



Gippsland Lakes ‘Snapshot’ – Nutrient Cycling and phytoplankton population dynamics

A report prepared for the Gippsland Lakes and Catchment Task Force

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Executive Summary

The continued presence of *Synechococcus* over winter of 2008 has led to concern about its persistence in the Gippsland Lakes. It has been hypothesised that the small size of this organism would prevent it settling out of the water column and would thus lead to an efficient recycling of nitrogen within the water column.

The following key points emerged from this field trip.

- Cells were nitrogen-limited, as indicated by NIFT bioassays. A large input of both nitrogen (N) and phosphorus (P) would however be required to trigger a bloom.
- Light was not a limiting factor.
- Zooplankton grazing exerted a strong control over *Synechococcus*, diatoms and dinoflagellates to the advantage of a small green alga.
- Benthic total carbon dioxide (TCO₂) fluxes are at historically low levels, possibly due to a lower than normal supply of organic matter. We ascribe this to a switch to relatively more recycling of organic matter in the water column (compared to the sediment) caused by the presence of small phytoplankton which settle out of the water column very slowly.
- Diatoms are the primary source of carbon (and nutrients) to the sediments. These organisms, therefore, play a critical role in nutrient removal from the water column.
- Total nitrogen (TN) is still high in the water column, and is being maintained by high water column recycling. At current rates of denitrification it is estimated that TN levels will return to background in 5 months time in the absence of any major inflows, and the phytoplankton will decline
- Phosphorus was strongly retained by the sediment rendering the chance of a *Nodularia* bloom low.

Introduction

Background

In November 2007 a cyanobacterial bloom occurred over the entire Gippsland Lakes and then persisted through much of 2008. 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.

In response to the *Synechococcus* bloom, 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.

The work reported here sought to answer the question: 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 a sediment dominated one to a water column dominated one, which means that nutrients and in particular nitrogen are retained in the system for a longer period.

Nutrient cycling in the Gippsland Lakes

Previous measurements of nutrient fluxes from the sediments within the Gippsland Lakes suggest that diatoms are important for the delivery of organic matter to the sediments 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 & 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 after the fires of 2006 and the floods of 2007, 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-N₂ 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.

Methods

Sites

Three sites were used (Figure 1): 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. These sites are regularly monitored by the EPA, and have previously been used for benthic chamber experiments.

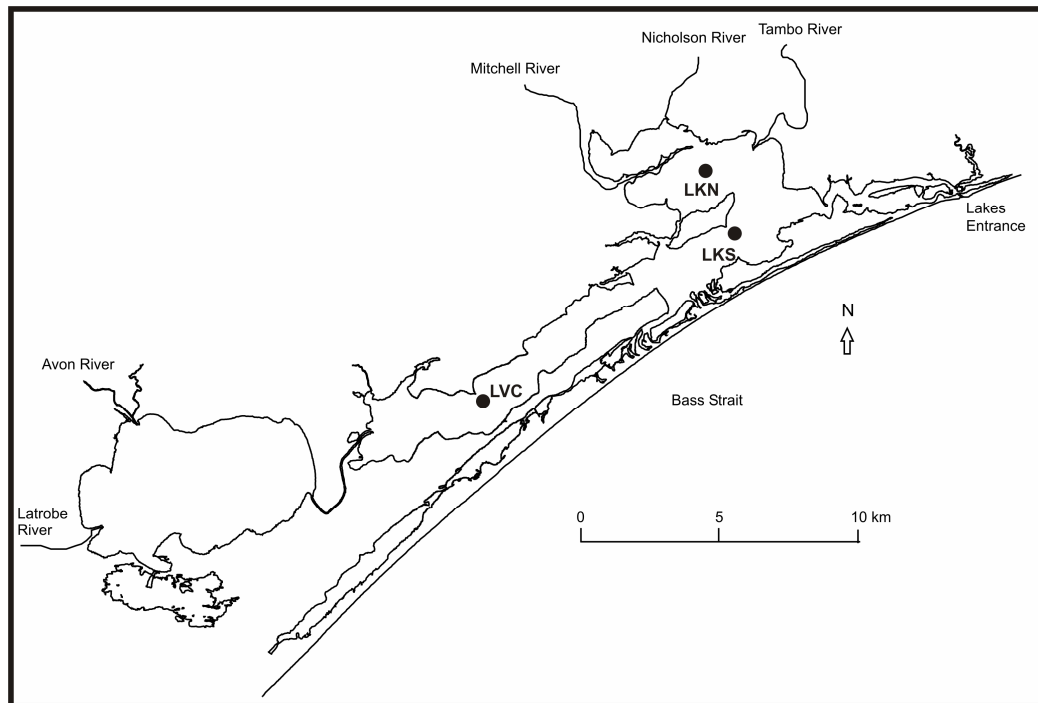


Figure 1. Map of the Gippsland Lakes, showing the location of the three sampling sites.

Zooplankton 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 & Hassett 1982). Plastic carboys (5 L) were filled with surface water at each of the three sites. Raw lake water was diluted with filtered site water (through 0.2 µm Supor filters) to a concentration of 0.05, 0.2 or 1.0 of the original sample. Triplicate 100 ml samples for each site and treatment were prepared in 150 ml Nalgene PETG (polyethylene terephthalate) bottles. The bottles were incubated in a temperature controlled water bath, held at 17 °C. This 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 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, although for a brief period each day (0.5-1 hr), direct sunlight would increase the incident light to approximately 500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. The experiment was run for 72 hours.

Growth was measured daily 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). Chlorophyll *a* was used as a proxy for biomass/productivity, with the assumption that chlorophyll *a* per cell would not change significantly, because ambient light and temperature were used.

In addition, samples were filtered onto Whatman GF/F filters at the start and end of the experiment for extractable chlorophyll *a* analyses, and at the end of the experiment 2 ml from each bottle was also preserved in Lugol's iodine, and stored for future enumeration and identification of the algae present.

Nutrient Induced Fluorescence Transients (NIFT)

Transient fluorescence signals 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.

Lake water (3 ml) was transferred to a glass cuvette and placed in the PhytoPAM. Fluorescence emission was recorded every 30 s at medium light (90 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and during a saturating pulse of light ($>400 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) 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, $\Phi_{\text{PSII}} = (F_t - F_m) / F_m$. 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 orthophosphate 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 & Beardall 2003). Fluorescence was then recorded for a further 10 min or longer, depending on whether a response was observed.

Lake Victoria water was thoroughly tested, because this site had the highest biomass, and was therefore considered more likely to be nutrient limited. Water from the other two sites was tested as time permitted. All measurements were performed within 8 hours of collection.

