Title: Stoichiometric Plasticity of Heterotrophic Bacteria in the Laurentian Great Lakes: The Impact of Nutrient Concentration on Microbial Community Adaptation

Background: The Laurentian Great Lakes represent the largest freshwater ecosystem on Earth and support around 34 million people and their economies, while providing crucial habitat for biological communities [1]. Influxes of nutrients and carbon (C) have impacted the lakes as a result of human population growth and land use change. The most affected lakes are Erie and Michigan, which receive the highest nitrogen (N) (61.5 and 62.9 kt/y, respectively) and phosphorus (P) (2.4 and 2.3 kt/y, respectively) inputs as particulate organic matter (POM), with the majority being runoff from manure and chemical fertilizers used for agriculture[2]. Changes in N and P cycling may lead to changes in C cycling, as C, N, and P cycles are often coupled [3], [4]. The impacts of rising nutrient inputs on biological communities and nutrient and carbon cycling are poorly understood. Therefore, we must investigate how microbially mediated ecological and biogeochemical processes concerning C, N, and P cycling affect the Great Lakes, and how changes induced by increased nutrient inputs will continue to impact the water quality of the Great Lakes. Microbial communities are of particular interest since they are heavily involved in nutrient cycling, carrying out processes such as nitrogen fixation, nitrification, and denitrification, as well as phosphate solubilization and organic matter decomposition, thereby making them essential controllers of nutrient availability and cycling. The composition of microbial assemblages can fluctuate [5], [6], along with their metabolisms [7], morphology [8], and stoichiometry [9], [10], as responses to environmental variables such as dissolved organic matter (DOM) and nutrients. Additionally, changes in stoichiometric ratios (C:N:P) can have impacts on the quality of food that lower trophic levels (bacteria and phytoplankton) provide for higher trophic levels (i.e., zooplankton and fish). It has been established that bacterial stoichiometry, metabolic capacity, and morphology change in response to particulate and dissolved C, N, and P in the environment, but these studies are often done in vitro, making it difficult to account for natural conditions. Here, I propose an *in situ* reciprocal transplant experiment where microbial communities from Lake Huron and Lake Superior will be collected and exposed to the nutrient conditions of each lake, to uncover the innate ability of microbial communities to adapt to changing nutrient conditions. In doing so, the proposed research will help inform management agencies on the present state of water quality in Lakes Huron and Superior while providing information on how increased nutrients into the Great Lakes may affect biological communities and important biogeochemical cycling.

Goals and Hypothesis: The goal of the proposed research is to investigate microbial community adaptation to different nutrient and DOM concentrations. Objective 1: Characterize the response of microbial stoichiometry to changes in POM and DOM. Hypothesis 1 (H1): Microbial communities from oligotrophic systems will be less flexible in their stoichiometry when compared to communities from eutrophic systems. Objective 2: Evaluate microbial community adaptation to altered nutrient concentrations and environmental conditions Hypothesis 2 (H2): Communities from oligotrophic systems will have lower functional redundancy compared to those from eutrophic systems, marked by the presence of more rare taxa and ability to metabolize a variety of carbon sources. Hypothesis 3 (H3): Communities taken from eutrophic environments will be less active in oligotrophic environments due to a limited range of OM substrates available.

Experimental Design: To investigate H1, water from Lakes Superior and Huron will be collected and used for an *in situ* reciprocal transplant experiment using dialysis bags (14,000 kDa MWCO). Water from one lake will be filtered using a 10 μm filter to remove grazers and placed

into dialysis bags, then incubated in the other lake for 14 days. Water samples taken from the bags will be analyzed for particulate C, N, and P of the bacterial communities and the seston. To measure DOM and dissolved nutrients, 0.45 um filtered water samples will be analyzed for dissolved organic carbon (DOC), total dissolved nitrogen (TDN), nitrogen species (nitrate, nitrite, and ammonium), and soluble reactive phosphorus (SRP). The quality of dissolved organic matter will be characterized by fluorescence excitation-emission matrix spectroscopy. To assess H2, morphological traits will be determined via flow cytometry, changes in community assemblage will be characterized using 16S rRNA gene sequencing, and functional capacity will be measured by carbon substrate utilization using BIOLOG Ecoplates. To explore H3, translationally active microbes will be quantified using biorthogonal amino acid tagging (BONCAT), which will provide information on microbial activity and whether the communities become more active or dormant in response to varying nutrient statuses. CTD and light profiles for each lake will also be collected at the beginning and end of each incubation for each host lake. Community adaptation in H1 will be measured via plasticity in stoichiometry and morphology, while H2 and H3 adaptation will be reflected by microbial community assemblages and functional redundancy. Bray-Curtis dissimilatory will be used to assess similarity across native lake communities and incubated communities. The ratio of C, N, and P in the seston and transplanted communities will be calculated and compared to their lake of origin and the other transplanted community. Cell abundances and cell DNA content will be determined via flow cytometry, and transplanted communities compared to host communities. Finally, the data generated from this experiment will be used to make ordinations fitted with environmental variables to assess which variables are responsible for microbial community adaptation. *Timeline:* Sampling will be conducted in May of 2026. Data analysis will be done in the fall of 2026. In Spring 2027, a manuscript will be drafted for publication. The results of this study will be included as a chapter in my dissertation, and I will tentatively defend it in spring 2029. **Products:** The outlined experiments and their findings will be incorporated into my dissertation as a chapter and presented at the 2028 IAGLR meeting. Finally, a manuscript will be prepared and submitted to the Journal of Great Lakes Research.

Relation to MISG strategic plan: The proposed research is directly related to the 2024-2027 Michigan Sea Grant Strategic plan Goal 1, Desired Outcome 1.2, "Educators, students, and lifelong learners have current information and innovative tools that meet or exceed relevant standards and practices". Goal 3, Desired Outcome 3.2, "Evidence-based science, traditional and local, and innovative solutions inform and improve management and conservation of coastal habitats". Goal 7, Desired Outcome 7.1, "Scientific understanding, including traditional and local knowledge, provides foundational information, and all community members understand the impacts of changing conditions and coastal hazards and can prepare, respond, and adapt". References:

E. Rau, C. Riseng, L. Vaccaro, and J. G. Read, "The Dynamic Great Lakes Economy: Employment Trends From 2009 To 2018," p. 19, Oct. 2020.

Md. Bodrud-Doza et al., "Evaluating best management practices for nutrient load reductions in tile-drained watersheds of the Laurentian Great Lakes Basin: A literature review," Sci. Total Environ., vol. 965, p. 178657, Feb. 2025, doi: 10.1016/j.scitotenv.2025.178657.

Z. Wang et al., "Coupling of Phosphorus Processes With Carbon and Nitrogen Cycles in the Dynamic Land Ecosystem Model: Model Structure, Parameterization, and Evaluation in Tropical Forests," J. Adv. Model. Earth Syst., vol. 12, no. 10, Sept. 2020, doi: https://doi.org/10.1029/2020MS002123.
 O. R. Anderson, "Evidence for Coupling of the Carbon and Phosphorus Biogeochemical Cycles in Freshwater Microbial Communities," Front. Mar. Sci., vol. 5, Feb. 2018, doi:

 ^[4] O. Ř. Anderson, "Evidence for Coupling of the Carbon and Phosphorus Biogeochemical Cycles in Freshwater Microbial Communities," Front. Mar. Sci., vol. 5, Feb. 2018, doi: 10.3389/fmars.2018.00020.
 [5] H. E. Adams, B. C. Crump, and G. W. Kling, "Metacommunity dynamics of bacteria in an arctic lake: the impact of species sorting and mass effects on bacterial production and

 ^[5] H. E. Adams, B. C. Crump, and G. W. Kling, "Metacommunity dynamics of bacteria in an arctic lake: the impact of species sorting and mass effects on bacterial production and biogeography," Front. Microbiol., vol. 5, Mar. 2014, doi: 10.3389/fmicb.2014.00082.
 [6] G. Michoud et al., "Mapping the metagenomic diversity of the multi-kingdom glacier-fed stream microbiome," Nat. Microbiol., vol. 10, no. 1, pp. 217–230, Jan. 2025, doi:

^[6] G. Michoud et al., "Mapping the metagenomic diversity of the multi-kingdom glacier-fed stream microbiome," Nat. Microbiol., vol. 10, no. 1, pp. 217–230, Jan. 2025, doi: 10.1038/s41564-024-01874-9.

