The Role of Nutrients and Light in the Growth of Phytoplankton

In Te Waihora/Lake Ellesmere, New Zealand.

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To my beloved father,

Graham Allan William M^{ac}Kenzie

(1961 - 2015)

Abstract

Te Waihora/Lake Ellesmere is a shallow, eutrophic, intermittently open lake/lagoon in Canterbury, New Zealand. It is considered one of the most polluted lakes in New Zealand due to high nutrient loading from its catchment. Efforts are underway to improve water quality, water clarity and reduce phytoplankton development. It is essential to understand the response of phytoplankton to nutrients and light, in order to guide management of phytoplankton. The focus of this study was to determine how light and nutrients control phytoplankton growth.

Nutrient limitation was determined using nutrient-addition mesocosm bioassays. 40 cm tall mesocosms were established five times through a year to which nitrate (N), phosphate (P), both nitrate and phosphate (NP) or no nutrients (control) were added to freshly collected lake water. Phytoplankton responses were followed using changes in chlorophyll *a*, quantum yield of photosynthesis and cell numbers. Results indicated that phytoplankton in Te Waihora are predominantly limited by nitrogen, but can at times of high ambient nitrate concentration become phosphorus-limited. Quantum yield responses indicated nutrient limitations did not usually affect photosystem II photochemical efficiency of phytoplankton cells. Nutrient additions commonly had no measureable effect on cell density, and community composition remained unchanged, with single-cell picocyanobacteria numerically dominant throughout.

Combined effects of light and nutrients were also determined using mesocosm experiments. No nutrients, or both nitrogen and phosphorus, were added to mesocosms of 80 cm, 40 cm, and 20 cm depth. Chlorophyll *a* responses indicated phytoplankton biomass in the 80 cm mesocosms were frequently unable to respond to nutrient enrichment, whereas the shorter mesocosms tended to show enhanced chlorophyll *a* after enrichment. The 20 cm mesocosms always had more chlorophyll *a* after enrichment, though sometimes reduced in overall chlorophyll *a* over time. Quantum yield decreased in the 20 cm mesocosms relative to both 40 and 80 cm, likely due to downregulation of the photosystem II protein complex under the higher irradiance prior to measurement. There was no clear effect of nutrients nor light on cell density, and single-celled picocyanobacteria was the dominant algae in these experiments, with neither light nor nutrient addition resulting in a community shift.

Light limitation of photosynthesis was explored by measuring light, photosynthesis, and respiration both in Te Waihora and in 80 cm mesocosms. Light is rapidly attenuated in Te Waihora, with both a shallow euphotic depth (0.5 m) and critical mixing depth (0.6 m). These confirmed that rate processes of phytoplankton can be severely light limited, however during calm weather and near the margins net growth can occur. Whole-mesocosm photosynthesis and respiration showed phytoplankton growth was likely light limited in the deepest mesocosms, confirming observations based on biomass accrual that light limitation prevented a response to nutrient enrichment.

The results suggest that phytoplankton growth and biomass in Te Waihora is controlled by both light and nutrient availability, and management actions for the reduction of phytoplankton need to focus on these key factors. Whole-catchment dual-nutrient control is highly recommended, as a reduction in nitrogen alone may allow potentially toxic picocyanobacteria to persist. Internal management of nutrients would likely be expensive or inefficient due to the size of the lake. Controlling phytoplankton by reducing light availability would be contradictory to the current management objective of increasing water transparency. However, increasing water column depth or reducing wave action may reduce sediments resuspension and internal nutrient loading, therefore reducing phytoplankton biomass.

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Chapter 1

Introduction

1.1 Intermittently Open/Closed Lakes and Lagoons

Transitional water bodies occupy the freshwater-marine interface (McLusky & Elliott, 2007; Tagliapietra et al., 2009). These waters, which range from coastal lagoons to estuaries to fjords (McLusky & Elliott, 2007), share certain common properties: strong water chemistry gradients, high productivity, socio-economic importance, and experience many anthropogenic stressors (Zaldívar et al., 2008). Intermittently closed and open lakes and lagoons (ICOLLs) are one such class of transitional waters (Tagliapietra et al., 2009). The term ICOLL was developed as a descriptor for coastal water bodies that have an intermittent connection with the ocean via the opening and closing of barriers (Roy et al., 2001). This class of transitional waters can therefore include a range of water bodies previously referred to as Temporarily Open/Closed Estuaries (Tagliapietra et al., 2009), coastal lagoons (Kjerfve, 1994), and some coastal lakes (Roy et al., 2001).

Ultimately the difference between estuaries and ICOLLs is the permanence of the connection to the sea: ICOLLs sometimes become disconnected by a barrier or berm developed from an accumulation of sand or gravels (Kjerfve, 1994). Natural openings can occur two ways; 1) if water, either from freshwater inputs to the ICOLL (Chuwen et al., 2009) or from stormy waves, exceeds the barrier level then a breach can form, and 2) seepage can scour a breach within the barrier itself (Rustomji, 2007). This barrier is usually reformed by the deposition of sand or shingle either from waves or long-shore drift (Kjerfve, 1994). The most important effect of closing is to increase the residence time of water, which allows the accumulation of inorganic and organic matter (Kjerfve, 1994). Managed barrier estuaries and ICOLLs are manually opened to the ocean, usually on a seasonal basis in response to rising water levels throughout winter and spring, to minimise impact on surrounding productive agricultural or urban landscapes (Chuwen et al., 2009).

ICOLLs are usually shallow, and can range in size up to 10,000+ km^2. Salinity can also vary greatly from freshwater due to dominating freshwater inputs, to hyper-saline (Kjerfve, 1994), the latter occurring where evaporation dominates hydrological balance in ICOLLs with high surface area to volume ratio (Brito et al., 2012). These shallow systems are also often light limited, due to high turbidity from suspended solids and high phytoplankton biomass.

ICOLLs cover more than 10% of the coastal area globally (Ortega-Cisneros et al., 2013). They, like other transitional water bodies, hold significant ecological value and perform a myriad of ecosystem processes and functions. Decomposition and nutrient cycling from complex sediment feedbacks is one such important function of ICOLLs (Brito et al., 2012; Levin et al., 2001). They are rich in valuable resources for human activities such as commercial fisheries, plant and algae harvesting, and salt mining (Duck & Da Silva, 2012; Gaertner-Mazouni & De Wit, 2012). Aquaculture can be important, as ICOLLs are nurseries to a range of fish species (Brito et al., 2012). These highly productive areas also attract migratory birds (Chuwen et al., 2009). Some ICOLLs are also considered aesthetically valuable (Levin et al., 2001).

Transitional waters are the most at-risk and degraded ecosystems in the temperate regions of the world (Chuwen et al., 2009). ICOLLs can be particularly sensitive to anthropogenic stressors (Kjerfve, 1994), especially during the closed phase as they can accumulate nutrients and other pollutants from the catchment. This can lead to eutrophication, resulting in phytoplankton blooms (Coutinho et al., 2012; Ortega-Cisneros et al., 2013; Schallenberg et al., 2010). However, there has been relatively little research on these systems (Everett et al., 2007; Ortega-Cisneros et al., 2013). It is important to understand and identify the key drivers of productivity to inform good management decisions. In turbid ICOLLS, the relationship between light and nutrient availability and productivity of phytoplankton is often particularly important.

1.2 Nutrient dynamics in ICOLLs

Nutrient dynamics influence ICOLLs through inputs from both the catchment and internal cycling. The nutrient conditions of receiving waters reflect catchment condition (Scanes et al., 2007). Land-use change from native vegetation to agricultural and urban lands alters catchment diffuse nutrient biogeochemistry and hydrology, causing increased nutrient loading and altered runoff intensity and frequency to ICOLLs (Young et al., 1996). Point-source discharges of stormwater runoff and treated and untreated effluent can also cause major disturbances to waterways (Schindler, 2006). Agricultural intensification, a growing global phenomenon, in particular the use of fertilisers has increased the inputs of nitrogen and phosphorus to soils (Smith et al., 1999). As these macronutrients leech from the soils into streams and rivers of each catchment, they can enter standing water bodies of high residence

time including lakes, reservoirs, and ICOLLs - or exit directly to the ocean (Schindler, 2006; Smith, 2003). Anthropogenic pollution via catchment land-use alterations and coastal zone development are considered to be the largest issue for coastal lagoon sustainability (Gaertner-Mazouni & De Wit, 2012).

Interactions between sediments and the water column are also important in determining nutrient dynamics in transitional water bodies (Smith et al., 2001; Spooner & Maher, 2009). Internal loading from sediments can contribute 3 - 4 times more nutrients on an annual basis than the catchment (Spooner & Maher, 2009). Part of this can be attributed to the mineralisation of autochthonous and allochthonous organic material through decomposition, although biogeochemical dynamics are profoundly affected by the oxic status of sediments (Harris, 1999). Under oxic conditions, sediments bind and retain phosphorus with iron hydrous oxides. However, anoxic zones in the sediments are created by bacteria as they utilize the oxygen to metabolise organic carbon, and reduction of iron oxides can release bound phosphates to the water column (Spooner & Maher, 2009). Immediate nitrogenous products of decomposition are reduced forms such as ammonium, which are oxidised and accumulate as nitrate in oxic sediments. Reduced oxygen in sediments leads to reduced nitrification, and nitrogen accumulates in the form of ammonia. Anoxia enhances the use of nitrate through denitrifying bacteria, a significant pathway in many ICOLLs for removing inorganic nitrogen. Bioturbation by invertebrates causes separation of the oxic and anoxic sediment zones, both of which drive the nutrient dynamics within these systems, and can create conditions that enhance the redox-related dynamics of nutrient biogeochemistry (Harris, 1999).

Nutrient dynamics within ICOLLs are also influenced by water retention time, hydrology (Coutinho et al., 2012), and geology of the area (Scanes et al., 2007). Longer residence time of the water usually means they accumulate nutrients (Coutinho et al., 2012). Openings can assist in flushing nutrients out of ICOLLs, but this is not always the case. If the freshwater inputs during closing, or the marine inputs during opening, are relatively low in nutrients, then this can assist in diluting the accumulated nutrients in ICOLLs (Haines et al., 2006). Due to the high surface area to water volume ratio typical of most extensive, shallow ICOLLs, the nutrient-sediment interactions are more pronounced, and can greatly influence nutrient concentrations (Spooner & Maher, 2009). In addition, these systems can have nutrient concentrations reflecting the geology of the area, so it can be difficult to determine the natural, baseline nutrient concentrations (Scanes et al., 2007).

There is usually a positive relationship between the addition of nutrients and primary productivity. This is well established, and is emphasised by many studies that have measured increased biomass with eutrophication (Jochimsen et al., 2013; Schindler, 2006; Watson et al., 1997). Eutrophication can be defined as an increase in the concentrations of organic materials in an ecosystem, caused by an increase in nutrient levels (Pinckney et al., 2001). The majority of transitional waters are eutrophic (Drake et al., 2011; Pinckney et al., 2001), although in some cases this is thought to be a natural, periodic process resulting from the mixing of terrestrial and marine subsidies in an accumulation setting (Pinckney et al., 2001).

Human activities have altered almost all major aquatic ecosystems by altering the flux of growth-limiting nutrients (Smith, 2003), and anthropogenic eutrophication has been deemed the primary cause of water quality issues globally (Abell et al., 2010). The effects of eutrophication can include a shift in community dominance to bloom-forming, and often toxic, cyanobacteria (Brauer et al., 2012), decreased fish abundance including fish kills, threats to endangered aquatic species, decreased water clarity, and depletion of oxygen (Pinckney et al., 2001; Smith, 2003). Cyanobacteria can also produce toxins which are dangerous for humans, domestic animals, and aquatic consumer species (Pinckney et al., 2001; Smith, 2003), although toxicity depends on which species of cyanobacteria is dominant (Smith, 2003).

Macronutrients are generally considered to most frequently limit primary productivity (Correll, 1998). The most limiting nutrient determines phytoplankton growth according to Liebig's Law of the Minimum (Dolman & Wiedner, 2015). Generally oceanic phytoplankton productivity is thought to be limited by nitrogen (Fong et al., 1993), whereas in lacustrine ecosystems phytoplankton are traditionally considered to be limited by phosphorus (Pennock & Sharp, 1994). However, Guildford and Hecky (2000) found that both marine waters and freshwater lakes are much more likely to be phosphorus-limited, while Lewis and Wurtsbaugh (2008) found that lakes are just as likely to be nitrogen limited as phosphorus limited. Some transitional waters can be highly variable in terms of whether nitrogen or phosphorus are limiting, both spatially and temporally (Correll, 1998; Fong et al., 1993).

Nutrient limitation is usually inferred from Redfield ratios or bioassays, involving following the response of phytoplankton to nutrient additions *in vitro* and whole-lake experiments (Abell et al., 2010). The Redfield ratio is based on cellular stoichiometry of phytoplankton. On average, phytoplankton conform to a molar ratio of 16:1 of N:P. Cells with a lower ratio are likely to be nitrogen limited, and a higher ratio phosphorus limited. However, Moss et al.

(2013) argue that the Redfield ratio is inconsequential to systems high in nitrogen and phosphorus. Instead, light is most likely to be the most limiting factor due to the very high biomass resulting in self-shading impacts on further growth. In turbid transitional waters this is even more likely to occur. However, even in cases where light is most limiting to phytoplankton growth, evaluating nutrient limitations in these systems can still be useful to determine ecosystem functioning and to determine how to manage excessive productivity.

Nutrient addition bioassays involve adding known nutrients to samples and measuring the response of biomass, chlorophyll *a*, or photosynthetic yield over days or weeks. Increases in these indices indicates that the added nutrient was limiting in the original sample (Beardall et al., 2001). These bioassays have provided a plethora of data on nutrient limitations in different marine and freshwater ecosystems (Howarth & Marino, 2006), but they have some limitations. For example, some experiments only include a single test phytoplankton species (Barbosa, 1989; Beardall et al., 2001), which may not reflect the response of whole phytoplankton communities. Conversely, using whole plankton communities may favour a subset of the population that grows well under culture conditions. There are also limitations in measuring the responses. For example, an increase in chlorophyll *a* may only be an increase in cellular chlorophyll *a* concentration rather than an increase in number of cells, and chlorophyll *a* responses may vary between species (Kruskopf & Flynn, 2006). If water for bioassays is obtained by fine filtration of natural waters in order to remove phytoplankton, then this can also remove natural sources of nutrients from the water (Wood & Oliver, 1995). In addition, lengthy bioassays could potentially show nutrient limitation where none existed initially (Hameed et al., 1999); conversely, the use of short incubations may not allow enough time for responses to become evident (Howarth & Marino, 2006).

Determining nutrient limitation in ICOLLs is important for a number of reasons. 1) Nutrients are an essential component of phytoplankton dynamics which underlies the process of eutrophication, determines the composition and biomass of unwanted phytoplankton blooms. Determination of the most limiting nutrient may also instigate further research into internal and external sources and cycling of nutrients, and assist in directing management practices both for the water body itself, and across the entire catchment involved.

1.3 Light dynamics in ICOLLs

Light is the source of energy used by phytoplankton and macrophytes for photosynthesis, and is essential to growth and productivity (Reynolds, 2006). Sufficient light for net photosynthesis by phytoplankton defines the euphotic zone (Gerbeaux & Ward, 1991), and this is frequently considered to be that part of the water column where photosynthetically active radiation (PAR, 400-700 nm wavelength) exceeds one percent of its incidence value (Davies & Smith, 2001). The depth of the euphotic zone depends on the rate of light attenuation with depth (Gerbeaux & Ward, 1991).

After light enters water, it is attenuated by the scattering and absorption of photons by water molecules, suspended particles and dissolved matter, setting the depth to which sufficient light penetrates for photosynthesis (Davies & Smith, 2001). Light attenuation is thus influenced by the inherent properties of water itself (Ganju et al., 2014), as well as the composition and concentration of materials in the water (Vant, 1990). These materials include suspended solids (non-living organic and inorganic matter), living phytoplankton and microbes, and coloured dissolved organic matter (CDOM - often also referred to as humic substances) (Davies & Smith, 2001; Ganju et al., 2014; Gerbeaux & Ward, 1991; Hughey & Taylor, 2009; Lawson et al., 2007; Verspecht & Pattiaratchi, 2010). Scattering of photons by the aforementioned optically active components increases the path length of photons, increasing the likelihood of absorption (van Duin et al., 2001). Light attenuation can be measured and compared between systems by determining the vertical attenuation coefficient (K), often in terms of down-welling (K_d) or scalar irradiance (K_o). Greater light attenuation is related to lower water clarity (Davies & Smith, 2001), and light limitation of phytoplankton is common in highly turbid aquatic systems (Sobolev et al., 2009). Optically active components can therefore ultimately control photosynthesis even when nutrients are plentiful (Foden et al., 2008).

In eutrophic systems with high phytoplankton productivity, phytoplankton can be the main contributor to light attenuation (Krause-Jensen & Sand-Jensen, 1998). Phytoplankton can both absorb and scatter light, and high concentrations of phytoplankton can induce self-shading in the water column, reducing the euphotic zone depth (Krause-Jensen & Sand-Jensen, 1998; van Duin et al., 2001). In deeper transitional waters phytoplankton is considered the major contributor to light attenuation, although in shallow waters, suspended sediment is considered the main contributor (Davies & Smith, 2001; Lawson et al., 2007).

Suspended sediments contribute mostly to scattering (van Duin et al., 2001), and Vant (1990) found scattering by inorganic suspended solids to be the main contributor of light attenuation in New Zealand estuaries. Wind forcing is the main cause of the resuspension of sediments (Lawson et al., 2007). It can have a significant effect on light attenuation in shallow systems, as it can increase the concentration of suspended solids in the water column (Mallin & Paerl, 1992). The likelihood of resuspension of sediments is affected by wind speed, water depth and sediment size. At shallow depths, and high wind speed the probability of bottom disturbance due to wave action increases (van Duin et al., 2001), and finer sediments are more prone to resuspension as they have a lower erosion threshold (Widdows et al., 2008). Fine sediments also take much longer to settle (van Duin et al., 2001) and have a higher impact of attenuation due to higher surface area of particles (Lawson et al., 2007). Shallow depths and accumulation of fine, river-delivered sediments, coupled frequently with exposed coastal locations may therefore result in high turbidity within ICOLLs.

Optically active components can affect the quality as well as quantity of light in the water column. Various wavelengths of light are not attenuated at the same rate, and different optically active components influence this in different ways. In pure water, longer-wavelength/lower-energy red light is more readily absorbed than shorter wavelength/high-energy blue light (van Duin et al., 2001). CDOM can also influence light quality, and readily absorbs shorter blue to ultraviolet wavelengths (Schubert et al., 2001). Suspended organic and inorganic matter is also associated with absorption of blue light (van Duin et al., 2001).

Phytoplankton species have developed different pigments in order to utilize the different light spectra available in the water column. These different pigments can provide phytoplankton with a competitive advantage over their peers (Vijaya & Anand, 2009). For example, eukaryotic algae use the pigment chlorophyll *a* and chlorophyll *c* or *d*, which are useful for capturing light in the red to near-infrared wavelengths (Vila & Abella, 2001). Cyanobacteria containing phycoerythrin can use the green-yellow wavebands, and phycocyanin-dominant cyanobacteria can utilize orange - red wavebands, and can occur in both red to infrared, as well as green-yellow light (Shui et al., 2009; Vila & Abella, 2001). The pigment composition within phytoplankton cells can adjust to changes in light quality and quantity. These changes can reflect photo-acclimation and photo-inhibition processes occurring within the cells (Borghini et al., 2009). For example, carotenoids work by relieving cells of excess energy and protect cells from photochemical damage (Sigaud-Kutner et al., 2002).

1.4 Light & phytoplankton

Phytoplankton have a variety of responses to changes in light intensity (Mallin & Paerl, 1992), and fluctuations can induce major changes to processes associated with photosynthesis and respiration (Litchman, 2000). Excess light can inhibit growth of phytoplankton at the surface (Peterson et al., 1987; Slagstad, 1982), usually due to cellular damage from light, and photo-oxidation (Han et al., 1999). Phytoplankton can also exhibit photo-acclimation (Han et al., 1999; Mallin & Paerl, 1992). This is characterised as a physiological change in cellular chlorophyll content whereby phytoplankton adapted to low light will contain more chlorophyll (Han et al., 1999; Mallin & Paerl, 1992; Slagstad, 1982). Shade-adapted phytoplankton are therefore able to more efficiently photosynthesise at a low light intensity than light-adapted phytoplankton (Slagstad, 1982). Falkowski and Owens (1980) found that shade-adapted phytoplankton also have reduced respiration rates in comparison to light-adapted phytoplankton. A low respiration rate is also often associated with cells in a dormant state. Under a slow mixing regime, phytoplankton are likely to exhibit a gradient of physiological light adaptation in the water column, which would manifest depending on recent light exposure history (Han et al., 1999). Alternatively, phytoplankton exposed to fast mixing regimes are likely to exhibit homogeneous physiological characteristics, and photoadaptation is unlikely to have much influence on phytoplankton population (Han et al., 1999).

Wind forcing has the effect of exposing phytoplankton to a variety of light regimes as they mix vertically through the water column (Litchman, 2000; Mallin & Paerl, 1992; Rhee & Gotham, 1981). The consequences of vertical mixing through steep gradients in light quality and quantity can challenge phytoplankton. In some instances, it can increase the exposure of cells to light over time, and can significantly increase phytoplankton productivity (Litchman, 2000; Mallin & Paerl, 1992). Other studies have found vertical mixing to either depress or have no significant effect on productivity (Kroon et al., 1992; Litchman, 2000). These differences in response to mixing may be due to growth responses to fluctuating light being species-specific (Litchman, 2000), different concentrations of suspended solids causing a different range of irradiance, or different levels of mixing used across the experiments. The essential issues with photosynthesis while circulating through a light field is the balance between photoinhibition at high irradiance, shade adaptation to ensure efficient use of low irradiance and minimisation of losses during episodes outside of the photic zone.

