ISAv Standard Sampling Protocol

What to Look for:

- Salmon that are dead but still silver and/or have not spawned (still contain eggs or milt)
- Salmon with "petechial hemorrhaging" (blood spots) on abdomen, in fins, or near the eyes
- Salmon with very pale gills (anemia) or a yellowish skin color ("jaundice")
- Salmon that are very near death (holding in the current but are slowly pushed downstream; see notes at end of this document regarding 4-point scale)- fresh fish are very important in order to obtain viable samples.
- If you are in marine waters and see herring or other baitfish with the above signs, we are interested in those fish as well- they may carry ISAv

Videos to Watch for Sampling Tips Before You Go: (from the "Salmon are Sacred" web page)
http://www.youtube.com/salmonaresacred#p/a/u/2/Z6JQ9je5LzE
http://www.youtube.com/salmonaresacred#p/a/u/1/u69MXbE0Mho
http://www.youtube.com/salmonaresacred#p/a/u/0/F2pfrFMG334

What to Bring:

- Digital camera (pics larger than 7MB are probably overkill; they need to be in focus!)
- Sharp knife/knives (to open fish only; use dissection tools for cutting samples)
- WFC sampling kit with tubes (containing RNALater, a non-toxic sample preservative), vials, labeling pens, dissection gear, household bleach, data sheets, etc.)
- Bottled water (either spring or water, for rinsing dissection gear; DO NOT USE RIVER WATER TO RINSE THE DISSECTION TOOLS as this may contaminate the samples)
- Rubber or latex gloves (available at most hardware stores, pharmacies)
- Paper towels
- Small garbage bag for gloves, paper towels, etc.
- Optional: GPS (record coordinates on data sheet, as well as general area, river reach, etc.)
- Optional: Measuring tape (tailor's tape, etc.) or string and a carpenter's retractable measuring tape (you don't want to place the retractable tape on the fish, it will quickly draw in fish slime, etc. and may contaminate future samples- measure the string instead). Goals is to measure the Fork Length of the fish; tip of snout to shortest point on the tail.
- Optional: small cooler with ice (for samples in tubes)
- Optional: long handled net or pole gaff for retrieving fish from the water

Sampling Steps: See other side of this page

Sample Storage: Samples should be placed on ice or in a refrigerator (4°C) as soon as possible; DO NOT FREEZE THE SAMPLES IN THE TUBES (which contain RNALater preservative; non-toxic).

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SAMPLING THE FISH

Cutting the fish: First, photograph the whole fish at close range; be sure to include the fish ID# in the picture (the space on the bottom of each datasheet is used for this- tear it off and write the sample ID# (it may already be printed there) in large print, then include that in all photos taken of that fish). Then use a knife to open the fish; slide the blade into the vent and cut forward, holding the knife at a very shallow angle to avoid having the tip of the blade slice internal organs. Once the fish is open, focus on "hand awareness"- you can touch the handles of the sampling instruments, but do not touch the blade or scissor points to avoid contamination. If you have an assistant, have them hold the fish open while you take a piece of the tissues; otherwise, you may want to remove part of the flank of the fish (muscle) with the knife so that you have access while the fish is laying on its side. Fill out the datasheet with species, location, date, etc. Remember to leave the carcass behind- dead salmon provide nutrients to the river.

How Much Tissue is Needed: VERY LITTLE. Ideally the piece of tissue should have a maximum thickness of 0.5 cm (e.g. 0.5 cm x 1 cm x 1 cm) in any one dimension [this is equal to 0.2 inches X 0.4" X 0.4"] . Note: 0.4" is about the width of the fingernail on an average little finger. Gills are easy- just snip 3-4 off with the scissors, use the tweezers to place them in tube. Use the dissection gear; avoid handling samples with fingers.

