Salmon in three PWS streams to collect tissues for DNA extraction and otolith samples for the ongoing fitness studies. The otolith sampling also allowed for estimation of the hatchery fractions of spawning salmon in two 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 2020, as they were for 2013 – 2015 (Knudsen et al. 2016, Knudsen et al. 2021).

The methods in this report reflect guidance in the RFP, some refinements made following the 2012 preliminary field season (Buckhorn et al. 2013), and the 2013-2015 full seasons (Knudsen et al. 2015a,b, and Knudsen et al. 2016), as well as changes made as a result of consultation with the Science Panel in November 2012, December 2013, December 2014, and April 2015. The 2020 field sampling protocol was unchanged from 2019 and is presented in Appendix A. This report includes summaries of sample collection during 2020 for the DNA-based fitness studies. Given the interest of the ADF&G Gene Conservation Lab in early sample processing, we arranged two separate sample shipments to Anchorage in 2020. The first transfer was 91 sample trays to ADF&G on 25 August 2020, followed by a final delivery of 319 sample trays to Anchorage on 26 September 2020. Otolith samples were subsequently sent by ADF&G to the lab in Cordova for processing. Otolith sample results were used to generate hatchery fraction estimates for streams sampled in 2020.

ADULT SAMPLING IN STREAMS

Background

Based on the original RFP from ADF&G, there were two primary purposes for sampling adult Pink Salmon and/or Chum Salmon in streams through the course of these studies: 1) to further assess the degree and the range of inter-annual variability in hatchery straying rates; and 2) determine the effects of hatchery fish spawning with wild populations on the fitness of wild populations. The former objective was addressed by collecting otoliths from spawned out adults in 64 streams across PWS and SEAK between 2013 and 2015. The otoliths were examined in ADF&G laboratories to determine whether the individuals were of hatchery or natural origin. The now-completed results are estimates of the percent of hatchery-origin fish for each stream, district, and regional spawning population over three years (Knudsen et al. 2016, Knudsen et al. 2021). The latter objective is being addressed by collecting tissues for DNA analysis, as well as otoliths, from adults in a subset of the same streams, referred to here as “fitness” streams. The DNA “markers” of these parents can be used to identify either their pre-emergent offspring collected the following spring, or progeny returning to the streams as adults, so that relative reproductive success (RRS) of hatchery- and natural-origin fish can be estimated for both males and females. This analysis is also referred to as pedigree analysis in this report.

Methods

Tissues for DNA extraction (primarily heart, but sometimes other tissues) were collected along with otoliths from adult Pink Salmon in 2020 in PWS. Samples taken from Chum Salmon in SEAK were managed by the Sitka Sound Science Center and are therefore reported elsewhere. This required repeated sampling of the two fitness study streams, Paddy and Erb Creeks) in PWS (Figure 1). The 2020 methods were identical to the methods used in 2019 and previous years (Knudsen et al. 2015a, b, Knudsen et al. 2016, Gorman et al. 2017, Gorman et al. 2018).

The RFP originally specified that fitness study streams have sampling targets of 500 individuals in high-stray-rate streams and 1,000 individuals in streams with lower stray rates. Subsequent discussions with ADF&G GCL staff and the Science Panel in late 2013, and again in December 2014 and April 2015, indicated the importance of exceeding the original sampling targets from these streams. Therefore, a strategy of maximizing the number of samples from fitness streams was implemented in 2015, and maintained in 2016, 2017, 2018, 2019, and 2020, to increase the statistical power of pedigree analyses through larger sample sizes.

Logistical Strategy

From August 9 through September 18 2020, three field crew members sampled two of the original six fitness streams for adult Pink Salmon otoliths and DNA, Paddy and Erb Creeks (Figure 1).

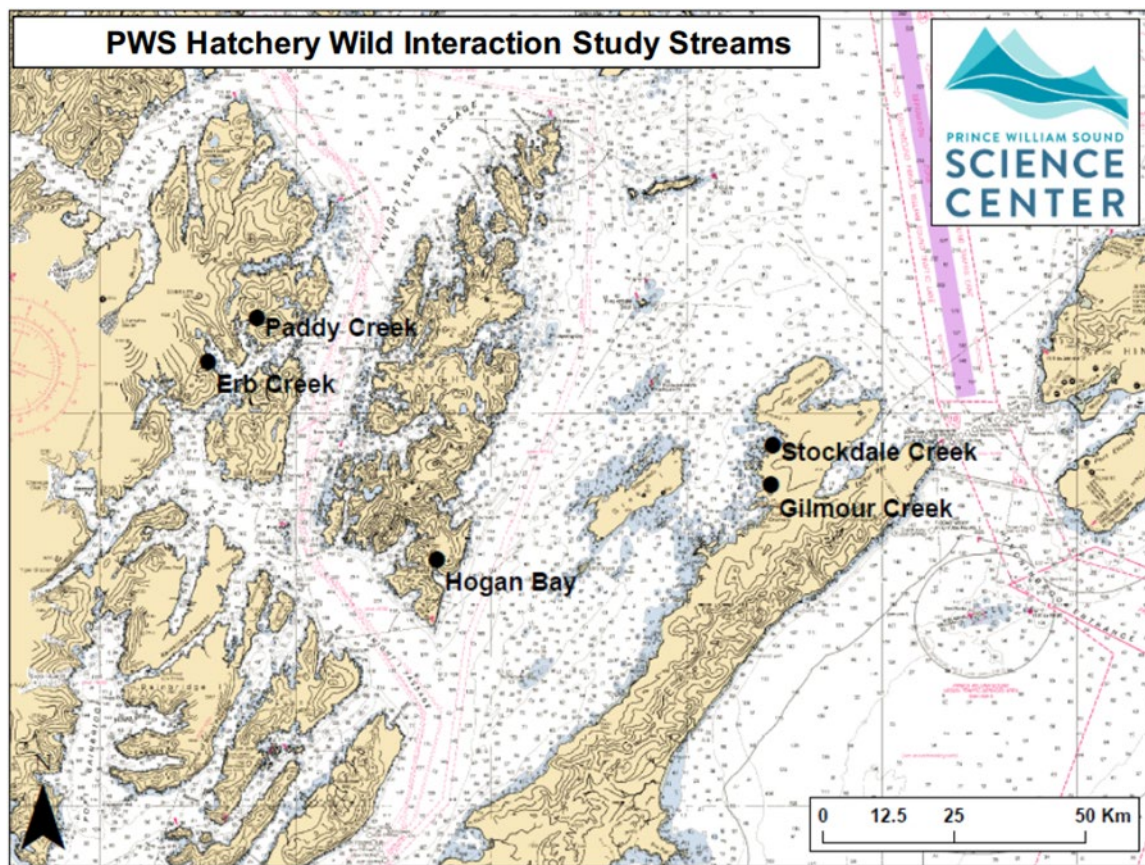


Figure 1. Pedigree stream locations in Prince William Sound for AHRP. During 2020, two streams were sampled (Paddy and Erb Creeks) for Pink Salmon otoliths and DNA tissues.

All PWS field crews were directly employed by PWSSC in 2020. Stream sampling is a dynamic process influenced by historic run timing, in-season fish abundance, weather, crew location, and distance between streams. Each field crew primarily worked independently and the 2020 crew leader was astute in making decisions to maximize efficiency and achieve sampling goals. Camp mobilization at Paddy Creek was supported by the Prince William Sound Aquaculture Corporation through the use of their landing craft, the M/V *Alaska Challenger*. Prior to mobilization, crew were trained in Cordova, included instruction on boating, bear and firearms safety, CPR and First Aid, protocol review, tablet use, and data entry.

Sampling effort was distributed between the two study streams. Early in the season, both streams were sampled on the same day, but by August 20th, the crew began alternating daily visits to the two streams to maximize sample collections. The total number of stream visits in 2020 was 25 days on Paddy Creek and 26 days on Erb Creek (Table 1). The number of live fish in the streams were declining sharply at the time field operations were terminated on September 18th (see Discussion for additional detail).

Stream Sampling Methods and Execution

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

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

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

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

Communication and Data Transmission

All crews filed float plans with PWSSC and checked in daily with the stream PI on Delorme inReach devices or personal cell phones. Satellite and cell phones were used when longer conversations were necessary. Each night, crews checked in 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 2020.

After completing a sample inventory, samples were delivered to ADF&G in two separate shipments during 2020 (described earlier). In Anchorage, tissues for DNA extraction were removed from the DWPs and otoliths were shipped back to Cordova ADF&G lab

for processing. Upon shipment of DWPs to ADF&G GCL, all field data had been transmitted to the project database. Electronic data delivery to ADF&G followed the quality control review so that otolith and DNA results could be matched to the field observation data.

Specific Biological Sampling Methods

Every effort was made to use consistent field methodologies throughout the data collection within and across years. Detailed methodological protocols were developed to guide the field data collection (Appendices A and B). The protocols were developed primarily from previous practices established within ADF&G, modified as necessary to facilitate the current study, and from experience during 2013 to 2019 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 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 2014-2019 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 - 2019, the objectives of the PWS field sampling in 2019 included estimates for the fractions of hatchery fish in each spawning population of Pink Salmon. However, unlike 2013-2015 when hatchery fractions were estimated for over two dozen streams, only two streams were visited in 2020. Each sampled stream was visited repeatedly from early August 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 2020 is the weighted average of the estimates across visits:

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

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.

