



**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

Volume 2

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APPENDIX A. OCEAN SAMPLING FIELD AND LAB PROTOCOL

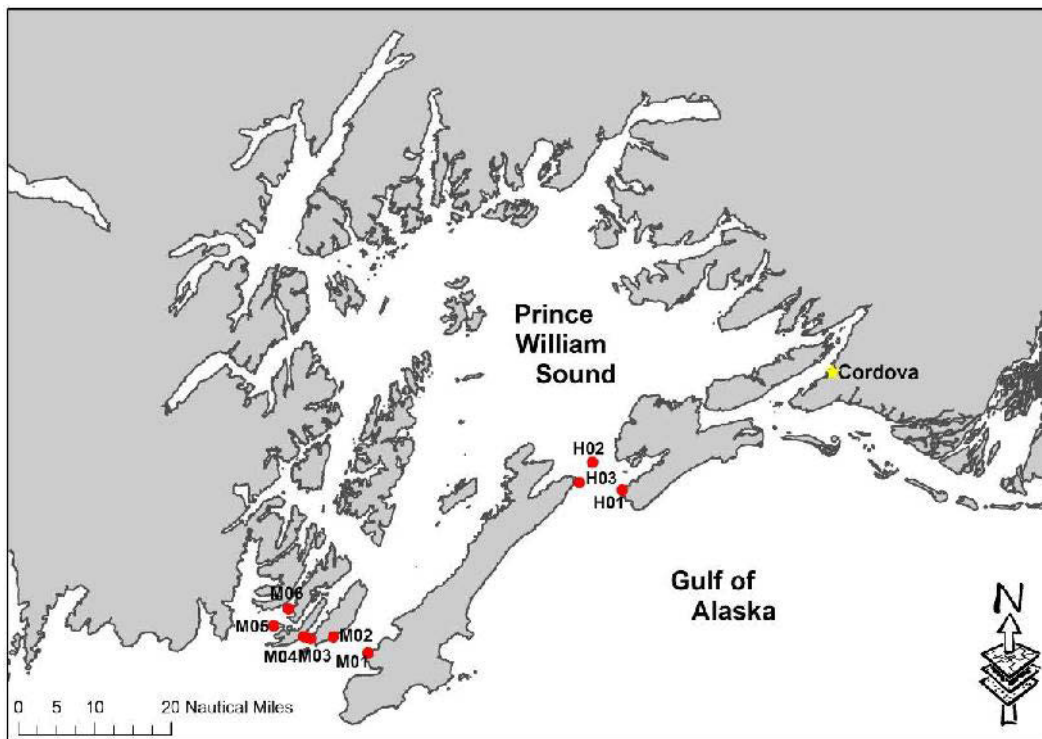
Always use the H-W data software to enter data. If the electronic data unit or software is malfunctioning, record all data manually for later entry.

Ocean Net Sets

Sampling Stations

The nine sampling stations are shown in Figure A1. There may be some latitude in station location, based on the relative catches at various stations, but only in consultation with the PI, Project Manager, and the Science Panel. The most important thing is to maintain as much consistency in station locations as is reasonable throughout the season.

Figure A1. PWS hatchery-wild interactions study ocean sampling station locations.



Sampling Schedule

Each set of stations will be fished twice a week, alternating between Hinchinbrook and Montague areas.

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Fish delivery to PWSSC	No fishing	Fish three Hinchinbrook stations	Fish six Montague stations	Fish delivery to PWSSC	Fish three Hinchinbrook stations	Fish six Montague stations

If the captain is unable to fish any of the stations at any of the scheduled times, the fishing days may be adjusted accordingly.

Any time a station is not sampled twice per week, a haul should be created in the database for each missed sample, with a comment explaining why the sampling was not completed, but no other information for that haul should be entered.

Net setting at each sampling station

At each sampling station location, set sufficient net, up to 160 fathoms, to capture and retain 20 pink and 20 chum salmon at each Hinchinbrook station and 10 pink and 10 chum salmon at each Montague station each sample day.

Four net panels of different mesh sizes (4-3/8, 4-3/4, 5-1/8, 5-1/2) will normally be shackled together in a prescribed order. If the same net configuration is fished for a week without using its full length in most sets, then it should be turned end-to-end at the beginning of the following week. There may be times when a panel is missing due to damage, etc. Regardless of the number or order of panels, the important thing is to record which panel went into the water first, second, etc. so that CPUE can be calculated later.

Use the HW laptop app to record:

- 1) the start and end of any periods of net setting,
- 2) the beginning and end of any drifts,
- 3) the start and end of any net retrievals, and
- 4) the end of retrieval.

Any pause in setting or retrieving greater than 2-3 minutes should be treated as a drift and the start and end times of the drift are recorded. The beginning of setting and the end of retrieval should be recorded using the software because the specific time and the geographic coordinates will be recorded automatically by the software. Drift start and end times can be recorded on paper and then entered to the software after the set is completed.

Ocean Netting Fog Protocol

Maintaining visibility of the gear is important for avoiding excessive catch and entangling whales; The fog protocol is as follows:

If foggy and winds are calm, arrive at site and shut down. Listen for whales, vessels. Use radar and AIS to detect approaching traffic.

If no traffic is detected, set 50 fathoms of gear or comfortable length so long as end buoy is visible. Shut down and listen except when gear tending is needed. Adjust net length according to visibility.

At-Sea Salmon Processing Protocol

If more than the required number of fish is captured, systematically select (i.e. not random) the sample from the catch (e.g., every other, every third, or every fourth, etc.,). Fish to be analyzed will be killed by concussion, bled, and stored on ice in slushy bags until delivery. Individuals will be marked by station using color coded floy tags, as follows (chum and pinks can be stored in the same bags):

H01-Black,
H02-Yellow,
H03-Pink,
M01-Dk. Green,
M02-Red,
M03-Baby Blue,
M04-Lime,
M05-Orange,
M06-Dk. Blue}

Disposition of Excess Catch

If salmon are captured in excess of the required samples, every effort will be made to bleed the fish and keep them iced and in marketable condition. Excess fish will be either sold or returned to PWSSC for donation to food programs. The captain has a permit card to sell fish; all proceeds from the sales go to ADFG.

Netting Steps and Data Entry

Use the "Ocean Haul" Tab to enter data

When to enter data	Software Entry	Info Recorded by software*	Comments
Arrive at station	Add New Haul	Station ID, Date	Select station and date from dialog box

	Net configuration	Panel mesh size in order of first, second, third. etc. panels out	Select a configuration from list, or use the "Settings" tab to create a new configuration, in order of deployment, regardless of whether they will all be deployed. Both "Code" and "Description" of the net configuration should be the numbers with no punctuation, e.g., 434500518 means three panels 4-3/4,5, and 5-1/8
Immediately before setting net	Set Begin	Time, Latitude, Longitude	If manual, use number pad to enter times and lat/lon degrees (to 3 decimal places)
	Cancel set begin		Use if false beginning net set – reset to begin net set
Start a drift	Start Drift	Time,	Record time. Repeat as necessary for any drift, or any setting or retrieving pause > 2-3 minutes.
	Fathoms	Net fathoms out	Length of net out
End a drift	End drift	Time	Repeat for end of each drift or pause
End of net retrieval	Retrieve End	Time, Latitude, Longitude	Enter as soon as the last of the net is pulled from water
After picking and sampling fish	Total pinks caught	Total pinks caught	Type number in
	Pinks retained	Pinks retained	Type number in
	Total chum caught	Total chum caught	Type number in
	Chum	Chum	Type number in

	retained	retained	
	Other species name	Other species name	Select from drop-down. If not on list, go to “Settings”
	Other species total catch	Other species total catch	Type number in
	Other species total retained	Other species total retained	Type number in
During net set or before leaving station	Sky cover	Sky cover	Select from drop-down
	Wind Direction	Wind Direction	Select from drop-down
	Wind speed	Wind speed	Select from drop-down
	Tide stage	Tide stage	Select from drop-down
	CTD	CTD	Check box if CTD was taken
	Haul Operator	Names of people on boat	Select from list, or use “Settings” tab to enter a new person. Repeat as necessary if more than one person on boat
	Comments	Comments	Any comments about the set

* Or by hand if software unavailable

After all the data is entered and double-checked for a given sampling trip, the last step is to conduct the QA process and “transmit” the data to the host database via the internet.