Target Organs to Sample: The goal is to take two samples of each organ from each fish (so 4 tubes total for each fish). Sample the <u>gills</u> and <u>heart muscle</u>. A few (3-4) of the gill filaments are all that is needed; place these in a tube and label with the fish ID#, then repeat into a second tube (be sure to label both the top and sides of the tube). For the heart (located below the gill covers ("operculum") and just below the esophagus), cut out a small cube, then slice the cube partway through the middle to allow better penetration of the preservative; same for posterior kidney (located along the spine). Place the fresh tissue into the sample tubes and screw on the cap; there should be at least 5x as much liquid as tissue in each tube. REMEMBER: very little tissue is needed, too much will result in a poorly preserved, and potentially useless, sample. You can use the fish itself as a "cutting board"- just don't allow instruments to contact another fish before disinfecting. Please photograph every fish after opening the abdomen (looking at internal organs) and the gills, with the fish ID# visible in each picture.

Disinfecting the Dissection Gear: Wipe down the dissection tools, and knife blade, then place into a container with a 20% bleach solution for at least 2 minutes between each fish sampled (a 20% solution is one part household bleach plus 4 parts water). Then, rise the gear twice in "clean" water- do not use river water, you need to use bottled spring water, tap water, or deionized water. If you have two sets of dissection gear, one can be soaking while you use the other set. Also perform this step after you are done for the day- remember, this is a CRITICAL STEP to prevent sample contamination. Discard paper towels and gloves between fish (if you are very careful and the gloves are still clean, and you only touch the handles of the dissection gear, they can be reused, but once removed they should be thrown out).

Other Things to Watch for and Photograph: If you observe internal organs that look unusual, photograph them and be sure to place the paper with the fish ID# on it so it's visible in the photo. All gills and hemorrhaging around the eyes or in the internal organs, swollen or pale hearts should be photographed.

Thank you for helping out!!!

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If You Don't Have a WFC Sampling Kit: If you find a fish that meets the description at the top of the previous page but don't have a sampling kit, you can take the heart and a section of the gills and place them in a small ziplock bag; they must go immediately on ice or they are not valuable (the ISAv test may cost up to $40 per sample, so we will not run "old" samples that are in poor condition). You need to use a separate knife, or bleach the same knife, for at least 2 minutes (20% bleach) between fish to avoid contamination. Each fish's organs need to be in a separate, clean ziplock bag. Contact me (Todd) ASAP so that we can preserve the samples.

How to determine the "Freshness" of the carcass: There is a box in the middle of each data sheet where you are asked to estimate the condition of the carcass "freshness" (on a 1-4 scale, with 1 being very fresh). It is important to sample only relatively fresh carcasses; if the fish is covered in a thick whitish slime, it is probably too old to be useful. Here are some tips to help determine which category to assign"

(1) Very fresh carcass. Spawned out, but still live, fish are ideal (if they are floating in the water upside down, or pinned among tree branches and not actively swimming, or other indications that they are close to death, you can kill the fish with several sharp blows to the head with a rock or branch, then sample. Make sure you're not violating any fishing regs if you do this, we don't want anyone getting in trouble with WDFW). If the fish is dead but still has clear eyes, flesh is firm, this could also be a #1 fish. Carcasses still in the water are best b/c the cold water slows decay.

(2) Fresh carcass. Eyes present but not entirely clear, or, if eyes are missing (scavengers, particularly birds, will take the eyes first), the eye socket will still be red or pink. Flesh is firm, internal organs still firm (particularly the heart) and gill filament edges are clearly defined.

(3) Eyes, if present, are opaque. Eye sockets are white. Flesh is beginning to soften. Gill filaments poorly defined and may be pale from age (rather than from disease).

(4) Flesh is mushy; internal organs beginning to break down – edges not well defined, organs soft. Gills eroded. Probably not worth sampling.

Note: If you find a fresh carcass with whitish areas in the gills, most of which are still reddish, sample from the white parts to increase chances of detecting disease.

Also: If the carcass has been partially eaten, it may still be OK to sample. Bears will often eat the brain first (and may leave the rest if they are well fed); other scavengers will often eat only the eggs.

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