1/16/15

Well the first week of the semester was busy as ever, but I did get a chance to start in the lab this week. I haven't started any bench research yet since I am currently trying to work my way through a backlog of scholarly articles that Andrew sent to me to read. I meant to read all of them over winter brake but I didn’t have a chance to get to two out of the four papers. In addition to the papers that Andrew sent to me when we first matched through the program, Andrew also gave me several other papers that are going to be relevant to my time in the lab to review. Since I have hours of reading to do Andrew is letting me use my lab time this week to do all of my reading. This is very different than what I experienced last year. Last year my lab provided me with four papers that I was expected to have read before I started in the lab. As of right now I don't feel like my understanding and awareness of the literature has improved much, since it takes me approximately two hours to read a twelve page paper ;however, I am retaining so much more of what I read than I was last year so I considered that to be a major success. As classes have just started there has been no connections between my lab work and classes. Although this experience as just begin I am cautiously optimistic about what this experience will hold for me.

Sincerely,

Megan Dunlevy

1/23/15

After hours and hours of reading I have finally escaped the paper ghetto! I spent most of my free time last week and this week reading and I can confidently said I know more about hard-bodied ticks than I ever thought I would. I can tell you about their physiology, how they interact with the environment, what effect climate change has and on them as well as several techniques to measure the metabolite content of the tick. In the end I read 15 papers on the topics listed above and on a couple of other things. After all of that I think my awareness of the literature in the field has widened exponentially. As I stated earlier I now know more about ticks and their physiology than I thought was humanly possible. I used my lab time to read Tuesday and Thursday but on Friday Andrew and I started on some bench research. We made stock solutions of glucose and sucrose that will be used as a blank in an assay to quantify the amount of carbohydrates found in the ticks. A assay is just a chemical test that answers some question. In this case the assay will tell us the amount of carbohydrates in the ticks using an spectrophotometric method. A spectro method means that the amount will be read on a spectrometer based on the extent of the color change in each well. The glucose and sucrose solutions will function as blank solutions that even out the reading by serving as a zero mark. The blank solutions should not change color and their readings can be subtracted from the sample values to obtain the sample values without any background interference. In addition to the stock solutions, Andrew and I made the reagent that this assay will use. I learned an interesting fact about adding acids and water. Apparently depending on which of the two you add first the reaction will either smoke and give off heat or the reaction will explode. We added acid to the water so the reaction simply gave off heat and smoke. I also started to practice weighing ticks this week. Weighing ticks is an interesting process as you have to place the tick onto the pestle, get the mass and get the tick off again all with tweezers and without letting the tick crawl everywhere. You really have to watch where the ticks crawl off to as there is a little hole in the balance that if they crawl into you can't pull them out and have to wait for them to come down on their own instead. Overall after a week things are progressing at about the same speed as they were last years but this year I am doing a lot more work with the animals which I appreciate as one of the things I hoped to get out of this experience was increased animal handling.

Until next time,

Megan Dunlevy

1/30/15

This week I got deeper into to the bench research. Using the stock solutions I prepared last week I did a serial dilution to obtain a gradient of molarities. A serial dilution is performed by first diluting the stock solution then using that diluting that dilution until you have either reached the concentration you desire or the number of dilutions that you desire. This is very helpful when you wish to reach a very small concentration that you are unable to pipette accurately. I used a series of 10 fold dilutions to reach .1ug/ml from 1 mg/ml. After the dilutions were made I followed the protocol for this assay and read the plate. These values were meant to serve as a calibration curve however this is science so it didn’t quite work out the first time…or the second time. I am starting to get the procedure down though. All of the dilution work is familiar to me from my lab work last semester as dilutions are I swear the cornerstone of most lab work. Seriously I everything I do in the lab requires some sort of dilution and I use them in Bio lab as well. On Friday Andrew and I went out to the field station to check on some ticks that Andrew had placed out there last Fall. I had been out to the field station before with my Bio class last semester so I knew that people placed samples out there. The ticks were placed to see if they would overwinter. Overwintering is the process by which ticks survive in the cold temperatures over winter. This process is very similar to bears hibernating. I also stated what I am calling Operation Secure Summer Funding. The goal of Operation Secure Summer Funding is to somehow receive a fellowship that will pay me to stay in Cincinnati over summer and do research. The first of these applications, SURF or Summer Undergraduate Research Fellowships, is due Monday so I will be spending the weekend completing that application.