Ocean Salmon Processing Protocols

Otolith Tray Preparation

Try to use otolith tray labels in sequential order. Before processing fish fill out the otolith tray label (shown below) with the appropriate data and adhere it to the tray. **Use #2 pencil to fill these out.** The date is the date the sample was taken from the water. In “Port/Location”, enter the site name (Hinchinbrook or Montague). In

“Districts/Subdistricts” write the station numbers. Write “PWSSC H-W Ocean sampling” in Comments.

Alaska Department of Fish and Game - Comm Fish	
Species: _____	Tray <u>794</u>
Sample Date: Month _____ Day _____	Year <u>2005</u>
Port/Location: _____	
District/Subdistricts: _____	
Collectors: _____	
Comments: _____	
ADF&G MTA Lab, Juneau - (907) 465-2306	
10107 Bentwood Place, Juneau, AK 99801	



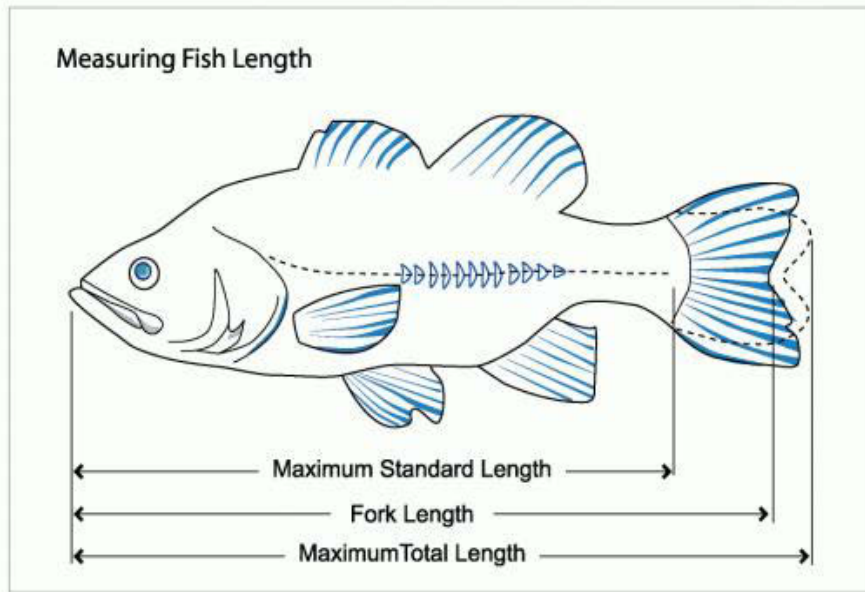
Processing Steps

Separate the fish by tag color in order to process one station at a time. **Always process batches by sampling date, species, and station.** This is because the otolith trays must be separate by date, species, and station, even if there is only or a few samples per tray.

Data collection order for each specimen:

1. Date of capture
2. Station ID
3. Species
4. Weight (g) -- Place a 5 gal bucket onto the Ohaus washdown bench scale and make sure the digital scale is zeroed. Then place the fish to be weighed inside bucket and take the measurement to the nearest gram.
5. Total Length (mm) -- Place the fish on the Ichthystick with the snout facing the black barrier at the zero mark. Make sure the cursor is in the cell for Total Length for the fish being measured. Lay the fish as flat as possible and place the magnetic stylus at the tip of the tail. The measurement has been taken if you see the blue light flash and/or the fish logo flash on the computer. If the sound is turned on the computer, a sound will emit as well and you can

proceed to the next measurement.



6. Standard Length (snout to hypural) -- Repeat above except make cursor is in the cell for Standard Length for the fish being measured and take the measurement at the end of the hypural bone. It may help visualize the hypural by folding the caudal fin towards the body.
7. Mid-eye-hypural length (mm) -- For this measurement, place the middle of the eye at the 10 cm mark and take the measurement at the end of the hypural bone, being sure the cursor is in the MEH cell. If the automatic Ichystick is unavailable, use a measuring tape from the mid-eye to the hypural bone, and enter the data into the software manually.
8. Mid-eye-Fork length (mm) -- Place the middle of the eye at the 15 cm mark and take the measurement at the closest point in the fork, being sure the cursor is in the MEF cell. If the automatic Ichystick is unavailable, use a measuring tape from the mid-eye to the closest point in the fork, and enter the data into the software manually.
9. Gonad weight (g) (if viable) -- If the fish appears in good condition and hasn't started to degrade, enlarge the slice made to examine the gonads and gently pull them out. Cut the ends to remove from the body, clean any obvious excess tissue from the gonads, and weigh on the digital balance to the nearest hundredth of a gram.
10. Sex -- Record male or female.
11. Otolith tray number (corresponds to white preprinted label)
12. Otolith cell number
13. Comments on fish or otoliths

Extracting otoliths

It is **extremely important** that the otolith tray and cell number correspond to the sex, length, etc. data so, if you cut heads in batches, you **MUST** keep the heads and bodies in the same order for which the sex and length data was taken until all the tray cells are filled and double-checked for that batch.

There are several methods for cutting the heads to access otoliths. This is one method:

- ◆ Grasp the fish by the eye sockets with the holding hand under the jaw. Scalp the top of the head off by cutting straight back (Figure A2). The best place to make the cut will be easy to determine after you've cut a few fish.



Figure A2. Example of where to make the first incision for collecting otoliths in the field.

- ◆ Cut far enough so that the scalp flap can be pulled back and out of the way to expose the brain cavity (Figure A3).



Figure A3. Slice far enough to expose the brain cavity – the depression in the center of the cut shown here.

Spread the head open and expose the brain cavity. If necessary remove the brain material to reveal the otolith wells (Figure A4).



Figure A4. Remove the brain tissue if necessary.

- ◆ With tweezers, pluck the left sagittal otolith from its sagittal well (Figure A5). The sagittal well is a depression in the most posterior-ventral portion of the brain cavity. (Pink salmon otoliths are about $\frac{1}{4}$ inch long, chum otoliths are

about 3/8-inch long.) Place the otolith onto the back of your hand and then recover the right otolith from the right sagittal well. It may take some probing, especially if the cut is not just right, or the carcass is old.



◆ **Figure A5. Removing otolith from well under brain cavity.**

- ◆ The otoliths are encased in a tissue bag surrounded by fluid. Gently rub or tweeze the tissue off the otoliths by rubbing them on a neoprene glove or cuff. Clean off any blood, and place both of them into the next well of the otolith tray.
- ◆ If an otolith is accidentally cut in half, place both halves in the well.
- ◆ If one of the two otoliths is lost during the process, place a colored bead into the well with the recovered otolith. If both are lost, place two beads in the cell for that fish. Always move to the next cell for the next fish.
- ◆ If an otolith or two are accidentally placed or fall into a cell that has previously been filled, do not guess which otolith is which. Instead, discard all those otoliths, clear the data for the suspect cells, and enter new otoliths and data for those cell (do not leave any cells empty).

Filling the Trays

Note that the first specimen for every tray should go into the highlighted cell, A1 (Figure A6). Cells in a tray should be filled left to right by row (like reading a book): A1-A12, B1-B12,.... H1-H12.

Repeat the dissection procedure until all the fish for that date, species, and station are processed. .Keep the heads and bodies in order until you have the batch of

otoliths in the tray. Before disposing of the heads and bodies, check the cells to be sure that every fish is represented in the tray and that tray cells match the fish.

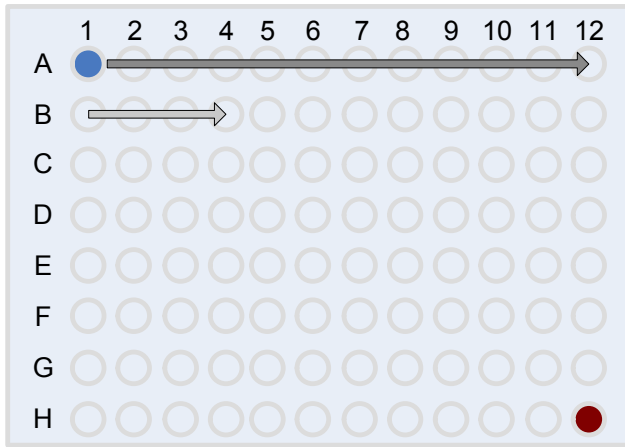


Figure A6. Fill otolith trays from left to right, top row first.

Finishing a Tray

Before ending a tray, check to be sure that every cell in the tray contains two otoliths, one otolith and one bead, or two beads. Check to be sure that the number of filled cells matches the number of fish entered into the data. If there are discrepancies that cannot be rectified by studying the number of fish processed, or by finding more than two items in one cell, make a note in the comments of every cell from the cell in question to the last cell in the tray.

