The complex web of ecological interactions in which hosts and their parasites are embedded has the potential to substantially alter patterns of infection. Mesocosms provide a manageable method for isolating and examining the effects of multiple species, in order to explore the importance of direct and indirect effects for parasite transmission in a multi-species planktonic system. The objective of this project is to examine the microbiomes of *Daphnia dentifera* that are infected with *Metschnikowia bicuspidata* and *D. dentifera* that are not infected with the fungus. The testable hypothesis is that infection of *Daphnia* with *Metschnikowia* leads to a change in the microbiome species composition of *Daphnia*. The specific aims for this summer project are to determine (a) if the microbiome of *Daphnia* change post-infection and (b) if the microbiomes of infected and non-infected *Daphnia* within the same mesocosm differ. Mesocosm experiments will be conducted to test the hypothesis. Mesocosms with and without the presence of *M. bicuspidata* will be established to sample *Daphnia*. Molecular techniques will be used to analyze the microbiomes from the samples collected, specifically, ribosomal intergenic sequence analysis (RISA). This technique creates community fingerprints from samples by creating PCR products from the space between the 16S and 23S rRNA genes, which vary in nucleotide length based on the species of bacteria. The community fingerprints can then be compared to determine if differences exist in terms of species diversity between the samples. If the hypothesis is correct, the fingerprints generated from microbiome samples collected from infected and non-infected *Daphnia* should differ. Microbiome analysis may lead to a better understanding of why some *Daphnia* are susceptible to infection by the parasite, while others can clear the infection.
Introduction and Background

Purpose and specific aims of project
This project is based on the work in Rapti et. al. (2019) published in Theoretical Population Biology of which I am a co-author and Merrill & Caceres (2018) published in Ecology. The general purpose is to learn more about how a parasite alters the host organism post infection in planktonic systems. The objective of this project is to test hypotheses that the infection of Daphnia with Metschnikowia leads to a change in the species composition of the Daphnia microbiome.

Specific aim: To determine if Metschnikowia alters the microbiome of Daphnia after infection using ribosomal intergenic spacer analysis to analyze species composition.

- The testable hypothesis is that infection of Daphnia with Metschnikowia leads to a change in the microbiome species composition of Daphnia.

Broader impacts on field of study and undergraduate research
The complex web of ecological interactions in which hosts and their parasites are embedded has the potential to substantially alter patterns of infection (Cáceres et al., 2014; Duffy et al., 2011; Searle et al., 2016). In the past 15 years, there has been a growing call for host–parasite models to embrace this community context (Hatcher et al., 2006; Johnson et al., 2015; Keesing et al., 2006; Tompkins et al., 2011).

The focal host, Daphnia, is a cyclically parthenogenetic zooplankton that is widely distributed across the Midwestern United States. The focal parasite, Metschnikowia, is a common ascomycete fungal pathogen that produces environmentally transmitted spores that infect filter-feeding Daphnia (Ebert, 2005). Following ingestion, Metschnikowia achieve high intensity infections which ultimately kill the host, as is required for this parasite’s transmission (Ebert, 2005).

At exposure, most Daphnia experience early infections with spores or hyphae; however, only a subset of early infections ultimately produce late infections (Merrill & Caceres, 2018). By tracking cohorts and individuals through time, it was found that Daphnia can clear spores, hyphae, and sporocysts. Clearance of early infections is common under laboratory conditions, but may be sensitive to environmental stressors (Merrill & Caceres, 2018). The immune defense responsible for clearing infections appears to be haemocytes, which were observed attacking spores, hyphae, and in some cases, sporocysts. Daphnia are highly variable in the number of spores they defend and in the magnitude of their haemocyte response (Merrill & Caceres, 2018).

Based on the literature, it is highly likely that there is a significant change in the microbiome of Daphnia. This is the goal of the project, to determine if infections lead to a change in the microbiome. Different microbiomes may lead to an explanation as to why some Daphnia are able to overcome infections and others are not.

This project builds new knowledge by exploring an ecological problem through traditional research. The work will contribute to the intellectual climate of the University and encourage other undergraduates to consider research projects.
Proposed Research Plan

**Experimental design and Methodology**
These experiments will utilize mesocosms in one liter buckets to test the hypothesis. Buckets will be used to simulate ecological interactions among the organisms, *Daphnia* and *Metschnikowia*. The host will be exposed to the parasite at a minimum final concentration of 30 spores per mL in the mesocosm for a period of 12 days. Infected and non-infected hosts will be sorted into separate pools. The host tissue will be grinded and DNA will be extracted.

Microbiome analysis will occur using ribosomal intergenic sequence analysis (RISA). The polymerase chain reaction will be used to amplify the intergenic space between the 16S and 23S ribosomal genes. The PCR product will be run on a 2% agarose gel which will allow for visualization of the number of bands and their fragment lengths according to size.

**Instrumentation required and accessibility**
The molecular techniques require standard instrumentation, which are available in the Biology Department. Through coordination with other research teams during the summer program, the project will be completed according to the timeline.

**Expected Outcomes and how you will determine success**
First, if the student is able to independently set up an experiment, collect and analyze data, and report results, then the project is a success. The real goal of this program is to train students to think like scientists and improve their inquiry skills.

In regards to the proposed project, the hypothesis is supported if the community fingerprint analysis determines a difference between infected and non-infected *Daphnia*.

**Mentorship Plan**

As a research assistant, the student will gain valuable research skills and learn the process of scientific inquiry by doing. The student is expected, under the supervision of the mentor, to plan experiments throughout the summer (time management), collect data, analyze and interpret results, and keep records of all work in a format expected for scientific research.

As the pandemic is still a factor, I will engage with the student in a hybrid format to reduce the time spent on campus. I will use video conferencing tools, such as Zoom or Collaborate, to meet with the student off campus. We will need to meet on campus to perform the experiments in the laboratory.

In May, before the start of the summer program, the student and I will meet, review the work to complete, and set a work schedule with deadlines. This way the student will know their work schedule before the program begins and can plan their summer accordingly. There will be weekly meetings to check progress.
By the end of the program, the student will be able to independently perform molecular biology techniques to profile microbial communities, cultivate stocks of *D. dentifera* and *M. bicuspidata*, and maintain a proper laboratory notebook. This will be accomplished through our weekly meetings when I mentor the student in the laboratory.

**Proposed Timeline that includes Aims and/or Goals**

| Weeks 1-2 | • Learn to culture stocks of *Daphnia* and *Metschnikowia*  
|           | • Learn to extract DNA from samples |
| Weeks 3-4 | • Set up experiment to infect Daphnia  
|           | • Learn to perform the polymerase chain reaction |
| Weeks 5-7 | • Collect samples from experiment  
|           | • Use ribosomal intergenic spacer analysis to test hypothesis |
| Weeks 8-10| • Analyze data and prepare presentation for concluding symposium |

**References** (not included in the 6 page limit)


Criteria for Student Applicants (Please report minimum criteria you will expect from student applicants, such as coursework that must be completed prior to starting work on this project):

The minimum criteria for applicants are:

1. The applicant should have a cumulative GPA of 3.0 or better and a cumulative science GPA of 2.75 or better based on a minimum of four semesters of coursework at Lewis University.

Preferred coursework includes General Microbiology and General Microbiology Lab.