Best,

Megan Dunlevy

2/6/15

So after a little bit of finagling I got everything turned in for SURF and now all that there is to do is wait. Thankfully I will not be idle long as the next round of applications are due the first week of March so next week I will start to work on the WISE ( Women in Science and Engineering) and the McMicken STEM fellowship applications. In the lab this week I continued to work on the carbohydrate assay (also known as the Anthrone assay). I did a couple more trials and on Thursday of this week I got the assay to work how we want it to. The entire time I have been working on this assay I have been trying to get all of the values on the calibration curve to be between 0 and 1 as this range is the range of which the spectrometer is most accurate. Thursday I found a set of dilutions that give me data all within this range. Yeah! Since I finally found a set of dilutions that give good data , on Friday I ran the assay fir the first time with ticks instead of just using the standard solutions. In order use ticks, the ticks first need to be cut up and then homogenized using the Bead Blaster which shakes the tubes to mix the solution and mush the ticks up. I also learned how we dehydrate the ticks. First you take the mass of the ticks and record the mass then you place the ticks in a plate to be placed in the oven (record which tick is in which well) and bake for several days. The ticks can be measured at any time however it is best to weigh the ticks at the same time every day to ensure that the measurements are accurate. So far things this year are very different than last year. Besides the fact that I am getting more experience handling the animals I am much more independent than I was last year. Andrew seems content to tell me what to do when I come in and let me go off and do it. This is very different than last year as last year I had someone looking over my shoulder every step of the way. Doing everything on my own is making me more confident than I was last year in the procedures that I am performing as well as in my basic lab skills such as pipeting.

Best,

Megan

2/13/15

This week I continued to dehydrate the ticks I started last week as well as took several measurement of the mass of the ticks over the period they were dehydrating. I also started to work on a new assay that Andrew and I had talked about earlier. As this experience continues I am becoming more and more able to follow along with what we are doing and how it fits into the big picture. Although there are several ways to measure all of the metabolites we are interested we are using these specific methods because the literature as well as Andrew's personal experience have proven that these assays work well with the amounts of metabolites and organisms that we are studying. This assay called the Vanillin Assay measures the lipid or fat content of the sample. The standard and reagents for this assay have already been made as this assay has been performed in my lab before. Although the Vanillin assay has been done in my lab before it has not been done with ticks. Andrew and I are also trying to get the total volume of the reaction down so the reaction will be easier to run and to mix. To start off I used the same serial dilution technique I described earlier to get another dilution set to run a calibration curve. As science always seems to go, there were several problems in the results. All of the readings I was getting both with a reduced volume and the full volume were way over the range I want data to be within. Despite these setbacks everything is going very well and I am excited to see what the future will bring.

Best,

Megan Dunlevy

3/6/15

Well it has just been a hectic couple of weeks! First the week of the 20th we got hit with a massive snow storm that closed UC for 4 whole days. That affectively made this week a wash in the lab. Snow week as we began to call it closed UC everyday that I was supposed to be in the lab. Then last week Andrew had me spend all of my lab time working on the rest of the summer fellowship applications as both the WISE and STEM applications are due this week. Then as luck would have it I got an email form SURF on Friday informing me that I had received a SURF fellowship! Operation Secure Summer Funding has officially been completed! I will be participating in SURF this summer and staying in Cincinnati to continue doing research in my lab. I would have loved to have gotten this news earlier as by now most of my materials for the other fellowships completed but what can you do. This week I am finally getting back to the science and started an new assay. This one called the Bradford assay is very easy and is used to measure the protein content of the tick. This assay is so easy in fact that this week I went from just learning to run the assay to doing the assay with ticks. We still aren’t sure what to homogenize the ticks in yet but I am comfortable running the assay. Again this is very different than what I experienced last year. Last year mastering a protocol took me much longer than I week. I don’t know if this is because I am more confident in myself or because my skills in the lab are improving.

Until the next time,

Megan Dunlevy

3/13/15

This week Andrew and I worked a combined assay protocol that will allow us to obtain carbohydrate, lipid and protein measurements all form one tick. This is a little bit ambitious as we don’t know if there will be enough of each metabolite within a single tick. This protocol is a long and because of that we broke it into several days work. The assay uses a fractional centrifugation method to separate all of the different metabolites. This means that the sample was spun several times to remove each of the metabolites at different times. After the metabolite was isolated then the normal assay I had been practicing could be run on the isolated sample. The data we got from this was a little hinky and because of this Andrew and I decided to abandon this combined assay as the data we get from running the assays separately is of better quality than trying to get all of the data at once. This week I finally got I tie in with one of my classes. My bio lab was using a fractional centrifugation to isolate different aspects of a mitochondria and then using the Bradford assay to quantify the amount of protein in each sample. This week was also very reminiscent of how last year went for me. Andrew and I worked side-by-side on this assay like I was use to doing from last year.