^[7] T. J. Kohler et al., "Global emergent responses of stream microbial metabolism to glacier shrinkage," Nat. Geosci., vol. 17, pp. 309–315, July 2025, doi: https://doi.org/10.5281/zenodo.10469376.

 ^[8] X. A. G. Morán et al., "More, smaller bacteria in response to ocean's warming?," Proc. R. Soc. B Biol. Sci., vol. 282, no. 1810, p. 20150371, July 2015, doi: 10.1098/rspb.2015.0371.
 [9] J. B. Cotner, E. K. Hall, T. Scott, and M. Heldal, "Freshwater Bacteria are Stoichiometrically Flexible with a Nutrient Composition Similar to Seston," Front. Microbiol., vol. 1, Dec. 2010. doi: 10.3389/fmicb.2010.00132.

^[10] C. M. Godwin and J. B. Cotner, "Stoichiometric flexibility in diverse aquatic heterotrophic bacteria is coupled to differences in cellular phosphorus quotas," Front. Microbiol., vol. 6, Feb. 2015, doi: 10.3389/fmicb.2015.00159.

I am a second-year PhD student in Biological Sciences at Michigan Technological University. I obtained my B.S. in Environmental Science from Northern Michigan University in 2023, and after graduation, I took a year off to gather knowledge and expertise in the fields of biogeochemistry and environmental science. I tentatively plan to defend my dissertation in the spring of 2029. The primary areas of research I will focus on in my PhD are microbial ecology, limnology, and biogeochemistry. Following a successful defense and graduation, I will participate in a post-doctorate program at either a federal or academic institution in a related field, which I expect to take 3-5 years to complete. Having held previous research positions in the federal government, I expect to do a postdoc in a federal facility. Following the completion of a post-doc, I plan to work for a federal agency such as the Department of Energy (DOE) as a scientific researcher. The total length of time should take about 10 years from now until my goal as a federal researcher. My anticipated career as a researcher would allow me to continue pursuing biogeochemistry and microbial ecology across a continuum of habitats. My overarching research interest is how biogeochemical and ecological processes change and impact one another from upland environments to freshwater environments. I have a special interest in the Great Lakes and would like to continue to incorporate the region into my future research because of the variability in lake processes, not just found between lakes but within them as well. The variation in land use, physical properties, and the differences in nutrient status between lakes offer unique insight into how biogeochemical and ecological processes interact with one another. The Great Lakes also follow a continuum of disturbance, which allows us to study a pristine system, like Lake Superior, and a heavily disturbed system like Lake Erie, which I hope to address with my proposed research and research I will conduct in my career.

The proposed research will help me reach my career goals in several ways. To begin with, just the act of submitting this proposal has given me valuable insight into what it is like being a PI and a researcher. This experience, regardless of the outcome, has provided me with the opportunity to develop methods and plan an experiment, all while considering how to budget and justify my research. This will become invaluable going forward as a researcher and is an experience I can draw from for the rest of my career. From the data I would collect, I would be able to conclude how microbial communities can possibly adapt when they experience nutrient concentrations that are different from what they typically exist in. I plan on looking at multiple ways in which communities adapt, which will guide my future research to ask more nuanced questions. I suspect that I will learn that the adaptive qualities of microbial communities are coupled, and that mechanistic lab studies may be necessary to specify how microbial communities adapt and why. Another advantageous outcome from receiving this fellowship would be the possibility of working with my identified NGO sponsor. I would effectively broaden my network and open the door to future collaborations in future projects. If given the opportunity, I believe that this fellowship would help propel me to my goal of becoming a research scientist, and allow me to conduct research in an area that I wish to establish a career in.

Date Issued: 31-JUL-2023 Institution Entity Code: 02759 OFFL Official Transcript

Record of: Connor Cian O'Loughlin Issued To: CONNOR C O'LOUGHLIN				HE A STATE OF PA	ge: 1
Course Level: Undergraduate Only Admit: Fall 2019	ERSITY	NORTHERN INVESTITY N NO.	COURSE TITLE	CRED GRD	NORTHERN PTS I
NORTHER WICHIGAN UNIVERSITY NO	5000	INO.	NIVER BY NO COURSE ITTHEN USE	CRED GRD	113 1
atest Program	Inst	itution I	nformation continued:	HERN SHAIR AN	
Bachelor of Science					
Program : BS-Environmental Science	Wint	er 2020			
College : College of Arts & Sciences	Со	llege of :	Arts & Sciences		
Major : Environmental Science	En	vironment	al Science THERN SAN U.S.		
Maj/Concentration : Natural Resources ASTY NORTHERN MICHIGAN UNIV	Co	ntinuing			
NORTHERN MICHMINORN: Biology RTHERN MICHIGAN UNIVERSITY NORTHERN MIC	BI	112 SITY N	Intro Biology: Diversity	4.00 B+	13.20
JNIVERSITY NORTHERN MICChemistry RSULY NORTHERN MICHIGAN UNIVERSITY NO	CH	111GAN U	General Chemistry 1	5.00 A	20.00
	EN	211	College Composition 2	4.00 B+	13.20
Degrees Awarded Bachelor of Science 09-MAY-2023	MU	125	Music In Society	3.00 A	12.00
Program : BS-Environmental Science		Ehrs:	16.00 GPA-Hrs: 16.00 QPts:	58.40 GPA:	3.65
College : College of Arts & Sciences	Dean	's List	MICHIGAN UNIVERSITY NORTHE IN MICH		
Major : Environmental Science	Good	Standing			
Maj/Concentration : Natural Resources		10AN 7			
Minor : Biology	Fall	2020			
Chemistry Chemistry	Co	llege of	Arts & Sciences		
Inst. Honors: Cum Laude		- FAIT 413 111	al Science		
	Co	ntinuing			
SUBJ NO. COURSE TITLE CRED GRD PTS	R CH	112	WEB: General Chemistry 2	5.00 B	15.00
	GC	202	Soils	4.00 B+	13.20
	PH	201	College Physics 1	5.00 B+	16.50
RANSFER CREDIT ACCEPTED BY THE INSTITUTION:			14.00 GPA-Hrs: 14.00 OPts:	44.70 GPA:	3.19
JICHAAN JINIVERSITA JARTUEDA MICHAAN AKIVERSITA NASTAEDI JIICHAAN VII	Good	Standing	MICHIGAN VERSIEVE VERN	TIETA SUPERSURA	Non
UL19-JUL19 APP-CEEB Advance Placement	יובאט ו	MIVED V			
Ehrs: 12.00 GPA-Hrs: 0.00 OPts: 0.00 GPA: 0.00 SPA: 0.00	Fall	2021			
MICHIGAN UNIVERSITY NORTHERN MICHIGAN UNIVERSITY NORTHERN MICHIGAN UNIV			Arts & Sciences V No. 2004		
INSTITUTION CREDIT: MARSEN NATIONAL CHICAS USAVE STAN NO SAMED NAME	100000000000000000000000000000000000000		al Science HEAN SHOER THE ORG		
INVERSITY KORTHERN MICHIGAN BINIVERSITY NORTHERN MICHIGAN UNIVERSITY RO		ntinuing	NIVE SI A NEED ET LE SE SE DE LA TIVE		
rall 2019 NVERSEY NORTHERN MICHGAN IN FER ANN ATRACHE BY HIGH	BI	210	Princ Of Ecology	4.00 A	16.00
College of Arts & Sciences	GC	210	WEB: Earth Hazards	4.00 A-	14.80
Denvironmental Science Delivers of Example 1991 United Street	GC	385	Weather And Climate	4.00 B	12.00
New First-time Freshman	(COCCO) (TOCCO)	401	Biogeography	4.00 A	16.00
BI 111 Intro Biology: Principles 4.00 A 16.00	/ /ei 🗀 u		16.00 GPA-Hrs: 16.00 OPts:	58.80 GPA:	3.67
GC 164 Human Geography 4.00 B- 10.80	Dean	's List	JIVL ISITE NORTHERN MICHGAN UNIVE	RISHTY NORTHERN	MIDHIGAN
IA 161 Calculus 1 4.00 C- 6.80		Standing			
0 101 Intro Sociology 4.00 A- 14.80	0000	Beamarns			
Ehrs: 16.00 GPA-Hrs: 16.00 OPts: 48.40 GPA: 3.02	Wint	er 2022			
ood Standing			Arts & Sciences		
*******************			al Science		
INTEREST MORE SELECTION OF WHAT COLOURS SELECTION OF SELE		ntinuing	MINISTER SERVICE IN SERVICE SERVICES		
	BI	466	WEB: Stream Ecology	4.00 A	16.00
	GC	205	Intro To Geographic Research		16.00
			THE TO GEOGRAPHIE RESEATER	1.00 A	10.00
	CC	225	Introduction To Maps ******* CONTINUED ON PAGE	2.00 A-	7.40