We pulled data from the database on June 13-14, 2021 to conduct the final analyses for 2020 reported here.

RESULTS

The 2020 stream surveys, sample sizes, and previous years’ results for estimated hatchery fractions for the two PWS fitness streams are reported below.

PWS Pink Salmon Fitness and Hatchery Fraction Sampling

Overall, 2020 sampling was successful at both of the selected Pink Salmon PWS fitness study streams where 14,121 DNA tissues and pairs of otoliths were taken (Table 1). The number of samples varied per stream visit (Appendix C). Foot survey-based live and dead counts were made on all stream surveys (Appendix C) and the dead counts were later used to weight the hatchery fraction estimates per visit. (See Appendix D for more details on the sampling of each PWS stream.)

Table 1. A stream-by-stream summary of sampling and hatchery fractions for three PWS Pink Salmon streams sampled in 2020, with previous data reported for earlier years for all five pedigree streams. Hatchery fractions for each stream were weighted by observed dead salmon counts taken during each visit. Note that the three streams sampled in 2020 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 2020	Samples collected 2020	Number of otoliths read 2020	Sampling Year							
					2013	2014	2015	2016	2017	2018	2019	2020
Paddy C	226-20-16010	25	6,656	6,076	0.154	0.595	0.328	0.206	0.352	0.076	NA	0.152
Erb C	226-20-16040	26	7,465	7,290	0.113	0.228	0.214	0.1	0.263	0.067	NA	0.115
Hogan Bay ^a	226-30-16810	NA	NA	NA	0.64	0.915	0.583	0.205	0.687	0.423	0.774	NA
Gilmour C ^{a,b}	227-20-17480	NA	NA	NA	NA	0.557	0.225	0.116	0.25	0.063	0.229	NA
Stockdale C ^a	227-20-17520	NA	NA	NA	0.163	0.735	0.24	0.078	0.095	0.057	0.147	NA

^aHogan Bay, Gilmour Creek, and Stockdale Creek were not sampled in 2020 to estimate the hatchery fraction of its spawning Pink Salmon population.

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

DISCUSSION

In comparison with previous years, the number of samples collected in 2020 (14,121) was lower than that sampled in 2019 owing to fewer streams sampled (2) and relatively low numbers of returning adult pink salmon.

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

2014: 7,939

2015: 46,386

2016: 37,549

2017: 58,688

2018: 36,550

2019: 30,661 (note Paddy and Erb Creeks were not sampled in 2019)

In general, the 2020 field season was marked by wetter conditions than that experienced during the prolonged draught and warm temperature experienced in 2019. Crews were very successful in surveying and sampling both streams during the field season. Black bears were commonly observed (as in previous years) at both streams. More detailed accounts in annual stream summaries are provided in Appendix D. In some cases, tissue samples were collected from carcasses that were depredated and missing otoliths, particularly earlier in the season.

The intention was to sample the entire spawning run in both streams. We were very successful at sampling the end of each run as indicated by the live counts and sample numbers in Appendix C. Sampling was terminated on September 18.

The results here indicate higher hatchery fraction values compared to the last year these streams were surveyed (2018), but generally lower than what was observed during the previous years (2013-2017, Table 1).

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APPENDIX A. STREAM SURVEYS AND ADULT SALMON COLLECTION

This protocol will be used to prepare for and conduct surveys of streams for the hatchery-wild interactions study in Prince William Sound (PWS) in 2020. 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 two PWS streams. Table A1 designates the streams and species to be sampled and the types of sampling at each stream in 2020. 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, while balancing sampling of the lower and upper reaches of the stream**. The three streams are sampled every other day (Table A1) although every day is preferable (Gilmour, Stockdale and Hogan Bay Creeks). These streams will be sampled every other day 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 2020

Stream name	Stream code	Target species	Fitness stream	Sampling events per season
Paddy Creek	226-20-16010	DNA pink	Yes	Daily or Alternate Days
Erb Creek	226-20-16040	DNA pink	Yes	Daily or Alternate Days

Dashboard

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 dashboard 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 dashboard.

Preparations for Surveys

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

✓ *Charge all tablets and scanners fully*

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

* To conserve tablet battery life,

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

✓ *Clean and inspect guns and other safety gear*

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

✓ *Dry and patch waders*

✓ *Prepare for the next sample visit*

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

✓ *Prepare sampling kits*

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

Stream Surveys

A stream survey is defined as a set of Processing Areas on one stream and one date, with a beginning and ending point along the stream. A survey includes information about a day's activity on a stream such as demarcating the beginning and end locations of the survey. A careful count of the total number of observed alive and dead target species in the survey area also will be made for each survey, with a few exceptions noted below. The survey can be conducted as the crew moves either upstream or downstream.

Arriving at the Study Stream

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

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

Starting a Survey

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

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

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

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

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

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

Depending on the number of fish that can be sampled, decide whether to split into two teams or all work together. You may use one or two tablets. **(If you use two tablets, add the live/dead counts to only one tablet, unless you are working on separate tributaries under separate surveys.)**

Designate one tablet as the primary survey tablet. If that tablet is lost, malfunctions, or the battery is close to dying, continue with another tablet. Re-establish the survey in the

second tablet. There is no need to enter the same data previously entered on the previously used tablet (except the live/dead counts – see below).

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

Data Entry

At the beginning point of the survey,

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

Counting Salmon

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

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

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

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

Data Entry

To accumulate salmon counts:

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

Processing Areas

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

Fish Collection

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

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

Completing the Survey

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

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

Data Entry

To finish a survey:

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

Post-Survey Data Management

Immediately upon return to your 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 ¾ with ethanol and that the entire tray is wrapped tightly with saran wrap. The ethanol WILL EVAPORATE if this is not done properly, which will compromise the integrity of the sample for DNA extraction. Store the DNA trays in a secure location for safe transport.

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

√ *Upload data to database*

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

✓ *Update the dashboard*

Also, whenever internet is available, access the dashboard 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 dashboard to help plan the next day's activities and sampling goals.

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

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

Background

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

Otoliths

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

DNA Tissues

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

Preparing for Specimen Sampling

Tray Preparation

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

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

Preparing DNA/Otolith 48dwp Trays

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



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

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



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

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

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

Field Equipment for Sampling Otoliths and DNA

Pack the following items for field sampling otoliths and DNA:

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

Processing Specimens

Processing Areas

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

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

Each Processing Area will be uniquely identified within a survey.

Data Entry

To establish a Processing Area:

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

Processing Samples

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

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

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

Data Entry

To establish a specimen tray:

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

Sex, Length, and Height Measurements

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

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

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

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

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

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

Data Entry

To record sex, length, and body height:

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

Cutting Heads for Sampling DNA and/or Otoliths

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

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



Figure B2. Cut head off.

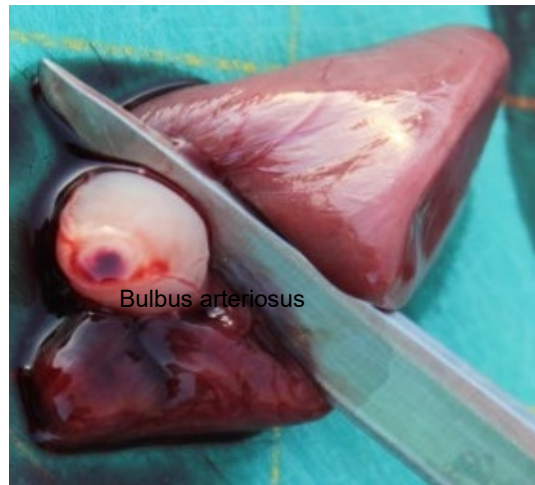


Figure B3. Bulbus arteriosus attached to heart.

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

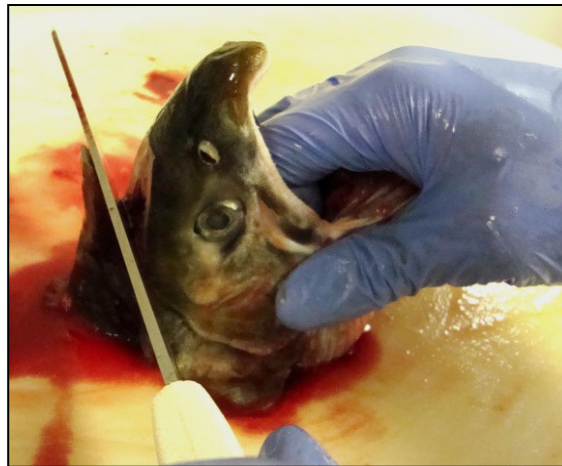


Figure B4. Slice the top of head off.



Figure B5. Remove brain tissue.

