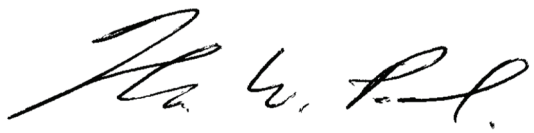
Using stable isotopes to identify fertilizer-derived nitrogen (N) assimilation in microcystins

Malcolm A. Barnard

Budget:

Budget Item	Cost Per Unit	Number of Units	Total Cost
^{15}N microcystins analysis	$100	21	$2100
1 g Sodium nitrate-^{15}N ≥98 atom % ^{15}N (Sigma-Aldrich 364606-1G)	$80.60	1	$80.60
1 g Ammonium-^{15}N chloride ≥98 atom % ^{15}N (Sigma-Aldrich 299525-1G)	$77.50	1	$77.50
250 mg Urea-^{15}N2 98 atom % ^{15}N, 99% (Sigma-Aldrich 316830-250MG)	$99.80	2	$199.69
Shipping Samples to UTK	$43	1	$43
Total			$2499.79
Total Requested			$2500.00



Dr. Hans Paerl

I acknowledge that the proposed activities would be beneficial to Malcolm Barnard's research and that funds for the proposed activities are not currently available.

Freshwater ecosystems are critical for sustaining life and establishing civilizations throughout history[1]. As global populations grow, increased urbanization, agricultural and industrial production, combined with poor wastewater treatment practices, have led to a widespread increase in pollution of freshwater bodies, and clean water scarcity globally. One major threat to many freshwater systems is the proliferation of toxic algal and cyanobacterial blooms. Western Lake Erie (WLE) has experienced a resurgence of toxic cyanobacterial harmful algal blooms (cyanoHABs) in the last few years, leading to major environmental and human health risks[2]. Prior to phosphorus (P) load reductions in the 1970s, cyanoHABs in Lake Erie were mostly N_2-fixers (e.g. *Aphanizomenon* and *Dolichospermum*[3], formerly *Anabaena*[4]). Today, summer cyanoHABs are primarily non-N_2-fixing *Microcystis*[5], which blooms in WLE and *Planktothrix*, which blooms in Sandusky Bay[6]: indicating that combined nitrogen (N) inputs play an increasingly important role in controlling cyanoHAB outbreaks.

A toxic *Microcystis* cyanoHAB in WLE in August 2014 created a water crisis that forced public water supplies to be shut down for over 400,000 people in Toledo, OH[2]. Blooms in Lake Erie have been occurring sporadically for decades, and have now become a regular occurrence. Climate change (e.g. warming and changing precipitation patterns) increases the likelihood of more expansive blooms, exposing larger human and animal (e.g. pets, cattle, fish, birds) populations to water-borne toxins[6,7]. Despite cyanoHAB toxicity being a major human and ecosystem health hazard, it is not fully understood what causes and influences the underlying toxicity[8]. **The shift from P limitation to N limitation in WLE is an important paradigm shift in lake phytoplankton communities. The dominant cyanotoxins, microcystins, are enriched in N, linking their synthesis to N availability; hence, there is a need to investigate the potential roles N fertilizers play in bloom dynamics and toxin production in Lake Erie. Due to the shift to non-N_2-fixing cyanoHABs, a persistent uncertainty with this shift in nutrient limitation is the impact to toxin production and how the toxin production relates to fertilizer runoff.**

I will deploy in situ bioassays, using 4L pre-cleaned polyethylene Cubitainers^R to which natural lake water will be added from the sites of interest to address this need. In situ bioassays are the best way to test nutrient enrichment (especially N) in bloom proliferation and toxin production as the method allows for controlled testing of natural phytoplankton communities. Triplicate controls and treatments will be performed, and their deployment at the Ohio State University Stone Laboratory on the shore of Lake Erie will be in accordance with Paerl *et al*.[9] and Xu *et al*.[10]. Individual treatments will receive fertilizer N as NH_4^+, NO_3^- and urea (Table 1), yielding similar total dissolved N concentrations falling within a range appropriate for Lake Erie nearshore waters. Individual and combined (with N) P treatments will also be examined. Incubations will run for 72 hours at ambient lake water temperatures and light conditions, using a layer of neutral density screening to prevent photoinhibition[6,9]. For a complete profile of microcystin variants from select samples, we will rely on LC-MS analyses[11] by Dr. Gregory Boyer at SUNY-ESF.

Since the addition bioassays only have the ability to show a correlation between different forms of N addition and toxin production, we need an isotopic tracer to show the direct incorporation of added N forms into the toxins. Using ^{15}N-labeled N as a tracer, I expect that any N treatment that leads to increased toxin production should see an increase in ^{15}N in microcystins. Using recently developed methods to measure ^{15}N incorporation into microcystins using HPLC-LC-MS techniques, I plan to measure the ^{15}N incorporation into microcystins[12,13]. **This will be the first non-culture based experiment using ^{15}N to track N from specific sources to microcystin assimilation. This experiment will quantitatively assess the direct impacts of N-based fertilizer pollution on toxin production in cyanoHABs.**

Table 1: Bioassay sample design is shown for additions of nutrients to test for toxicity and growth responses in naturally-occurring Lake Erie cyanoHABs. Field work and access to lab instrumentation for fertilizer addition experiments are available through an existing project. This request for funding covers the purchase of ^{15}N labeled substrates and analyses (bottom row) and will be the first to test the hypothesis that fertilizer N is directly responsible for toxin production in cyanoHABS.

Nutrient Added	Control	$+PO_4^{3-}$	$+NH_4$	$+NO_3$	$+NH_4$ and $+NO_3$	+Urea	Status
+ 40 μM N	n/a	X	X	X	X	X	Funded
+2 μM PO_4^{3-}	n/a	n/a	X	X	X	X	Funded
^{15}N label ($+PO_4^{3-}$)	**n/a**	**n/a**	**$^{15}NH_4$**	**$^{15}NO_3$**	**$^{15}NH_4$** / **$^{15}NO_3$**	**$^{15}N_2$-Urea**	**Proposed Here**

References:

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