Targeted monitoring of algal bloom potential in the Gippsland Lakes 2010-2011

A report prepared for the Gippsland Lakes and Catchment Task Force

Gippsland Lakes Algal Bloom Assessment

13 July 2011

Daryl Holland[1,2], Perran Cook[2] and John Beardall[1,2]

[1]School of Biological Sciences, Monash University, Clayton 3800, Victoria

[2]Water Studies Centre, Monash University, Clayton 3800, Victoria

Table of Contents

Table of Contents 2
Executive Summary 3
Introduction 4
Methods 5
Sampling regime 5
Chlorophyll fluorescence 6
Bioassays and Grazing experiments 6
Loggers 7
Results 7
In situ monitoring data 7
Weather 12
Nodularia 12
Bioassays – Lake King South 13
Grazing 13
Lake Surveys 16
Nitrogen fixation 17
Discussion 18
What stopped a major *Nodularia* bloom? 18
What role do grazers play in *Nodularia* growth? 18
What is the limiting nutrient? 19
Conclusion 19
Acknowledgements 20
References 20

Executive Summary

Following a detailed review of water quality data in the Gippsland Lakes, we proposed a critical sequence of events that leads to summer *Nodularia* blooms. Winter/spring floods bring nutrients (primarily nitrogen) into the lakes which lead to a bloom of diatoms and dinoflagellates. Through a cascading series of biogeochemical and ecological events (see Cook et al. 2008) this bloom may transition into a summer *Nodularia* bloom. At present our understanding of this process is based on a retrospective analysis of data, and has many associated uncertainties. We presently lack detailed understanding on which nutrient (N versus P) is limiting, how this changes from winter to summer and the role this plays in the development of *Nodularia*. Grazing has also been proposed as a major control over cyanobacterial growth, yet there are no measurements of grazing and the interaction with *Nodularia* growth in the Gippsland Lakes.

The present study was initiated following a large inflow event in September 2010, which we speculated could lead to a *Nodularia* bloom. Continued rain throughout spring led to ideal growth conditions for *Nodularia* in December (low surface salinities, strong stratification and high concentrations of phosphorus relative to nitrogen). This led us to warn of the very high probability of a *Nodularia* bloom that summer. In February, a *Nodularia* bloom did develop (fortunately minor) which gave us an unprecedented insight into the role of nutrients, grazing and climate in *Nodularia* bloom development. Key findings include:

- In agreement with low nitrogen:phosphorus ratios in the water column, bioassays showed that phytoplankton were co-limited by N and P immediately following the September flood, and subsequently transitioned to N limitation until February. The appearance of *Nodularia* in February marked a transition to P limitation, when *Nodularia* could out-compete other algal species due to their ability to fix N_2 from the atmosphere.
- Bioassays showed that maximum potential phytoplankton biomass occurred in early spring and steadily declined into summer. This was caused by a concomitant steady increase in grazing rates from spring through to summer. *Grazing thus plays a significant role in controlling maximum phytoplankton biomass in the Gippsland Lakes.*
- Grazing was shown to be highly selective for diatoms and dinoflagellates over *Nodularia*, giving *Nodularia* a competitive advantage. *We conclude that grazing plays an important role in the transition from diatoms and dinoflagellates to* Nodularia *in summer*.
- The addition of nitrogen was shown to inhibit *Nodularia* growth, most likely as a consequence of this treatment being advantageous to faster growing, but non-N_2 fixing, taxa such as dinoflagellates. This observation is consistent with our hypothesis that higher nitrogen loads (caused by extensive bushfires in the catchment) in the 2007 floods led to a *Synechococcus* (non-N_2 fixing species) bloom rather than a *Nodularia* bloom occurring.
- *Nodularia* growth in control bioassays (no N or P addition) was significantly higher than in the field. The *in situ* weather conditions in February were unusually cool, overcast and wet, and we hypothesise that this was the key factor that prevented a major *Nodularia* bloom.

Introduction

Nodularia spumegina is a toxic, filamentous cyanobacteria. It has a cosmopolitan range, occurring in certain brackish, coastal waters and saline inland lakes in northern Europe, Australia and North America (Bolch et al. 1999). *Nodularia* filaments contain heterocysts, cells that are specialised at fixing atmospheric nitrogen (N_2) into bioavailable forms such as nitrate (NO_3^-) and ammonium (NH_4^+) (Adams and Duggan 1999). This ability to fix nitrogen gives *Nodularia* a distinct competitive advantage when nitrogen levels are low and other nutrients, such as phosphorus, are plentiful (Mazur-Marzec et al. 2006). The other adaptation that *Nodularia* has is its ability to produce akinetes, which are dormant cells that can remain in the sediment for many years (Yamamoto 1975), before germinating when conditions are right (Huber 1985).

In the Gippsland Lakes, *Nodularia* blooms occurred sporadically between 1988 and 2002 (Cook and Holland 2011), but between 2003 and the start of 2011 there was no bloom, and, moreover, *Nodularia* was almost completely absent from the lake phytoplankton (it was observed in a single sample in 2007 and again in 2009, and minor amounts were observed during the *Synechococcus* bloom of 2008, but otherwise nothing – J. Smith, pers. comm., 20/7/2011).

Work undertaken by Monash University and the Department of Primary Industries' Fisheries Research Branch over the past two years has resulted in a detailed hypothesis on the processes resulting in cyanobacterial blooms in the Gippsland Lakes (Cook and Holland 2011, Cook et al. 2010, Holland et al. 2010). The primary trigger in almost all cases is a period of high river inflows with large nitrogen inputs in winter or spring.