1.5 Te Waihora

Te Waihora is an ICOLL located in Canterbury, New Zealand. During the present interglacial period and up to about 500 years ago, Te Waihora naturally switched from being the estuary of a large mountain river to extensive wetlands and a coastal lagoon as the braided Waimakariri River changed between its current outflow north of Banks Peninsula to flowing south of the peninsula through the current location of the lake (Soons et al., 1997). Since European arrival, the Waimakariri has been managed in its northern course, which has turned the lake into a large coastal lagoon of interconnecting wetlands (Hughey & Taylor, 2009). Since then, the draining of lowland wetland areas to provide productive and extensive agricultural land has led to control of the lake level so that at its maximum it is approximately 20,000 ha in area and only 2.5m maximum depth (Gerbeaux & Ward, 1991). The lake is now a managed ICOLL; it is manually opened to ensure the lake does not flood surrounding land (Gerbeaux & Ward, 1991). Te Waihora is important for its biodiversity as well as for cultural, and recreational values, but is under pressure from historical and present contaminating sources, and this is putting the values of the lake at risk (Hughey & Taylor, 2009).

Like many ICOLLs, Te Waihora is extremely turbid, with a very shallow euphotic zone (Gerbeaux & Ward, 1991). Wind-induced mixing of the shallow water column re-suspends sediments which increases nutrient availability, and may ultimately increase phytoplankton biomass, although some studies show that phytoplankton is limited by turbidity through light attenuation (Carrick et al., 1993). Light is attenuated rapidly in Te Waihora mainly because of scattering by the large amount of suspended solids in the water column (Gerbeaux & Ward, 1991; Larned & Schallenberg, 2006), which induces high concentrations of planktonic chlorophyll *a* (Gerbeaux & Ward, 1991).

The euphotic zone is usually 0.3-0.5m deep, and its contraction to less than 0.3m was not found to reduce chlorophyll *a* concentrations (Gerbeaux & Ward, 1991). Similar results were found after 15 years of monitoring (1993 - 2007), during which water clarity did not correlate with phytoplankton biomass (Hughey & Taylor, 2009). This phenomenon is likely to have occurred because the windy conditions at the lake induces mixing of the water column, which may expose the phytoplankton to enough light to maintain high biomass (Gerbeaux & Ward, 1991). In contrast, a short period of reduced turbidity (increased water clarity) was found to lead to an increase in phytoplankton biomass (Larned & Schallenberg, 2006). These different results may be due to spatial and temporal heterogeneity inherent in both nutrient supply and phytoplankton, but are as yet unexplained.

Te Waihora is estimated to receive 90% of its total phosphorus and 98% total nitrogen loading from its tributaries (Hughey & Taylor, 2009). Despite an overall decrease in nitrogen loading from 1993-2007, there was no change in chlorophyll *a* (Hughey & Taylor, 2009). This suggested that nitrogen was therefore not likely to be the most limiting factor in the growth of phytoplankton, as light is considered primarily limiting. However nitrogen could become limiting during calm weather, when water clarity is increased. There has been speculation that nitrogen entering the system is likely to be quickly taken up by phytoplankton, as dissolved inorganic nitrogen was usually less than 10% of the tributary concentrations (Larned & Schallenberg, 2006). Conversely, phosphorus levels in the lake were found to be approximately 10x higher than in the tributaries, which is likely attributable to wind-induced mixing of the water column and cycling of phosphorus from the bottom sediments (Larned & Schallenberg, 2006).

There have been no bioassays completed for Te Waihora since 1996, the results of which aligned with those studies of Larned & Schallenberg (2006) and Hughey & Taylor (2009). This suggested that nitrogen was more commonly a limiting factor than phosphorus (Hawes & Ward, 1996). This may no longer be true (Larned & Schallenberg, 2006). In times of relative water clarity, it has been suggested that nitrogen will be the next most important limiting factor, although nutrient levels in the lake are beyond the requirements of phytoplankton the majority of the time (Larned & Schallenberg, 2006). The factors controlling phytoplankton growth and biomass in Te Waihora are poorly understood.

The present light and nutrient environment of Te Waihora is influenced by historical outside sources, and positive feedback forcing from within the lake. Prior to the late 1960's, Te Waihora was deeper, and water clarity was much higher. In 1968 the great Wahine storm came through the lake, and the majority of the macrophyte beds were destroyed (Gerbeaux & Ward, 1991). Unfortunately, macrophytes previously played a large role in stabilizing the lakebed, providing habitat for zooplankton and fish, and potentially competing with phytoplankton for nutrients. The loss of macrophyte beds coupled with nutrient addition following agricultural development of the catchment led to a regime shift in the lake from macrophyte dominance and high water clarity to an alternative state of high turbidity and phytoplankton dominance (Schallenberg et al, 2010).

1.6 Research aims and objectives

There is much interest in returning Te Waihora to its historical state, of higher water clarity and macrophyte dominance, by improving water quality and clarity (Larned & Schallenberg, 2006). Re-establishment of macrophytes is being explored as a management option to improve water clarity by increasing lakebed stability, which would reduce resuspension of sediments (Drake et al., 2011; Schallenberg et al., 2010). This could have both positive and negative effects (Hughey & Taylor, 2009). For example, stabilising the lake bed may lead to an extended euphotic zone, which may in turn lead to increased phytoplankton productivity (Hughey & Taylor, 2009). Phytoplankton biomass could be controlled by reducing nutrient loads to the lake (Larned & Schallenberg, 2006), but the reduction required in nutrient concentrations is unknown (Hughey & Taylor, 2009). A major question is whether restoration efforts for increased water clarity need to address both resuspension of sediments and nutrient loading from the catchment. My project is directly relevant to this management issue, as it will assess whether reduced turbidity allows light-limited, nutrient replete phytoplankton to increase in biomass. The following objectives were used to explore nutrient and light limitation in this study:

- To determine, using laboratory experiments, the short-term response of phytoplankton productivity to increased nutrients. I hypothesize that phytoplankton productivity and biomass will increase rapidly with increased nutrient availability, and that nitrogen will be the primary limiting nutrient.
- To determine the response of phytoplankton to increased light availability. I hypothesize that phytoplankton productivity and biomass will increase with increased light availability.
- To explore the interaction between light availability, nutrient availability, and phytoplankton productivity. I hypothesize that as light becomes increasingly available, nutrients will become increasingly limiting to phytoplankton productivity.
- To explore the aforementioned relationship in the context of the euphotic zone and critical depth. I hypothesize that with decreasing depth of the water column, the increase in light availability will result in the limiting nutrients determining the growth of phytoplankton.

Chapter 2

Methods

2.1 Water collection and sampling at Te Waihora

The field collection site was located at Timberyard Point, an accessible point on the western side of Te Waihora (Figure 1). 100 L of lake water was collected by wading out to chest-height, and collecting the water in five clean 20 L plastic jerry cans. Water to be used for experiments was collected two times in winter (May and July 2015), once in spring (September 2015) and two times in summer (December 2015 and January 2016) approximately six weeks apart.

On each occasion basic water quality variables were measured at the same location using an YSI EXO Sonde (www.YSI.com). Parameters measured were turbidity, optical dissolved oxygen, conductivity, temperature, pH, chlorophyll *a*, phycocyanin, and salinity.

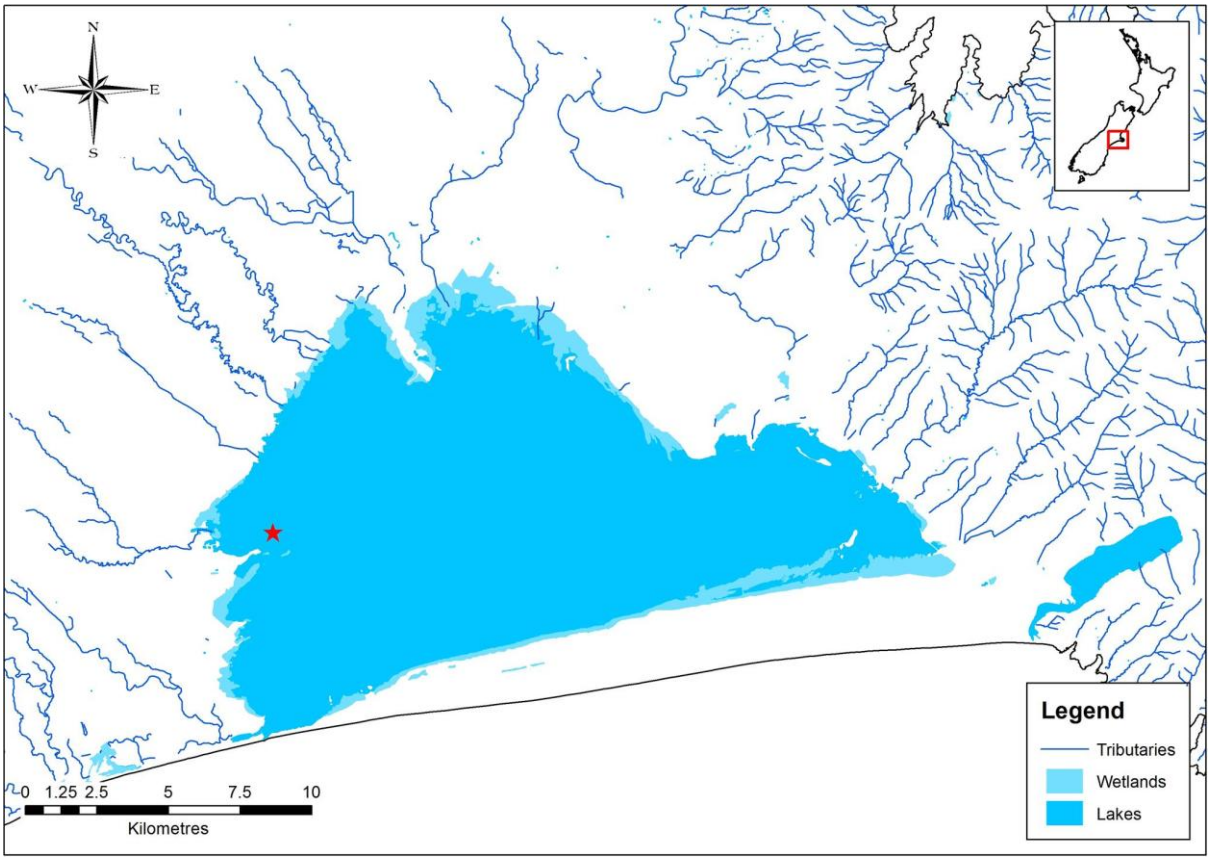

Figure 1. Map of Te Waihora/Lake Ellesmere, located in Canterbury, New Zealand. Approximate site of water collection and sampling off Timberyard Point denoted by a star symbol.

2.2.1 Light environment

Incident and underwater Photosynthetically Active Radiation (PAR) was measured at the Timberyard Point site on February and March 2016. PAR was measured using a LiCor Li190 sensor (air) and Li192 (water) sensors connected to a Li1400 data logger. Underwater PAR was measured every 2.5 cm below the surface until 10 cm depth, and thereafter every 5 cm.

The PAR was adjusted using the following equation to allow for any changes in surface PAR over the sampling period:

$$I_{adj} = I_{0i} * I_z / I_0 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, I_{0i} = Initial surface PAR

I_z = PAR measured at a specific depth

I_0= surface PAR measured at the time of the depth-specific measurement

The adjusted PAR was then plotted against depth in Excel to assess the relationship, and to determine the attenuation/extinction coefficient. The following equation was fitted to the data using least-squares regression and the Excel curve fitting routine:

$$I_z = I_0 \cdot \exp(-K_d \cdot z)$$

Where, I_z = the PAR at a depth (z) in the water column,

K_d = attenuation coefficient for down-welling irradiance

I_0= the average light at the surface

Which can be rearranged to,

$$K_d = (\ln I_0 - \ln I_z) \cdot z^{-1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

2.2 Mesocosm experiment setup and design

In total 24 mesocosms were made and used across the experiments. Mesocosms were made out of lightweight PVC storm-water tubing of 10cm diameter. The base was made of thicker PVC, glued using waterproof sealant. Twelve mesocosms were made to a depth of 45 cm, six to 25 cm, and six more to 85 cm. Each experiment was run in a temperature-controlled growth room set to 20^{o}C. Photosynthetically Active Radiation (PAR) at the surface of the mesocosms

was set to 350 – 400 µmol $m^{-2}s^{-1}$. Piped air supply ran through clear plastic tubing to an air stone attached to the end, which was placed at the bottom of each mesocosm in order to ensure efficient mixing, and mesocosms were manually mixed daily.

Lake water was filtered through 80 µm Nybolt mesh to exclude larger zooplankton before it was added to the mesocosms. While zooplankton are often important contributors to phytoplankton dynamics, they were outside the scope of this research. The effect of Nybolt filtering on phytoplankton species composition and biomass was examined in a pilot experiment, and was found to be negligible.

At the beginning of each experiment, nutrients were added to the nutrient addition treatments. For the first and second experiments (May 2015 and July 2015), nutrients (K_2HPO_4 and $NaNO_3$) were added in excess of the targets, and starting dissolved reactive phosphorus (DRP) and nitrate- and nitrite-nitrogen (NNN) concentrations in addition treatments were over 500 µg L^{-1} and 5000 µg L^{-1} respectively. For experiments of all other months, a lower concentration of nutrients was added (50 µg L^{-1} DRP, and 500 µg L^{-1} NNN).

The first set of mesocosm experiments were designed to compare the effects of nutrient additions of phytoplankton growth, where no nutrients (control), nitrate-only (N), phosphate-only (P), or a combination of nitrate of phosphate (NP) were added to 40 cm mesocosms. Three replicate mesocosms were used for each treatment and level of each experiment. Water chemistry, chlorophyll a, cell counts, and photosynthetic efficiency were measured in these experiments.

The second set of mesocosm experiments were designed to compare and analyze the effects of nutrient and light on phytoplankton growth, where no nutrients (control), or a combination of nitrate and phosphate (+NP), were added to water columns of 20 cm, 40 cm, and 80 cm depth. Three replicate mesocosms were used for each treatment and level of each experiment. Following is a description of the different parameters measured in the mesocosms. Water chemistry, chlorophyll a, cell counts, and photosynthetic efficiency were measured in these experiments.

In the third set of experiments, light attenuation, rate of photosynthesis and respiration were determined at depths of 10 cm increments down 80 cm deep mesocosms using a light/dark bottle technique.

2.2.1 Water chemistry

Dissolved oxygen, pH, conductivity, temperature, and salinity were measured using the YSI EXO Multi-parameter Sonde on the final day of the experiment. Turbidity was measured every day using a Thermo Scientific Orion AQUAfast AQ4500 turbidity meter to ensure appropriate mixing of the water column. This was important because the suspended solids were to assist in creating the light gradient within the mesocosms, and it was necessary to ensure phytoplankton were actively circulated.

Inorganic nutrient samples were collected on days 0 and 5. This was to indicate how much of these nutrients have been assimilated or converted to other entities in the mesocosms over the duration of the experiment.

Nitrate- and nitrite-nitrogen (NNN) were measured together using the method from Makareth et al. (1978). In brief, nitrate was reduced to nitrite in the presence of cadmium. Nitrite was diazotized with sulfanilamide, and coupled with NED dihydrochloride to form an azo dye. The samples were then measured via a spectrophotometer at 543 nm.

Ammoniacal nitrogen (NH_3-N) was measured using the phenate method, where indophenol was formed by the reaction of ammonia, hypochlorite, and phenol, catalysed by sodium nitroprusside. The absorbance was then measured via spectrophotometry at 640 nm (Makareth et al., 1978)

Dissolved reactive phosphorus (DRP) was analysed according to Eaton (2005). In this method, ammonium molybdate and antimony potassium tartrate reacted with orthophosphate to form phosphomolybdic acid. The acid was then reduced to molybdenum blue by ascorbic acid, and the sample was read using the spectrophotometer at 880 nm.

2.2.2 Chlorophyll *a*

Spectrophotometric determination is usually used for samples containing a relatively high concentration of chlorophyll *a*. However, low concentrations may be measured if the light path is increased. The fluorometric method is more sensitive in comparison, and therefore lower concentrations can be measured appropriately using this method (Yentsch & Menzel, 1963). Best practice for calibration of the fluorometer is by spectrophotometry, with a concentrated sample from the same source and producing a stepwise dilution series (Eaton, 2005).

Extracted chlorophyll *a* was analysed by using the fluorometric method. Samples were taken on all days for this method, where 10mL sample was pushed through a Whatmans GF/C filter to trap cells. Each filter was then placed in a 15 mL sample tube. 5 mL of 96% ethanol was added, the tube stoppered and shaken, and the tube placed in a hot water bath (70+ ^{o}C) for 2 minutes. The extract was then re-filtered through a membrane filter (pore size 0.45 µm). Extracted chlorophyll *a* was then measured using 3 mL of extract in a Turner Designs AquaFluor handheld fluorometer (www.turnerdesigns.com).

The fluorometer was calibrated against a spectrophotometer by concentrating cells from approximately 500 mL water from Te Waihora using centrifugation, extraction in ethanol, and measuring the absorption at 665 and 750 nm in a 1 cm cuvette. The chlorophyll *a* of the calibration sample (in µg L^{-1}) was determined using the equation:

$$\text{Concentration} = \frac{12.2 \cdot A \cdot v}{d \cdot V} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where, A = corrected absorbance at 665 nm ($A_{665} - A_{750}$)

v = volume of ethanol in mL

V = volume of initial filtered sample in L

D = cell path-length in cm

A serial dilution of this standard was created as a stepwise dilution series and measured using the handheld AquaFluor (as relative fluorescence units – RFU) to develop a calibration curve for converting RFU to µg L^{-1}.

2.2.3 Cells

Chlorophyll *a* is often used as a proxy measure for biomass. However, cells may have different amounts of chlorophyll *a* depending on species and photoacclimation (Kasprzak et al., 2008). Light limited cells may contain more chlorophyll *a*, and because light limitation is very important to this study, cell counts were also taken as a second metric for algal biomass.

Sedimentation is a commonly used method for concentrating cells for counting, as it is relatively non-destructive. However, smaller phytoplankton cells and motile species may not settle completely (Eaton, 2005). At the beginning and end of each experiment (Day 0 and Day 5), 15 mL sample was taken and the cells were centrifuged at 1000 RPM for 1 minute to concentrate the cells. The supernatant was then carefully removed to leave 1 mL volume. In

order to ensure concentration of motile species, the samples were preserved in Lugol's prior to centrifugation (Furet & Benson-Evans, 1982).

Cell counts were performed on a cell-counting chamber under a light microscope, whereby each grid square represents a known volume. Counts from each grid square were then converted to cells per 1 mL volume and divided by the settled volume (15 mL) to get the original concentration of cells per mL. The number of cyanobacteria, green algae, and diatoms were recorded for each grid. For each sample, the number of cells on each of five grids-squares were counted. Counting continued until 100 cells had been enumerated, and cells per mL calculated.

2.2.4 Photosynthetic efficiency

PSII quantum yield can be measured via pulse amplitude modulation fluorescence. As phytoplankton absorb light, chlorophyll reaction centres become excited. Energy absorbed can either be passed on to another molecule for electron transport, be released as heat, or emitted as fluorescence (Maxwell & Johnson, 2000). PAM fluorescence measures the energy absorbed which is not used for photosynthesis. PSII quantum yield can be used as a relative measure of the recent light history of phytoplankton, as well as nutrient stress under unbalanced growth conditions (Harrison & Smith, 2013; Maxwell & Johnson, 2000; Parkhill et al., 2001).

Pulse amplitude modulation (PAM) was used in order to measure the photosynthetic efficiency of phytoplankton, using a Walz Toxy-PAM (www.walz.com). Essentially, PAM techniques measure Photosystem II photochemical efficiency (Schreiber 2004). Photosynthetic efficiency was measured using the Toxy-PAM chlorophyll fluorometer for all treatments on all days of the experiment, with samples taken during the light period, and immediately transferred to the PAM cuvette for measurements. Samples were thus all acclimated to ambient irradiance.

2.2.5 Light environment

In order to determine the light environment within the mesocosms, PAR was measured using a Lambda L1-185 Quantum Photometer. Measurements were taken at 1 cm below the surface, and at 10 cm increments down the mesocosms.

2.2.6 Rate of photosynthesis

In the 80 cm mesocosms, photosynthesis was measured at the end of each experiment by measuring the change in dissolved oxygen over time using a Presens Microx 4 logger with a fibre optic oxygen microsensor in a flow-through cell (www.presens.de). The initial dissolved oxygen was measured in each mesocosm, and 10 mL incubation tubes were filled with the mesocosm water. Three capped, airtight tubes were wrapped in tinfoil (dark treatment). The other capped, airtight tubes were horizontally suspended down the mesocosms at 1 cm below the surface, and at 10 cm increments (light treatments). The time of deployment was recorded. The tubes remained in the mesocosms for 1 – 6 hour durations, with shorter stays for tubes exposed to more light, and the time recorded when the tubes were retrieved. The dissolved oxygen was again measured in the tubes. After all light-exposure tubes were measured, the oxygen concentrations in the dark tubes were also measured. The net photosynthetic rate was determined by dividing the increase in dissolved oxygen in the tube (mg L^{-1}) by the time deployed (hours). Respiration was calculated based on the rate of oxygen consumption in the dark tubes. The gross photosynthesis was determined as the sum of dissolved oxygen gained by photosynthesis and respiration.

