# Rationale and Design of a Phytoplankton Nutrient Limitation Study

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## INTRODUCTION

Nitrogen (N) and phosphorus (P) are key macronutrients required by phytoplankton for growth. When these nutrients are abundant (i.e., under eutrophic conditions), phytoplankton primary production can be excessive resulting in decreased water quality, toxic algal blooms and reduced aesthetic appearance of aquatic areas (Abell et al. 2010).

Phosphorus is the most commonly targeted macronutrient for management in order to limit phytoplankton growth (Schindler et al. 2008). Phosphorus loading reductions are typically achieved through measures such as land use modification, improved pastoral management practices (Özkundakci et al. 2010) and lake alum dosing (Reitzel et al. 2005). Phosphorus-reduction-only advocates argue that strategies to reduce N in surface waters are often confounded by the ability of some cyanobacteria species to fix atmospheric nitrogen and thus offset N reduction strategies (Schindler 1974; Schindler et al. 2008; Wang and Wang 2009; Welch 2009). However, there is also evidence that dual N and P control strategies can be effective due to the susceptibility of some lakes to become limited by N as well as P (Elser et al. 2007; Lewis and Wurtsbaugh 2008). Aquatic systems may become N limited due to an absence of $N_2$ fixation by resident cyanobacteria species (Howarth et al. 1988), the failure of $N_2$ fixation to always balance N load reductions (Scott and McCarthy 2010) and the need to consider downstream impacts on marine systems which are typically limited by N (Paerl 2009). Therefore, if an eutrophication management strategy is to be successful, determination of the limiting (or con-limiting) macronutrient(s) is imperative for management strategies to be optimally effective.

Alum (aluminium sulphate) dosing is a proven effective method for removing P from freshwater systems, and is currently being employed by the Bay of Plenty Regional Council to help meet water quality targets for Lake Rotorua. Continuous alum dosing to the Utuhina Stream started in mid-2006, and the Puarenga Stream in mid-2010, both of which discharge into Lake Rotorua. Hamilton et al. (2014) reported that the alum dosing was highly effective in adsorbing dissolved reactive phosphorus (DRP) in the stream inflows and that some in-lake improvements were also observed in total phosphorus (TP) and DRP from 2007.

Alum dosing in the Utuhina and Puarenga stream inflows is currently regulated to maintain 3-month surface TP concentrations at 20 mg $m^{-3}$ in Lake Rotorua, i.e., around one-half of the levels observed in the lake in the mid-2000s. In-lake P concentrations now show less seasonal variability than before alum dosing. Of considerable importance is whether alum dosing has brought about a transition in nutrient limitation status of phytoplankton in Lake Rotorua. Studies of nutrient limitation in the mid-2000s have commonly shown addition of both N and P to have had the greatest growth-stimulation effect on phytoplankton (i.e. 'co-limitation'). However a nutrient limitation study by Abell et al. (2010) found N limitation in the lake following periods of high rainfall. Further modelling by Hamilton et al. (2014) suggests that this N limitation may have been transient and the lake can shift in relative nutrient limitation in response to environmental conditions.

Based on these findings, the Bay of Plenty Regional Council requested the University of Waikato to develop a regular, repeatable monitoring protocol to determine seasonal phytoplankton nutrient limitation in Lake Rotorua in order to maximise the impact of current nutrient management programmes such as alum dosing of the Utuhina and Puarenga stream inflows.

## STUDY OBJECTIVES

1. Determine the limiting macronutrient (N or P) or macronutrients (N and P) of the phytoplankton community assemblage in Lake Rotorua on a seasonal scale.
2. Determine the concentrations of inorganic and total nutrients in relation to phytoplankton community composition at a seasonal scale.
3. Based on the findings of objectives 1 and 2 make recommendations as to the alum dosing rates of the Utuhina and Puarenga stream inflows.