Place two plastic inserts on top of the cells (or one if the inserts are thick enough) to avoid otoliths jumping from one cell to another during handling. Then cover the tray with a lid, and seal it tightly with three rubber bands. Be sure all trays are handled and stored right side up to avoid jostling the otoliths to adjacent cells,

Data Entry – Ocean Specimens

All data will be entered into the project software for uploading to the project database. There are two steps for entering data for a given sample date, species, and station.

1. Go to the “Ocean Haul” tab.

When to enter value	Software Entry	Info Recorded by software*	Comments
Before processing a sample for one date, species,	Ocean Haul	Station, Date	Highlight haul from pre-recorded list on left side of screen. If the haul is not displayed, click “Add New Haul” at bottom and select the transect (station) and date

station			that the sample was collected. Click “Save”. Highlight the Haul to be processed.
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* Or by hand if software unavailable

2. Do not change anything else on the “Ocean Haul” screen, but then switch to the “Ocean Specimens” tab.

When to enter value	Software Entry	Info Recorded by software*	Comments
Before starting to process fish	Ocean Haul		Be sure the correct Ocean haul station and date are displayed in the upper left. If not, go back to “Ocean Haul” tab and select or creat correct haul.
	Species	Species code	Select from drop-down
	Add specimens - count and species	Specimen ID and species code	Count and enter the number of fish to be processed.
	Add specimens – otolith tray number	Otolith tray number	Enter the full tray label number beginning with 2014 (eg 20148000)
	Samplers	Samplers name	Select from list, or use “Settings” tab to enter a new person. Repeat as necessary if more than one person is working on process
Processing fish-by-fish	Sex	Sex	Select from drop-down
	Weight	Weight	Nearest gram
	Total length	Total length	Nearest mm
	Standard length	Standard length	Nearest mm
	Mid-eye – hypural length	Mid-eye – hypural length	Nearest mm
	Mid-eye – fork length	Mid-eye – fork length	Nearest mm

	Otolith tray number	Otolith tray number	Entered in software
	Otolith cell number	Otolith cell number	Should be auto-filled by software. If not, enter cell number: A1 through H12. (Every fish has a cell number, even if no otoliths are recovered).
	Gonad weight	Gonad weight	To the nearest hundredth of a gram
	Fish comments	Fish comments	Any comments about individual fish, their measurements, or otolith peculiarities

* Or by hand if software unavailable

Sample Fish Disposition

Each week, depending on the run strength, up to 240 chum salmon and 240 pink salmon will be delivered to the portable fish processing lab on the PWSSC dock in Cordova. The fish will be kept on ice to maintain their freshness for sampling. After sampling, the fish will be sold to local buyers if they are marketable or given to local food banks. If there is no buyer or outlet for the sample fish, they will be taken back out to sea for disposal.

APPENDIX B. ESTIMATING VARIANCES OF THE PROPORTION OF HATCHERY CHUM SALMON AND PINK SALMON ENTERING PRINCE WILLIAM SOUND

Variance of CPUE

Catch in a standardized unit of fishing effort is considered to be distributed according to a Poisson probability distribution with mean μ and variance μ . If C_j is the catch in a standardized drift j during a trip to a specific station, the expected value of C_j is μ , which makes

$$\hat{z} = \frac{\sum_{j=1}^n C_j}{n}.$$

where n is the number of drifts during that trip. In our study, the variable $CPUE$ for a specific trip to a specific station is not based on specific drifts, but on summing catches of the target species over several drifts at each trip to each station such that

$$CPUE = \frac{\sum_{j=1}^n C_j}{E} = \frac{n\hat{z}}{E}$$

where E is the sum of the fishing effort in fathom-hours across drifts. Remembering that the variance of a Poisson variate is equal to its mean, the variance for $CPUE$ for a specific trip to a specific station is

$$v(CPUE) = \frac{n^2 \hat{z}}{E^2} = \frac{n}{E} \left(\frac{n\hat{z}}{E} \right) = \frac{n}{E} CPUE. \quad (A1)$$

The mean of $CPUE$ across trips for a station in our study is the arithmetic mean

$$\overline{CPUE}_s = \frac{\sum_{t=1}^{T_s} CPUE_{st}}{T_s}.$$

Equation 7 is the above formulation only adapted for the occurrence of missing data. Because estimates of $CPUE$ were independent across trips, the variance of mean $CPUE$ is the sum of the trip variances multiplied by the square of the reciprocal of the number of trips:

$$v(\overline{CPUE})_s = \frac{\sum_{t=1}^{T_s} v(CPUE_{st})}{T_s^2}.$$

Equation 15 is the above formulation only adapted for the occurrence of missing data.

Variance of \hat{W}_{st}

By the delta method the variance of a non-linear function of variables $g[\mathbf{X}]$ where \mathbf{X} is the vector $[x_1, x_2, \dots, x_n]$ can be approximated with the non-quadratic terms in a Taylor series expansion of $g[\mathbf{X}]$ as follows:

$$v(g[\mathbf{X}]) = \sum_i v(x_i) \left(\frac{\partial g}{\partial x_i} \right)^2 + 2 \sum_{i < j} \text{Cov}(x_i, x_j) \left(\frac{\partial g}{\partial x_i} \right) \left(\frac{\partial g}{\partial x_j} \right).$$

Here the non-linear function is \hat{W}_{st} and the x 's are the $CPUE_{st}$ as given in Equation 3. The derivatives under these circumstances are:

$$\frac{\partial \hat{W}_{st}}{\partial CPUE_{st}} = \frac{\sum_{t', t' \neq t} CPUE_{st'}}{\left(\sum_{t'} CPUE_{st'} \right)^2} \quad \frac{\partial \hat{W}_{st}}{\partial CPUE_{st'}} = - \frac{CPUE_{st}}{\left(\sum_{t'} CPUE_{st'} \right)^2}.$$

Note that summation over subscript t indicates a sum over all trips including trip t ; the summation with configuration $t \neq t$ indicates a sum over all trips excluding trip t . With substitution of the derivatives and some rearranging of summations, the result is Equation 11:

$$v(\hat{W}_{st}) \cong v(CPUE_{st}) \left(\frac{\sum_{t', t' \neq t} CPUE_{st'}}{\left(\sum_{t'} CPUE_{st'} \right)^2} \right)^2 + \left(- \frac{CPUE_{st}}{\left(\sum_{t'} CPUE_{st'} \right)^2} \right)^2 \sum_{t', t' \neq t} v(CPUE_{st'}).$$

Because catch per unit of effort was measured independently across trips within a station, there are no covariances involved in the above formulation.

Variance for PWS

Equation 13 can be formulated as follows to simplify derivation of an approximate variance:

$$v(\hat{p}) = v \left(\sum_{s=H01}^{H01 \dots M06} \frac{\overline{CPUE}_s}{\sum_{s'} CPUE_{s'}} \hat{p}_s \right)$$

By the delta method the variance of a function of variables $g[\mathbf{X}]$ where \mathbf{X} is the vector $[x_1, x_2, \dots, x_n]$ can be approximated with the non-quadratic terms in a Taylor series expansion of $g[\mathbf{X}]$ as follows:

$$v(g[\mathbf{X}]) \cong \sum_i v(x_i) \left(\frac{\partial g}{\partial x_i} \right)^2 + 2 \sum_{i < j} \sum_{j < i} \text{Cov}(x_i, x_j) \left(\frac{\partial g}{\partial x_i} \right) \left(\frac{\partial g}{\partial x_j} \right)$$

The x 's here are the set of 18 variables $\{\overline{CPUE}_{H01}, \dots, \overline{CPUE}_{M06}, \hat{\rho}_{H01}, \dots, \hat{\rho}_{M06}\}$. Because each variable (estimate) in this set came from different samplings, the variables are independent with $\text{Cov}(x_i, x_j) = 0$. The derivatives under these circumstances are:

$$\frac{\partial g}{\partial \overline{CPUE}_s} = \frac{(\sum_{s'} \overline{CPUE}_{s'} - \overline{CPUE}_s) \hat{\rho}_s}{(\sum_{s'} \overline{CPUE}_{s'})^2} - \frac{\sum_{s', s' \neq s} \overline{CPUE}_{s'} \hat{\rho}_{s'}}{(\sum_{s'} \overline{CPUE}_{s'})^2} = \frac{\hat{\rho}_s - \hat{\rho}}{\sum_{s'} \overline{CPUE}_{s'}}$$

$$\frac{\partial g}{\partial \hat{\rho}_s} = \frac{\overline{CPUE}_s}{\sum_{s'} \overline{CPUE}_{s'}} = \hat{W}_s$$