Joshua L. Santiago, Registrar Northern Michigan University Date of Birth: 26-SEE

Date Issued: 31-JUL-2023 Institution Entity Code: 02759 OFFL Official Transcript

GPA

3.61

Points

404.50

404.50

0.00

Page: 2

Record of: Connor Cian O'Loughlin Level: Undergraduate MICHIGAN UNIVERSITY NORTHERN MICHIGAN UNIVERSITY I			**************************************	TRANSCI ned Hrs 112.00	RIPT TOTALS GPA Hrs 112.00	
			TOTAL TRANSFER	12.00	0.00	
NOTTHERN MICHIGAN UNIVERSITY NORTHERN MICHIGAN UNIVERSITY NORTHERN SUBJ NO. NVETSITY NORTHE COURSE TITLE	CRED GRD	RSII F PTS R	OVERALL ***********************************	124.00 END OF	112.00 TRANSCRIP	
Institution Information continued: GC 235 Quantitative Methods Ehrs: 14.00 GPA-Hrs: 14.00 QPts: Dean's List Good Standing	4.00 A 55.40 GPA:	1016.00 VE H 3.95 OH H SITY NOR	THEPN MERICAN UNIVERSITY PROPERTY NORTHERN MICHIGAN L IGAL VIVERSITY NORTHERN I THE CHIGAN UNIVERSITY THERN MICHIGAN L			
Summer 2022 College of Arts & Sciences Environmental Science Continuing GC 320 WEB: Environ Policy & Reg Ehrs: 4.00 GPA-Hrs: 4.00 QPts: Good Standing	4.00 A 16.00 GPA:	16.00 4.00				
Fall 2022 College of Arts & Sciences Environmental Science Continuing						
BIOH 230 UNIVERS Plant Kingdom CHICAL MINUERSHY	4.00 A	16.00				
CH = 220 MCHICAIntro Organic Chem MCHIGAN U	5.00 A	20.00				
CHIVE 2417 NORTH Chemical Æquilibrium NORTHERN	3.00 A	12.00				
GC 335 WEB:Geographic Info Systems Ehrs: 16.00 GPA-Hrs: 16.00 QPts: Dean's List with 4.0 Good Standing	4.00 A 64.00 GPA:	16.00				
Winter 2023 College of Arts & Sciences Environmental Science Continuing						
BI 215 Principles of Evolution BI 218 Intro To Cell & Molecular Bio CH 450 Biochemistry I GC 488 Earth/Env Science Capstone Res Ehrs: 16.00 GPA-Hrs: 16.00 QPts: Dean's List Good Standing Last Standing: Good Standing	4.00 A-	12.00 16.00 14.80 16.00 3.67				
**************************************	N ******	*****),		



This official transcript does not require a raised seal.

Joshua L Santiago

Joshua L. Santiago, Registrar Northern Michigan University

Michigan Technological University

Student No: M53035278 Date of Birth: 26-SEP Date Issued: 07-AUG-2025

Record of: Connor Cian O'Loughlin

Issued To: CONNOR O'LOUGHLIN

Course Level: Graduate

Current Program

College : College of Sciences & Arts

Major : Biological Sciences

SUBJ COURSE TITLE CRED GRD PTS R

INSTITUTION CREDIT:

Summer 2024

College of Sciences & Arts

Biological Sciences

BL 5990 Master's Research in Bio 1.00 S

Sci

Total Earned Credits 1.00

Fall 2024

College of Sciences & Arts

Biological Sciences 4400

Microbial Ecology 12.00 BL 3.00 A $_{\mathrm{BL}}$ Graduate Seminar in 1.00 A 5012 4.00

2.00 A

3.00 A

8.00

12.00

Biology Scientific 5051

Writing/Publishing

MA 3740 Stat Programming and

Analysis

Total Earned Credits 9.00

Spring 2025

College of Sciences & Arts Biological Sciences

4100 Limnology 3.00 A The Scientific Profession 2.00 AB 5025 5411

Applied Data Analysis 3.00 A 12.00 5412 Data Analysis in R 1.00 A 4.00

Total Earned Credits

Fall 2025

IN PROGRESS WORK

1.00 IN PROGRESS BT. 5012 Graduate Seminar in

Biology Applied Bacterial BL 5300 3.00 IN PROGRESS Genomics

5990 Master's Research in Bio 5.00 IN PROGRESS

Sci

9.00

***** TRANSCRIPT TOTALS
Earned Hrs GPA Hrs Points
71.00 TOTAL INSTITUTION 19.00 18.00 71.00 TOTAL TRANSFER 0.00 0.00 0.00 0.00

OVERALL 19.00 18.00 ************ END OF TRANSCRIPT

> Theresa Geogues Theresa Jacques, Registrar Michigan Technological University

Page: 1





August 08, 2025

Dear selection committee,

I am writing to share my recommendation for Connor O'Loughlin as he applies for the Michigan Sea Grant Graduate Student Research Fellowship. I am a principal investigator at the Cooperative Institute for Great Lakes Research (CIGLR) and a member of the research faculty at the University of Michigan. My research group studies nutrient biogeochemistry in the Great Lakes. I reviewed Connor's proposal and prospectus and have agreed to assist his research by providing feedback and suggestions on his plans and approaches, as well as helping connect him with information resources and specialists. We have discussed several ways his proposed research could draw upon existing products from CIGLR and other regional partners, synergize with ongoing projects and monitoring, and help inform future predictive models for Great Lakes nutrient issues.