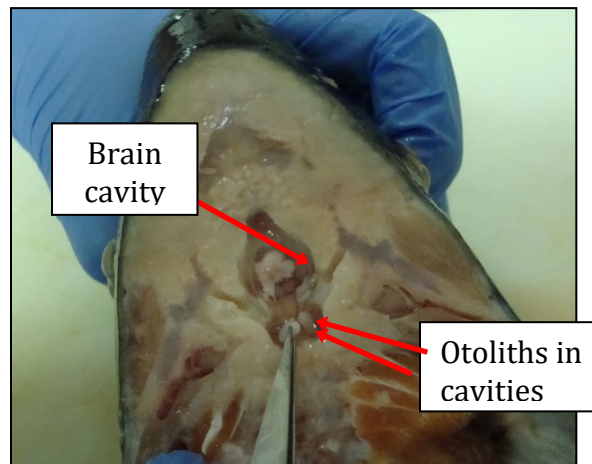


Figure B6. Otoliths in two lateral wells posterior to brain cavity.

Using 48dwp Trays for DNA Tissue and Otoliths

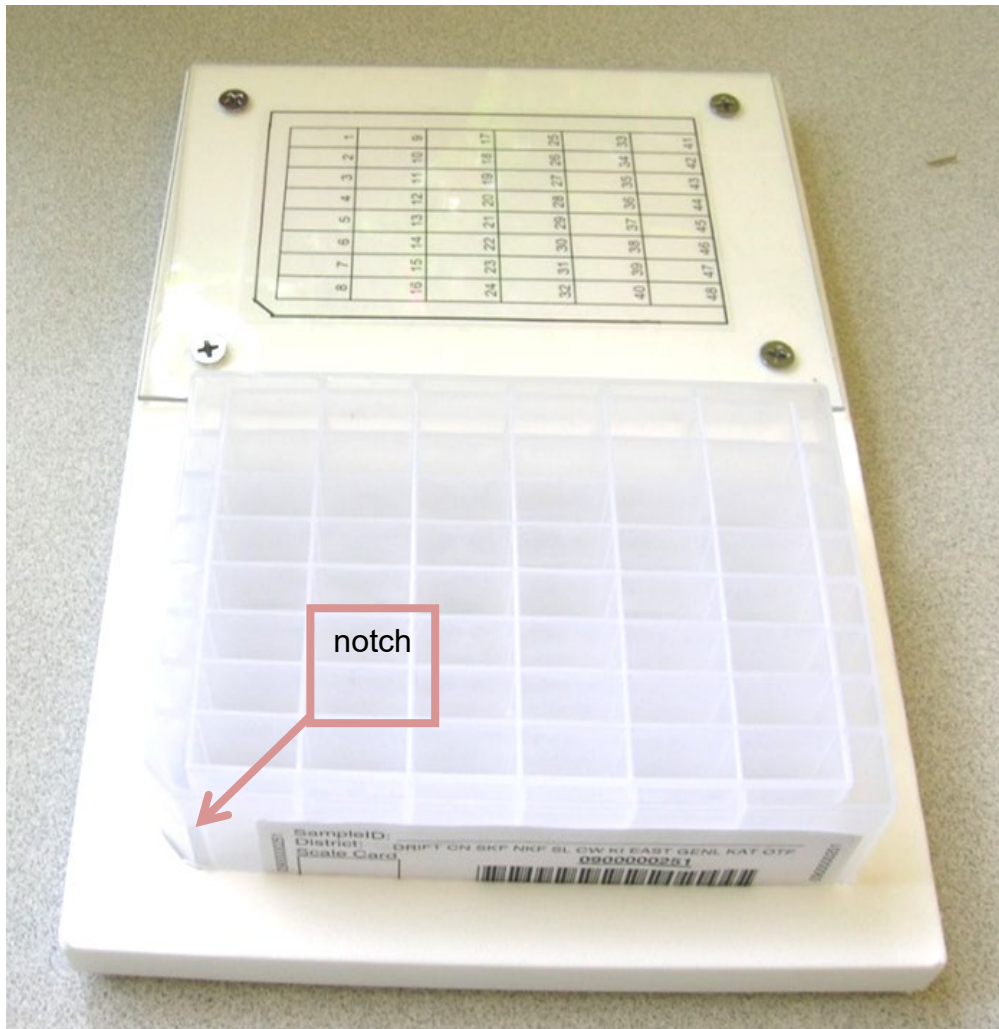
It is important to try to collect the best **quality DNA tissue samples to ensure reliable results**. Tissue quality degrades after death, so moribund and recently dead fish will yield best results. Frequent visits to streams will provide better opportunity to sample fresher spawn-outs. However, sample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

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

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

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

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



Set-up for sampling:

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

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

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

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

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

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

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

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

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

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

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



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

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

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

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

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

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

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

Cleaning, Storing, and Shipping Samples

Inspecting DNA and Otolith Samples

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

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

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

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

Pre-Shipment QA Process

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

Data Entry

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

SHIPPING

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

48dwp Trays

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

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

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

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

Person responsible for delivery:

Recipient:

Date sent to Cordova Lab:

Date received by Cordova Lab:

APPENDIX C. PWS PINK SALMON SURVEY SUMMARY

Stream Name	AWC	Date	Samples	Live	Dead
Paddy C	226-20-16010	8/9/2020	6	137	10
Paddy C	226-20-16010	8/10/2020	15	63	16
Paddy C	226-20-16010	8/12/2020	20	150	20
Paddy C	226-20-16010	8/13/2020	30	32	25
Paddy C	226-20-16010	8/14/2020	21	21	16
Paddy C	226-20-16010	8/15/2020	20	236	20
Paddy C	226-20-16010	8/16/2020	89	193	89
Paddy C	226-20-16010	8/17/2020	73	774	73
Paddy C	226-20-16010	8/18/2020	97	294	180
Paddy C	226-20-16010	8/20/2020	231	667	240
Paddy C	226-20-16010	8/22/2020	269	599	157
Paddy C	226-20-16010	8/24/2020	232	1360	211
Paddy C	226-20-16010	8/26/2020	139	1229	150
Paddy C	226-20-16010	8/28/2020	186	1397	190
Paddy C	226-20-16010	8/30/2020	240	2340	553
Paddy C	226-20-16010	8/31/2020	144	780	185
Paddy C	226-20-16010	9/1/2020	192	1212	335
Paddy C	226-20-16010	9/3/2020	384	980	631
Paddy C	226-20-16010	9/5/2020	48	230	115
Paddy C	226-20-16010	9/7/2020	432	202	528
Paddy C	226-20-16010	9/9/2020	192	209	384
Paddy C	226-20-16010	9/11/2020	192	72	192
Paddy C	226-20-16010	9/13/2020	81	48	81
Paddy C	226-20-16010	9/16/2020	38	4	38
Paddy C	226-20-16010	9/17/2020	0	0	0
Paddy Left Trib	226-20-16010	8/9/2020	0	0	0
Paddy Left Trib	226-20-16010	8/10/2020	0	0	0
Paddy Left Trib	226-20-16010	8/12/2020	0	0	0
Paddy Left Trib	226-20-16010	8/17/2020	0	0	0
Paddy Left Trib	226-20-16010	8/18/2020	0	0	0
Paddy Left Trib	226-20-16010	8/22/2020	0	0	0
Paddy Left Trib	226-20-16010	8/26/2020	38	80	38
Paddy Left Trib	226-20-16010	8/28/2020	39	83	39
Paddy Left Trib	226-20-16010	9/1/2020	46	29	75
Paddy Left Trib	226-20-16010	9/3/2020	28	42	33
Paddy Left Trib	226-20-16010	9/7/2020	30	4	30
Paddy Left Trib	226-20-16010	9/9/2020	0	1	3
Paddy Left Trib	226-20-16010	9/11/2020	0	0	0
Paddy Lower Right Trib	226-20-16010	8/9/2020	5	13	7

