

# Seasonal changes in phytoplankton nutrient limitation: Lake Rotorua

---



ERI Report Number 135  
Client report prepared for Bay of Plenty Regional Council  
By Grant Tempero  
2020

Environmental Research Institute  
School of Science  
University of Waikato, Private Bag 3105  
Hamilton 3240, New Zealand

Cite report as:

Tempero, G.W. 2020. Seasonal changes in phytoplankton nutrient limitation: Lake Rotorua. ERI Report No. 135. Client report prepared for Bay of Plenty Regional Council. Environmental Research Institute, School of Science, University of Waikato, Hamilton, New Zealand. 41 pp.

Disclaimer:

The information and opinions provided in the Report have been prepared for the Client and its specified purposes. Accordingly, any person other than the Client, uses the information and opinions in this report entirely at their own risk. The Report has been provided in good faith and on the basis that reasonable endeavours have been made to be accurate and not misleading and to exercise reasonable care, skill and judgment in providing such information and opinions.

Neither The University of Waikato, nor any of its employees, officers, contractors, agents or other persons acting on its behalf or under its control accepts any responsibility or liability to third parties in respect of any information or opinions provided in this Report.

*Reviewed by:*



**Prof Ian Hawes**

Environmental Research Institute

University of Waikato

*Approved for release by:*



**Dr John Tyrrell**

Research and Innovation Manager

University of Waikato

# Executive Summary

The management of macronutrient availability is central for reducing harmful algal blooms and the restoration of aquatic ecosystems. While there is some dispute as to the need for phosphorus (P) only control versus P and nitrogen (N) control, scientific opinion generally supports dual nutrient control in New Zealand lakes. Previous nutrient limitation studies of Lake Rotorua conducted during the 1970s and 1980s reported N limitation to varying degrees. This period coincided with the disposal of sub-optimally treated municipal wastewater to the lake, resulting in significant P loading and resultant algal blooms. Land disposal of wastewater was initiated in 1991 reducing nutrient loads to the lake, however, no further nutrient limitation studies were conducted until the summer of 2004 when N and P co-limitation was reported. From the mid-2000s water quality improved from a Trophic Lake index (TLI) of approximately 4.8 to 4.2, a change likely driven, in part, by sediment P depletion following the change to land disposal of wastewater and the initiation of alum dosing in 2006.

Reduced P availability suggests that the observed improvements in Lake Rotorua water quality may have resulted in a regime shift from N limitation towards more frequent nutrient co-limitation or even P-limitation. The University of Waikato was contracted to develop a suitable phytoplankton nutrient limitation protocol to determine the most likely limiting macronutrient(s) of the phytoplankton community in Lake Rotorua on a seasonal scale. Effects of nutrient concentrations of phytoplankton community composition were also investigated. These findings would then be used to provide recommendations as to the alum dosing rates of inflows to Lake Rotorua.

Previous studies have found there is no single satisfactory method to identify the limiting nutrient(s). Therefore, a range of assays were employed to determine nutrient limitation in Lake Rotorua on a seasonal basis. Assays were conducted in March, June, September and December 2019 and included lake total nitrogen to total phosphorus ratios, particulate nutrient concentration ratios, nutrient debt determined from individual nutrient uptake rates, and phytoplankton biomass responses to nutrient addition (+N, +P and +NP). Phytoplankton biomass was determined from chlorophyll *a* concentration and cell biovolume increases following 7-day *in vitro* incubation. Changes in phytoplankton community composition in response to nutrient addition were also determined from the nutrient addition assays.

Assay results were variable and in some cases contradicting, the summarised results are presented in the table below. Nitrogen limitation was primarily indicated for the March assay and nutrient co-limitation for the December period. The March N limitation was likely due to epilimnetic nutrient depletion during periods of summer stratification followed by release of hypolimnetic P during breakdown of stratification in the autumn. Similarly, co-limitation in December may also be due to epilimnetic nutrient depletion associated with stratification,

combined with reduced availability of dissolved organic nitrogen and dissolved organic phosphorus. The June 2019 phytoplankton growth assay did not respond to any of the nutrient addition treatments and growth responses were likely limited by light availability producing inconclusive responses. However, large growth responses occurred in the both the control and nutrient addition treatments in the September 2019 assay. When these results are considered in conjunction with the nutrient ratio indicators and the nutrient uptake assay, it is likely that nutrients were not limiting during the September period.

**Summary of seasonal nutrient limitation assays for Lake Rotorua. Limitation for each measure is indicated by P (phosphorus), N (nitrogen), CO (nutrient co-limitation) or Inconclusive.**

Assay Period	TN:TP	Particulate		Chlorophyll yield	Chlorophyll growth rate	Biovolume yield	Biovolume growth rate
		nutrient ratios.	Nutrient uptake				
March 2019	CO	N	N	N	CO	N/CO	CO
June 2019	CO	P	CO	Inconclusive	Inconclusive	Inconclusive	Inconclusive
September 2019	P	P	N	Inconclusive	Inconclusive	P	Inconclusive
December 2019	CO	CO	CO	CO	CO	P/CO	Inconclusive

There was little variation in phytoplankton community composition between the assay periods at the family taxonomic level with either diatoms or green algae being the dominant taxon. Initial phytoplankton community composition was typically numerically dominated by five or six species, however, these species rarely responded to nutrient addition with the maximum observed growth responses. Across all assay periods green algae species had the greatest growth response to nutrient addition. Cyanobacterial species were sparse for all but the March assay period and either declined or responded minimally to nutrient addition.

Currently, Lake Rotorua appears to undergo seasonal changes in nutrient limitation, including no nutrient limitation. This suggests that the Bay of Plenty Regional Council’s target of nutrient co-limitation for Lake Rotorua is partially effective. However, growth assay results from the winter and spring periods were somewhat inconclusive and there was no indication that strong P limitation was occurring as a result of the alum dosing programme. In fact, P release during post-stratification lake mixing appears sufficient to produce a N limitation response in the phytoplankton community.

Further investigation of nutrient limitation at smaller time scales over the spring-summer-autumn period would provide improved temporal resolution of nutrient availability in relation to environmental perturbation and allow for assessment of more responsive nutrient limitation indicators such as DIN:TP. Cyanobacterial species are more prevalent when water temperatures are higher in the summer and autumn. Increasing the number of nutrient limitation assays during this period would provide improved understanding into cyanobacterial growth responses to nutrient addition and the dynamics between phytoplankton taxonomic groups.

# Acknowledgements

I would like to thank Ian Hawes and John Tyrrell for reviewing this document. Joe Butterworth and Wendy Paul provided technical support. Chris McBride provided monitoring buoy data and historical environmental data for Lake Rotorua. Funding for this work was provided by the Bay of Plenty Regional Council and the Waikato University Chair in Lake Restoration.

# Table of Contents

Executive Summary .....	3
Acknowledgements .....	5
Table of Contents .....	6
Introduction .....	10
Phytoplankton nutrient limitation .....	10
Historical changes in Lake Rotorua nutrient limitation .....	11
Methods .....	15
Study site .....	15
Lake sampling .....	16
Carbon, phosphorus and nitrogen particulate ratios .....	17
Nitrogen and phosphorus debt assays .....	17
Phytoplankton growth assay .....	17
Results .....	19
Vertical lake profiles .....	19
Lake nutrient concentrations .....	20
Light availability and suspended particles .....	20
Particulate nutrient concentrations .....	21
Nutrient uptake assay .....	22
Phytoplankton growth assay .....	22
Discussion .....	29
Individual nutrient limitation indicators .....	29
Seasonal changes in nutrient limitation .....	32
Phytoplankton community changes .....	33
Conclusions .....	34
Recommendations .....	35
References .....	36
Appendices .....	41

## LIST OF FIGURES

- Figure 1. Annual (previous July to present June) Trophic Level Index (TLI) at mid-lake for Lake Rotorua. Each coloured circle is the mean of seasonal means, with the TLI equation (Burns et al. 1999) applied. Large black circles are the annual TLI (average of four components). Solid circles denote years for which at least one measurement was available for all four seasons (solid black circles denote that all four component variables of the TLI were sampled each season). Open circles denote that measurements were missing for at least one season. The TLI target (4.2) for Lake Rotorua is shown by the dashed red line. Reproduced with permission from McBride et al. (2018a).....12
- Figure 2. Relationship between alum dosing and in-lake median monthly dissolved reactive phosphate (DRP) concentrations in Lake Rotorua. Alum dosing of the Utuhina (since 2006) and Puarenga (from 2010) inflows has been combined into a 10-day rolling average of aluminium. ....14
- Figure 3. Lake Rotorua with locations of alum dosing stations on the Utuhina and Puarenga Streams. Sampling for nutrient limitation assays were taken from the Lake Rotorua monitoring buoy, apart from September 2019 when sampling was conducted at the indicated alternative site due to adverse weather conditions.....15
- Figure 4. Temperature (°C) (top) and dissolved oxygen (% saturation) (bottom) profiles for Lake Rotorua on the four sampling dates. Profiles were taken at the Lake Rotorua monitoring buoy using a CTD, data presented for 23 September is from the lake monitoring buoy as a CTD cast could not be safely undertaken on this date. ....19
- Figure 5. Phytoplankton nutrient (phosphate-P and ammonium-N) uptake rates per  $\mu\text{g}$  of chlorophyll *a* under 24 h dark incubation at 21°C (March) and 11°C (June), 11.5°C (September) and 21°C (December). The green dashed line indicates threshold value for nitrogen limitation of  $>0.15 \mu\text{mol N } \mu\text{g}^{-1}$  Chlorophyll *a* and the red dashed line the threshold value for phosphorus limitation of  $>0.075 \mu\text{mol P } \mu\text{g}^{-1}$  Chlorophyll *a* as proposed by Rattan (2017).....22
- Figure 6. Changes in mean ( $n=3$ ) chlorophyll *a* concentration in response to the addition of either phosphorus ( $10 \text{ mg-P m}^{-3}$ ), nitrogen ( $100 \text{ mg-N m}^{-3}$ ) or nitrogen + phosphorus ( $10 \text{ mg-P m}^{-3}$ ,  $100 \text{ mg-N m}^{-3}$ ) after 7-days. Assays were conducted in (A) March 2019, (B) June 2019, (C) September 2019 and (D) December 2019. Error bars indicate  $\pm 95\%$  confidence intervals. Within groups, treatments significantly different (ANOVA  $<0.05$ ) to the control are indicated by \* .....23
- Figure 7. Phytoplankton growth rates determined from chlorophyll *a* fluorescence for (A) March 2019, (B) June 2019, (C) September 2019 and (D) December 2019. Treatments of either phosphorus ( $10 \text{ mg-P m}^{-3}$ ), nitrogen ( $100 \text{ mg-N m}^{-3}$ ) and nitrogen + phosphorus ( $10 \text{ mg-P m}^{-3}$ ,  $100 \text{ mg-N m}^{-3}$ ). Treatment groups with significantly higher (ANOVA,  $P<0.05$ ) growth rates compared to control groups are indicated by \*. Error bars indicate  $\pm$  standard error.....24

Figure 8. Change in mean ( $n=3$ ) total phytoplankton biovolume in response to the addition of either phosphorus ( $10 \text{ mg-P m}^{-3}$ ), nitrogen ( $100 \text{ mg-N m}^{-3}$ ) or nitrogen + phosphorus ( $10 \text{ mg-P m}^{-3}$ ,  $100 \text{ mg-N m}^{-3}$ ) after 7-days. Assays were conducted in (A) March 2019, (B) June 2019, (C) September 2019 and (D) December 2019. Significantly different biovolumes between nutrient addition and control groups are indicated by \* at the  $P<0.05$  level and \*\* at the  $P<0.01$  level. Error bars indicate  $\pm 95\%$  confidence interval. ....27

Figure 9. Phytoplankton relative growth rates in response to the addition of either phosphorus ( $10 \text{ mg-P m}^{-3}$ ), nitrogen ( $100 \text{ mg-N m}^{-3}$ ) or nitrogen + phosphorus ( $10 \text{ mg-P m}^{-3}$ ,  $100 \text{ mg-N m}^{-3}$ ) after 7-days. Significantly different growth rates to the control group for each assay period are indicated by \*. Error bars indicate  $\pm 95\%$  confidence intervals. ....28

Figure 10. Example biomass response patterns. These patterns correspond to a subset of the nutrient limitation categories defined by Harpole et al. (2011). A) Single N limitation: a response to only one of the single treatments, in this example +N and the response to the +NP treatment is no different. B) Serial P limitation: a response to only one of the single nutrient treatments, in this example +P and a larger response to the +NP treatment. C) Independent co-limitation (primary P): a response to both single nutrient treatments with a larger response to +P and an even larger response to the +NP treatment; D) Simultaneous co-limitation: a response only to the +NP treatment. No nutrient limitation: no response to any nutrient treatment (not shown). ....31

## LIST OF TABLES

Table 1. Stoichiometric molar ratios of lake phytoplankton as indicators of relative nutrient limitation (Healey and Hendzel 1980). ....10

Table 2. Summary of published nutrient (nitrogen and phosphorus) limitation studies conducted on Lake Rotorua. ....13

Table 3. Timing and conditions of experimental growth assays to determine phytoplankton nutrient limitation in Lake Rotorua. Temperature (2 m depth) and light regimes were set to match prevailing seasonal conditions. ....16

Table 4. Lake Rotorua nutrient concentrations from 2 m and 15 m depth at the time of water sampling for nutrient limitation assays. ....20

Table 5. Seasonal observations of Photosynthetically Active Radiation (PAR), water temperature, light extinction coefficient ( $K_d$ ), chlorophyll  $a$  concentrations, total suspended solids (TSS), volatile suspended solids (VSS) and inorganic suspended solids (ISS) for Lake Rotorua. Note that PAR and  $K_d$  are not presented for 23 September and 16 December due to adverse weather conditions and sensor availability, respectively. Chlorophyll  $a$ , TSS, VSS and ISS samples were not retrieved from 15 m depth on 23 September. ....21