Connor's research proposal focuses on a key connection in biogeochemistry, namely the exchange of nutrients and carbon between plankton and their environment. The elemental content of plankton, in this case free-living heterotrophic bacteria, is integral to mechanistic models used to predict how the Great Lakes food webs respond to changing nutrient concentrations. This property represents a yield term (i.e., biomass per nutrient) that determines how much biological material is produced at a given nutrient concentration. Connor's proposed work could improve our understanding of this key linkage, particularly the extent to which it varies between nutrient-poor, oligotrophic environments such as Lake Superior and comparatively nutrient-rich environments in regions of Lake Huron. I expect that the product of his work will be of interest to applied researchers and modelers working to provide decision support for Great Lakes management.

Connor's proposal includes diverse and up-to-date methodological approaches. The incorporation of these tools will help connect his findings to other adjacent research areas that are important to agencies and academic researchers, including the microbial food web, 'omics-informed approaches, and modeling. His dissertation advisor and research center have the disciplinary expertise and resources to help him pursue his research aims. Connor's plans to communicate his findings and engage through conferences and publications are commensurate with his career stage and will help extend the impact of the proposed work. In his Career Goals Statement, Connor conveyed a sense of responsibility to the Great Lakes and a desire to join the regional science and management community as an agency researcher.

In working with Connor over the past several months, I have found him to be a strong communicator, an eager and thoughtful self-directed learner, and responsive to feedback. I believe these attributes will make him a productive and successful recipient of the Graduate Student Research Fellowship. Please contact me if you require additional information.

Sincerely,

Canth

Casey M. Godwin, Ph.D. Associate Research Scientist University of Michigan, School for Environment and Sustainability cgodwin@umich.edu, 734-741-2282



8 August 2025

Michigan Sea Grant Graduate Student Research Fellowship Review Committee 400 North Ingalls, Suite G241 Ann Arbor, MI 48109

Dear Review Committee,

It is my pleasure to provide this letter of recommendation for Connor O'Loughlin, a graduate student in my group who is applying for the 2025 Michigan Sea Grant Graduate Student Research Fellowship. Connor joined my lab in the summer of 2024 to begin his M.S. work on our MISG-funded project, "An Ecosystem-Scale Approach to Understanding Changing Winters in the Great Lakes". Beginning in the Fall of 2025, Connor will transition to the Ph.D. program, where he will continue his work on our MISG project.

Our project requires the coordination of multiple collaborating research teams, including the organization and supply of sampling material kits. Connor has successfully carried out that significant task, while also moving forward in his research. This demonstrates his ability to plan and coordinate complex research tasks, which will serve him well if awarded the GSRF. In addition, Connor took on methods development tasks that both serve his research goals and those of the lab, deriving a method for the BONCAT analyses which are included in his proposed GSRF work, and contributing to the lab development of organic matter analysis pipelines, which are also included in the GSRF work. Connor's progress over the past year demonstrates his ability to problem solve and ask questions that will move his work forward as he transitions into the Ph.D. program.

Connor's proposed work integrates well with our funded MISG project but adds a new and exciting dimension to it, which will inform our understanding of the responses of bacterial communities to biogeochemical change in the Great Lakes. He will be well-supported in our lab, and our location within the Michigan Tech Great Lakes Research Center means that he will have access to field logistics and equipment support to ensure the successful completion of his research.

In closing, I am pleased to support Connor's efforts as he embarks on his doctoral studies, and I am excited to see his ideas and work move forward. Since joining the Iab, Connor has demonstrated, curiosity and a desire to learn. Combined with his strong background in the environmental sciences, I believe that he is well-positioned to embark on a path to a successful research career, with support from the GSRF. I recommend him without hesitation. Please let me know if I can be of further assistance.

Sincerely,

Trista J. Vick-Majors Assistant Professor

Department of Biological Sciences Michigan Technological University

906-487-1848 tjvickma@mtu.edu



Sponsored Programs

Lakeshore Center 1400 Townsend Drive Houghton, Michigan 49931-1295 906-487-2228 • Fax 906-487-2245

August 13, 2025

Lauren Mullenbach Research Program Manager Michigan Sea Grant 400 North Ingalls, Suite G241 Ann Arbor, MI 48109

Email: lemullen@umich.edu

RE: Michigan Tech Proposal #26-0061, Title: Stoichiometric Plasticity of Heterotrophic Bacteria in the Laurentian Great Lakes: The Impact of Nutrient Concentration on Community Resilience, submitted by PhD student Connor O'Loughlin

Dear Dr. Mullenbach,

On behalf of Connor O'Loughlin, Michigan Technological University is pleased to submit a proposal for the Michigan Sea Grant 2025 Graduate Student Research Fellowship funding opportunity. The anticipated period of performance for this effort is the two-year period from September 1, 2025, through August 31, 2027.

The total proposed budget for this project is \$89,986 which includes \$59,990 requested from the sponsor in addition to \$29,996 in cost share committed as follows: Michigan Technological University, Department of Biological Sciences committing \$14,998 and Michigan Technological University, Great Lakes Research Center committing \$14,998.

For any questions, please contact Anna Walls, Pre-Award Analyst at (906) 487-2226 or sponsored-programs-l@mtu.edu.

Sincerely,

Sherri Peters Digitally signed by Sherri Peters Date: 2025.08.13 15:23:46 -04'00'

Sherri Peters

Sr. Pre-Award Analyst, Sponsored Program

Connor O'Loughlin

Michigan Technological University Biological Sciences 1400 Townsend Dr. Houghton, MI 49931 (630)-750-1165 ccolough@mtu.edu

Education

Michigan Technological University - PhD, 2029 (expected)

• Dissertation Advisor: Trista Vick-Majors.

Northern Michigan University - B.S., 2023

- Major: Environmental Science.
- Minor(s): Biology and Chemistry.
- Wildcat Achievement Award, National Academic Award.

Professional Experience

Graduate Research Assistant - Michigan Technological University, 2024 - Present

Student Research Intern - Pacific Northwest National Laboratory, 2024

Student Research Intern – Argonne National Laboratory, 2022

Fellowships and Awards

- 1st Place Paper Presentation, American Association of Geographers, 2022 (\$1,000 travel award).
- Most Outstanding Undergraduate Presentation, American Association of Geographers, 2022 (\$100 award).
- GLRC Travel Award, 2025 (\$750 travel award).

Publications and Scholarly Work

• O'Loughlin, C.C., Flynn, T.M., Kemner, K.M., Weishorn, P.B., O'Loughlin, E.J.: Inhibition of methanogenesis by 9,10-Anthraquinone-2-carboxylic acid (AQC). In preparation for *Microorganisms*.

Selected Conferences and Presentations

 "Seasonal Variation in Microbial Community Dynamics and Organic Matter in the Great Lakes", International Association of Great Lakes Researchers (IAGLR) Annual Meeting, Milwaukee, WI, June 3, 2025.

- "Chemical Inhibitor of Methanogenesis: The Complete Story", American Association of Geographers (AAG) Annual Meeting, Denver, CO, March 24, 2023.
- "Chemical Inhibitor of Methanogenesis: How Does It React in Different Environments?"
 East Lakes Division of the American Association of Geographers (ELDAAG) Annual Meeting, Muskegon, MI, October 28, 2022.
- "Investigating the Hydrological Connectivity of Forested Mitigation Wetlands Between 2019 2021, in Marquette, Michigan, USA" Joint Aquatic Sciences Meeting, Grand Rapids, MI, May 18, 2022.

Service

- Exhibitor Great Lakes Research Center (GLRC), International Association of Great Lakes Researchers (IAGLR) Annual Meeting, Milwaukee, WI, 2025.
 - I volunteered for 6 cumulative hours and was responsible for replenishing materials and answering questions concerning the GLRC.
- Poster Judge Senior Capstone, April 14, 2025
 - I volunteered for 2 cumulative hours and was tasked with judging three poster presentations.