Photosynthesis-Irradiance (PI) curves

PI curves were measured using the Phytopam, where measurements of fluorescence excitation with increasing irradiance is 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 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$), and ran a separate experiment measuring the change in dissolved oxygen (DO) in a sealed 260 ml glass bottle 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} .

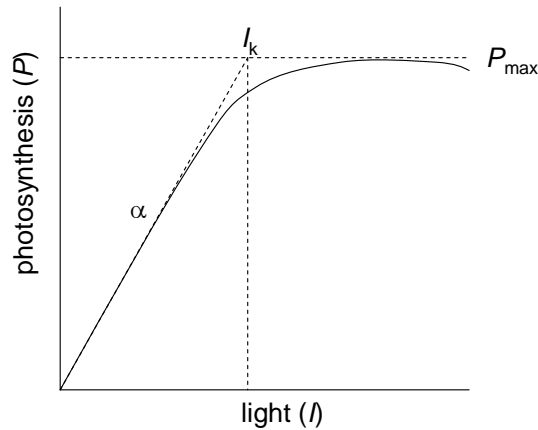


Figure 2. Stylised *PI* curve. I_k is the extrapolated irradiance where the linear portion of the curve would reach the maximum photosynthesis rate (P_{max}). α is the slope of this line (I_k/P_{max}).

Bioassay

Lake water samples (150 ml) were transferred to 200 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. Three different temperatures were also used. Treatments were as follows (in triplicate):

Temperature	+N	+P	+N+P	control
17 °C	✓	✓	✓	✓
23 °C	×	×	✓	×
10 °C	×	×	✓	×

Bottles were incubated in temperature controlled water baths (for the 17 °C and 10 °C treatments) or at ambient laboratory temperature (23 °C treatment; this was the temperature through most of the day although it varied from 21 °C to 24 °C due to heat from the lights), under a 14:10 hour light:dark cycle and illumination of approximately 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Chlorophyll *a* fluorescence was measured every three to four days using the Phytopam, and growth was followed until either a steady state or decline was observed (about three weeks). At the beginning and end of the experiment, samples were passed through GF/F filters, which were frozen for later chlorophyll *a* extraction.

Benthic flux (from cores)

The cores used were cylindrical polypropylene tubes of length 30 cm and internal diameter 6.7 cm. The tube was inserted into the sediment up to approximately half its length, with the remainder of the core containing overlying lake water. Four cores were collected from each site, and then transferred to a water bath held at ambient lake temperature (17 °C). A 5 L carboy of bottom water was also collected at each site, and this water was used to flush the cores, removing any water that may have been affected by the disturbance of core collection and transport. Cores were then allowed to settle overnight while air bubbled site water was circulated through them.

On the day after collection, each core was fully sealed. DO and pH were measured on four occasions over 5 hrs and at the same time water samples were collected for later analysis of NO_x , phosphate, ammonium and alkalinity.

On the second day after collection, the denitrification rate in the cores was measured using the ^{15}N isotope pairing technique (Dalsgaard et al. 2000).

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, later verified by measurement of caesium injected into the chamber at the start of the deployment. 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 ($0.45 \mu\text{m}$), frozen in the field, and analysed at the Fisheries Research Branch (FRB) 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. Caesium concentrations were measured using flame atomic absorption analysis. 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

Water samples were collected from the top and bottom of the water column at each of the three sites. These water samples (150 ml) were incubated for 4-6 hours after the addition of $0.1 \mu\text{M}$ of either $^{15}\text{NO}_3^-$ or $^{15}\text{NH}_4^+$. Surface samples were incubated in the light at between 100 and $200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, while bottom samples were kept in the dark. A second set of bottom samples from Lake Victoria were kept in low light ($\sim 10 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) as this site was shallower than the Lake King sites, and light would reach the bottom during the day. 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

Caution: The chlorophyll a fluorescence is only a relative measure until properly calibrated by comparison with extracted chlorophyll a measured by standard spectrophotometric methods. There was insufficient biomass in most samples for the accurate measuring of chlorophyll a using acetone extraction, so a full calibration could not be attained. Those samples that did contain enough biomass for accurate extraction analysis did, nevertheless, correlate with the fluorescence data approximately 1:1. Chlorophyll a fluorescence is therefore presented in $\mu\text{g L}^{-1}$, but with the proviso that these results are not entirely quantitative.

Likewise, the deconvoluted taxon data (Green, Brown and Cyan) are based on standard reference spectra pre-loaded onto the PhytoWIN software, and may not be precisely representative of the fluorescence spectra of the relevant groupings in the samples being analysed for this report.

Phytoplankton community

Chlorophyll *a* was elevated at all three sites (background levels at this time of year are typically 1-2 $\mu\text{g L}^{-1}$), and was highest in Lake Victoria, at almost 8 $\mu\text{g L}^{-1}$, which was twice as high as LKN, and 50% higher than LKS (Table 1). F_v/F_m was approximately 0.6 at all sites. Typically, F_v/F_m values greater than 0.6 are considered to come from populations that are not overly stressed, however the values vary widely between different taxa, and without a baseline measurement for the dominant species, this value is difficult to interpret. Further measurements of this value over the coming months will provide good information on whether the population is becoming more or less healthy.

Table 1. Phytoplankton in the Gippsland Lakes as measured using the Phytopam.

Site	Surface Chl <i>a</i> ($\mu\text{g/L}$)	F_v/F_m	% Green
LKN	3.58 \pm 0.04	0.603 \pm 0.007	91
LKS	4.52 \pm 0.10	0.613 \pm 0.003	100
LVC	7.76 \pm 0.06	0.597 \pm 0.009	96

The most significant and unexpected aspect of the community was that it appeared to be dominated almost entirely by green algae. This finding has since been confirmed (personal communication 19 Nov 2008, Jonathon Smith, consultant and Guillaume Martinez, Vic EPA). The species present is similar in size to *Synechococcus* (~1 μm diameter) and can therefore not be easily identified or counted under a light microscope.