Table 6. Lake Rotorua surface (2 m) particulate carbon (C), nitrogen (N) and phosphorus (P) concentrations and atomic ratios for the four seasonal nutrient limitation assays. Values marked with a \* were higher than reported total phosphorus concentrations and were omitted from further analysis. Green highlight indicates no nutrient deficiency and orange moderate nutrient deficiency based on the proposed thresholds by Rattan (2017) (refer Table 1 for threshold bands). .....21

Table 7. Phytoplankton species initial percentage community composition and percentage change in biovolume after 7-day incubation in control (no nutrient addition) and nutrient (nitrogen and phosphorus) addition treatment groups. Highlighted values indicate the initial top five abundant species and the five species with the largest growth response for each assay period. ....25

Table 8. Summary of seasonal nutrient limitation results for Lake Rotorua. Limitation for each measure is indicated by P (phosphorus), N (nitrogen), CO (nutrient co-limitation) or Inconclusive.....32

# Introduction

## Phytoplankton nutrient limitation

Carbon (C), nitrogen (N) and phosphorus (P) are key macronutrients required by phytoplankton for growth (Smith et al. 2006). When these nutrients are abundant (i.e., under eutrophic conditions), phytoplankton primary production can be excessive, resulting in decreased water quality, cyanobacterial harmful algal blooms (cyanoHABs) and reduced aesthetic qualities of aquatic systems (Smith et al. 2006, Abell et al. 2010). Optimal nutrient supply was first proposed following the observation that marine algal growth was not nutrient limited when the C:N:P molar ratio is 106:16:1 (i.e. the Redfield ratio), this ratio is present with little variation in deep ocean environments (Geider and La Roche 2002). However, large variations in limnetic phytoplankton C:N:P ratios have been reported, with N:P ratios often strongly correlated with N:P loading rates to lakes (Healey and Hendzel 1980, Hecky et al. 1993). Stoichiometric ratios of C:N, C:P and N:P have been developed as indicators of phytoplankton nutrient deficiency. The particulate stoichiometric ratios of C:P and N:P have been the most widely used as indicators of P status (Rattan 2017). When P or N become limited in supply, cell division will be reduced after depletion of stored nutrients, but phytoplankton can still store excess photosynthetic carbon. C:P and C:N ratios are then expected to be considerably higher compared to the ratios under nutrient-sufficient conditions (Leonardos and Geider 2004). This has made nutrient ratios useful for determining which nutrients are limiting in a localised system as outlined in Table 1.

**Table 1. Stoichiometric molar ratios of lake phytoplankton as indicators of relative nutrient limitation (Healey and Hendzel 1980).**

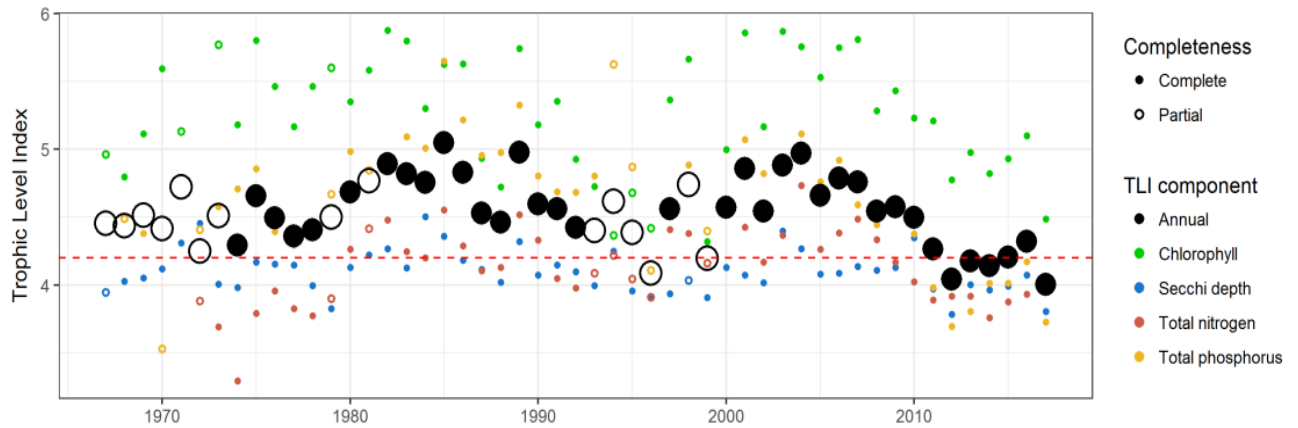
Indicator	Nutrient	No deficiency	Moderate deficiency	Extreme deficiency
C:N (atomic ratio)	N	<8.3	8.3 - 14.6	>14.6
N:P (atomic ratio)	P	<23		>23
C:P (atomic ratio)	P	<133	133 - 258	>258

The management of macronutrient availability is axiomatic for the control of lake eutrophication. However, there is persistent debate as to the need for N and P control versus control of P alone (Schindler et al. 2008, Schindler et al. 2016, Smith et al. 2016, Paerl et al. 2020). Schindler et al. (2008) stated that many lakes rendered eutrophic by the addition of anthropogenic P contain phytoplankton communities that show signs of extreme N limitation in short-term bioassays such as N debt (Healey and Hendzel 1980, Hendzel et al. 1994) or nutrient addition bioassays (Schindler 1971). Schindler posited that N limitation was the result of over-fertilization with P and proposed that short-term N limitation was not necessarily a reliable indication that N must be controlled to reverse eutrophication (Schindler et al. 2008).

Phosphorus-reduction-only advocates also 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). In contrast, others have argued that both N and P limitation is required to reverse lake eutrophication citing the resurgence of non-N<sub>2</sub>-fixing *Microcystis* blooms in lakes such as Erie and Balaton which have undergone extensive management actions for P control (Paerl et al. 2014, Paerl et al. 2020). Similarly, Abell et al. (2010) concluded from the analysis of 121 New Zealand lakes that 53% were P limited, whereas only 14% of lakes were potentially N limited. When compared to data from 689 European lakes, N had a greater role in determining lake productivity in New Zealand than in Europe, concluding that differences in lake nutrient sources occur between the two regions. There is also direct evidence that dual N and P control strategies can be effective due to the susceptibility of some lakes to become N as well as P limited (Elser et al. 2007, Lewis Jr and Wurtsbaugh 2008). If an eutrophication management strategy is to be successful, determination of the limiting (or co-limiting) macronutrient(s) is imperative for management strategies to be optimally effective in controlling eutrophication and associated cyanoHABs.

#### Historical changes in Lake Rotorua nutrient limitation

Lake Rotorua has undergone significant anthropogenic eutrophication since the 1960s, with annual Trophic Level Index (TLI) varying from around 4.4 in the late 1960s to close to 5.0 in the 1980s before recent reductions to less than the lake management target of 4.2 since 2010. The increases in TLI were initially driven by urban development and then land use intensification within the catchment (McBride et al. 2018a). These changes were recently reviewed by McBride et al. (2018a), with the authors concluding that surface inflow P concentrations have been comparatively stable since the 1970s, however, lake external P load has fluctuated considerably over time, due to changes in particulate P loads and the change to land disposal of Rotorua City treated wastewater in 1991. In contrast, the external nitrogen load has increased substantially and steadily since the 1960s, primarily due to increases in nitrate concentrations associated with agricultural land use intensification. Nitrogen loads are now well in excess of the sustainable target (McBride et al. 2018a). A summary of the changes in Lake Rotorua TLI are presented in Figure 1.



**Figure 1. Annual (previous July to present June) Trophic Level Index (TLI) at mid-lake for Lake Rotorua. Each coloured circle is the mean of seasonal means, with the TLI equation (Burns et al. 1999) applied. Large black circles are the annual TLI (average of four components). Solid circles denote years for which at least one measurement was available for all four seasons (solid black circles denote that all four component variables of the TLI were sampled each season). Open circles denote that measurements were missing for at least one season. The TLI target (4.2) for Lake Rotorua is shown by the dashed red line. Reproduced with permission from McBride et al. (2018a).**

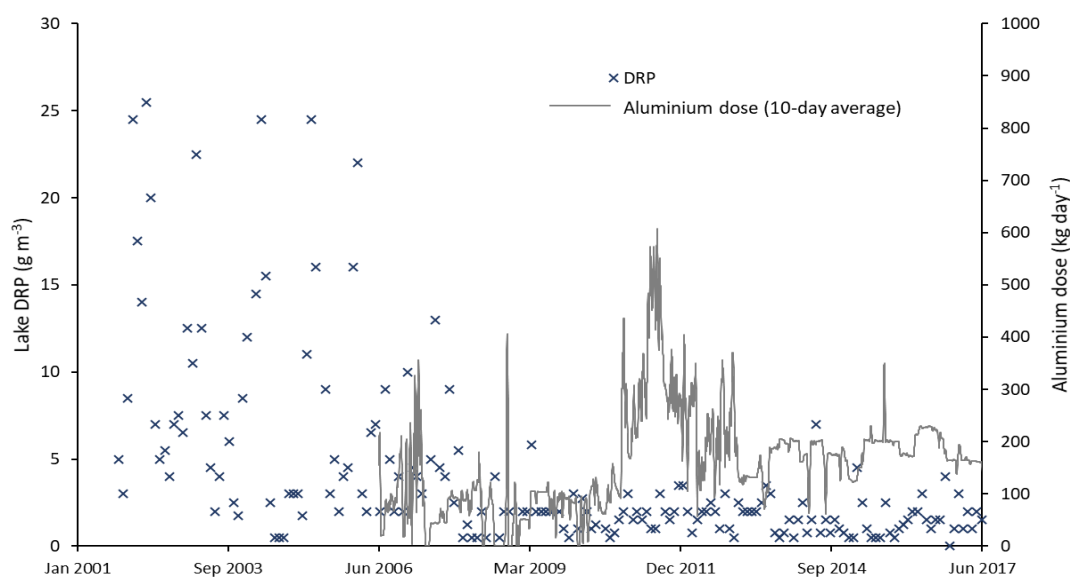
A number of phytoplankton nutrient limitation studies have been conducted for Lake Rotorua and are summarised in Table 2. Early nutrient limitation studies by White and Payne (1978) in 1975-76 and Vincent (1981b) in 1979 indicated that Lake Rotorua was distinctly nitrogen limited. A comparative lake study using various assays indicated that Lake Rotorua was either not nutrient limited or slightly N limited in 1982 (White et al. 1985), while a follow-up study conducted in December 1984 and February 1985 found strong nitrogen limitation in the lake (White et al. 1986). This period of nitrogen limitation coincided with significant P loading from sub-optimally treated municipal wastewater discharged to the lake (Rutherford et al. 1989, McBride et al. 2018a). No nutrient limitation studies were conducted in the period directly following the switch to land disposal of city wastewater in 1991, however, nutrient co-limitation was reported for the summer of 2004 by Burger et al. (2007) and March 2012 by Abell (2013), a change likely driven, in part, by sediment nutrient depletion following the change to land disposal of wastewater and stream alum dosing initiated in 2006.

**Table 2. Summary of published nutrient (nitrogen and phosphorus) limitation studies conducted on Lake Rotorua.**

Assay date	Study type	Assay(s)	Response measured	Limiting nutrient	Reference
June 1975 - May 1976	<i>In-vitro</i>	Nutrient addition	Chlorophyll	Nitrogen	White and Payne (1978)
March 1979	<i>In-vitro</i>	Ammonium uptake	Ammonium uptake capacity	Nitrogen	Vincent (1981a)
	<i>In-vitro</i>	Nutrient addition	Seston N:P	Nitrogen	Vincent (1981a)
	<i>In-vitro</i>	<sup>32</sup> P-PO <sub>4</sub> <sup>3-</sup> kinetics	<sup>32</sup> P-PO <sub>4</sub> <sup>3-</sup> uptake	Inconclusive	Vincent (1981b)
	<i>In-vitro</i>	Nutrient addition	Seston N & P	Nitrogen	Vincent (1981b)
	<i>In-vitro</i>	ATP response to P	ATP	Inconclusive	Vincent (1981b)
<i>In-vitro</i>	Alkaline phosphatase activity	Fluorescence	Nitrogen	Vincent (1981b)	
March 1982 - December 1982	<i>In-vitro</i>	Nutrient addition	Seston change in P and N	Not limited	White et al. (1985)
	<i>In-vitro</i>	Nutrient addition	Seston N:P	Slight Nitrogen	White et al. (1985)
	<i>In-vitro</i>	Dark CO <sub>2</sub> fixation	<sup>14</sup> C CO <sub>2</sub> water uptake	Not limited	White et al. (1985)
	<i>In-vitro</i>	<sup>32</sup> P-PO <sub>4</sub> <sup>3-</sup> uptake	Seston P uptake	Not limited	White et al. (1985)
December 1984 - February 1985	<i>In-vitro</i>	Nutrient addition	Seston change in P and N	Nitrogen	White et al. (1986)
	<i>In-vitro</i>	Nutrient addition	Chlorophyll	Nitrogen	White et al. (1986)
January & February 2004	<i>In-situ</i>	Nutrient addition	Chlorophyll, cell abundance and biomass	Co-limitation	Burger et al. (2007)
March 2012	<i>In-vitro</i>	Nutrient addition	Chlorophyll	Co-limitation	Abell (2013)

Notably, only one of the studies (Burger et al. 2007) summarised in Table 2 examined changes in phytoplankton cell abundance or biovolume in relation to nutrient limitation. Vincent (1981a) concluded that luxury nutrient uptake incubations were the most reliable guide to N versus P demand, although phytoplankton species composition did play a significant role, with diatom and chlorophyte compositions being more reliable indicators than heterocystous cyanobacterial species. Analysis of phytoplankton species composition is therefore an important requirement when assaying natural communities (Vincent 1981a).