The lack of *Nodularia* blooms during the period 2002-2010 is attributed to the long-term drought in this region. Inflows of water – and thus nutrient loads – were low throughout this period, apart from 2007, when large winter and spring inflows occurred following the widespread fires that burnt 60% of the catchment in the summer of 2006-2007. Rather than leading to a *Nodularia* bloom, these two events (fire and flood) combined to cause an extensive and persistent bloom of *Synechococcus*. The primary reason for this is hypothesised to be the unprecedented input of dissolved inorganic nitrogen (DIN) into the system. DIN remained elevated throughout the summer of 2007-2008, giving the small, fast growing *Synechococcus* a competitive advantage over the relatively slower growing, nitrogen-fixing *Nodularia* (Cook et al. 2010).

There was some concern at the time that the *Synechococcus* bloom and the persistently high DIN concentrations would change the lakes to a permanently eutrophic state, where phytoplankton would dominate while seagrass and other in-lake macrophytic vegetation would die off. This did not eventuate, since 2008 and 2009 were low flow years, and by the summer of 2009-2010 the lakes had returned to a similar state to that they were in prior to the fires and floods of 2007 (Holland et al. 2010). Denitrification within the sediments was the major contributor to the removal of water column DIN and a return to the typical nitrogen limited system (Holland et al. 2010).

Beginning in the winter of 2010, a strong La Niña event in the Pacific Ocean, coupled with a strongly negative Indian Ocean Dipole led to widespread and persistent rainfall over eastern Australia (http://www.bom.gov.au/climate/enso/archive/ensowrap_20100929.pdf). The Gippsland Lakes received large inflows in late August 2010, followed by regular rainfall events throughout the spring

and following summer. This spring rainfall provided a potential trigger for a summer *Nodularia* bloom, and a study was initiated to answer the following questions:

- Did the flood waters of August 2010 provide a non-limiting amount of nutrients for phytoplankton growth?
- Does the observed nutrient limiting growth of phytoplankton agree with the nutrient limitation implied by *in situ* nutrient concentrations?
- Are phytoplankton blooms controlled solely by nutrient availability, or does grazing play a major role?

Methods

Sampling regime

The lakes were visited fortnightly between September 2010 and April 2011. The following sampling and experiments were undertaken:

- Nutrient collection (surface and bottom) and water column profiles (pH, salinity, temperature, turbidity and dissolved oxygen) at two sites, Lake King South (LKS) and Lake King North (LKN) – EPA sites 002314 and 002316 (Figure 1). Dissolved inorganic nitrogen (DIN), filterable reactive phosphorus (FRP), total nitrogen (TN) and total phosphorus (TP) concentrations were measured in a NATA accredited analytical laboratory.
- Surface water was collected (in 20 L or 5 L carboys) for experimental manipulation at LKS and on two occasions at a number of other sites (see below).
- Nutrient addition bioassays.
- Grazing experiments.
- Multi-channel loggers (measuring temperature, pH, salinity, dissolved oxygen (D.O.) and chlorophyll-*a* fluorescence) were attached to the channel markers closest to LKS and LKN.
- When Nodularia appeared (from the 2nd of February onwards), additional assays were undertaken
- Nitrogen fixation was calculated at the end of the bioassays.
- On the 2nd of March, bioassays were conducted on water collected from eight sites around the lakes – Eagle Bay (EB), Lake King North (LKN), Mid-Lake King (LKM), Lake King South (LKS), Lake King West (LKW), Newlands Arm (NA) and McMillan straight (MS). See Figure 1.
- On the 16th of March, bioassays were conducted on water collected from Eagle Bay (EB), Lake King North (LKN), Mid-lake King (LKM) and Lake King South (LKS). See Figure 1.

Additionally, weather information (maximum daily temperature, daily rainfall and monthly mean rainfall) was obtained from the Bureau of Meteorology (http://www.bom.gov.au/), and river flow data was obtained from the Victorian Water Resources Data Warehouse (http://www.vicwaterdata.net/).