Note that a summation over subscript $s \not\equiv$ indicates a sum over all stations including station s ; the summation with configuration $s \not\equiv s \not\equiv s$ used below indicates a sum over all stations excluding station s . With substitution of the derivatives and some rearranging of summations, the result is Equation 14:

$$v(\hat{\rho}) \cong \sum_{s=H01}^{H01 \dots M06} [\hat{W}_s^2 v(\hat{\rho}_s) + \left(\frac{\hat{\rho}_s - \hat{\rho}}{\sum_{s'} \overline{CPUE}_{s'}} \right)^2 v(\overline{CPUE}_s)].$$

APPENDIX C. 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 Southeast (SEAK) and Prince William Sound (PWS) in 2015. There are two major purposes of the study: 1) to collect salmon otolith samples, and data on sex, fish length, and fish height, as a basis for estimating the fraction of hatchery-origin salmon in 64 streams (32 each in SEAK and PWS) and 2) to additionally collect DNA tissue samples on a subset six “fitness” streams in PWS for estimating the relative survival of hatchery- and wild-origin Pink Salmon. Selected PWS streams will also be sampled for other purposes: additional DNA samples for the ADF&G genetic stock structure study and a suite of tissues for stable isotope analyses for a preliminary analysis of foraging ecology. Tables B1 and B2 designate the streams and species to be sampled and the types of sampling at each stream in 2015. Details about collecting measurements and biological samples from each specimen are described in the next protocol (Appendix C). This protocol is about collecting fish from streams.

Targeted Number of Fish to Sample

Otolith-only Streams

For each SEAK otolith-only stream, a minimum of two visits per season are required, but more will be conducted if needed and if time allows. In PWS, at least three visits per otolith-only stream per species are required, but more are preferable. The targeted number of individual samples for analysis in each otolith-only stream (both SEAK and PWS) is 384 over all the seasonal visits. 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 over the multiple visits to each stream and throughout the stream length as much as possible. More stream visits are preferable to less. The crew leader can elect to spend more than one day per stream visit if that will help to complete sampling goals.**

When collecting samples on otolith-only streams, only carcasses or post-spawners are eligible for processing. Live, pre-spawned fish must be released to continue spawning.

Although the overall target number of samples in otolith-only streams is set at 384 spread over all the visits, the crew should always attempt to collect as many samples as possible in the first and second visits to compensate for the possibility of a lack of fish on subsequent visits. If there are enough fish available on the first visit, try to sample 300 fish. On the second visit, if between 192 and 300 were collected on the first visit, collect approximately 192 on the second visit. If less than 192 were collected on the first visit, collect 300 on the second visit if possible. During the third and subsequent visits, sample 200 or all spawned-out salmon present, whichever is the smaller number.

Table B1. Streams to be sampled in Southeast Alaska in 2015. No fitness sampling will be conducted in Southeast Alaska in 2015.

Stream name	Stream code	Sampling events per season
Carroll Creek	101-45-10780	2-4
Chaik Bay Creek	112-80-10280	2-4
Chuck River	110-32-10090	2-4
East of Snug Cove*	110-23-10210	2-4
Ford Arm Creek	113-73-10030	2-4
Freshwater Creek	112-50-10300	2-4
Game Creek	114-31-10130	2-4
Glen Creek	110-34-10060	2-4
Greens Creek	112-65-10240	2-4
Harding River	107-40-10490	2-4
Hidden Inlet*	101-11-11010	2-4
Johnston Creek	110-23-10100	2-4
Kadashan River	112-42-10250	2-4
King Creek	101-71-10040-2006	2-4
King Salmon River	111-17-10100	2-4
Little Goose Creek	112-48-10190	2-4
Marten River	101-30-10600	2-4
North Arm Creek	108-40-10150-2007	2-4
Petrof Bay W Head*	109-62-10240	2-4
Ralphs Creek	112-21-10060	2-4
Rodman Creek	113-54-10070	2-4
Saginaw Bay S Head*	109-44-10370	2-4
Seal Bay Head*	112-46-10070	2-4
Sister Lake SE Head*	113-72-10040-2025	2-4
Swan Cove Creek	111-16-10450	2-4
Ushk Bay W End*	113-56-10030	2-4
W Crawfish NE Arm Hd*	113-32-10050	2-4
Whitewater Creek	112-90-10140	2-4
Admiralty Creek	111-41-10050	2-4
Prospect Creek	111-33-10100	2-4
Fish Creek	111-50-10690	2-4
Sawmill Creek	115-20-10520	2-4

* These streams are not officially named

Table B2. Streams and species to be sampled in PWS in 2015, indicating the fitness streams, the number of sampling events per season, and the targeted number of samples for the associated stock structure study (see also the “Tote Board” on Google Drive).

Stream name	Stream code	Target species	Fitness stream	Sampling events per season	DNA for stock structure*
Hartney C	221-10-10020	pink, chum		3 to 5	240
Sheep R	221-20-10360	Pink		3 to 5	
Beartrap R	221-30-10480	pink, chum		3 to 5	
Fish C	221-40-10890	Pink		3 to 5	240
Lagoon C	221-40-10990	Pink		3 to 5	240
Short C	221-40-10880	Pink		3 to 5	
Sunny R	221-40-10875	pink, chum		3 to 5	
Long C	222-10-12140	pink, chum		3 to 5	260
Spring C	221-20-10200	DNA pink	Yes	Daily	
Spring C	222-10-12170	pink, chum		3 to 5	
Vanishing C	222-10-12157	Chum		3 to 5	
Siwash R	222-20-12640	pink, chum		3 to 5	200
Surplus C	222-20-12338	Pink		3 to 5	
Wells R	222-20-12340	Chum		3 to 5	
Hummer C	224-10-14250	Pink		3 to 5	
Mill C	224-10-14210	Chum		3 to 5	
Coghill R	223-30-13220	pink, chum		3 to 5	240
Blackstone C	224-10-14510	Chum		3 to 5	
Tebenkof C	224-10-14500	Chum		3 to 5	
Paulson C	224-10-14550	pink, chum		3 to 5	240
W. Finger C	224-40-14850	pink, chum		3 to 5	
Comstock C	225-20-15040	Pink		3 to 5	
Bainbridge C	226-20-16300	Pink		3 to 5	
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	
Johnson C	226-40-16269	Pink		3 to 5	
Cabin C	227-20-17464	pink, chum		3 to 5	260
Gilmour C	227-20-17480	DNA pink	Yes	Every other day	
Stockdale C	227-20-17520	DNA pink	Yes	Every other day	
Swamp C	227-20-17390	pink, chum		3 to 5	
Double C	228-40-18310	pink, chum		3 to 5	
Constantine C	228-60-18150	pink, chum		3 to 5	240
Humpback C	221-10-10110	pink		2**	240
Windy C	228-30-18610	pink		2**	240
Snug Harbor C	226-30-16820	pink		3**	371

* Approximately half of the samples taken early in the run, the other half taken late in the run

** Stock structure sampling only

In many cases the total number of carcasses and post-spawners in a stream will be small enough that the crew can process them all during that visit. If the resulting number of samples collected that day is very small, it may be necessary to amend the crew schedule and prioritize re-visiting these streams in which the fewest samples have been collected. On other occasions, especially during the peak stages of the run, there may be more carcasses and post spawners in the stream than the crew can process on that visit. On these occasions, the crew will need to collect as many samples as possible, while keeping sampling effort spread out across the entire stream reach.

Fitness Streams

The analytical goal for otolith and DNA tissue samples from each fitness stream is the maximum number of samples possible. Three of the fitness study streams will be sampled every day (Spring, Paddy, Erb). The three other streams may be sampled every other day (Table B2) 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.

PWS Pink Salmon Stock Structure Sampling

Twelve streams will be sampled for Pink Salmon genetic tissues in PWS in addition to the fitness streams. The sampling targets are shown in Table B2 except Snug Harbor Creek (below). The methods for the Stock Structure sampling are the same as for the fitness sampling, as described below. In the Stock Structure streams (except Snug), try to take the first half of the goal early in the run, and the second half of the goal in the later part of the run. All stock structure samples should be taken upstream of the intertidal zone. All stock structure samples should be from either live fish or fish with pink gills – no deteriorated fish please. This can include pre-spawn fish. Because the stock structure samples will include otoliths, they will be counted as part of the otolith-only sampling targets. At Snug Harbor Creek, the 1st collection of 96 samples is targeted for the last week of July (early run). The 2nd collection of 125 fish will happen in the middle of August (2~2.5 weeks after the 1st collection). The 3rd collection of 150 fish (late run) will happen in the end of August or early September.