Improvements in the TLI of Lake Rotorua since 2010 have been attributed to continuous alum (aluminium sulphate) dosing of the Utuhina (since 2006) and Puarenga (from 2010 to 2018) inflows, although the proportion of the response directly due to alum dosing is uncertain (McBride et al. 2018, Tempero 2019). Alum dosing is intended to sequester dissolved reactive phosphorus (DRP), removing it from the water column, thereby reducing primary production and improving water clarity. The University of Waikato modelled the effects of stream alum dosing on Lake Rotorua; the results predicted extremely low DRP concentrations in the stream inflows below the dosing point, although total phosphorus (TP) remained unchanged (Hamilton et al. 2015). In-lake DRP concentrations also significantly declined in parallel with alum dosing (Figure 2). It was suggested that the observed effects were greater than what could be expected from P sequestration of the Utuhina and Puarenga Steams alone and 'excess' alum entering the lake was sequestering additional phosphorus and removing it from the water column (Hamilton et al. 2015). These results, along with findings by Abell (2013) and Abell and Hamilton (2013) suggest that Lake Rotorua may have shifted from nitrogen limitation to co-limitation or even periodic P limitation.



**Figure 2. Relationship between alum dosing and in-lake median monthly dissolved reactive phosphate (DRP) concentrations in Lake Rotorua. Alum dosing of the Utuhina (since 2006) and Puarenga (from 2010) inflows has been combined into a 10-day rolling average of aluminium.**

The possibility that recently observed improvements in Lake Rotorua water quality were a result of a regime shift towards more frequent P-limitation is an important consideration for management of the lake. Specifically, the intensity and sustainability of alum dosing needs to be carefully weighed against the management of present and future loads of both nitrogen and phosphorus from catchment land use (Hamilton et al. 2015). It was therefore proposed that a monitoring protocol for determining phytoplankton nutrient limitation in Lake Rotorua be developed. This proposal was supported through the Plan Change 10 hearings process, with the need to identify if the lake was N-limited, P-limited, or co-limited emphasised.

The University of Waikato was contracted to develop a regular, repeatable phytoplankton nutrient limitation protocol to meet the following objectives.

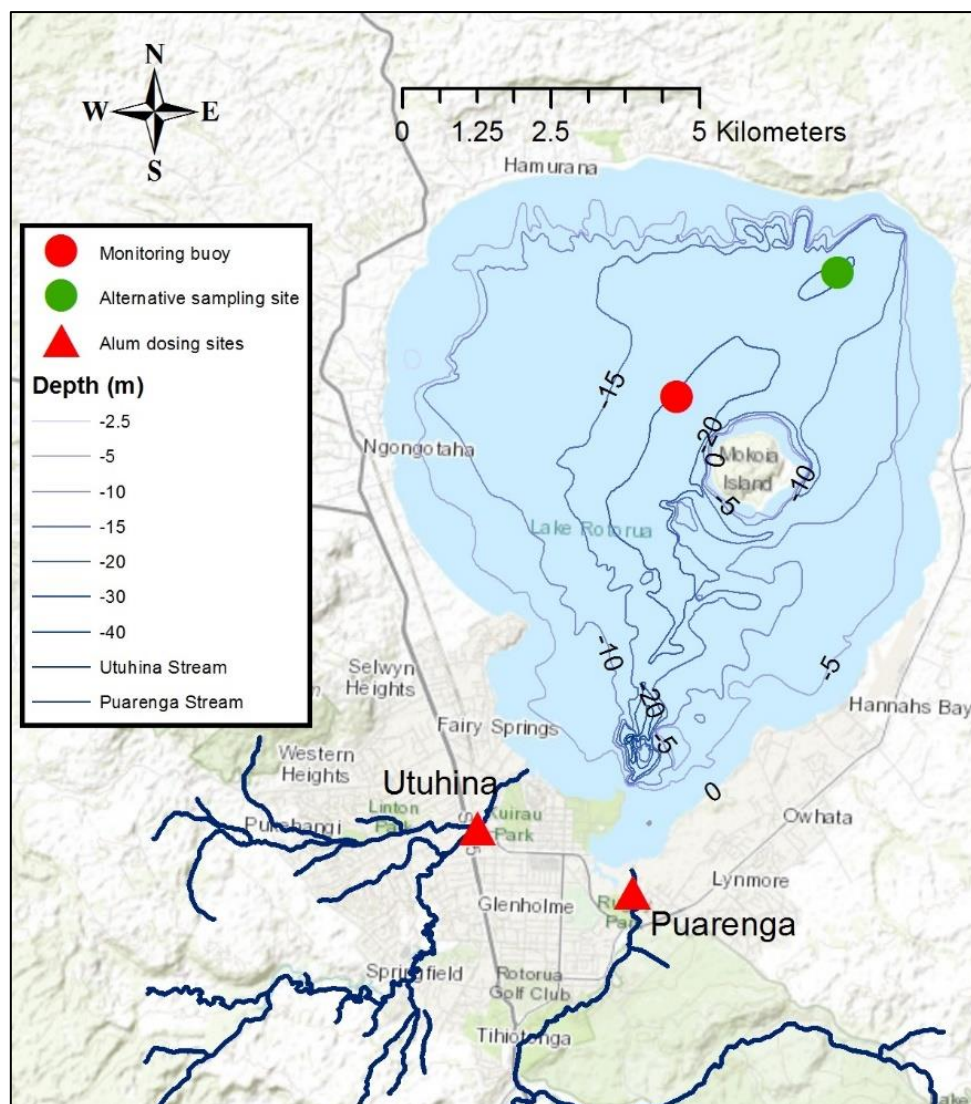
1. Determine the limiting macronutrient (N or P) or macronutrients (N and P) of the phytoplankton community assemblage in Lake Rotorua on a seasonal scale.
2. Determine the concentrations of inorganic and total nutrients in relation to phytoplankton community composition at a seasonal scale.
3. Based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

This report presents findings from the first year of monitoring, covering nutrient limitation results from four seasons in 2019, discusses the suitability of the initial nutrient assays and provides recommendations as to potential improvements in future nutrient limitation monitoring of Lake Rotorua.

# Methods

## Study site

Lake Rotorua (Figure 3) is the largest of 12 lakes jointly managed under the Rotorua Te Arawa Lakes Programme. It has a surface area of 80.6 km<sup>2</sup>, a mean depth of 10.8 m (maximum depth 45 m), a total water volume of 0.85 km<sup>3</sup> and polymictic stratification patterns (Burger et al. 2007). A TLI target of 4.2 has been set for Lake Rotorua, which is considered to be representative of the lake's tropic state in the early 1960s, and considerably lower than that of the early 2000s (McBride et al. 2018a).



**Figure 3. Lake Rotorua with locations of alum dosing stations on the Utuhina and Puarenga Streams. Sampling for nutrient limitation assays were taken from the Lake Rotorua monitoring buoy, apart from September 2019 when sampling was conducted at the indicated alternative site due to adverse weather conditions.**

In addition to in-lake nutrient and chlorophyll concentrations, phytoplankton nutrient limitation was assessed using three experimental assays, particulate ratios of C:P, N:P and C:N, phosphorus and nitrogen uptake assays and phytoplankton growth following nutrient addition. Use of multiple assays allowed for the determination of N and P status indicators as demonstrated by Rattan (2017). Assays involving the addition of both N and P were also conducted to determine nutrient co-limitation. Lake sampling and nutrient assays were conducted a total of four times during the year, on a seasonal basis (Table 3). Lake water sampling was conducted 25 m from the Lake Rotorua automated monitoring buoy (-38.071479, 176.270123), apart from 23 September when weather conditions made the lake unsafe to reach the normal sampling site and samples were taken from the alternative site indicated in Figure 3.

**Table 3. Timing and conditions of experimental growth assays to determine phytoplankton nutrient limitation in Lake Rotorua. Temperature (2 m depth) and light regimes were set to match prevailing seasonal conditions.**

Date	Water temperature (°C)	Light regime (Light:Dark)	Sampling site
25 March 2019	21.0	12:12	Monitoring buoy
24 June 2019	11.0	9:15	Monitoring buoy
23 September 2019	12.2	12:12	Alternative
16 December 2019	20.5	15:9	Monitoring buoy

### Lake sampling

Mid-lake water samples (Figure 3) from the near-surface (2.0 m depth) and hypolimnion (15 m depth) of Lake Rotorua were taken for analysis of nutrients (total nitrogen (TN), total phosphorus (TP), dissolved reactive phosphate (DRP), nitrate, total suspended solids (TSS), inorganic suspended solids (ISS) and volatile suspended solids (VSS) and chlorophyll *a* concentrations. A CTD (conductivity, temperature, depth) profile (SBE 19 plus SEACAT Profiler, Seabird Electronics Inc.) was conducted, with additional mounted sensors for dissolved oxygen (DO) concentration (Seabird Electronics), chlorophyll fluorescence (Chelsea MiniTracka II) and beam transmittance (WetLabs C-star). Approximately 20 L of water retrieved from the near-surface (2 m depth) and was immediately filtered using a 100 µm net to remove large zooplankton capable of reducing phytoplankton biomass due to grazing. The nutrient samples and filtered water were then placed in a chilled dark container and transported back to the laboratory for analysis.

## Carbon, phosphorus and nitrogen particulate ratios

One litre of unfiltered lake water from 2 m depth was provided to NIWA, Hamilton for triplicate analysis of particulate nutrient ratios of C:P, N:P and C:N.

## Nitrogen and phosphorus debt assays

Nitrogen and phosphorus uptake assays were conducted following the methodology of Rattan (2017) and using nutrient concentrations employed by Vincent (1981a,b). Nitrogen and phosphorus uptake were determined by the addition of  $\text{NH}_4\text{Cl}$  (final concentration  $100 \text{ mg-N m}^{-3}$ ) and  $\text{KH}_2\text{PO}_4$  (final concentration  $10 \text{ mg-P m}^{-3}$ ). Triplicate 1 L aliquots of lake water were incubated for 24 h in the dark at in-lake temperature. The experimental control consisted of triplicate 1 L lake water aliquots with no nutrient addition followed by 24 h dark incubation. Nutrient (TN, TP,  $\text{NO}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$ ,  $\text{NH}_4\text{-N}$ , DRP) and chlorophyll *a* concentrations were measured at the beginning and end of the incubation. Nutrient uptake was taken as the difference in N and P at the beginning and end of the 24 h incubation and normalised to the mean of the beginning and end chlorophyll *a* concentrations. Nutrient debt was then calculated as the amount of N and P removed per unit chlorophyll *a* over the 24 h period.

## Phytoplankton growth assay

Triplicate phytoplankton growth assays were incubated for 7-days under a single light intensity of  $100 \mu\text{mol m}^{-2}\text{s}^{-1}$  PAR (Solar system 550, California Lightworks) with day length adjusted to match seasonal changes along with equivalent in-lake temperatures (2 m depth). Phosphorus as  $\text{K}_2\text{HPO}_4$  was added to a final concentration of  $10 \text{ mg-P m}^{-3}$  to the +P treatment and N as  $\text{NaNO}_3$  to a final concentration of  $100 \text{ mg-N m}^{-3}$  to the +N treatment. An additional combined +N and +P treatment group was conducted for assessment of co-limitation. Samples were kept in suspension using an orbital shaker and once-daily hand mixing.

Nutrient concentrations (TN, TP, DRP,  $\text{NO}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$  and  $\text{NH}_4\text{-N}$ ) were determined at the beginning (following nutrient addition) and end of the growth assay. Nutrient concentrations were analysed using a Flow Injection Analyser 8500 Series II (FIA+ 8000 Series, Zellweger Analytix, Inc. Hach). Phosphate was analysed using LACHAT QuickChem method 31-115-01-1-H; ammonium was analysed using LACHAT QuickChem method 31-107-06-1-B and LACHAT QuickChem Method 31-107-04-1-A was used to analyse nitrate/nitrite. Limits of detection were  $0.001 \text{ mg N L}^{-1}$  for  $\text{NO}_2$ ,  $\text{NO}_3$ ,  $0.002 \text{ mg N L}^{-1}$  for  $\text{NH}_4$  and  $0.001 \text{ mg P L}^{-1}$  for DRP.

For chlorophyll *a* analysis, 50 mL water samples were filtered onto glass fibre filters (GF/C: nominal pore size  $0.2 \mu\text{m}$ , 25 mm) that were stored in the dark and stored frozen ( $-20 \text{ }^\circ\text{C}$ )

before extraction with 90% buffered acetone. The samples were extracted for 24 h. Chlorophyll *a* concentrations were then estimated fluorometrically using a Turner Designs 10-AU-005-CE fluorometer (Sunnyvale, CA, USA) calibrated against pure chlorophyll *a*. Growth rate was estimated by linear regression of the natural log of in vivo fluorescence versus time between days 0 and 4. Differences in linear growth rate were determined using one-way ANOVA followed by Tukey's post-hoc tests.