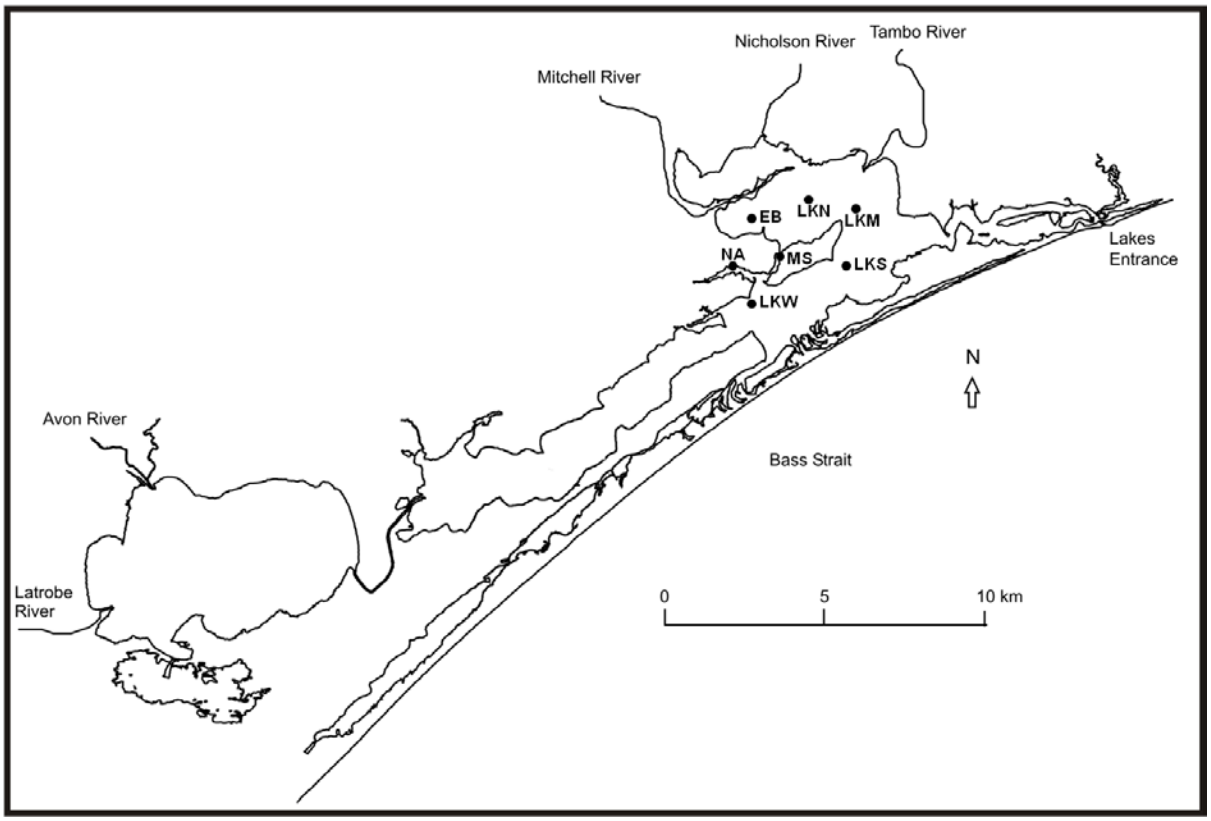

Figure 1. Map of the Gippsland Lakes, with the Lake King sampling sites marked.

Chlorophyll fluorescence

Chlorophyll-*a* was used as a proxy for biomass, and was estimated using a non-destructive fluorometric approximation (Jakob et al. 2005), in a Phytopam Phytoplankton Analyzer (Heinz Walz, GMBH, Germany) connected to a PC running PhytoWIN software. This device allows the fluorescence output to be deconvoluted into three major phytoplankton groups: Green (Chlorophytes), Brown (diatoms and dinoflagellates) and Cyan (cyanobacteria). This deconvolution process 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.

The total chlorophyll-*a* calculated by the Phytopam was calibrated against samples filtered for extractible chlorophyll-*a*. Representative samples were filtered onto Whatman GF/F filters at the start and end of each experiment, and the chlorophyll-*a* was extracted in 90% acetone and analysed spectrophotometrically.

Bioassays and Grazing experiments

Nutrient additions were 100 µM ammonium (N) and 10 µM phosphate (P). Four treatments were used: C (control), N (just N added), P (just P added) and A (both N and P added).

Grazing pressure can be reduced by diluting the lake samples with filtered water (Landry and Hassett 1982). Unfiltered lake water was diluted with filtered lake water (filtered through Whatman GF/F filters) to a concentration of 5% or 20% of the original sample. For each concentration, C and A treatments were prepared.

Triplicate 100 ml samples for each treatment were prepared in 150 ml Nalgene PETG bottles. Bottles were incubated in a temperature controlled water bath at ambient site temperature ±1 °C, under an ambient light:dark cycle (adjusted for the time of year) and illumination of approximately 100 µmol photons m^{-2} s^{-1}. Chlorophyll *a* fluorescence was measured every 1-3 days using the Phytopam.

Growth was followed for between seven and thirteen days. In many cases, total chlorophyll *a* began to decline after five days, so growth rates for total chlorophyll *a* were calculated from the total chlorophyll signal over the first five days. For determining growth rates of *Nodularia*, the rate was calculated over the whole incubation, as the *Nodularia* chlorophyll was very low to begin with, and rose exponentially over the whole incubation.

The dominant grazer populations were identified via microscopy.

Loggers

Hydrolab DS5X multi-probe loggers were placed in the lakes at the start of December and set to log temperature, pH, salinity, dissolved oxygen (D.O.) and chlorophyll *a* fluorescence at 15-30 minute intervals. These were attached to channel markers in Lake King South and Lake Kind North, approximately 500 m from the deep water sampling sites used for other measurements. The loggers were swapped with clean and freshly calibrated loggers every two weeks.

Results

In situ monitoring data

The surface water temperature steadily increased until December, and remained above 19 °C through to March at both sites (Figure 2 and Figure 3).

The water column at both LKS and LKN was salinity stratified throughout this study, with high salinity bottom waters (>30 [NB: salinity was calculated on the practical salinity scale, which is a unit-less ratio, although it is often approximated to mg L^{-1} or ppt NaCl]) and medium salinity surface waters (between 15 and 22). The bottom waters at LKN were anoxic throughout the summer, while at LKS the bottom waters remained oxic apart from two periods, in mid December and again in mid February (Figure 2 and Figure 3). The DIN and FRP concentrations in the bottom waters were elevated at times of anoxia (Figure 4 and Figure 5).

Surface chlorophyll-*a* peaked in mid January and again in March at around 10 µg L^{-1} at both sites (Figure 2 and Figure 3). Chlorophyll was spread throughout the water column, and often was at the highest concentration at between 2 and 5 metres deep (e.g. Figure 6).

Surface water FRP was high, at ~1 µM throughout January and February, while DIN was low, also around 1 µM. The DIN:FRP ratio was thus approximately 1:1, which indicates extreme nitrogen limitation, given that phytoplankton require these nutrients at the Redfield ratio, which is 16:1 (Figure 4 and Figure 5).

The surface TN:TP ratio was consistently close to the Redfield ratio of 16:1 from December onwards, indicating that most of the nitrogen in the water column was contained in phytoplankton (Figure 4 and Figure 5).

