***Title***: Stoichiometric Plasticity of Heterotrophic Bacteria in the Laurentian Great Lakes: The Impacts of Winter and Nutrient Concentration on Community Resilience

***Background:*** The Laurentian Great Lakes represent the largest freshwater ecosystem on Earth and support countless human populations and their economies [1]. Of particular concern are the commercial fisheries that have been in decline since the 1940s, which have been attributed to overfishing, invasive species, chemical pollution, and declining nutrient levels [2]. Much of what we know about nutrient levels and cycling in the Great Lakes is limited to spring and summer, with few *in situ* Great Lakes studies representing winter processes [3]. Recent work has brought to light the vital role that winter ecological and biogeochemical processes play in year-round conditions, with impacts that are felt in the subsequent spring and summer [4], [5], [6], [7]. Changing winter conditions can upset normal lake processes and can have cascading effects on ecological and biogeochemical processes that can, in turn, threaten the Great Lakes’ water quality and health of biological communities [4]. It is important to understand how microbial communities will react to different environmental conditions because microbes exist at the base of many food webs and are responsible for recycling nutrients and organic matter in aquatic systems (need a citation). It is because of their trophic position and integral part of biogeochemical cycling, potential changes to those communities can be felt throughout the ecosystems they reside in (need another citation here). Microbial communities can fluctuate in their assemblages [8], [9], their metabolism [10], morphology [11], and stoichiometry [12], [13] as responses to environmental variables such as temperature, dissolved organic matter (DOM), and nutrient availability. Globally, DOM inputs into northern lakes have increased, and the consequences are relatively unknown [14]. The current evidence shows that increased DOM inputs are expected to change the availability of macronutrients (C, N, P) and several micronutrients (Fe, Mn, etc.) [15], which in turn influence the stoichiometry of biological communities such as phytoplankton and bacteria. Additionally, the stoichiometry of bacterial communities has also been shown to be coupled with temperature [16]. The interplay between annual variation in winter variables (such as ice cover and water temperature), seasonal variation, and increased DOM inputs is not well understood, and we hope to provide additional insight with our research.Winter is an integral component of annual limnological processes, the better we will be able to apply effective measures to manage the Great Lakes and maintain healthy

***Goals and Hypothesis:*** The goal of my proposed research is to investigate how seasonality impacts microbial communities, with an emphasis on the transition from winter to spring. **Objective 1:** Characterize the response of microbial stoichiometry to changes in particulate organic matter in conjunction with seasonal changes in temperature **Hypothesis 1 (H1):** Oligotrophic systems will be more susceptible to temperature effects on bacterial stoichiometry, shown by an increase in P content and cell size. **Hypothesis 1 (H2):** Less severe winters, will show a moderate response in stoichiometric plasticity. **Objective 2:** evaluate microbial community resilience to altered nutrient concentrations and environmental conditions **Hypothesis 3 (H3):** oligotrophic systems will have a lower functional redundancy when compared to eutrophic systems, marked by more specialized taxa when compared to eutrophic systems making oligotrophic systems less resilient to changes in environmental conditions.

***Relation to MISG strategic plan:*** The proposed research 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, Desirec 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”.

***Experimental Design:* Experiment 1:** To investigate **H1 and H2,** bacterial communities from Lake Superior, Huron, and Erie will be collected, and an *in situ* reciprocal transplant experiment using dialysis bags will be conducted. Lake Superior and Huron communities will be swapped as well as Lake Huron and Lake Erie, to establish a trophic gradient. The bacterial response to being transplanted into a different environment will be tracked throughout 48 hrs with sub-sampling taken at intervals within that time. Samples will be analyzed for particulate C, N, and P in the bacterial communities and the water inside the dialysis bag. Water samples from the host lake will also be collected for analysis at the beginning and end of the incubation period. Additionally, morphological traits will be determined via flow cytometry, and changes in community assemblage will be analyzed using 16S rRNA gene sequencing. Physical and chemical parameters such as ice cover (when applicable), dissolved oxygen, pH, and water temperature will also be measured. To measure DOM, 0.45 mm filtered water samples will be analyzed for dissolved organic carbon (DOC) and total nitrogen (TN) using a Shimadzu TOC-L, and fluorescent dissolved organic matter will be measured by fluorescent microscopy and excitation-emission matrices using a Horiba Aqualog. **H3** will be conducted similarly to **H1** and **H2**, but field work will not be conducted until the following winter (2027) to record annual variation in winter conditions. Winter severity will be measured in both experiments 1 and 2 and will be classified as ice depth (cm), as ice depth is closely linked to water temperature.