3/27/15

The week of the 20th was Spring Break and therefore I wasn’t in the lab. In fact I was spending an absolutely wonderful week in Paris with another one of my classes but that’s a story for another time and you will be able to read all about it on my learning portfolio. Getting back in to the swing of things in the lab things week I learned how to do a RNA extraction. We extract RNA in order to later perform QPCR or quantitative PCR on the sample. PCR is used to make more DNA from an existing sample of DNA. Fun fact in *Jurassic Park* PCR is how they created the DNA necessary to re-create the dinosaurs. QPCR keeps track of how much DNA is made after each cycle of the PCR reaction. QPCR also allows us to compare the relative amounts of DNA thus allowing us to compare how much each sample has been amplified. In order to do this first the RNA must be extracted from the entire tick. Then after the RNA has been extracted and the concentration of the RNA obtained you need to treat the samples with DNase to remove any DNA contamination. Then the treated RNA is purified to remove any left over Dnase. This as you can imagine is a very lengthy process. This entire process took me an entire week to complete and several of these steps Andrew had to complete steps for me. I also tried a new buffer for the Bradford assay this week and got more hinky results. My wells were very cloudy and all of the readings were off the chart. Next week Andrew and I will be investigating why this is.

Best,

Megan Dunlevy

4/3/15

This week I continued to work towards running QPCR on the RNA that I extracted last week. In order to run QPCR first you need to make some DNA from the extracted RNA as PCR machines can only read DNA. In order to make DNA from the cleaned RNA I preformed a simple PCR reaction to create DNA that is complementary to the RNA. After the DNA is made finally you can run the QPCR, which I did this week. Setting up a QPCR reaction I have discovered is a marathon not a sprint. I am just talking about the QPCR step. Currently I takes me about an hour to set up the tubes containing the reaction. Up until the QPCR portion of this process everything had been done in tubes but the QPCR machine that my lab has uses plates for the QPCR. Once I have the tubes set up I have to set the computer program to read the wells that contain samples. The tubes I was talking about earlier contain three replications of the same reaction, so one tube equals three wells in the QPCR plate. This week I also learned how to make primers. In order to run PCR you need primers to tell the enzymes that allow for DNA replication where to start and where to stop. These primers can be ordered commercially and come to you as a powder. In order to use the primers in PCR reaction they need to be in liquid form so first you need to add water to them. Then the primers need to be diluted like everything else I work with to keep their concentration relative to everything else in the reaction.

Sincerely,

Megan Dunlevy

4/10/15

This week I did some troubleshooting on the QPCR as one of our primers didn’t give us any amplification and another primers gave very low amplification. I order to fix this I tried increasing the amount the primer in the reaction, increasing the amount of DNA in the reaction, and I tried a new primer set. The data I got from this round of QPCR showed that the one primer set didn’t work and the other set just needed to have the primer concentration increased. After developing a protocol that will work for all of the primers I started to work on a set of RNA samples that Andrew had from a previous experiment. Andrew had previously dehydrated a batch of ticks that he had sent to Cincinnati Children's to be sequenced. Now he wants me to run QPCR to verify the data from the RNAseq as the same genes that are upregulated in the RNAseq will show a greater increase in the QPCR. These samples are already in RNA form so they don’t need to be extracted but the DNase, clean-up and DNA synthesis step still need to be preformed. Some of the samples had enough DNA for me to work with but I needed to DNase treat and clean up a couple before I could make the DNA I needed for the QPCR. Once that was done I started to set up the largest QPCR that I have done yet. This reaction was 12 tubes to be spread into 36 wells. This week has been very different from last year. After showing me what to do at each step when I ran my first QPCR last week Andrew is letting me do all of this myself. I come into the lab and we go over the data from last time and then Andrew tells me what to run this time. Next week I will continue to work on the QPCR for Andrew's samples. We have to spread the samples into 3 runs because not all of the reactions for each sample will fit in one of the plates.

Megan Dunlevy

4/26/15

A week ago I ran QPCR on the rest of Andrew's samples. Once I got data on all of the samples it became apparent that I would need to run some addition runs to get better data on a couple of the primers. So I spent the rest of last week and Tuesday of this week doing that. This started a series of QPCR reactions that I am calling the mother of all QPCR reactions. One of these mothers was 45 wells and the other was 48 wells. All of the set up of the reactions needs to be done in an ice bucket to ensure the enzymes will not denature or fall apart. When I was doing the mother reactions I started to run out of room in my ice bucket because there were simply too many tubes in the bucket. The day I ran my biggest reaction, the 48 well, I had been up both super late and super early due to an O Chem test so I was very sleep deprived. In order to make sure that I didn’t add the wrong thing to a tube I color coordinated my tubes so each color represented a different DNA sample. I also make a chart that I could check off what I had added to each tube, but I always do that as if you lose track of what you have added you have to throw the tube away and start over. I also did a extraction myself which got some mixed results. I got RNA but the concentration was so low that I had to make some adjustments when I went to DNase treat and clean up the samples . I don’t know how the RNA turned out since this was the last thing I did before leaving to study for finals. Andrew and I are meeting tomorrow to discuss what the summer will bring and I am very excited to see what I will be doing this summer. Over all this has been I great experience I feel that I made some serious progress towards the learning objectives I wished to accomplish and I think I have finally found I lab in which I want to stay while in. I will see how the summer goes but pending anything major I think I will try and stay in this lab until I graduate.

Signing off,

Megan Dunlevy