Gippsland Lakes & Catchment Taskforce

Nutrient Cycling and Phytoplankton population Dynamics in the Gippsland Lakes

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Executive Summary

This study was initiated to monitor the factors controlling phytoplankton growth and nutrient recycling following an unprecedented input of nitrogen and subsequent cyanobacterial bloom (*Synechococcus* spp.) in 2007-2008. We had hypothesised that the small cell size of the *Synechococcus* bloom led to a dominance of water column recycling of nitrogen over benthic recycling, hence reducing denitrification, which is the major nitrogen (generally the limiting nutrient) removal mechanism in the Gippsland Lakes.

The results of this study show that the ecological status of the Gippsland Lakes in terms of phytoplankton dynamics and nutrient cycling has seemingly returned to a state similar to that prior to the *Synechococcus* bloom as indicated by the following observations.

- The TN (total nitrogen) content of the water column has now returned to long term average baseline values.
- Benthic nutrient recycling rates are similar to before the bloom. Importantly, denitrification rates and efficiencies remain relatively unaltered. We estimate denitrification rates within the lakes were able to remove the pulse of nitrogen from the 2007 floods in 466 days, a figure backed up by water column total nitrogen measurements collected by the Victorian EPA. Diatoms still appear to be the major vector for carbon delivery to the sediment as indicated by stoichiometric total carbon dioxide to silica (TCO_2:Si) ratios.
- Phytoplankton growth is N limited. Fluorescence measurements indicate that following a brief domination by green algae in late 2008, the lakes have returned to dominance by low-medium concentrations of diatoms and dinoflagellates, a situation that is typical of non-bloom periods.

This study has also generated important baseline data on the influence of grazing and nutrient controls on phytoplankton biomass. Phytoplankton biomass was more or less in equilibrium with grazing pressure. Adding nutrients to the system disrupts this equilibrium, leading to excess phytoplankton growth. This indicates that bottom-up processes are more important than top-down processes in determining phytoplankton biomass; phytoplankton are likely to react to an increase in nutrients significantly more quickly than the grazers can react to an increase in phytoplankton biomass.

Water column nitrogen recycling appears to be a key process that helps maintain water column TN concentrations. Comparing these recycling rates with rates that occur during blooms would provide important insights into the biogeochemical processing of nitrogen under various bloom scenarios.

We conclude that there is minimal risk of a recurrence of the *Synechococcus* bloom in the absence of any large nitrogen inputs. The risk of a *Nodularia* bloom in the immediate future is low, but in the event of a large freshwater input over winter/spring, this risk will be elevated, particularly given the recent input of phosphorus which was most likely stored in the sediments and is released under conditions of low dissolved oxygen and increased sediment respiration.

Since both nitrogen and phosphorus are important in bloom development, future management of the Gippsland Lakes needs to focus on limiting inputs of both of these

nutrients, especially given the increased likelihood of more frequent bushfires in the catchment as the region becomes drier and warmer.

One possible future climate change scenario sees the lakes becoming a marine-dominated system. Under this scenario, *Nodularia* blooms, which thrive in brackish water, will occur less often, but other problem species may take their place. Denitrification potential is unlikely to be adversely affected under this scenario, making it unlikely that the lakes will ever become permanently hypereutrophic.

Introduction

Background
The Gippsland Lakes has a history of problematic cyanobacterial blooms (Stephens et al. 2004). In November 2007 a cyanobacterial bloom occurred over the entire Gippsland Lakes and then persisted through much of 2008. Until then, the small, single-celled cyanobacterium of the genus *Synechococcus* had never been observed to bloom in this system. Previous cyanobacterial blooms were usually of the genus *Nodularia* – although isolated blooms of *Anabaena* and *Microcystis* species have also been observed – and these blooms always occurred in the summer, and would last a maximum of a few months (Stephens et al. 2004).

The Gippsland Lakes and Catchment Task Force commissioned this study to answer the questions: what initiated this bloom, and what led to *Synechococcus* dominating for such a sustained period? In a previous report (Cook et al. 2008) we hypothesized that the large influx of nutrients, especially nitrate, following the 2007 bushfires and floods provided conditions favourable to the fast growing *Synechococcus*, and, because of the small size of the cells (~1µm), it has shifted the usual nutrient cycling regime from being sediment dominated to water column dominated, which means that nutrients, in particular nitrogen, are retained in the system for a longer period. This study attempts to test this hypothesis and to determine whether there has been a long-term effect on the lakes from the unusual conditions present in 2007-2008.

Nutrient cycling in the Gippsland Lakes
Previous measurements of sediment fluxes within the Gippsland Lakes suggest that diatoms are an important vector for organic matter delivery to the sediment as indicated by carbon to silicon flux ratios close to that expected for diatoms (~7C:1Si) (Cook et al. 2008). The benthic recycling of organic matter is accompanied by a loss of nitrogen through the process of denitrification, which takes place within the sediment. In previous years, winter/spring algal blooms (usually triggered by inflow events) were short-lived and dominated by diatoms and dinoflagellates, which then sank to the benthos, and hence the large incoming nitrogen loads from the catchment were rapidly delivered to the sediment and denitrified (i.e. lost from the system). This resulted in a severely N-limited system over the summer months which was highly conducive to blooms of *Nodularia*, which can fix atmospheric nitrogen (Moisander and Paerl 2000). The ability to fix atmospheric nitrogen, however, comes at a cost. The process of N-fixation is highly energy intensive and requires large, specialized cells, which are relatively slow growing, are sensitive to physical factors such as salinity, temperature and turbulence and have high requirements for micro nutrients such as iron and molybdenum (Howarth et al. 1988; Marino et al. 2002; Marino et al. 2006). This means that whilst blooms of *Nodularia* are economically, socially and environmentally disastrous, they are usually relatively short-lived, because the conditions conducive to their proliferation are restricted to short periods over the summer months. As such, a combination of nitrogen-limitation and physical conditions ensured that phytoplankton biomass was previously kept in check.

The dominance of *Synechococcus* may have changed this. We believe that this bloom was triggered by unusually high nitrogen loads entering the lakes, which may have been so high that diatom and dinoflagellate growth was unable to remove it before the water temperature rose and light availability increased, favouring cyanobacterial

growth; in this case the non-N_2 fixing but fast growing *Synechococcus* (Beardall 2008; Cook et al. 2008). The small size of *Synechococcus* cells means that they do not sink to the bottom, resulting in the recycling of dead algal cells being shifted from the sediment to the water column. This may have resulted in a short-circuiting of denitrification, because instead of nitrogen being permanently lost after cells die, it will be efficiently recycled, thus allowing the high algal biomass to be perpetuated.

Study

In September-October 2008, the Gippsland Lakes and Catchment Task Force commissioned a 'snapshot' of the Lakes, taking in seagrass, fish, nutrients, and phytoplankton. The purpose was to obtain some preliminary data on whether there has been a shift in the lakes to a new state, and if so, whether this state is likely to persist. As part of this 'snapshot' we investigated nutrient cycling (both benthic and water-column), denitrification, phytoplankton nutrient limitation, and grazing of phytoplankton. We concluded from this investigation that the lakes were returning to a nitrogen limited state (Holland et al. 2009). To test this conclusion, we have repeated these measurements on three more occasions: October 2009, December 2009 and March 2010.

Methods

Sites

Three sites were used: LKN (EPA site 2316) in deep water (7 m) in central-northern Lake King; LKS (EPA site 2314) in deep water (8 m) off Raymond Island in southern Lake King; LVC (EPA site 2311) in 4 m deep water off Storm Point in Lake Victoria. These sites are regularly monitored by the Victorian EPA, and have previously been used for benthic chamber experiments Figure 1. Field trips were undertaken in the weeks beginning 27 October 2008, 19 October 2009, 14 December 2009 and 1 March 2010.

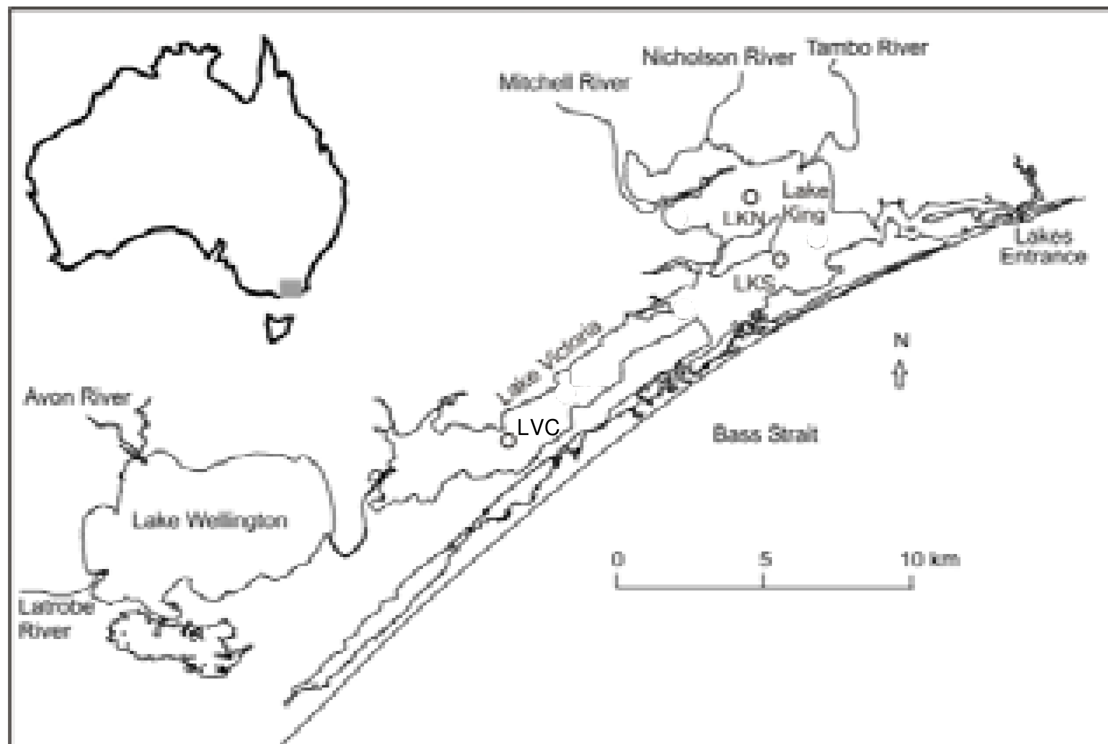

Figure 1. Map of the Gippsland Lakes with sampling sites marked with open circles.