Phytoplankton samples (50 mL) were taken at the beginning (day 0) and end (day 7) of the growth assay, and preserved with Lugol's iodine. Phytoplankton species abundance was determined from 10 mL aliquots allowed to settle in a tubular plankton chamber (Hydro-bios, Denmark) for 24 hours prior to counting. Species were identified to the lowest possible taxonomic level using an inverted microscope (Olympus, Ix71, Japan) at either 400X or 200X magnification in a single transect. The 20 most numerically dominant species for each replicate were identified and enumerated. Where filamentous and colonial species were highly abundant cell counts from were derived from the average number of cells in a subsample of 30 plankton units. Species densities were converted to algal biomass ( $\mu\text{m}^3 \text{mL}^{-1}$ ) using standard values from published literature (Dryden and Vincent 1986, Olenina et al. 2006, Rimet and Druart 2018). Net rates of phytoplankton growth over the incubation period, based on species biovolume, were calculated using the equation:

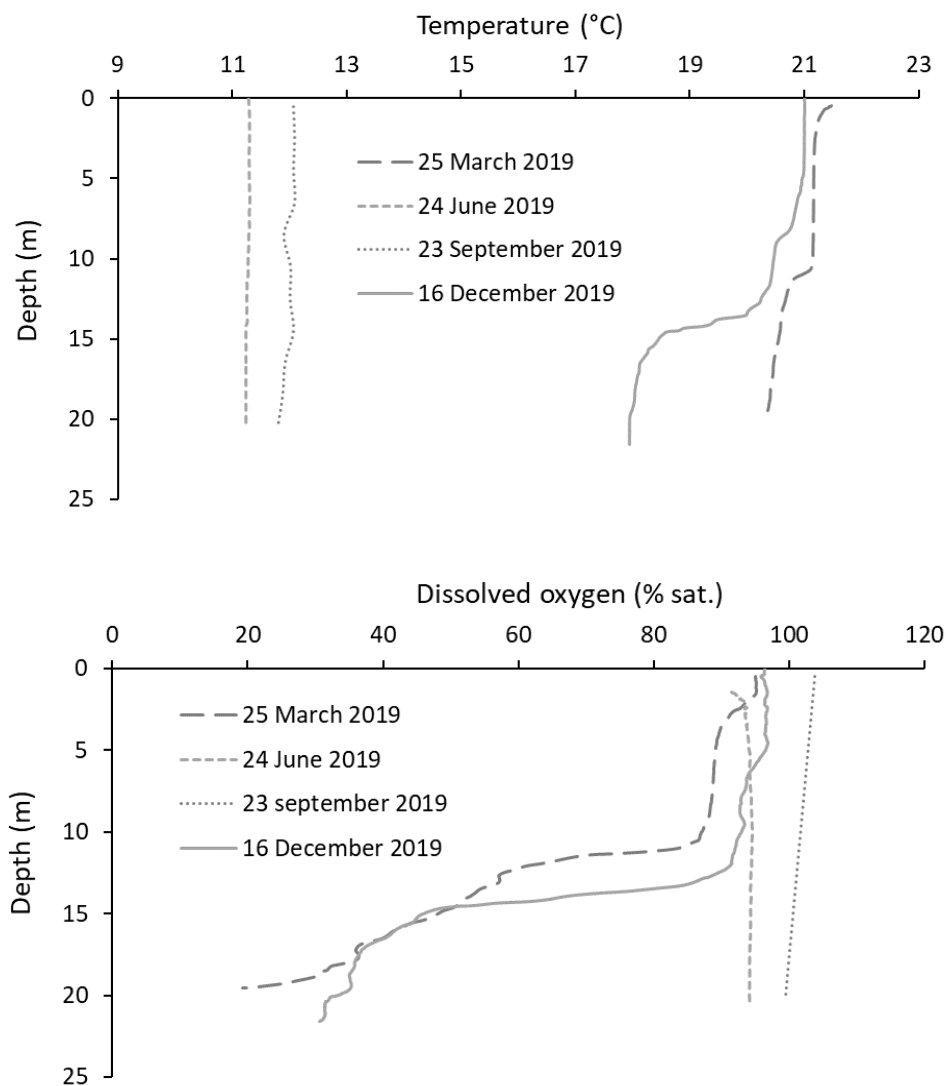
$$r_n = \frac{\ln(N_t/N_0)}{t} \text{ (Reynolds 1997)}$$

where  $r_n$  is the growth rate ( $\text{day}^{-1}$ ) and  $N_t$  and  $N_0$  are cell densities at times  $t$  and 0, respectively. Differences in biovolume and phytoplankton biovolume linear growth rate were determined using one-way ANOVA followed by Tukey's post-hoc tests

# Results

## Vertical lake profiles

Vertical temperature and oxygen profiles from CTD casts indicated strong stratification on 16 December and moderate stratification on 25 March, while the lake was mixed on 24 June and 23 September (Figure 4). Data for 23 September is taken from the lake monitoring buoy as weather conditions prevented safe deployment of the CTD. September dissolved oxygen was interpolated from surface (0.5 m) and bottom (20.5 m) oxygen sensors.



**Figure 4. Temperature (°C) (top) and dissolved oxygen (% saturation) (bottom) profiles for Lake Rotorua on the four sampling dates. Profiles were taken at the Lake Rotorua monitoring buoy using a CTD, data presented for 23 September is from the lake monitoring buoy as a CTD cast could not be safely undertaken on this date.**

## Lake nutrient concentrations

In-lake nutrient concentrations were higher in March compared to the other sampling periods (Table 4). Ammonium and DRP concentrations were notably elevated in the bottom waters in March, likely related to lake stratification. In comparison, surface DRP and nitrite levels were always below detection limits and nitrate was only notably elevated in September. Nutrient concentrations for 15 m depth on 23 September were not sampled due to adverse weather conditions.

**Table 4. Lake Rotorua nutrient concentrations from 2 m and 15 m depth at the time of water sampling for nutrient limitation assays.**

Sample Date	Depth (m)	TN:TP	Total	Total	Ammoniacal-N (g m <sup>-3</sup> )	Nitrate-N (g m <sup>-3</sup> )	Nitrite-N (g m <sup>-3</sup> )	DRP (g m <sup>-3</sup> )
			Nitrogen (g m <sup>-3</sup> )	Phosphorus (g m <sup>-3</sup> )				
25 March	2	14.4:1	0.26	0.018	0.018	0.002	< 0.002	< 0.004
	15	14.5:1	0.42	0.029	0.220	0.011	< 0.002	0.008
24 June	2	14.6:1	0.19	0.013	< 0.010	< 0.002	< 0.002	< 0.004
	15	18.5:1	0.24	0.013	< 0.010	< 0.002	< 0.002	< 0.004
23 September	2	17.5:1	0.21	0.012	0.014	0.038	< 0.002	< 0.004
	15	-	-	-	-	-	-	-
16 December	2	10:1	0.14	0.014	< 0.010	< 0.002	< 0.002	< 0.004
	15	14.3:1	0.20	0.014	0.026	0.006	< 0.002	< 0.004

## Light availability and suspended particles

Photosynthetically Active Radiation (PAR) and water temperature (2 m) were notably different between March and June (Table 5) due to differences in incident solar radiation. PAR and associated light extinction coefficient ( $K_d$ ) were not able to be determined in September and December due to adverse weather conditions and sensor availability, respectively. Chlorophyll  $a$  concentrations varied seasonally, with December surface concentrations notably higher than concentrations measured earlier in the year. Total suspended solids (TSS), volatile suspended solids (VSS) and inorganic suspended solids (ISS) were also seasonally variable, with June observations higher than comparative seasons.

**Table 5. Seasonal observations of Photosynthetically Active Radiation (PAR), water temperature, light extinction coefficient ( $K_d$ ), chlorophyll  $a$  concentrations, total suspended solids (TSS), volatile suspended solids (VSS) and inorganic suspended solids (ISS) for Lake Rotorua. Note that PAR and  $K_d$  are not presented for 23 September and 16 December due to adverse weather conditions and sensor availability, respectively. Chlorophyll  $a$ , TSS, VSS and ISS samples were not retrieved from 15 m depth on 23 September.**

Date	$K_d$ ( $m^{-1}$ )	Depth (m)	Temperature ( $^{\circ}C$ )	PAR ( $\mu mol\ m^{-2}\ s^{-1}$ )	Chlorophyll $a$ ( $mg\ m^{-3}$ )	TSS ( $g\ m^{-3}$ )	VSS ( $g\ m^{-3}$ )	ISS ( $g\ m^{-3}$ )
25 March	0.71	2	21.2	48.0	5.26	4.3	1.4	2.9
		15	20.5		2.66	3.0	0.8	2.2
24 June	0.61	2	11.3	4.0	4.93	7.7	4.2	3.5
		15	11.2		2.62	7.2	4.2	3.0
23 September	-	2	12.1	-	2.22	3.2	1.9	1.3
		15	12.1		-	-	-	-
16 December	-	2	21.0	-	10.07	3.5	3	0.5
		15	18.5		5.13	4.3	2.7	1.6

#### Particulate nutrient concentrations

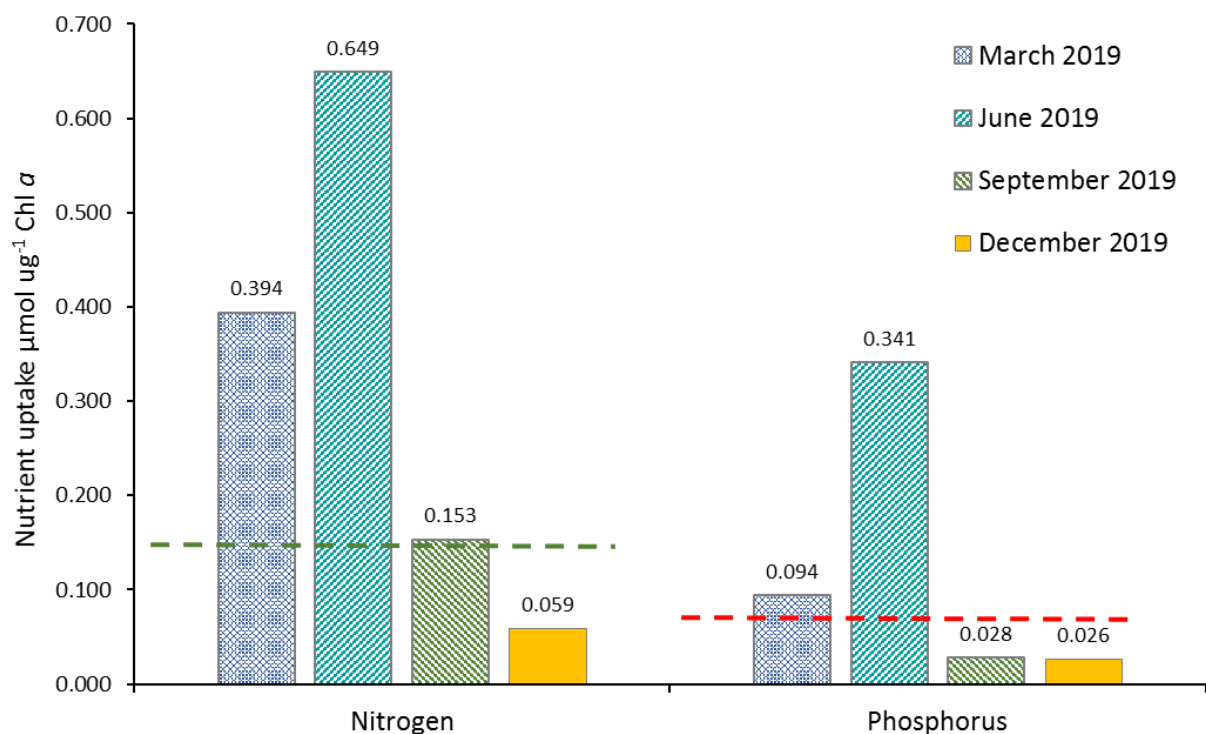
Particulate nutrient concentrations were highly variable throughout the year, which was reflected in the variation of nutrient atomic ratios (Table 6). Reported particulate phosphorus concentrations were significantly higher than total phosphorus concentrations (Table 4) for March and June periods and were therefore omitted from further analysis.

**Table 6. Lake Rotorua surface (2 m) particulate carbon (C), nitrogen (N) and phosphorus (P) concentrations and atomic ratios for the four seasonal nutrient limitation assays. Values marked with a \* were higher than reported total phosphorus concentrations and were omitted from further analysis. Green highlight indicates no nutrient deficiency and orange moderate nutrient deficiency based on the proposed thresholds by Rattan (2017) (refer Table 1 for threshold bands).**

Date	Particulate Carbon ( $mg\ m^{-3}$ )	Particulate Nitrogen ( $mg\ m^{-3}$ )	Particulate Phosphorus ( $mg\ m^{-3}$ )	C:N (atomic ratio)	C:P (atomic ratio)	N:P (atomic ratio)
March 2019	931.5	129.5	139.0*	8.4	-	-
June 2019	1115.0	164.0	20.3*	7.9	-	-
September 2019	445.0	65.9	7.0	7.9	163.9	20.8
December 2019	729.0	69.8	8.0	12.2	235.0	19.3

## Nutrient uptake assay

Nitrogen and P uptake assays were also variable over the year with both N and P limitation indicated for March and June and no limitation or slight nutrient limitation (N, September) in September and December. Nutrient analytical detection limits reduced the sensitivity of the December DRP assay, as DRP-P detection limit concentrations of  $0.004 \text{ g m}^{-3}$  were assumed for samples below those detection limits.