In mesocosms, depth versus $\Delta DO/\Delta t$ plots were used to determine approximate whole-mesocosm photosynthesis and respiration. The following equation was used to calculate whole-mesocosm photosynthesis, by calculating the cumulative photosynthesis and respiration within 1 cm deep segments of mesocosms:

$$M_{PS} = \Sigma\, PS * (I_o * \exp(Z/100*K)) * V_s$$

Where, PS = rate of photosynthesis from the depth versus $\Delta DO/\Delta t$ curve

I_o = Irradiance at the surface

Z = depth of the mesocosm segment

K = light attenuation

Vs = volume of the mesocosm segment

The following equation was used to calculate whole-mesocosm respiration:

$$M_R = \Sigma R * V_s$$

Where, R = rate of respiration from the depth versus $\Delta DO/\Delta t$ curve

V_s = volume of the mesocosm segment

2.2.7 Critical depth

The critical depth is the depth of mixing at which the photosynthetic gains of pytoplankton are equal to respiratory losses. When the mixing depth exceeds the critical depth, net respiratory losses occur, whereas when the mixing depth is shallower than the critical depth, net population growth occurs (Behrenfield, 2010; Nelson & Smith, 1991). Critical depth was calculated using the aforementioned measurements of light and rate of photosynthesis. Critical depth can be determined using the simplified version of Sverdrup's equation as follows (Nelson & Smith, 1991):

$$\text{Critical Depth } Z_c = 0.8(\bar{I}_o/KI_c)$$

Where, 0.8 = correction term for surface reflectance

$\bar{I}_o$= average irradiance at the surface

K = light attenuation coefficient of the water

I_c= compensation irradiance

For the lake measurements, $\bar{I}_o$ was calculated by retrieving 24-hour irradiance data from Lincoln. The compensation depth irradiance is the irradiance at the depth at which rates of photosynthesis and respiration are equal.

2.3 Statistical analyses

2.3.1 Nutrient Enrichment Experiments

Chlorophyll *a*

A one-way between subjects ANOVA was conducted to compare the effect of different nutrient addition combinations on chlorophyll *a* in the following treatments: control (no nutrients added), nitrate addition, phosphate addition, and enriched (where both were added). A one-way ANOVA of this type was performed for each individual month, using the chlorophyll *a* results of day 2 and of the final day of each experiment.

Cell counts

A one-way ANOVA was performed to determine if there is any effect of different nutrient addition combinations on the concentration of cells on the final day of these experiments. One test was performed for each experiment. A post-hoc Tukey multi-comparison test was then performed when ANOVA detected a significant effect of treatment.

Photosynthetic efficiency

The yield data collected was non-normal and non-transformable, so a Kruskal-Wallis non-parametric alternative was used to compare the effect of different treatments on the photosynthetic efficiency of cells. A Kruskal-Wallis test was employed for each individual experiment, where day 2 and day 5 were tested individually.

2.3.2 Enrichment versus Depth Experiments

Chlorophyll *a*

Although the nutrient versus depth experiments were designed as a two-way ANOVA, some of the chlorophyll a data were non-normal which made using a 2-way ANOVA inappropriate. Instead, Kruskal-Wallis tests were performed, with each individual treatment types used (80 cm control, 80 cm enriched, 40 cm control, 40 cm enriched, 20 cm control, and 20 cm enriched). Subsequently a Tukey's multiple comparison test was performed to compare each treatment type.

Cell counts

A two-way ANOVA was performed to determine if there was any effect of depth, enrichment, and an interaction between the two, on the number of cells per mL on day 5 of these experiments. A post-hoc Tukey multiple comparison test was performed on significant effects to compare each treatment type.

Photosynthetic efficiency

The yield data collected for these experiments were also non-normal, so again a Kruskal-Wallis test was performed with treatment types treated individually (80 cm control, 80 cm enriched, 40 cm control, 40 cm enriched, 20 cm control, and 20 cm enriched). A Tukey test was performed to compare treatment types.

Chapter 3

Results

3.1 Overview

This chapter begins by describing the control treatments for all experiments, to allow differences in conditions between months to be identified, and then moves on to examine each series of experiments. The nutrient addition experiments in 40 cm water columns determine nutrient most limiting nutrients, and responses are described for chlorophyll *a*, cell count data, and photosynthetic efficiency. Following the nutrient addition experiment results, the nutrient depth experiments examine interactions between nutrients and depth. This section follows the same format as the nutrient addition experiments.

The light quality and quantity measurements taken in the lake are then described to give an indication of the light environment of Te Waihora, and in situ photosynthetic responses are described. The light versus photosynthesis results from the mesocosms in July, September, and January are presented to show how the light environment determined rate of photosynthesis, and therefore potential growth of phytoplankton.

3.1.1 Water quality in mesocosms

Water quality parameters measured in the control treatments on the final day of each experiment show similarities in conductivity, dissolved oxygen content and temperature (Table 1). Notable difference were low turbidity in September, high turbidity in May, while low pH values occurred in the December, and to a lesser extent January, experiments. Turbidity decreased substantially over the duration of the July, September, and January experiments.

Table 1. Turbidity on the first day of experiments, and turbidity as well as other water quality parameters measured in the 40 cm control treatments on the final day of experiments in May, July, September and December 2015, and January 2016.

Date	Turbidity (NTU)	Turbidity (NTU)	Conductivity (mS cm^{-1})	pH	Dissolved Oxygen (mg L^{-1})	Temperature (^{o}C)
Month	**Day 0**	**Day 5**	**Day 5**	**Day 5**	**Day 5**	**Day 5**
May 2015	193.3	186.7	8.83	8.49 - 8.51	9.10	17.91
July 2015	99.0	46.2	8.37	8.54 - 8.56	9.06	18.42
September 2015	61.1	12.9	10.01	8.73 - 8.83	8.82	18.23
December 2015	61.4	53.1	9.52	6.95 - 6.97	8.92	19.58
January 2016	197.7	78.4	11.24	7.48 - 7.59	8.94	19.66

Initial nutrient concentrations in controls varied amongst experiments (Table 2). In January, September, and July the NNN concentrations were below detection limits (<0.025 mg L^{-1}) at the beginning of experiments and remained so at the end. In contrast, the December and May NNN concentrations were initially high (>0.5 mg L^{-1}) but were partially depleted during the experiment. No DRP concentrations were initially above detection limits, except in July (0.025 mg L^{-1}). Depletion of DRP was seen during January and May, and possibly in September, but not in July or December. The NH_3-N concentrations where high in all months (>0.120 mg L^{-1}) except December when the concentration was below detection limits (<0.03 mg L^{-1}). NH_3-N was significantly depleted by the end of the experiments in January, September and July.

Table 2. Nutrients concentrations in the 40cm control mesocosms on the first and final day of each experiment.

Date	NNN (mg L^{-1})	NNN (mg L^{-1})	NH_3-N (mg L^{-1})	NH_3-N (mg L^{-1})	DRP (mg L^{-1})	DRP (mg L^{-1})
	Start	**End**	**Start**	**End**	**Start**	**End**
May 2015	0.584	0.226	<0.03	<0.03	0.008	0.003
July 2015	<0.025	<0.025	0.123	<0.03	0.025	0.029
September 2015	<0.025	<0.025	0.143	0.114	0.002	<0.001
December 2015	0.811	0.400	<0.03	<0.03	0.007	0.009
January 2016	<0.025	<0.025	0.159	<0.03	0.007	0.003

3.2 Nutrient addition experiments

3.2.1 May 2015

Nutrients did not deplete evenly across treatment types (Table 3). At the beginning of the experiment, the NNN concentration was high, DRP was relatively low, and NH_3-N below limits of detection. Both NNN and DRP depleted in the Control treatment by the end of the experiment, but were still above limits of detection. The NNN and DRP concentrations were depleted more in the +NP treatment than in controls. NNN was depleted substantially in the +P treatment then the control.

Table 3. Nutrient concentrations on day 0 of the experiment in the control, and day 5 of all treatments in May. Treatments include the control, nitrate addition (+N), phosphate addition (+P), and a combination of both nitrate and phosphate addition (+NP).

Treatment	NNN (mg L^{-1})	DRP (mg L^{-1})	NH_3-N (mg L^{-1})
Control day 0	0.584	0.008	<0.03
Control day 5	0.226	0.003	<0.03
+N day 5	96.17	0.011	<0.03
+P day 5	0.169	8.967	<0.03
+NP day 5	88.59	6.888	<0.03

Chlorophyll *a* concentrations increased in all treatments over the duration of the experiment (Figure 2). Chlorophyll *a* concentrations were generally higher in +P treatments on days 2 and 5, but due to high variability there was no statistically significant effect of nutrient additions (Appendix A) on day 2 ($F(3,8)= 3.31$, $p = 0.078$) or day 5 ($F(3,8)= 2.34$, $p = 0.150$).

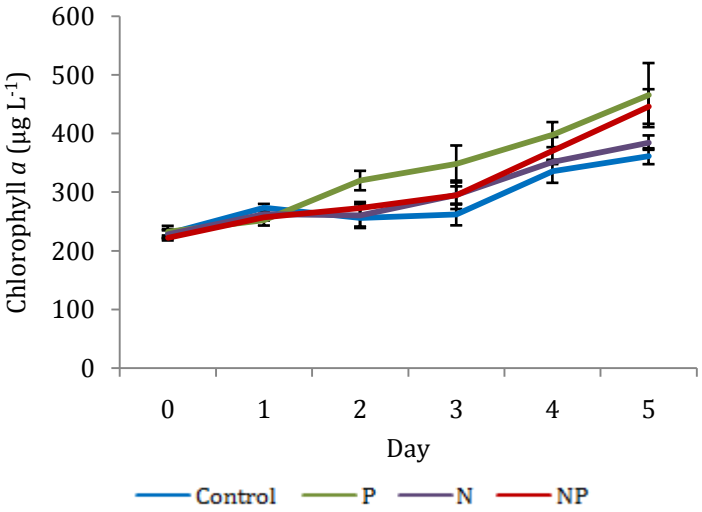

Figure 2. Chlorophyll *a* concentrations during the May experiment in the control, nitrate-addition (N), phosphate-addition (P), and combined addition (NP) treatments. Differences amongst treatments were insignificant on day 2 (p = 0.078) and day 5 (p = 0.150).

Nutrient additions had a significant effect on cell density($F(3,8)= 10.55$, $p < 0.05$) (Figure 3). The +NP treatments resulted in a significantly higher cell density than +P and Control treatments ($p < 0.05$). The +N treatment stimulated the second highest cell density, though this effect was not statistically greater than the Control or +P treatments. Picocyanobacteria was the dominant algae across all treatments.

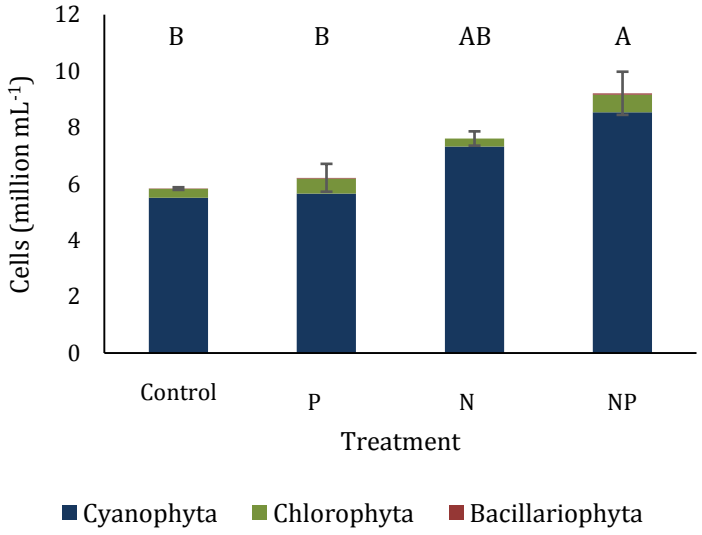

Figure 3. Cell counts of different algae in the control, nitrate-addition (+N), phosphate-addition (+P), and combined additions (+NP) treatments on day 5 of the December experiment. Treatments with different letters were statistically significant (p <0.05).

Nutrient additions did not have an effect on photosynthetic efficiency (Figure 4 & Appendix B) on day 2 (H= 6.90, DF = 3, p = 0.075) or day 5 (H= 2.28, DF = 3, p=0.516).

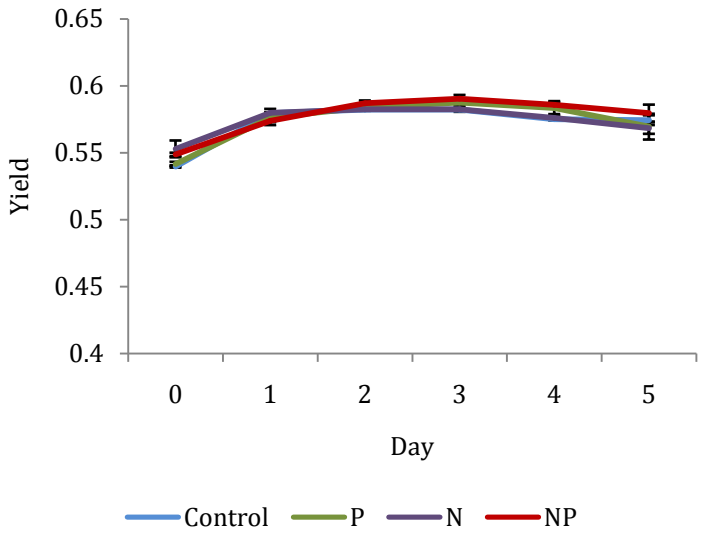

Figure 4. Photosynthetic efficiency (dimensionless ratio) in different treatments on each day of the May experiment. Treatments include control, added phosphate (P), added nitrate (N), and both nitrate and phosphate added in combination (NP). All treatments were in statistically similar groupings. Differences amongst treatments were insignificant on day 2 (p = 0.075) and day 5 (p = 0.516). Note the y-axis does not start at 0.

3.2.2 July 2015

Initial DRP and NH_3N concentrations were high, and the NNN concentration was below detection limits (Table 4). In the Control treatment, ambient DRP remained high and NH_3-N had depleted to below detection limits at the end of the experiment. In the +N treatment, DRP concentrations were considerably depleted, but remained high in the +P and +NP treatments. NNN was depleted in the treatments where phosphate was added (+P & +NP). NH_3-N was consumed in the Control and +P treatments.

Table 4. Nutrient concentrations on day 0 of the experiment in the control, and day 5 of all treatments in July. Treatments include the control, nitrate addition (+N), phosphate addition (+P), and a combination of both nitrate and phosphate addition (+NP).

Treatment	NNN (mg L^{-1})	DRP (mg L^{-1})	NH_3-N (mg L^{-1})
Control day 0	<0.025	0.025	0.123
Control day 5	<0.025	0.029	<0.03
+N day 5	2.156	0.012	0.101
+P day 5	<0.025	0.785	<0.03
+NP day 5	<0.025	1.262	0.082

Initial chlorophyll *a* concentrations (Figure 5) were similar to those in May. On day 2 the chlorophyll *a* concentrations were significantly different between treatments ($F(3,8)= 7.25$, $p < 0.05$). The concentrations in the +NP treatment was greater than the Control and +P treatments on day 2 ($p < 0.05$) (Appendix A), and continued to substantially increase until the end of the experiment ($p < 0.05$). The +N treatment was significantly different to the Control and +P treatments by day 5 ($p < 0.05$), as the Control and +P treatment concentrations decreased over the duration of the experiment. This means in both the short and long-term nitrate and phosphate added in combination had the greatest effect on chlorophyll *a*. Nitrate-only additions also resulted in increased chlorophyll *a*, though to a lesser extent.

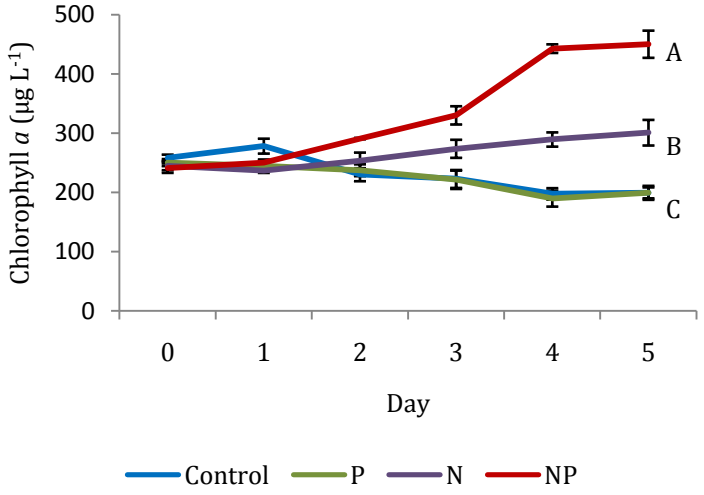

Figure 5. Chlorophyll *a* concentrations at the start of the July experiment and every 24 hours thereafter in the control, nitrate-addition (N), phosphate-addition (P), and combined addition (NP) treatments. Treatments with different letters are statistically different (A, B, C).

Similar to May, single-celled picocyanobacteria had the highest abundance in all treatments (Figure 6). Nutrient additions had a significant effect on cell density ($F(3,8)= 55.42$, $p < 0.05$). The +P treatment had a much greater cell density ($p < 0.05$). The Control treatment also had a higher cell density than the +N and +NP treatments ($p < 0.05$), indicating nitrate addition led to lower cell densities, the opposite of what occurred in May.

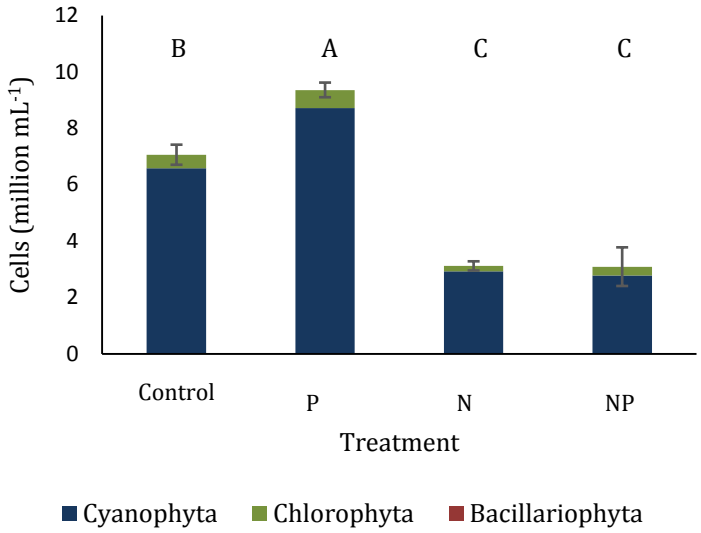

Figure 6. Cell counts of different algae in the control, nitrate-addition (+N), phosphate-addition (+P), and combined additions (+NP) treatments on day 5 of the July experiment. There was a significant difference between treatments (p <0.05). Treatments with different letters are statistically different (A, B, C).

Nutrient additions had an effect on photosynthetic efficiency (H= 8.74, DF = 3, p <0.05), whereby the +N and +NP had the greatest yields by day 2 (p <0.05) (Figure 7 & Appendix B). Yield dropped overall in the Control treatments by day 5, and only nutrient additions had sustained increased photosynthetic efficiency, with the +N treatment having the greatest yield (p<0.05). The unusual +NP results on day 4 is due to one extremely low replicate, and may be an artefact.

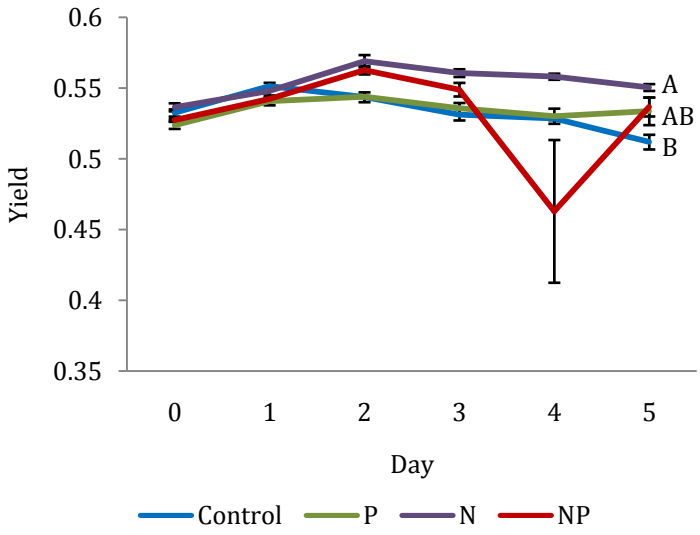

Figure 7. Photosynthetic efficiency (dimensionless ratio) in different treatments on each day of the July experiment. Treatments include the Control, nitrate addition (+N), phosphate addition (+P), and both added in combination (+NP). Treatments with different letters are statistically different (A, B). Note the y-axis does not start at 0.

3.2.3 September 2015

Initial concentrations of NNN and DRP were particularly low, whereas NH_3-N concentrations were high (Table 5). By day 5, ambient NH_3-N concentrations remained high, except in the +P treatment where it was severely depleted. NNN concentrations depleted in +N and +NP treatments. DRP concentrations had also depleted in the +NP treatment, and the +P treatment to a lesser extent.

Table 5. Nutrient concentrations on day 0 of the experiment in the control, and day 5 of all treatments in September. Treatments include the control, nitrate addition (+N), phosphate addition (+P), and a combination of both nitrate and phosphate addition (+NP).

Treatment	NNN (mg L^{-1})	DRP (mg L^{-1})	NH_3-N (mg L^{-1})
Control day 0	<0.025	0.002	0.143
Control day 5	<0.025	<0.001	0.114
+N day 5	<0.025	<0.001	0.216
+P day 5	<0.025	0.005	0.015
+NP day 5	<0.025	<0.001	0.189

Chlorophyll *a* concentrations at the start of the experiment (Figure 8) were lower than in May and July. There was a significant effect of nutrient additions on day 2 ($F(3,8)= 13.88$, $p <0.05$), where the +NP treatment had significantly increased ($p <0.05$) (Appendix A). There was also a significant effect by day 5 ($F(3,8)= 14.59$, $p <0.05$), where the +NP treatment concentration was greater than the +P and Control treatments ($p <0.05$), both of which showed a gradual decline. The chlorophyll *a* concentrations of the +P and Control treatments decreased over the duration of the experiment. This means in both the short and long-term the combined additions of nitrate and phosphate had the greatest effect on chlorophyll *a*. Nitrate added in the +N treatment allowed consistent chlorophyll *a* concentrations over the duration of the experiment. These results are similar to those found in July, except there was less of an increase in +NP treatments, which may related to depletion of DRP to below detection limits.