Memberships in Associations and Institutional Affiliations

- Gamma Theta Upsilon (2022 present).
- International Association of Great Lakes Researcher (2025-Present).
- American Geophysical Union (2025-Present).
- American Association of Geographers (2022-2024).
- Society of Wetland Scientists (2022-2023).

Primary Advisor Contact Information

- Trista Vick-Majors
 - tjvickma@mtu.edu
 - 906-487-1848



MICHIGAN SEA GRANT UNIVERSITY OF MICHIGAN + MICHIGAN STATE UNIVERSITY

NOAA DATA SHARING DIRECTIVE POLICY

Data and information collected and/or created under NOAA grants and cooperative agreements must be made visible, accessible, and independently understandable to general users, free of charge or at minimal cost, in a timely manner (typically no later than two years after the data are collected or created), except where limited by law, regulation, policy or by security requirements. The requirement has two basic parts: (1) environmental data generated by a grant project must be made available after a reasonable period of exclusive use, and (2) the grant application must describe the plan to make the data available (Principal Investigators are expected to execute the plan).

If your project produces environmental data, it must conform to NOAA's Data Sharing Directive for Grants, Cooperative Agreements, and Contracts. For detailed guidance, you can view the current version of the policy, including a definition of environmental data (which can include socioeconomic and model data), download any updates and access additional implementation resources at the following permanent URL (Appendix B outlines requirements):

https://seagrant.noaa.gov/Portals/1/Guidance/Data Sharing Directive v3.0 remediated 20 18-06-07.pdf.

Proposals submitted in response to this Announcement must include a Data Management Plan describing how these requirements will be satisfied. To comply with this requirement, the Principal Investigator must use the form below to explain how the data and metadata will be provided. Please complete the form, including information for all applicable datasets related to your project(s). If funding is required for data curation and archiving, please make sure that funds are budgeted in the project proposal for data management. All data generated through Sea Grant funded projects are required to be completely QA/QC'ed (Quality Assurance and Quality Control) and made publicly accessible **by two years after the end date of the project**. If the proposed research will not generate environmental data then a Data Management Plan will need to be stated as such: "This project will not generate any environmental data."

Sea Grant Data Management Plan Form Proposal Submission Phase

Title of the Proposal (required answer):

Stoichiometric Plasticity of Heterotrophic Bacteria in the Laurentian Great Lakes: The Impact of Nutrient Concentration on Microbial Community Adaptation

Name of lead PI (required answer): Sea Grant requires that the lead PI serve as the data steward.

Connor O'Loughlin

Contact Information (required answer):

Email: <u>ccolough@mtu.edu</u>

Cell: 630-750-1165

Address: 1400 Townsend dr. Houghton, MI 49931

Dataset Description(s) (required answer): What data will the dataset(s) contain? This includes descriptive details on data types, inclusion of metadata, data format(s), collection times / date ranges, etc. What name(s), if any, will be designated to the dataset(s)?

The dataset for this project will comprise limnological, chemical, and biological data collected from the proposed experiment, and will be presented in a tabular format. Specific data types include water depth profiles of light (photosynthetically active radiation), conductivity, temperature, and dissolved oxygen, collected via handheld sensors and sondes, microbial community assemblage data from 16S rRNA gene sequencing, dissolved organic matter (mg C/L), TDN (mg N/L), organic and inorganic nitrogen (mg/L), soluble reactive phosphorus (μ g P/L), absorption values from Biolog EcoPlates, tabulated microbial abundance (cells/mL) and absorption data from flow cytometry, particulate carbon, nitrogen, and phosphorus (mg/L), translationally active cells (cells/mL) and inactive cells (cell/mL) from BONCAT, and dissolved organic matter quality (excitation-emission matrix spectroscopy shown as fluorescence indices).

Do you agree to release all data no later than 2 years after the end-date of the project? (required answer):

Yes

Issues (required answer): Are there any legal, access, retention, etc. issues anticipated for the dataset? If yes, please explain.

None

Data Size: What will be the estimated size of the dataset? Please report estimated number of MB, GB, TB, etc., collected.

Sequence data: 6.2 GB; All other data 1 GB

Data Format: What format will the dataset utilize? (i.e., Excel file, model code, audio/video recording, etc.)

16S sequence data will be FASTA, .csv, and all other data will be .csv or .xlsx

Ownership (required answer): Who will own the dataset, if not the lead PI?

Trista Vick-Majors (CO-PI)

Post-Processing: What post-processing, QA/QC will this dataset undergo? Who will be responsible for performing this post-processing and QA/QC to prepare the dataset for its deposition into a repository?

Data from field and laboratory notebooks, and experimental data will be checked for outliers using R statistical software. Post-processing will be the responsibility of Connor O'Loughlin (PI). The 16S rRNA sequence data will be QC'd and processed using the DADA2 pipeline in the R software package. The PI is responsible for deposition into a repository.

Preservation Plan (required answer): What data repositories will be used to host the dataset? If none, how will the data be preserved?

The data will be made available in 2 years following the completion of the study, or when the results get published. A public repository on GitHub will be made available, with the URL link being provided in the publication.

Products: Will any information or data products be developed from this dataset? How will the related costs be supported? Which organization(s) will be producing these products?

We anticipate one peer-reviewed publication to be submitted to

Other Comments: Are there any additional comments related to the data that will results from your Sea Grant-funded study?

Sea Grant Data Management Form *Project Completion Phase*

Date Submitted (required answer):
Title of the Proposal (required answer):
Name of lead PI (required answer): Sea Grant requires that the lead PI serve as the data steward.
Contact Information (required answer):
Dataset Description(s) (required answer) : What data do the dataset(s) contain? This includes details on data type, format, collection times / date range, etc. What name(s), if any, will be designated to the dataset(s)?
Issues : Are there any legal, access, retention, etc. issues existing for the dataset(s) (i.e.; IRB restrictions)?

Data Size : What is the estimated size of the dataset? Please report estimated number of MB, GB, TB, etc., collected.
Data Format : What format(s) do(es) the dataset(s) utilize? (i.e., Excel file, model code, audio/video recording, etc.)
Ownership (required answer): Who owns the data, if not the lead PI?
Post-Processing : What post-processing, QA/QC has this data undergone? What organizations performed this post-processing and QA/QC to prepare the data for its deposition into a repository?
Preservation Plan (required answer) : What data repositories were used to host the dataset? If none, how was the data preserved? Please provide URL for any data repositories that were used to preserve this data and any necessary information needed to extract the data.
Keywords (required answer) : Please provide a list of terms used to query the database.
Release Date (required answer) : When will this dataset be available to the public? Reminder: the release date must be no later than 2 years after the end of the project.

Products (required answer) : Have any information or data products been developed from this dataset? Which organization(s) produced these products? Please provide a location for any products that were produced as a result of this project.
Preferred Data and Product Citations (required answer) : How to reference data, publications, or any other project outcomes?
Other Comments: Are there any additional comments related to the data that you produced with your Sea Grant funding?

ABBREVIATED NOAA ENVIRONMENTAL COMPLIANCE QUESTIONNAIRE

Instructions: Answer EVERY question in the yellow square below it.

Questions are selected from the full 81-question NOAA Environmental Compliance Questionnaire (available at www.noaa.gov/nepa), as such the numbers below are for reference here and do not reflect the numbering in the full questionnaire.