Sedimentation rates

The sinking rate (u) of small spherical objects falling in a viscous medium (e.g. phytoplankton cells in water) can be calculated using the Stokes Equation (Stokes 1901).

$$u = \frac{2r^2\Delta\rho}{9\eta}$$

r is the radius of the object, $\Delta\rho$ is the excess density (the difference between the density of the cell and the water) and η is the viscosity of the water. Small phytoplankton cells typically

have buoyant densities up to approximately 1100 kg m^{-3} (Reynolds 1984); we use this value as a conservative upper boundary. Assuming that the water contains 25 g L^{-1} NaCl, at $17 \text{ }^\circ\text{C}$ the water density will be $\sim 1025 \text{ kg m}^{-3}$ and the viscosity will be $\sim 1.10 \times 10^{-3} \text{ kg m}^{-1} \text{ s}^{-1}$. The *Synechococcus* cells reported in the Gippsland Lakes are $1\text{-}2 \text{ }\mu\text{m}$ diameter (as are the current green algal cells), so taking a conservative upper boundary we will make the radius $1 \text{ }\mu\text{m}$. The maximum likely sinking velocity of single cells is therefore $1.5 \times 10^{-8} \text{ m s}^{-1}$, or 1.3 mm day^{-1} . What this essentially means is that *Synechococcus* cells are neutrally buoyant and will never sink out of the water column, unless large aggregations occur. In contrast, diatom cells are generally much larger, $>20 \text{ }\mu\text{m}$ diameter, and a spherical cell of this diameter and the same density as above will sink 100 times faster, i.e. 130 mm day^{-1} . Diatoms are generally denser than cyanobacteria and will often form long filaments (Reynolds 2006), increasing their potential sinking rate to 1 m day^{-1} or greater.

Zooplankton grazing

An experimental manipulation of grazing indicated that significant grazing pressure currently exists in the lakes. In all cases, samples with the biggest dilution (and hence the smallest grazing pressure) exhibited the highest growth rate (Figure 3). Consistent growth was also seen in the 0.2 dilution, whereas samples grown with no manipulation showed little growth; the LKS and LVC samples in fact showed reduced chlorophyll *a* after 72 hours (Figure 3). The approximate contribution to the total chlorophyll pool of green algae (chlorophytes), ‘brown’ algae (diatoms and dinoflagellates) and blue-green algae (cyanobacteria) changed significantly over the course of the incubation, especially in the diluted samples, with an increase in the proportion of Brown and Cyan signals over the 72 hour incubation. This indicates that perhaps the grazing community had a preference for, and was therefore suppressing, phytoplankton taxa other than the dominant green alga. It is interesting to note that in the diluted LVC samples, there was little or no growth in the green algae, but considerable growth in both the Cyan and Brown channels (Figure 3). In this case removing grazers did not appear to affect the green algae, but it enhance growth of the other two taxa,. This suggests that zooplankton grazing of the green algae may be inconsequential, whereas grazing of the other taxa appears to be high.

The increase in total chlorophyll *a* (used as a proxy for growth rate) was calculated in each case, and a regression line was used to estimate the growth rate in the absence of all grazing (Figure 4). The theoretical growth rate in the absence of grazers is the y-intercept (i.e. the growth rate at $0 \times$ dilution), and the grazing rate is the gradient of the regression line. LVC, which had the highest initial biomass, had the slowest potential growth rate of 0.2 day^{-1} , whereas the Lake King sites had potential growth rates between 0.3 and 0.4 day^{-1} . One possible explanation for this discrepancy is that LVC samples may have their growth rates suppressed by other factors, such as lack of nutrients. Grazing rates were similar to growth rates in undiluted LKS and LVC samples and this explains the lack of growth in these samples. The grazing rate in LKN samples was around 0.28 , compared to a growth rate of 0.38 , leaving a positive growth rate of 0.1 in undiluted samples. While the maximum growth rates (the y-intercept) should be more or less independent of nutrient availability, it is likely that the grazing rate (the slope) is over-estimated, as a proportion of the suppressed growth in the undiluted samples was likely due to nutrients running out, although the strong linear fits in Figure 4 suggest that this effect may be small over the time period measured.

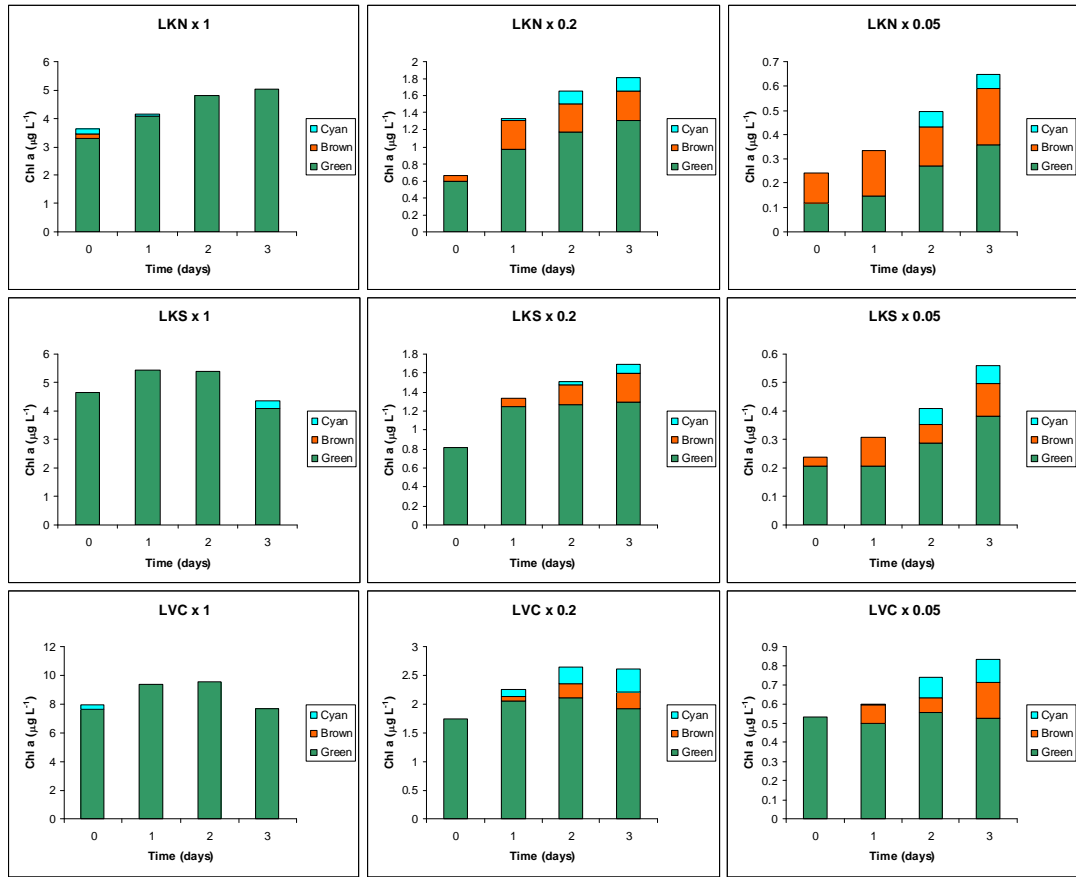


Figure 3. Increase in chlorophyll *a* fluorescence in samples diluted with site water in order to reduce grazing pressure. The number next to the site name indicates the dilution (e.g. 0.2 is equivalent to 20 ml raw site water diluted with 80 ml filtered site water). The different coloured bars represent the amount of chlorophyll *a* associated with the three main phytoplankton groupings, as indicated.

