

Pacific salmon from many countries including Korea, Japan, Russia, Canada and the US spend a large portion of their marine life in the north Pacific. A small number of these salmon have been tagged providing information on the stream or river they originated from. However, most of the fish are unmarked.

Knowing the origin of the fish is critical in research designed to understand what is regulating survival and variability between stocks. DNA stock analysis is now a common tool used in both fisheries in season management and in fisheries science. Using a small piece of tissue collected from any fish, labs around the Pacific are able to identify the region and even the river or stock the fish originated from.

New technology being developed, parental based tagging, even allows a fish to be connected back to its individual parents if tissue samples were taken from them at the time of spawning .

DNA analysis for research is a process that is typically completed weeks or months after we return from the field. The many pieces of fin clips or other tissues that have been collected for stock identification are submitted to a highly specialized laboratory where they are processed. Walking into one of the laboratories is like walking into a specialized medical lab. High tech equipment, spacious areas to work, and many people in lab coats. In this expedition we are testing new technology that would give scientists the ability to identify the stock origin of the fish in almost real time – at least during field expeditions.

Dr. Christoph Deeg is currently doing post doctoral work with Dr. Kristi Miller at the Pacific Biological Station, DFO, Nanaimo. Christoph, originally from Germany, came to Canada in 2013 where he completed his PhD at UBC. For this expedition, he has spent months preparing and testing methodologies that would allow the DNA analysis to be conducted while we are at sea.

During the first eight days of the expedition, about 30 coho salmon were caught and small pieces of their fins were collected to test of this new "at sea" process. Christoph's diagnostic lab is set up in the lower levels of the research vessel. This lab looks nothing like those back on shore. Although behind a closed door and away from other fish samples to ensure no contamination of his process, the room is small and rather dark. Totes of dry supplies are stacked around the room. In addition, he shares this small space with spectral analysis, water processing for eDNA and chlorophyll filtration. Therefore, at times there can be up to four of the science team sharing this small area.

This is where we found Christoph in the early morning of February 26<sup>th.</sup> He was looking rather tired after a late night of fish processing the evening before but was eager to get the first analysis started. It is fair to say he was also feeling a little pressure of ensuring the process went smoothly as this was the 'proof of concept' that this work could be done at a remote site and in almost 'real time'. We say "almost" as the work would consume most of Christoph's next 24-36 hours and final results could take from 48 to 60 hours.

In addition, the rest of the science team was eager to learn the results as it had been a bit of a surprise that coho salmon were only second in the catch to chum salmon. Although we had expected to catch a few in our survey, this species is generally considered to reside on the shelf or closer to shore.

When we arrived with the cameras, Christoph was already cutting very small pieces of each of the previously collected fin clips and placing them onto a specialized analysis plate with a buffer solution that would extract DNA that is required for the analysis from the fish tissue. This is the first of over ten steps that Christoph would undertake over the next day and a half. This is meticulous work as ensuring no cross contamination between fish is essential. In fact, all of the steps that Christoph would undertake over the same level of attention.

The process is really quite amazing. The DNA that Christoph obtains from the fin clip will have markers attached to both ends. These markers will be unique to each sample. They are in essence a name tag and allow the DNA from all fish to be combined into a single sample. Christoph will then conduct a process that replicates the DNA to increase the size of the sample. Concatenation will then join all these microscopic bits of DNA together into a long strand. With the exception of the magnetic beads that help retain the DNA in the vials during the various processes, there is very little to see.

We visited Christoph throughout the day to check up on the progress of the analysis. You could see the stress or slight anxiety continued to exist. He would not know if all these hours of work would provide results until the very last step. A simple oversite or mistake in any of the steps could mean no amplification of the DNA or contamination of the sample and therefore no results. These many steps and a lot of patience from Christoph, results, hopefully, in a strand of DNA from the sample fish that can be compared with the baseline database of known stocks. The analysis looks at a specific segment of the DNA that remains relatively consistent but is unique between stocks.

After about 16 hours in the lab Christoph has done all he can do. It is now up to computer software for the next 24 hours. The process is similar to facial recognition. A specific segment of the sample DNA is compared with the same segment from all known fish. The computing power required for this step is massive and although the seagoing laptop that Christoph is using can do the job, it is not fast. And so we all wait...and wonder. Will it work? Where are these coho from? Why are there coho, a species generally thought of as coastal, so far offshore. Once Christoph receives the sequences from the analysis he is back to work again, analyzing the results. This requires an additional six hours.

About 50 hours after the first step of the process Christoph enters the galley with an expression that can only mean success. He looked tired but stress free. DNA had been recovered and successfully amplified. About one-third of the sample had results that were significant. The remaining samples have some results but additional work is required to verify them. This could be for several reasons from poor amplification to insufficient sequencing depth. However, Christoph has demonstrated, for the first time, that DNA analysis can be conducted in the field in "almost" real-time.

What is equally impressive, are the results. The analysis identified coho salmon from both Puget Sound in Washington State to the Stikine River in northern BC. Therefore, these high seas migrating coho salmon were not from a specific area but from a wide geographical distribution. New science, new discoveries, new knowledge. The successes of this expedition continue!