

UREAP APPLICATION FORM

First Name: Tallis

Last Name: Dixon

Student ID: T00696719 Start Date of Project: 01/05/2024 (DD/MMM/YYYY)

Please complete all sections of this application form.

1. FACULTY MENTORS INFORMATION

1.1 Who is your Primary Faculty Mentor? **Dr. Louis Gosselin**

1.2 Who is your Secondary Faculty Mentor? **Dr. Eric Bottos**

NOTE: Your Primary and Secondary Faculty Mentors must each complete a Faculty Mentor Support Form. Forms can be found under the attachments tab within your TRU Romeo UREAP application and on the TRU UREAP webpage under information and Forms for Faculty Mentors..

2. PROJECT DESCRIPTION

2.1 Provide an abstract of your proposed research: (maximum 1500 characters)

We aim to identify the microbial community present on the marine intertidal snail *Nucella ostrina* using polymerase chain reactions (PCR). The microbiome plays an important role in maintaining the health and fitness of marine invertebrates by facilitating immune defence, aiding in digestion, and enabling adaptation to environmental conditions (Khan et al. 2018). Carried out at the Bamfield Marine Sciences Center, located on the West Coast of Vancouver Island, this research will examine the microbial communities associated with marine invertebrates. The study will determine (1) how the microbial community changes as the animal grows from juvenile to adult, (2.1) across local habitats that differ in temperature, salinity and wave action, and (2.2) the effect of elevated seawater temperature on the microbial community associated with juvenile invertebrates. DNA samples of the microbial community from the snails will be returned to the Microbial Ecology and TRUGen sequencing laboratory at TRU, where the DNA will be analyzed to determine species variation between samples. Identifying the microbial species present on the bodies of these animals will increase understanding of how their microbiomes respond to change.

2.2 Provide a brief literature review for your proposed research: (maximum 3500 characters)

Marine invertebrates harbour a diverse variety of bacterial communities, predominantly within their digestive systems as well as on the external surfaces of the body. Some bacteria found on the body surface of marine invertebrates have beneficial properties, others can be harmful pathogens, and some may have no functional benefit to the animal at all. The bacteria associated with marine invertebrates have been found to alter the animal's vulnerability to pathogens and to predators (Menabit et al. 2024). In addition, studies using PCR sequencing and synthesis techniques have identified bioactive metabolites with antimicrobial properties (El Samak et al. 2018), an important consideration given the current increase in antibiotic resistance among medically important pathogens. There is also a growing concern

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that climate change, which is causing a rise in seawater temperature (Iwabuchi & Gosselin 2019), could disrupt the natural microbial communities associated with marine invertebrates. For instance, Khan et al. (2018) discovered that bacteria in genera Clostridiales and Verrucomicrobiales were present in high densities on the gills and in the gut of heat-resistant oysters, and those bacteria improved the oyster's ability to respond to heat; however, Acidobacteriales and Betaproteobacteriales were potential pathogens and were found more frequently on heat-susceptible oysters. Additionally, PCR analysis of sponge microbiomes before and after heat stress events revealed the presence of transient bacteria on vulnerable sponges, resulting in fatalities of some of those sponges (De Castro-Fernández et al. 2024). Warming oceans can also affect the gut microbiome of marine invertebrates, particularly the abundance of disease-causing bacteria (Liu et al. 2023); Liu et al. (2023) found microbial diversity and abundance increased under warming temperatures, in the laboratory and slightly in field sites. Evidence to date therefore indicates that the microbial community associated with marine invertebrates plays an important role in the survival and growth of these animals.

Though several studies have examined microbial diversity and activity on marine invertebrates, most have focussed on the gut microbiome, and all studies have focussed on adult invertebrates. No study to date has investigated the outer microbiome of early juveniles, which is known to be the most vulnerable phase of the life cycle of marine invertebrates (Gosselin & Qian 1997). In addition, a majority of microbial studies have focussed on invertebrates reared in aquaculture facilities; far fewer studies have examined the microbial community associated with wild invertebrates in natural habitats.

2.3 What is the hypothesis or research question for your proposed research? Include any specific objectives: (maximum 500 characters)

This project seeks to identify factors influencing the microbiome of early juvenile marine invertebrates living in natural habitats. The specific goals of the project will be to: (1) determine how the microbiome of the intertidal snail *Nucella ostrina* changes as the snail grows from newly hatched juvenile to adult; and (2) determine the extent to which the early juvenile microbiome varies as a function of the local habitat.