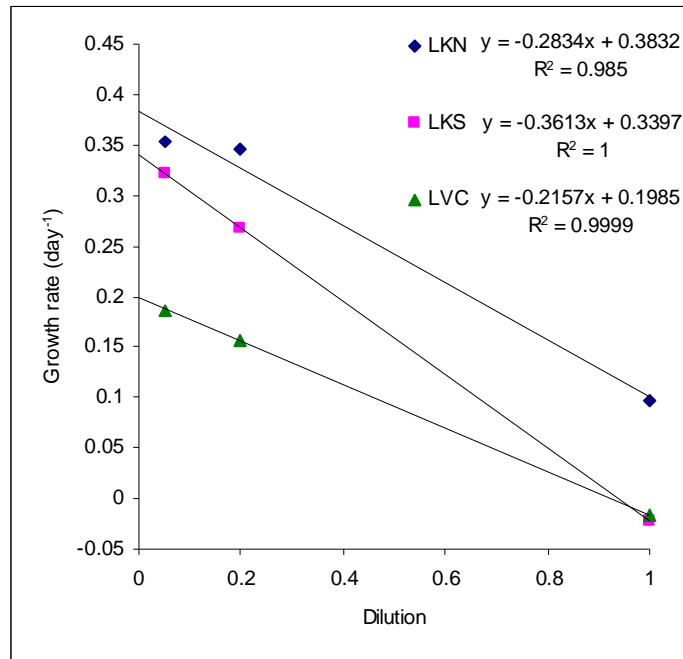


Figure 4. Growth rate (increase in total chlorophyll *a* fluorescence) at three different dilutions. The y-intercept of regression lines through the data for each site indicates the theoretical growth rate under zero grazing pressure.

Nutrient Induced Fluorescence Transients

No NIFT was observed with the addition of nitrate, orthophosphate or water (2 attempts of each on LVC water). The addition of ammonia, on the other hand, produced unambiguous NIFTs on three tests on LVC water on two separate days (Figure 5). This response was, however, only observed in the outputs from the 645 nm and 665 nm excitation channels on the PhytoPAM, wavelengths that correspond to the phycocyanin excitation peak (characteristic of cyanobacteria), and one of the chlorophyll *b* peaks (characteristic of green algae), respectively. The larger signals at 470 nm and 520 nm showed no response. No response was seen when ammonia was added to samples from LKS or LKN.

These results suggest that the LVC phytoplankton population is nitrogen limited, and that the population is pre-adapted to take-up ammonia rather than nitrate. If the phytoplankton are using ammonia as their major source of nitrogen, then their ammonia uptake mechanism may react fast when a pulse is added, leading to the NIFT response seen. The uptake mechanism for nitrate is different and may not be active in these cells.

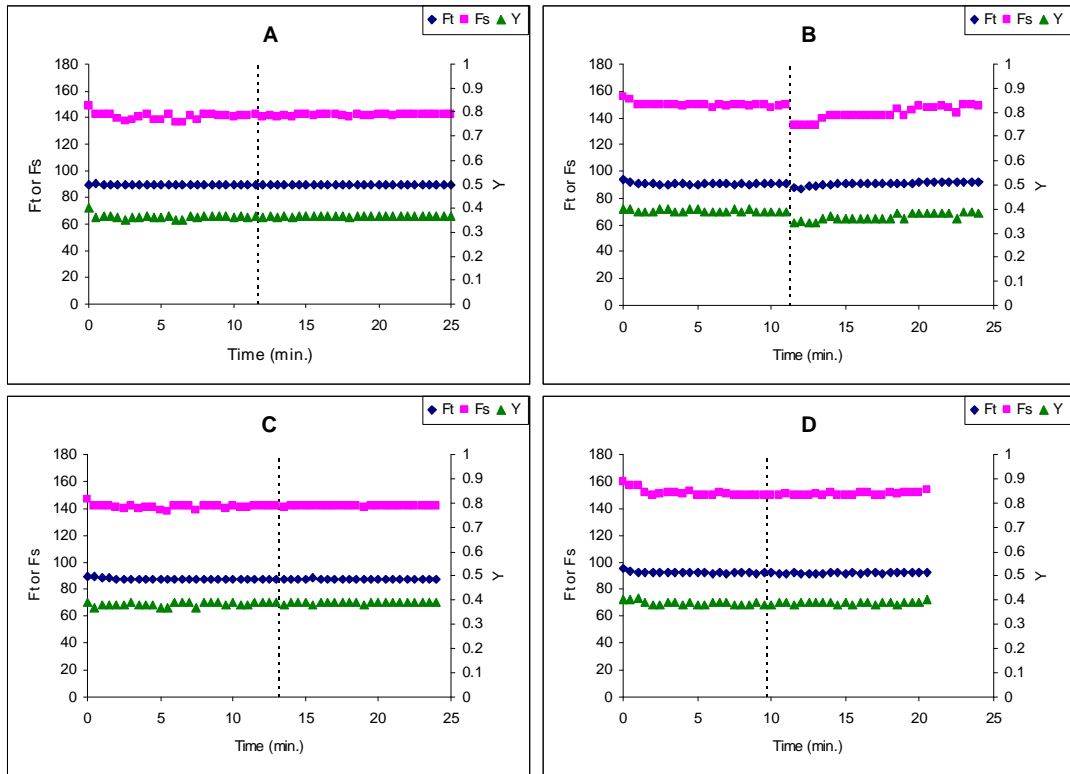


Figure 5. Fluorescence response (at 620 nm excitation) to nutrient addition of LVC surface water: a) nitrate; b) ammonia; c) phosphate; d) water. F_t is the variable fluorescence signal (under $1 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ irradiance), F_s is the fluorescence signal immediately after a flash of saturating light (at $> 400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ irradiance) and Y is the quantum yield (Φ_{PSII}) calculated from $(F_t - F_i)/F_s$. 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 similar for each site, in the range $0.4\text{-}0.6 \text{ mmol O}_2 \text{ L}^{-1} \text{ day}^{-1}$ (Table 2). The productivity of LVC water was only slightly higher than that of LKN water, even though it had nearly twice the chlorophyll *a*. The other *PI* parameters were also similar among sites. The samples had maximum photosynthesis rates at an irradiance of $300\text{-}400 \mu\text{mol photons m}^{-2}$, and values of the light saturation parameter, I_k (see Figure 2 for definition) were high, $\sim 170 \mu\text{mol photons m}^{-2}$, indicating that these populations were adapted to high light (Table 2). I_k in the LVC bottom water, while still relatively high, was lower than in the surface, indicating adaptation to slightly lower light levels (Table 2). These results indicate that light is not likely to be a limiting factor in phytoplankton growth in the lakes.