PWS Pink Salmon Foraging and Breeding Performance Sampling

Ten streams will be sampled for additional attributes to investigate the relative biological and ecological performance of fish from different streams and hatcheries. A separate protocol will guide this sampling.

Priorities for Stream Visits

In pedigree/fitness streams, continue sampling your streams as often and as much as possible, or at least according to the schedule shown in Table B2. The more samples the better for the fitness analysis.

For otolith-only streams, the overall goal is to sample each stream over the run timing and over the entire accessible extent of each stream throughout the season. The priorities for sampling depend on a mix of the status of previous sampling, fish availability (run timing), and your total stream assignments (schedule). This information will be in flux as the season progresses and as we learn from additional ADF&G aerial survey information. The tote board is your basic guide and tracking system.

The relative priorities as established by the Science Panel are:

- Try to meet the overall sampling goals on all streams
- If necessary, prioritize getting a good sampling on all streams over getting maximum numbers on fewer streams.
- Upon reaching the goal in some streams, increase effort on the less sampled streams.
- Try to get a thorough sampling of the entire accessible reach at least once per season (if not during every visit).

Tote Board

Individual sampling goals for each visit to a stream, and a method for tracking progress toward the goals, is established in separate spreadsheets for PWS and SEAK. These are each located in their respective folders of the project 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 their 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 in 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 method. 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*

Study maps and other info for streams to be visited. The crew leader will consult tote board or, if in doubt, call the project coordinator to ascertain species to be collected, number of each, sample types, and gear needed to accomplish task. Crew leader will consult tide graph and based on that (and the target species), 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 otolith and/or DNA 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 other protocols).

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 will also be made for each survey. 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 need 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 pink and chum 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 and/or fitness stream mapping 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, by logging beginning and ending survey points for the tributary or separate stream (see the special case below).

The survey Begin Location and End Location to be entered into the tablet are meant to describe the linear extent of your daily survey on each stream. The Begin Location or End Location do not need to be at the most downstream or upstream point in your survey, as long as, between the two, they described the entire survey extent.

In PWS, both pink and chum otoliths will be collected in some streams. 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 tablet for both species, being very careful that data are entered under the correct species, or you may use two separate 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.)**

If it hasn't already been established, try to ascertain the upper access extent of pink and chum salmon. Pink salmon and chum salmon usually do not jump more than about 3-4 foot vertical falls or ascend long, steep cascades, but watch for narrow slots that are accessible. If necessary, try to assess this during your first visit so that you and other teams will know how far to go in future surveys. Only mark the upper access extent in the tablet if you actually observe an obvious barrier (do not try to guess where it is). The following streams' extents are already documented, so no need to re-do:

Southeast		PWS	
107-40-10490	Harding River	226-40-16269	Johnson C
109-62-10240	Petrof Bay W Head	227-20-17480	Gilmour C
110-23-10210	East of Snug Cove	226-20-16300	Bainbridge C
110-32-10090	Chuck River	226-30-16810	Hogan Bay
111-16-10450	Swan Cove Creek	227-20-17464	Cabin C
111-50-10690	Fish Creek	227-20-17480	Gilmour C
115-20-10520	Sawmill Creek	227-20-17520	Stockdale C
		226-20-16040	Erb C
		226-20-16010	Paddy C
		221-10-10020	Hartney C
		225-20-15040	Comstock C
		221-20-10200	Spring C
		224-10-14550	Paulson C
		221-20-10360	Sheep R
		224-10-14510	BlackStone C
		221-40-10890	Fish C
		221-40-10880	Short C
		221-40-10990	Lagoon C
		224-10-14210	Mill C
		222-20-12640	Siwash R
		222-10-12157	Vanishing C
		223-30-13220	Coghill R

Some streams are too large to effectively survey on foot and may need to be surveyed by drifting in a raft or by running a jetboat upstream. In those cases, be observant for accumulation of spawn-outs. In turbid waters, watch for clearer side sloughs and small tributaries as good locations to sample.

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 stream. 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 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 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 Southeast, count only chum and Chinook 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 dead of each species. Allow the people counting to do so before the fish are disturbed by collecting fish for sampling. In many, but not all, lighting conditions, it will help to wear polaroid glasses. Include all dead fish from previous sampling in your dead count if their carcasses are still visible on the survey.

Use hand tally-whackers, 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 data. 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, you may skip counting live/dead salmon for two days, but do a complete count of the entire stream every third day. If you are visiting a fitness stream every-other-day, you may skip live/dead counts every other visit (i.e., do a complete count every fourth day). This is because getting more 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 C.

Fish Collection

Fish to be sampled can be collected by any practical means. **Only collect spawned out, dying, or dead adults** (except on PWS fitness and stock structure streams, as noted below). Use gaffs, spears, snagging gear (rod and reel with treble hooks), dip nets, 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.

Unlike in previous years' sampling in fitness streams, if a fish's gonads have been removed by predators, sample it. (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.

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 the events of the day are still fresh. This is important to complete the following list immediately to ensure that the data are of highest quality and any errors that could've occurred during the day are addressed immediately.

√ Enter data if necessary

Enter any data that was necessary to have been 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 to the laptop from the tablet.

2) Using Windows Explore 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 all 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 **otolith** or **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 reviewing otolith trays for the QA process, leave them in a very secure and stable place with the lids off so the samples will dry for at least 24 hours. Then replace the lids securely with rubber bands and pack them away in a safe storage location.

After inspecting DNA trays, fill every cell with alcohol 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 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 separate containers from otolith trays 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 no internet, call, text, or in-reach your project coordinator to report daily survey numbers. Then use the tote board to help plan the next day’s activities and sampling goals.

APPENDIX D. 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, measuring, and for collecting, processing, and shipping otolith and DNA tissue samples from streams in the Hatchery-Wild Interactions Study. See the Appendix B for protocols on which streams and how to collect fish.

Background

Otoliths removed from specimens will be used to differentiate “stray” hatchery-reared salmon from salmon that originated from natural spawning in all our study streams. DNA tissues will be collected in a sub-set of “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 (All Streams)

Thermal marking is one of the methods being utilized to identify and manage hatchery-released Pink Salmon and chum 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 to ADF&G’s Thermal Mark Laboratory (SEAK crews) or to the Cordova ADFG lab (PWS crews) 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 (Fitness Streams)

The ADFG Gene Conservation Lab (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 otolith-only streams, you will use the 96-cell otolith trays. In “fitness” streams, use 48 deep well plates (dwp) or trays. When using

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


Preparing Otolith Trays

Please adhere the adhesive tray labels to the sides and bottom of the 96-cell otolith sampling tray (Figures C1 and C2) before it is filled with otoliths. **This step is critically important to maintaining the integrity of each sample!** Keep the tray numbers in sequential order throughout a given week and sampling season. Because the sample number on each tray label is unique, you cannot use a label to identify more than one tray of 96 otoliths. **Do not place sample labels on the otolith tray lid because the lids can get separated from the sample tray!**

Before processing fish, fill out one otolith tray label (Figure C1) with the appropriate data. Use number 2 pencil to fill these out. The date is the date the sample was taken from the stream. In “Port/Location”, enter the stream name. In “Districts/Subdistricts” write the stream number (see Table B1 or B2 or your tablet app). Put all the team members’ names in the Collectors space. Write “PWSSC H-W Stream Sampling” in Comments. To avoid spilling otoliths from a tray, the labels must be affixed to the bottom of a tray and completely filled out **before** filling them with otoliths. Take extra trays into the field but only fill out the data just before beginning a new tray.

Alaska Department of Fish and Game - Comm Fish
Species: _____ Tray 8016
Sample Date: Month _____ Day _____ Year 2015
Port/Location: _____
District/Subdistricts: _____
Collectors: _____
Comments: _____

ADF&G MTA Lab, Juneau - (907) 465-2306
10107 Bentwood Place, Juneau, AK 99801



← TRAY NUMBER:
provides a unique
sample number for

Figure C1. Example of otolith tray label – note that the tray is pre-numbered.



Figure C2 Vertical barcode to be cut away and affixed to side of otolith tray (opposite of notched side). Remainder of label, including horizontal barcode is to be affixed to bottom of tray.