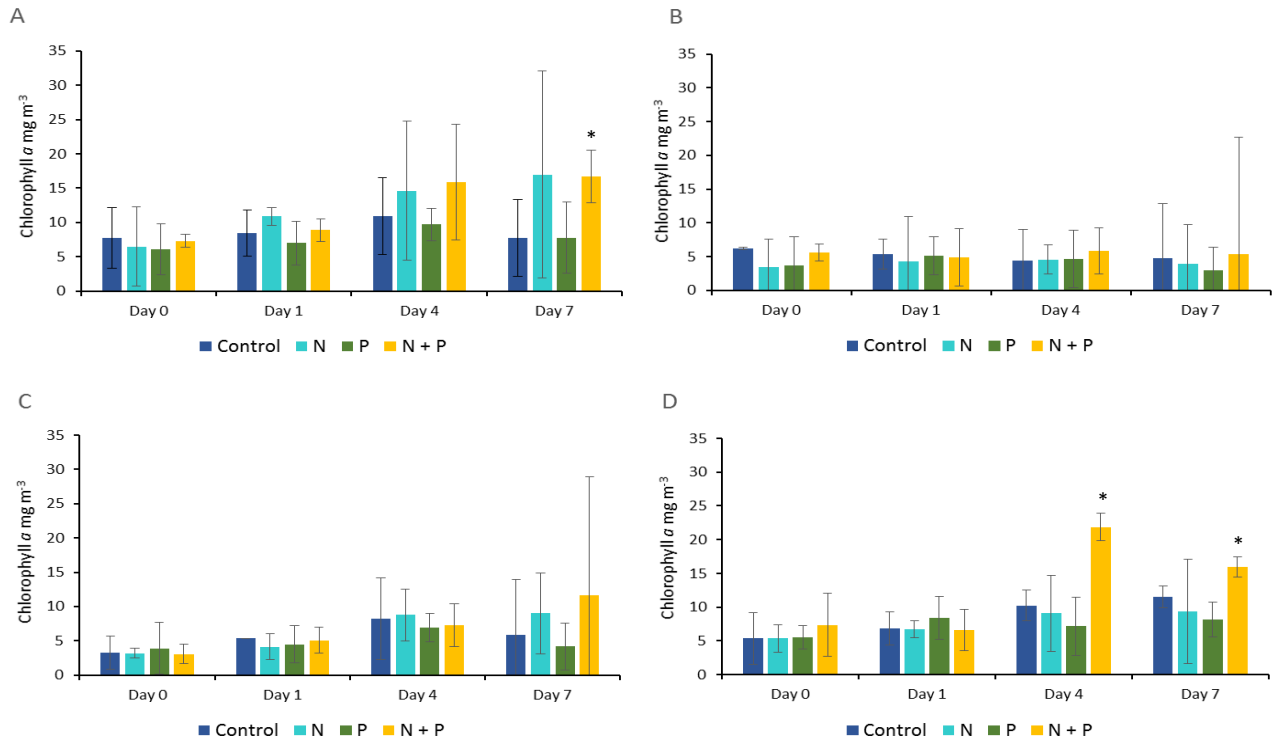


**Figure 5. Phytoplankton nutrient (phosphate-P and ammonium-N) uptake rates per  $\mu\text{g}$  of chlorophyll *a* under 24 h dark incubation at  $21^\circ\text{C}$  (March) and  $11^\circ\text{C}$  (June),  $11.5^\circ\text{C}$  (September) and  $21^\circ\text{C}$  (December). The green dashed line indicates threshold value for nitrogen limitation of  $>0.15 \mu\text{mol N } \mu\text{g}^{-1}$  Chlorophyll *a* and the red dashed line the threshold value for phosphorus limitation of  $>0.075 \mu\text{mol P } \mu\text{g}^{-1}$  Chlorophyll *a* as proposed by Rattan (2017).**

## Phytoplankton growth assay

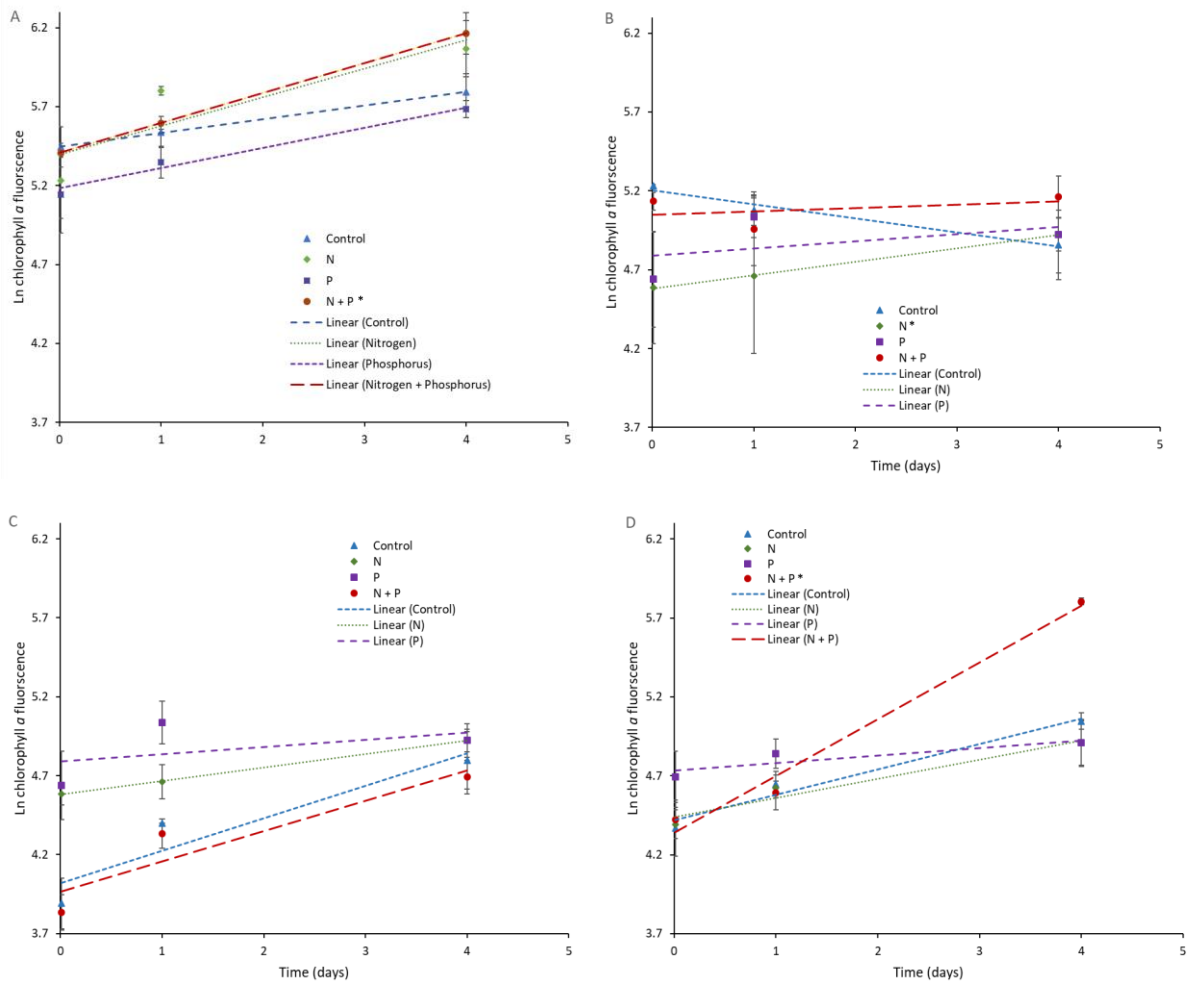
Increasing trends in chlorophyll *a* concentrations were observed in all sampling periods except June. For the March growth assay, mean chlorophyll *a* concentrations in the N + P treatment were significantly larger (ANOVA,  $P < 0.05$ ) compared to the control group by Day 7 (Figure 7A). The March nitrogen treatment produced a similar chlorophyll *a* response but this was not statistically different to the control group. There were no significant changes in

chlorophyll *a* concentrations for any treatments in the June and September growth assays (Figure 7B&C). The December 2019 assay (Figure 7D) produced significant differences (ANOVA,  $P < 0.05$ ) in chlorophyll *a* concentrations in the N + P treatment compared to the control.



**Figure 6. Changes in mean ( $n=3$ ) chlorophyll *a* concentration in response to the addition of either phosphorus ( $10 \text{ mg-P m}^{-3}$ ), nitrogen ( $100 \text{ mg-N m}^{-3}$ ) or nitrogen + phosphorus ( $10 \text{ mg-P m}^{-3}$ ,  $100 \text{ mg-N m}^{-3}$ ) after 7-days. Assays were conducted in (A) March 2019, (B) June 2019, (C) September 2019 and (D) December 2019. Error bars indicate  $\pm 95\%$  confidence intervals. Within groups, treatments significantly different (ANOVA  $< 0.05$ ) to the control are indicated by \*.**

Phytoplankton growth rates estimated from linear regression of the natural log of in vivo fluorescence between days 0 and 4 are presented in Figure 7. Treatment groups with significantly higher ( $P < 0.05$ ) growth rates compared to control groups were observed for N + P March 2019, N June 2019 and N + P December 2019.



**Figure 7. Phytoplankton growth rates determined from chlorophyll *a* fluorescence for (A) March 2019, (B) June 2019, (C) September 2019 and (D) December 2019. Treatments of either phosphorus ( $10 \text{ mg-P m}^{-3}$ ), nitrogen ( $100 \text{ mg-N m}^{-3}$ ) and nitrogen + phosphorus ( $10 \text{ mg-P m}^{-3}$ ,  $100 \text{ mg-N m}^{-3}$ ). Treatment groups with significantly higher (ANOVA,  $P < 0.05$ ) growth rates compared to control groups are indicated by \*. Error bars indicate  $\pm$  standard error.**

A total of 36 phytoplankton species were recorded in Lake Rotorua during the four seasonal growth assays. A comparison between initial community composition and growth responses in the control group and nutrient (N + P) addition treatments found phytoplankton species that were the most abundant at the beginning of the growth assay generally did not respond with the greatest growth response (Table 7). Species with the largest growth responses in the control and nutrient treatment groups partially coincided, but overall growth responses were greater in the nutrient treatments. Growth responses to nutrient addition were strongest in the green alga (Chlorophyta) species and the diatom species *Fragilaria crotonensis*. All other taxonomic groups either declined or experienced small increases in abundance. No cyanobacterial species were observed in the December assay.

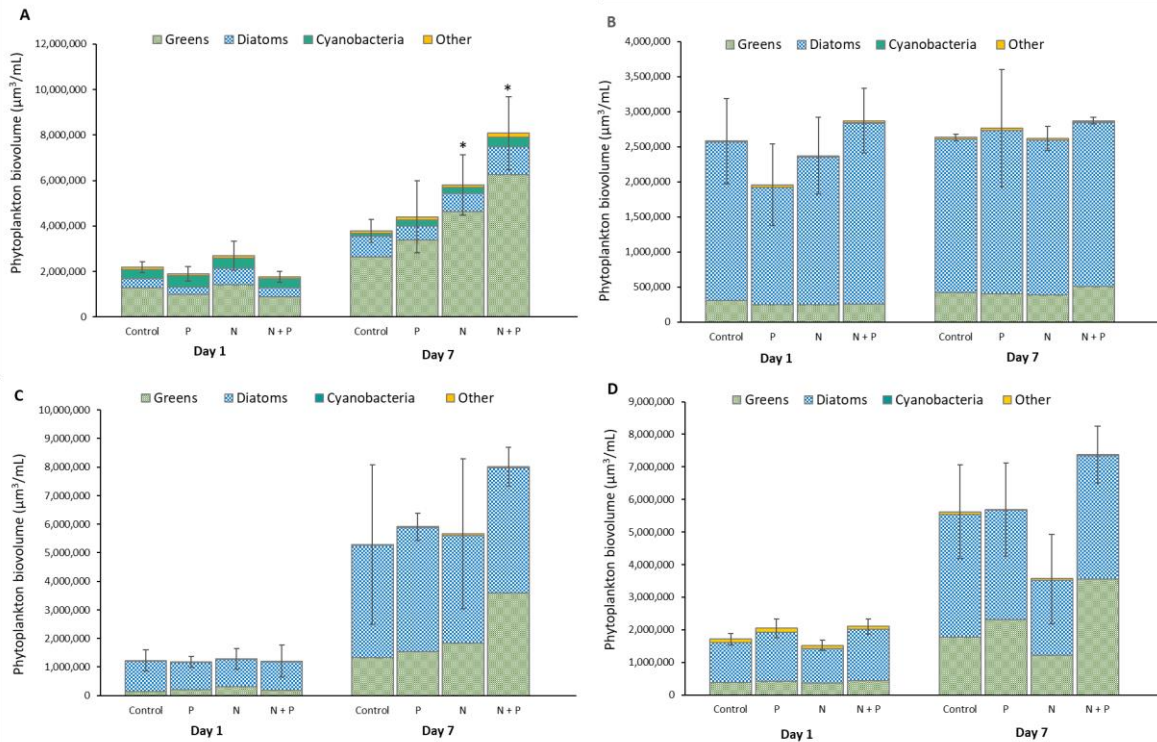
**Table 7. Phytoplankton species initial percentage community composition and percentage change in biovolume after 7-day incubation in control (no nutrient addition) and nutrient (nitrogen and phosphorus) addition treatment groups. Highlighted values indicate the initial top five abundant species and the five species with the largest growth response for each assay period.**

Species	March 2019			June 2019			September 2019			December 2019		
	Initial percentage composition	Control Percentage change	N + P Percentage change	Initial percentage composition	Control Percentage change	N + P Percentage change	Initial percentage composition	Control Percentage change	N + P Percentage change	Initial percentage composition	Control Percentage change	N + P Percentage change
<b>Chlorophyta (Greens)</b>												
<i>Actinastrum hantschii</i>	-	-	-	0.72	178.3	178	-	-	-	-	-	-
<i>Closterium acutum var. variable</i>	<0.01	70948.3	19793	-	-	-	0.83	243.8	3111	0.62	-68.0	83
<i>Closterium aciculare</i>	-	-	-	-	-	-	3.90	1119.6	4216	1.17	40.0	110
<i>Coelastrum sphaericum</i>	11.41	1.0	523	-	-	-	4.56	1325.7	1200	-	-	-
<i>Dictyosphaerium sp.</i>	10.63	186.6	1236	2.43	-30.3	24	1.00	37.0	715	1.16	31.9	380
<i>Elakotothrix gelatinosa</i>	-	-	-	-	-	-	-	-	-	0.51	-19.2	195
<i>Kirchneriella obesa</i>	17.27	108.1	326	3.02	29.3	15	-	-	-	0.22	311.1	505
<i>Monoraphidium mirabile</i>	0.07	885.7	3277	1.01	208.6	450	1.90	551.7	960	0.93	228.6	728
<i>Mougeotia sp.</i>	3.04	170.4	1038	0.10	366.7	211	1.52	678.6	415	8.36	781.6	1515
<i>Oocystis sp.</i>	-	-	-	0.35	51.9	-37	-	-	-	-	-	-
<i>Pediastrum duplex</i>	-	-	-	-	-	-	-	-	-	0.63	-100.0	166
<i>Quadrigula lacustris</i>	4.96	261.4	290	0.12	-80.0	53	-	-	-	-	-	-
<i>Sphaerocystis sp.</i>	2.71	-21.4	323	0.66	17.7	130	0.12	1406.6	2900	4.93	-41.1	-10
<i>Spondylosium sp.</i>	-	-	-	0.02	-89.1	2225	0.31	-100.0	333	<0.01	-100.0	-100
<i>Staurastrum sp.</i>	-	-	-	0.50	238.1	103	0.97	520.0	203	3.33	92.8	44
<b>Euglenophyta</b>												
<i>Trachelomonas volvocina</i>	0.93	18.3	225	0.36	28.0	-23	0.35	-10.0	7	-	-	-
<b>Cryptophyta</b>												
<i>Chroomonas sp.</i>	2.09	-65.0	-46	0.46	63.0	-7	-	-	-	-	-	-
<i>Cryptomonas sp.</i>	-	-	-	0.14	35.8	-100	0.93	-42.9	176	-	-	-
<b>Chrysophyta</b>												
<i>Mallomonas sp.</i>	<0.01	<1.0	<1	-	-	-	0.28	140.0	200	-	-	-
<i>Dinobryon sp.</i>	-	-	-	-	-	-	-	-	-	3.93	-29.0	-41