There was a distinct drop in both TN and TP at both sites (surface and bottom) between March 2 and March 15. This coincided with a distinct decrease in surface chlorophyll *a*, and increase in bottom water chlorophyll *a*, suggestive of a loss of phytoplankton to the sediment (Figure 6)

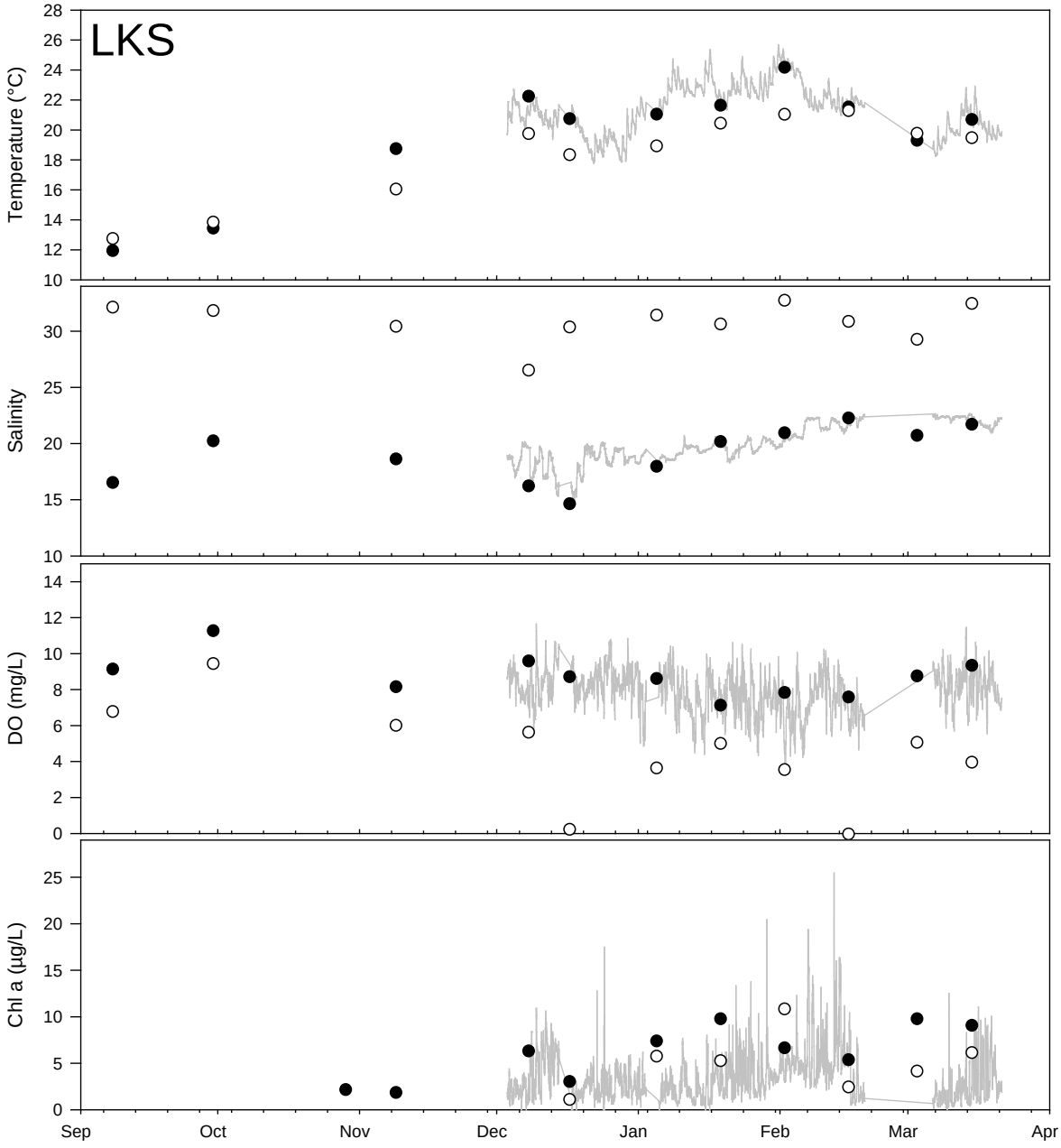

Figure 2. ***In situ*** **measurements of temperature, salinity, dissolved oxygen (DO) and Chlorophyll** ***a*** **in the bottom (open symbol) and surface (closed symbol) waters of Lake King South. The continuous lines are data from** ***in situ*** **loggers recording at 15-30 min intervals.**

