Sampling the WHOLE salmon!

The primary objective of the expedition is to document the abundance, ecology, and health of salmon across the northeast Pacific during the winter, a potentially critical period of the salmon life cycle that we know little about. To achieve our objective, in addition to counting the salmon, we take a wide range of tissue samples from outside and inside each fish. Some of these samples are processed on board the *Professor Kaganovskiy* within hours, while others require large (and often expensive) equipment and instead will be analyzed in laboratories around the Pacific Rim. By the time we're done processing each fish in each haul, there isn't much left except the head, skeleton, and a few bits of flesh.

Each tissue type we collect only tells us about a single aspect of the salmon, much like an individual jigsaw puzzle piece shows only a small fraction of the picture. By assembling the many pieces each analysis provides, we create the "big picture," namely a comprehensive determination of how each fish is doing. Because so little salmon research has been conducted in this area at this time of year, our collective results fill an important gap in our knowledge of the salmon life cycle.

When the fish come on board the ship, the first thing we do is identify them to species: pink, chum, sockeye, coho, and Chinook salmon. We do this using a suite of characteristics, including body size and shape, the size of gill rakers and scales, and the shape and coloration of the tail, back, anal fin, and color inside the mouth. Distinguishing between species requires attention to detail, which can be hard when you've left your warm cozy bunk at 3 am to process the catch. Having several people--even sleepy ones--examine each fish ensures we get the species right, a critical first step in processing salmon.

We then examine the outside of the fish, noting whether the adipose fin has been clipped (indicating either a wild fish or the presence of an internal coded wire tag), any obvious scars or wounds, the presence of sea lice, and external signs of disease such as black spots (caused by an internal parasite). We secure a tag containing a unique number around the tail of each fish so we can trace all samples collected during processing back to each individual fish, or find the fish when we realize hours later that we forgot to take some tissue sample. Carefully recording the sample numbers that came from each fish is essential to fully assemble our jigsaw puzzle—if we don't know which sample came from which fish, we lose a lot of information.

Next, we measure the length and weight of each fish, a deceptively simple procedure that provides extremely useful but rough information about approximate fish age, growth rate, and feeding success (skinny or fat). We also get detailed age and growth information from scales

and otoliths tissues we collect, but they take longer to process and will be analyzed back on shore. For example, we've been seeing several sizes of chum salmon (small, medium, and large), which we expect the scale data will show are fish that have spent 1, 2 and 3 winters in the ocean.

We then take several small fin clips that will be used to genetically determine where each fish originated from. Because salmon home to their natal streams, populations are genetically distinct and can be assigned to the geographic region or river of origin. For example, we can genetically distinguish between 24 populations of sockeye salmon in the Fraser River. Using the same methodology popular for human ancestry tests, we do this by comparing the genetic "finger print" of each fish to a baseline composed of genetic characterizations of hundreds of populations from rivers around the Pacific Rim.

We have the equipment and supplies on board the ship to make an initial genetic analysis for coho and Chinook salmon, a process that takes roughly two to three days to complete. The first batch of coho salmon was started yesterday and we eagerly await the results. Some of the questions the genetic data should answer are where each salmon came from, and whether the coho we've caught so far represent mixtures of stocks or largely come from a single population. If all goes well, we'll have answers by dinner!

We can also learn where fish came from two "tags" we're collecting: internal tags (coded wire and PIT tags) and otolith thermo-marks, or bar coding of the ear bones, which will be processed on shore. Both techniques are largely restricted to hatchery fish and applied while young fish are still in the hatchery. These are also important tools for salmon management, such as estimating harvest rates and catch distributions. However, we take advantage of the information they contain to tell us which specific hatchery each tagged fish comes from.

Once the external examination is complete, we put on the latex gloves and begin the dissections. Stomachs are removed and analyzed within hours of collection to determine both the amount and type of prey in their stomachs. Once the stomach is out of the way, we record whether the fish is male or female, and weigh the eggs or testes to document whether the fish will spawn in the coming year (maturing) or in will wait for future years (immature) before it returns to its home stream.