2.4 Provide a description of the research methodology/methodologies and analysis that you intend to employ in completing this research: (maximum 1500 characters)

All fieldwork will be carried out at the Bamfield Marine Science Centre, located in Barkley Sound on the West Coast of Vancouver Island. The study will examine the snail *Nucella ostrina*, a model organism with 30 years of research history in the Gosselin Lab. For goal (1), 200 newly hatched juveniles and 20 adults will be collected from Dixon Island and their microbiomes will be compared. For goal (2), 200 juveniles will be collected from each of five different sites in Barkley Sound, differing in environmental conditions (salinity, temperature, wave action), and their microbiome will be compared. Also, an experiment will be conducted to determine the effects of increased seawater temperature on the microbiome using snails from Dixon Island. 50 early juvenile snails will be exposed for 10 days to one of three treatments: a control (same as field conditions), as well as +2° C, and +4° C above field conditions to simulate climate change effects. All samples will be frozen at -80° C for transport to the Microbial Ecology and TRUGen sequencing laboratories at TRU.

Microbial analysis will involve sonication for surface DNA removal, DNA extraction using the DNeasy PowerBiofilm Kit, PCR amplification of the 16S rRNA gene, and sequencing on the Ion S5 sequencer.

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Microbial community composition will be analyzed through amplicon sequencing with comparisons made across different ages and environmental conditions using statistical methods.

2.5 Provide a description of how your research will significantly impact your field of study:

(maximum 1500 characters)

The microbiome within marine invertebrates has been extensively studied over the years for a variety of reasons including antimicrobial compounds, disease-causing bacteria, and invertebrate survival in warming oceans (Khan et al. 2018; El Samak et al. 2018). These studies primarily concentrated on identifying the bacterial species linked with the digestive tract and internal systems of adult marine invertebrates. However, the surface microbiome of these organisms remains largely unexplored, and the microbial communities present on early juvenile invertebrates are entirely unknown. The microbial diversity associated with early juveniles as well as the consistency of the microbial community as the juvenile grows to the adult stage, remain unknown. By examining the microbiome of intertidal marine invertebrates, a better understanding can be gained of the associated microbial community that resides on them and whether it is beneficial or harmful to the organism. If the microbiome were to change over time and space, these studies could help predict potential impacts on the health and survivorship of marine invertebrates in natural habitats.

2.6 Describe your plans to disseminate your research findings: (maximum 500 characters)

The findings of this research will be presented at the TRU Undergraduate Research and Innovation Conference in March 2025, and the TRU Science Undergraduate Poster Exhibition of Research (SUPER) Conference in April 2025. The findings will also be written as a Directed Studies report, which will be made available on TRUspace. The project is also designed to be published in a scientific journal if the findings are sufficiently conclusive.

2.7 List the references that you have cited throughout your research proposal observing the appropriate citation style for your discipline: (maximum 3500 characters)

De Castro-Fernández P, Ballesté E, Angulo-Preckler C, Biggs J, Avila C, García-Aljaro C. 2023. How does heat stress affect sponge microbiomes? Structure and resilience of microbial communities of marine sponges from different habitats. *Frontiers in Marine Science*. 9. doi: <https://doi.org/10.3389/fmars.2022.1072696> [Accessed Feb 1st 2024]

El Samak M, Solyman SM, Hanora A. 2018. Antimicrobial activity of bacteria isolated from Red Sea marine invertebrates. *Biotechnology Reports*. 19. doi: <https://doi.org/10.1016/j.btre.2018.e00275> [Accessed Feb 1st 2024]

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Gosselin Louis, Chia Fu-Shiang. 1993. Feeding habits of newly hatched juveniles of an intertidal predatory gastropod, *Nucella emarginata* (Deshayes). *Journal of Experimental Marine Biology and Ecology*. 176. doi:

http://www.tru.ca/faculty/lgosselin/publications/gosselin&bourget_1989_jouranimacol_58_287-303.pdf [Accessed Feb 10th 2024]