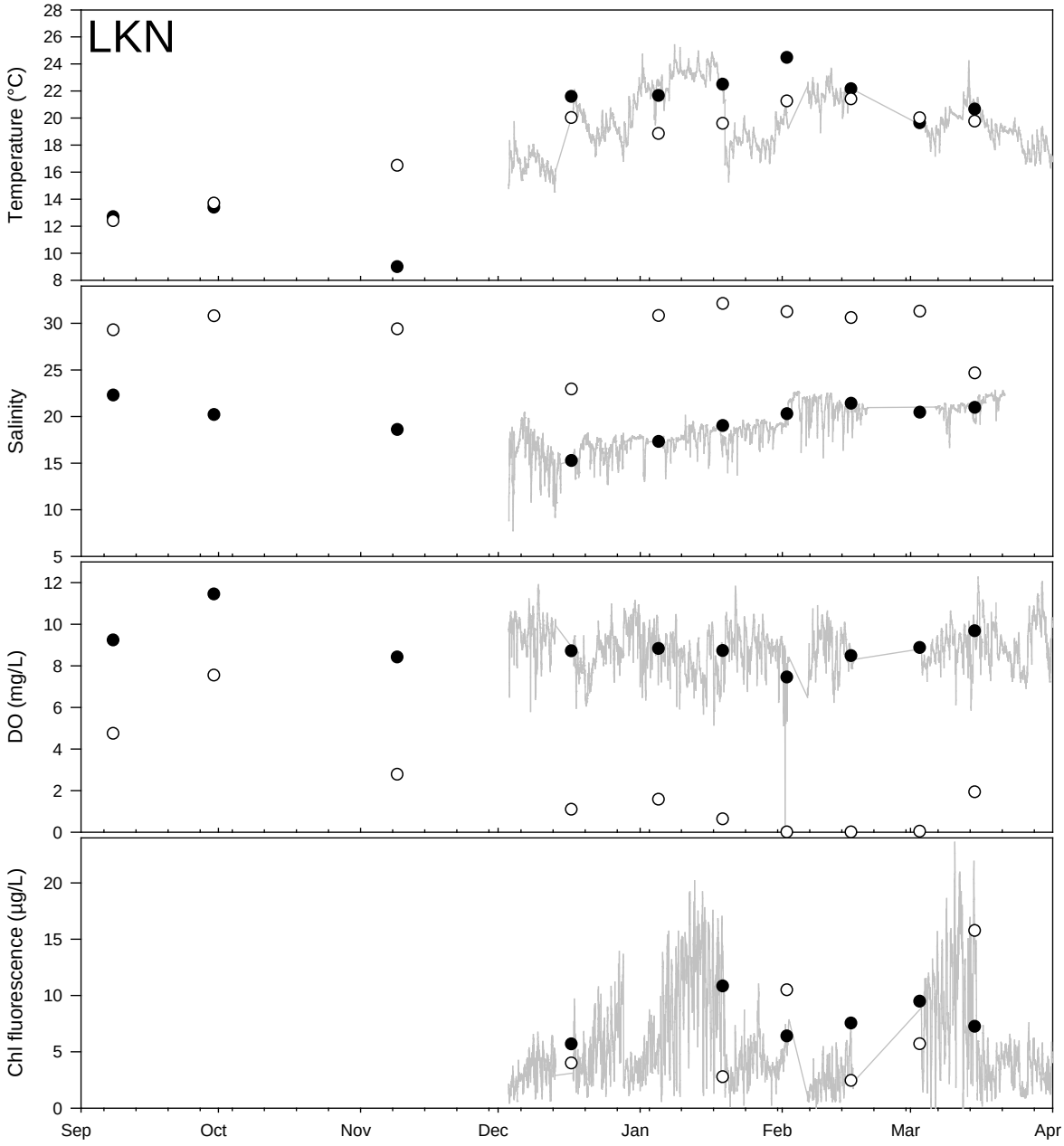

Figure 3. ***In situ*** **measurements of temperature, salinity, dissolved oxygen (DO) and chlorophyll** ***a*** **in the bottom (open symbol) and surface (closed symbol) waters of Lake King North. The continuous lines are data from** ***in situ*** **loggers recording at 15-30 min intervals.**

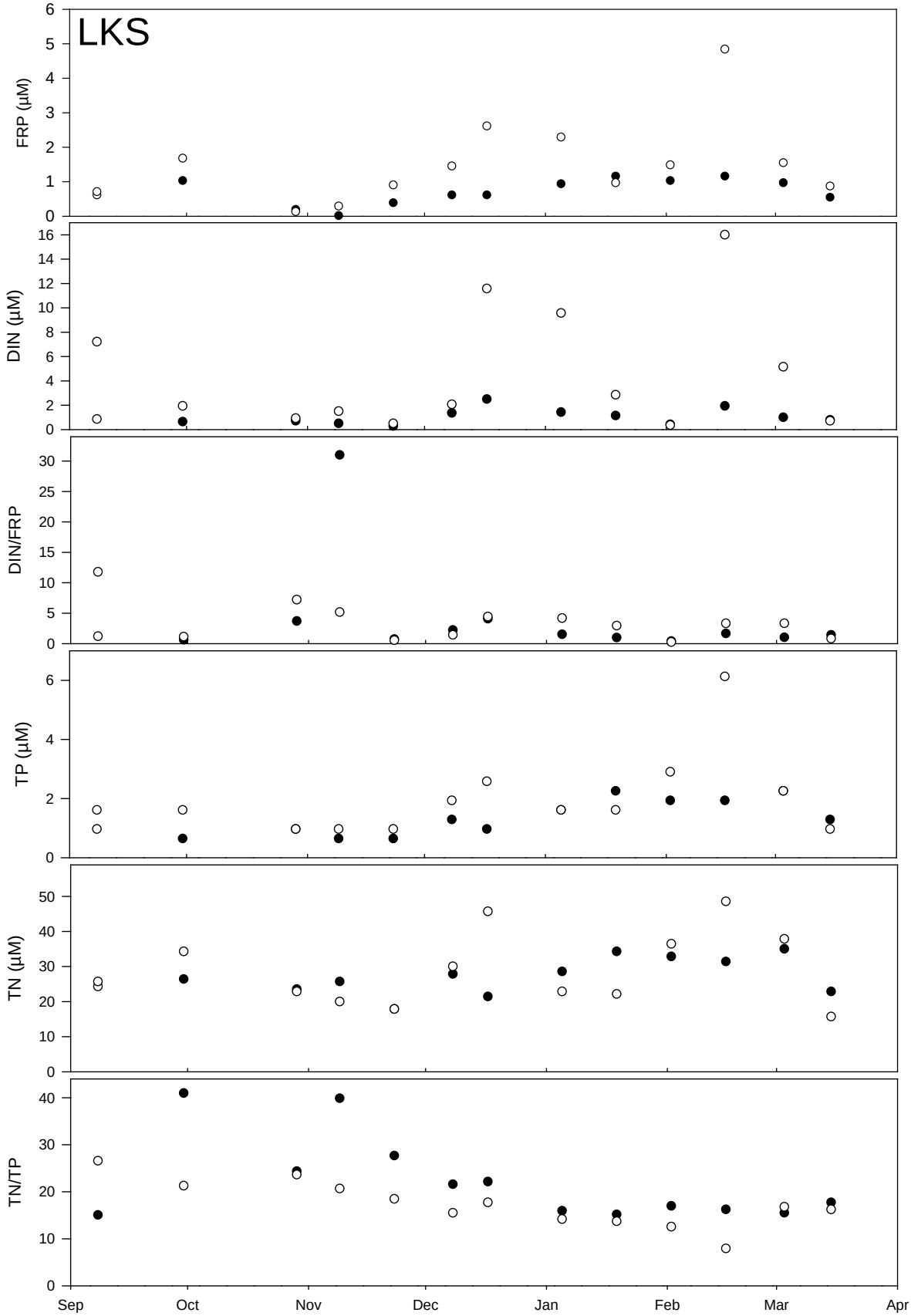

Figure 4. ***In situ*** **nutrients in the bottom (open symbols) and surface (closed symbols) water of Lake King South.**