Paddy Lower Right Trib	226-20-16010	8/10/2020	0	4	0
Paddy Lower Right Trib	226-20-16010	8/12/2020	0	4	0
Paddy Lower Right Trib	226-20-16010	8/13/2020	14	0	14
Paddy Lower Right Trib	226-20-16010	8/14/2020	3	0	3
Paddy Lower Right Trib	226-20-16010	8/15/2020	19	136	19
Paddy Lower Right Trib	226-20-16010	8/17/2020	113	134	113
Paddy Lower Right Trib	226-20-16010	8/18/2020	96	247	101
Paddy Lower Right Trib	226-20-16010	8/20/2020	117	48	115
Paddy Lower Right Trib	226-20-16010	8/22/2020	126	117	126
Paddy Lower Right Trib	226-20-16010	8/24/2020	133	52	140
Paddy Lower Right Trib	226-20-16010	8/26/2020	227	987	275
Paddy Lower Right Trib	226-20-16010	8/28/2020	336	1300	625
Paddy Lower Right Trib	226-20-16010	8/30/2020	447	1115	490
Paddy Lower Right Trib	226-20-16010	9/1/2020	480	1230	555
Paddy Lower Right Trib	226-20-16010	9/3/2020	96	490	110
Paddy Lower Right Trib	226-20-16010	9/5/2020	192	269	450
Paddy Lower Right Trib	226-20-16010	9/7/2020	96	138	134
Paddy Lower Right Trib	226-20-16010	9/9/2020	432	99	516
Paddy Lower Right Trib	226-20-16010	9/11/2020	109	33	109
Paddy Lower Right Trib	226-20-16010	9/13/2020	48	22	48
Paddy Lower Right Trib	226-20-16010	9/16/2020	13	5	13
Paddy Lower Right Trib	226-20-16010	9/17/2020	2	0	2
Erb C	226-20-16040	8/9/2020	54	1413	61
Erb C	226-20-16040	8/10/2020	96	1578	159
Erb C	226-20-16040	8/11/2020	88	1612	24
Erb C	226-20-16040	8/12/2020	116	2090	120
Erb C	226-20-16040	8/13/2020	68	3131	75
Erb C	226-20-16040	8/14/2020	187	4137	197
Erb C	226-20-16040	8/15/2020	133	1467	140
Erb C	226-20-16040	8/16/2020	157	2109	161
Erb C	226-20-16040	8/17/2020	175	3164	185
Erb C	226-20-16040	8/19/2020	544	3790	506
Erb C	226-20-16040	8/20/2020	144	3397	258
Erb C	226-20-16040	8/21/2020	460	4210	465
Erb C	226-20-16040	8/22/2020	29	2967	211
Erb C	226-20-16040	8/23/2020	624	3140	727
Erb C	226-20-16040	8/25/2020	443	3490	472
Erb C	226-20-16040	8/27/2020	566	3045	570
Erb C	226-20-16040	8/29/2020	480	1860	560
Erb C	226-20-16040	8/31/2020	528	2245	625
Erb C	226-20-16040	9/2/2020	720	675	825
Erb C	226-20-16040	9/4/2020	240	692	300

Erb C	226-20-16040	9/6/2020	624	429	718
Erb C	226-20-16040	9/8/2020	480	233	574
Erb C	226-20-16040	9/10/2020	346	158	301
Erb C	226-20-16040	9/12/2020	101	55	101
Erb C	226-20-16040	9/15/2020	48	12	48
Erb C	226-20-16040	9/18/2020	14	0	14

Appendix D. PWS Stream Summaries

Paddy Creek
226-20-16010
Pink Salmon Fitness

Crew members: Dan Dessent, Chandler Sharp, Darby Finnegan, and Eva Bingham

We observed low flows in the early season that became more extreme throughout the early two-week period of sunny weather. This was especially evident in the tributaries, Paddy Left and Paddy Right, where flow was not high enough for fish to pass above the high tide line. More large woody debris had accumulated in the stream since 2018 and the general topography of the stream seemed different, leading us to question whether a seismic event or several major storms disturbed the area. It began raining on August 24th, giving salmon an opportunity to push up Paddy Main and tributaries. The storm on September 2-3rd flushed out many possible samples. The last rainstorm beginning on September 16th rose water levels again, forming new temporary channels, but by this time there were very few live or dead fish in the stream.

The run seemed to be smaller than in the past. We never observed the numbers we expected to, which was especially apparent in the bay during the early season. The schools of fish holding in the bay were always relatively small. Once the rains came, we tended to have more samples on Paddy Right tributary than on Paddy Main.

We consistently observed bears on the stream until the last couple weeks of the season. They generally ignored our presence, and one bear in particular often shared the stream with us. No bears were aggressive. During the first couple weeks of the season, nearly all of our samples were preyed upon by bears, and these bears tended to eat the brains. As such, we often were not able to obtain otoliths in the early season. We found many of these preyed-upon samples piled up in riparian areas, but we noticed that as the season progressed, bears began stashing fish in different locations.

Erb Creek
226-20-16040
Pink Salmon Fitness

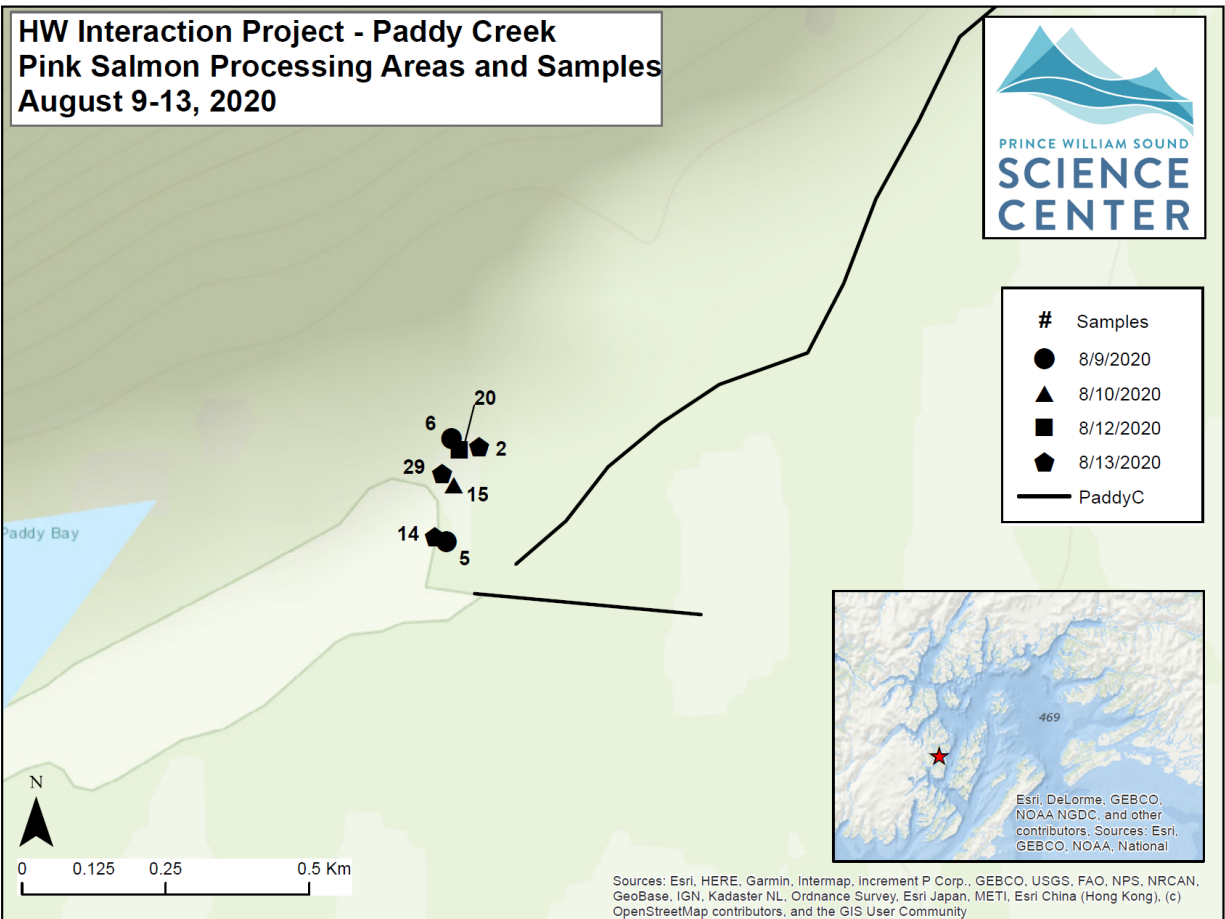
Crew members: Dan Dessent, Chandler Sharp, Darby Finnegan, and Eva Bingham

We observed low flows during the early season, but salmon could still pass above the high tide line during the lowest flows. The weather was sunny and warm throughout the first two weeks of our season in Early August, but rain became more consistent after that. A storm beginning on August 24th increased water flow and initially flushed out many fish we hoped to sample. The last major rainstorm, beginning on September 16th, also rose water levels and washed out any remaining samples.

The number of live fish in the stream consistently increased over the first couple weeks of the season, though the daily counts likely varied due to differences in survey length from day to day. The run seemed to end before it even started. We expected a peak in dead fish, but it never came. By the second week of September, there were few live fish in the stream, and carcasses were very spread out.

After our first few days of sampling, the bears tended to make themselves scarce while we surveyed and were easily scared off whenever we encountered them. Many of our samples were preyed upon by bears and were found piled up in the riparian areas. Bears on this stream did not bite off heads of fish as frequently as the Paddy Creek bears.

