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Longwood Senior Thesis
Fall Semester Summary of Progress
The Effects of Impaired Water on Zebrafish Muscle Development and Startle Responses

This semester has been very productive so far. As my project is a bit daunting it was imperative to stay on schedule. This being said, there were points when the project had some bumps along the way and yet I am still very proud of the work I have completed so far. In my outline of the timeline, I wanted to complete several tasks before winter break. This includes first being able to quantify the coliform and *Escherichia coli* present in the impaired waterways we are investigating. Using the Colilert ® defined substrates test from IDEXX the samples from Gross Creek and Appomattox River were enumerated and recorded. We found what we had expected, that Gross Creek had a little over 15 times the amount of total coliform and 4 times the amount of E. coli than Appomattox had.

Before being able to study specific changes in genes, the list of genes needed to be narrowed down using qRT-PCR. First I researched a list of 11 genes and their forward and reverse primers so that they could be ordered to test on our previously made cDNA. For two time points (24 and 52 hours post fertilization) each gene was run in triplicate to be able to observe any trends in expression compared to a control. Before the last plate was completed we ran out of cDNA and had to completely start from scratch in order to obtain new DNA samples from embryos as well as perform RNA extraction before more cDNA could be made to use. This did set us back a little but was a good learning experience. This data collection has been completed and analysis of the plates has begun. Once the plates are done being analyzed then it will be possible to narrow down the list to 3-4 genes to use in this study.

During this semester the fish in the lab were also able to be bred several times for house-keeping as well as to have embryos to test our camera set-up on for tracking. These fish are doing quite well and next semester when we need more embryos for treatment groups it will be easier to complete and most likely the fish will again produce healthy viable eggs. We also needed to establish proper tracking methods for the second half of this project, and Dr. Jackson was able to create a fantastic rig in order to capture and save data seamlessly and efficiently. Dr. Jackson also created a Notion website for us to use for lab meetings, notes, research notes and to keep our data organized. This semester I worked on becoming more familiar with using this

resource and I now feel comfortable using it. This will be extremely helpful next semester for ensuring all of the data collected is able to be kept together and organized.

Next semester it will be time to make the probes for the specific genes chosen from the qRT-PCR and use *in situ* hybridizations to illustrate the gene expression in embryos. Additionally we will begin to test the control embryos for response against the sound stimulus using the camera set up while we wait for the right timing to collect water samples after crops are being planted. This is important because the runoff from the farms greatly contributes to the health of the waterways and collecting the water too early will not give us an accurate sample of the contamination. Then the rest of the camera trials can be completed with embryos raised in these samples. After that there is still plenty of time for data quantification and preparation for the oral defence. I am so excited to continue this project and I cannot wait for next semester to continue investigating.