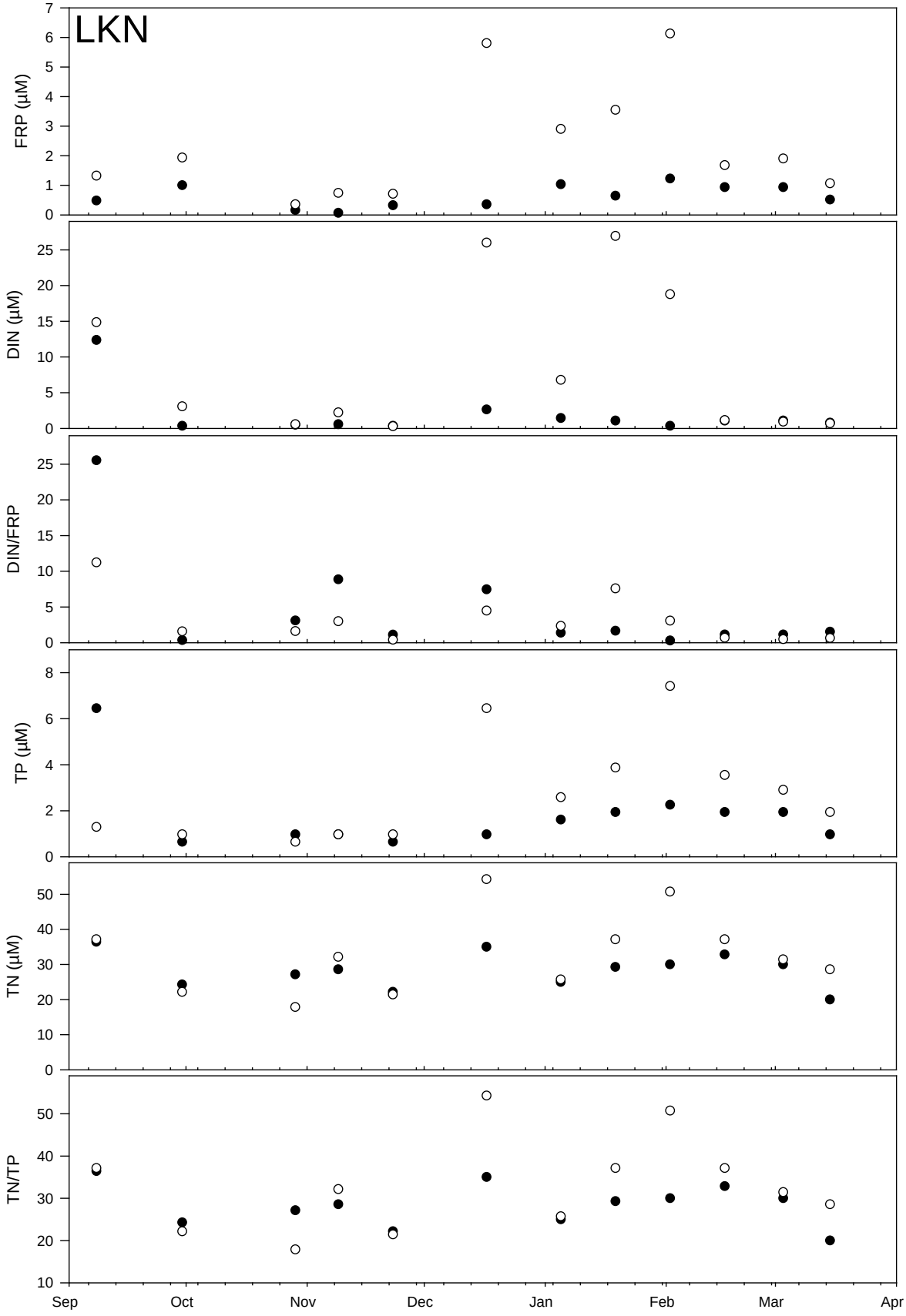

Figure 5. ***In situ*** **nutrients in the bottom (open symbols) and surface (closed symbols) water of Lake King North.**