Chlorophyll fluorescence

Chlorophyll *a* was used as a proxy for biomass/productivity, with the assumption that chlorophyll *a* per cell would not change significantly during experimental incubations, because ambient light and temperature were used. Biomass was therefore estimated using a non-destructive fluorometric approximation of chlorophyll *a* (Jakob et al. 2005), in a Phytopam Phytoplankton Analyzer (Heinz Walz, GMBH, Germany) connected to a PC running PhytoWIN software. This device allows deconvolution of the fluorescence output into three major phytoplankton groups, Green (Chlorophytes), Brown (diatoms and dinoflagellates) and Cyan (cyanobacteria). This deconvolution is based on reference species for each group, and provides a useful comparison, but may not entirely accurately represent the proportions of the same groups in natural populations. In order to calibrate the total chlorophyll *a* calculated by the Phytopam, representative samples were filtered onto Whatman GF/F filters at the start and end of each experiment for extractable chlorophyll *a* analyses.

Grazing
Grazing pressure (the rate of grazing per phytoplankton cell) can be measured by serial dilution of a sample, which reduces the number of grazers per ml, and hence reduces the likelihood that a particular phytoplankton cell will be eaten (Landry and Hassett 1982). 5 or 20 L plastic carboys were filled with surface water at each of the three sites. Unfiltered lake water was diluted with filtered lake water (through 0.2 μm Supor filters) to a concentration of 0.05, 0.2 or 1.0 of the original sample. For each concentration, two nutrient treatments were also prepared (except in October 2008 when no nutrients were added); C (no added nutrients) and A (100 μM ammonium and 10 μM phosphate added). Triplicate 100 ml samples for each site and treatment were prepared in 150 ml Nalgene PETG bottles. The bottles were incubated in a temperature controlled water bath, held at the current lake temperature ±2 °C. In the October 2008 experiment, the water bath was kept outside (in Paynseville, beside Lake King), under partial shade, and was subject to ambient day-night lighting conditions. During the day, the light was generally between 100 and 200 μmol photons m^{-2} s^{-1}, although for a brief period each day (0.5-1 hr), direct sunlight would increase the incident light to approximately 500 μmol photons m^{-2} s^{-1}. In the subsequent experiments, the water bath was kept in an air-conditioned laboratory and lit by fluorescent lighting – generating approximately 100 μmol photons m^{-2} s^{-1} – for 14 hours per day. The experiment was run for between 3 and 7 days. Biomass was measured between 3 and 5 times per incubation using the Phytopam.

Bioassay
100 ml lake water samples were transferred to 150 ml Nalgene PETG bottles. Nutrients were added in the form of either ammonium (N) or phosphate (P), increasing the sample concentration of these elements by 100 μM and 10 μM respectively. Four treatments were used: C (control), N (just N added), P (just P added) and A (both N and P added). Bottles were incubated in temperature controlled water baths at ambient site temperature ±2 °C under a 14:10 hour light:dark cycle and illumination of approximately 100 μmol photons m^{-2} s^{-1}. Chlorophyll *a* fluorescence was measured every 1-4 days using the Phytopam; in the October 2008 experiment, growth was followed until either a steady state or decline was observed (about three weeks), while subsequent incubations were terminated after seven days.

Nutrient Induced Fluorescence Transients
NIFTs occur when samples are sufficiently nutrient limited that a spike of nutrients triggers a reallocation of energy from carbon fixation to nutrient uptake, thus altering the fluorescence output.

3 ml of lake water was transferred to a glass cuvette and placed in the Phytopam. Fluorescence emission was recorded every 30 s at medium light (90 μmol photons m^{-2} s^{-1}) and during a saturating pulse of light (>400 μmol photons m^{-2} s^{-1}) giving the fluorescence values known as F_t and F_m respectively. These two values can be used to calculate the effective quantum yield of Photosystem II, $\Phi_{PSII} = (F_t - F_m)/F_m$. After approximately 10 minutes, a 10 μl spike of a solution of phosphate, nitrate, ammonium or distilled water was added to the cuvette, increasing the nutrient concentration by either 10 μM (if nitrate or phosphate was added) or 100 μM (if ammonium was added). These concentrations have previously been shown to produce

strong NIFTs in nutrient limited cultures (Holland et al. 2004; Roberts et al. 2008; Young and Beardall 2003). Fluorescence was then recorded for a further 10 min or longer, depending on whether a response was observed.

Due to time constraints, NIFTs were not tested for all nutrients at all sites. Lake Victoria water was the most thoroughly tested, because this site consistently had the highest biomass, and was therefore considered more likely to have sufficient biomass to produce a NIFT. Water from the other two sites was tested as time permitted. All measurements were performed within 48 hours of collection.

Photosynthesis-Irradiance curves

PI curves were measured using the Phytopam, where measurements of fluorescence excitation with increasing irradiance are used to calculate the maximum electron transport rate (ETR_{max}), the light saturation parameter (I_k), and the slope (α) of the linear portion of the curve (see Figure 2). From these curves we also estimated the irradiance where maximum photosynthesis occurred (in this case it was around 300-400 µmol photons m^{-2} s^{-1}), and ran a separate experiment measuring the change in dissolved oxygen (DO) in sealed 260 ml glass bottles over time either in the dark or at saturating irradiance. From this we calculated the maximum photosynthesis rate (P_{max}), and then recalculated α as a function of this, rather than ETR_{max}.

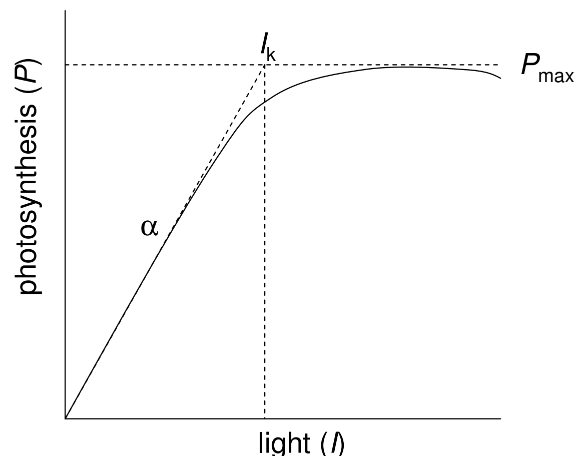

Figure 2. Stylised *PI* curve. I_k is the extrapolated irradiance where the linear portion of the curve would reach the maximum photosynthesis rate (P_{max}). α is the slope of this line (I_k/P_{max}).

Benthic flux (from *in situ* chambers)

Benthic fluxes were measured using automated benthic chambers deployed *in situ*. The chamber design has been previously described in detail (Pomc 2008). Briefly, each chamber enclosed 10-15 L of water over an area of 0.07 m^2. The chambers were stirred with a paddle stirrer at a rate sufficient to create a diffusive boundary layer thickness of 0.3-0.4 mm. The volume of the chamber was calculated from video observation of the depth of penetration into the sediment. Two benthic chambers were deployed at each site, and benthic fluxes were estimated by the change in concentration of metabolites within the chambers over time. Both chambers were transparent. Chambers were deployed for about 20 hours, and collected samples over 16 hours. All nutrient samples (NO_x, NH_4^+, RP and RSi) were filtered (0.45 µm), frozen in the field, and analysed at the Monash University Water Studies Centre using

standard colorimetric methods (Grasshoff 1983). Samples for pH were analysed in the field using a high-precision electrode and meter. Alkalinity was estimated by Gran titration of samples with dilute standardised HCl. Benthic fluxes were calculated by linear least-squares regression of metabolite concentration over time; only linear portions of the concentration/time plots were used to estimate fluxes.

Nitrogen cycling in the water column

Surface-water samples were collected from the each of the three sites; bottom water was also collected in October 2008 only. 150 ml samples were incubated for 4-6 hours after the addition of 0.1 µM of either $^{15}NO_3^-$ or $^{15}NH_4^+$. Samples were incubated either in the light at between 100 and 200 µmol photons m^{-2} s^{-1}, or the dark. In October 2008 surface water was used in the light and bottom water in the dark incubations, and a second set of bottom samples from Lake Victoria were kept in low light (~10 µmol photons m^{-2} s^{-1}). At other times, surface water was used for both light and dark incubations. At the end of the experiment, the samples were filtered onto ashed GF/F filters, and frozen. ^{15}N retained on the filter and thus incorporated into the phytoplankton was measured using a stable isotope mass spectrometer at Griffith University. N-uptake rates were calculated using the technique of Dugdale and Wilkerson (1986).

Results and Discussion

Phytoplankton community

A comparison between chlorophyll *a* values measured with the Phytopam and those measured by chlorophyll extraction showed that, while the correlations within each sampling trip were generally quite good, the relationship was not 1:1 and changed over time. To calibrate the fluorescence output we therefore multiplied each of the chlorophyll *a* fluorescence measurements by the calibration factor shown in Figure 3 to estimate the true chlorophyll *a*. The correlation for October 2008 was poor. While the exact reason cannot be ascertained we believe that there were some errors in the spectroscopic analysis of the extracted chlorophyll, due to very low readings. This was rectified for the latter trips by combining the triplicate bioassay samples onto one filter.