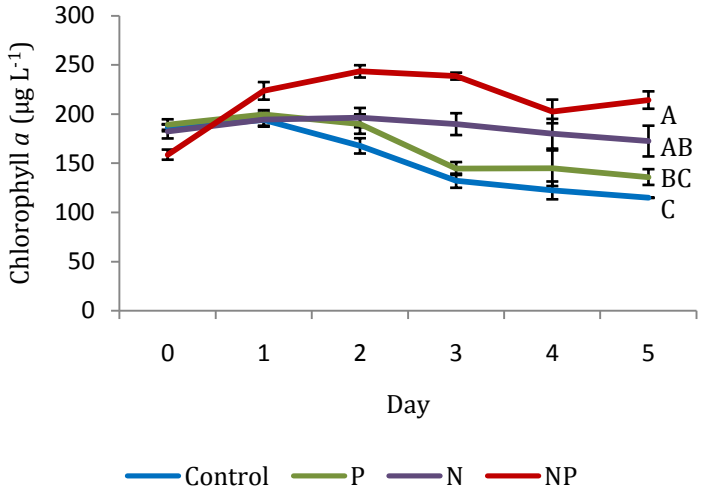

Figure 8. Chlorophyll *a* during the September experiment in the control, nitrate-addition (N), phosphate-addition (P), and combined addition (NP) treatments. Differences amongst treatments were significant (p <0.05), and treatments with different letters are statistically different (A, B, C).

Cyanobacteria had the greatest abundance in all treatments (Figure 9). Nutrient additions had no significant effect on phytoplankton cell density ($F(3,8)= 1.33$, $p = 0.330$). However, the +N mesocosms generally had much greater cell densities.

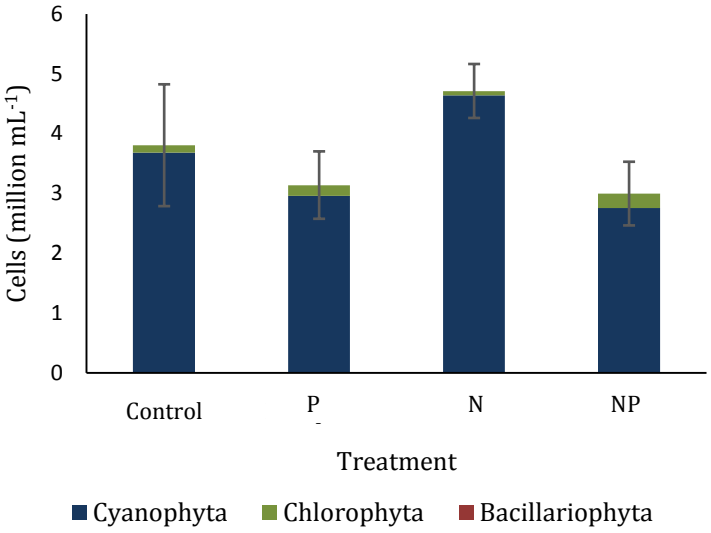

Figure 9. Cell counts of different algae in the control, nitrate-addition (+N), phosphate-addition (+P), and combined additions (+NP) treatments on day 5 of the September experiment. There was no significant difference between treatments (p = 0.330).

On day 2 the photosynthetic efficiency between treatments was significantly different ($H= 8.90$, $DF = 3$, $p <0.05$) (Figure 10). The +NP treatment had the highest yield (Appendix B), and was significantly different to the control ($p <0.05$). Phosphate additions were enough to maintain the yield in the +NP and +P treatments until day 5, where both treatments had significantly greater yields than the Control ($p <0.05$).

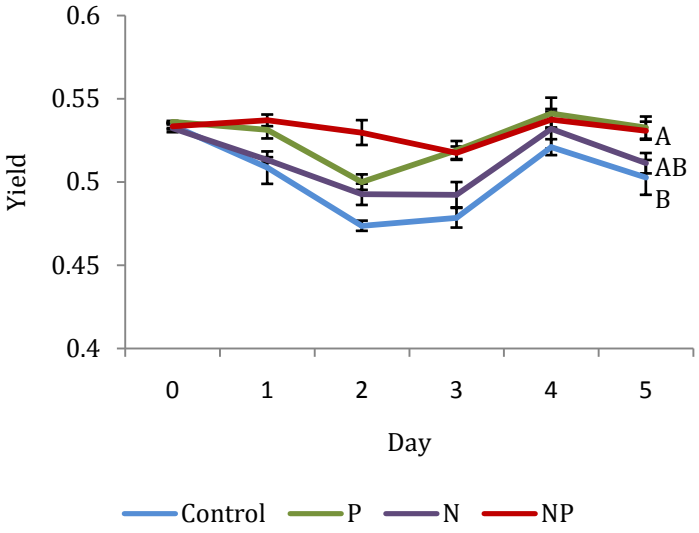

Figure 10. Photosynthetic efficiency (dimensionless ratio) in different treatments on each day of the September experiment. Treatments were the Control, nitrate addition (+N), phosphate addition (+P), and both added in combination (+NP). Treatments with different letters were statistically different (A, B). Note the y-axis does not start at 0.

3.2.4 December 2015

The nutrient concentrations in the Control treatment at the start of the experiment (Table 6. were similar to those found in May: very high NNN concentrations, low DRP concentrations, and NH_3-N below detection limits. By the end of this experiment, NNN concentrations had depleted in the Control, +P and +NP treatments. DRP concentrations depleted to Control levels in phosphate addition treatments (+P and +NP). These results are similar to the May experiment.

Table 6. Nutrient concentrations on the first day of the experiment in the control, and day 5 of all treatments in December. Treatments include the control, nitrate addition (+N), phosphate addition (+P), and a combination of both nitrate and phosphate addition (+NP).

Treatment	NNN (mg L^{-1})	DRP (mg L^{-1})	NH_3-N (mg L^{-1})
Control day 0	0.811	0.007	<0.03
Control day 5	0.400	0.009	<0.03
+N day 5	2.946	0.008	<0.03
+P day 5	0.113	0.009	<0.03
+NP day 5	2.168	0.008	0.035

Initial chlorophyll *a* concentrations (Figure 11) were lower than prior experiments. On day 2 there was a significant effect of treatment type on chlorophyll *a* concentration ($F(3,8)= 13.01$, $p < 0.05$). The +NP treatment had the highest chlorophyll *a* concentration on day 2 ($p < 0.05$),

and the concentrations in the +P treatment had also increased, though not significantly so (Appendix A). The control and +N treatments showed little change. The +P and +NP treatments had significantly higher chlorophyll *a* concentrations day 5 ($F(3,8)= 12.52$, $p <0.05$). This means in both the short and long-term added phosphate had the greatest effect on chlorophyll *a* for the December experiment.

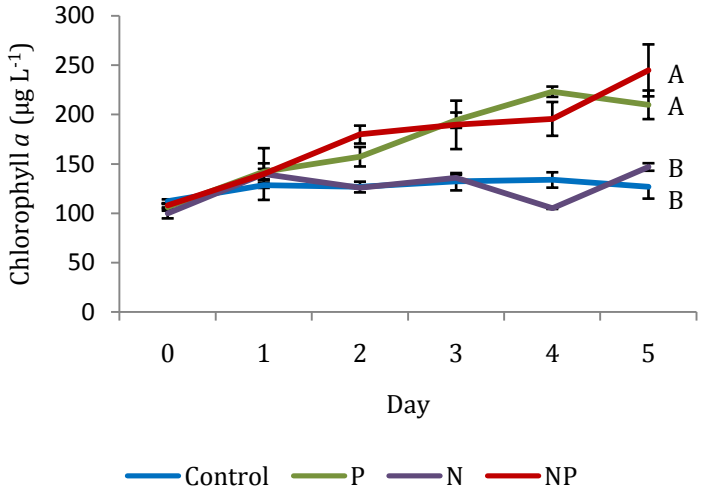

Figure 11. Chlorophyll *a* during the December experiment in the control, nitrate-addition (N), phosphate-addition (P), and combined addition (NP) treatments. Differences amongst treatments were significant ($p <0.05$). Treatments with different letters were statistically different (A, B) at the end of the experiment.

Cyanobacteria was the dominant phytoplankton group in December (Figure 12). Nutrient additions had no effect on cell density ($F(3,8)= 0.51$, $p = 0.687$).

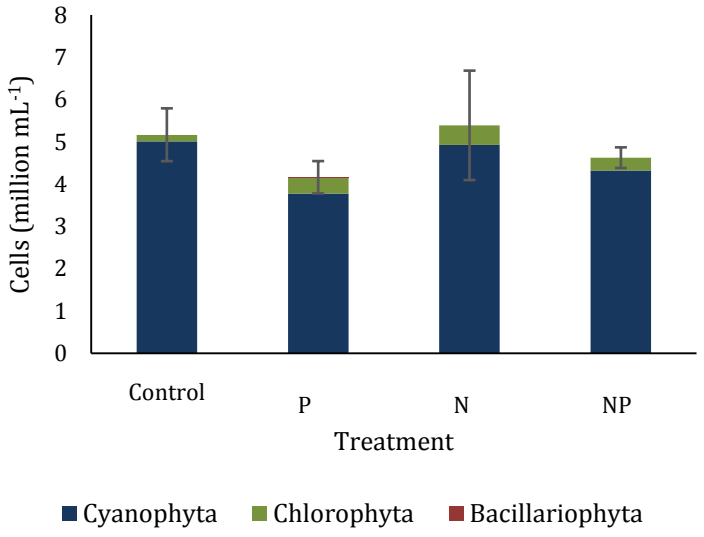

Figure 12. Cell counts of different algae in the control, nitrate-addition (+N), phosphate-addition (+P), and combined additions (+NP) treatments on day 5 of the December experiment. There was no statistical difference between treatments ($p = 0.687$).

Nutrient additions also had no effect on photosynthetic efficiency (day 2: H= 7.62, DF = 3, p = 0.55; day 5: H= 6.28, DF = 3, p = 0.099) (Appendix B), though there was a general increase in all treatments toward day 2, and decrease towards day 5 (Figure 13).

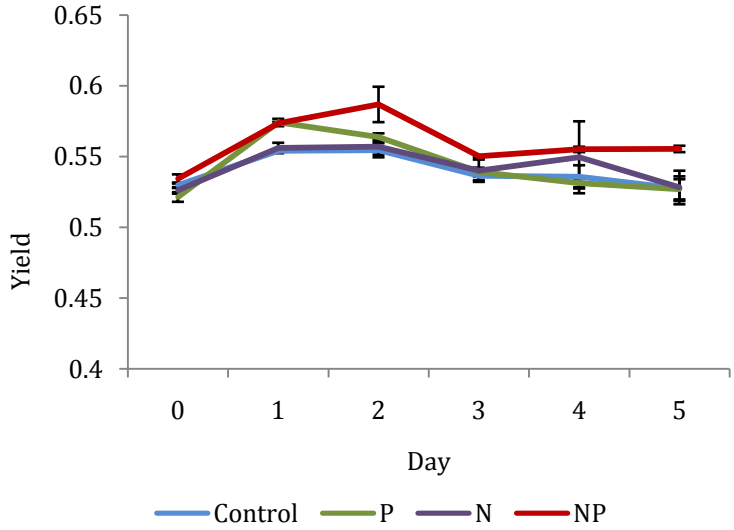

Figure 13. Photosynthetic efficiency (dimensionless ratio) over the duration of the December experiment. Yield measured at the start of the experiment, and every subsequent 24 hours. Treatments were: Control, nitrate addition (+N), phosphate addition (+P), and both added in combination (+NP). All treatments were statistically similar. Note the y-axis does not start at 0.

3.2.5 January 2016

The nutrient concentrations at the start of the experiment (Table 7) were similar to those in September: NNN concentrations below limits of detection, low DRP, and high NH_3-N concentrations. By the end of the experiment, DRP and NH_3-N had both depleted in the Control treatment, and NNN was also depleted to below detection limits in the +N and +NP treatments. DRP concentrations were depleted in the +N and +P treatments, and the +NP treatment to a lesser extent. NH_3-N was depleted in the +P and +NP treatments.

Table 7. Nutrient concentrations on day 0 of the experiment in the control, and day 5 of all treatments in January. Treatments include the control, nitrate addition (+N), phosphate addition (+P), and a combination of both nitrate and phosphate addition (+NP).

Treatment	NNN (mg L^{-1})	DRP (mg L^{-1})	NH_3-N (mg L^{-1})
Control day 0	<0.025	0.007	0.159
Control day 5	<0.025	0.003	<0.03
+N day 5	<0.025	<0.001	0.039
+P day 5	<0.025	0.003	<0.03
+NP day 5	<0.025	0.036	<0.03

Initial chlorophyll *a* concentrations (Figure 14) were similar to those at the start of December. On day 2 of the experiment there was a significant effect of treatment type on chlorophyll *a* concentration ($F(3,8)= 13.01$, $p < 0.05$), whereby the +NP treatment had the greatest chlorophyll *a* concentration ($p < 0.05$) (Appendix A). This was also true for day 5 ($F(3,8)= 9.26$, $p < 0.05$). Nitrate additions (+N and +NP) led to increased chlorophyll *a* concentrations over the duration of the experiment. These results were similar to those found in July and September.

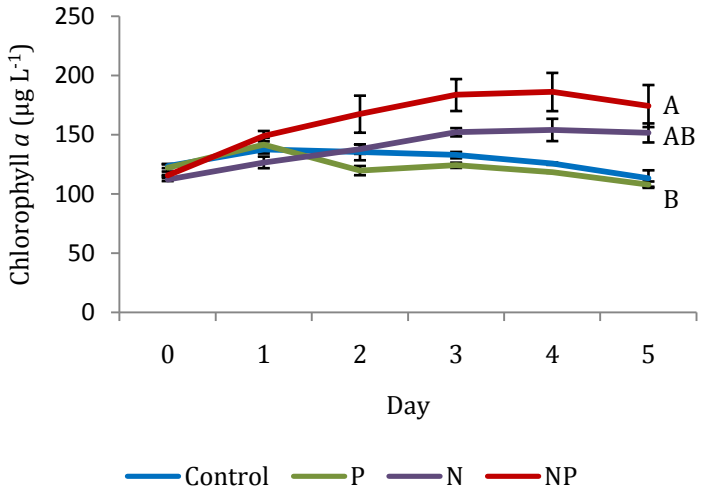

Figure 14. Chlorophyll *a* concentrations at the start of the January experiment and every 24 hours thereafter in the control, nitrate-addition (N), phosphate-addition (P), and combined addition (NP) treatments. Treatments with different letters are statistically different (A, B).

Cyanobacteria was the dominant phytoplankton group (Figure 15). Nutrient additions had no significant effect on phytoplankton cell density ($F(3,8)= 0.71$, $p = 0.573$), although the +N treatments generally had lower densities.

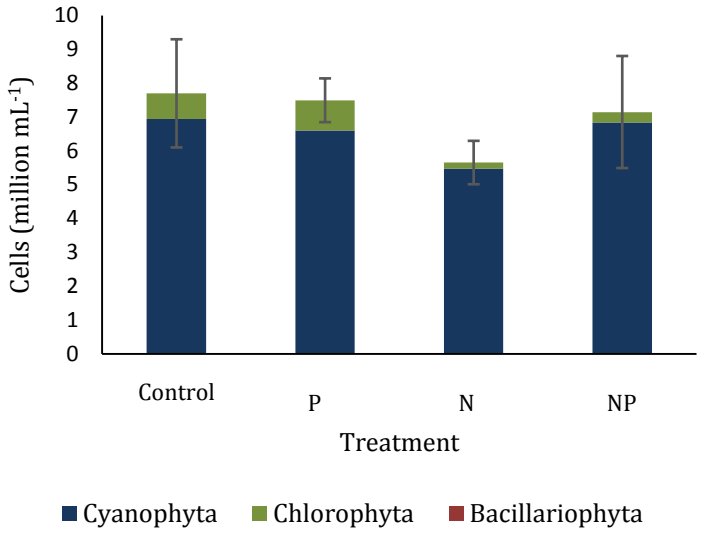

Figure 15. Cell counts of different algae in the control, nitrate-addition (+N), phosphorus-addition (+P), and combined addition (+NP) treatments on day 5 of the January experiment. There was no significant difference between treatments (p = 0.573).

Nutrient additions had no significant effect on photosynthetic efficiency (Appendix B) on day 2 (H= 5.56, DF = 3, p = 0.135) or day 5 (H= 5.56, DF = 3, p = 0.135) (Figure 16).

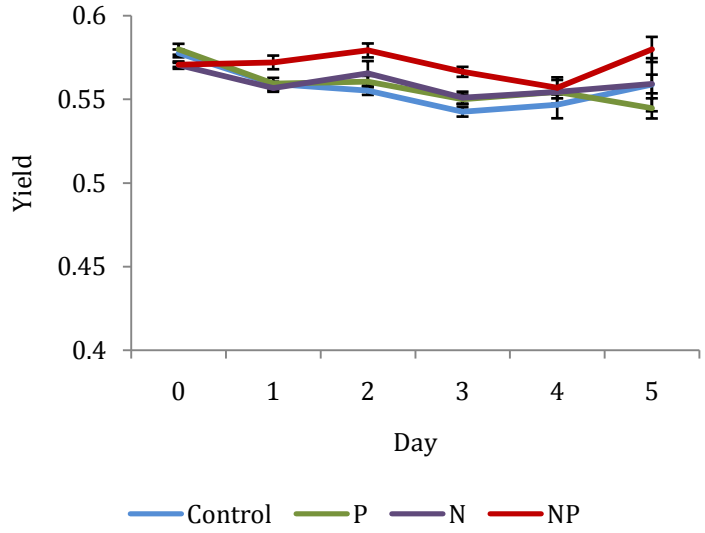

Figure 16. Photosynthetic efficiency (dimensionless ratio) over the duration of the January experiment. Yield measured at the start of the experiment, and every subsequent 24 hours. Treatments are: Control, nitrate addition (+N), phosphate addition (+P), and both added in combination (+NP). There was no significant difference between treatments on day 5 (p = 0.135). Note the y-axis does not start at 0.

3.3 Nutrient versus depth experiments

3.3.1 May 2015

Nutrients were not depleted evenly across treatment types (Table 8). In the enriched treatments, NNN reduced to a greater extent in shallow than deep mesocosms, whereas DRP depleted more in the deepest mesocosms. The NNN concentrations and DRP concentrations in control treatments were universally low. DRP was reduced to the greatest extent in the deepest mesocosms in both enriched and control treatments. These results suggest there was higher uptake of NNN and lower uptake of DRP in shallow treatments. The NH_3-N concentrations were all below limits of detection in all treatments.

Table 8. The nutrient concentrations on day 0 of the control treatments, and day 5 of all treatments in May. Treatments include the 20 cm, 40 cm, and 80 cm control, as well as 20 cm, 40 cm, and 80 cm enriched.

Treatment	NNN (mg L^{-1})	DRP (mg L^{-1})	NH_3-N (mg L^{-1})
Day 0 Control	0.584	0.008	<0.03
80cm Control	0.175	0.002	<0.03
80cm Enriched	92.02	5.067	<0.03
40cm Control	0.226	0.003	<0.03
40cm Enriched	88.59	6.888	<0.03
20cm Control	0.080	0.011	<0.03
20cm Enriched	68.64	6.791	<0.03

On day 2, there was no significant difference in chlorophyll *a* concentrations between treatment types ($H(5)= 8.04$, $p = 0.154$), although the shallow enriched treatment exceeded all others (Figure 17 & Appendix C). However, by day 5 there was a significant difference between treatments ($H(5)= 13.30$, $p <0.05$), as the 20 cm enriched treatment had almost 3x more chlorophyll *a* than control treatments ($p <0.05$). There was no significant effect of mesocosm depth in the control incubations, nor of nutrient additions in the 40 cm and 80 cm mesocosms.

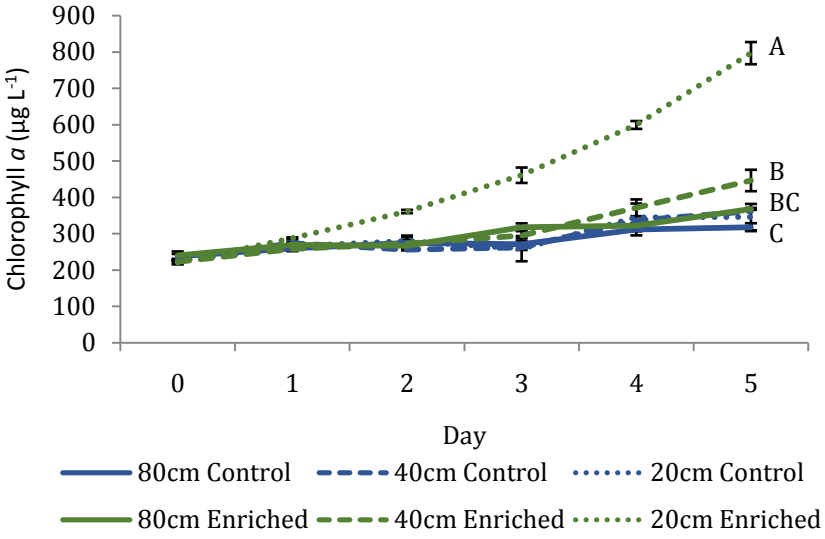

Figure 17. Chlorophyll *a* concentrations over the duration of the experiment in May in the 20 cm, 40 cm, and 80 cm control, and 20 cm, 40 cm, and 80 cm enriched treatments. Treatments with different letters were statistically different (A, B, C).