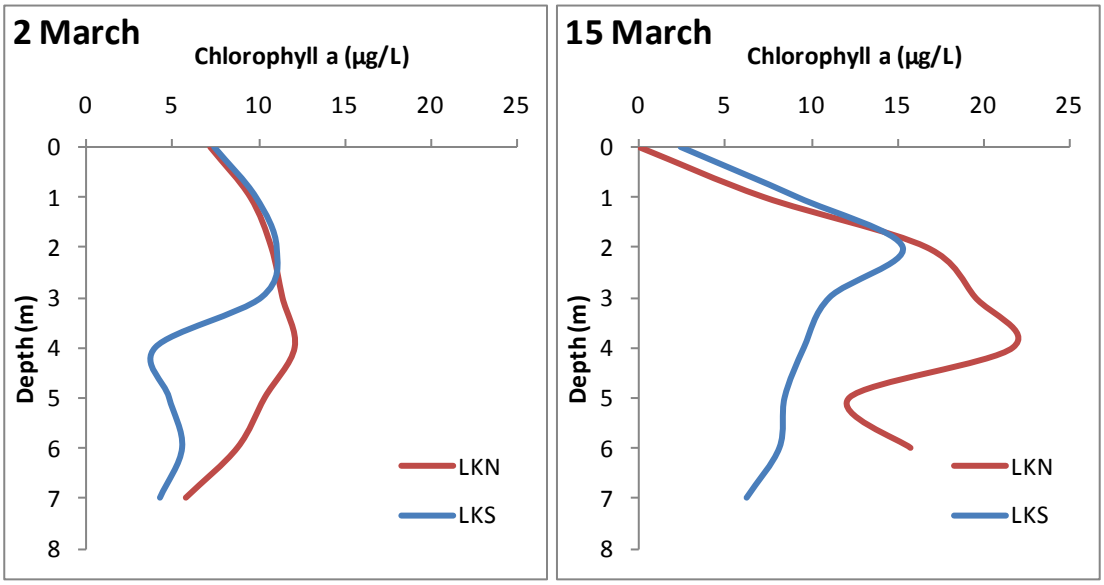

Figure 6. Chlorophyll profiles at LKS and LKN on the 2nd and 15th of March. There chlorophyll maximum is sub-surface, and there is an almost total loss of chlorophyll *a* from the surface on 15 March, and an increase at depth.

Weather

At Bairnsdale Airport, the temperature was generally mild over the summer of 2010-2011, apart from four days above 35 °C, three of those consecutive – from January 30 to February 1 (Figure 7). By comparison, 2009-2010 had ten days with temperatures greater than 35 °C. Rainfall events occurred regularly, with many large inflow events from August 2010 to April 2011 (Figure 7). By comparison, winter/spring inflows were lower in 2009 and the summer of 2009-2010 the inflows rarely exceeded baseline flows.

Nodularia

Nodularia appeared in low concentrations in the Gippsland Lakes at the beginning of February, following a three day heatwave. *Nodularia* was visible in the water column (as individual filaments), throughout February and March, and formed a technical bloom (biovolume >0.2 mm^3L^{-1}) in Lake King and Eagle Bay on 22/2/2011 (C. Garland, EPA Victoria pers. com., 25/2/2011). A significant bloom did not eventuate.

The onset of *Nodularia* coincided with a peak in surface water temperature (24 °C) and a salinity of 20-21. This also coincided with the lowest surface water DIN:FRP ratios seen over the entire summer (0.3 at LKN and 0.4 at LKS; see Figure 4 and Figure 5).

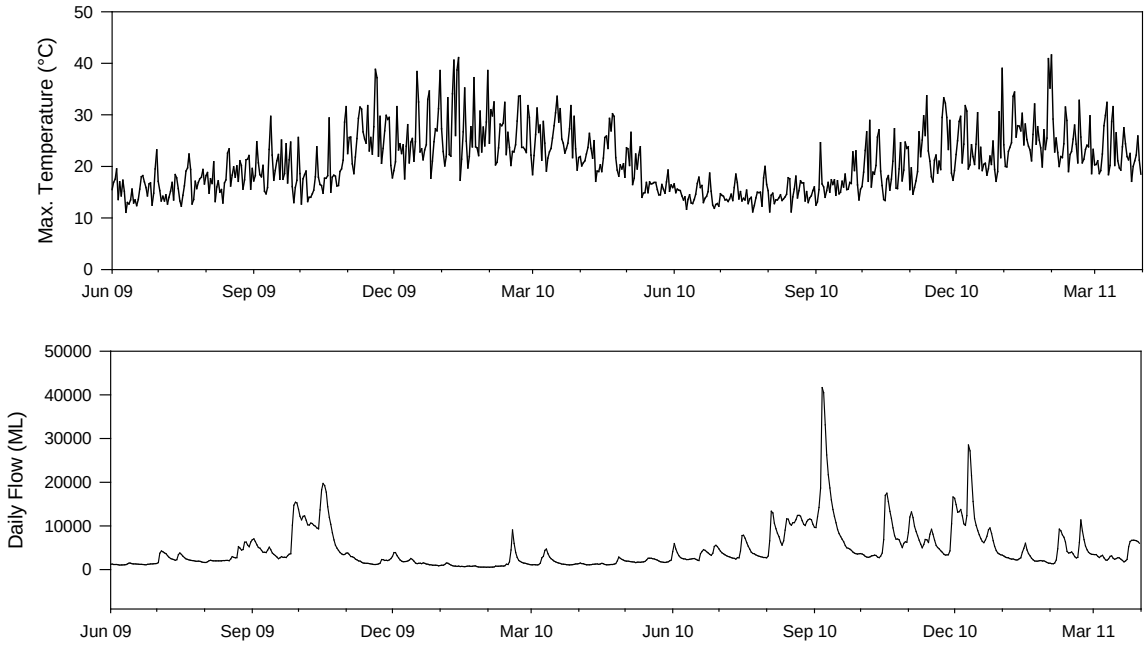

Figure 7. Daily maximum daily temperature at Bairnsdale Airport and total inflows between June 2009 and April 2011.

Bioassays – Lake King South

The total phytoplankton growth rate and maximum biomass reached were, broadly speaking, in line with what was expected from the *in situ* nutrient concentrations (i.e. growth was nitrogen limited), until February, when *Nodularia* appeared (Figure 8).

The addition of nitrogen alone led to enhanced 5-day growth in eight of the thirteen bioassays, while phosphorus additions were indistinguishable from the control in all bioassays apart from two occasions, the 16th of February and the 15th of March. This indicates that the phytoplankton were either nitrogen limited or nitrogen and phosphorus co-limited at all times up until the 16th of February. On the 16th of February growth was clearly phosphorus limited. Nitrogen limitation of the growth rate resumed on the 3rd of March, and the growth was again phosphorus limited on the 15th of March.