We also note the presence of large parasitic round worms, called nematodes, which inhabit the stomach, body cavity, and other parts of the body. These nematodes have complex life cycles that involve being passed from host to host, as the first animal they infect is eaten by the next host and the worm hitchhikes along. Most of the worms we've see use salmon as a temporary intermediate host; the worm's ultimate host—where they complete their life cycle--is typically a marine mammal. At low abundances these nematodes probably don't hurt the fish, but some of the high levels we've observed—up to thirty 4 cm-long worms in a single individual--probably have negative health effects.

We then sample the various internal organs for several different purposes. The most extensive collection of tissues are used to assess how healthy each fish is and the presence of any diseases the fish might carry, whether or not it looks sick. Small pieces of heart, liver, spleen, muscle, brain, blood, and kidney are carefully removed, preserved on board, and will be analyzed back in the lab both microscopically and genetically for the presence of viruses and other diseases. We will also determine overall fish health, as shown by the up- or down-regulation of specific genes. This technique will allow us to determine if, for example, large nematode infestations are affecting the health of the fish. Some diseases that fish acquire in freshwater as juveniles go into remission while in saltwater but can then flare up again when the fish return to freshwater to spawn. Our samples help us understand this process.

We're also collecting tissue samples to construct an energy budget for salmon overall: how much energy they consume in their food, how much they use for metabolism, how much they store as muscle or fat, and how much they devote to reproduction. To do this, we collect tissue samples from the muscle, liver, eggs, and stomach contents from each salmon, all of which are frozen on board. Back on shore, each tissue type will be analyzed for total energy and fat content. This information is entered into a bioenergetics model, which accounts for temperature-based metabolic rates. Once the energy budget is complete, we can estimate the energy demand of salmon across the Northeast Pacific, and how it varies by species or location. We can also use this framework to explore how energy budgets vary under different scenarios, such as the effects of elevated ocean temperatures (since salmon metabolism increases with water temperature) or changes to the abundance of particularly high or low energy prey.

In a parallel study, we're using naturally occurring stable isotopes of carbon and nitrogen, and fatty acids (think Omega 3 fatty acids), to trace the flow of energy throughout the food web. These trophic biomarkers get passed from prey to predator more or less intact, allowing us to study the flow of energy at time scales of weeks to months. To assemble this energy flow diagram, we're collecting muscle tissue from each salmon, as well as tissue samples of potential salmon prey items ranging from zooplankton and jelly fish to small fishes, and also muscle from salmon predators (so far just sharks). Like the energy analysis, all the lab work for this study will be conducted back on shore. And like the energy analysis, it allows us to trace the flow of energy across the entire food web, from photosynthetic phytoplankton to salmon prey to salmon themselves and then their predators. We can then use the model to explore how energy flow will likely change under different scenarios.

Finally, we're left with a carcass that consists of the head, backbone, a few bits of muscle, and the intestines. Each carcass goes into a labeled plastic bag and gets frozen in case we need to take additional types of samples (e.g. kidney tissues) or realize we missed something in the frenzy of fish processing. We are slowly filling the ship's freezers with hundreds if not thousands of carefully numbered tissue samples in labeled vials, bags, and boxes, and an equal number that are chemically preserved. Although they don't look particularly impressive at this point, they are invaluable for what they will tell us about the salmon we catch.

When we reach Vancouver in three weeks, we will get off the *Professor Kaganovskiy* with many completed analyses in hand, namely detailed information about the abundance, distribution, size, and diets for the five species of Pacific salmon across our study area. In the coming weeks and months, the thousands of tissue samples collected at sea will be analyzed at laboratories across the Pacific Rim. If all goes as planned, in a year or two many of the puzzle pieces should be complete and we will begin to assemble this jigsaw puzzle that tells the story of salmon marine ecology. We expect the scientific contribution of this expedition will be huge, and shape our thinking about salmon in the ocean for decades to come.