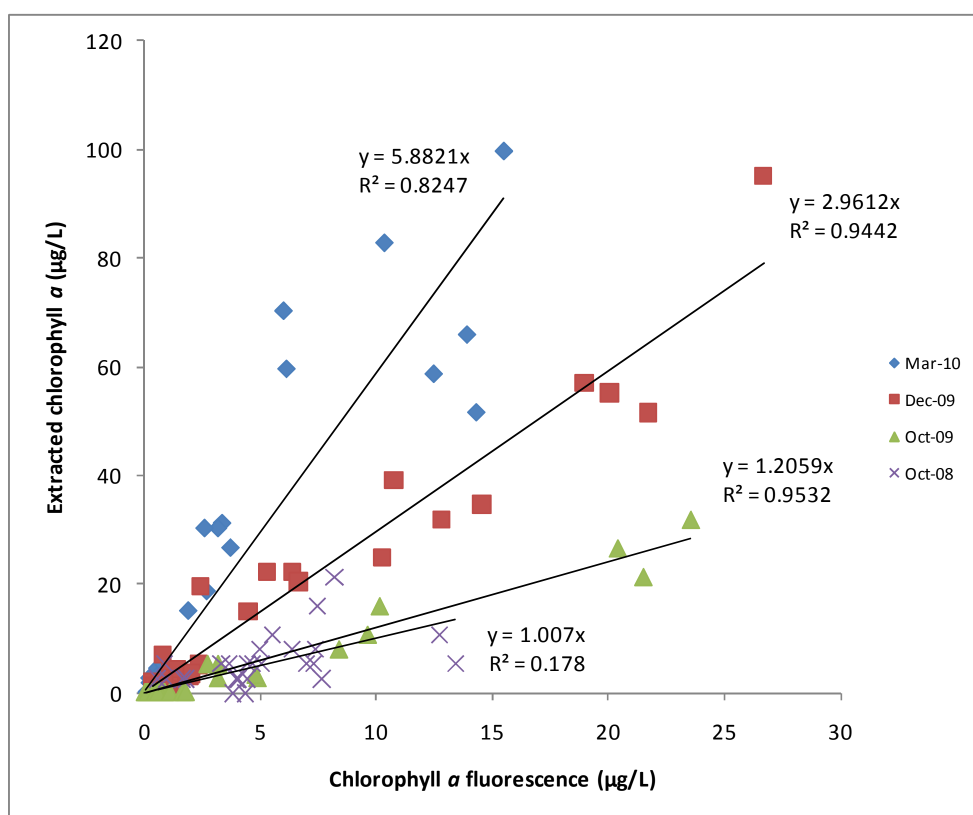

Figure 3. Chlorophyll *a* fluorescence (calculated by the Phytopam) versus extracted chlorophyll *a*. Note that the high chlorophyll *a* concentrations are from samples collected at the end of the nutrient addition bioassay incubations.

In October 2008, chlorophyll *a* was elevated at all three sites (background levels at this time of year are typically 1-2 µg L^{-1}), and was highest in Lake Victoria, at almost 8 µg L^{-1}, which was twice as high as LKN, and 50% higher than LKS (Figure 4). Chlorophyll *a* was considerably lower than this the following October and December (0.5-4 µg L^{-1}), and by March 2010 it had increased to similar levels to those in October 2008. Green algae dominated in October 2008, while brown algae (diatoms and/or dinoflagellates) dominated at other times. Cyanobacteria were undetectable in the majority of cases and a minor component of the flora in those cases where it was detected.

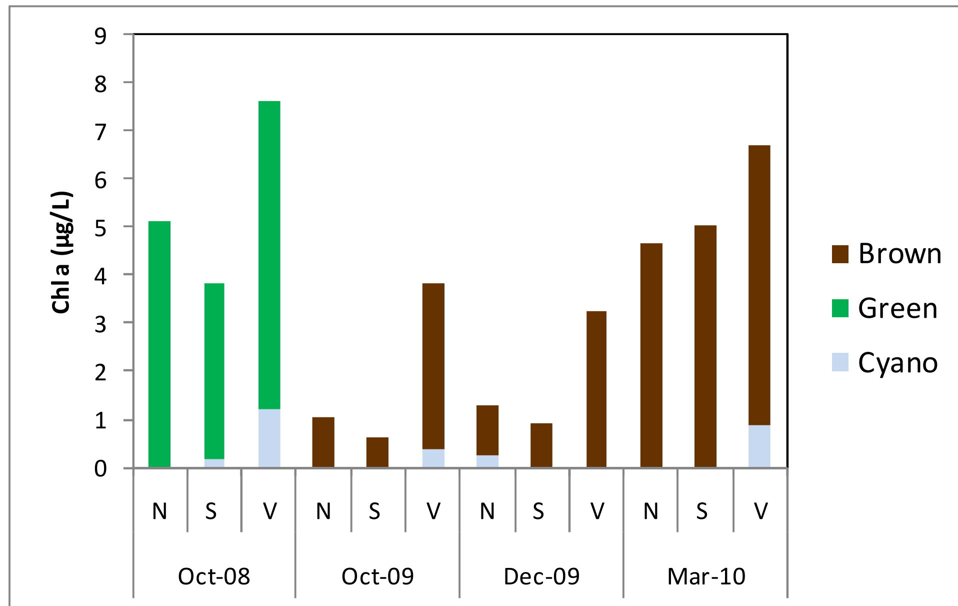

Figure 4. Chlorophyll *a* in the Gippsland Lakes, divided into Brown (diatoms and dinoflagellates), Green (green algae) and Cyano (cyanobacteria), as measured by the Phytopam. Bars labelled N represent LKN, S represents LKS and V represents LVC.

Grazing

An experimental manipulation of grazing indicated that significant grazing pressure currently exists in the lakes. In almost all cases, samples with the largest dilution (and hence the smallest grazing pressure) exhibited the highest growth rate. The exceptions were some of the nutrient addition samples from Lake King in October and December 2009. Samples with no nutrients added (labelled C in Figure 5) tended to show no growth when undiluted, the hypothetical growth rate in the absence of grazing was therefore approximately equal to the grazing rate. In the samples with nutrients added, the grazing rate was typically around half of the maximum growth rate. The conclusion from this is that during the periods when the field trips were undertaken, there was an equilibrium, where nutrient availability and grazing by zooplankton kept the phytoplankton at a constant biomass. Adding nutrients or removing grazers disrupts this equilibrium, leading to increased growth of phytoplankton.

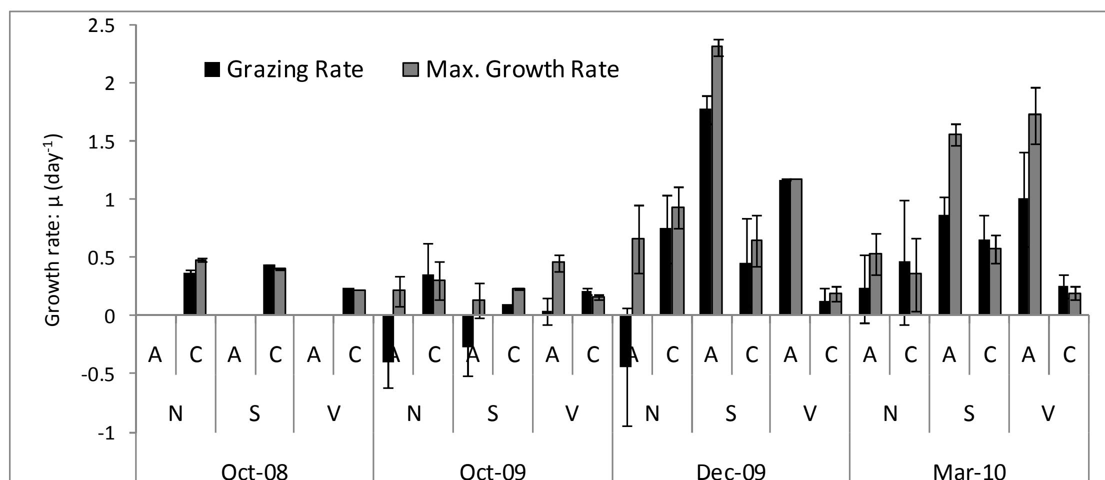

Figure 5. Grazing rate and maximum growth rate (growth rate in the absence of grazing) calculating from a dilution experiment. N, S and V represent sites LKN, LKS and LVC respectively, and A represents the treatment with added nitrogen and phosphorus and C the control.

Nutrient-Induced Fluorescence Transients

NIFTs were observed with the addition of ammonia to water from LVC in October 2008, but not for nitrate, phosphate or water (Figure 6). No NIFTs were observed for the two other sites at this time, and for the following three trips, NIFT testing was only done on LVC water. No NIFTs were observed in the three subsequent field trips. These results are consistent with the current knowledge of NIFTs, in that they tend to occur relatively consistently under nutrient limitation in green algae or cyanobacteria, the two dominant taxa in October 2008, but not with diatoms, which dominated during 2009-2010. This clearly indicates that NIFTs have little utility in this system during regular sampling. They could, however, potentially provide a rapid indication of nutrient limitation during a bloom of either green algae or cyanobacteria.