There was a main effect of enrichment on cell density ($F(1,12)= 8.14$, $p <0.05$) but no interactive effect of depth and enrichment on phytoplankton cell density ($F(2,12)= 1.47$, $p = 0.269$). The 80 cm and 40 cm enriched mesocosms had significantly more cells compared with controls (Figure 18).

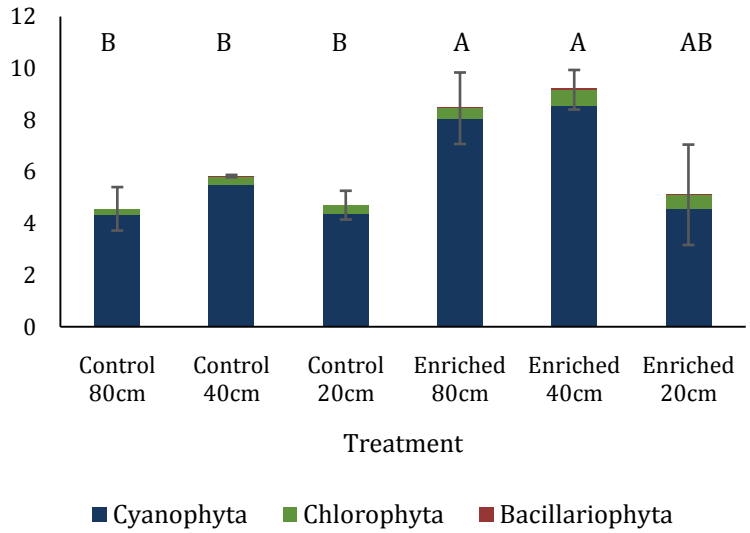

Figure 18. Cell counts of different algae in the 20 cm, 40 cm, and 80 cm control, and 20 cm, 40 cm, and 80 cm enriched treatments on day 5 of the May experiment.

Photosynthetic efficiency decreased substantially in the shallowest mesocosms (Figure 19). There was a significant effect of treatment type on photosynthetic efficiency by day 2 ($H(5)= 16.06$, $p <0.05$). The 20 cm control treatment had a much lower yield than deeper treatments

(p <0.05) (Appendix D). By day 5, both the 20 cm enriched and 20 cm control treatment yields were significantly lower than all other treatment types (p <0.05).

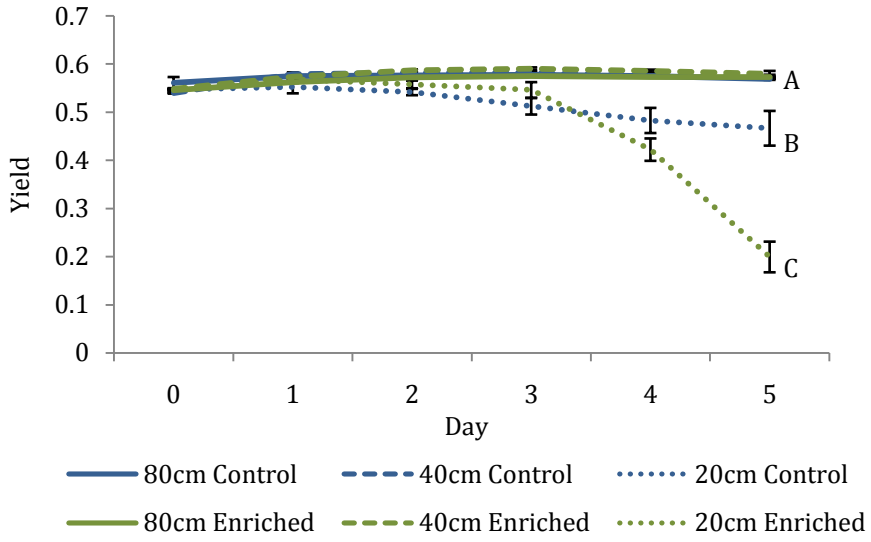

Figure 19. Photosynthetic efficiency (dimensionless ratio) over the duration of the May experiment. Treatments include 20 cm, 40 cm, and 80 cm Control, as well as 20 cm, 40 cm, and 80 cm Enriched. Treatments with different letters were statistically different (A, B, C). Note y-axis does not start at 0.

3.3.2 July 2015

At the end of the experiment, the control treatments all had NNN and NH_3-N concentrations below detection limits (Table 9), whereas the DRP concentrations remained high. Of the enriched treatments, NNN was depleted to the greatest extent in the 80 cm mesocosms, and DRP depleted in the 80 cm and 20 cm mesocosms. In addition, in the enriched treatments NH_3-N depleted to the greatest extent in the 80 cm mesocosms.

Table 9. The nutrient concentrations on day 0 of the control treatments, and day 5 of all treatments in July. Treatments include the 20 cm, 40 cm, and 80 cm control, as well as 20 cm, 40 cm, and 80 cm enriched.

Treatment	NNN (mg L^{-1})	DRP (mg L^{-1})	NH_3-N (mg L^{-1})
Day 0 Control	<0.025	0.025	0.123
80cm Control	<0.025	0.074	<0.03
80cm Enriched	2.488	1.041	0.047
40cm Control	<0.025	0.025	<0.03
40cm Enriched	3.404	1.262	0.082
20cm Control	<0.025	0.078	<0.03
20cm Enriched	3.108	1.006	0.125

By day 2, chlorophyll *a* concentrations in the 20 cm enriched mesocosms were significantly greater than shallow control treatments ($p < 0.05$) (Appendix C). On day 5, there was still a significant difference between treatments ($p < 0.05$). The chlorophyll *a* concentrations increased in all enriched treatments, substantially exceeding all control treatments (Figure 20). There was a gradual decrease in control treatments, with no significant difference between lengths. There was a general trend of increasing chlorophyll *a* concentrations in shallow enriched mesocosms, and a trend of declining chlorophyll *a* concentrations within shallow control mesocosms.

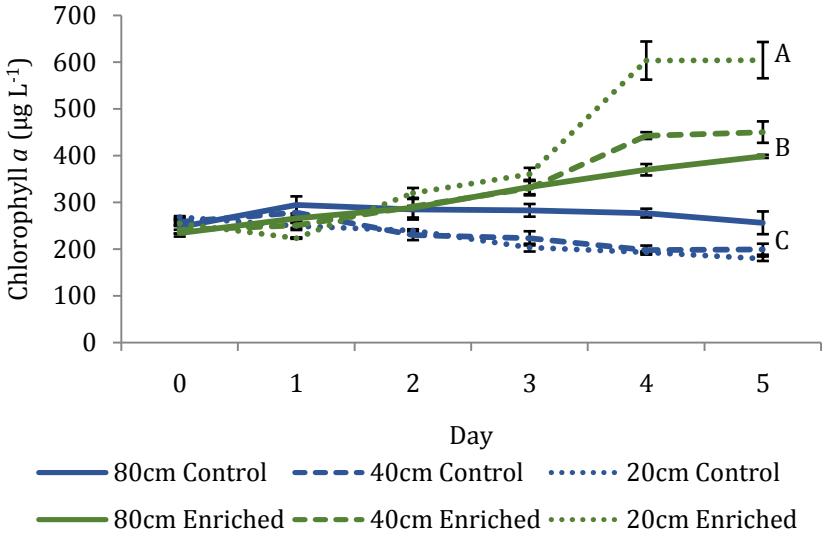

Figure 20. Chlorophyll *a* concentrations over the duration of the experiment in July in the 20 cm, 40 cm, and 80 cm control, and 20 cm, 40 cm, and 80 cm enriched treatments. Treatments with different letters were statistically different (A, B, C).

There was a significant effect of enrichment on cell density ($F(1,12) = 133.80$, $p < 0.05$), but no significant interactive effect of depth and enrichment on day 5 ($F(2,12) = 1.31$, $p = 0.307$). There were significantly higher cell densities in control mesocosms in comparison with enriched mesocosms.

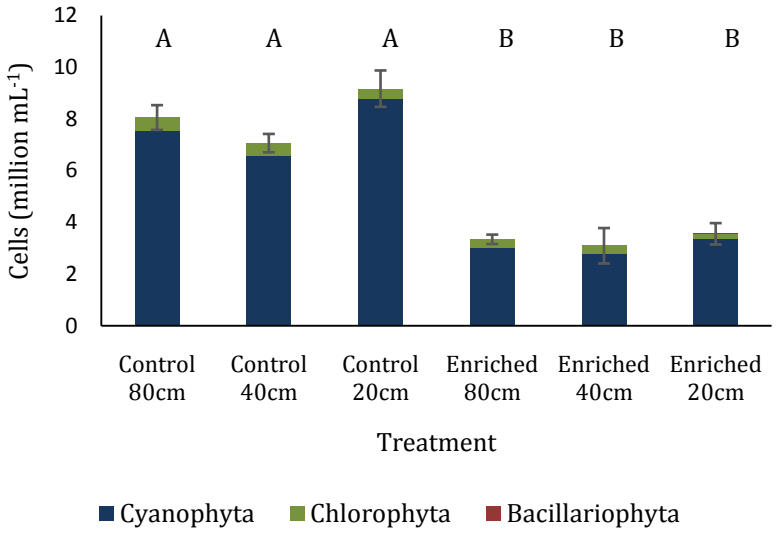

Figure 21. Cell count results of different cell types in 20 cm, 40 cm, and 80 cm controls, and 20 cm, 40 cm, and 80 cm enriched treatments on day 5 of the July experiment.

By day 2, the photosynthetic efficiency in the 20 cm control was significantly lower than in deeper mesocosms ($H(5)= 14.68$, $p < 0.05$). In the enriched treatments, yield was lower in the shallow mesocosms (Appendix D). This trend continued towards the final day of the experiment (Figure 22), where yield declined substantially in shallow mesocosms in both control and enriched treatments.

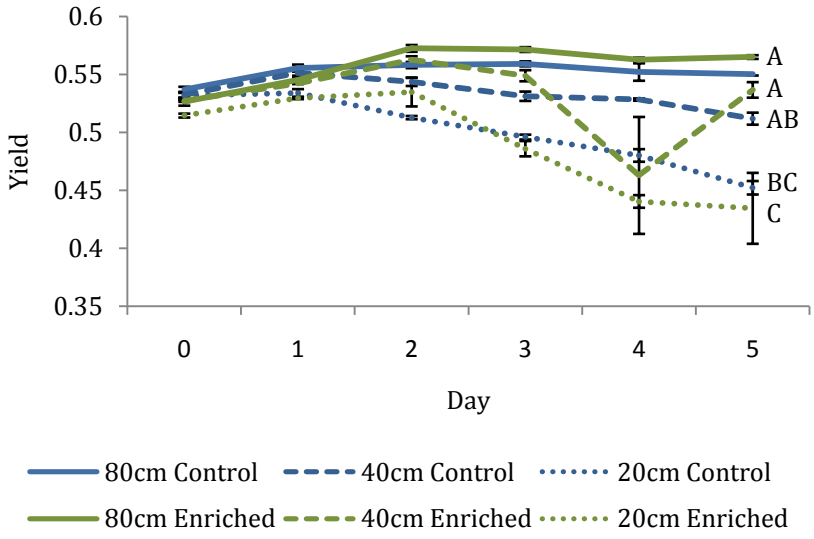

Figure 22. Photosynthetic efficiency (dimensionless ratio) measured over the duration of the July experiment. Treatments include 20 cm, 40 cm, and 80 cm control, as well as 20 cm, 40 cm, and 80 cm enriched. Treatments with different letters are statistically different (A, B, C). Note the y-axis does not start at 0.

3.3.3 September 2015

By the end of incubations, NNN concentrations were depleted in the 20 cm and 40 cm enriched, and to a lesser extent in the 80 cm treatment (Table 10). The DRP concentrations in the 20 cm and 40 cm treatments were depleted to below detection limits, and DRP was also substantially depleted in the 80 cm enriched treatment. NH_3-N concentrations depleted in the 20 cm treatments. These results suggest there was a high uptake of both NNN and NH_3-N in shallower mesocosms, and DRP in all enriched treatments.

Table 10. The nutrient concentrations on day 0 of the control treatments, and day 5 of all treatments in September. Treatments include the 20 cm, 40 cm, and 80 cm control, as well as 20 cm, 40 cm, and 80 cm enriched.

Treatment	NNN (mg L^{-1})	DRP (mg L^{-1})	NH_3-N (mg L^{-1})
Day 0 Control	<0.025	0.002	0.143
80cm Control	<0.025	0.004	0.122
80cm Enriched	0.112	0.002	0.203
40cm Control	<0.025	<0.001	0.114
40cm Enriched	<0.025	<0.001	0.189
20cm Control	<0.025	<0.001	0.086
20cm Enriched	<0.025	<0.001	0.092

On day 2 there was a significant increase in chlorophyll *a* concentrations in all enriched mesocosms, which were greater than controls ($H(5)= 14.81$, $p < 0.05$). A trend emerged in the control treatments (Figure 23), with declining chlorophyll *a* concentrations in shallower mesocosms. On day 5 there was still a significant difference between treatments ($H(5)= 15.59$, $p < 0.05$), as chlorophyll *a* declined substantially in the 20 cm enriched treatment after day 2 (Appendix C), as well as the 20 cm and 40 cm control treatments after day 1. Chlorophyll a concentrations in 80 cm and 40 cm enriched mesocosms gradually increased over time. The general trend for the September experiment appears to be much higher chlorophyll *a* concentrations in enriched mesocosms and with higher concentrations in the deeper mesocosms.

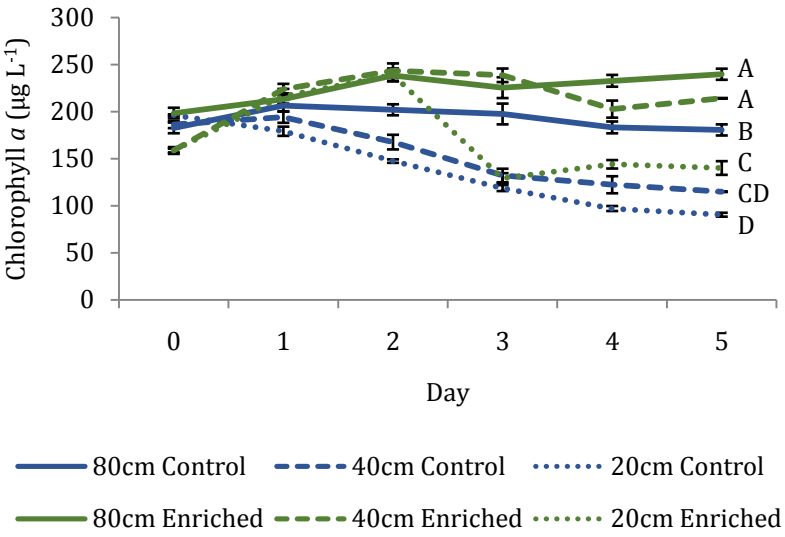

Figure 23. Chlorophyll *a* concentrations over the duration of the experiment in September in the 20 cm, 40 cm, and 80 cm control, and 20 cm, 40 cm, and 80 cm enriched treatments. Treatments with different letters were statistically different (A, B, C, D).

There was no significant interactive effect of depth and enrichment on phytoplankton cell density on day 5 ($F(2,12)= 1.31$, $p = 0.307$). Nor was there a significant effect of depth, or of enrichment by themselves, though cell densities in the 80 cm enriched mesocosms were generally higher.

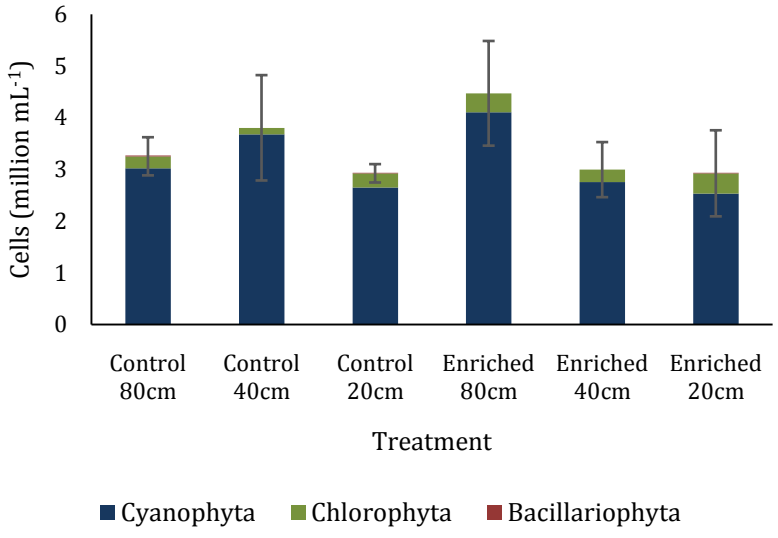

Figure 24. Cell counts of different algae in 20 cm, 40 cm, and 80 cm control and 20 cm, 40 cm, and 80 cm enriched treatments on day 5 of the September experiment.

By day 2, there was a significant difference between treatment types on photosynthetic efficiency ($H(5)= 15.97$, $p <0.05$), as yield declined in all control treatments and the 20 cm enriched mesocosms (Figure 25). There was also a significant difference between treatments

on day 5 (H(5)= 16.18, p <0.05). In the controls, yield declined substantially more in 20 cm mesocosms (Appendix D). This trend continued in 20 cm control mesocosms by day 5, and emerged in 20 cm enriched mesocosms. Again, the trend for September appears to be shallower mesocosms having reduced photosynthetic efficiency.

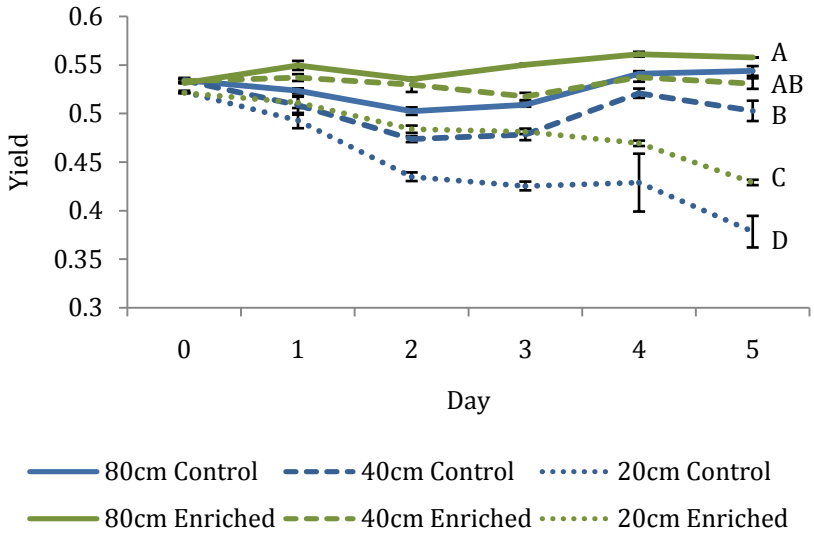

Figure 25. Photosynthetic efficiency (dimensionless ratio) measured over the duration of the September experiment. Treatments include the 20 cm, 40 cm, and 80 cm control, as well as 20 cm, 40 cm, and 80 cm enriched. Treatments with different letters are statistically different (A, B, C, D). Note the y-axis does not start at 0.

3.3.4 December 2015

By day 5, in both control and enriched treatments, NNN concentrations depleted much further in shallow treatments when compared with their deeper counterparts, though all remained detectable (Table 11). The DRP concentrations depleted in enriched treatments to the same concentrations found in control mesocosms over the duration of the experiment. This indicates the depth of mesocosms may have played a role in the uptake of NNN, but not of DRP. NH_3-N concentrations were all below limits of detection except in the 80 cm and 40 cm enriched treatments.

Table 11. The nutrient concentrations on day 0 of the control treatments, and day 5 of all treatments in December. Treatments include the 20 cm, 40 cm, and 80 cm control, as well as 20 cm, 40 cm, and 80 cm enriched.

Treatment	NNN (mg L^{-1})	DRP (mg L^{-1})	NH_3-N (mg L^{-1})
Day 0 Control	0.811	0.007	<0.03
80cm Control	0.660	0.009	<0.03
80cm Enriched	2.804	0.009	0.043
40cm Control	0.400	0.009	<0.03
40cm Enriched	2.168	0.008	0.035
20cm Control	0.282	0.008	<0.03
20cm Enriched	1.082	0.008	<0.03

Day 2 revealed a significant difference between the chlorophyll *a* concentrations of different treatments ($H(5)= 13.96$, $p <0.05$), where the 20 cm and 40 cm enriched treatment concentrations had increased (Appendix C), while other treatments had not (Figure 26). There was also a significantly difference on day 5 ($H(5)= 15.73$, $p <0.05$). The control treatment chlorophyll *a* concentrations all remained low. However, there was a substantial increase in enriched treatments, with a general trend of greater chlorophyll *a* concentrations in shallower mesocosms.

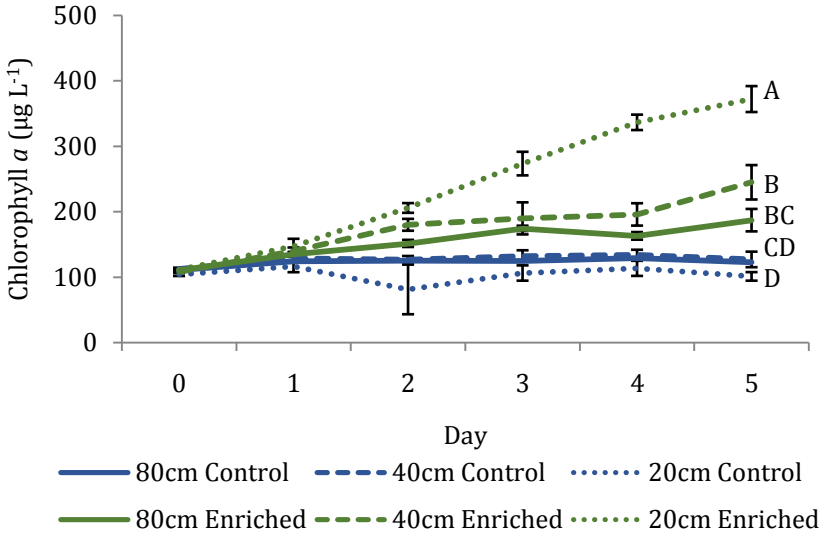

Figure 26. Chlorophyll *a* over the duration of the December experiment. Treatments are: 20 cm, 40 cm, and 80 cm Control, as well as 20 cm, 40 cm, and 80 cm Enriched. Treatments with different letters are statistically different (A, B, C, D).