***Timeline:*** Sampling for experiment 1 will be done in January and May of 2026. Data analysis for experiment 1 will be done in the fall of 2026. Field work for experiment 2 will be conducted during January and May of 2027, with data analysis being conducted in the fall of 2027. In Spring 2028, a manuscript will be drafted. I will tentatively defend my dissertation in spring 2029.

***Products:*** The outlined experiments and their findings will be incorporated into my dissertation as a chapter and presented the 2028 IAGLR meeting. Finally, a manuscript will be prepared and submitted to the *Journal of Great Lakes Research.*

***References:***

[1] E. Rau, C. Riseng, L. Vaccaro, and J. G. Read, “The Dynamic Great Lakes Economy: Employment Trends From 2009 To 2018”, Accessed: Jul. 11, 2025. [Online]. Available: https://repository.library.noaa.gov/view/noaa/38612

[2] J. C. Hudson and S. S. Ziegler, “Environment, Culture, and The Great Lakes Fisheries\*,” *Geogr. Rev.*, vol. 104, no. 4, pp. 391–413, Oct. 2014, doi: 10.1111/j.1931-0846.2014.12041.x.

[3] G. Pu *et al.*, “The Great Lakes Winter Grab: Limnological data from a multi-institutional winter sampling campaign on the Laurentian Great Lakes,” *Limnol. Oceanogr. Lett.*, vol. 10, no. 1, pp. 37–61, 2025, doi: 10.1002/lol2.10447.

[4] S. E. Hampton *et al.*, “Ecology under lake ice,” *Ecol. Lett.*, vol. 20, no. 1, pp. 98–111, 2017, doi: 10.1111/ele.12699.

[5] D. Özkundakci, A. S. Gsell, T. Hintze, H. Täuscher, and R. Adrian, “Winter severity determines functional trait composition of phytoplankton in seasonally ice-covered lakes,” *Glob. Change Biol.*, vol. 22, no. 1, pp. 284–298, 2016, doi: 10.1111/gcb.13085.

[6] U. Sommer *et al.*, “Beyond the Plankton Ecology Group (PEG) Model: Mechanisms Driving Plankton Succession,” *Annu. Rev. Ecol. Evol. Syst.*, vol. 43, no. Volume 43, 2012, pp. 429–448, Dec. 2012, doi: 10.1146/annurev-ecolsys-110411-160251.

[7] Z. Wen *et al.*, “Variability of chlorophyll and the influence factors during winter in seasonally ice-covered lakes,” *J. Environ. Manage.*, vol. 276, p. 111338, Dec. 2020, doi: 10.1016/j.jenvman.2020.111338.

[8] 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.

[9] 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.

[10] “Global emergent responses of stream microbial metabolism to glacier shrinkage | Nature Geoscience.” Accessed: Jul. 14, 2025. [Online]. Available: https://www.nature.com/articles/s41561-024-01393-6

[11] 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, Jul. 2015, doi: 10.1098/rspb.2015.0371.

[12] 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.

[13] 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.

[14] “Global Change Biology - 2018 - Creed - Global change‐driven effects on dissolved organic matter composition Implications (1).pdf,” Google Docs. Accessed: Jul. 11, 2025. [Online]. Available: https://drive.google.com/file/d/1KbxTHAGoV\_vm-eiIJz7KaVglZzLbarnI/view?usp=sharing&usp=embed\_facebook

[15] “A test of the subsidy–stability hypothesis: the effects of terrestrial carbon in aquatic ecosystems - Jones - 2015 - Ecology - Wiley Online Library.” Accessed: Jul. 14, 2025. [Online]. Available: https://esajournals.onlinelibrary.wiley.com/doi/10.1890/14-1783.1

[16] K. N. Phillips, C. M. Godwin, and J. B. Cotner, “The Effects of Nutrient Imbalances and Temperature on the Biomass Stoichiometry of Freshwater Bacteria,” *Front. Microbiol.*, vol. 8, Sep. 2017, doi: 10.3389/fmicb.2017.01692.