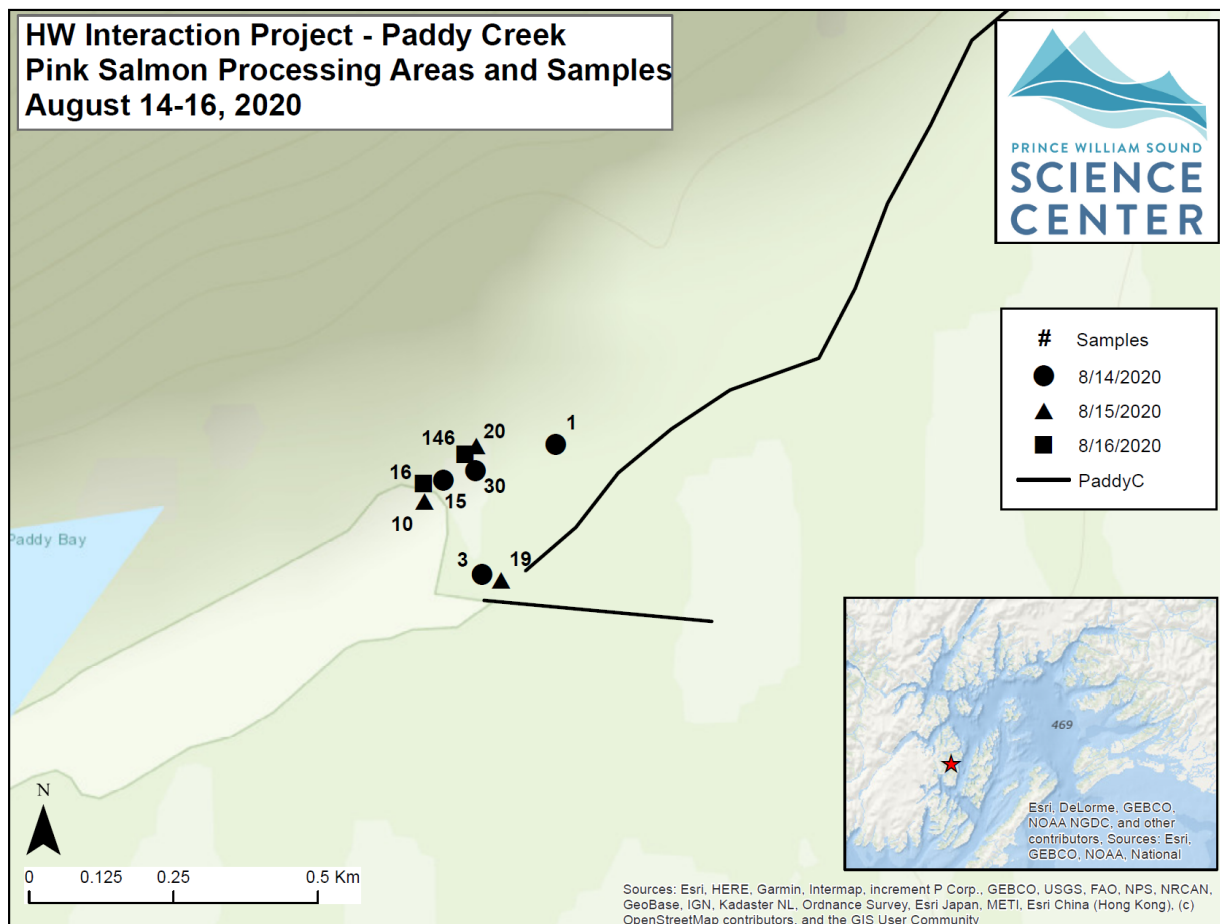
APPENDIX E. STREAM MAPS



HW Interaction Project - Paddy Creek Pink Salmon Processing Areas and Samples August 14-16, 2020

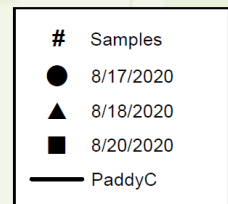


#	Samples
●	8/14/2020
▲	8/15/2020
■	8/16/2020
—	PaddyC



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

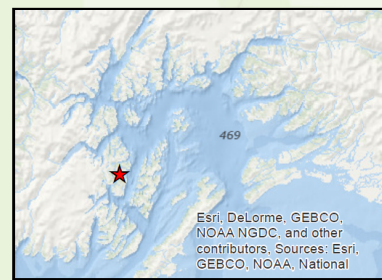
HW Interaction Project - Paddy Creek Pink Salmon Processing Areas and Samples August 17-20, 2020



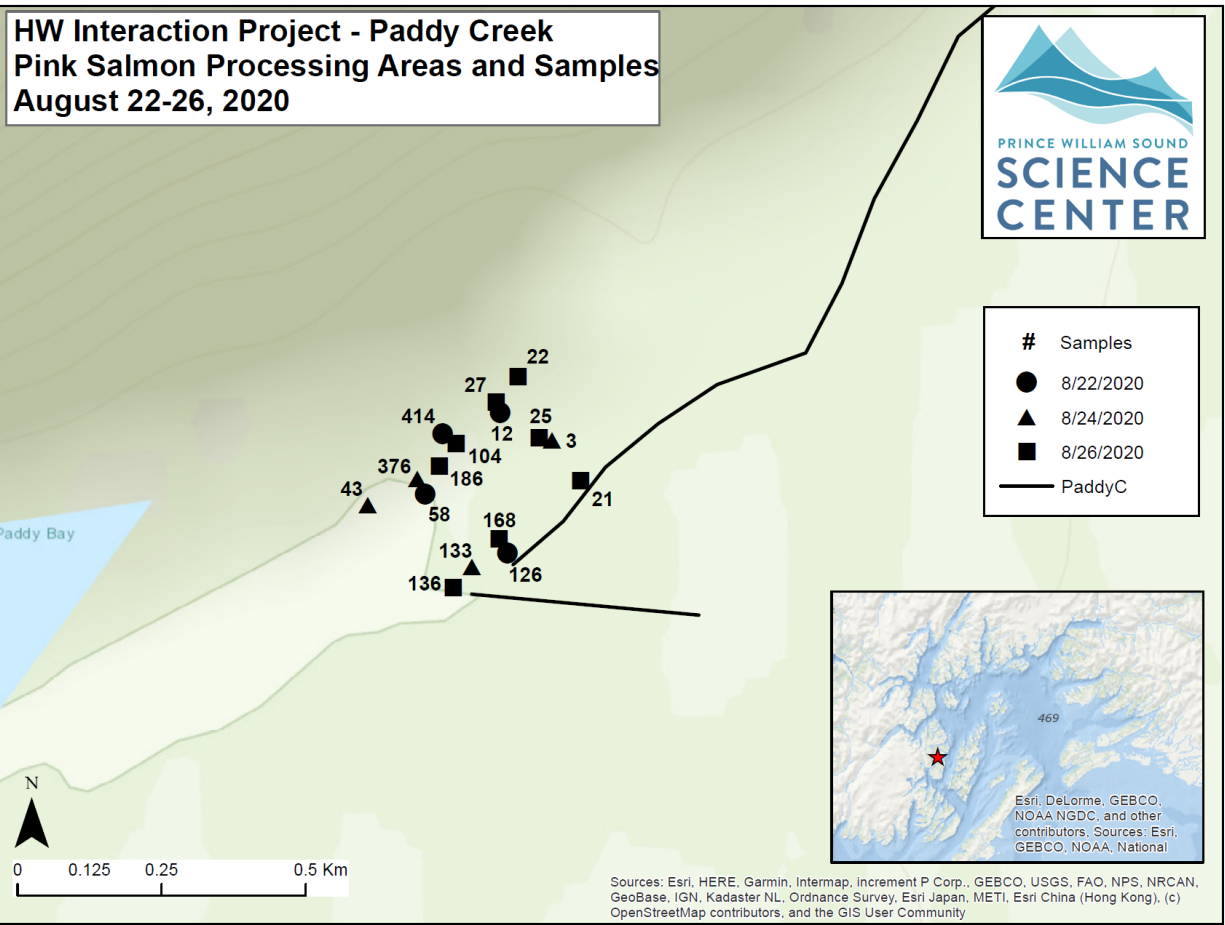
Paddy Bay



0 0.125 0.25 0.5 Km



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



HW Interaction Project - Paddy Creek Pink Salmon Processing Areas and Samples August 28-31, 2020

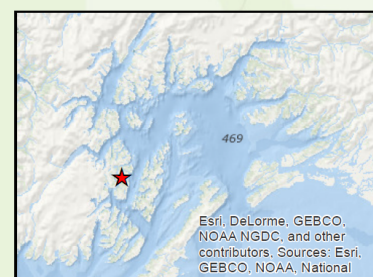


#	Samples
●	8/28/2020
▲	8/30/2020
■	8/31/2020
—	PaddyC

Paddy Bay



0 0.125 0.25 0.5 Km



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

HW Interaction Project - Paddy Creek Pink Salmon Processing Areas and Samples September 1-5, 2020

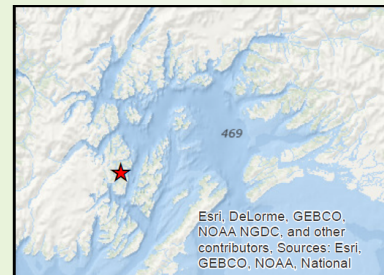


#	Samples
●	9/1/2020
▲	9/3/2020
■	9/5/2020
—	PaddyC

Paddy Bay



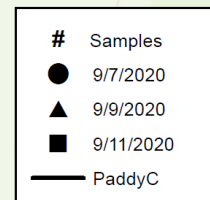
0 0.125 0.25 0.5 Km



Esri, DeLorme, GEBCO, NOAA NGDC, and other contributors. Sources: Esri, GEBCO, NOAA, National