Labels should be attached on the 96-well trays as shown in Figure C3. Use clear packing tape to cover and protect the bar-code labels only. It will be critical in the field to keep the labels as dry as possible. When it rains a lot the written information on the label can get smudged and become illegible. So, be sure to double check the written information on all labels once a tray has been completed.



Figure C3. A plastic 96-cell otolith tray filled with label n the bottom and side.

Preparing DNA/Otolith 48dwp Trays

Trays for DNA sampling in fitness streams (Figure C4) are different than otolith-only trays. They 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.



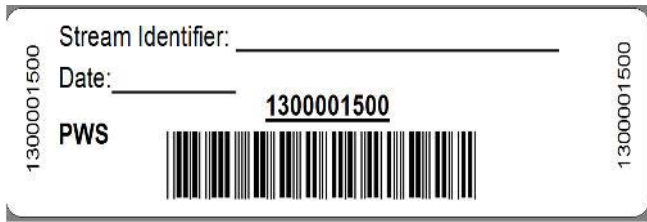
Figure C4. An empty 48dwp tray for DNA tissue and otoliths (cover off).

Pre-printed barcode labels for each 48dwp have been provided by GCL. Keep label sheets packed in 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). 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 for some reason leaks out. Further, during very

rainy conditions, having tape on the label will help preserve the label information. 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 Only or Otoliths/DNA

Pack the following items for field sampling otoliths (and DNA in fitness streams):

OTOLITH DISSECTION TOOLS

- | | |
|--|----------------------------|
| 1) Butcher knives with deep 6-8" blade | 7) Pencils – No.2 soft |
| 2) Forceps (fine point) | 8) Cutting boards |
| 3) Cotton gloves | 9) Knee pads |
| 4) 96-cell otolith trays with compression plates & lids, labels attached | 10) Brightly colored beads |
| 5) Carrying case for trays – spill prevention | 11) Neoprene cuffs |
| 6) Calipers and Measuring tape | 12) Tablets |

ADDITIONAL DNA DISSECTION TOOLS – FITNESS STREAMS

- | | |
|-------------------------------------|--|
| 1) 48dwp trays with labels attached | 5) DNA tray carrying case for trays – spill prevention |
| 2) Tray cover mats | 6) Tray holders |
| 3) EtOH in large nalgene bottle | 7) Plastic wrap for sealing full trays |
| 4) Nalgene squirt bottles for EtOH | |

Processing Specimens

Processing Areas

Collect spawn-outs or carcasses from the study stream (as described in Appendix B) into an area where you can work on cutting the heads and extracting the otoliths. 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 no more than approximately 200 meters 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 groups of 12 per row for processing otoliths only (by separate species, if working on a stream where both pinks and chum are being sampled) or in groups of 8 if working on otoliths and DNA. 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 96-cell or 48dwp trays.

At each Processing Area, **work through collecting specimen information in the following order: sex, length, height, DNA tissues (fitness only), otoliths, and morbidity condition (fitness only).** 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 one. **After you have collected the required samples from a fish, leave it in place within its row.** 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 recording 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. For otolith tray numbers, be sure to add “2015” before the 4-digit tray number. (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, and length before dissecting for otoliths and DNA.

Male chum are usually identifiable by their kype (a secondary characteristic of extension of jaw and enlargement of teeth). Male Pink Salmon also have a kype, but are more noticeable by the hump in their dorsal outline. Sometimes the sex is not obvious so, if in doubt, squeeze the area just anterior of the vent to see evidence of milt or eggs.

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, grab the caudal fin and flex it 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, 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).

Please also measure and record body height unless there is damage or advanced deterioration that would compromise the measurement. Measure 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.

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
- 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)
- At anytime, you can return to the SPECIMEN LIST (upper right) 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 C5). If sampling for DNA tissue, this will expose the heart with the bulbus arteriosus attached (Figure C6). See description below for further instructions on sampling DNA tissue.



Figure C5. Cut head off.

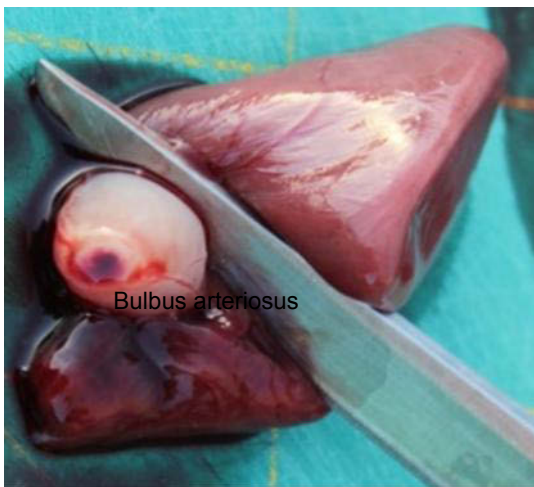


Figure C6. Bulbus arteriosus attached to heart.

Place the cut head on the cutting board and slice the top off (Figure C7). This should reveal the brain cavity. Remove the brain tissue (Figure C8). The otoliths will be in two wells just posterior of the brain cavity (Figure C9).



Figure C7. Slice the top of head off.



Figure C8. Remove brain tissue.

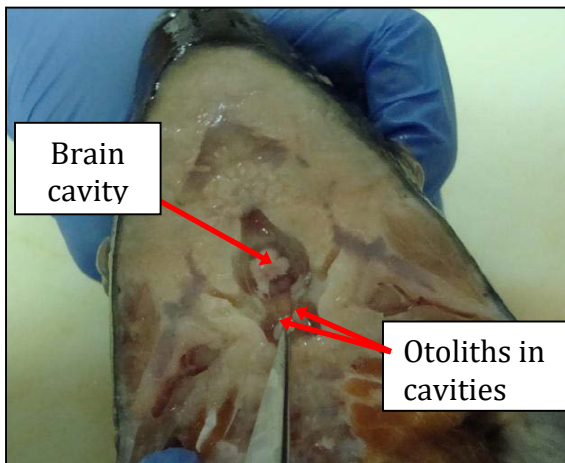


Figure C9. Otoliths in two later wells posterior to brain cavity.

Using 96-cell Plates for Otoliths Only

Use one 96-cell tray per stream, date, and species (or more if necessary). **Never place otoliths from more than one stream or sampling visit into the same tray!** Mixing otoliths from various streams or visits results in massive confusion and an irrevocable loss of data. Even if you have a tray containing only ONE otolith for a stream visit, send it that way.

- ◆ 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 C9). The otoliths are about ¼ inch long in Pink Salmon and 1/3-inch long in chum 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, clean off any blood and place both of them into the next well of the otolith tray (Figure C10).



Figure C10. Cleaning an otolith on a neoprene glove or sleeve.

- ◆ Place the pair of otoliths from one fish into the tray wells following the left-to-right, top-to-bottom scheme shown in Figure C11 so that when you are done, the tray looks like Figure C12.
- ◆ 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 (Figure C12).
- ◆ After each row is complete, check all the cells in that row to be sure none were skipped and that every cell has two otoliths (or beads). If you find discrepancies, go

back to the last known accurate sample, and start over with new fish. Discard any otoliths that may be confused. Do not try to guess which otoliths in the confused cells go with which fish.

- ◆ Be sure to close the otolith tray properly each time you finish adding samples. Use the plastic inserts to cover the otolith wells and place the lid on top to ensure that otoliths will not shift cells when transporting. Secure the lid to the tray with at least two tight rubber bands crosswise to each other (Figure C13). Any time you are opening an otolith tray that contains samples, slide the plastic sheet across the surface of the tray so that any otoliths that got stuck to the sheet will fall back into their respective wells.

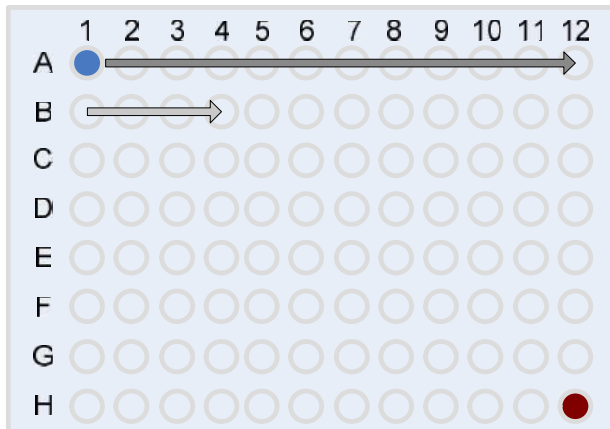


Figure C11. How to fill a 96-cell otolith-only tray.



Figure C12. An otolith tray filled to cell G8. Note yellow beads to represent missing otoliths.