## PROPOSED STUDY DESIGN

Phytoplankton nutrient limitation will be assessed using three experimental assays, particulate ratios of C/P, N/P and C/N, phosphorus and nitrogen debt assays and phytoplankton growth following nutrient additions. Use of multiple assays allows for the determination of N and P status indicators as demonstrated by Rattan (2017). Assays involving the addition of combined N and P will not be conducted as these commonly result in a confounding result of nutrient co-limitation and therefore are not a cost-effective measure for nutrient limitation. Lake sampling and nutrient assays will be conducted on a seasonal basis (i.e., four times per year at 3-monthly intervals).

### Lake Sampling

Mid-lake water samples from the surface (0.5 m depth) and hypolimnion (15 m depth) of Lake Rotorua will be taken for analysis of nutrients (TN, TP, DRP, $NO_3$, $NO_2$ and $NH_4$), total suspended solids and chlorophyll *a* concentrations. In addition, a CTD (conductivity, temperature, depth) profile will be conducted and 20 L of water retrieved from the surface (0.5 m depth). This water will be immediately filtered using a 100 µm net to remove large cladocerans and copepods capable of reducing phytoplankton biomass due to grazing. The samples and filtered water will then be transported back to the laboratory for analysis and use in nutrient limitation assays, respectively.

### Carbon, Phosphorus and Nitrogen Particulate Ratios

One litre of unfiltered lake water will be provided to NIWA, Hamilton for triplicate analysis of particulate ratios of C/P, N/P and C/N.

### Nitrogen and Phosphorus Debt Assays

Nitrogen and phosphorus debt assays will be conducted following the methodology of Rattan (2017). Nitrogen and phosphorus debt will be determined by addition of $NH_4Cl$ (final concentration 5 µmol $L^{-1}$–N) and $KH_2PO_4$ (final concentration 5 µmol $L^{-1}$–P) to triplicate 1 L aliquots of lake water, followed by 24 h incubation in the dark at a water temperature corresponding to the site and depth of sample

collection. The experimental control will consist of triplicate 1 L lake water aliquots with no nutrient addition followed by 24 h dark incubation at a water temperature corresponding to the site and depth of sample collection. Nutrient and chlorophyll *a* concentrations will be measured at the beginning and end of the incubation.

Nitrogen debt and P debt will be calculated by the amount of N or P removed over the 24-h period normalised to the initial chlorophyll *a* concentration (Healey and Hendzel 1979).

### Phytoplankton Growth Assay

Phytoplankton growth assays control and treatment aliquots will be incubated for 3 days under a single light intensity (100 µmol $m^{-2}s^{-1}$) and a water temperature corresponding to the site and depth of sample collection. Twelve µmol $K_2HPO_4$–P $L^{-1}$ will be added to the +P treatment and 400 µmol $NaNO_3$–N $L^{-1}$ will be added to the +N treatment at the start of the incubation. Growth assays will be carried out under 12/12 h light/dark lighting provided by white LED strips with light diffused by a plexiglas acrylic sheet and light intensity monitored with a LI-COR light sensor.

Daily chlorophyll *a* concentrations will also be measured and chlorophyll *a* rate and yield limitation (sensu O'Brien, 1972) will be determined. Growth rate will be estimated by linear regression of the natural log of in vivo fluorescence versus time between days 0 and 3. Yield will be estimated from the concentration of chlorophyll *a* on day 3.

Phytoplankton species counts and biovolumes will be determined using 100 mL aliquots taken and preserved in Lugol's iodine from each replicate for identification and abundance count at day 0 and day 3. Samples will be observed under an inverted microscope using sedimentation chambers. Cell counts will be performed using the Utermöhl (1958) methodology, and phytoplankton identified to species level when possible. At least 100 individuals of the most frequent species or 400 individuals in total will be counted in random fields. Following the end of the incubation period all replicates will be sampled to determine total and dissolved nutrient concentrations.

## JUSTIFICATION AND LIMITATIONS OF STUDY DESIGN

Culturing and quantification of algal species to determine nutrient limitation have significant challenges. *In situ* experiments provide the benefit of being more representative of the environment but also provide logistical challenges with repeated sampling on a compact time-scale. Associated with this are difficulties in controlling factors such as light and temperature between replicates. Therefore, we have proposed a laboratory based approach that will reduce sampling costs, reduce chances of replicate contamination during incubation and sampling, and allow greater control of environmental variables. However, this does increase the risk of producing results not representative of the original environment, and therefore, extrapolation of laboratory results to understanding of nutrient limitation to larger scales (i.e., whole lake) must be conservative (Beardall et al. 2001).