Table 2. Photosynthesis-Irradiance parameters for surface water for three sites in the Gippsland Lakes, plus bottom water from site LVC only. Standard Errors are indicated (n=3 for surface waters and n=1 for LVC bottom). O₂ production rates and hence α were not measured in LVC bottom water.

Site	Max. O ₂ production rate mmol L ⁻¹ day ⁻¹	α m ² .(mmol O ₂) L ⁻¹ photon ⁻¹	I_k μmol photons m ⁻² s ⁻¹
LKN	0.53 ± 0.12	0.036 ± 0.008	170 ± 3
LKS	0.43 ± 0.07	0.029 ± 0.005	172 ± 2
LVC	0.57 ± 0.06	0.037 ± 0.004	178 ± 4
LVC bottom			138

Bioassay

Similar results were observed for the three sites (Figure 6). Samples incubated at the higher temperature (23 °C with added nitrogen and phosphorus) grew rapidly early in the incubation, but then decreased rapidly so that by the 21 day mark the chlorophyll fluorescence was close to where it was at time zero. A similar trend was observed in the 17 °C treatment with added nitrogen and phosphorus, although the rise was slower and the decrease less pronounced. In both cases the rise and then decrease occurred earlier in the LVC samples than in those from Lake King. The population crash seen in the nutrient replete treatments may have been a result of all of the added nutrients being used up, when the chlorophyll *a* reached 20-25 μg L⁻¹. Failure of a waterbath within the first five days of the 10 °C treatment forced the abandonment of this experiment, although all samples had shown a decrease in chlorophyll fluorescence over this period (data not shown).

The control and plus phosphorus treatments all showed an initial decline over the first week of the incubation and then no further change. The plus nitrogen treatment tracked the control and plus phosphorus treatments for the first 10 days (i.e. no change) and then began to grow, reaching levels almost on par with the plus N and P samples, especially in the LKS samples (Figure 6). This data indicates that N is more limiting than P, but that both are 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 indicates that no nitrogen fixation is occurring in the water column at this time. It is apparent that without an increase in external nutrient supply (from either the catchment or benthos), the phytoplankton are unlikely to increase in biomass over the summer, but that a flush of nutrients could cause a rapid increase. The data from the four channels of the PhytoPAM indicated that most of the phytoplankton throughout the incubation were green algae, and that the green alga that dominated the lake fauna at the time of collection probably remained dominant throughout the experiment.

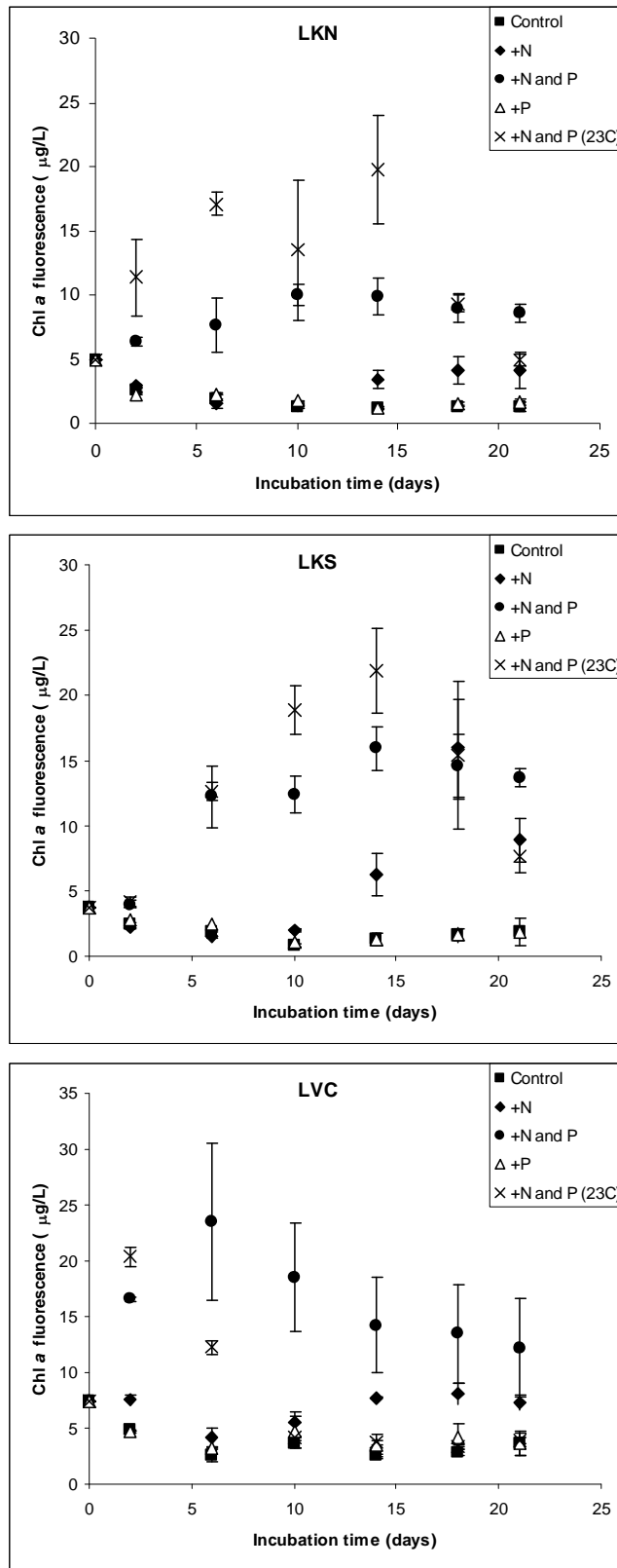


Figure 6. Results of a nutrient enrichment bioassay conducted on Gippsland Lakes surface water samples. Treatments are as indicated, and results include standard error bars.