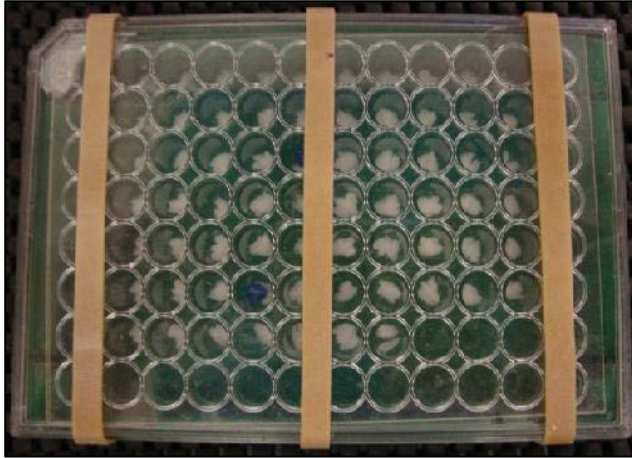


Figure C13. Proper arrangement of otolith tray for storage and movement. One thick sheet of thick plastic lie over the top of the otolith wells with the plastic tray lid on top. Secure tightly with three rubber bands as illustrated.

Data Entry

The tray and cell numbers have already been entered into the HW tablet app:

- Be extremely careful that the otoliths are deposited in the cell that corresponds to the correct fish
- For any fish missing one or two otoliths, check the missing otolith boxes on the specimen card for that specific fish (and add beads to the cell).
- Add any comments in the comment space pertinent for the fish being sampled

Using 48dwp Trays for DNA Tissue and Otoliths

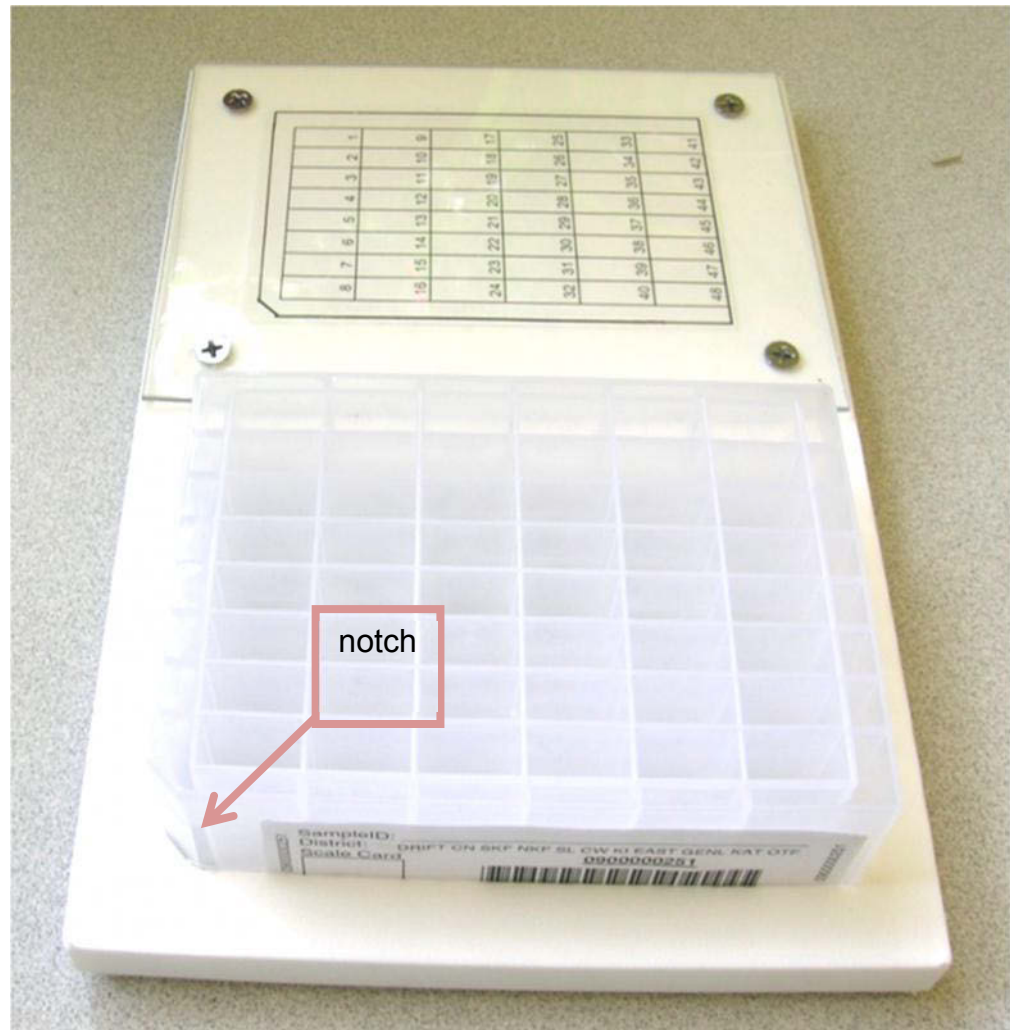
If you are working on a pedigree-fitness stream, or if you are collecting DNA tissue in PWS for the stock structure study, use a 48dwp tray for both the DNA tissue and the otoliths (Figure C4).

It is important to try to collect the best **quality DNA tissue samples to give quality 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. Sample any previously unsampled 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 after sampling. Add EtOH from the squirt bottle after 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.

As with otolith trays, use one (or more) 48dwp trays per stream, date, and species. **Never place otoliths and DNA tissue from more than one stream or sampling visit into the same 48dwp!** Even if you have a 48dwp containing only ONE otolith/DNA tissue sample for a stream visit, send it 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 C14).



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 sampling pattern.

Figure C14. proper set-up of 48dwp in sampling tray holder.

- b. 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 C6).

c. **FROM SAME FISH**, follow the steps above for otolith excision and deposit the otoliths into the well that corresponds to the fish number. Put **two** otoliths in **same** well as bulbus arteriosus.

d. Deposit otoliths and DNA tissue 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) rows 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

e. If one or both otoliths are lost or missing, place one or two beads into the well, respectively. If the bulbus arteriosus is lost, 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.

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

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

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.

- h. After tissue samples are taken, make sure **all tissues are covered with EtOH** before sealing plates with rubber mats. 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.
- i. 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.
- j. The squirt bottle should be emptied after **each sampling session; it will leak** if not emptied.

Data Entry

Tray and cell numbers have already been entered for 48dwp otolith and DNA tissues. However, please enter:

- In the Card View, Select the correct morbidity state for each fish sampled
- Add comments about missing otoliths and/or DNA issues in the Card View Comments

Cleaning, Storing, and Shipping Samples

Inspecting DNA and Otolith Samples

Soon after return to the live-aboard boat or other lodging, while performing the data QA on the HW laptop, carefully inspect the 96-cell and 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 separate from otolith trays until delivery to the Science Center.

Drying 96-cell Otolith-Only Trays

The otoliths in 96-well trays will be shipped dry so, if there is any water in the cells the tray must be allowed to dry. When opening the otolith tray for the first time since sample

collection, first inspect the cells through the lids to see if any otoliths are trapped between the rim and the lid. Tap the lid and then tap the acetate to get sticky otoliths to drop into the correct cells. If any otoliths are still stuck to the acetate, carefully remove the lid and then slide the plastic sheet across the surface of the tray so that any otoliths that got stuck to the sheet will fall back into their respective wells. Once open, visually ensure each pair of otoliths is at the bottom of the cell. Push any otoliths that are along the edge of the cell to the bottom of the cell using forceps. Set the tray on anti-skid matting and allow to dry uncovered in a location where they will not get bumped or spilled. It is very important to let the tray of otoliths dry before they are stored or shipped. Dry otoliths are more likely to stay in their cells.

After 24 hours of drying, replace the plastic sheets, the tray lids, and rubber bands and store the trays, preferably in order, in boxes for delivery to the respective Science Centers. After replacing the lids securely, carefully write the number of otoliths in the tray on the label.

Pre-Shipment QA Process

It is critically important for all otolith trays, and especially DNA trays, to be delivered to each study's respective 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.

When the trays are returned to the respective science centers, it is important that each tray is documented to have been 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 a center by:

1. Download the most recent version of the Tray Inventory report from the keta web site and save it as an Excel spreadsheet.
2. Sort the tray inventory so it's convenient to compare to the batches of trays.
3. Create two new columns in the spreadsheet: "Tray received" and "Samples verified".
4. Scan the first tray code into the first "Tray received" cell.
5. 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.
6. 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 transfers of trays from PWSSC/SSSC 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. See separate file for the Cordova Delivery Slip packing list.