There was no significant interactive effect between enrichment and depth of mesocosms on cell density ($F(2,12)= 3.52$, $p = 0.063$), however shallow enriched mesocosms generally had lower cell densities (Figure 27).

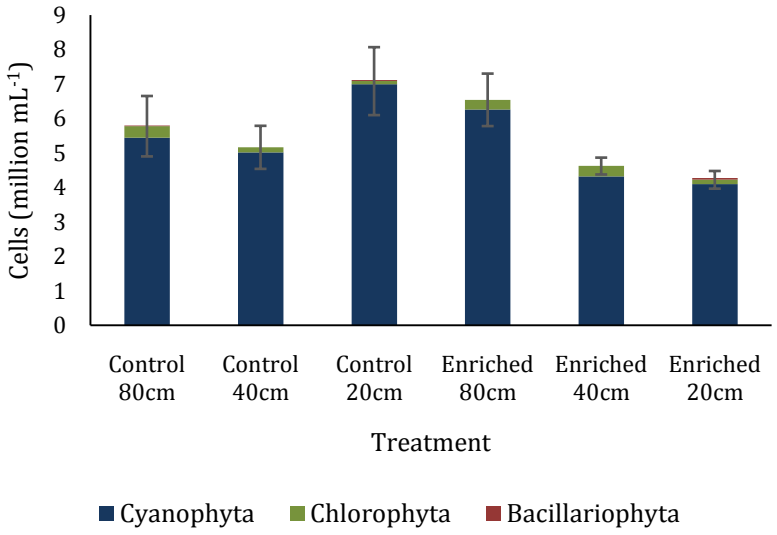

Figure 27. Cell counts of different algae in the 20 cm, 40 cm, and 80 cm controls, and the 20 cm, 40 cm, and 80 cm enriched treatments on day 5 of the December experiment.

There was a significant difference between treatment types on photosynthetic efficiency on day 2 ($H(5)= 15.69$, $p <0.05$), where yield measured in the 20 cm control and 20 cm enriched treatments declined significantly compared with other treatments ($p <0.05$) (Appendix D). This trend continued through to the final day ($H(5)= 16.16$, $p <0.05$), where shallow mesocosms had a lower yield than deeper treatments. Control mesocosms generally had a reduced photosynthetic efficiency compared to their enriched counterparts of equal depth on day 5.

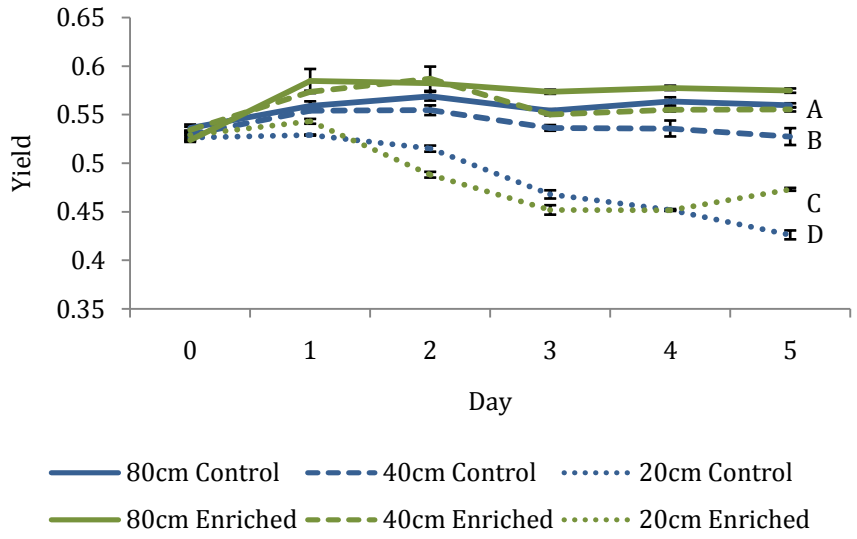

Figure 28. Photosynthetic efficiency (dimensionless ratio) over the duration of the December experiment. Treatments include 20 cm, 40 cm, and 80 cm control, as well as 20 cm, 40 cm, and 80 cm enriched. Treatments with different letters are statistically different (A, B, C, D). Note y-axis does not start at 0.

3.3.5 January 2016

By the end of the experiment, NNN concentrations were depleted to limits of detection in 20 cm and 40 cm enriched mesocosms, and 80 cm mesocosms to a lesser extent (Table 12). The DRP concentrations were all also depleted substantially in 20 cm and 80 cm enriched treatments. The DRP concentrations depleted further in shallow controls when compared with deep control treatments. NH_3-N concentrations depleted across all treatments by the end of this experiment. This indicates that depth of mesocosms may have played a role in influencing the uptake of both NNN and DRP in January.

Table 12. The nutrient concentrations on day 0 of the control treatments, and day 5 of all treatments in January. Treatments include the 20 cm, 40 cm, and 80 cm control, as well as 20 cm, 40 cm, and 80 cm enriched.

Treatment	NNN (mg L^{-1})	DRP (mg L^{-1})	NH_3-N (mg L^{-1})
Day 0 Control	<0.025	0.007	0.159
80cm Control	<0.025	0.006	<0.03
80cm Enriched	0.041	0.004	<0.03
40cm Control	<0.025	0.003	<0.03
40cm Enriched	<0.025	0.036	<0.03
20cm Control	<0.025	<0.001	<0.03
20cm Enriched	<0.025	0.002	<0.03

By day 2 of the experiment, photosynthetic efficiency was significant different between treatment types ($H(5)= 13.88$, $p < 0.05$), with increases in yield in enriched and deep control mesocosms (Figure 29 & Appendix C). The yield of the 20 cm control concentrations reduced by day 2. This trend continued through to the final day of the experiment, which also showed a significant difference between treatments ($H(5)= 15.13$, $p < 0.05$). The general trend for January appears to be greater chlorophyll *a* concentrations in deep mesocosms with nutrient enrichment, and a decline in shallow mesocosms with no nutrient enrichment. Concentrations in enriched mesocosms consistently tended to be greater than controls of the same depth, thought this difference was not statistically significant.

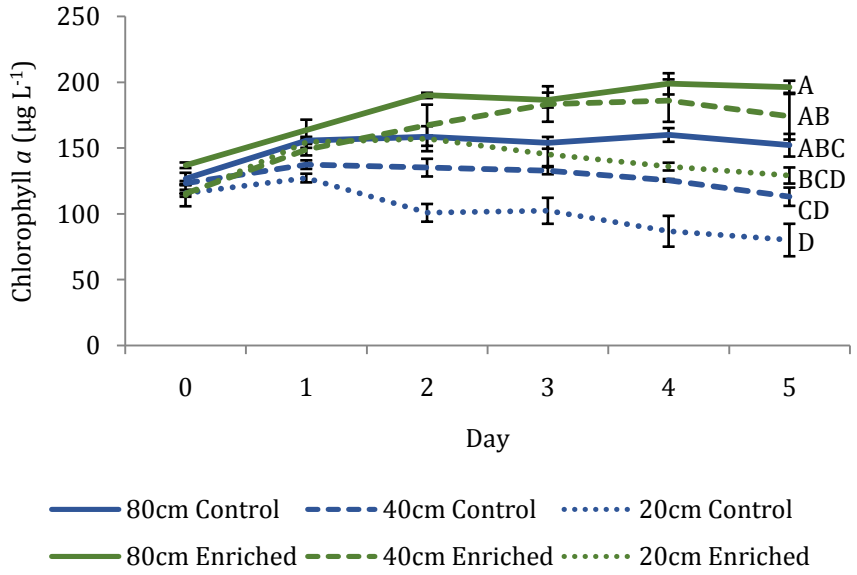

Figure 29. Chlorophyll *a* over the duration of the January experiment. Treatments include the 20 cm, 40 cm, and 80 cm control, as well as 20 cm, 40 cm, and 80 cm enriched. Treatments with different letters are statistically different (A, B, C, D).

There was no significant interactive effect of depth and enrichment on phytoplankton cell density on day 5 ($F(2,12)= 0.40$, $p = 0.679$). Neither depth nor enrichment had a statistically significant effect on phytoplankton density, though the cell densities were generally higher in control mesocosms (Figure 30).

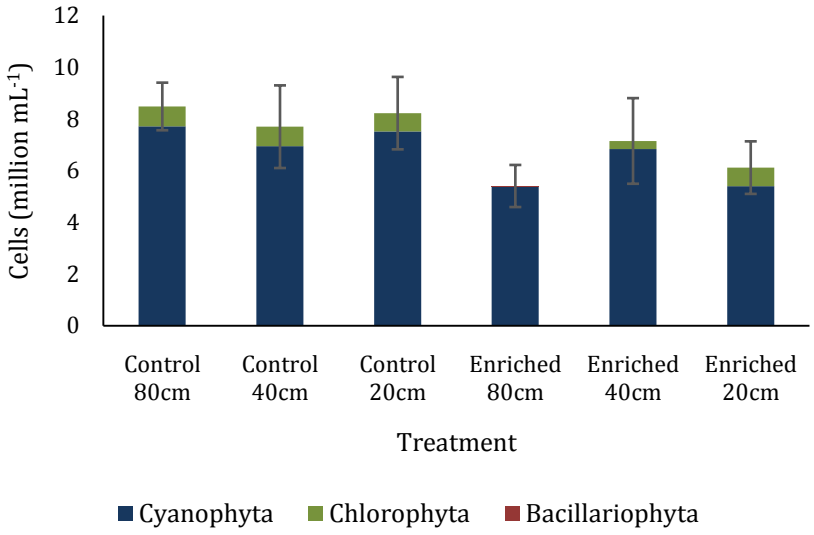

Figure 30. Cell count results of different algae among different treatments on day 5 of the January experiment. All treatments were statistically similar.

Photosynthetic efficiency on day 2 was significantly different between treatment types ($H(5)= 15.13$, $p < 0.05$). Yield in the 20 cm and 40 cm control, and 20 cm enriched conditions had dropped significantly by day 2 (Figure 31 & Appendix D). However, by day 5 of the experiment, there was less disparity between treatments ($H(5)= 13.26$, $p < 0.05$), where two statistically similar groups of treatments had developed. The 20 cm control and 20 cm enriched treatment had a significantly lower yield when compared with the other treatment types ($p < 0.05$). Generally, the photosynthetic efficiency of deeper mesocosms did not appear to change much between days 2 and 5.

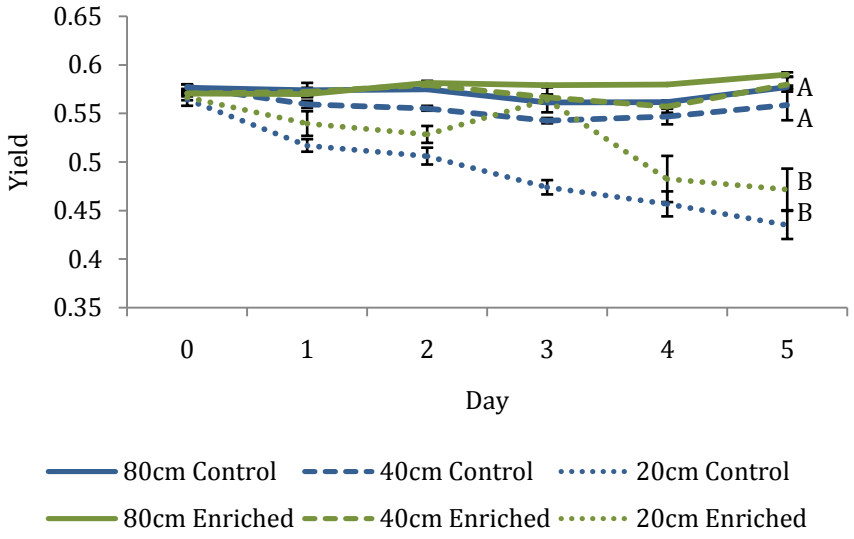

Figure 31. Photosynthetic efficiency (dimensionless ratio) over the duration of the January experiment. Treatments are: 20 cm, 40 cm, and 80 cm Control, as well as 20 cm, 40 cm, and 80 cm Enriched. Treatments with different letters are statistically different (A, B). Note y-axis does not start at 0.

3.4 Light & photosynthesis

The light environment in Te Waihora on February 27th 2016 had average surface PAR of 690 µmol $m^{-2}s^{-1}$, and a down-welling attenuation coefficient of 9.3 m^{-1} (Figure 32). The euphotic depth (1% of surface PAR) was approximately 50 cm. The high-frequency, high-energy 412 nm and 470 nm light attenuated more quickly (attenuation coefficients of 21.5 and 14.9 m^{-1} respectively) than the lower-frequency, lower-energy wavelengths of 532, 566, 624, and 671 nm (attenuation coefficients between 9.4 and 10.3 m^{-1}).

The photosynthesis, respiration and PAR fitted to the following hyperbolic model using least-squares regression:

$PS = Resp + (PS_{max} \times PAR)/(PAR_{50} + PAR)$

whereby, PS_{max} is the maximum rate of photosynthesis,

and PAR_{50} is the PAR at which half of PS_{max} occurs

According to this model, in Te Waihora the respiration rate was -0.192 mg O_2 L^{-1} h^{-1}, the maximum photosynthetic rate was 3.24 mg O_2 L^{-1} h^{-1}, and the PAR at which 50 % of the maximum rate of photosynthesis was 206 µmol $m^{-2}s^{-1}$. The compensation depth was approximately 0.45 m depth (33), the compensation irradiance 10 µmol $m^{-2}s^{-1}$ and the critical depth, assuming the water column was fully mixed, was 5.95 m. Critical depth greatly exceeds actual depth, and an overall net photosynthesis would seem probable.

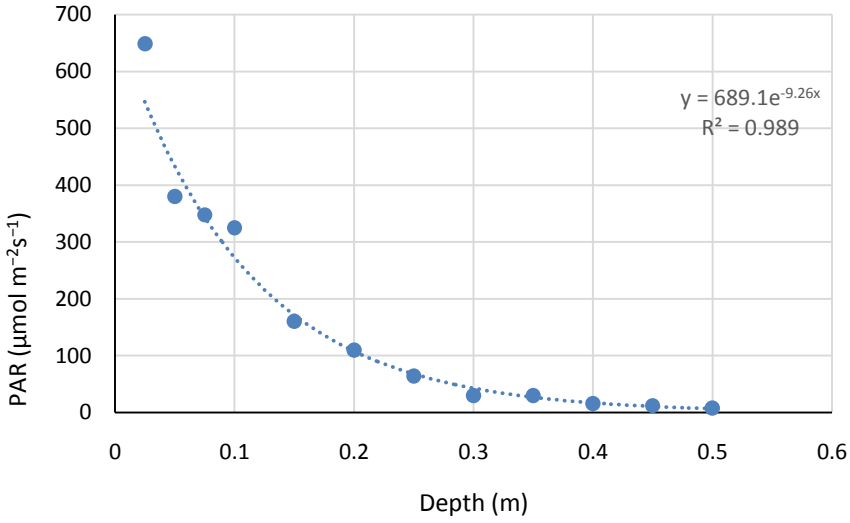

Figure 32. Light attenuation in Te Waihora: Photosynthetically Active Radiation at different depths in the water column.

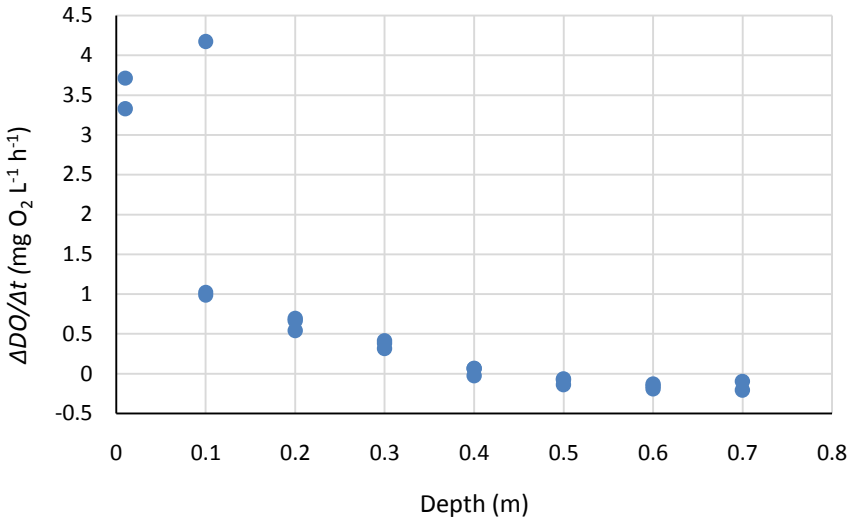

Figure 33. Net photosynthesis at different depths in Te Waihora in February. Where the points reach zero change in dissolved oxygen over time shows the depths at which photosynthesis is equal to respiration (compensation depth).

Photosynthesis in mesocosms

Attenuation of PAR within the mesocosms was exponential (Figure 34), and allowed Kd, and the PAR for each incubation depth, to be calculated. Measured gross photosynthesis typically followed a linear trend with irradiance (Figure 35), which allowed an estimation of respiration rate (photosynthesis at PAR = 0) and compensation irradiance (PAR at PS = 0). The average light at the surface of mesocosms was substantially lower than at the lake. On average, July and January received similar PAR at the surface, while September was lower. In July, rate of photosynthesis was higher than other experiment months, and was the only month where photosynthesis exceeded respiration in the entire 80 cm mesocosms (Table 13). Rate of photosynthesis was lowest, and respiration greatest, in the January experiment, despite having similar average surface PAR and down-welling light attenuation. In the September experiment, average surface PAR was lower than other experimental months, but light attenuation was similar to the lake in February.

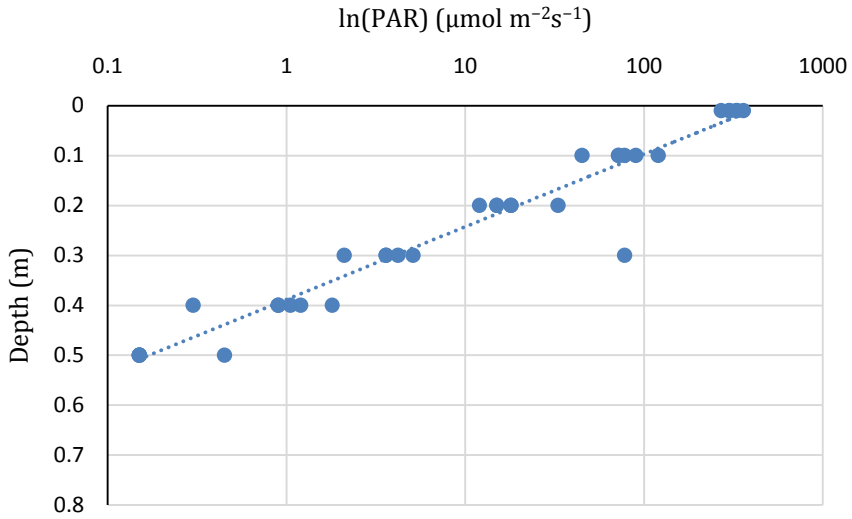

Figure 34. Photosynthetically Active Radiation at different depths in the July experiments. Note logarithmic x scale.

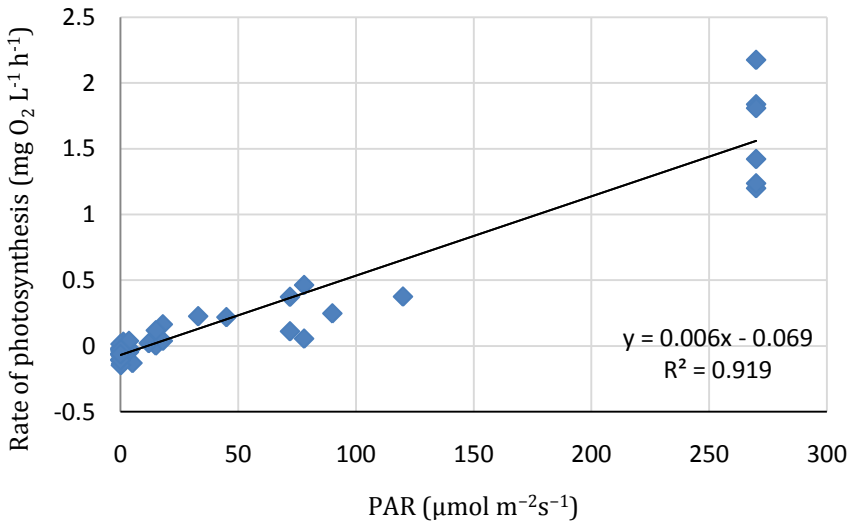

Figure 35. Rate of photosynthesis at different intensities of photosynthetically active radiation in the July experiment.

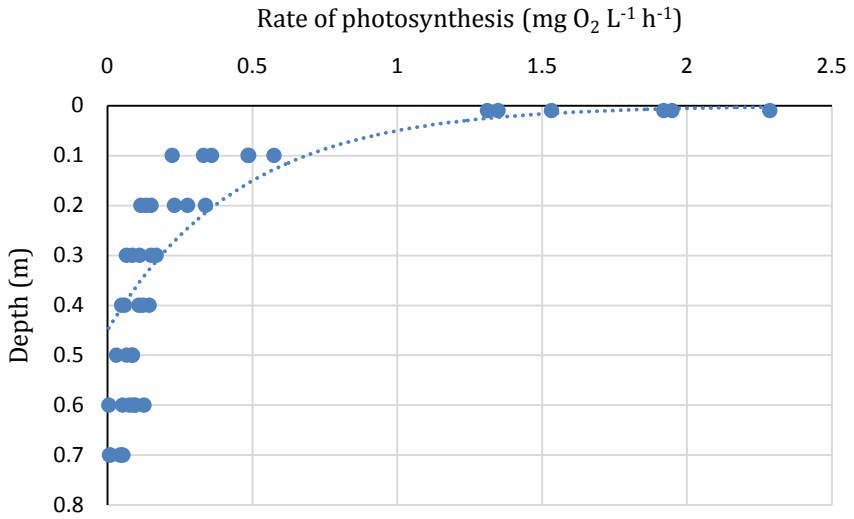

Figure 36. Rate of photosynthesis at different depths in the July experiment.