Water column nutrients

Samples collected at the same time as this study was conducted (Table 3) show that filterable reactive phosphorus (FRP) levels were low, being at or near the detection limit (0.03-0.06 μM), whereas ammonia and NO_x concentrations were more elevated (0.6-1.1 μM) and were nearly equal. TN and TP were much higher (>30 and >1.6 μM respectively), indicating that most of the water column nutrients were in organic forms, either dissolved, detrital or incorporated into plankton. The elemental ratios of TN:TP and DIN:FRP suggest that nitrogen may be slightly in excess compared to phosphorus relative to that required by phytoplankton growing at Redfield proportions. Four weeks prior to this study, DIN was lower whereas FRP was higher, with DIN:FRP clearly indicating nitrogen limitation. Between the 2nd and 30th of October, TN at all sites dropped by approximately 7 μM , whereas TP did not change. While the TN:TP ratios suggest slight N-limitation, the fact that TN was dropping while TP was not suggest that nitrogen was becoming limiting if not already so.

Table 3. Surface water nutrient concentrations (μM) and N:P elemental ratios. Water column sampled on 30/10/08.

Site	NH_4^+	NO_x	FRP	TN	TP	DIN:FRP	TN:TP
30/10/08							
LKN	0.79	0.71	0.03	30.0	1.6	47	19
LKS	0.71	0.64	0.06	30.7	1.6	21	19
LVC	1.07	0.79	0.06	50.0	2.9	29	17
2/10/08							
LKN	0.29	0.36	0.06	37.1	1.3	10	29
LKS	0.21	0.21	0.10	37.4	1.6	4	23
LVC	0.21	0.43	0.19	57.1	2.9	3	20

Benthic flux

We calculated fluxes of total CO_2 , O_2 , NO_x , phosphate, ammonia and silicate from the change in water column concentration of benthic cores kept at a constant 17 °C in low light (<5 $\mu\text{mol photons m}^{-2} \text{ s}^{-2}$) over approximately 5 hrs, and from benthic chambers deployed *in situ* for approximately 18 hours.

There were some differences between the fluxes measured in the chambers compared to the cores, with considerably higher ammonia and phosphate fluxes in the chambers, especially at LKS and LKN, and considerably lower respiration (measured as TCO_2) at LVC (Figure 7).

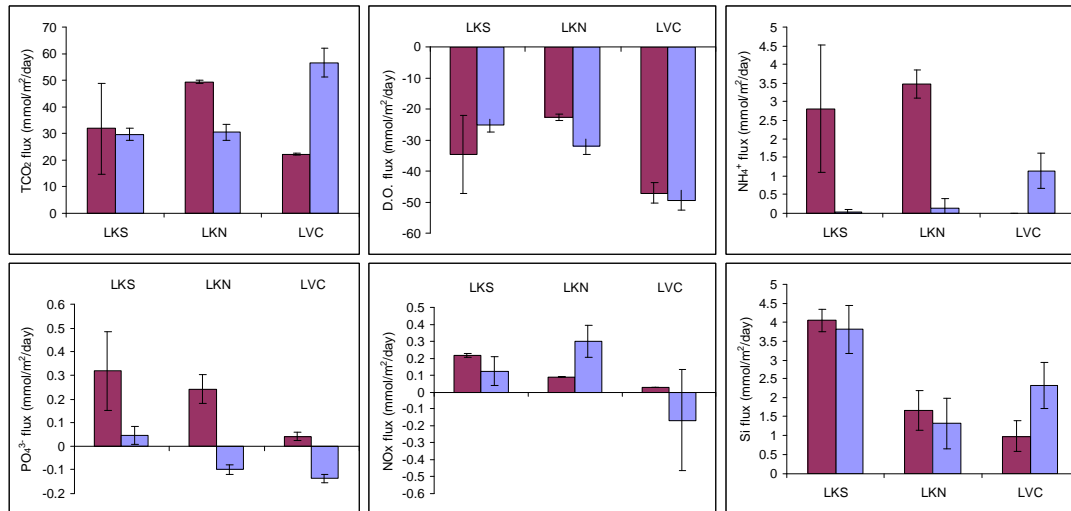


Figure 7. Nutrient exchange between the benthic sediment and overlying water column in *in situ* chambers (plum) or cores (blue) collected from three sites in the Gippsland Lakes, with one standard error. Each graph shows a different chemical, as indicated on the y-axis.

MAFRI have deployed benthic chambers in the lakes in November or December on three other occasions (1997, 1998 and 2002). The DO, NO_x and SiO₄ fluxes for 2008 are similar to previous years. TCO₂ fluxes are lower than in previous years, and ammonia and phosphate fluxes in the *in situ* chambers are at the low end, and in the cores they are an order of magnitude smaller in the 2008 samples than in the three other years (Table 4). The C:P ratio of the fluxes measured in the chambers in this study was 175, which indicates a net storage of P within the sediments when compared with the expected “Redfield” C:P flux ratio of 106C:1P. This compares with a similar net storage observed in 1997 when benthic TCO₂ fluxes were also low, and no net storage in 1998 and 2002 when the TCO₂ fluxes were high. The observations are consistent with the paradigm that an oxidising sediment will store more P than a reducing sediment. In previous years, TCO₂:SiO₄ was approximately unity with the Redfield ratio of diatoms (15:1), which we suggested was due to the majority of nitrogen and silicate released from the sediment being due to the breakdown of diatom blooms. In 2008, this ratio was maintained, even though the water column was dominated by green algae and cyanobacteria. This supports our hypothesis that while ‘heavy’ organisms such as diatoms will sink out of the water column (to be broken down in the sediment) the small green and cyanobacterial cells will be broken down within the water column.

Given the rather large variability in the flux data, we can at best suggest that nitrogen and phosphorus fluxes and sediment respiration are lower and that the sediments are more oxic than at the same time in previous years, but the confidence with which we make these claims is not high. There are clearly some non-systematic differences between the fluxes measured using *in situ* chambers compared with cores, and until these differences are reconciled, it would be unwise to rely on the results of core incubations alone.

Table 4. Mean (+S.E.) benthic flux rates ($\text{mmol m}^{-2} \text{ day}^{-1}$) for Lakes Victoria and King combined, measured in November or December in four different years. Core and chamber results are presented separately for 2008.