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

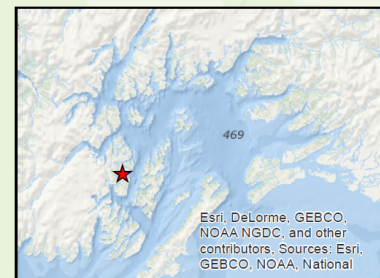
HW Interaction Project - Paddy Creek Pink Salmon Processing Areas and Samples September 7-11, 2020



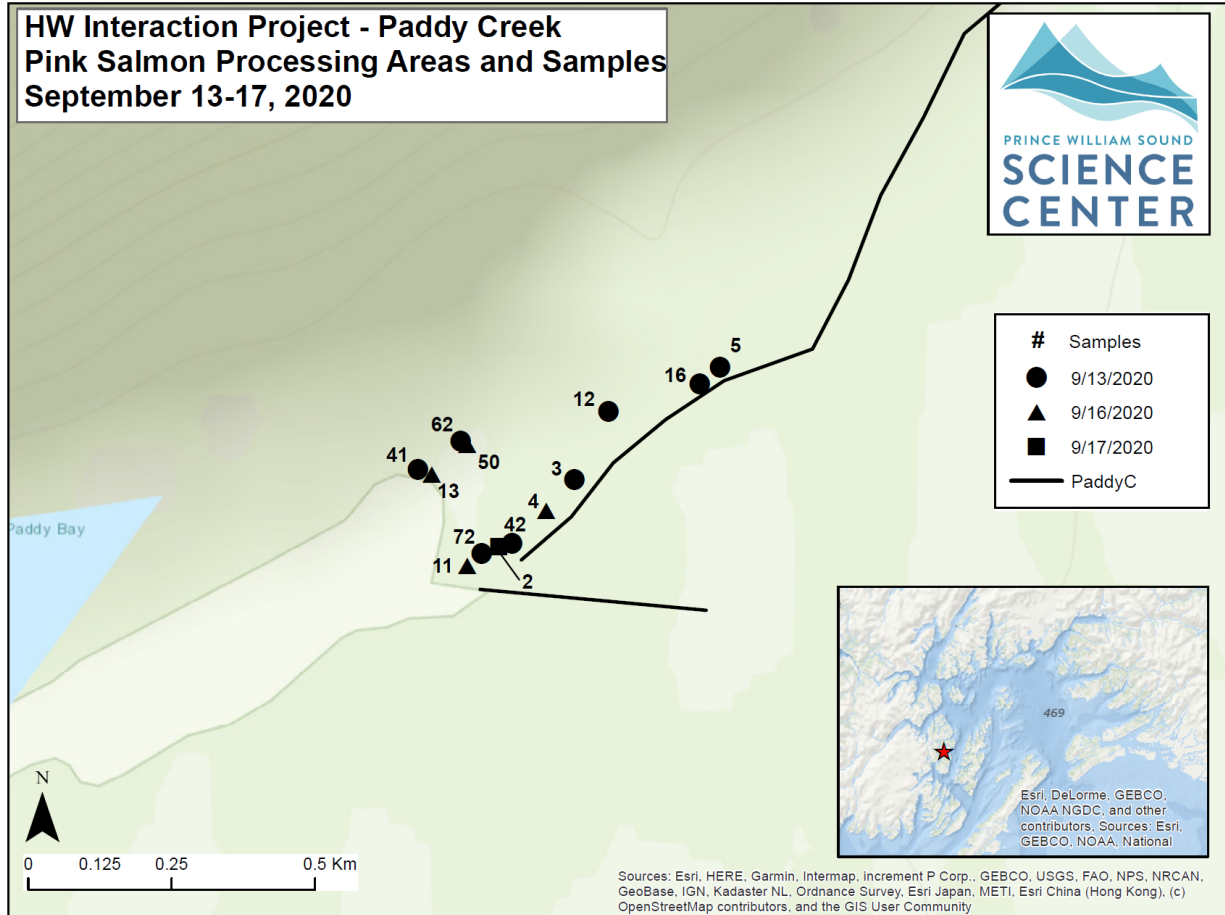
Paddy Bay



0 0.125 0.25 0.5 Km



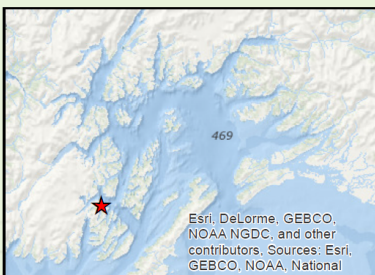
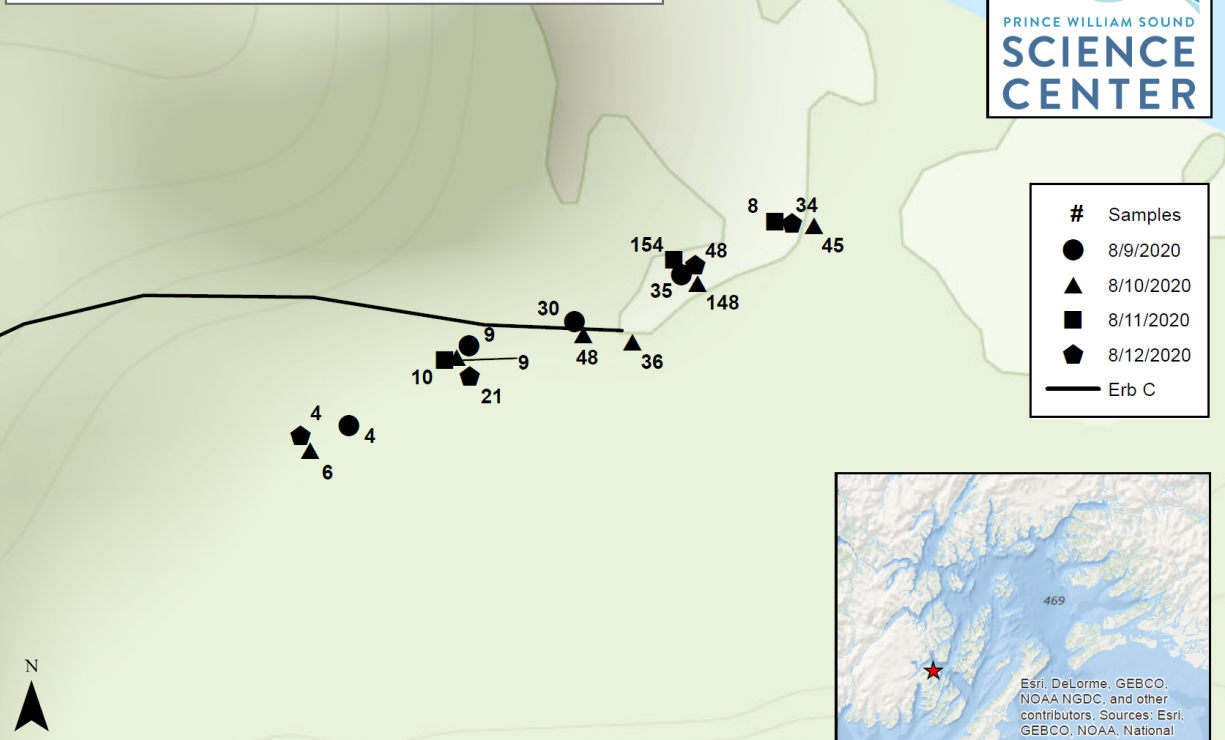
Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community



HW Interaction Project - Erb Creek Pink Salmon Processing Areas and Samples August 9-12, 2020



