NDnano Undergraduate Research Fellowship (NURF)
2015 Project Summary

1. Student name: Megan Kilbride

2. Faculty mentor name: Molly Duman-Scheel

3. Project title: A screen for siRNA nanoparticle pesticides to target mosquito vectors of human disease

4. Briefly describe any new skills you acquired during your summer research: I learned a lot about different websites and how to use them. Specifically, I became very competent in BLAST, VectorBase, and IDT. I also had to perform a lot of data analysis and use statistical software and testing. I also learned how to make chitosan nanoparticles and many different mosquito rearing techniques.

5. Briefly share a practical application/end use of your research: Once successfully completed, our research could be taken to the field and the siRNAs we designed could be used in place of the current pesticides on the market.

Begin two-paragraph project summary here (~ one type-written page) to describe problem and project goal and your activities / results:

Aedes aegypti is the primary vector of dengue fever, and is also a vector of the viruses that cause yellow fever and chikungunya. Currently there is no treatment for these diseases and there is only a vaccine for yellow fever. Thus, the focus is on controlling *A. aegypti* populations so they are unable to transmit these diseases. This is done through larviciding, which is the application of microbial or chemical agents to kill mosquito larvae before they develop into reproducing adults that vector human disease. However, there is a push to identify new environmentally safe larvicidal agents due to concerns of pesticide resistance and the off-target effects of pesticides, with pesticides often killing birds and bees. Previous studies have demonstrated that larval ingestion of chitosan nanoparticles delivering small interfering RNA (siRNA) can be used for selective targeting of *A. aegypti* larval genes. Thus, our project goal is to utilize siRNAs as mosquito larvicidal agents. We aim to develop larvicides that are species-specific and to develop a repertoire of these larvicides in order to combat larval resistance. The overarching goal is to create larvicides that are environmentally safe and that will be effective in stopping the spread of multiple arboviral diseases.

Our project entailed a large-scale screen to test the larvicidal potential of hundreds of siRNAs corresponding to putative A. aegypti larval lethal genes. I designed the siRNAs by using a list of 2500 genes we found to be larval lethal in *Drosophila melanogaster* and expressed throughout the larval stages of *A. aegypti*. We tested about 300 of these siRNAs this summer.

We did this by microinjecting the siRNAs into third instar larvae and then assaying for mortality. The siRNAs that gave a mortality rate of 50% or greater were classified further with chitosan nanoparticles and we also used a soaking procedure to confirm our "hits." We still have yet to discover the most effective method for introducing the siRNAs to the larvae in the field. We began a bit of research on this over the course of the summer through the use of chitosan nanoparticles, but we will do further work when the initial screen is complete. Furthermore, I also developed siRNAs corresponding to the larval lethal genes of interest in *Anopheles gambiae* so we could test our hits in other species in order to discern if the design of siRNA nanoparticle larvicides for species-specific control could be used in additional container-breeding insect vectors of human disease. Since many of the genes we are targeting in this investigation are conserved in other insects, we believe this study could be effective in looking at how to better control other insect vectors of human disease. Some of the siRNAs targeting *A. gambiae* genes have been ordered and are being tested currently. Overall, we worked our way through over 20% of the genes we started with and have done many repeat tests on siRNAs that looked promising. There is still much work to accomplish, but luckily I will be working in Dr. Duman-Scheel's lab this year so we can continue progressing towards our goal of developing multiple larvicides.

As part of our soaking procedure, we soaked the larvae for two hours in an siRNA solution. In this image I am transferring the larvae into cups after the soaking procedure was completed.

Publications (papers/posters/presentations): We didn't publish any papers this summer, but the hope is we will be publishing a paper or two by the end of next year.

I was able to present multiple times during the summer. I presented at our NURF gathering, and I also presented at lab meetings and meetings with the different mosquito labs on campus.