

December/January Update

I'm very excited for this report because I have some results from our work in November and December. We're getting closer to wrapping up one branch of the study and preparing the next. One of the slowest parts of this project has been the lack of molecular information about fish. That is something we've been doing some deep digging about.

November was all about proteins. A vastly underrated but quickly becoming a popular means of disease identification are proteasome studies. Proteins can be expressed differently in an organism that is sick compared to one that is healthy, especially if cancer is involved. To do one of these studies, a researcher needs to take out proteins from different types of tissue throughout the body. One of the hurdles for us was that there was very little literature about pulling proteins out of fish tissue. This meant that we had to find some instructions for one type of tissue and adapt it to work with fish.

We spent the first week dissecting two crappies. One was brought from Bone Lake and the other was from a lake system north of Polk County that did not have any known cases of Black Crappie Sarcoma. Because we do not have a detection method yet, we cannot be sure that the 'uninfected' lake was actually uninfected, but it was far enough from the hotspot to



Image 1. Kayla Boyd and group member, Maggie Freiermuth, dissecting an infected black crappie.

be the best candidate we could get. We collected tissues that was reported to have tissue damage in BCS infected fish. These were the heart, spleen, muscle, and fin. Each of these organs were separated

into three sections of equal weight. These samples were then crushed, liquified, and dissolved to try and find the best way to get the proteins out from each tissue. One way was crushing the tissue with a mallet after freezing it in liquid nitrogen. That was the most fun, in my opinion. The picture to the right shows myself and one of my professors crushing fish tissue.

The other two methods weren't so visual but one involved a small glass homogenizer which rips the tissue apart using pressure and the other let the tissue stew at high temperature for a while and shaking it up. After all that work, we found that the liquid nitrogen did the best with releasing the proteins from within the tissue. The set of three images below shows what some of our

results looked like. Each of the dark bands is a specific protein that was stained blue. We cut out these bands and we're going to send a few of them off in February to be sequenced. We're hoping to find some viral protein in the sick fish but that's an overly optimistic goal. Regardless if we find viral proteins or not, we will be able to sequence some new proteins that have never been described before.



Image 2. Kayla Boyd and professor Jen Grant crushing fish tissue using liquid nitrogen chilled crusher and a mallet.



I'm hoping to sequence four or five proteins of interest. That information will be taken to a national conference in Orlando this spring.

We'll be using the rest of January to finish the protein work and we're going to try get two other subprojects up and running. Once we get our hands on some fresh fish in the spring, we may grow some tissue in the lab to watch for cancer-like behavior to confirm that the raised tissue is cancer. The other path is searching through the genetic databases online to find specific parts in virus genomes that are the same in each group of viruses. We'll then search through the tissue to find evidence of any of viruses. It's a round-about way of searching but the more standard method had come back negative in the past. If something is hiding, this might be the way to find it!

Funding

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| November total | \$4,925 |
| Beaver Dam Lake Association | \$1,000 |
| Private Donation | \$200 |
| Updated total: | \$6,125 |