#	Samples
●	8/9/2020
▲	8/10/2020
■	8/11/2020
◆	8/12/2020
—	Erb C

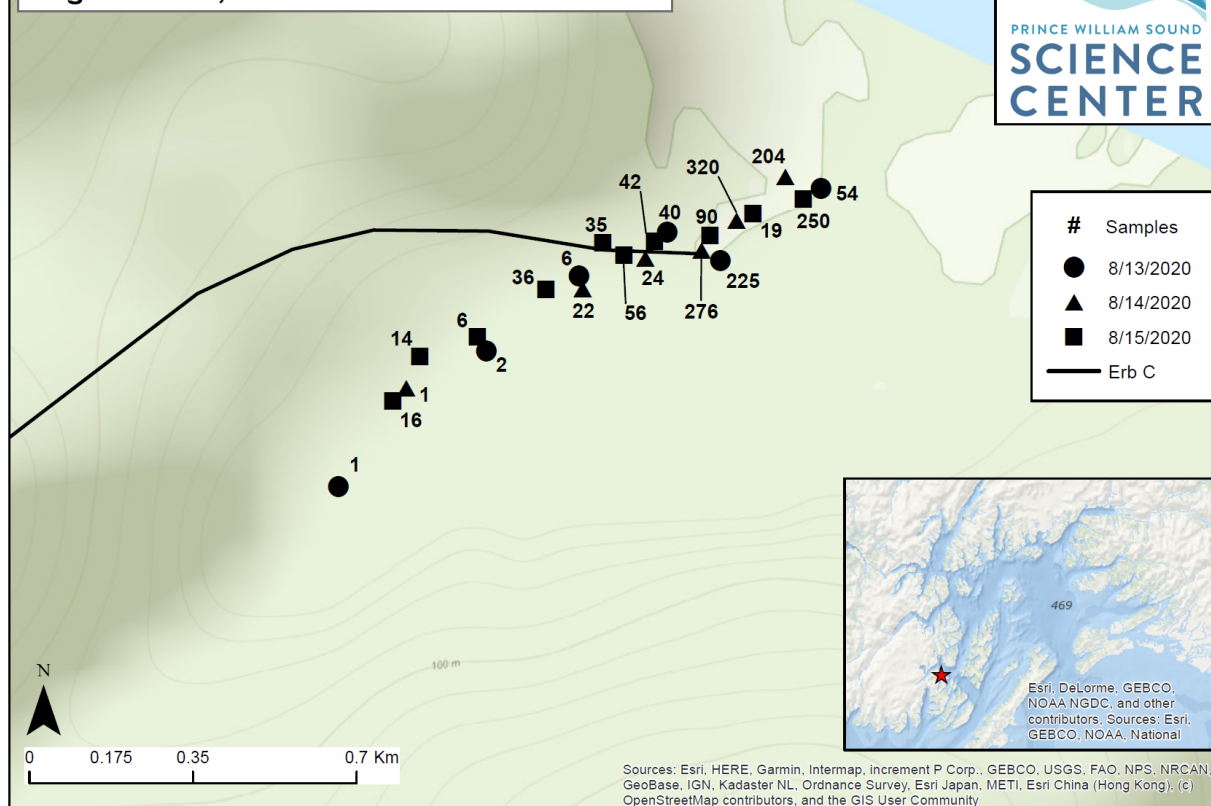


Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

HW Interaction Project - Erb Creek Pink Salmon Processing Areas and Samples August 13-15, 2020



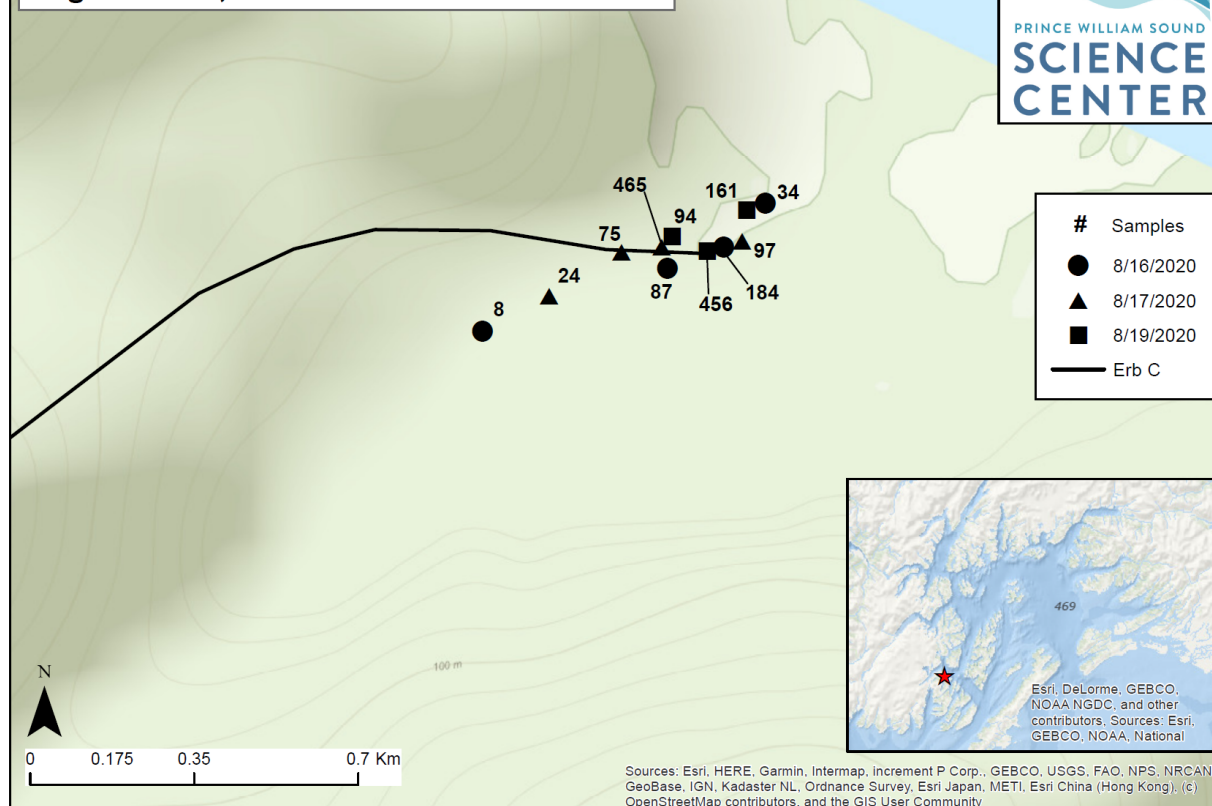
#	Samples
●	8/13/2020
▲	8/14/2020
■	8/15/2020
—	Erb C



HW Interaction Project - Erb Creek **Pink Salmon Processing Areas and Samples** **August 16-19, 2020**



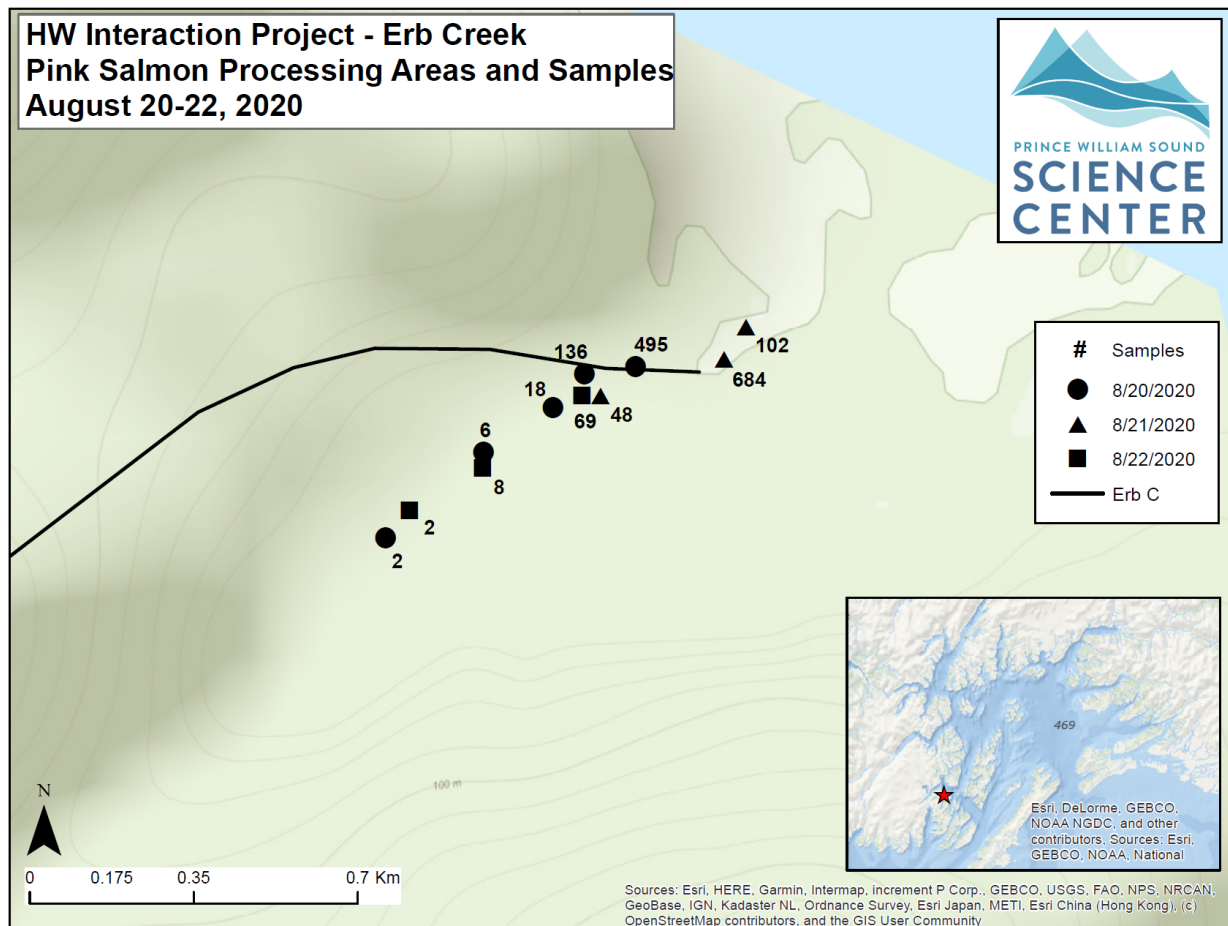
#	Samples
●	8/16/2020
▲	8/17/2020
■	8/19/2020
—	Erb C