Table 13. Calculated light and photosynthetic parameters in July 2015, September 2015, and January 2016 in mesocosms, and in Te Waihora for February 2016 for the equivalent of mesocosm volume and depth. Parameters include average surface PAR ($\bar{I}_o$), down-welling light attenuation, rate of photosynthesis (in whole mesocosm), and respiration rate (in whole mesocosm).

Month	$\bar{I}_o$ (µmol $m^{-2}s^{-1}$)	Kd (m^{-1})	Rate of PS (mg hr^{-1})	Respiration (mg hr^{-1})
July 2015	233	14.67	0.69	0.43
September 2015	150	11.49	0.28	0.35
January 2016	244	14.22	0.14	0.69
In lake February 2016	689.1	9.27	1.61	0.11

Chapter 4

Discussion

4.1 Water chemistry/quality in Te Waihora

Water quality parameters in Te Waihora varied temporally, with conductivity ranging from 8.4 to 11.2 mS cm^{-1}, pH from 6.95 to 8.83, and turbidity from 61 to 198 NTU. This range of values is generally consistent with prior studies (Environment Canterbury, unpublished data; Wood, 2008). Schallenberg & Schallenberg (2012) attributed changes in conductivity to saline water intrusions during opening events, and waves over-washing the barrier. pH in the range of near-neutral to alkaline has been found in prior studies (Wood, 2008). Variable turbidity in Te Waihora has been widely reported, and Gerbeaux & Ward (1991) found turbidity was high in winter due to sediment resuspension from high winds, and lower in summer as wind and wave-action reduced. My data do not concur with this seasonality, with low lake turbidity in winter and spring, though the dependence of turbidity on wind events does mean that any given year may not correspond to long term trends. Turbidity tended to decrease over the duration of the experiments, indicating that manual mixing and air bubbling did not prevent sedimentation of some suspended solids. However, even the lowest turbidity values in my experiments can also be found in Te Waihora (Wood, 2008).

Initial nitrate, ammonia and phosphate concentrations in control mesocosms varied from sampling to sampling. A general pattern emerged where one or more nutrients were close to detection limits during sampling, but occasionally very high concentrations did occur. Nitrate in particular was either high (>0.5 mg L^{-1}), or below detection limits (<0.025 mg L^{-1}), with no clear seasonal trend, and DRP was unusually high on one occasion. Concentrations in this study were within the ranges previously found in the lake. For example, Schallenberg et al. (2010) found DRP ranged from 0.002 - 0.058 mgL^{-1}, NNN ranged from 0.005 - 1.000 mg L^{-1}, and NH_3-N ranged from 0.003 - 0.220 mg L^{-1}. The tendency for occasional very high concentrations of nutrients is also evident in Environment Canterbury monitoring data. At Timberyard Point, nitrate peaks above 0.5 mg L^{-1} tend to occur between May and November (Environment Canterbury, unpublished data). The ability of phytoplankton rapidly to respond to and deplete these pulses of nutrients is evident in my data, as initially high nitrate concentrations depleted substantially over the duration of experiments.

Single-celled picocyanobacteria are currently the dominant algae in Te Waihora, and were dominant in all of my experiments. A small colonial *Microcystis* species were also observed,

though were not as abundant. This represents a substantial shift since the 1980's, when Lineham (1983) found *Dictyosphaerium, Oocystis, Planctonema,* and *Microcystis* to be the most dominant phytoplankton genera. At that time cyanophyta were associated with low nitrate and high pH conditions, with *Anabaena* and *Nodularia* forming blooms in summer months (Lomax et al., 2015; Te Waihora Joint Management Plan, 2005). In the more recent past, *Microcystis minutissima* was identified as the most abundant cyanobacteria in Te Waihora (Te Waihora Joint Management Plan, 2005). Two long opening events of 2013 resulted in the shift in algal dominance to picocyanobacteria, which was likely due to extensive disturbance from increased salinity and persistently low lake levels (Lomax et al., 2015). Median salinity in my experiments (9.52 mS cm^{-1}) remained higher than that of the 1990-2000 period (6.4 mS cm^{-1} – Environment Canterbury, unpublished data). There have been no comprehensive taxonomic phytoplankton studies for Te Waihora since 1983.Single-celled marine picocyanobacteria consist of the genera *Prochlorococcus* and *Synechococcus* (Jakubowska & Szelag-Wasielewska, 2015), and freshwater picocyanobacteria are more diverse (Callieri et al., 2012). It is unknown which picocyanobacteria species proliferate in Te Waihora.

4.2 Nutrient limitation in Te Waihora

There were three distinct responses of chlorophyll *a* to various combinations of nutrient additions in the 40 cm mesocosms across all months.

1) Chlorophyll *a* concentrations increased equally in all treatments over the entire period, with no significant differences between treatments. This response suggests that nutrient concentrations were not limiting at that time. This was observed in May, when high initial concentrations of NNN and detectable DRP were observed, and when detectable amounts of both were still present in controls at the end of incubations.

2) An increase in chlorophyll *a* concentrations in +NP treatments throughout the incubations, with a slower increase in nitrate-only treatments. This was the most common response, having occurred in three of the five experimental months (July, September and January), and is taken to indicate primary limitation by nitrogen, with a secondary limitation by phosphorus once nitrogen limitation is satisfied. In most cases where this occurred, NNN was undetectable in controls at the start of the experiments, and remained at detection limits in controls at the end.

3) An equal increase in chlorophyll *a* in the +NP and +P-only addition treatments, which continued throughout the incubation. This was observed only in December, and is indicative of phosphate being the most limiting nutrient. This result was consistent with high concentrations of NNN throughout the December incubations. Surprisingly, DRP was above detection limits in the controls.

The chlorophyll *a* responses in this study began immediately in the 40 cm incubations, and there is no evidence to suggest that nutrient limitation was an artefact of the incubations. My data suggests that Te Waihora phytoplankton is most frequently nitrogen-limited, at times co-limited by both nitrogen and phosphorus or, when ambient nitrate concentrations are high, limited by phosphorus alone. Prior studies based on bioassays and water stoichiochemistry have also suggested nitrogen to be the most limiting nutrient in Te Waihora (Hamilton, 2008; Hawes & Ward, 1996; Schallenberg et al., 2010). High concentrations of dissolved inorganic nitrogen (DIN; NNN and NH_3-N) in Te Waihora have previously been associated with tributary inputs (Hughey & Taylor, 2009). High DIN concentrations have been found in the major tributaries, the Selwyn River and LII (0.44+ mg L^{-1}), and were attributable to the high intensity land use of the catchment (Stevenson et al., 2010). My observations that there is a rapid uptake of nitrate by phytoplankton once it enters the lake are supported by other analyses of nutrient dynamics (Larned & Schallenberg, 2006), which have supported the view that nitrate is the most frequently limiting nutrient. An additional sink for nitrate may be high denitrification rates, though this has not yet been fully studied (Renwick et al., 2010), and would not apply in my mesocosms where an anoxic sediment sink for nitrate was not present.

Bioavailable phosphate concentrations are usually low in Te Waihora, which may be why co-limitation of both nutrients can occur. Enhanced quantum yield in September in +P treatments may also indicate nutrient stress, as this effect has been observed in studies of other phosphorus-limited systems (Harrison & Smith, 2013). Phytoplankton in Te Waihora have been found to be occasionally limited by phosphorus (Lineham, 1983), and high chlorophyll *a* concentrations were associated with high phosphorus concentrations in summer months (Gerbeaux, 1989; Lineham, 1983), though my data suggest that phosphorus limitation alone is rare. DRP concentrations of the Selwyn River and LII can be high (0.009+ and 0.03+ mg L^{-1} respectively) due to effluent, fertiliser, stock access and storm water (Stevenson et al., 2010), though these are not the only contributors. The lake is very shallow, and the geology of the area is volcanic, and these factors may contribute to phosphorus availability (Lomax et al., 2015; Schallenberg, 2004). Internal phosphorus loading is therefore likely to play a role in Te

Waihora. Waters (2016) found high internal phosphorus loading in neighbouring Wairewa (Lake Forsyth), and increases in salinity and pH were associated with DRP release to the water column. The same may be true for Te Waihora. The high DRP loads and DRP release to the water column may explain why the phytoplankton biomass in Te Waihora is predominantly nitrogen limited.

Co-limitation of both phosphorus and nitrogen does occur in New Zealand lakes (33% of lakes studied in Abell et al., 2010), but nitrogen is usually the primarily limiting nutrient (Abell et al., 2010; Schallenberg, 2004). The primary limiting nutrient in other ICOLLs also varies both spatially and temporally. Studies on New South Wales ICOLLs found phytoplankton were limited by nitrogen only, phosphate only, alternating between the two, or co-limited. For example, phytoplankton in Lake Illawara is most likely nitrogen limited (Liu, 2008; Liu et al., 2013). Lakes Coila and Smiths were both limited by phosphorus (Everett et al., 2007; Liu et al., 2013). Liu et al. (2013) showed of the seven ICOLLs studied, five (St Georges Basin, and lakes Burril, Conjola, Durras, and Swan) either alternated between nitrogen and phosphorus limitation, or were co-limited. In a study on Wilson Inlet (Twomey & Thompson, 2001), bioassays provided evidence of nitrogen limitation, however Redfield ratios predicted phosphorus to be the most limiting. It was concluded that Wilson Inlet was mostly nitrogen limited, but was at times co-limited. In the alternating- or co-limitation ICOLLs of New South Wales, higher DRP concentrations appear to lead to nitrogen limitation, and those with lower DRP concentrations are more likely to be phosphorus limited.

There was usually no response of quantum yield (photochemical efficiency) to nutrient additions. Two experiments did not follow this trend, as in July there was an increase in +N treatments, and in September an increase in +P treatments (both of which were predominantly nitrogen-limited). However, across all treatments the quantum yield remained high (0.45+). This means that the capability of phytoplankton to photosynthesise was not inhibited by nutrient limitation in Te Waihora. This is an unusual response, as phytoplankton stress due to nutrient limitation often results in a decline in photochemical quantum yield (Steglich et al., 2001). Parkhill et al. (2001) state that the decrease in PSII quantum yield is dependent on how long the cells had been limited by the nutrient, and also on the growth rate.

Nutrient additions either caused no change, a decline, or an increase, in cell density over different experimental months. Two particular tendencies were evident. Firstly, no change or a decline was observed in cell density with added nutrients while the chlorophyll *a* concentration increased, suggesting cells utilized the nitrogen and/or phosphorus to increase

the concentration of intracellular chlorophyll *a*. Secondly, cell densities increased with nutrient additions while chlorophyll *a* concentrations also increased, which suggests that nutrient additions stimulated reproduction of cells. Halsey et al. (2010) also found variable responses of cell numbers to nitrogen additions, and noted that intracellular chlorophyll *a* concentrations increased with nitrogen additions. It must be noted that cell counts are highly variable, and results may be an artefact of inaccurate counting methodology. In addition, cell counts were only performed on the final day in this study, so any trends over the duration of experiments are unknown, and cell counts rather than cell biovolumes were recorded, whereby the contribution to biomass of many picocyanobacteria, 1-2 µm in diameter, is quite different to that of a few chlorophytes of 10-20 µm diameter.

Across all treatments and experiments, picocyanobacteria had the highest cell densities. Lineham (1983) found cyanophyta in Te Waihora were abundant in low nitrogen, high pH conditions. This may also be the case today, with nitrogen limitation allowing picocyanobacteria to proliferate. Single-celled picocyanobacteria dominated all cell counts in these experiments, although experiments may not have run long enough for any observable community shifts to take place. Wehr (1989) concluded that picoplankton hold a competitive advantage over larger species in nutrient limiting circumstances. Picoplankton have a high surface area to volume ratio, potentially giving them a higher intake of nutrients across the cell membrane (Maranon, 2009). Timmermans et al. (2005) found picoplankton were able to maintain growth when phosphate or ammonia were limiting, because they have such low nutrient requirements. Additionally, some cyanobacteria are able to supplement nitrogen limitation through phycocyanin degradation and nitrogen fixation (Allen & Hutchison, 1980). *Synechococcus* can also utilize organic nutrients in the water column (Donald et al., 1997) Likewise, single-celled freshwater picocyanobacteria are more likely to be found in oligotrophic lakes (Irwin et al., 2006), with colonial varieties more common in shallow eutrophic lakes (Callieri et al., 2012).

It is at first glance counter-intuitive that, despite the "eutrophic" status of Te Waihora, picocyanobacteria dominance may persist in a frequent state of nitrogen and, at times, phosphorus limitation. It is, however, important to distinguish between total nitrogen and phosphorus concentrations, the metrics typically used to calculate trophic indices (Burns et al. 2000), and available nitrogen and phosphorus for new growth. Where nitrogen or phosphorus is already tied up as organic material, or in the case of phosphorus potentially bound tightly to

suspended sediments (Schallenberg, 2004), then the availability of new nutrients for new growth can be low.

4.3 Light limitation in Te Waihora

In addition to nutrients, the high turbidity of shallow, wind-swept ICOLLs is also often implicated in controlling phytoplankton growth. The accumulation and resuspension of fine sediments, and associated high light attenuation, in Te Waihora has frequently been thought to limit phytoplankton growth (Gerbeaux and Ward 1991; Schallenberg et al., 2010). The euphotic depth of Te Waihora that was estimated in this study was 0.5 m, which is within the range of 0.3 – 0.5 m normally found in the lake (Gerbeaux & Ward, 1991; Schallenberg et al., 2010). The estimated critical mixing depth of Te Waihora was 0.6 m in February 2016. Te Waihora has an average depth of only 1.4 m (Hughey et al., 2013). This means that with sufficient vertical mixing and sufficient suspended solid concentrations (Gerbeaux, 1989), as is common in Te Waihora, phytoplankton can be expected to be severely light limited due to the long time spent in darkness or below the compensation irradiance. However, in calmer weather, when resuspended sediment concentrations are lower (Gerbeaux, 1989), the mixing depth can exceed the critical depth, resulting in a possibility of net growth of phytoplankton. The proportion of lake depth to critical mixing depth changes spatially and temporally in the lake. Net productivity, and therefore growth, will not occur evenly throughout the lake. In the shallow margins, net productivity will occur, but in the deeper parts of the lake (maximum depth 2.5 m, Gerbeaux & Ward, 1991) there may be no net productivity when mixing reaches the lakebed. Likewise net production may be possible during short periods of time, and cells may need to tolerate prolonged unfavourable conditions before growth may again be possible. Thus the interaction between light and nutrient limitation of growth becomes an important consideration for Te Waihora phytoplankton.

My results suggest that, of the 80 cm mesocosms in July, September, and January experiments, net photosynthesis on a whole water column basis was possible only in July. This means that light was likely to have significantly impeded the growth of phytoplankton in September and January. While the photosynthetic parameters of the populations growing in shorter tubes was not measured, for logistic reasons, if the photosynthetic performance of cells in the 80 cm tubes, is used to estimate the potential for light availability to impede growth in shorter columns, it appears that growth would be possible in 40 cm and 20 cm

mesocosms in September, and 20 cm mesocosms in January. The ability of 40 and 20 cm mesocosms to respond to nutrient enrichment in those months, when the 80 cm columns did not, is consistent with these observations of insufficient light to allow net growth in longest mesocosms.

The photosynthetic efficiency of phytoplankton was stable over the duration of the experiments in the deeper mesocosms, but decreased substantially in the shallow mesocosms in both control and enriched treatments. It was universal for yield to decline over time in the short mesocosms. To understand this it is necessary to consider the physiological basis of yield estimations, whereby the value returned is dependent on the immediate prior light history of the cells (Schreiber, 2004). The enhanced ambient irradiance in the short mesocosms is likely to be leading to a slight reduction in quantum yield. However, the small reduction in quantum yield compared to 40 cm mesocosms is likely to be offset by the substantial increase in irradiance in the 20 cm tubes, since the actual rate of photosynthetic activity is given by the product of irradiance and quantum yield (Schreiber 2004).

When the multiple nutrient addition experiments in 40 cm mesocosm experiments were considered above, the only occasion on which chlorophyll *a* was able to increase without any nutrient enrichment was in May. In that month, responses of control treatments in the three lengths of mesocosm were essentially similar – a small increase from 250 – 300 μg L^{-1}. The absence of a significant effect of irradiance on controls suggests that this was not limiting to growth, though it was clear that the amount of available nitrate that was consumed increased as mesocosms depth declined, suggesting that the time until nutrient limitation began was affected by irradiance. When nutrients were added to the mesocosms in May, while no stimulation was observed within 5 days of incubation in 40 and 80 cm mesocosms, cell counts increased suggesting growth did occur. However, a rapid chlorophyll *a* response was evident in the short mesocosms. Together these observations suggest that the ability to deplete nutrients to limiting concentrations depended on the light received.

For all other sets of combined light plus nutrient incubations, control chlorophyll *a* concentrations did not greatly increase over the duration of experiments, and in some shallow mesocosms there was a decrease. There were two distinct responses of chlorophyll *a* to enrichment: 1) an increase in shallow enriched treatments while deeper ones remained rather constant (July & December 2015), and 2) an initial increase in all enriched treatments, followed by a decline in shallow enriched treatments, while deeper ones again remained constant (September 2015 & January 2016). In every case, the trajectory of enriched

treatments involved higher concentrations of chlorophyll *a* than respective controls. Declining chlorophyll *a* concentrations in controls is consistent with a reduced cell pigment content, as there was no obvious trend in cell density, which may be expected to accompany photoacclimation to increased irradiance in the short mesocosms. These results do suggest, however, that increasing the irradiance in Te Waihora water, for example through interventions that enhanced water clarity, would only allow an enhancement of phytoplanktonic chlorophyll *a* if nutrient availability were also to increase. An increase in nutrient concentrations could allow an increased biomass to accumulate. The worst combination for phytoplankton chlorophyll *a* would appear to be a reduction in clarity and an increase in nutrients. This is partially consistent with inferences of Gerbeaux (1989), who noted increased chlorophyll *a* concentrations during low lake levels, but my findings suggest that this would not usually occur if nutrient limitations were not also relieved.

The light and nutrient addition experiments suggest that increased light availability can quickly lead to nutrient limitation of phytoplankton biomass in Te Waihora. It was previously proposed that there was no seasonal variability in chlorophyll *a* due to alternating nutrient and light limitations. This hypothesis suggested that: 1) in winter, phytoplankton in Te Waihora is primarily light limited, as wind-induced resuspension of sediments contracts the euphotic depth, and 2) in summer, wind-induced mixing is reduced, light availability increases, and phytoplankton could become primarily nutrient-limited (Hawes & Ward, 1996). However, this study suggests that the processes of light and nutrient limitation may not be so straightforward. Experimental evidence suggests that light limitation occurred in winter as well as summer months (May, July and December). Of these, July and December showed no change in chlorophyll *a* concentrations in mesocosms where more light was available but nutrients were not added. This means that either 1) phytoplankton in Te Waihora can be co-limited by both light and nutrients, or 2) the nutrient limitation observed in control mesocosms was an artefact of the experiment design, if water used lacked the natural supply of nutrients from sediments. Chlorophyll *a* concentrations have been known to increase with sediment resuspension events in Te Waihora, due to this increase in available nutrients (Gerbeaux, 1989). It is a common phenomenon in shallow systems (Hansen et al., 1997; Søndergaard et al., 1992).

In two light limitation experiments (September & July), the chlorophyll *a* concentrations were higher in deeper mesocosms, and reduced in shallow mesocosms. This may be due to: 1) phytoplankton cells increasing (in deeper mesocosms) or reducing (in shallower mesocosms)

in chlorophyll *a* content in order to adapt to the imposed light regimes, 2) the phytoplankton community composition shifting from smaller to larger cells, thereby increasing chlorophyll *a* concentrations, or 3) cellular storage of nitrogen in the form of chlorophyll. Kasprzak et al. (2008) and Vidal et al. (2007) note that chlorophyll *a* concentrations are usually lower in smaller cells, so there may have been a community composition shift from smaller to larger cells in the deep mesocosms. Additionally, some studies have found carbon to chlorophyll *a* ratios decreased with decreasing light (Taylor et al., 1997; Wang et al., 2009).

A significant caveat of my experiments is, as mentioned above, that chlorophyll *a* is not necessarily an accurate measure of biomass. Phytoplankton are known to adapt to light environments by changing their cellular concentrations of chlorophyll *a*. In particular, phytoplankton increase intracellular chlorophyll *a* when exposed to low light climates. Nitrate is also known to stimulate intracellular chlorophyll *a* production. This means that chlorophyll *a*, which is a commonly included in water quality indicators for monitoring and lake profiling, may overestimate the biomass of phytoplankton in systems with low light or high nitrate, or underestimate biomass where high light and low nitrate conditions occur. Further investigation is therefore required to evaluate the usefulness of chlorophyll *a* as a proxy for biomass in Te Waihora.