Note: The state Sea Grant program is responsible for ensuring that the information below is filled out appropriately. It is the responsibility of the program to review materials submitted by applicants and ensure that the questionnaire provides an adequate level of detail. Please use the <u>step by step guide</u> (on <u>Inside Sea Grant</u>) to determine if an adequate level of detail has been provided for each question. If an applicant states that a permit is needed, ensure a copy of the full permit is submitted with the questionnaire (as required per the permit question).

Refer to the relevant Notice of Funding Opportunity for when this questionnaire must be completed and submitted with your application package.

Grant number and/or Project ID (if available)

26-0061-P0001

Project Title

Stoichiometric Plasticity of Heterotrophic Bacteria in the Laurentian Great Lakes: The Impact of Nutrient Concentration on Community Resilience

Name and contact information for the person completing this form

Connor O'Loughlin email: ccolough@mtu.edu

State Sea Grant Program

Michigan

PROPOSED ACTIVITY

- 1. Describe the proposed activity, including:
 - Explain the purpose, objectives, and goals; and
 - Explain whether the proposed activity would occur in different locations and/or have multiple phases.

We are proposing an *in situ* reciprocal transplant experiment that will take place in Lakes Superior and Huron. The goal of this project is to evaluate microbial community adaptation to changes in nutrient conditions by collecting water from one lake and transplanting it into a different lake with different nutrient conditions. The objectives are to characterize the response of microbial stoichiometry to changes in particulate and dissolved organic matter, and to evaluate microbial community adaptation to altered nutrient concentrations and environmental conditions. The purpose behind all of this is to provide additional knowledge relating to biogeochemical and ecological processes in the Great Lakes and to help inform resource management efforts regarding water quality.

- 2. Is the proposed activity a continuation or part of an ongoing activity? If yes, then:
 - Describe any changes to the proposed activity since it was initiated, including progress toward achieving its objectives/goals; and
 - Provide any additional information, previous environmental review documents, and/or reports from previous years.

No

3. Describe sampling, collecting, or observation protocols and operational procedures.

Initial samples will be collected at the start of the incubation at each lake. Approximately 6 L will be filtered through a 10 μ m filter (to remove larger particulate and grazers), and subsequently through a 3 μ m filter to collect particulate carbon, nitrogen, and phosphorus in the seston fraction. Samples will be collected to perform BONCAT and to evaluate carbon substrate utilization and microbial abundance. The remaining water will be filtered again through a 0.22 μ m Sterivex to be analyzed for DNA, and the rest of the water will be filtered with a 0.7 μ m filter to collect particulate carbon, nitrogen, and phosphorus in the bacterial fraction. The remaining water will contain the dissolved fraction and will be analyzed in triplicate for dissolved organic carbon, fDOM, total dissolved nitrogen, soluble reactive phosphorus, and nitrogen species. Then, water will be collected from one lake and transported to another lake. The water will be filtered through a 10 μ m filter to remove grazers and larger particulate matter. The filtered water will then be placed into 12-14 kDa MWCO dialysis bags, measured to hold 500 mL each. At the end of the incubation, the dialysis bags will be destructively sampled in the same fashion as described above. The fieldwork will be conducted at Whitefish Bay and St. Martin's Bay and will last for a total of 14 days.

4. Will the proposed activity require the cataloging and compiling of sources of socioeconomic data? If yes, then please explain.

No

5. Does the proposed activity consist solely of software research and manipulation? If yes, please explain.

No

6. Does the proposed activity utilize a new or untested scientific technology or method? If yes, then describe briefly the technological process or methodology and potential environmental effects of the proposed activity.

No

7. For the proposed action, what amount (total numbers and/or weight) of fish or invertebrates are proposed to be caught? What is the size (weight, length, and age class) of each species targeted for capture?

NA

8. List non-target species that may occur in the proposed sampling area and specify how many of each non-targeted species are expected to be caught.

NA

9. Would the proposed activity introduce genetically modified organisms, species bred for specific traits (e.g. disease resistant stocks), or non-indigenous species into an area?

No

10. Describe the data processing methods to be used to conduct the research.

The particulate carbon and nitrogen will be sent to Timothy Wahl at the University of Wisconsin-Milwaukee, DNA samples will be analyzed using 16S rRNA gene sequencing at Integrated Microbiome Research. The particulate phosphorus, DOC, TDN, SRP, and nitrogen species samples will be sent to the AQUatic Analysis lab. Finally, the samples collected for BONCAT, Biolog Ecoplates, flow cytometry, and fDOM will be analyzed by the PI. All data will undergo QA/QC before undergoing data analysis.

LOCATION

11. Describe the proposed activity location, including, if available and appropriate, geographic coordinates (latitude, longitude in DD MM.MMM), river mile markers, etc. for all distinct phases of the proposed activity.

The two lakes that we decided to experiment on are Lake Superior and Huron. The proposed site for Lake Superior is located at Whitefish Bay, which is roughly 46° 26.80914' north and -84° 48.01644' west. The proposed site for Lake Huron is St. Martin's Bay, which is roughly 46° 2.14506' north and -84° 37.02054' west.

12. Are there pre-existing or ongoing uses at the location of the proposed activity? If yes, then describe and explain the previous or ongoing uses at the location of the proposed activity or, if not known, describe how previous or ongoing uses will be determined.

To our current knowledge, both sites are used for recreation and have been used for scientific research.

- 13. Describe the characteristics of the location of the proposed activity:
 - Indicate degree to which the location has been disturbed. Examples include highly developed, light development, active harbor use, public beach, open space, etc.
 - Indicate whether the area is a unique geographic area of notable recreational, ecological, scientific, cultural, historical, scenic, economic, or aesthetic importance;
 - Identify ESA-listed and/or MMPA species that may occur and overlap with the proposed activity;
 - Describe any anticipated changes over time to the natural landscape and/or viewshed that would result from the proposed activity;
 - List any ecologically significant or critical (e.g., spawning, nursery, or foraging grounds) areas in the location of the proposed activity, including areas that are normally inundated by water (wetlands including permanent or temporary wetlands) or other aquatic habitat or areas within the 100-year flood plain;
 - List any designated Essential Fish Habitat and Habitat Areas of Particular Concern designated under the Magnuson-Stevens Fishery Conservation and Management Act;
 - List any critical habitat areas for Endangered Species Act-listed species;
 - List any marine protected areas including national marine sanctuaries and national marine monuments in the location of the proposed activity;
 - List any National Wildlife Refuge areas, wild or scenic rivers, wetlands, or prime/unique farmland in the location of the proposed activity;
 - List any properties listed or eligible for listing on the National Register of Historic Places,

- National Historic Landmarks, or National Monuments; and
- List any religious or cultural sites of any federally recognized Indian Tribes or Native Hawaiian organizations in the proposed activity area.

Whitefish Bay is minimally disturbed. There are some public beaches along the coast and one USDA campsite. The Whitefish Bay site is also located close to the Pendills Creek National Fish Hatchery, which is listed as a National Wildlife Refuge.

- St. Martin's Bay is located close to a major interstate (I-75). There are residential homes along the shore and a highway that follows the coastline.
- 14. Are there minority or low-income communities located in the area of the proposed activity? If yes, then describe how the minority or low-income communities may be impacted by the proposed activity.

No

PROJECT PARTNERS, PERMITS AND CONSULTATIONS

15. List all other interested or affected Federal, state, and local agencies, Native American tribes or Native Hawaiian organizations, non-governmental organizations, and private individuals that may potentially be interested and/or affected by the action.

NA

- 16. Are Federal, state, or local permits, authorizations, waivers, determinations, or consultations required in order for the proposed activity to begin? If yes, then:
 - List and provide the status of all required Federal, state, or local permits, authorizations, waivers, determinations, conditions, and consultations, as applicable; and
 - Provide copies of all required Federal, state, or local permits, authorizations, waivers, or determinations that you have secured.