**Table 7. Continued**

Species	March 2019			June 2019			September 2019			December 2019		
	Initial percentage composition	Control Percentage change	N + P Percentage change	Initial percentage composition	Control Percentage change	N + P Percentage change	Initial percentage composition	Control Percentage change	N + P Percentage change	Initial percentage composition	Control Percentage change	N + P Percentage change
<b>Bacillariophyceae (Diatoms)</b>												
<i>Acanthoceras zachariasii</i>	1.98	-50.0	33	3.67	35.0	-27	0.71	340.0	-67	15.43	149.5	40
<i>Asterionella formosa</i>	-	-	-	-	-	-	2.47	463.2	952	0.65	300.0	176
<i>Aulacoseira distans</i>	0.53	-59.4	313	0.63	1.1	18	0.15	137.5	194	0.26	3.7	61
<i>Aulacoseira granulata</i>	2.73	-48.5	29	12.40	51.5	1	3.04	11.6	114	0.55	203.4	348
<i>A. granulata</i> var. <i>angustissima</i>	0.37	-75.4	385	12.82	-45.5	-49	4.51	27.8	67	0.67	300.0	154
<i>A. granulata</i> var. <i>spiralus</i>	-	-	-	42.80	3.5	9	6.35	78.7	24	2.26	67.6	6
<i>Cyclotella stelligera</i>	<0.01	-100.0	-100	0.38	-53.2	57	-	-	-	24.80	12.4	-34
<i>Fragilaria crotonensis</i>	3.35	717.9	1272	0.65	68.7	232	60.14	291.2	395	25.77	414.7	395
<i>Melosira</i> sp.	14.07	-77.3	-1	16.24	-41.4	-40	5.63	2250.0	193	3.82	441.2	-26
<i>Urosolenia eriensis</i>	-	-	-	0.17	-21.4	-83	-	-	-	-	-	-
<b>Dinoflagellata</b>												
<i>Peridinium</i> sp.	1.75	0.6	122	0.07	-100.0	100	-	-	-	-	-	-
<b>Cyanobacteria</b>												
<i>Dolichospermum planctonicum</i>	0.04	-100.0	-100	<0.01	-100.0	-100	0.31	466.7	-29	-	-	-
<i>Aphanizonmenon issatschenkoi</i>	1.39	-100.0	-83	-	-	-	-	-	-	-	-	-
<i>Aphanocapsa</i> sp.	18.03	-55.6	-32	0.28	-66.2	-67	-	-	-	-	-	-
<i>Pseudanabaena mucicola</i>	2.67	-97.4	-98	-	-	-	-	-	-	-	-	-

Apart from the June assay, total phytoplankton biovolumes increased over the 7-day incubation period for all control and treatment groups. Significantly (ANOVA,  $P < 0.05$ ) greater increases in total phytoplankton biovolume after 7 days were observed for the March N treatment and N + P treatment compared to the control group. The September and December N + P treatments did respond with increased growth but this was not significantly different to the control group (Figure 8).

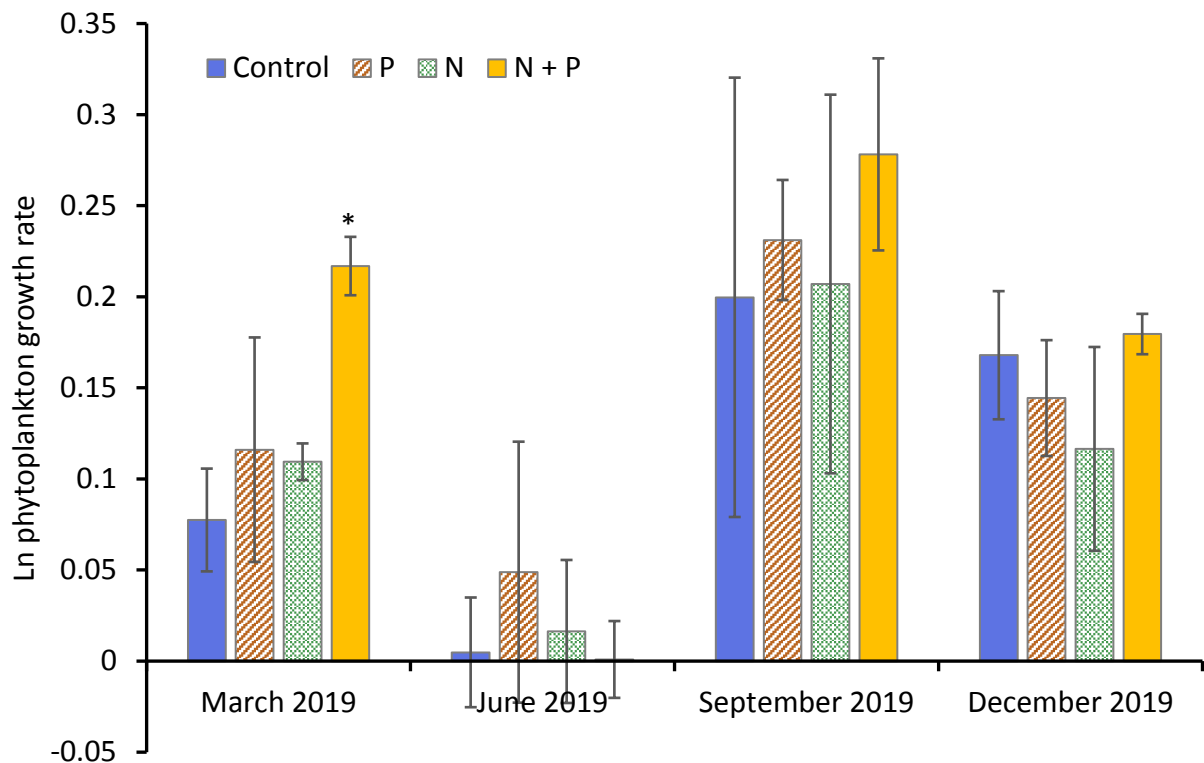


**Figure 8. Change in mean ( $n=3$ ) total phytoplankton biovolume in response to the addition of either phosphorus ( $10 \text{ mg-P m}^{-3}$ ), nitrogen ( $100 \text{ mg-N m}^{-3}$ ) or nitrogen + phosphorus ( $10 \text{ mg-P m}^{-3}$ ,  $100 \text{ mg-N m}^{-3}$ ) after 7-days. Assays were conducted in (A) March 2019, (B) June 2019, (C) September 2019 and (D) December 2019. Significantly different biovolumes between nutrient addition and control groups are indicated by \* at the  $P < 0.05$  level and \*\* at the  $P < 0.01$  level. Error bars indicate  $\pm 95\%$  confidence interval.**

Green algae (Chlorophyta) were the dominant taxonomic group in March with 53% of the total phytoplankton biomass in the initial samples (Figure 8). This dominance was supplanted by diatoms (Bacillariophyceae) constituting  $>70\%$  of the total phytoplankton biomass in the subsequent assays. Cyanobacterial (Cyanophyceae) species were most abundant in March composing 21% of the total phytoplankton biomass. *Aphanizonmenon issatschenkoi* and *Pseudanabaena mucicola* were the two most abundant cyanobacterial species but neither respond positively to any of the nutrient addition treatments. Notably, *P. mucicola* abundance declined from a mean of  $4723 \text{ cells mL}^{-1}$  at the start of the incubation to  $103 \text{ cells mL}^{-1}$  on day-

7. Mean cyanobacterial biomass was <0.2% of the initial total biomass in the June, September and December assays.

Comparisons of seasonal phytoplankton relative growth rates in the assay experiments are presented in Figure 9. March was the only period with a statistical difference (ANOVA,  $P < 0.05$ ) in phytoplankton growth rate between the control and treatment groups, and this was only for the N+P treatment.



**Figure 9. Phytoplankton relative growth rates in response to the addition of either phosphorus ( $10 \text{ mg-P m}^{-3}$ ), nitrogen ( $100 \text{ mg-N m}^{-3}$ ) or nitrogen + phosphorus ( $10 \text{ mg-P m}^{-3}$ ,  $100 \text{ mg-N m}^{-3}$ ) after 7-days. Significantly different growth rates to the control group for each assay period are indicated by \*. Error bars indicate  $\pm 95\%$  confidence intervals.**

# Discussion

## Individual nutrient limitation indicators

Phytoplankton nutrient debt and nutrient growth assays were conducted in March, June, September and December 2019 to determine whether seasonal shifts in nutrient limitation were occurring in Lake Rotorua. Phytoplankton species responses to nutrient addition were also examined. Indicators of nutrient limitation were calculated including TN:TP and particulate nutrient ratios (C:N, C:P and N:P). In addition, direct measured responses to nutrient addition including nutrient uptake rates, inferred growth rate from chlorophyll *a* change, phytoplankton biovolume change and relative phytoplankton growth were also conducted.

Individual phytoplankton species each evolve physiological responses to cope with the limiting nutrient at a given point in time. Therefore, nutrient ratios and nutrient uptake reflect the status of phytoplankton cells at the time of sampling. Species able to maximise uptake of transiently available nutrients in response to shifting ratios will be more successful (Healey and Hendzel 1980, Hecky et al. 1993). In comparison, growth responses to transient enrichment indicate whether the community responds, under culture conditions, to an increase in N and/or P supply (Rattan 2017).

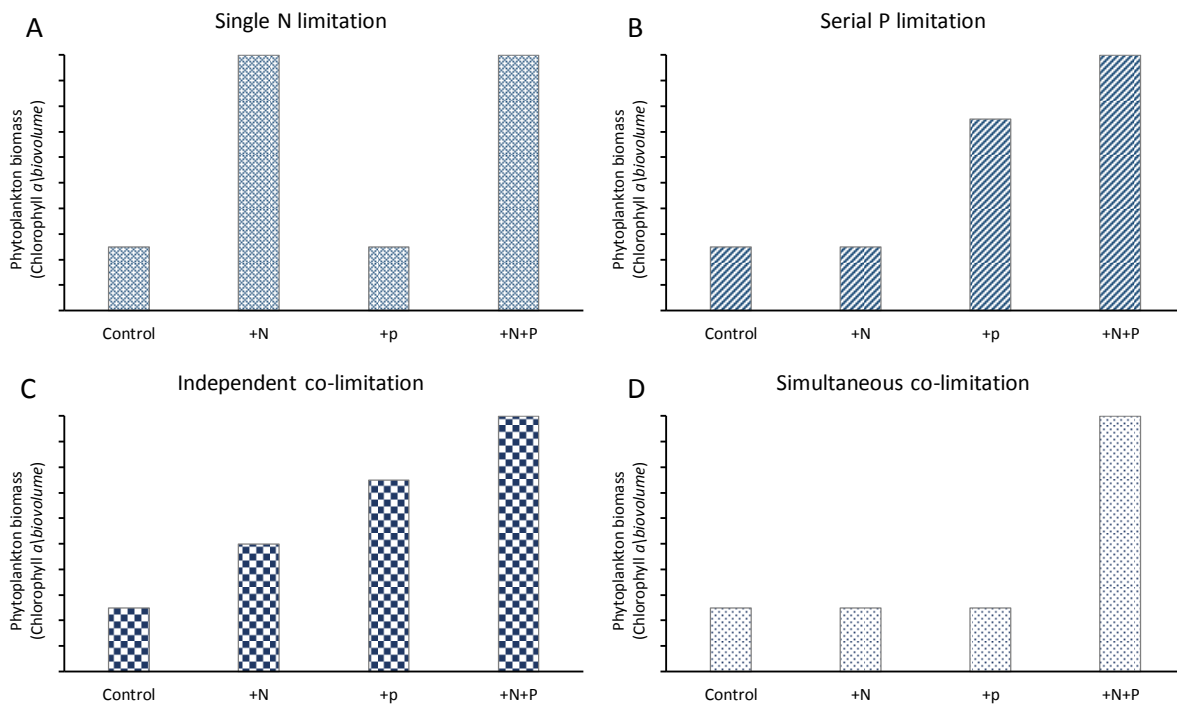
In-lake nutrient concentrations indicated that readily available inorganic forms of N (nitrate and ammonium) and P (DRP) were restricted for most of the assay periods, while particulate forms constituted approximately half of the TN and TP. The remaining total nutrients can be inferred as mostly dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP). DON and DOP are a complex mixture of compounds, which differ in chemical structure and bioavailability but represent a potentially bioavailable source of N and P (Boëchat et al. 2019). Depending on their lability, DON and DOP components can be quickly assimilated by phytoplankton (Fellman et al. 2009), although species assimilation efficiencies differ between molecules of low and high molecular weight (Fiedler et al. 2015). These organic compounds can also affect phytoplankton community composition, as different species have different abilities to use DON compounds (Fiedler et al. 2015). In-lake total N and P concentrations were higher in March compared to the other seasons (Table 4), likely due to diffusion of ammonium and DRP out of the bottom sediments during stratification (Burger et al. 2005, Smith et al. 2016). The polymictic nature of Lake Rotorua produces short periods of stratification followed by lake overturn during the autumn, resulting in pulses of inorganic nutrient release to the surface waters (Burger et al. 2005). In contrast, June and December TN and TP surface (2 m) concentrations were lower and consisted of higher fractions of inorganic N and P and less available DON and DOP. However, the December bottom (15 m) nutrient concentrations were slightly elevated in response to stratification and developing hypoxia in the hypolimnetic zone. The September surface (2 m) nitrate concentration was

higher compared to the other sampling periods but was within the normal seasonal range. Employing the assumptions that TN:TP >15:1 is indicative of potential P-limitation, TN:TP <15:1 and >7:1 is indicative of potential N- and P co-limitation, and TN:TP < 7:1 is indicative of potential N-limitation (Abell et al. 2010) classifications of nutrient co-limitation in March, June and December and P limitation in September can be applied.