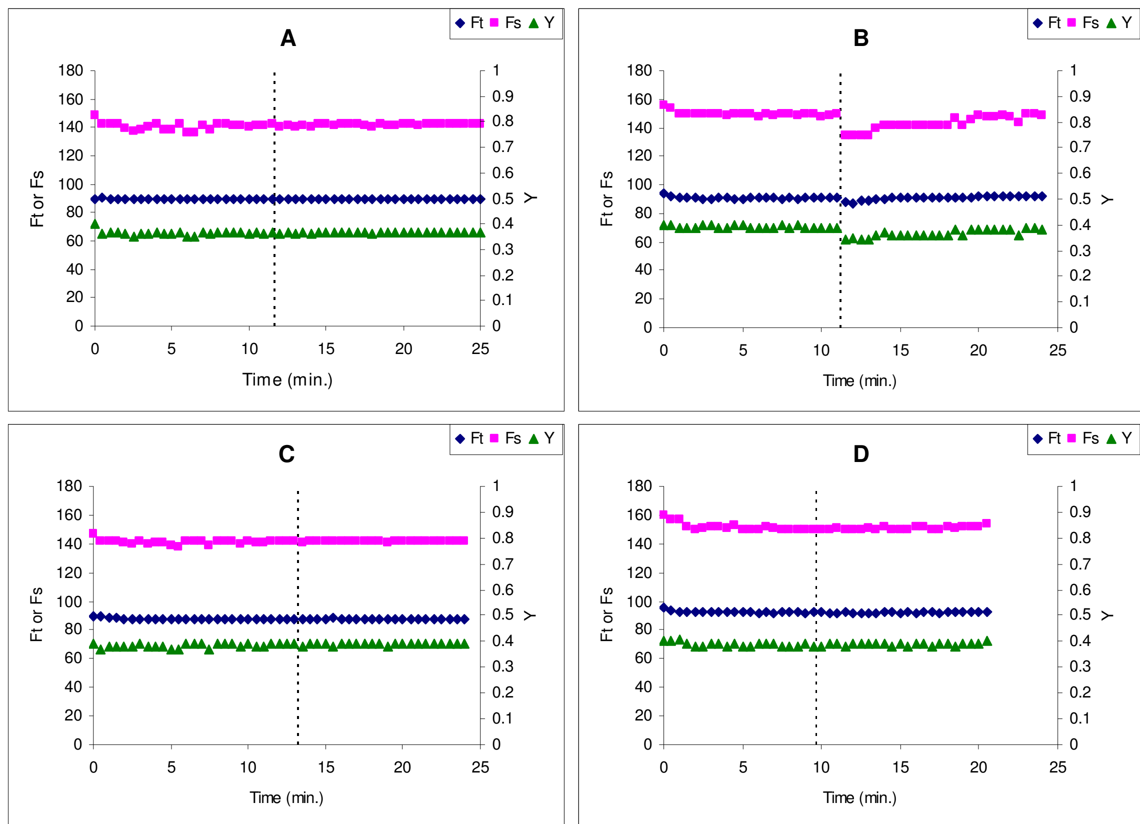

Figure 6. Fluorescence response (at 620 nm excitation) to nutrient addition of LVC surface water in October 2008: A) nitrate; B) ammonia; C) phosphate; D) water. F_t is the variable fluorescence signal (under 1 µmol photons m^{-2} s^{-1} irradiance), F_s is the fluorescence signal immediately after a flash of saturating light (at > 400 µmol photons m^{-2} s^{-1} irradiance) and Y is the quantum yield (Φ_{PSII}) calculated from $(F_s-F_t)/F_s$. The nutrient was added at the time indicated by the dashed line. Note the characteristic quenching and then recovery of the fluorescence following addition of ammonia.

Photosynthesis-Irradiance curves

The gross oxygen production rate of the surface water was calculated from the difference between the rate of DO change in high (saturating) light and no light. The rates were generally highest when the biomass was highest, e.g. in October 2008 (Figure 7).

The other *PI* parameters were similar among sites (apart from two unusually high values, which cannot be explained and are probably due to measurement errors). The samples had maximum photosynthesis rates at an irradiance of 300-400 µmol photons m^{-2}, and values of the light saturation parameter, I_k (see Figure 2 for definition) were high, generally >170 µmol photons m^{-2}, indicating that these populations were adapted to high light. These results indicate that light is not likely to be a limiting factor in phytoplankton growth in the surface waters of the lakes.

Bioassay

The October 2008 bioassay was run for 21 days, whereas the other three bioassays were run for 7 days. For this report, therefore, the results of this first bioassay have been re-analysed using only the first 10 days of measurements (no reading was taken on day 7).

Similar results were observed for the three sites (Figure 8). In all cases, the full nutrient treatment (nitrogen plus phosphorus) had the highest growth rate. This shows that nutrients were limiting growth in this system, assuming the *in situ* growth conditions matched the experimental conditions. There was no significant difference between the control and phosphorus treatments in any of the bioassays. Nitrogen treatments generally had higher growth rates than the controls, but lower than the full treatment. Combined, this data indicates that at the time these waters were collected, N was more limiting than P, but that both were in short supply. The phytoplankton appear to be able to adapt to an increase in N (through either changes in the population structure or cellular nutrient content) but not to P. The data also suggests that little or no nitrogen fixation was occurring in the water column at these times, in agreement with the lack of cyanobacteria.

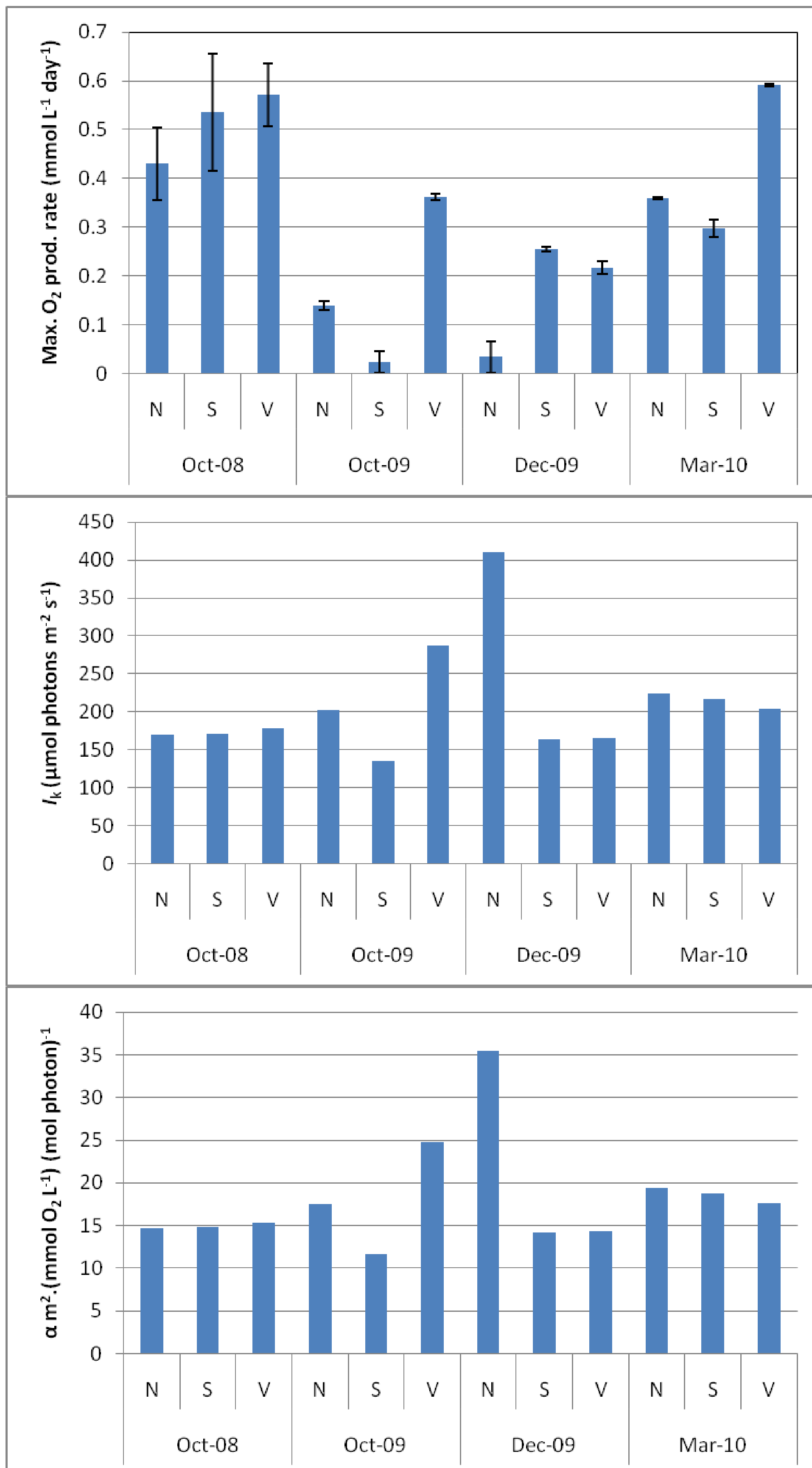

Figure 7. Photosynthetic parameters at each site over time. N, S and V represent sites LKN, LKS and LVC respectively.

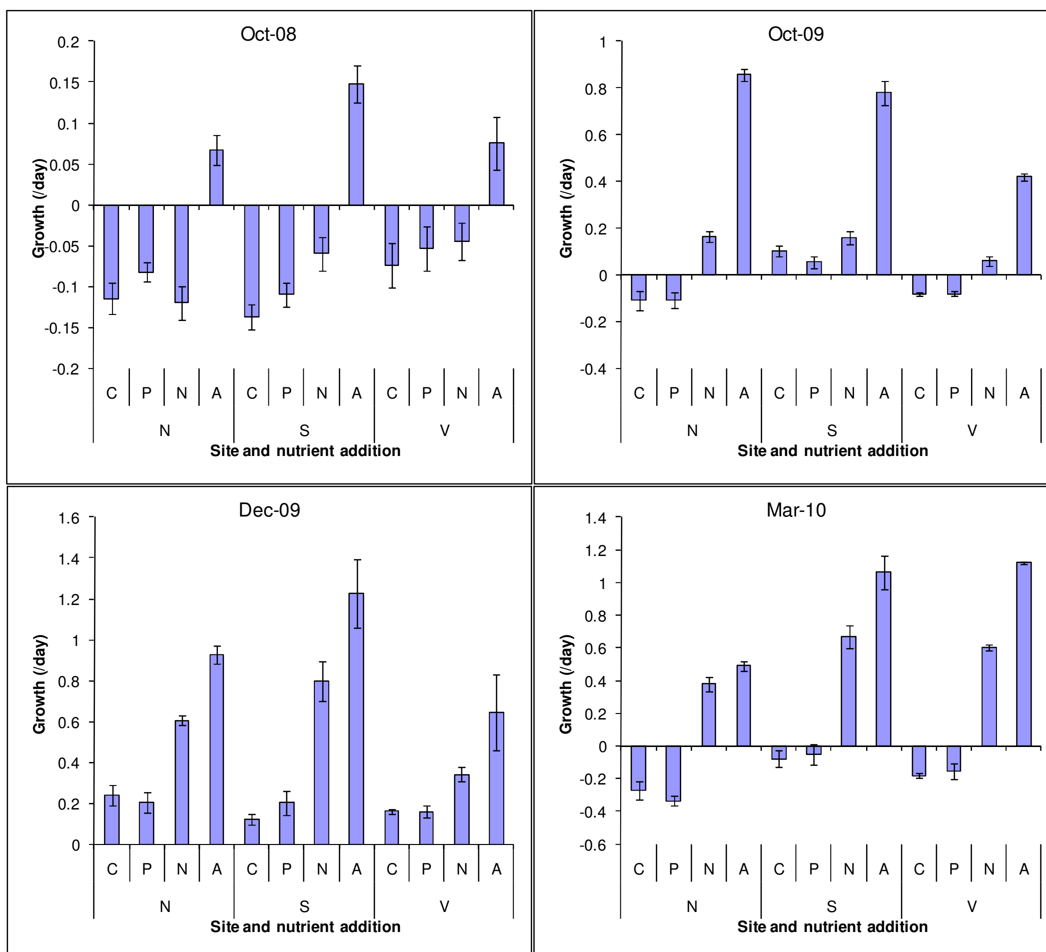

Figure 8. Results of nutrient enrichment bioassays conducted on Gippsland Lakes surface water samples. N, S and V represent sites LKN, LKS and LVC respectively, and A represents the treatment with added nitrogen and phosphorus and C the control. Results include standard error bars.