Date	TCO ₂	DO	NH ₄ ⁺	NO _x	PO ₄ ³⁻	SiO ₄	TCO ₂ : DIN	DIN :FRP	TCO ₂ : FRP	TCO ₂ : SiO ₂
Dec 1997	49 ± 8	-13 ± 14	4.0 ± 1	-0.01 ± 0.00	0.21 ± 0.06	2.8 ± 0.45	12	19	233	18
Nov 1998	71 ± 6	-26 ± 2	6.5 ± 0.8	-0.03 ± 0.03	0.64 ± 0.16	6.1 ± 1.7	11	10	110	12
Nov 2002	71 ± 19	-40 ± 5	4.3 ± 1.9	-0.01 ± 0.03	0.58 ± 0.29	5.4 ± 2.3	17	7	122	13
2008 core	40 ± 5	-37 ± 4	0.5 ± 0.2	0.08 ± 0.12	-0.07 ± 0.03	2.4 ± 0.5	72	-8	-571	17
2008 cham.	35 ± 7	-35 ± 6	2.1 ± 0.8	0.11 ± 0.04	0.20 ± 0.07	2.2 ± 0.6	16	11	175	16

Denitrification rates were calculated directly from cores using the isotope pairing technique, but there were inconsistencies within and between cores that made these rate calculations unreliable. Approximate denitrification rates were instead calculated from the expected nitrogen flux (based on a stoichiometric ratio of carbon to nitrogen of 6.625:1, Table 5).

In the summer of 2007-8, the TN in Lakes King and Victoria peaked at approximately 100 μM on the 22nd of February. If we assume that denitrification rates were constant throughout (at 3.0 $\text{mmol m}^{-2} \text{ day}^{-1}$, see Table 5), then denitrification should have removed 750 mmol m^{-2} nitrogen in the 250 days up until the sampling trip for the current study. Given that Lake King has a surface area of 98 km^2 and a mean depth of 5.4 m, and Lake Victoria has a surface area of 75 km^2 and a mean depth of 4.8 m (Webster et al. 2001), this denitrification rate would reduce the water column nitrogen by 146 μM , or 0.58 μM per day. The average TN in the lakes on 30/10/08 was around 40 μM (see Table 3). Therefore, either the average denitrification in the lakes is lower than this by approximately 50% or nitrogen is being added to the system. The average annual riverine TN load to the system is equivalent to $\sim 1 \text{ mmol m}^{-2} \text{ d}^{-1}$, but given the relatively low flows throughout this period we suggest that this is a maximum for TN inputs over this period. Assuming that all nitrogen loss is through denitrification, the nitrogen cycle would be balanced with a lakewide denitrification rate of approximately 2.5 $\text{mmol m}^{-2} \text{ day}^{-1}$ (Figure 8). This is within 1 standard error of the rate measured in the chambers (Table 5). This rate is also consistent with the drop in water column TN of 7 μM over the month of October (Table 3). If this rate were to continue and inputs of nitrogen continued at $\sim 1 \text{ mmol m}^{-2} \text{ d}^{-1}$, then the current water column nitrogen would essentially disappear in approximately 5 months (138 days). The increased nitrogen loads from the late November 2008 floods will obviously delay any reduction in nitrogen.

Other sources of N could include N₂-fixation and precipitation, and the other major sink for N is loss to Bass Strait. Incorporation of these elements is beyond the scope of this study but would be a requirement of a formal nutrient budget for the lakes.

Table 5. Mean (+S.E.) denitrification rates ($\text{mmol m}^{-2} \text{ day}^{-1}$) calculated by stoichiometric balance, the isotope pairing technique or direct measurement of N_2 flux (chambers). Efficiency calculated from the stoichiometric technique.

Date	Denit. (stoichiometric)	Denit. (measured)	Denitrification efficiency
Dec 1997	3.4 ± 0.9		47 ± 10
Nov 1998	4.2 ± 0.6		38 ± 5
Nov 2002	6.5 ± 1.4		68 ± 10
2008 core	5.5 ± 0.7	8.1 ± 5.8	91 ± 4
2008 cham.	3.0 ± 0.5	3.7 ± 0.5	63 ± 12

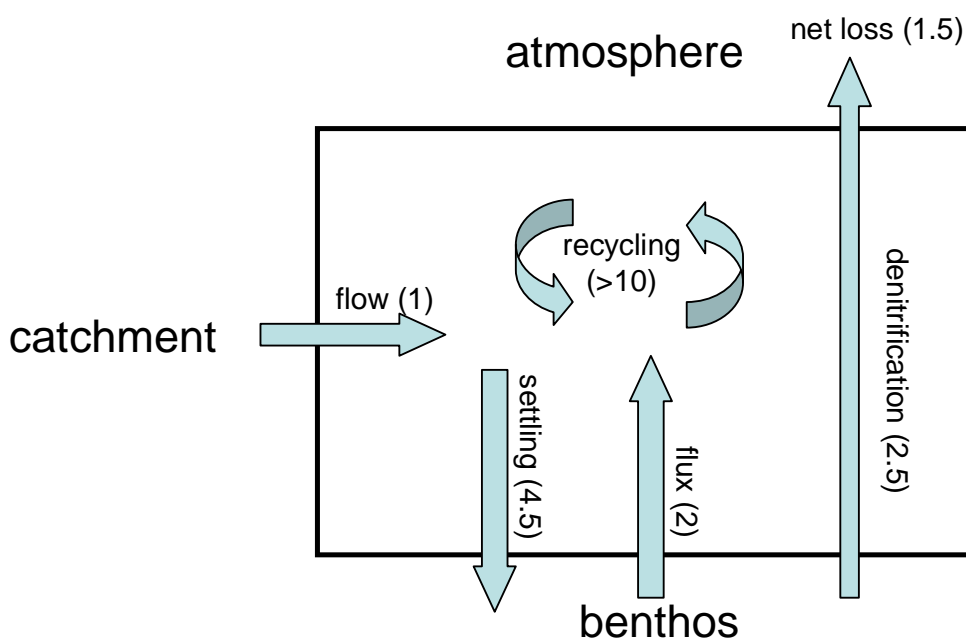


Figure 8. Simplified nitrogen cycle for the Gippsland Lakes, where the only inputs are catchment derived and the only loss is denitrification. C:Si ratios imply that the settling organic matter is largely diatoms. The numbers in brackets indicate the measured or theoretical rates of movement of N (in $\text{mmol m}^{-2} \text{ day}^{-1}$), as described in the text. Under this scenario, there is a net loss of $1.5 \text{ mmol N m}^{-2} \text{ day}^{-1}$.

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 intermediate at low light (Figure 9). Uptake of nitrate was slightly greater than the uptake of ammonia. The results from the different sites were quite similar, apart from LVC-ammonia which was approximately 1/3 of the LKS and LKN.