None

SAFETY

17. Describe potential unique or unknown risks to human health or the environment from the proposed activity.

None

- 18. Describe the potential to generate, use, store, transport, or dispose of hazardous or toxic substances. Please include the following:
 - A list of any hazardous substances (as defined by 29 C.F.R. 1910.120(a)(3)) that will be involved in this project and any hazardous wastes (as defined by 40 C.F.R. 261.3) that could potentially be generated during the proposed activity;
 - Any hazardous contaminants that may be uncovered and/or disturbed by the proposed activity;
 - A list of the procedures/protocols that will be followed to ensure safe handling, storage, use, collection and transport of hazardous substances and proper disposal of all hazardous wastes.

None

EQUIPMENT

19. If the proposed activity involves the use of any specialized equipment that may introduce sound into the environment, then provide a description of the noise(s), including frequency (Hz), sound pressure level (dB), amplitude (dB), angle (or degrees) radius the noise may travel from the source, and other relevant technical specifications. Compare the noise(s) generated by the proposed activity with ambient noise conditions, if known. Also, discuss the length of time and frequency of occurrence that the noise is expected to be introduced into the environment. In addition, the introduction of anthropogenic sound sources into the aquatic environment has the potential to modify the acoustic soundscape of the environment, as well as result in the direct exposure of fish, ESA-listed species, MMPA protected species, and/or other marine species, to elevated levels of underwater noise, which in turn, has the potential to result in physiological and behavioral impacts to these species. Given this, an assessment of the acoustic impacts to these species, as well as the acoustic soundscape of the affected environment, will need to be provided.

NA

AQUACULTURE (IF APPLICABLE)

20. Would the proposed activity be conducted in a closed system mesocosm/aquaculture facility or in open water (coastal or Federal waters), or in a Hawaiian fishpond?

NΑ

21. If using aquaculture gear, describe whether gear would be deployed short-term (1-2 years) or long-term (2+ years) and describe the number of cages/nets, lines, anchors, etc. that would be used during the course of the study. What type and size of cages/nets, lines, anchors, etc. would be used? How often would the aquaculture facility be serviced or tended?

NA

22. What amount (total numbers and/or whole weight in pounds) of fish or invertebrates are proposed to be captured for culture purposes (i.e., broodstock)? What is the target size (weight and length) and age class of each species to be captured for culture purposes?

NA

23. What amount (total numbers and/or whole weight in pounds) of fish or invertebrates are proposed to be cultured? What is the estimated size (weight and length) and age class of each species targeted for harvest at the end of each culture period?

NA

MARINE DEBRIS (IF APPLICABLE)

24. Will the proposed activity identify, determine sources of, assess, prevent, reduce, remove, dispose, or recycle marine debris? If yes, then describe the targeted debris type, debris condition, and why the proposed action is needed in the project area.

NA

25. Describe the disposal technique and the extent to which recycling, reuse, or other sustainable disposal alternatives were considered.

NA

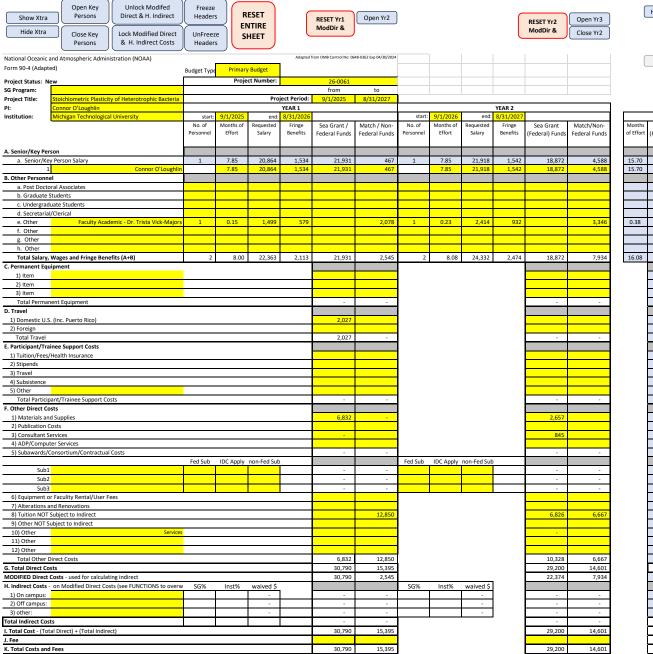
26. If marine debris is to be collected in a single area or facility, describe who would be responsible for disposal and what permits would be required to collect, store, and dispose of the collected marine debris.

NA

Paperwork Reduction Act Statement

Because this Questionnaire is intended for members of the public, NOAA must use the Questionnaire in accordance with the Paperwork Reduction Act ("PRA"; 44 U.S.C. §§ 3501–3521). Congress passed the PRA to minimize the paperwork burden for nonfederal entities and members of the public that can result from the collection of information by or for the federal government. The PRA is administered by the Office of Management and Budget (OMB), which has reviewed and approved the Questionnaire (OMB Approval No. 0648-0538).

Notwithstanding any other provisions of the law, no person is required to respond to, nor shall any person be subjected to a penalty for failure to comply with, a collection of information subject to the requirements of the Paperwork Reduction Act, unless that collection of information displays a currently valid OMB Control Number.



HIDE FUNCTIONS

SHOW

	TOTAL	
Months	Sea Grant	Matching/Non-
of Effort	(Federal) Funds	Federal Funds
	(reactar) runus	i ederari dilas
15.70	40,803	5,055
15.70	40,803	5,055
	-	-
	-	-
	-	-
		-
0.38	-	5,424
	-	
16.08	40,803	10,479
10.00	40,003	10,473
	-	-
	-	-
	-	-
	-	-
	2,027	-
	-	-
	2,027	-
	,	
		_
	-	-
		-
	-	-
	-	-
	-	-
	9,489	-
	-	-
	845	-
	-	-
	-	-
		-
	-	-
	6,826	19,517
	-	-
	-	-
	-	-
	-	-
	17,160	19,517
	59,990	29,996
	53,164	10,479
	-	-
	-	-
	-	-
	-	-
	59,990	29,996

59,990

29 996

A. Personnel:

Position Title Name	Year 1 Salary % of Time # of Mos Amount	Year 2 Salary % of Time # of Mos Amount		Cost Share
	\$29,979	\$31,479		
	34.6%	34.6%		
PI: Connor O'Loughlin	4.16 mos	4.16 mos		
Graduate Research Assistant	\$10,432	\$10,493	\$16,518	\$4,407
	\$22,168	\$24,317		
	30.8%	30.8%		
PI: Connor O'Loughlin	3.69 mos	3.69 mos		
Hourly Graduate Student	\$10,432	\$11,425	\$21,857	
	\$87,714	\$87,714		
	1.3%	1.9%		
	.15 mos	.23 mos		
Co-PI: Dr. Trista Vick-Majors	\$1,499	\$2,414		\$3,913

Personnel Justification:

<u>PI – Connor O'Loughlin:</u> This position oversees the general operation of the project and is responsible for executing fieldwork and data analysis. Responsibilities include sample materials acquisition, sample collection, data analysis, data formatting, and data interpretation. This position is responsible for making sure data is made available and that the project meets all deadlines and relates to all program objectives. Connor O'Loughlin will work as a Graduate Research Assistant during the spring of Y1 and Y2, and as an hourly graduate student during the summers of Y1 and Y2, for a total of \$42,782 over 24 months. Co-PI, Dr. Vick-Majors will advise the PI during the project for a total of \$3,913. The total requested amount is \$38,375 with cost share of \$8,320. These rates include a 5% annual escalation for stipends and a 5% escalation for tuition costs for budget estimating. A 5% increase is also included for the Co-PI for year 2 for standard budget estimating.