Water column nutrients

Dissolved inorganic nitrogen (DIN, NH_4^+ and NO_x) was elevated in October 2008, at between 1.3 and 2 µM (Table 1). This elevation in DIN was a result of the floods of June 2007. These nitrogen levels had been declining as time went by, and in our previous report we predicted that denitrification would remove this excess DIN by early in 2009. By October 2009, DIN had dropped to around 0.4 µM, a more typical value for the lakes, when not influenced by floods. In December 2009 and March 2010 DIN values were slightly higher, at around 0.7 µM. FRP was higher at each successive sampling trip, rising from almost undetectable, at 0.03-0.06 µM in October 2008, to >0.4 µM in March 2010. DIN:FRP was higher than the Redfield ratio in October 2008, but was very low during the other field trips (Table 1). On their own, these results would suggest that phosphorus was limiting in October 2008, whereas nitrogen was limiting at the other times. Total Nitrogen (TN) and Total Phosphorus (TP) were measured during the first two field trips; the values were much higher than the inorganic nitrogen and phosphorus and the ratio was only slightly higher than the Redfield ratio (Table 1). Bottom water dissolved oxygen was close to saturation, except at LKN in December 2009 and in March 2010, when all sites had low D.O., particularly LKN and LVC. These low D.O. values could be indicative of high FRP

flux, which would explain the increase in FRP over the summer of 2009-2010 (*c.f.* FRP flux values in Figure 9).

Table 1. Surface water nutrient concentrations (µM) and N:P elemental ratios, plus bottom water dissolved oxygen (D.O., % saturation).

Site	NH_4^+	NO_x	FRP	TN	TP	DIN:FRP	TN:TP	D.O.
30/10/08								
LKN	0.79	0.71	0.03	30.0	1.6	47	19	81
LKS	0.71	0.64	0.06	30.7	1.6	21	19	93
LVC	1.07	0.79	0.06	50.0	2.9	29	17	123
20/10/09								
LKN	0.21	0.14	0.10	15.7	0.7	4	24	86
LKS	0.29	0.14	0.10	15.7	0.3	4	49	115
LVC	0.29	0.14	0.10	36.4	1.3	4	28	74
15/12/09								
LKN	0.07	0.64	0.13	NA	NA	6	NA	29
LKS	0.04	0.71	0.13	NA	NA	6	NA	106
LVC	0.21	0.86	0.19	NA	NA	6	NA	98
03/03/10								
LKN	0.43	0.43	0.39	NA	NA	2	NA	7
LKS	0.04	0.14	0.39	NA	NA	0.5	NA	62
LVC	0.43	0.21	0.55	NA	NA	1	NA	19

Benthic flux

Nutrient fluxes were consistent over the first three sampling trips (apart from a spike in activity in Lake Victoria in December 2009): DO uptake and TCO_2 loss were consistently in the range 20-50 mmol m^{-2} day^{-1}; DIN flux was between 0 and 4 mmol m^{-2} day^{-1}; FRP (phosphate) flux was between 0 and 0.25 mmol m^{-2} day^{-1} and silicate flux was between 1 and 6.5 mmol m^{-2} day^{-1} (Figure 9). Respiration (TCO_2 flux) was five-fold higher in Lake Victoria in December 2009 compared to the previous two trips, and the fluxes of DIN, FRP and silicate were similarly higher. TCO_2 flux was elevated at all sites in March 2010 (when the bottom water D.O. was low), with DIN and FRP fluxes especially high. Cook et al. (2010) showed that FRP fluxes in the lakes were generally higher than expected by Redfield stoichiometry at low respiration, and higher at high respiration. This new data shows a similar trend (Figure 10), further supporting the hypothesis that sediment bound phosphorus is released as a result of the reduction of Fe(III) to Fe(II) due to sulphide production associated with benthic respiration.

Denitrification rates were calculated two different ways; stoichiometrically, from the difference between the measured DIN flux and the flux expected from the TCO_2 flux given the Redfield ratio – N:C – of 106:16 and directly from measurements of N_2 gas over time in the chambers (Figure 11). The stoichiometric denitrification rate was remarkably consistent, running at between 1.8 and 4.3 mmol m^{-2} day^{-1} at all sites and trips, apart from a spike in December 2009 at Lake Victoria, and a large negative rate at Lake King North in March 2010. The spike in December 2009 is consistent with the spike in respiration and mineralisation at this time, suggesting that coupled nitrification-denitrification was enhanced by these conditions. The negative value in March 2010 (when the bottom water was anoxic) is clearly erroneous, and is caused by a flux of nitrogen that cannot be explained by the breakdown of phytoplankton (i.e. around three times more DIN was released than would be expected from the TCO_2 flux, if these nutrients were being released through the breakdown of phytoplankton).

A similar unexplained flux of nitrogen was seen in Lake King North in August 1998 (Longmore et al. 2001). The direct N_2 measurements were, overall, quite similar, apart from some unexplained negative fluxes, including a large negative flux at Lake King North in October 2008.

The mean denitrification rate across the lakes was 2.5 ± 1.5 mmol m^{-2} day^{-1} using the stoichiometric method, or 1.5 ± 0.6 mmol m^{-2} day^{-1} using the direct method; the mean denitrification efficiency (the proportion of nitrogen released from the sediment as N_2 gas) was 26 ± 14 % using the stoichiometric method and 42 ± 17 %. Excluding the probably erroneous negative N_2 flux values (see Figure 11) gives the higher means of 4.5 ± 0.7 mmol m^{-2} day^{-1} and 65 ± 4 % for the stoichiometric method, and 2.4 ± 0.4 mmol m^{-2} day^{-1} and 52 ± 7 % for the direct method. These are consistent with the denitrification rates measured previously (Roberts et al. 2003).

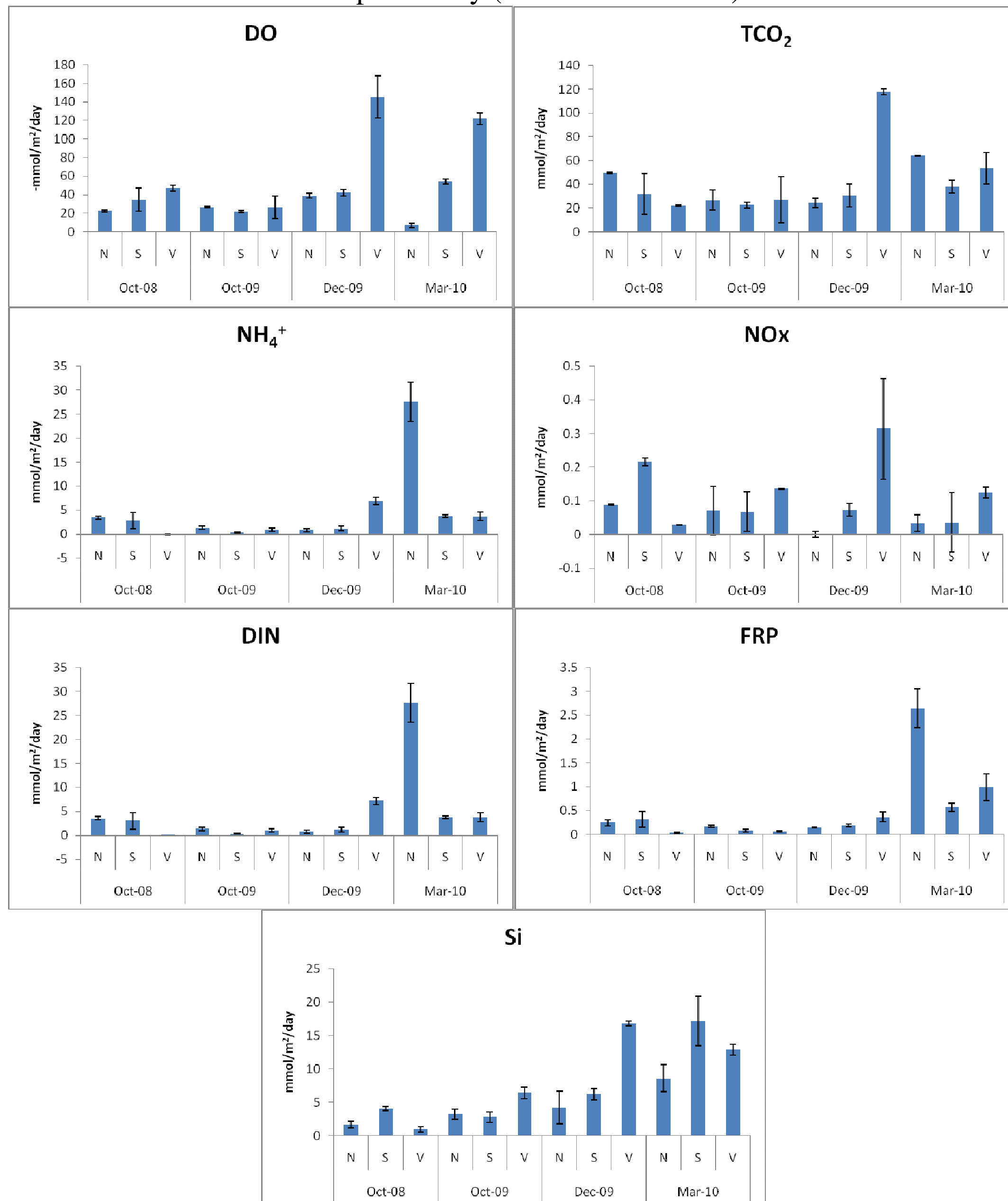

Figure 9. Sediment nutrient fluxes measured *in situ* in chambers. N, S and V represent sites LKN, LKS and LVC respectively. Standard error bars are included.