Taking the average of the light (surface) and dark (bottom) results (or in the case of LVC, the average of the light, dark and dim results) gives a rough approximation of the 24 hour uptake rates through the water column (Table 6). The disparity between benthic flux and uptake rates must be from either new nitrogen entering the system, the depletion of water column

dissolved nitrogen, or from regeneration of dissolved nitrogen from particulate nitrogen in the water column. If we assume a steady state (conditions were stable at the time of sampling, flows into the system were low and there were no nitrogen fixing cyanobacteria present), then the disparity would be caused almost entirely by regeneration, i.e. recycling of nitrogen within the water column. Greater than 75% of the ammonia and almost all of the nitrate that was taken up by plankton would therefore have come from the degradation of other plankton. An addition of $1 \text{ mmol N m}^{-2} \text{ day}^{-1}$ from the catchment would make little difference to these recycling rates (Figure 8).

It is interesting that ammonia uptake was lower than nitrate uptake in LVC, considering that the NIFT experiment indicated that ammonia was the most readily taken up. Likewise, the surface water ammonia and NO_x concentrations were almost identical (Table 3), and in this situation we would expect ammonia to be preferentially taken up, because it requires less energy to incorporate ammonia into cellular material than NO_x . Certain species of phytoplankton may, however, have a preference for nitrate over ammonia (Dortch 1990), and this would explain the result of the uptake experiment, but not the discrepancy with the NIFT experiments. Insufficient information is known about the physiology of the dominant species at the time of this study and therefore further work would be required for a definitive explanation of this discrepancy to be made.

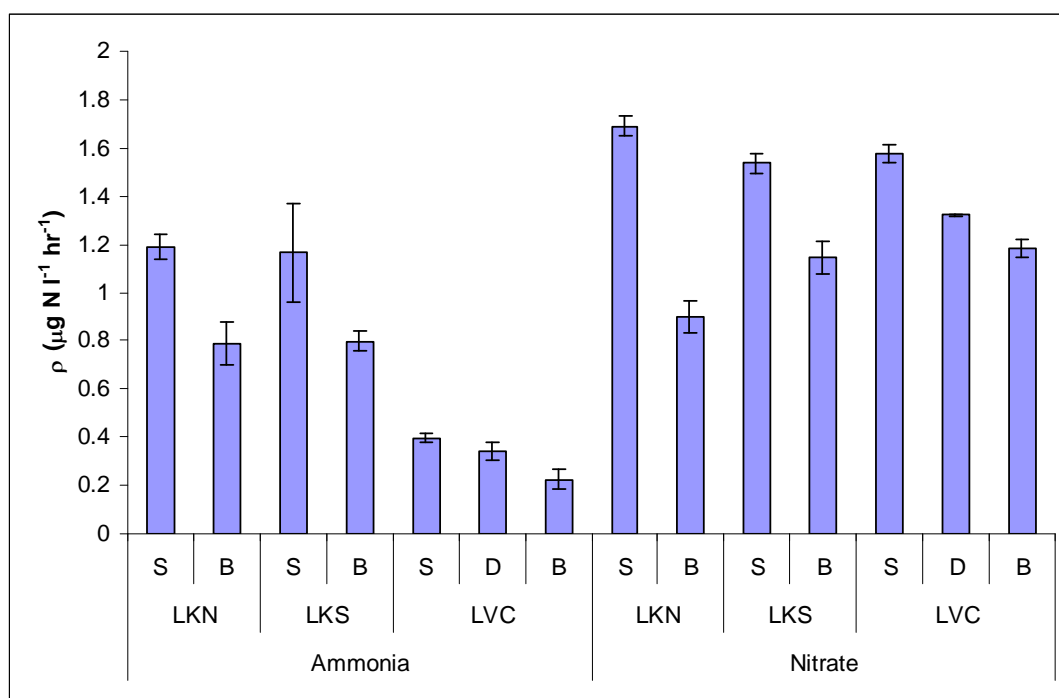


Figure 9. Uptake of ^{15}N -labelled ammonia and nitrate on water from three sites on the Gippsland Lakes (with standard error bars). S represents surface water, incubated in the light, B is bottom water incubated in the dark, and D is bottom water incubated in low light.

Table 6. Nutrient uptake in the water column integrated through the water column. Units are mmol m⁻² day⁻¹, including one standard error.

Site	NH ₄ ⁺ uptake	NH ₄ ⁺ benthic flux	NH ₄ ⁺ water column regeneration	NO ₃ ⁻ uptake	NO ₃ ⁻ benthic flux	NO ₃ ⁻ water column regeneration
LKN	11.9 ± 0.6	3.5 ± 0.4	8.4	15.6 ± 0.5	0.98 ± 0.02	14.6
LKS	13.5 ± 1.4	2.8 ± 1.7	10.7	18.4 ± 0.5	0.04 ± 0.00	18.4
LVC	2.2 ± 0.1	-0.02 ± 0.03	2.2	9.3 ± 0.1	0.01 ± 0.00	9.3

General discussion

Both nitrogen and phosphorus are in somewhat short supply in the Gippsland Lakes, with nitrogen being the most immediately limiting nutrient. Most of the nitrogen and phosphorus available for growth is probably already locked up in algal biomass, and supply of nitrogen and phosphorus from the sediment is low compared with other years. Thus without a significant input of ‘new’ nutrients from the catchment, phytoplankton biomass seems unlikely to increase significantly. Likewise, if the negative flux of phosphate seen in the benthic cores is indicative of the lakes as a whole, a bloom of N₂-fixing cyanobacteria such as *Nodularia* is unlikely. Grazing is a significant source of phytoplankton mortality, and may be selectively suppressing diatoms, dinoflagellates and cyanobacteria.

This data provides no evidence to suggest that *Synechococcus* will again dominate the phytoplankton over the coming summer, as the bioassay run at summer temperatures showed continued dominance of the currently unidentified green species. There are, however, many unknown factors, and small bottle experiments, whilst they can give a general indication of immediate nutrient limitation, are not representative of the ecosystem as a whole.

The high flow event in a large part of the catchment at the end of November 2008 is a cause for concern. A flush of extra nutrients combined with high concentrations of organic matter and lake-wide stratification could potentially lead to a situation where benthic processes again dominate, leading to a high phosphorus-low nitrogen environment conducive to *Nodularia*. If, however, this flush of nutrients is largely consumed by the picoplankton, these will not sink out of the water-column and the benthos will remain relatively benign. A situation similar to the summer of 2007-8 will occur, with a high phosphorus-very high nitrogen environment conducive to *Synechococcus*. The latter appears to be occurring, with the EPA data from the 17th of December showing a marked increase in picoplanktonic cyanobacteria. We advise close monitoring of the nutrients and processes throughout the coming summer (2008-9) as this may be a key period for the lakes, and information collected at this time will inform future predictive capacity and indicate future research directions.

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