In the proposed study mixed phytoplankton species taken from Lake Rotorua will used in determining nutrient limitation. Phytoplankton species are known to respond differentially to given nutrient concentrations and environmental conditions. This can lead to confounding results, for example, some cyanobacterial species are able to thrive under low N conditions by fixing atmospheric $N_2$, while other species may be limited in their ability to compensate for low N

concentrations. Similarly, diatoms may have sufficient N and P for growth but could be limited by silicate availability. Alternatively, environmental conditions such as light intensity may not be optimal for all phytoplankton species, resulting in a reduction or even inhibition of growth in some species (Beardall et al. 2001). Differential growth responses by phytoplankton under laboratory conditions may result in unexpected growth by some species or inhibition of growth which could confound interpretation of the results and increase uncertainty around recommendations for lake management.

Determination of phytoplankton growth presents a number of challenges. Phytoplankton biomass proxies using both *in vivo* and *in vitro* fluorescence quantification of chlorophyll *a* have been shown to be unreliable (Beardall et al. 2001; Kruskopf and Flynn 2006). This is due to variation in phytoplankton cell chlorophyll *a* content with regard species, stage of growth, nutrient status and colony formation among other factors (Kruskopf and Flynn 2006). Regardless, fluorescence quantification of chlorophyll *a* remains a commonly employed supporting measure in determining relative changes in phytoplankton biomass when coupled with cell enumeration. Direct determination of species abundance and biovolume is essential for assessment of nutrient limitation as it provides an absolute quantification of phytoplankton growth. However, phytoplankton species identification and enumeration requires a highly specialised skill set and is relatively time consuming resulting in elevated analysis costs. For this reason we have attempted to minimise the number of variables and replicates.

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Proposed study design for the determination of phytoplankton nutrient limitations in Lake Rotorua. Study will be conducted on a seasonal basis (i.e., four times per year at 3-monthly intervals).

| Day 0 | Day 1 | Day 3 | Day 4 |
|---|---|---|---|
| **Lake sampling** | | | |
| CTD cast | | | |
| 0.5 m Total nutrients x1 | | | |
| Dissolved nutrients x1 | | | |
| Chlorophyll *a x1* | | | |
| TSS x1 | | | |
| 20 L water filtered through 100 µm net | | | |
| 15 m Total nutrients x1 | | | |
| Dissolved nutrients x1 | | | |
| Chlorophyll a x1 | | | |
| TSS x1 | | | |
| **Particulate nutrient ratios** | | | |
| 1 L lake water to NIWA for triplicate analysis | | | |
| **N & P Debt Assays** | | | |
| 1 L aliquots of lake water | Sampling following 24 h dark incubation | | |
| 3 controls (no nutrient addition) | Total nutrients x 9 | | |
| 3 +N treatments | Dissolved nutrients x 9 | | |
| 3 +P treatments | Chlorophyll *a* x 9 | | |
| Sampling at 0 hour | | | |
| Total N & P x 9 | | | |
| Dissolved nutrients x 9 | | | |
| Chlorophyll *a* x 9 | | | |
| **Phytoplankton growth assay** | | | |
| 1 L aliquots of lake water | Sampling following 24 h incubation | Sampling following 48 h incubation | Sampling following 72 h incubation |
| 3 controls (no nutrient addition) | Chlorophyll *a* x 9 | Chlorophyll *a* x 9 | Phytoplankton cell count x 9 |
| 3 +N treatments | | | Total N & P x 9 |
| 3 +P treatments | | | Dissolved nutrients x 9 |
| Sampling at 0 hour | | | Chlorophyll *a* x 9 |
| Total N & P x 9 | | | |
| Dissolved nutrients x 9 | | | |
| Chlorophyll *a* x 9 | | | |
| Phytoplankton cell count x 9 | | | |