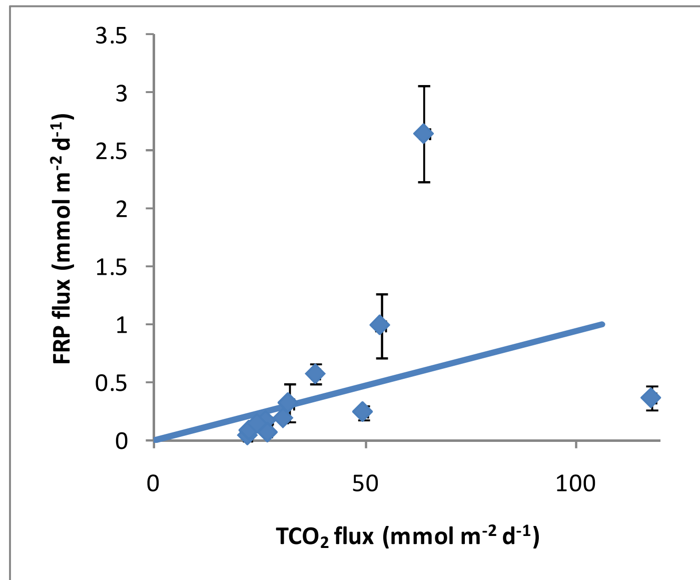

Figure 10. FRP flux plotted against TCO_2 flux, with the blue line indicating the Redfield ratio of 106:1. FRP flux is enhanced at high respiration rates (one outlier notwithstanding).

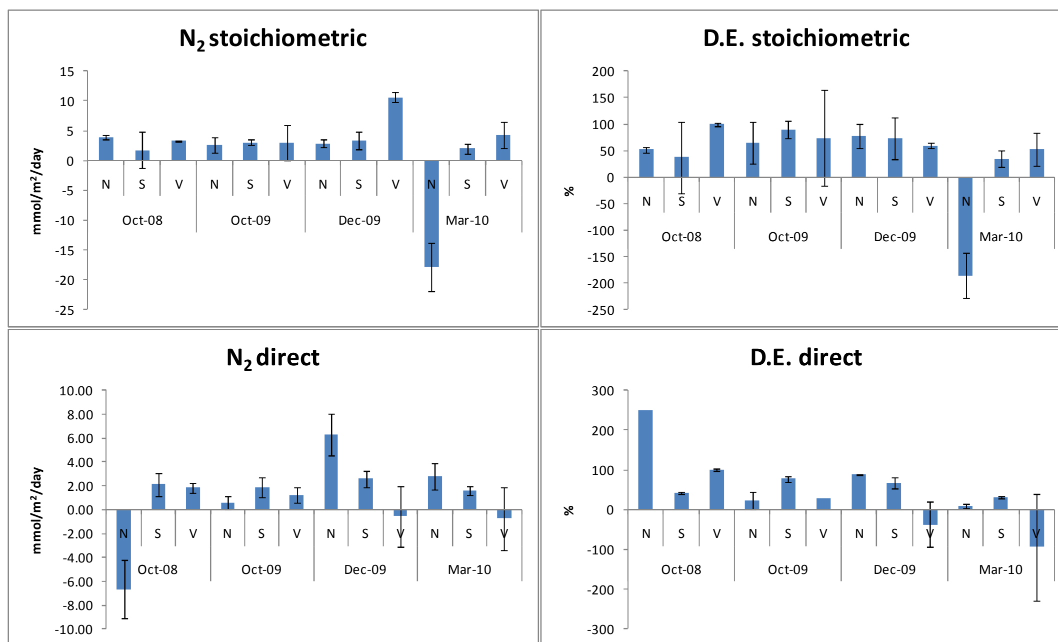

Figure 11. Sediment denitrification measured *in situ* in chambers. The N_2 flux and denitrification efficiency (D.E.) have been calculated from the expected stoichiometric relationship between TCO_2 flux and nitrogen flux, and from direct measurements of N_2 over time in the chambers. N, S and V represent sites LKN, LKS and LVC respectively. Standard error bars are included.

Nitrogen cycling in the water column

The uptake rate of ^{15}N from labelled ammonia or nitrate provides a measure of the nitrogen cycling within the water column. Uptake, which is an energy requiring process, was, as expected, greater in the light than the dark, and the average of these gives a rough estimation of daily uptake (Figure 12). Uptake of nitrate was higher than the uptake of ammonia in October 2008, with the reverse occurring at the other three times. Uptake was higher in October 2008 and December 2009 than at the other two times.

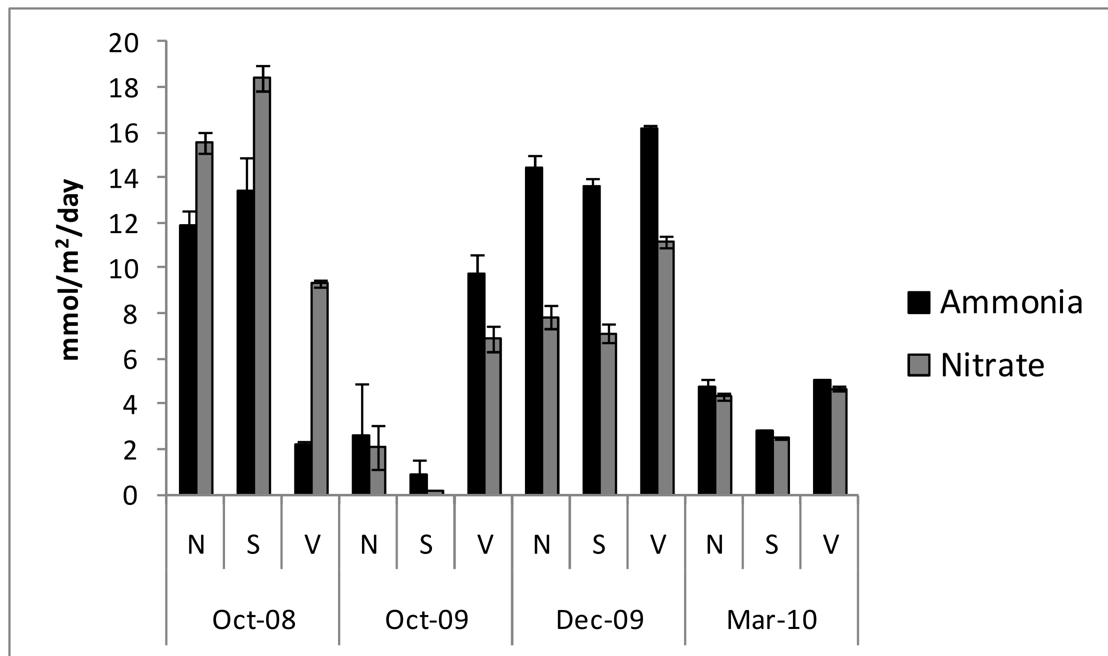

Figure 12. Daily uptake of ^{15}N-labelled ammonia and nitrate integrated through the water column (with standard error bars). N, S and V represent sites LKN, LKS and LVC respectively.

General discussion

Lake Wellington used to be dominated by macrophytes, but a drought in 1968 led to an influx of saline water from Lake Victoria, which killed these plants. The lake never recovered, and has since been dominated by phytoplankton (Harris et al. 1988). The *Synechococcus* bloom in lakes Victoria and King severely reduced light penetration and there were anecdotal reports of seagrass loss, and subsequent measurements showed a reduction in seagrass cover compared with 10 years previously (Hindell and Warry 2009). The concern was that this could provide a similar “tipping point” to what occurred in Lake Wellington, consigning the entire Lakes Victoria and King region to a long period dominated by nanoplankton with a concomitant reduction in important habitats. It is now apparent that while the effect of fires and floods was quite significant during late 2007 and 2008, leading to this widespread and persistent phytoplankton bloom, this impact has now subsided. When the seagrass was resampled in April 2009, significant recovery was seen, and in the October 2009 to March 2010 field season for this study, the lakes had returned to what we would consider to be typical, low flow, pre-*Synechococcus* conditions. These conditions include relatively low dissolved nutrient concentrations and nitrogen limitation of phytoplankton growth.

Nitrogen

The year from 1 June 2007 – 31 May 2008 saw the introduction of an unprecedented 6060 tonnes of nitrogen into the Gippsland Lakes – more than four times the annual average for the previous 20 years, and twice the next highest load. The major mechanism for nitrogen removal in the Gippsland Lakes is likely to be through denitrification within the sediments (Webster et al. 2001). The mean denitrification rate measured in the current study was 2.5 mmol m^{-2} day^{-1}, or ~13 tonnes day^{-1} (based on a lakes-wide surface area of 364 km^2), which would have removed this nitrogen in around 470 days. We note that this estimate of denitrification is biased by the fact that it was measured at the deep sites within the lakes. In 2002, DIN fluxes were measured in both shallow and deep chambers, and the deep sites had more than four times the fluxes than the shallow sites (6.2 mmol m^{-2} day^{-1} compared with 1.4 mmol m^{-2} day^{-1}). This is likely a result of lower organic loading to these shallow sites, and pore water analysis suggests that this is the case – Longmore (2000) found 118 mmol m^{-2} ammonia in the top 20 cm of pore water in deep water sites compared with 38 mmol m^{-2} in shallow sites. If we assume that denitrification efficiency is the same across the lakes, we can estimate that shallow sites will have an average denitrification rate close to 0.6 mmol m^{-2} day^{-1}. Given that all of Lake Wellington, all of Jones Bay and the western third of Lake Victoria – representing more than half of the surface area of the lakes – are < 4 m deep (estimated from Fig. 2.2 in Webster et al. 2001), a realistic average lake-wide denitrification rate is probably somewhere between the deep and shallow values; the average of these is 1.6 mmol m^{-2} day^{-1}, i.e. 8 tonnes day^{-1}. This rate would increase the time taken to remove the 6060 tonnes of nitrogen to around 760 days. Denitrification within the lakes, therefore, has the capacity to remove a substantial fraction of catchment derived nitrogen, even in years of extreme loading.

One of the critical questions to be investigated by this study is whether or not the 2007 flood and subsequent *Synechococcus* bloom affected denitrification rates. We hypothesised that the small *Synechococcus* cells would not sink, therefore slowing the

rate of nitrogen supply to the sediment, and hence the rate of N removal through denitrification. Denitrification rates were not measured during the bloom itself, but inspection of in-lake TN in the water column at Lake King South shows that total nitrogen concentrations remained elevated in the water column of the Gippsland Lakes for much longer than a previous comparable flood in 1999 (Figure 13). Total N concentrations, did, however, return to the long term baseline concentration of ~20-30 µM by the summer of 2009-2010 (Figure 13). This steady decline in TN back to baseline conditions closely matches the trend expected from the denitrification rates.

