Research objective

Overarching question: Which nutrients are controlling primary production and when?

1. Nutrient Limitation and HAB Primary Production
Determine the potential for N, P, and/or N+P limitations to influence the primary production/growth of algal and cyanobacterial species.

2. Seasonal Nutrient Limitations of HAB Primary Production
Determine if there is a seasonal component (i.e., spring, summer, and fall) driving the potential nutrient limitations and algal and cyanobacteria species growth.

3. Spatial Nutrient Limitations of HABs
Determine whether there is a spatial aspect to the nutrient limitations of algal or cyanobacterial growth (i.e., Provo Bay; main body of lake, east; main body of lake, west).
We completed We quantified phytoplankton nutrient limitation and response to different N forms as the growth response ($\Delta R$) during the 48 h incubation period. Growth response was calculated for each taxonomic group as:

$$\Delta R = \log_{10} \left( \frac{\text{avg chl treatment}}{\text{avg chl control}} \right) / t$$
<table>
<thead>
<tr>
<th>Measurement</th>
<th>Completed for bioassays (summer = S, late summer = LS, and fall = F)</th>
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</thead>
<tbody>
<tr>
<td>Water chemistry sonde: pH, temperature, electrical conductivity, dissolved oxygen, turbidity, dissolved organic matter, blue green algae-phycocyanin *, and chlorophyll-a *</td>
<td>S, LS, F</td>
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<tr>
<td>Chlorophyll-a (ethanol extraction) *</td>
<td>S, LS</td>
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<tr>
<td>ELISA toxins (microcystin, anatoxin-a, and cylindrospermopsin)</td>
<td>S, LS</td>
</tr>
<tr>
<td>TSS and VSS to estimate photosynthetic biomass *</td>
<td>S, LS, F</td>
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<tr>
<td>TP and TN SRP, and ammonium and nitrate</td>
<td>S, LS</td>
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<tr>
<td>Cyanobacterial species and algal division composition direct microscopy *</td>
<td>S, almost all LS</td>
</tr>
<tr>
<td>RNA transcript extractions</td>
<td>started S</td>
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<tr>
<td>RT-qPCR cyanobacteria biomass *</td>
<td>-</td>
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<tr>
<td>RT-qPCR of nifH to estimate biological nitrogen fixation</td>
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<tr>
<td>Sequencing of cyanobacterial composition</td>
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Fig. 8. Threshold relationship between DIN (the sum of NO$_3$-N and NH$_4$-N) and the nitrogen (N) addition $\Delta R$ limitation response ($\log_{10}$ ratio of nutrient treatment growth relative to the control per day) for the total phytoplankton assemblage, chlorophytes, cyanobacteria, and diatoms (a). Threshold relationship between the DIN to SRP ratio and the nitrogen (N) addition $\Delta R$ limitation response (b). Threshold relationship between SRP concentration and the P addition $\Delta R$ limitation response (c), and the threshold relationship between the DIN:SRP ratio and the P addition $\Delta R$ limitation response (d). Note: Solid vertical lines indicate statistical significance ($\alpha < 0.05$), and dashed vertical lines indicate marginal significance ($\alpha > 0.05$ and < 0.10).
We completed bioassays in summer (22-26 July), late summer (26-30 August), and fall (7-11 October) across the three lake locations. To date, we have completed three of the five bioassays. We will add a zooplankton “grazer” removal trial to the spring and nutrient dilution trial to early summer bioassays.
Probe readings
- 200 mL rinse
- 300 mL for measurement
- poured into EXO storage cup

Microscopy evaluation of Cyanobacteria and algae
- 100 mL
- Preserved with 2 mL Lugol’s fixing agent

Nutrients and pigments
- 1 L
- Kept on ice

RNA filtering
- 1 L
- Kept on ice

Toxin (ELISA)
- 2 60-mL amber vials
- One vial for anatoxin A receives 4.5 mL due to added preservative
- Kept on ice

TSS/VSS, Chl 𝛼, & phycocyanin

Filtered SRP, DIN

Unfiltered TN/TP

Frozen -80°C for future
Cyanobacterial biomass estimates via quantitative PCR, bacterial community composition, and nifH gene quantification

DNA/RNA filtering
- 1 L
- Kept on ice

Toxin (ELISA)
- 2 60-mL amber vials
- One vial for anatoxin A receives 4.5 mL due to added preservative
- Kept on ice

Frozen -80°C for future
Cyanobacterial biomass estimates via quantitative PCR, bacterial community composition, and nifH gene quantification