Gosselin LA, Qian PY (1997) Juvenile mortality in benthic marine invertebrates. *Marine Ecology Progress Series* 146: 265-282 http://faculty.tru.ca/lgosselin/publications/gosselin&qian_1997_meps_146_265-282.pdf [Accessed Feb 19th 2024]

Iwabuchi BL, Gosselin LA (2019) Long-term trends and regional variability in sea surface temperature, salinity, and intertidal rock temperature on Vancouver Island, Canada. *Bulletin of Marine Science* 95: 337-354

<https://www.ingentaconnect.com/content/umrsmas/bullmar/2019/00000095/00000003/art00001;jsessionid=sado8t88efn9.x-ic-live-01> [Accessed Feb 19th 2024]

Khan B, Clinton SM, Hamp TJ, Oliver JD, Ringwood AH. 2018. Potential impacts of hypoxia and a warming ocean on oyster microbiomes. *Marine Environmental Research*. 139:27–34. doi:

<https://doi.org/10.1016/j.marenvres.2018.04.018> [Accessed Feb 1st 2024]

Liu M, Li Q, Tan L, Wang L, Wu F, Li L, Zhang G. 2023. Host-microbiota interactions play a crucial role in oyster adaptation to rising seawater temperature in summer. *Environmental Research*. 216. doi:

<https://doi.org/10.1016/j.envres.2022.114585> [Accessed Feb 9th 2024]

Menabit S, Lavin P, Begun T, Mihaela Mureşan, Teacă A, Purcarea C. 2024. First screening of bacteria assemblages associated with the marine polychaete *Melinna palmata* Grube, 1870 and adjacent sediments. *Frontiers in Marine Science*. 10. doi: <https://doi.org/10.3389/fmars.2023.1279849> [Accessed Feb 19th 2024]

3. PROJECT TIMELINE WITH BENCHMARKS

3.1 Provide a timeline for your project that includes key benchmarks: (maximum 1000 characters)

May 1 - 14

-Logistics planning and preparation

-Traning in DNA extraction techniques in TRUGen Sequencing Laboratory

May 15

-Travel to BMSC (Bamfield Marine Science Center)

May 16 - 31

-Goal 1: Sampling of juveniles from different field sites and DNA extraction

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May 28 - June 12

-Goal 2.1: Sampling of juveniles from different field sites and DNA extraction

June 4-30

-Goal 2.2: Testing effects of seawater temperature on microbial community - lab experiment followed by DNA extraction

July 1

-Travel from BMSC to Kamloops

July 2 - August 15

-Microbial analysis and PCR sequencing at Microbial Ecology and TRUGen Sequencing Laboratories

August 16 - September 15

-Interpretation of Research Results

-Writing of Report

NOTE: Please refer to the UREAP Help Guide for a project timeline example. Students must demonstrate a willingness to engage in 12 weeks or equivalent of sustained research per the Terms of Reference.

4. OPERATING GRANT BUDGET PROPOSAL

4.1 The UREAP award offers up to \$1000 toward direct research expenses. These expenses must be preapproved by the UREAP committee in the adjudication phase. Use the provided template under the Attachments tab in the TRU Romeo UREAP application to complete your budget proposal. Copy amount from the TOTAL AMOUNT line of the budget here. Total Amount: \$ 1,000.00

4.2 Additional budget information: (maximum 500 characters)

Any research costs exceeding the \$1000.00 UREAP grant will be covered by the NSERC Discovery Grant held by the proposed supervisor, Dr. Louis Gosselin.

5. CONTRIBUTION TO ACADEMIC/PROFESSIONAL GOALS

5.1 Describe how this project will contribute to your academic and/or professional goals:

(maximum 1000 characters)

During my last three years at Thompson Rivers University, I have had the opportunity to explore various branches of science within my program. I am currently in my 3rd year of studies in Cellular, Molecular and Microbial Biology and am very interested in mixing different branches of study such as microbiology, animal biology and ecology, which is how this project came to be.

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After graduation, I hope to pursue further research and attend graduate school. Participating in this UREAP would be invaluable, offering first hand experience in designing and executing a research project. The process will equip me with essential skills such as proposal writing, laboratory work, organization, collaboration, presentations, and data collection.

NOTE: Include your role in conceiving of the project, your role in the implementation of the project, and your overall academic objectives – explaining how this project will help to advance those objectives.