Work for my thesis project began in the summer of 2017. For my project, I will characterize the microbiome of migratory (*Selasphorus rufus*) and resident hummingbirds (*Calypte anna*) in the Pacific Northwest to investigate how fattening cycles, parasite load, age, and sex of the hummingbirds interact with their microbiomes. This will provide insight into the role of the microbiome in the metabolism and nutrition of their bird hosts, with particular focus on energy-costly behaviors such as migration. While using wild animals for this study creates the additional challenges of capturing and tracking birds, it avoids confounding our results with changes to the bird microbiome that may be caused by captivity. My goal for this summer was to begin work necessary to complete my thesis project by the end of the fall September. To do this, I wanted to establish and validate the protocols that I will use.

My lab was sent samples of hummingbird urine and feces collected from the field in San Juan Island and the Pacific Northwest. I used the QIAamp® PowerFecal® DNA Kit to purify DNA from the samples, following the standard instructions from the kit. Next, in order to amplify DNA that would allow me to identify members of the microbiome community, I ran PCR reactions with the purified DNA and primers that provide broad coverage for the domains Bacteria and Archaea and for the fungus kingdom. These primers targeted the V4 region of the 16S rRNA gene in bacteria and archaea and the V4 region of the 18S rRNA gene in fungi. Next, I found primers that provided broad coverage for the phyla nematoda and insecta. Identifying members of these communities present in hummingbird guts will allow me to control for parasite
load of and the amount of protein consumed by each hummingbird. I validated that all five sets of primers amplify DNA in my samples.

In order to gain a preliminary understanding of the bacterial residents in hummingbird guts, I carried out a TOPO ligation and cloning reaction on the PCR products from one fecal sample. This reaction created plasmids that carried the gene fragments I amplified from the fecal DNA. I then purified the resulting plasmids and had them sequenced. This allowed me to identify seven candidate bacterial community members from this hummingbird’s microbiome: five *Staphylococci*, one *Clostridium*, and one *Brevundimonas*.

Once I have finished amplifying fecal DNA, I will send the gene fragments to be sequenced using Next Generation Sequencing technology. This technology uses a high-throughput, massively parallel reaction that will allow me to sequence huge numbers of amplicons instead of the ten or so I am able to do at a time through the TOPO cloning procedure. This will allow me to characterize the hummingbird microbiome much more thoroughly. This summer’s work allowed me to do all the troubleshooting, preparation, and procedure validation that I needed in order to complete my thesis project by the end of the semester.