The use of stoichiometric molar ratios of particulate nutrients to infer nutrient status relies on the deviation of cellular C, N and P content from optimal values in planktonic algae and bacteria, reflecting the relative supply of each. These were assessed against bands proposed by Healey and Hendzel (1980) (Table 1) to indicate N and P deficiency in “average” freshwater algae. These threshold values indicated N limitation in March, P limitation in June and September and co-limitation in December.

The nutrient uptake assay uses the extent to which cells have invested in nutrient uptake and assimilation, by determining the rate of dark uptake of nutrients. As with stoichiometry, this assay indicated N being the primary limiting nutrient in March, but identified N and P as both limited in June and N marginally limiting in September. Nitrogen and P uptake was comparatively minor in the December assay indicating either no nutrient limitation or co-limitation of either the primary macronutrients or a micronutrient (Healey 1973).

Phytoplankton bioassay outcomes were classified according to the nutrient limitation categories defined by Harpole et al. (2011) and illustrated in Figure 10. The responses include single nutrient (N or P) limitation; a response to only one of the nutrient treatments (+N or +P) and the response to the combined treatment (+N+P) is no different (Figure 10A). Serial limitation (N or P); the response is to only one of the single nutrient treatments (+N or +P) but a larger response to the +N+P treatment (Figure 10B). Independent co-limitation (primary N or P); a response to both single nutrient treatments and a larger response to the +N+P treatment; the single treatment with the larger response indicates the primary limiting nutrient (Figure 10C). Simultaneous co-limitation; response only to the +N+P treatment (Figure 10D).



**Figure 10. Example biomass response patterns. These patterns correspond to a subset of the nutrient limitation categories defined by Harpole et al. (2011). A) Single N limitation: a response to only one of the single treatments, in this example +N and the response to the +NP treatment is no different. B) Serial P limitation: a response to only one of the single nutrient treatments, in this example +P and a larger response to the +NP treatment. C) Independent co-limitation (primary P): a response to both single nutrient treatments with a larger response to +P and an even larger response to the +NP treatment; D) Simultaneous co-limitation: a response only to the +NP treatment. No nutrient limitation: no response to any nutrient treatment (not shown).**

Apart from the June period, increases in chlorophyll *a* concentrations were observed in all phytoplankton growth assay periods, indicating conditions were favourable to growth for some taxa. While increases in chlorophyll *a* concentrations were generally observed, the degree of sample variation often resulted in poor statistical differentiation. For example, in the March assay chlorophyll *a* increased over time in both the N and N + P treatments while the P treatment response was similar to the control group (Figure 6a), however, only the N + P treatment was statistically different from the initial chlorophyll concentration. In addition, there is some evidence that exhaustion of the nutrient supply may have occurred, resulting in slowed or even declining growth after day 4. There does appear to be general agreement between chlorophyll *a* concentrations and biovolumes for treatment groups, indicating that the pattern of response is consistent, if statistically variable for treatment groups (Appendix 1). From these results it appears that N limitation was present in March and simultaneous co-limitation was occurring in December.

Phytoplankton growth rates determined from chlorophyll *a* concentrations and total phytoplankton biomass provided little additional insight. When treatment growth rates were compared to control rates only +N+P treatments for the March and December assays were positively significantly different. The September chlorophyll *a* N treatment was significantly different from the control, however this was primarily due to decreasing chlorophyll *a* concentrations in the control group, a pattern which was not observed in the total biovolumes. A summary of conclusions from the nutrient limitation measures is presented in Table 8 below.

**Table 8. Summary of seasonal nutrient limitation results for Lake Rotorua. Limitation for each measure is indicated by P (phosphorus), N (nitrogen), CO (nutrient co-limitation) or Inconclusive.**

Assay Period	TN:TP	Particulate		Chlorophyll yield	Chlorophyll growth rate	Biovolume yield	Biovolume growth rate
		nutrient ratios.	Nutrient uptake				
March 2019	CO	N	N	N	CO	N/CO	CO
June 2019	CO	P	CO	Inconclusive	Inconclusive	Inconclusive	Inconclusive
September 2019	P	P	N	Inconclusive	Inconclusive	P	Inconclusive
December 2019	CO	CO	CO	CO	CO	P/CO	Inconclusive

### Seasonal changes in nutrient limitation

The December and March periods provide the clearest indications for nutrient limitation, with N limitation occurring in March and independent co-limitation in December. Lake Rotorua is polymictic, with summer stratification periods of up to 3–4 weeks reducing to intermittent periods of a few days by March–April (Burger et al. 2007, McBride et al. 2018b). During periods of stratification nutrients often become depleted in the epilimnion due to uptake by actively growing phytoplankton and loss of nutrients by sedimentation of seston to the hypolimnion (Scholten et al. 2005). These factors could explain the occurrence of nutrient co-limitation for the March and December assays. The occurrence of N limitation in March is likely due to episodic release of P from the hypolimnion as lake stratification weakens during the autumn period as alum dosing is insufficient to mitigate internal P release. In contrast, co-limitation in December may reflect depletion of epilimnetic nutrient pools and reduced P availability due to alum dosing.

The June phytoplankton growth assay resulted in no significant increase in either chlorophyll *a* or biovolume despite indications of strong N and P debt from the nutrient uptake assay (Figure 5). Phytoplankton in high latitudinal environments experience significant seasonal changes in day length. Studies have shown that day length affects growth of algae and these effects may be species-specific. For example, shorter day length decreased growth rates of cyanobacteria and diatoms, with the decrease being greater in cyanobacteria (Foy and Gibson

1993). This effect was apparent in the phytoplankton community composition for the June assay; mean cyanobacteria biovolume decreased by from 2014 to 933 mm<sup>3</sup> mL<sup>-1</sup> over the 7-day incubation period. Similar species-specific growth rate declines have been reported for diatoms with the reduced day length effect being greater than nutrient limitation effects (Litchman et al. 2003, Shatwell et al. 2013). Another potential factor limiting growth may have been depletion of micronutrients such as silicon. Diatoms were the most abundant taxonomic group for the June period, the growth rates of which are known to be negatively impacted under reduced silicon availability (Sommer and Stabel 1983). However, an investigation examining silicon availability as a limiting nutrient in Lake Rotorua found that while diatom productivity is sufficiently high to keep the concentrations of silicon <2 mg L<sup>-1</sup>, recycling rates are likely sufficient for diatom growth (Pearson et al. 2016). As silicon concentrations were not measured in this study the potential effects of silicon limitation is speculative.

As with the June assay, the September phytoplankton growth assay was somewhat inconclusive. Unlike the June assay, large increases in phytoplankton biovolume were observed for all treatment and control groups, although only comparatively small increases in chlorophyll *a* concentrations occurred. The N + P treatment did produce the largest absolute growth responses in terms of chlorophyll *a* concentration and biovolume, however the response was not significantly different from either the control or individual nutrient growth rates. Other measures such as nutrient ratios and nutrient debt assay also failed to provide consistent indications of nutrient limitation. The magnitude of the September biovolume growth response was the largest of the four assays and coupled with the higher in-lake nitrate concentration at the time may represent a case of neither nutrient being a limiting factor to growth.

### Phytoplankton community changes

There was little variation in phytoplankton community composition between the assay periods at the phyla taxonomic level with either diatoms or green algae being the dominant taxon (Figure 8). Cyanobacterial species were the next most abundant taxon but were only present in ecologically relevant abundance in the March assay where they constituted approximately 21% of the initial biovolume, for the remaining assays periods they were present at <0.2% of the total biomass. Initial phytoplankton community composition was typically numerically dominated by five or six species, however, these species rarely responded to nutrient addition with the maximum observed growth responses. Across all assay periods green algae species had the greatest growth response to nutrient addition. This is a common response to nutrient addition experiments, as green algae appear to be optimally adapted to respond to nutrient influxes in controlled mesocosms compared to slower growing diatoms and cyanobacterial species (Sakshaug and Olsen 1986, Dauta et al. 1990, Jensen et al. 1994, Lürling et al. 2013). The differential growth response of phytoplankton species

should be kept in mind when evaluating nutrient addition experiments, as N and P addition represents a stochastic event to the phytoplankton community often resulting in a shift community composition as different species are able to take advantage of the disturbance. It is significant that the response observed in the bioassay containers may not mimic the lake environment.

The abundance of diatoms and green algae is typical for Lake Rotorua (Burger et al. 2007, Paul et al. 2012) or eutrophic lakes in general (Kalff and Knoechel 1978, Ryan et al. 2006), however, cyanobacterial species are a common feature of large eutrophic lakes (Paerl et al. 2020). The comparative scarcity of cyanobacterial species in the assays conducted subsequent to the March assay is expected as cyanobacterial species rarely proliferate during the winter and spring when temperatures are cooler and there is less available light (Bellinger and Sigeo 2015), although their absence from the December assay was unexpected. This may represent a potential underrepresentation of a key taxonomic group when assessing nutrient limitation responses in Lake Rotorua and further investigation is recommended.

There is no single satisfactory method to identify the limiting nutrient. The average elemental ratio may be unreliable if detrital matter constitutes a significant part of the total particulates. The average ratio may also be unreliable if a few species dominate a community, because the relative requirement for N and P is quite variable between species. For example, when the dominant species has an N:P requirement of less than 7:1, a ratio of 10: 1 for the community should be a sign of P limitation, rather than N limitation, as the Redfield ratio would suggest. Nutrient debt assays are also limited by the fact that phytoplankton can uptake and store P relative to their growth requirements (White et al. 1986), likely a response to environmental pulses of available P (White et al. 1985). This means that P debt responses may indicate that phytoplankton phosphorus storage pools are less than full, but not necessarily that growth is being reduced or prevented by shortage of phosphorus (White et al. 1985). The results of bioassays also cannot be accepted uncritically. Whether one uses the response of a single organism or of natural populations to nutrient additions as a measure of limitation, problems can arise from, among others, species differences in nutrient requirements and artificial successions.

## Conclusions

The results from the current nutrient limitation study indicate that no single nutrient emerged as limiting for all or most of the time. From the various lines of evidence it appears that here nutrients were limiting to growth, both N and P were variously implicated and at times co-limitation may be occurring. This suggests that current nutrient loading mitigation measures undertaken by the Bay of Plenty Regional Council, including alum dosing, are intermittently achieving the targeted co-limitation response. However, assay results from the winter and

spring periods were inconclusive with the June assay indicating light to be the limiting factor and that there was no limiting nutrient in the September assay. It is notable that there was comparatively little variation in surface TP and DRP concentrations across the four assay periods and changes in the TN:TP were driven by TN which was in-turn driven by variation in dissolved inorganic nitrogen (DIN). It has been proposed that DIN:TP may be a better predictor of nitrogen limitation (Kolzau et al. 2014) and could be investigated as a more efficient indicator of nutrient limitation. Phytoplankton community composition was dominated by diatoms for the majority of the study although growth responses to nutrient addition primarily occurred in the green algal species. When present, cyanobacterial species biovolume declined in both control and nutrient addition treatments, indicating experimental conditions were either not favourable for growth or they were out competed by more optimally adapted species.

While some variation in nutrient status and community composition were observed at a seasonal scale further investigation may provide insight into the environmental drivers and the time scales at which these changes occur. Determination of these drivers will assist in developing suitable indicators of nutrient limitation that can be measured and reported on at time-scales appropriate for short-term (days-weeks) management responses such as adjustment of alum dosing rates.

## Recommendations

1. Current alum dosing appears to provide effective mitigation of external P loading to Lake Rotorua for most of the year. However, the efficacy of alum dosing in relation to P internal loading following stratification requires further investigation at smaller time scales than the seasonal assessments conducted in this study.
2. Further nutrient limitation assays conducted at 2-weekly intervals over the spring-summer-autumn period will provide finer-scale temporal resolution of nutrient availability in relation to environmental perturbation and allow for assessment of more responsive nutrient limitation indicators such as DIN:TP.
3. Additional nutrient limitation assays conducted during the summer and autumn period when conditions are more favourable to cyanobacterial growth should be conducted. This will provide guidance as to the likely growth responses of cyanobacterial species to varying nutrient addition.

# References

Abell JM (2013). Variability in nutrient loading to lake ecosystems and associated impacts on water quality. PhD thesis, University of Waikato.

Abell JM and Hamilton DP (2013). Bioavailability of phosphorus transported during storm flow to a eutrophic, polymictic lake. *New Zealand Journal of Marine and Freshwater Research* **47**: 481-489.

Abell JM, Özkundakci D and Hamilton DP (2010). Nitrogen and phosphorus limitation of phytoplankton growth in New Zealand lakes: implications for eutrophication control. *Ecosystems* **13**: 966-977.

Bellinger EG and Sigeo DC (2015). Freshwater algae: identification, enumeration and use as bioindicators, John Wiley & Sons.