96-cell Trays

1. Pack the trays into a box, cushioning them with packing material.
2. Seal the box with tape and affix with the adhesive shipping labels provided:

SHIPPING SUPPLIES

- | | |
|------------------------|--|
| 1) Ziploc plastic bags | 4) Pre-addressed and numbered shipping labels* |
| 2) Packing tape | 5) Inventory list |
| 3) Packing boxes | 6) 4G fibreboard box (for 48dwp trays only) |

SEAK 96-cell trays will be shipped as follows:

SHIP TO: Alaska Department of Fish and Game
Otolith Processing Lab
ATTN: Megan Lovejoy
10107 Bentwood Place
JUNEAU, ALASKA 99801

PWS 96-cell trays will be delivered to the ADF&G office in Cordova, attention Elena Fernandez. These can be delivered directly and do not need to be shipped to ADF&G in Cordova.

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.

Date sent to Cordova Lab:

Date received by Cordova Lab:

Person responsible for delivery:

Recipient:

APPENDIX E. METHODS FOR ALEVIN SAMPLING

Our goal in sampling alevins is to collect 1-25 fry in at least 250 redd samples in each stream by hydraulic sampling (“fry-pumping”) in March and early April. The reason for collecting a large number of alevins is because only some of the parents were sampled for genetic tissue, and there may be many other alevins of unknown parentage mixed in with those whose parentage can be identified.

General Approach

Sites will be sampled with a standard redd pump sampler to collect alevins. It is important that the sampling be distributed approximately in proportion to spawning distribution in the previous summer. A stratified sampling grid will be developed for each stream with a total of 1,000 sample sites equally spaced to cover the entire area of spawning mapped the previous summer. This includes all spawning areas, whether large, mass spawning areas, small patches of multiple redds, or single redds.

Because some sample sites will produce no target alevins, it will be important to “oversample” so the target of 250 positive samples can be attained. Therefore, the initial sampling distribution will include 300 sampling sites. A successful sample will contain up to 25 alevins of the target species.

It is possible that, after sampling the first 300 sites distributed throughout the stream, the target of 250 positive samples will not be met. In that case, determine how many more positive samples are required and estimate the percentage of sampling success in the first pass. Use this information to estimate how many more samples are required and approximately how they should be distributed throughout the stream to make another pass through the stream.

Selecting Sample Locations

The spawning areas from which adult DNA was collected the previous summer will be provided on a list with the GPS locations and descriptions of the starting point and dimensions of the spawning area. Each spawning area will be assigned a proportional number of the preliminary 300 sample sites. Roughly spread the designated number of samples across the area to be sampled.

Sampling will begin at the lower left “corner” of the designated spawning area (facing downstream). The lower left “corner” will be provided to you as a combination of GPS location, and descriptions of references, bearings, and distances to nearby markers or obvious landmarks.

Sampling will normally proceed in a left to right and upstream direction. Estimate the spacing distance to the right, or if no more sites are spaced to the right then upstream, to establish the second sample site location, and so on.

Place the sampling net hoop over the substrate wherever you can get a reasonable “seal” of the bottom ring of the net to prevent escape of alevins under the bottom of the ring. If the net does not lay flat on the substrate, move it slightly sideways, or up- or downstream, until it sits as flat as possible. Caution should be taken to not disturb the next sampling site to the right or upstream until that sample is taken.

Record the location of each sample with a GPS location. Use the position averaging feature of the GPS to get a better position. Sample sites will be numbered sequentially for all sample sites in one stream in chronological order.

Pumping to Collect Alevins

At each sample site, one or two team members will work the 0.5-m net frame down into the substrate as far as practical so that alevins cannot escape underneath the frame during pumping. The codend of the net should be on the downstream flow side of the net frame.

Work the injector probe 6-8” into the stream substrate inside the net frame. Start the water pump, being sure that the intake is in sufficiently deep water and that the pump is primed according to directions. Submerge the probe into multiple locations within the net frame, to 12-18” deep if possible, repeating this action until all possible fry have been released. Record the amount of time the substrate was probed with pumped water from start to end.

If alevins are observed on the surface within the net frame, they may be scooped with a dip-net and retained in a water-filled container. Additionally, remove the net frame and wash materials into the codend. Dump the contents of the codend into a round container and swish the contents until the alevins come to the surface. Scoop them out with an aquarium net and retain in the same container as any previous alevins from that sample. Be sure to keep alevins from one pump sample separate from another sample.

Alevin Samples

All alevins from each sample site will be sorted and counted by species and recorded. (See salmonid fry keys for species recognition.) All non-target species, and excess target species, should be released alive into the stream. If no target species are captured, record zero for the target species and move to the next sample site.

Up to 25 of the target species (if available in the sample) will be retained for genetic analysis in sample-specific, pre-labeled, ethanol-filled vials (use Isopropanol/Methanol/Ethanol (EtOH)). The vials should contain 4:1 EtOH to fish tissue. Minimize excess water in the vials and top them off from a squirt bottle of EtOH. On a small, write-in-the-rain sample label,

record, the date, stream, and sample number and place it inside the bottle. The sample number corresponds to the last four digits from the vial's bar code. Write the number of fish on the outside of the bottle. Store the sample bottle in a safe location.

Record or scan the sample vial number on the data sheet, being certain that the vial number is associated with the GPS data for the same pump sample.

LETHAL WHOLE ALEVIN TISSUE SAMPLING FOR DNA ANALYSIS

ADF&G Gene Conservation Lab, Anchorage

Section 1.01

Section 1.02 I. General Information

We use the whole alevin as tissue samples from individual fish to determine the genetic characteristics and profile of a particular run or stock of fish. The most important thing to remember in collecting samples is that **only quality tissue samples give quality results**. If sampling from carcasses: tissues need to be as "fresh" and as cold as possible and recently moribund, do not sample from fungal alevin.

Preservative used: Isopropanol/Methanol/Ethanol (EtOH) preserves tissues for later DNA extraction. Avoid extended contact with skin.

II. *Sampling Method*



- Wipe excess water and/or slime off the whole alevin prior to placing into bulk bottle to avoid getting either water or fish slime to reduce dilution of preservative (see diagram to left).
- Repeat procedure for **up to 25 individual fish** into the same bottle. If you don't reach this number of fish, that's ok, it's the limit for proper preservation of whole alevin.
- Using pencil, record on pre-printed label on outside of bottle: sample date (mm/dd/yy), total number of fish placed in each bottle. This ensures correct data with each collection.

- Using pencil, record the last 4 digits of the barcode on Rite-in-Rain paper and insert inside each bottle with samples.
- After collection is complete and 24 hours have passed since the last alevin was sampled, “refresh” the samples as follows: carefully pour off $\frac{3}{4}$ EtOH and pour fresh EtOH into the bottle containing the alevin. Cap and invert bottle twice to mix EtOH and alevin.
- If collection occurs over 4-5 day period, “refresh” and end of the collection.
- **Tissue samples must remain in 125ml EtOH at all times.** Store sample bottle containing alevin at room temperature but away from heat. In the field: keep samples out of direct sun, rain and store capped bottle(s) in a dry, cool location. Freezing not required.

- Place 2 labels/bottle in vertical position (barcode, date/ # fish) before each sampling event. This should be done in dry area.
- Pre-filled bulk bottle with EtOH.
- Pat slime/water off alevin.
- Place whole alevin into 125ml bulk bottle until sample goal met.
- Capacity: 25 alevin/125ml bulk.
- Pencil; record date, # fish sampled.



III. Supplies included in sampling kit:

1. 125ml bulk bottle – pre-filled with EtOH
2. EtOH –in bulk bottle(s) for “refresh” step.
3. Sampling instructions - laminated.

Section 1.03 IV. Shipping: **HAZMAT paperwork is required for return shipment of these samples.**

Section 1.01	Return to ADF&G Anchorage lab: ADF&G - Genetics	Lab staff: 907-267-2247
	333 Raspberry Road	Judy Berger: 907-267-2175
	Anchorage, Alaska 99518	Freight code: _____

Shipping samples

As soon as possible, store sample vials at room temperature, away from heat, until shipping to ADFG.

Paying attention to Hazmat issues, ship samples to:
ADF&G – Genetics Conservation Laboratory
333 Raspberry Road
Anchorage, Alaska 99518
Attn: Judy Berger

ADFG Lab staff: 907-267-2247
Judy Berger: 907-267-2175