B. Fringe:

Category	Regular Non-Student Employees	Graduate Students
FICA	7.6%	0.0%
TIAA and Fidelity Retirement	7.7%	0.0%
MPSERS (State Retirement)	0.2%	0.0%

Health Care	18.3%	0.0%
Life, Disability, Unemployment, WC,		
Other	1.3%	0.0%
VHS and Short Term Disability	3.5%	0.0%
Graduate Student Health Insurance	0.0%	14.7%
Total Fringe Benefit Expenses	38.6%	14.7%

		Year 1 Salary	Year 2 Salary		
Personnel (Role)	% Rate	Fringe	Fringe	Request	Cost Share
		\$10,432	\$10,493		
PI: Connor O'Loughlin	14.7%	\$1,534	\$1,542	\$2,428	\$648
		\$1,499	\$2,414		
Co-PI: Trista Vick-Majors	38.6%	\$579	\$932	\$0	\$1,511

Fringe Justification:

Michigan Tech will collect fringe benefits at rates approved by the University's federal cognizant agency, the Office of Naval Research.

<u>PI – Connor O'Loughlin:</u> The fringe benefit rate for graduate students is 14.7 % when paid as a GRA. No fringe is requested for hourly pay during Y1 and Y2.

<u>Co-PI – Dr. Trista Vick-Majors:</u> The fringe rate for faculty is 38.6%.

C. Domestic Travel:

Expense Item	Request
Gas - 18 mpg, \$3.20 per gallon, 86 gallons, 1490 miles	\$275
Car Rental – Husky Motors, 3 trips, 1st trip: 4 days total, 2nd trip: 2 days total, 3rd trip: 2 days total; \$95/day rental fee	\$760
Lodging (5 nights @ \$110/night) 3 nights on trip 1 and 1 night on trips 2 and 3.	\$550
Meal Allowance (\$55.25/day)	\$442

Domestic Travel Justification:

Trip 1: The PI will travel from Houghton to Whitefish Bay, MI and Saint Martin's Bay, MI, over 4 days, to begin the incubations and collect samples. There will be two additional trips to the field sites to gather samples. One will be at the midpoint of the incubations, and one final trip to

terminate the incubations, each being 2 days in duration. A compact SUV will be rented from Michigan Tech Husky Motors and driven to the field sites each trip. Total travel expenses requested are \$2,027. Expenses are based on online rates, GSA for lodging, and historical information.

D. Equipment:

None Requested.

E. Supplies:

Expense Item	Count	Unit Cost	Request
Sterivex Filter	50	\$10.22	\$511.00
60 mL Luer Lock Syringe	2	\$40.95	\$81.90
40 mL Amber vial	72	\$1.37	\$98.64
3 μm filter (47 mm)	100	\$2.45	\$245.00
60 mL Luer slip syringe	2	39.25	\$78.50
25 mm filter holder	2	\$40.00	\$80.00
10 μm PC filter (47 mm)	100	\$1.84	\$184.00
0.7 μm GF/F filter (25 mm)	100	\$2.17	\$217.00
Biolog Ecoplate	2	\$221.40	\$442.80
47 mm filter holder	18	\$96.00	\$1,728.00
50 mL centrifuge tubes	1	\$367.50	\$367.50
12-14 kDa MWCO, 75mm Dialysis tube	2	\$328.00	\$656.00
ZymoBiomics DNA Kit	1	\$319.70	\$319.70
CuSO4 solution	5	\$8.96 (per mg)	\$44.80
Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine	3	\$142.50	\$427.50
Sodium Ascorbate	1	\$126.50	\$126.50
Aminoguanidine	25	\$2.94 (per gram)	\$73.50
Carboxyrhodamine 110 Alkyne	1	\$103.00 (per mg)	\$103.00
L-Azidohomoalanine	5	\$78.80 (per mg)	\$394.00
15 mL centrifuge tubes	1	\$465.50	\$465.50
2 mL centrifuge tubes	1	\$418.48	\$418.48
2 mL centrifuge tubes	2	\$46.33	\$92.66
Universal Dialysis Tubing Closure	16	\$145.80	\$2,332.80

Total \$9,48	89
--------------	----

Supplies Justification

All supplies listed will be used for sample collection, processing, and analysis. Each supply item is essential to the study. The prices were obtained from the supplier's website.

F. Contractual:

Service	Request
16S rRNA Analysis – Integrated Microbiome Resource (\$30.00 per sample X 18 samples)	\$540.00
Particulate C & N – AQUatic Analysis Lab (\$6.00 per sample X 6 samples)	\$36.00
Particulate P – AQUatic Analysis Lab	\$37.20
Soluble reactive Phosphorus – AQUatic Analysis Lab (\$6.20 per sample X 18 samples)	\$111.60
DOC/TN – AQUatic Analysis Lab (\$6.65 per sample X 18 samples)	\$119.70

Contractual Justification

A total of \$845 is requested for services needed to meet the project's needs. The following services will be provided by the AQUatic Analysis lab: particulate C, N, & P, soluble reactive phosphorus, and DOC/TN. Integrated Microbiome Resource will provide service for 16S rRNA analysis.

G. Construction:

None Requested.

H. Other direct costs:

Other Budget Items	Year 1	Year 2	Request	Cost Share
Tuition	\$ 12,850	\$ 13,493	\$ 6,826	\$ 19,517

Other Justification

Tuition expenses and fees are requested as a component of Graduate Research Assistant (GRA) compensation. Graduate student tuition for the Spring semesters of 2026 and 2027 totals \$26,343 to support Michigan Technological University's PhD program requirements. A cost-share of

\$12,850 will be provided by the Great Lakes Research Center in year 1 and \$6,667 by the Department of Biological Sciences in year 2 to off-set total tuition costs. Total support requested in Year 1 is \$0 in Sea Grant funds, and in year 2 is \$6,826.

I. TOTAL DIRECT COSTS:

A total of \$59,990 is requested for direct costs, and \$29,996 is provided in cost share.

Budget Category	Request	Cost Share
A. PERSONNEL	\$38,375	\$8,320
B. FRINGE	\$2,428	\$2,159
C. TRAVEL	\$2,027	
D. EQUIPMENT	\$0	
E. SUPPLIES	\$9,489	
F. CONTRACTUAL	\$845	
G. OTHER	\$6,826	\$19,517
	\$59,990	\$29,996

J. INDIRECT COSTS

No indirect costs are requested.

K. TOTAL DIRECT AND INDIRECT COSTS:

Total direct and indirect costs are \$59,990.

L. COST SHARE:

Budget Category	Year 1	Year 1 Source	Year 2	Year 2 Source
		Department of		Department of
Personnel	\$1,906	Biological Sciences	\$6,414	Biological Sciences
		Department of		Department of
Fringe Benefits	\$639	Biological Sciences	\$1,520	Biological Sciences
		Great Lakes		Great Lakes
Other - Tuition	\$12,850	Research Center	\$2,148	Research Center
				Department of
Other - Tuition	\$0	N/A	\$4,519	Biological Sciences
Total Cost Share	\$15,395		\$14,601	
Total Cost Share by Source	\$14,998	Great Lakes Research Center	\$14,998	Department of Biological Sciences

Cost Share Justification

Cost share will be provided for a portion of the GRA's stipend (\$4,407), faculty academic time (\$3,913), the associated fringe benefits (\$2,159), and a portion of tuition (\$19,517) for a total of \$29,996. Cost share will be split between the Department of Biological Sciences and the Great Lakes Research Center.