Boëchat IG, Paiva-Magela ABdM, Reis HR and Gücker B (2019). Dissolved organic N in shallow tropical lakes and reservoirs: Contribution to total dissolved N and relationships with eutrophication. *International Review of Hydrobiology* **104**: 106-115.

Burger D, Hamilton D, Hall J and Ryan E (2007). Phytoplankton nutrient limitation in a polymictic eutrophic lake: community versus species-specific responses. *Fundamental and Applied Limnology/Archiv für Hydrobiologie* **169**: 57-68.

Burger DF, Hamilton DP, Pilditch CA, Gibbs MM and Hall JA (2005). Sediment phosphorus release during stratification in polymictic Lake Rotorua, New Zealand. *SIL Proceedings, 1922-2010* **29**: 811-814.

Burns NM, Rutherford JC and Clayton JS (1999). A monitoring and classification system for New Zealand lakes and reservoirs. *Lake and Reservoir Management* **15**: 255-271.

Dauta A, Devaux J, Piquemal F and Boumnic L (1990). Growth rate of four freshwater algae in relation to light and temperature. *Hydrobiologia* **207**: 221-226.

Dryden SJ and Vincent WF (1986). Phytoplankton species of Lake Okaro, Central North island. *New Zealand Journal of Marine and Freshwater Research* **20**: 191-198.

Elser JJ, Bracken ME, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB and Smith JE (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* **10**: 1135-1142.

Fellman JB, Hood E, D'amore DV, Edwards RT and White D (2009). Seasonal changes in the chemical quality and biodegradability of dissolved organic matter exported from soils to streams in coastal temperate rainforest watersheds. *Biogeochemistry* **95**: 277-293.

- Fiedler D, Graeber D, Badrian M and Köhler J (2015). Growth response of four freshwater algal species to dissolved organic nitrogen of different concentration and complexity. *Freshwater Biology* **60**: 1613-1621.
- Foy R and Gibson C (1993). The influence of irradiance, photoperiod and temperature on the growth kinetics of three planktonic diatoms. *European Journal of Phycology* **28**: 203-212.
- Geider R and La Roche J (2002). Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. *European Journal of Phycology* **37**: 1-17.
- Hamilton DP, McBride CG and Jones H (2015). Assessing the effects of alum dosing of two inflows to Lake Rotorua against external nutrient load reductions: Model simulations for 2001-2012. ERI Report No. 56. Hamilton, New Zealand, University of Waikato: 56p.
- Harpole WS, Ngai JT, Cleland EE, Seabloom EW, Borer ET, Bracken ME, Elser JJ, Gruner DS, Hillebrand H and Shurin JB (2011). Nutrient co-limitation of primary producer communities. *Ecology Letters* **14**: 852-862.
- Healey F (1973). Inorganic nutrient uptake and deficiency in algae. *CRC Critical Reviews in Microbiology* **3**: 69-113.
- Healey F and Hendzel L (1980). Physiological indicators of nutrient deficiency in lake phytoplankton. *Canadian Journal of Fisheries and Aquatic Sciences* **37**: 442-453.
- Hecky R, Campbell P and Hendzel L (1993). The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. *Limnology and Oceanography* **38**: 709-724.
- Hendzel L, Hecky R and Findlay D (1994). Recent changes of N<sub>2</sub>-fixation in Lake 227 in response to reduction of the N: P loading ratio. *Canadian Journal of Fisheries and Aquatic Sciences* **51**: 2247-2253.
- Jensen J, Jeppesen E, Olrik K and Kristensen P (1994). Impact of nutrients and physical factors on the shift from cyanobacterial to chlorophyte dominance in shallow Danish lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **51**: 1692-1699.
- Kalff J and Knoechel R (1978). Phytoplankton and their dynamics in oligotrophic and eutrophic lakes. *Annual Review of Ecology and Systematics* **9**: 475-495.
- Kolzau S, Wiedner C, Rucker J, Köhler J, Köhler A and Dolman AM (2014). Seasonal patterns of nitrogen and phosphorus limitation in four German lakes and the predictability of limitation status from ambient nutrient concentrations. *PloS one* **9**.
- Leonardos N and Geider RJ (2004). Effects of nitrate: phosphate supply ratio and irradiance on the C: N: P stoichiometry of *Chaetoceros muelleri*. *European Journal of Phycology* **39**: 173-180.
- Lewis Jr WM and Wurtsbaugh WA (2008). Control of lacustrine phytoplankton by nutrients: erosion of the phosphorus paradigm. *International Review of Hydrobiology* **93**: 446-465.

Litchman E, Steiner D and Bossard P (2003). Photosynthetic and growth responses of three freshwater algae to phosphorus limitation and daylength. *Freshwater Biology* **48**: 2141-2148.

Lürling M, Eshetu F, Faassen EJ, Kosten S and Huszar VL (2013). Comparison of cyanobacterial and green algal growth rates at different temperatures. *Freshwater Biology* **58**: 552-559.

McBride CG, Abell J and Hamilton DP (2018a). Long-term nutrient loads and water quality for Lake Rotorua: 1965 to 2017. ERI Report No. 127. Hamilton, New Zealand, University of Waikato: 70p.

McBride CG, Allan MG and Hamilton DP (2018b). Assessing the effects of nutrient load reductions to Lake Rotorua: Model simulations for 2001-2015. ERI Report No. 128. Hamilton, New Zealand, University of Waikato: 64p.

Olenina I, Hajdu S, Edler L, Andersson A, Wasmund N, Busch S, Göbel J, Gromisz S, Huseby S, Huttunen M, Jaanus A, Kokkonen P, Ledaine I and Niemkiewicz E (2006). Biovolumes and size-classes of phytoplankton in the Baltic Sea Helsinki, Baltic Marine Environment Protection Commission: 144.

Paerl HW, Gardner WS, McCarthy MJ, Peierls BL and Wilhelm SW (2014). Algal blooms: noteworthy nitrogen. *Science* **346**: 175-175.

Paerl HW, Havens KE, Xu H, Zhu GW, McCarthy MJ, Newell SE, Scott JT, Hall NS, Otten TG and Qin BQ (2020). Mitigating eutrophication and toxic cyanobacterial blooms in large lakes: The evolution of a dual nutrient (N and P) reduction paradigm. *Hydrobiologia*: 17.

Paul WJ, Hamilton DP, Ostrovsky I, Miller SD, Zhang A and Muraoka K (2012). Catchment land use and trophic state impacts on phytoplankton composition: a case study from the Rotorua lakes' district, New Zealand. *Phytoplankton Responses to Human Impacts at Different Scales*. N. Salmaso, L. Naselli-Flores, L. Cerasino et al., Springer: 133-146.

Pearson LK, Hendy CH and Hamilton DP (2016). Dynamics of silicon in lakes of the Taupo Volcanic Zone, New Zealand, and implications for diatom growth. *Inland Waters* **6**: 185-198.

Rattan KJ (2017). Comparative analyses of physiological assays and chlorophyll a variable fluorescence parameters: investigating the importance of phosphorus availability in oligotrophic and eutrophic freshwater systems. *Aquatic Ecology* **51**: 359-375.

Reynolds CS (1997). *Vegetation processes in the pelagic: a model for ecosystem theory*. Germany, Ecology Institute Oldendorf.

Rimet F and Druart J-C (2018). A trait database for phytoplankton of temperate lakes. *Annales de Limnologie-International Journal of Limnology*, EDP Sciences.

Rutherford J, Pridmore R and White E (1989). Management of phosphorus and nitrogen inputs to Lake Rotorua, New Zealand. *Journal of Water Resources Planning and Management* **115**: 431-439.

- Ryan E, Duggan I, Hamilton D and Burger D (2006). Phytoplankton assemblages in North Island lakes of New Zealand: is trophic state, mixing, or light climate more important? *New Zealand Journal of Marine and Freshwater Research* **40**: 389-398.
- Sakshaug E and Olsen Y (1986). Nutrient status of phytoplankton blooms in Norwegian waters and algal strategies for nutrient competition. *Canadian Journal of Fisheries and Aquatic Sciences* **43**: 389-396.
- Schindler D (1971). Carbon, nitrogen, and phosphorus and the eutrophication of freshwater lakes 1. *Journal of Phycology* **7**: 321-329.
- Schindler DW (1974). Eutrophication and recovery in experimental lakes: implications for lake management. *Science* **184**: 897-899.
- Schindler DW, Carpenter SR, Chapra SC, Hecky RE and Orihel DM (2016). Reducing phosphorus to curb lake eutrophication is a success. *Environmental Science and Technology* **50**: 8923-8929.
- Schindler DW, Hecky RE, Findlay D, Stainton M, Parker B, Paterson M, Beaty K, Lyng M and Kasian S (2008). Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. *Proceedings of the National Academy of Sciences* **105**: 11254-11258.
- Scholten MC, Foekema EM, Dokkum HP, Jak RG and Kaag NH (2005). *Eutrophication Management and Ecotoxicology*, Springer Science & Business Media.
- Shatwell T, Köhler J and Nicklisch A (2013). Temperature and photoperiod interactions with silicon-limited growth and competition of two diatoms. *Journal of Plankton Research* **35**: 957-971.
- Smith VH, Joye SB and Howarth RW (2006). Eutrophication of freshwater and marine ecosystems. *Limnology and Oceanography* **51**: 351-355.
- Smith VH, Wood SA, McBride CG, Atalah J, Hamilton DP and Abell J (2016). Phosphorus and nitrogen loading restraints are essential for successful eutrophication control of Lake Rotorua, New Zealand. *Inland Waters* **6**: 273-283.
- Sommer U and Stabel H-H (1983). Silicon consumption and population density changes of dominant planktonic diatoms in Lake Constance. *The Journal of Ecology*: 119-130.
- Vincent WF (1981a). Rapid physiological assays for nutrient demand by the plankton. I. Nitrogen. *Journal of Plankton Research* **3**: 685-697.
- Vincent WF (1981b). Rapid physiological assays for nutrient demand by the plankton. II. Phosphorus. *Journal of Plankton Research* **3**: 699-710.
- Wang H and Wang H (2009). Mitigation of lake eutrophication: Loosen nitrogen control and focus on phosphorus abatement. *Progress in Natural Science* **19**: 1445-1451.

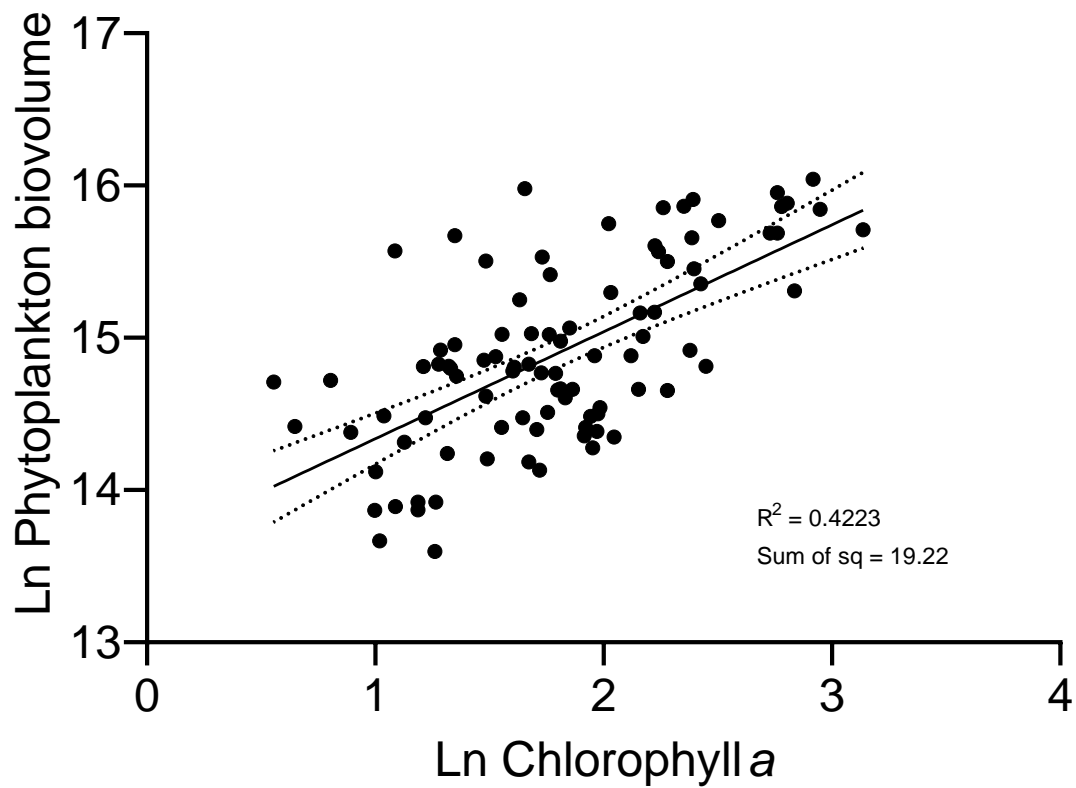
Welch EB (2009). Should nitrogen be reduced to manage eutrophication if it is growth limiting? Evidence from Moses Lake. *Lake and Reservoir Management* **25**: 401-409.

White E, Law K, Payne G and Pickmere S (1985). Nutrient demand and availability among planktonic communities—an attempt to assess nutrient limitation to plant growth in 12 central volcanic plateau lakes. *New Zealand Journal of Marine and Freshwater Research* **19**: 49-62.

White E and Payne G (1978). Chlorophyll production, in response to nutrient additions, by the algae in Lake Rotorua water. *New Zealand Journal of Marine and Freshwater Research* **12**: 131-138.

White E, Payne G, Pickmere S and Woods P (1986). Nutrient demand and availability related to growth among natural assemblages of phytoplankton. *New Zealand Journal of Marine and Freshwater Research* **20**: 199-208.

# Appendices



**Appendix 1. Ln regression of phytoplankton biovolume and chlorophyll *a* concentration from all growth assay treatment groups. Solid line indicates linear regression, dotted lines indicate upper and lower error.**