Single-celled picocyanobacteria was the dominant algae in all treatments across all experiments. Morris & Glover (1981) found restrictive light environments can contribute to the prevalence of *Synechococcus*, which are most productive under light intensities as low as 45 μmol photon m^{-2} s^{-1}. Wehr (1993) states that low-light adapted picocyanobacteria, such as *Synechococcus*, also have a competitive advantage during nutrient limitation. Maranon (2009) notes that smaller cells are more efficient at absorbing light because here is less self-shading of pigments within smaller cells than from within larger cells. Accessory pigments such as phycoerythrin and phycocyanin can aid in photosynthesis, allowing the absorption of green to blue-green, and yellow to red wavelengths, allowing efficient use of available light energy (Caroppo, 2015). Caroppo (2015) highlights that picocyanobacteria are likely more productive under lower irradiances due to sensitivity to photodamage. In a lake with severe light limitation such as Te Waihora, picocyanobacteria may have a competitive advantage over other phytoplankton. The coupled low light environment and frequent nutrient limitation in Te Waihora may be why picocyanobacteria have continued to dominate.

4.4 Management of Te Waihora

At present, Te Waihora is characterised as a hypertrophic ICOLL, and is a highly turbid, high-pH, saline ecosystem, with high total N and P, substantial nutrient and sediment loading from an agricultural catchment and dominated by picoplanktonic cyanobacteria. It is a physically diverse ecosystem due to its nature as a transition zone between fresh and marine waters. This is also the case for other ICOLLs, and is generally reflected in the water quality and chemistry parameters, where horizontal and vertical mixing creates temporal variability of physico-chemical properties (Hawden & Arthington, 2006).

Historically, the margins of the lake were quite different with extensive beds of rooted macrophytes and associated clear water (Gerbeaux, 1989). There is much interest in returning Te Waihora to a clear-water, macrophyte-dominated ICOLL. Management approaches need to be tailored to suit both the specific system and the main objectives set for recovery and restoration. The main objectives for restoration of Te Waihora include: reducing phytoplankton biomass, increasing water transparency, and restoration of macrophytes to the lake (Mahaanui Iwi Management Plan, 2013; Te Waihora Joint Management Plan, 2005). It is therefore important to determine the extent to which phytoplankton growth and biomass is reliant on light and nutrient availability, and whether reduction of phytoplankton may be most likely achieved through these key factors. However, a reduction in light availability is contradictory to the key objective of increasing water transparency and management to reduce phytoplankton therefore need to focus on reducing nutrients in Te Waihora at the same time as increasing lake water transparency.

My results confirm that phytoplankton appear to be nutrient limited most of the time, and provide some reassurance that, while light appears to play a major role in the productivity of phytoplankton, an increase in water clarity without an increase in nutrient availability may not lead to intensification of chlorophyll *a* concentration and algal blooms. Bioassays are not, however, necessarily informative of the overall response of the lake, since they deliberately focus on a single element of the lake ecosystem. My experiments deliberately removed large zooplankton and sediments, and both have a role in phytoplankton growth through nutrients, grazing and sediment suspension/settling. At best my experiments show that nitrogen is often available in concentrations that are limiting to amount of cells, and that at the same time light can be limiting to the rate of activity, photosynthesis and potential growth.

There has been much debate on single-nutrient control versus dual nutrient control (Wang & Wang, 2009). The merits of single-nutrient control has been arguable, and has been found inadequate to return eutrophic lakes to a clear, macrophyte-dominated state (Schindler et al., 2008). Nitrogen and phosphorus limitations can alternate both spatially and temporally in freshwater lakes (Davies et al., 2004; Kolzau et al., 2014), and coastal lakes and lagoons (Liu et al., 2013; this study). Although my results suggest that nitrogen is the most limiting nutrient, except when pulses of nitrate have entered the lake, reduced overall nitrogen loads from the Te Waihora catchment from 1993 – 2007 did not reduce overall phytoplankton biomass (Hughey et al., 2013). This is likely to be because phytoplankton are also light limited much of the time. While it is unlikely that the picocyanobacteria in Te Waihora are capable of nitrogen fixation, future developments could allow the return of colonial nitrogen fixers, such as *Nodularia* that historically were common (Lineham, 1983). Reducing the concentrations of both nitrogen and phosphorus may therefore be necessary to elicit a response in Te Waihora. Dual nutrient control is also important for receiving marine waters, as single-nutrient control can exacerbate eutrophication downstream where the alternative nutrient may be most limiting (Paerl, 2009).

Limiting nutrient loading from the catchment has been successful in numerous river, lake, and transitional waters. In lakes, reduced nutrient loads can lead to reduced phytoplankton biomass and chlorophyll *a*, and changes in phytoplankton bloom dynamics (Jeppesen et al., 2005; Philips et al., 2005). However, in some cases nutrient load reductions were not enough to return the system to a clear-water state (Jeppesen et al., 2007). Some systems can take years to recover from historical nutrient loads. Internal cycling of phosphate can delay recovery by up to 10 to 15 years after reduction of phosphorus loading, and recovery after nitrogen load reductions can take up to five years (Jeppesen et al., 2005). Upwelling groundwater with high nitrate concentrations is the source of many tributaries leading to Te Waihora (Lomax et al., 2015). The lag time between nitrogen leaching to groundwater, and upwelling into tributaries is thought to be decades. Any improvement in catchment-wide nitrogen controls will likely have a large lag in responses in the lake in Te Waihora.

Alternative, internal management options for lakes include physicochemical alum-, iron-, or calcium-capping, or dredging of sediments, oxygenation of bottom waters, or biomanipulation. For example, iron capping works by binding phosphorus in the sediments, and has been successful in reducing chlorophyll *a* concentrations (Bakker et al., 2016). Dredging of sediments can remove the main source of internal phosphorus and reduce organic

matter remineralisation (Zhang et al., 2010). Dredging of sediments can remove the main source of internal phosphorus and reduce organic matter remineralisation (Zhang et al., 2010). Hypolimnetic oxygenation, which works by preventing anoxic conditions as the sediment-water interface, can reduce ammonia, phosphorus, and chlorophyll a concentrations (Beutel & Horne, 1999). However, some of these management actions have been criticized as not being successful, or cost-effective in large, shallow and polymictic lakes (Beutel & Horne, 1999; Kuha et al., 2016; Liboriussen et al., 2009). However, directly influencing nutrient concentrations in these systems has been most successful where reducing nutrient loading from the catchment has also been employed as a management strategy (Bakker et al., 2016).

Biomanipulation of higher trophic levels can lead to flow-on effects down the food chain, reducing the biomass of phytoplankton. For example, the removal of planktivorous fish and addition of piscivorous fish in a shallow prairie lake led to a dramatic decrease in chlorophyll *a* concentrations due to increased zooplankton populations, which reduced turbidity and allowed macrophytes to flourish (Hanson & Butler, 1990). This has been observed in many lake case studies, with variable success (Hanson & Butler, 1990; Søndergaard et al., 2007). Biomanipulation would not likely be successful in Te Waihora as planktivorous fish are a minor component of the resident fish community (Jellyman et al., 2009).

Restoration of Te Waihora to a clear-water state would require a reduction in suspended solids, which contributes 80% of the turbidity (Gerbeaux, 1991). Inorganic suspended solids can potentially be managed directly by reducing the load from the catchment, increasing the depth of the ICOLL, and/or restoring the macrophyte beds. Reduced suspended solid loading from the catchment can be achieved through the addition of riparian buffer zones or constructed wetlands for tributaries, both of which can also reduce nutrient loading. Constructed wetlands are usually designed to effectively remove suspended solids (both abiotic and biotic) and particle-bound phosphorus (Dunne et al., 2012). As vegetation grows it can be removed, which permanently removes phosphorus and nitrogen from the system (Martin et al., 2013).

Another way to reduce suspended solid in the water column would be to increase the depth of the water, which would in turn potentially reduce resuspension of sediments. This can be especially applicable to managed ICOLLs, which are often opened to the sea once they reach an allotted depth. Historically Te Waihora was twice as deep during the closed phase as the current trigger level for opening used by Environment Canterbury for lake openings (Hemmigsen, 1997). Lake openings would naturally occur where the lake reaches

approximately 2.7 - 3.6 m above mean sea level (Gerbeaux, 1989). Increasing lake depth may reduce the effects of wind forcing by reducing sediment shear stress, and potentially aide the return to a clear water state. However, this may prove a contentious issue in Te Waihora, as openings occur to stop flooding of surrounding agricultural and residential land, and may negatively impact foreshore vegetation values (Te Waihora Joint Management Plan, 2005).

The re-establishment of macrophyte beds in Te Waihora is both an objective and a potential tool for restoration. Currently macrophytes are restricted to sheltered embayments. Macrophytes are associated with clear-water states as they provide stability for sediment beds by preventing the resuspension of sediments. They also compete with phytoplankton for resources, can reduce phytoplankton via allelopathy (Vanderstukken et al., 2011), provide habitat for macroinvertebrates and fish species, and provide refugia for young fish from piscivorous fish (Jellyman et al., 2009).

The main issues restricting macrophyte growth in Te Waihora are light availability, potentially unviable seed bank (Gerbeaux, 1993), and dewatering (Jellyman et al., 2009). The euphotic depth in Te Waihora is very shallow in comparison to other lakes where macrophytes are dominant, and light is likely to be too limiting for seed germination. However, the germination experiments showed limited seed viability (Gerbeaux, 1993). Light limitation, sediment shear stress, and water level fluctuations would likely make planting of adult macrophytes a non-practical option.

An increased, stable lake depth would be beneficial for macrophytes. Lakebeds may be more stable or at less risk of being uprooted by prevailing winds, and would make macrophytes less accessible by grazing waterfowl (Jellyman et al., 2009). The current managed opening regime with lower and more frequently fluctuating water levels has a negative impact on macrophytes, due to dewatering and desiccation (Jellyman et al., 2009; Robertson & Funnell, 2012). The rise in salinity during opening events was also found to inhibit the growth of *Ruppia* species, and was associated with a decline of *Ruppia* in Waituna Lagoon of Te Waihora from 2008 - 2011 (Gerbeaux, 1989; Robertson & Funnell, 2012).

Two tools, which may successfully provide refugia for macrophyte growth, are wave barriers (currently being implemented) and the planting of artificial macrophytes. Wave barriers work by reducing wave action and in turn reducing resuspension of sediments. In 2016, a 100 m long wave barrier was constructed and installed in Te Waihora (Whakaora Te Waihora, 2016). Artificial macrophytes could potentially work in a similar way, by reducing the shear

stress on the sediment bed and reduce turbidity, allowing natural macrophytes to grow. Studies have found artificial macrophytes create refugia for macroinvertebrates (Gerrish & Bristow, 1979) and fish species (Jenkins & Sutherland, 1997; Santos et al., 2011), which could have flow-on effects to lower trophic levels, promoting top-down control of phytoplankton biomass. This has not been studied as a macrophyte restoration tool in Te Waihora.

4.5 Conclusions

Phytoplankton growth was frequently limited by nitrogen and occasionally limited by phosphorus in Te Waihora. Phosphorus-only limitation occurred when ambient nitrate concentrations were high. Phytoplankton responded rapidly to nutrients in these experiments, which contributes to the evidence that nitrate entering the lake via tributaries is quickly utilised by phytoplankton. Although phytoplankton were frequently limited by nutrients, there was no evidence of nutrient stress when photochemical efficiency was assessed.

High denitrification rates acting as an additional sink may occur in Te Waihora, which has not yet been explored, and could lead to phytoplankton nutrient limitation. In addition, measuring rates of dissolved reactive phosphorus release at the sediment-water column interface and through sediment resuspension would contribute significantly to the understanding of nutrient cycling, and the availability of nutrients to phytoplankton.

Phytoplankton growth in Te Waihora is likely to be severely light limited due to the shallow critical depth. With sufficient mixing, phytoplankton would spend a substantial amount of time in darkness. It is assumed that net growth would occur in the lake during calm weather, and around the lake margins at points where the depth of the lake is shallower than the critical depth.

The results of the nutrient and light experiments showed that phytoplankton biomass did not increase with increasing exposure to light in the absence of additional nutrients. However, in a low light environment with enrichment, there was a marked increase in phytoplankton biomass. This means that management actions employed to increase water clarity may not have the negative unintended consequence of increasing phytoplankton growth. Also, single-celled picocyanobacteria were dominant in Te Waihora, and persisted through nutrient and light limitation and additions in experiments. Prior studies have found picocyanobacteria are

able to adapt well to shade and low nutrient environments, which may account for their dominance.

A major caveat of this study was that chlorophyll *a* may not be an accurate measure of phytoplankton biomass. Phytoplankton can adapt to a variety of light regimes and nutrient availabilities by increasing or reducing intracellular chlorophyll *a* concentrations. Future research is recommended on the consistency of chlorophyll *a* as a measure of biomass in Te Waihora. In addition, cell counts instead of cell biovolumes were used in this research. A more quantitative approach is recommended in future studies.

Management actions for the reduction of phytoplankton in Te Waihora need to focus on both light and nutrients. Dual nutrient control across the entire catchment is recommended, as potentially toxic cyanobacteria may persist if only nitrogen is managed. Internal management of nutrients is not likely to be practical in Te Waihora due it its size and the expense. Reducing sediment resuspension via reduced wave action or increasing the depth of the water column may reduce internal nutrient loading, and therefore reduce phytoplankton biomass.

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Appendix A: Chlorophyll *a* in Bioassay Experiments

Chlorophyll *a* results for different treatment types on days 2 and 5 of each experimental month in the nutrient addition bioassay experiments. Treatment types are: control, nitrate addition (+N), phosphate addition (+P), and combined addition of nitrate and phosphate (+NP). Statistical groupings denoting significance of different treatment types are also shown ($\alpha = 0.05$).

Treatment	Mean (SD) ($\mu g\ L^{-1}$)	ANOVA Groups	Mean (SD) ($\mu g\ L^{-1}$)	ANOVA Groups
May	**Day 2**		**Day 5**	
Control	256 (30)	A	362 (24)	A
+N	261 (32)	A	385 (21)	A
+P	320 (29)	A	466 (95)	A
+NP	273 (18)	A	446 (51)	A
July 2015				
Control	230 (19)	B	200 (20)	C
+N	254 (24)	AB	301 (37)	B
+P	238 (17)	B	199 (16)	C
+NP	291 (2)	A	450 (40)	A
September 2015				
Control	168 (13)	B	123 (15)	C
+N	196 (17)	B	172 (27)	AB
+P	190 (17)	B	136 (14)	BC
+NP	243 (11)	A	214 (15)	A
December 2015				
Control	127 (9.40)	B	127 (21)	B
+N	126 (1)	B	135 (7)	B
+P	157 (17)	AB	210 (25)	A
+NP	180 (16)	A	245 (46)	A
January 2015				
Control	135 (12)	AB	113 (12)	B
+N	138 (6)	AB	152 (14)	AB
+P	120 (7)	B	108 (5)	B
+NP	167 (27)	A	174 (31)	A

Appendix B: Yield in Bioassay Experiments

Yield (dimensionless ratio) results for different treatment types on days 2 and 5 of each experimental month in the nutrient addition bioassay experiments. Treatment types are: control, nitrate addition (+N), phosphate addition (+P), and combined addition of nitrate and phosphate (+NP). Statistical groupings denoting significance of different treatment types are also shown (α = 0.05).

Treatment	Mean (SD)	ANOVA Groups	Mean (SD)	ANOVA Groups
May	**Day 2**		**Day 5**	
Control	0.58 (0.00)	A	0.57 (0.01)	A
+N	0.58 (0.00)	A	0.57 (0.01)	A
+P	0.59 (0.00)	A	0.57 (0.02)	A
+NP	0.59 (0.01)	A	0.58 (0.01)	A
July 2015				
Control	0.54 (0.01)	AB	0.55 (0.00)	AB
+N	0.57 (0.01)	C	0.45 (0.01)	A
+P	0.54 (0.00)	AB	0.51 (0.01)	AB
+NP	0.56 (0.01)	BC	0.57 (0.00)	B
September 2015				
Control	0.47 (0.01)	A	0.54 (0.01)	A
+N	0.49 (0.01)	AB	0.51 (0.01)	AB
+P	0.50 (0.01)	AB	0.43 (0.05)	B
+NP	0.53 (0.01)	B	0.53 (0.01)	A
December 2015				
Control	0.55 (0.01)	A	0.53 (0.02)	A
+N	0.56 (0.01)	A	0.53 (0.02)	A
+P	0.56 (0.02)	A	0.53 (0.01)	A
+NP	0.59 (0.00)	A	0.56 (0.00)	A
January 2015				
Control	0.56 (0.00)	A	0.56 (0.03)	A
+N	0.57 (0.01)	A	0.56 (0.01)	A
+P	0.56 (0.01)	A	0.54 (0.01)	A
+NP	0.58 (0.01)	A	0.58 (0.01)	A

Appendix C: Chlorophyll *a* in Nutrient vs. Depth Experiments

Chlorophyll *a* results for different treatment types on days 2 and 5 of each experimental month in the nutrients versus depth experiments. Treatments types are: 20 cm, 40 cm, 80 cm control, and 20 cm, 40 cm, and 80 cm enriched (with nitrate and phosphate). Statistical groupings denoting significance of different treatment types are also shown ($\alpha = 0.05$).

Treatment	Mean (SD) ($\mu g\ L^{-1}$)	ANOVA Groups	Mean (SD) ($\mu g\ L^{-1}$)	ANOVA Groups
May	**Day 2**		**Day 5**	
80cm Control	275 (30)	A	318 (19)	C
80cm Enriched	267 (23)	A	368 (5)	BC
40cm Control	256 (30)	A	362 (24)	BC
40cm Enriched	273 (18)	A	446 (51)	B
20cm Control	280 (26)	A	346 (63)	BC
20cm Enriched	361 (8)	A	797 (53)	A
July	**Day 2**		**Day 5**	
80cm Control	285 (38)	AB	256 (42)	C
80cm Enriched	288 (36)	AB	398 (6)	B
40cm Control	230 (19)	B	200 (20)	C
40cm Enriched	291 (2)	AB	450 (40)	B
20cm Control	240 (4)	B	179 (8)	C
20cm Enriched	320 (19)	A	604 (67)	A
September	**Day 2**		**Day 5**	
80cm Control	202 (20)	B	180 (11)	B
80cm Enriched	238 (10)	A	240 (10)	A
40cm Control	168 (13)	C	115 (0)	CD
40cm Enriched	243 (11)	A	214 (15)	A
20cm Control	147 (3)	C	90 (4)	D
20cm Enriched	239 (12)	A	140 (13)	C
December	**Day 2**		**Day 5**	
80cm Control	126 (0.708)	C	123 (5)	CD
80cm Enriched	151 (9.44)	BC	187 (30)	BC
40cm Control	127 (9.40)	C	127 (21)	CD
40cm Enriched	180 (15.71)	AB	245 (46)	B
20cm Control	119 (14.2)	C	101 (11)	D
20cm Enriched	206 (12.86)	A	372 (34)	A
January	**Day 2**		**Day 5**	
80cm Control	159 (1.475)	AB	152 (15)	ABC
80cm Enriched	190 (3.51)	A	196 (9)	A
40cm Control	135 (11.61)	BC	113 (12)	CD
40cm Enriched	167 (27.1)	AB	174 (31)	AB
20cm Control	101 (11.65)	C	80 (21)	D
20cm Enriched	157 (16.42)	AB	129 (11)	BCD

Appendix D: Yield in Nutrient vs. Depth Experiments

Table 2. Yield (dimensionless ratio) results for different treatment types on days 2 and 5 of each experimental month in the nutrients versus depth experiments. Treatments types are: 20 cm, 40 cm, 80 cm control, and 20 cm, 40 cm, and 80 cm enriched (with nitrate and phosphate). Statistical groupings denoting significance of different treatment types are also shown (α = 0.05).

Treatment	Mean (SD)	ANOVA Groups	Mean (SD)	ANOVA Groups
May	**Day 2**		**Day 5**	
80cm Control	0.58 (0.00)	AB	0.57 (0.00)	A
80cm Enriched	0.57 (0.00)	AB	0.57 (0.00)	A
40cm Control	0.58 (0.00)	A	0.57 (0.01)	A
40cm Enriched	0.59 (0.00)	A	0.58 (0.01)	A
20cm Control	0.54 (0.01)	C	0.47 (0.06)	B
20cm Enriched	0.56 (0.01)	BC	0.20 (0.06)	C
July				
80cm Control	0.56 (0.01)	ABC	0.55 (0.00)	A
80cm Enriched	0.57 (0.00)	A	0.57 (0.00)	A
40cm Control	0.54 (0.01)	BC	0.51 (0.01)	AB
40cm Enriched	0.56 (0.01)	AB	0.54 (0.01)	A
20cm Control	0.51 (0.00)	D	0.45 (0.01)	BC
20cm Enriched	0.53 (0.02)	CD	0.43 (0.05)	C
September				
80cm Control	0.50 (0.01)	B	0.54 (0.01)	A
80cm Enriched	0.54 (0.00)	A	0.56 (0.00)	A
40cm Control	0.47 (0.01)	C	0.50 (0.02)	B
40cm Enriched	0.53 (0.01)	A	0.53 (0.01)	AB
20cm Control	0.43 (0.01)	D	0.38 (0.03)	D
20cm Enriched	0.48 (0.01)	BC	0.43 (0.00)	C
December				
80cm Control	0.57 (0.01)	AB	0.56 (0.00)	A
80cm Enriched	0.58 (0.00)	AB	0.57 (0.00)	A
40cm Control	0.55 (0.01)	B	0.53 (0.02)	B
40cm Enriched	0.59 (0.02)	A	0.56 (0.00)	A
20cm Control	0.52 (0.01)	C	0.43 (0.01)	D
20cm Enriched	0.49 (0.01)	C	0.47 (0.00)	C
January				
80cm Control	0.57 (0.00)	AB	0.58 (0.00)	A
80cm Enriched	0.58 (0.00)	A	0.59 (0.00)	A
40cm Control	0.56 (0.00)	B	0.56 (0.03)	A
40cm Enriched	0.58 (0.01)	AB	0.58 (0.01)	A
20cm Control	0.51 (0.02)	C	0.44 (0.03)	B
20cm Enriched	0.53 (0.02)	C	0.47 (0.04)	B