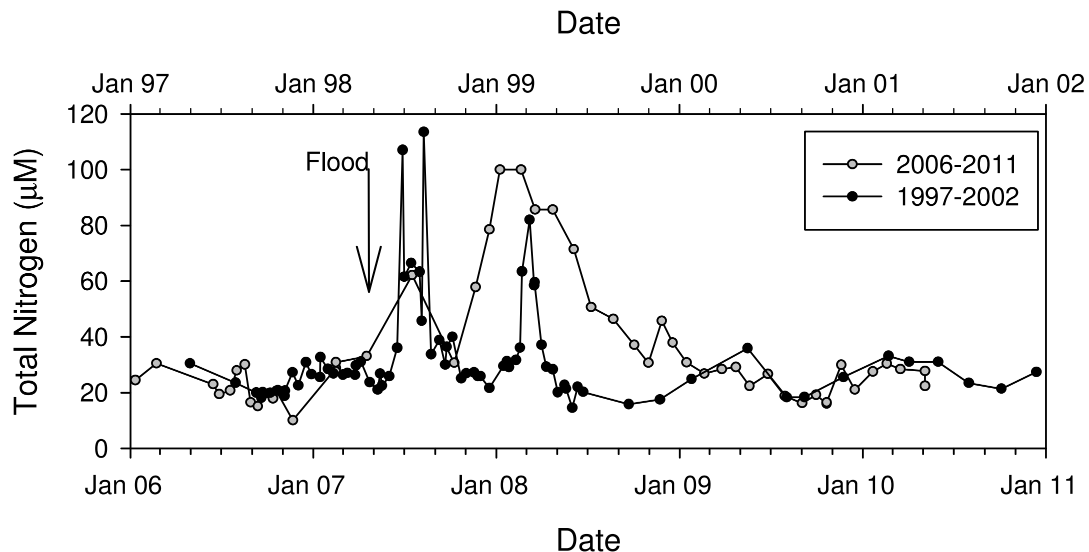

Figure 13. Comparison of surface water total nitrogen concentrations for the period 1997-2002 (top axis) and 2006-2011 (bottom axis). A flood occurred in the middle of the second year in each range, leading to a spike in nitrogen levels. The peak in March 1999 was the result of a *Nodularia* bloom, while the high levels throughout 2008 track the *Synechoccocus* bloom.

Measurements of benthic respiration (TCO_2 flux), SiO_4 fluxes and denitrification all suggest benthic recycling rates and processes are not markedly altered from those prior to 2007 (Table 2). The delivery of organic material to the sediment in 2008-2009 and 2009-2010 (as indicated by the TCO_2 fluxes) is remarkably consistent with rates measured previously at similar sites. Diatoms are generally thought to be an important vector for carbon delivery to the sediment because of their relatively rapid sinking velocity, and we can use SiO_4 fluxes as a tracer for their importance (Haese et al. 2007). The recent SiO_4 fluxes and the TCO_2:SiO_4 flux ratios in Table 2 are within the range of those observed prior to 2007, suggesting that the importance of diatoms as a vector for organic carbon delivery to the sediment remains unaltered. Consistent with these observations, denitrification rates within the sediment (both in terms of the absolute amount of nitrogen and the efficiency of the process) are not markedly different to prior to the 2007 flood event.

Table 2. Mean (+S.E.) fluxes and denitrification rates (mmol m^{-2} day^{-1}) calculated by stoichiometric balance or direct measurement of fluxes (in chambers). Denitrification Efficiency (D.E.) calculated from the stoichiometric technique.

Date	Site	TCO_2 flux	SiO_4 flux	TCO_2:SiO_4	Denit. (stoich)	Denit. (measured)	D.E.
1997-98	N	36 ± 16	1.6 ± 0.6	23 ± 14	2.4 ± 1.8		71 ± 10
	S	83 ± 21	2.8 ± 0.8	30 ± 11	10.3 ± 3.6		63 ± 12
	V	2 ± 17	1.8 ± 0.7	1 ± 10	-0.7 ± 2.5		83 ± 10
	Average	39 ± 12	2.0 ± 0.4	19 ± 7	3.9 ± 1.7		73 ± 6
1998-99	N	25 ± 14	3.3 ± 0.4	7 ± 4	-1.3 ± 1.9		21 ± 29
	S	48 ± 8	3.9 ± 0.3	12 ± 2	2.6 ± 0.8		32 ± 9
	V	47 ± 7	7.1 ± 1.1	7 ± 1	2.3 ± 0.7		18 ± 16
	Average	40 ± 6	4.8 ± 0.4	8 ± 1	1.2 ± 0.8		24 ± 11
2002-03	N	65 ± 22	5.0 ±1.4	13 ± 6	5.2 ± 2.1	4.2 ± 1.4	49 ± 7
	S	30 ± 5	3.2 ± 0.6	9 ± 2	2.6 ± 1.3	4.6 ± 1.2	26 ± 28
	V	85 ± 14	8.1 ± 1.2	10 ± 2	4.8 ± 1.2	4.0 ± 1.2	40 ± 7
	Average	64 ± 9	5.9 ± 0.8	11 ± 2	4.2 ± 0.8	4.2 ± 0.8	38 ± 9
2008-09	N	49 ± 1	1.7 ± 0.5	30 ± 9	7.7 ± 1.3	-6.7 ± 0	70 ± 8
	S	32 ± 17	4.1 ± 0.3	8 ± 4	2.7 ± 0.6	2.1 ± 1.0	51 ± 20
	V	22 ± 0.5	1.0 ± 0.5	22 ± 9	8.0 ± 2.0	1.8 ± 0.4	100 ± 0
	Average	35 ± 7	2.3 ± 0.6	15 ± 5	6.2 ± 1.3	0.3 ± 1.8	74 ± 11
2009-10	N	39 ± 8	6.2 ± 1.4	6 ± 2	-4.1 ± 4.5	3.6 ± 1.1	-15 ± 55
	S	31 ± 4	8.7 ± 2.9	3 ± 1	2.8 ± 0.4	2.0 ± 0.3	65 ± 10
	V	66 ± 18	13.1 ± 1.8	5 ± 2	5.4 ± 1.9	-0.0 ± 1.0	39 ± 14
	Average	46 ± 8	9.2 ± 1.5	5 ± 1	1.3 ± 1.9	1.8 ± 0.6	28 ± 21

Finally, we calculate a rough snapshot of nitrogen movement for each of the field trips (Figure 14). For each trip in Figure 14 we have presented both the high load year of 2007-2008 and the low load year 2008-2009, and the subsequent net nitrogen gain (or loss) from the system based on these extremes. This has been done because nitrogen loads enter the system in bursts over a long period, while the flux and recycling rates that are presented are from a single moment in time. These approximations show that in low flow years there is likely to be an overall loss of nitrogen from the system (a negative net gain of N), and in high flow years there is potential for a net gain of N. Overall, it seems that denitrification has the potential to remove all of the nitrogen inputs in an average year. Nitrogen recycling was an order of magnitude greater in the water column than in the sediment, except in March 2010, where DIN flux rates appear to be higher than water column nitrogen uptake rates; this suggests a net increase in water column DIN at this time.

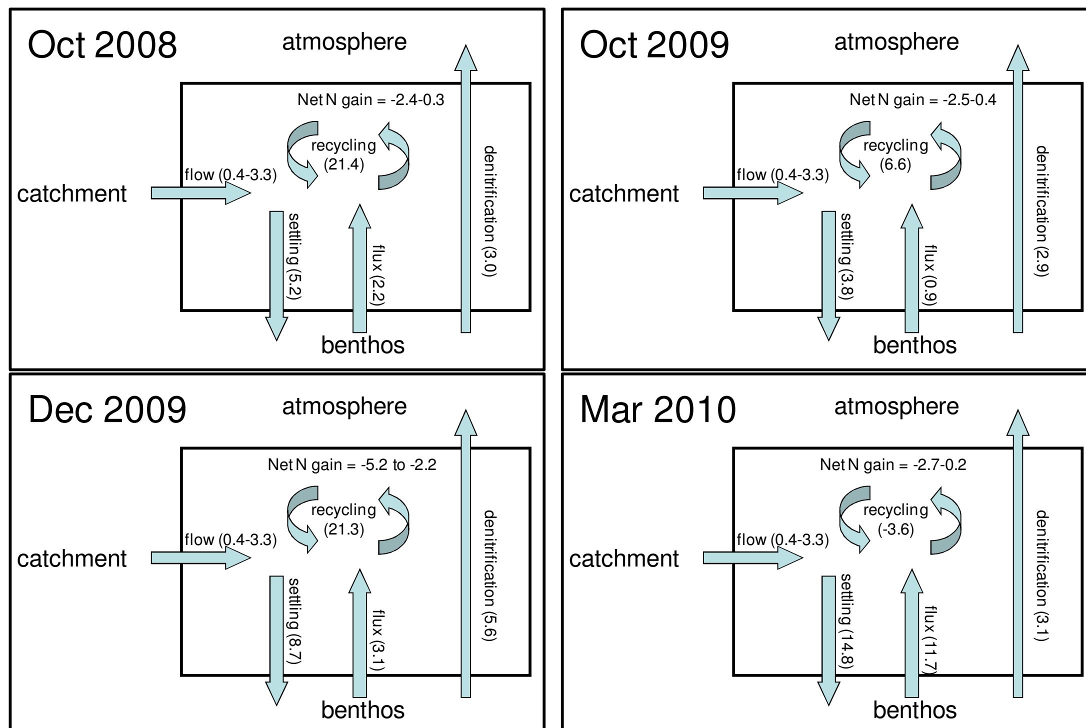

Figure 14. Nitrogen budgets for the Gippsland Lakes based on recycling, flux and denitrification rates calculated at the given date, and the minimum and maximum annual nitrogen loads for the period 2007-2009.The denitrification rate for March 2010 neglects the rate calculated for Lake King North as this leads to an unrealistic value of -3.9 mmol m^{-2} day^{-1}. Recycling rates are the nitrogen uptake rates minus the DIN flux rates; the negative recycling rate in March 2010 may indicate a disequilbrium between benthic flux and uptake at this time.