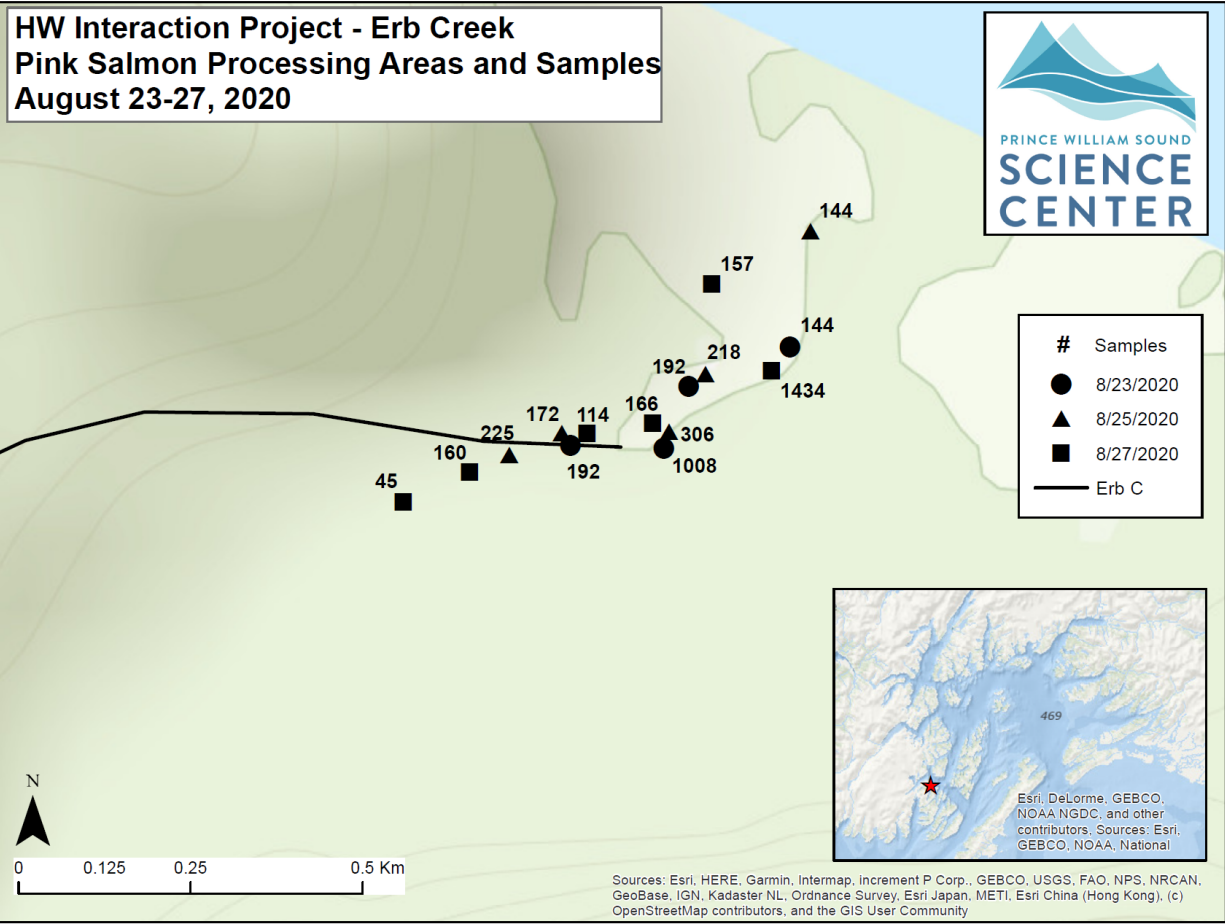


HW Interaction Project - Erb Creek Pink Salmon Processing Areas and Samples August 20-22, 2020

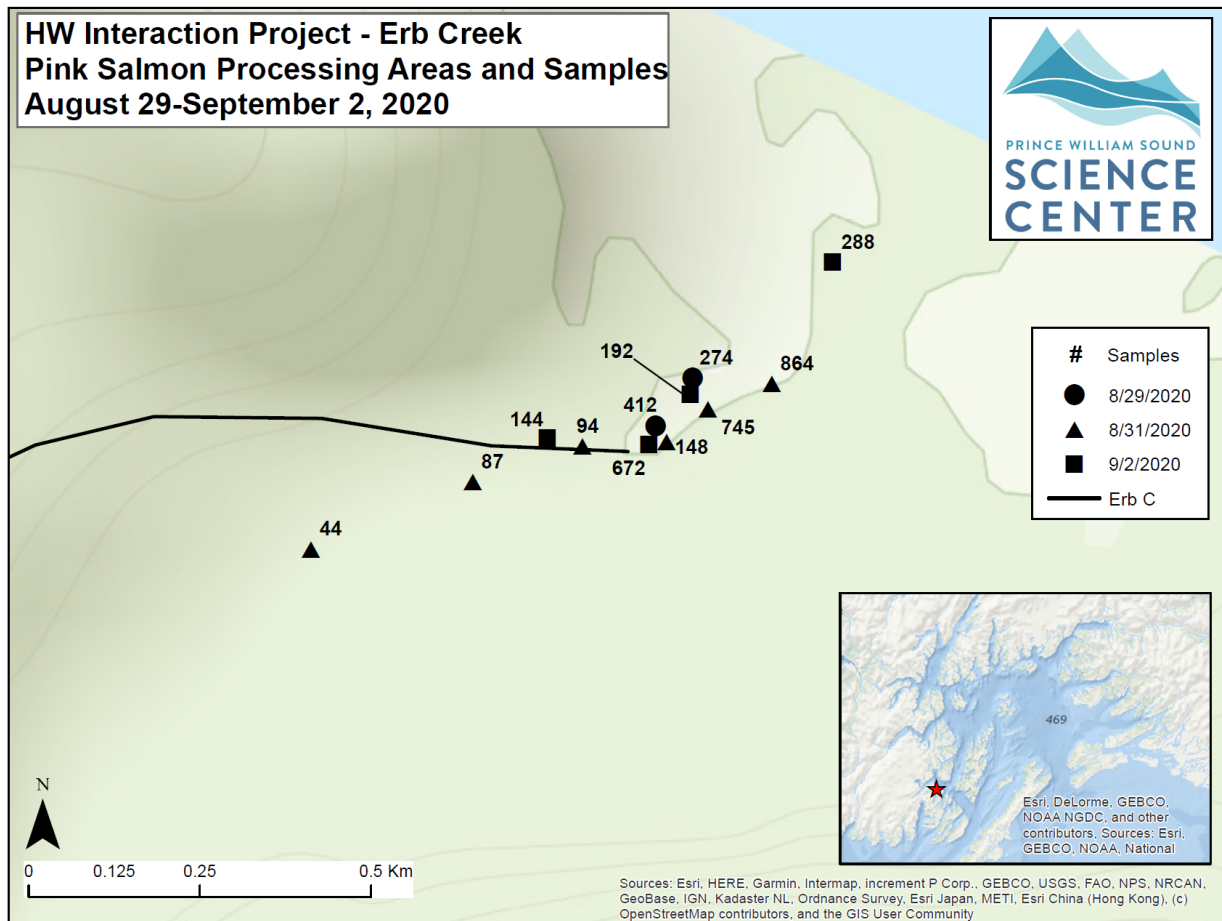
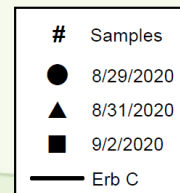


#	Samples
●	8/20/2020
▲	8/21/2020
■	8/22/2020
—	Erb C





HW Interaction Project - Erb Creek Pink Salmon Processing Areas and Samples August 29-September 2, 2020



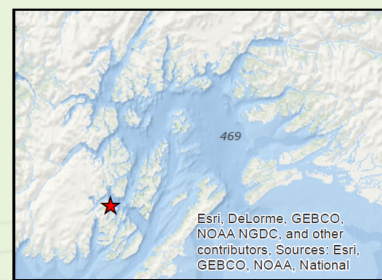
HW Interaction Project - Erb Creek Pink Salmon Processing Areas and Samples September 4-8, 2020



#	Samples
●	9/4/2020
▲	9/6/2020
■	9/8/2020
—	Erb C



0 0.125 0.25 0.5 Km



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

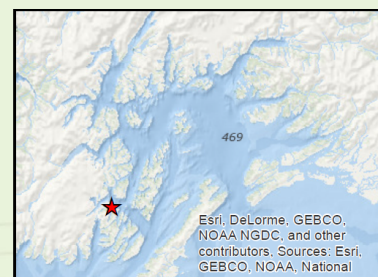
HW Interaction Project - Erb Creek Pink Salmon Processing Areas and Samples September 10-18, 2020



#	Samples
●	9/10/2020
▲	9/12/2020
■	9/15/2020
◆	9/18/2020
—	Erb C



0 0.125 0.25 0.5 Km



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