Phosphorus

The ultimate fate of phosphorus in the lakes is either to be buried in the sediment or to move out to the ocean. This makes phosphorus a much more persistent element, and hence has been of greater concern to managers of the Gippsland Lakes. The hydrologic year 1 June 2007 – 31 May 2008 saw the introduction of 870 Tonnes of phosphorus into the Gippsland Lakes, while the average annual input in the 20 years previous to this was 156 Tonnes.

FRP fluxes from the sediment were relatively low in the first three field trips, but were higher in March 2010, in agreement with the higher water column concentrations. It has been demonstrated that the sediment is the primary source of phosphorus for *Nodularia* blooms (Cook et al. 2010), and so the large addition of phosphorus in 2007-2008 (now in the sediment) may be available for future blooms if the right conditions occur. The results of this study in combination with our previous studies suggest that high respiration leads to increased phosphorus flux, so it may be that another winter flood, followed by a diatom and/or dinoflagellate bloom followed by a calm summer will lead to the mobilisation of this extra phosphorus, potentially increasing the magnitude of the *Nodularia* bloom.

Phytoplankton Growth – Bloom potential

From October 2009-March 2010 Lakes King and Victoria were once again dominated by low to moderate levels of diatoms and dinoflagellates, as they tend to be outside of cyanobacterial algal blooms. At the times that these experiments were

run, there was an equilibrium between nutrient supply and grazing, such that phytoplankton biomass was constant. The experimental addition of nitrogen and phosphorus led to an increase in both the growth rate and of the grazing rate, but under nutrient replete conditions the growth rate outpaced the grazing rate by approximately 2:1. Presumably over time the grazers would further adapt to the increased growth and the grazing rate would again balance or exceed the growth rate, but it is clear that influxes of nutrients give the phytoplankton a head start. It thus appears likely that bottom-up processes (benthic flux and nutrient loads) are the key drivers of bloom potential in this system, although top-down processes such as grazing may have the effect of minimizing the size and duration of blooms.

High rates of water column nitrogen recycling will prolong a bloom by reducing the supply of nutrients to the sediment, where denitrification occurs. The rates measured in this study provide a basis for future comparisons with recycling rates that could be measured during blooms of various taxa, such as diatoms, dinoflagellates, *Synechococcus* and *Nodularia*, which may then provide further insights into the dynamics of such blooms, and the overall biogeochemical processing within the lakes.

Risk of a *Synechococcus* bloom

It is clear that *Synechococcus* is, and will remain, a part of the phytoplankton community in the Gippsland Lakes. While it is difficult to draw conclusions from a single event, it is apparent that there is a risk of further *Synechococcus* blooms if conditions similar to those that occurred in 2007-2008 recur. Given that this bloom was sustained by large amounts of DIN, and that in a typical year most, if not all of the DIN that enters the lakes is removed via denitrification, then it will take another massive influx of nitrogen to initiate a similar bloom. The only possible avenue for this is influx is the combination of widespread fires followed by widespread flooding.

Risk of a *Nodularia* bloom

The evidence suggests that the lakes have returned to the point that they were prior to the fires and floods of 2007. The risk factors for *Nodularia* blooms therefore remain where they have been for the last 20-30 years, with one potential further risk: the fate of the massive load of phosphorus that entered the lakes following the bushfires is unknown. Any phosphorus held in the water column will slowly be removed via exchange with Bass Straight, but this is likely to be a slow process, and most of the phosphorus will instead be sediment bound, or dissolved in the pore water. This is therefore an additional source of phosphorus to that which was available prior to the fires, and may exacerbate any *Nodularia* blooms that occur in the near future.

Management implications and climate change

If the Gippsland Lakes become a marine dominated system, which could occur through a combination of rising sea levels and reduced freshwater inflows, this will clearly have implications for the future risk of algal blooms. Historically, *Nodularia* blooms occur when the lake surface water salinity is between 15 and 20 (Cook et al. 2008), and these conditions, along with suitable temperature, light and nutrients, will be unlikely to occur very often under this future scenario, where the salinity will be close to twice this range. There may be an increased risk of blooms of other, potentially problematic species under this scenario, but analyses of these possibilities is beyond the scope of the current study.

Denitrification efficiently removes nitrogen from the lakes, and is probably a key factor in keeping the lakes from becoming permanently hypereutrophic, such as they were following the fires and flood of 2006-2007, when the *Synechococcus* bloom occurred. The bottom waters of the lakes are already largely marine, and further changes in this direction are unlikely to change the denitrification potential of this system, although we advise vigilant monitoring.

While phosphorus is the nutrient that limits *Nodularia* bloom formation, our recent research found, somewhat ironically, that it is the nitrogen load that provides the initial trigger for a string of processes ultimately leading to a mass release of phosphorus from the sediment. Therefore, while long-term steps to reduce the load of phosphorus to the lakes will ultimately reduce the sediment phosphorus pool, these steps will have little effect in the short-medium term, and there is the risk that bushfires, which are inevitable, and likely to occur more frequently in the future, will negate any reduction in baseline phosphorus inputs. It may therefore be necessary to focus nutrient-reduction strategies on nitrogen as well as phosphorus.

References

Beardall, J. 2008. Blooms of *Synechococcus*: An analysis of the problem worldwide and possible causative factors in relation to nuisance blooms in the Gippsland Lakes. Monash University.

Cook, P. L. M., D. P. Holland, and A. R. Longmore. 2008. Interactions between phytoplankton dynamics, nutrient loads and the biogeochemistry of the Gippsland Lakes. Monash University, http://www.gippslandlakestaskforce.vic.gov.au/.

---. 2010. Effect of a flood event on the dynamics of phytoplankton and biogeochemistry in a large temperate Australian lagoon. Limnology and Oceanography **55:** 1123-1133.

Dugdale, R. C., and F. P. Wilkerson. 1986. The use of ^{15}N to measure nitrogen uptake in eutrophic oceans; experimental considerations. Limnology and Oceanography **31:** 673-689.

Grasshoff, K. 1983. Methods of Seawater Analysis, 2nd edition. Verlag Chemie.

Haese, R. R., E. J. Murray, C. S. Smith, J. Smith, L. Clementson, and D. T. Heggie. 2007. Diatoms control nutrient cycles in a temperate, wave-dominated estuary (southest Australia). Limnology and Oceanography **52:** 2686-2700.

Harris, G., G. Batley, I. T. Webster, R. Molloy, and D. Fox. 1988. Gippsland Lakes Environmental Audit: Review of water quality and status of the aquatic ecosystems of the Gippsland Lakes. CSIRO.

Hindell, J. S., and F. Y. Warry. 2009. Fish assemblages and seagrass condition of the Gippsland lakes. p. 43. Arthur Rylah Institute for Environmental Research, Victorian Department of Sustainability and Environment.

Holland, D. P., P. Cook, J. Beardall, and A. Longmore. 2009. Gippsland Lakes 'Snapshot' - nutrient cycling and phytoplankton population dynamics. Monash University Water Studies Centre.

Holland, D. P., S. C. Roberts, and J. Beardall. 2004. Assessment of the nutrient status of phyoplankton: a comparison between conventional bioassays and nutrient-induced fluorescence transients (NIFTs). Ecological Indicators **4:** 149-159.

Howarth, R. W., R. Marino, and J. J. Cole. 1988. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical controls. Limnology and Oceanography **33:** 688-701.

Jakob, T., U. Schreiber, V. Kirchesch, U. Langner, and C. Wilhelm. 2005. Estimation of chlorophyll content and daily primary production of the major algal groups by means of multiwavelength-excitation PAM chlorophyll fluorometry: performance and methodological limits. Photosynthesis Research **83:** 343-361.

Landry, M. R., and R. P. Hassett. 1982. Estimating the grazing impact of marine micro-zooplankton. Marine Biology **67:** 283-288.

Longmore, A. R. 2000. Gippsland Lakes sediment nutrient inventory., Marine and Freshwater Resources Institute Report No. 21. Fisheries Victoria, Victoria, Australia.

Longmore, A. R., G. J. Nicholson, and B. Abbot. 2001. Effects of water quality on black bream habitat in the Gippsland Lakes. p. 87. Marine and Freshwater Resources Institute.

Marino, R., F. Chan, R. W. Howarth, M. Pace, and G. E. Likens. 2002. Ecological and biogeochemical interactions constrain planktonic nitrogen fixation in estuaries. Ecosystems **5:** 719-725.

Marino, R., F. Chan, R. W. Howarth, M. L. Pace, and G. E. Likens. 2006. Ecological constraints on planktonic nitrogen fixation in saline estuaries. I. Nutrient and trophic controls. Marine Ecology-Progress Series **309:** 25-39.

Moisander, P. H., and H. W. Paerl. 2000. Growth, primary productivity, and nitrogen fixation potential of *Nodularia spp.* (cyanophyceae) in water from a subtropical estuary in the United States. Journal of Phycology **36:** 645-658.

Pomc. 2008. Nutrient cycling (denitrification) - detailed design - CDP ENV MD 019 Rev 1. www.channelproject.com. Port of Melbourne Corporation.

Roberts, S., B. T. Hart, D. P. Holland, A. Longmore, G. Nicholson, and B. Light. 2003. Algal blooms in the Gippsland Lakes: Assessment of the importance of sediment-bound nutrients. Technical report fo Coast and Clean Seas project.

Roberts, S., K. Shelly, and J. Beardall. 2008. Interactions among phosphate uptake, photosynthesis, and chlorophyll fluorescence in nutrient-limited cultures of the chlorophyte microalga *Dunaliella tertiolecta*. Journal of Phycology **44:** 662-669.

Stephens, A., N. Biggins, and S. Brett. 2004. Algal bloom dynamics in the estuarine Gippsland Lakes. Victorian Environmental Protection Agency.

Webster, I. T., J. S. Parslow, R. B. Grayson, R. P. Molloy, J. Andrewartha, P. Sakov, K. S. Tan, S. J. Walker, and B. B. Wallace. 2001. Gippsland Lakes Environmental Study: Assessing options

for improving water quality and ecological function. Gippsland Lakes Environmental Study. CSIRO.

Young, E. B., and J. Beardall. 2003. Rapid ammonium- and nitrate-induced perturbations to chl *a* fluorescence in nitrogen-stressed *Dunaliella tertiolecta* (Chlorophyta). Journal of Phycology **39:** 332-342.