There was a decline in the maximum biomass reached in the summer compared to the spring.

Visual inspection of the lake and bioassay samples yielded no detectable *Nodularia* until February, and the cyanobacteria detected in December and January was, we believe, primarily *Synechococcus*, and the growth rate of this cyanobacterium was, as would be expected, nitrogen limited (Figure 8). From February onwards, the cyanobacteria was primarily *Nodularia*, and the cyan channel of the phytopam was well correlated with the measured *Nodularia* biomass (R^2 = 0.83). It is apparent that on the 2nd of February, the 3rd of March and the 15th of March, nitrogen addition inhibited growth of the *Nodularia*, as the growth rates in N-treatments were lower than in the control treatments. Phosphorus, on the other hand, had little or no effect on the growth of *Nodularia*.

Grazing

The dominant zooplankton were mussel and copepod larvae, as well as a lesser number of adult copepods.

Grazing rates of the total phytoplankton population were higher in summer than in winter (Figure 8). Grazing rates of *Nodularia*, however, were unusual in that the *Nodularia* showed the highest growth rates in the undiluted samples, i.e. when the grazing pressure was the highest. Contrast this with the other dominant phytoplankton (diatoms and dinoflagellates), which showed a classic dilution response, wherein the highest dilutions and therefore the lowest grazing pressure induced the highest growth rates (Figure 9).

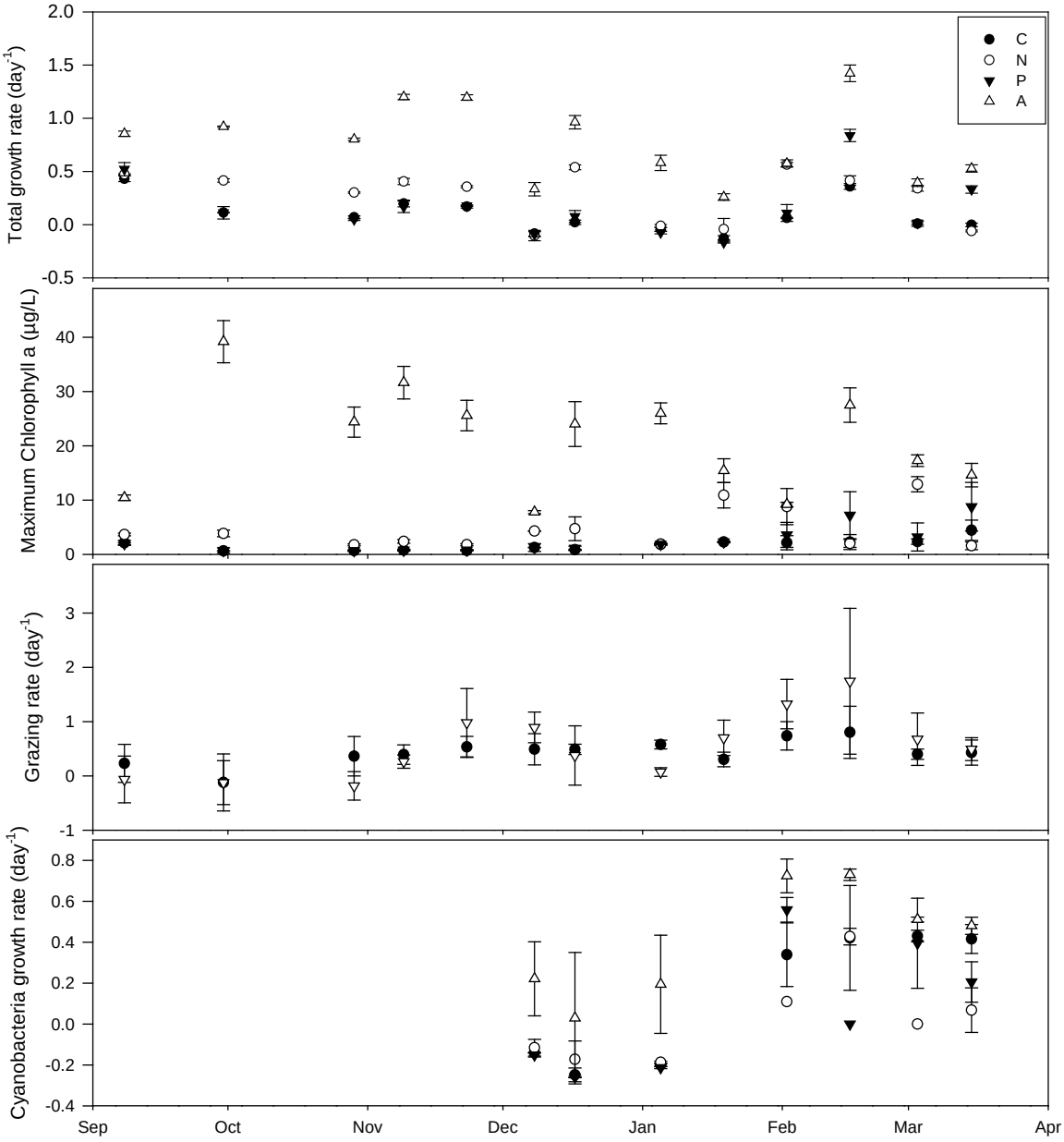

Figure 8. Bioassay growth experiments using Lake King South water. The top panel shows the growth rate after 5 days, the 2nd panel shows the maximum Chlorophyll a, panel 3 shows the grazing rate after 5 days, and panel 5 shows the cyanobacterial growth rate for the full incubation. C=control treatments, N=nitrogen addition, P=phosphorus addition, A=nitrogen and phosphorus addition.

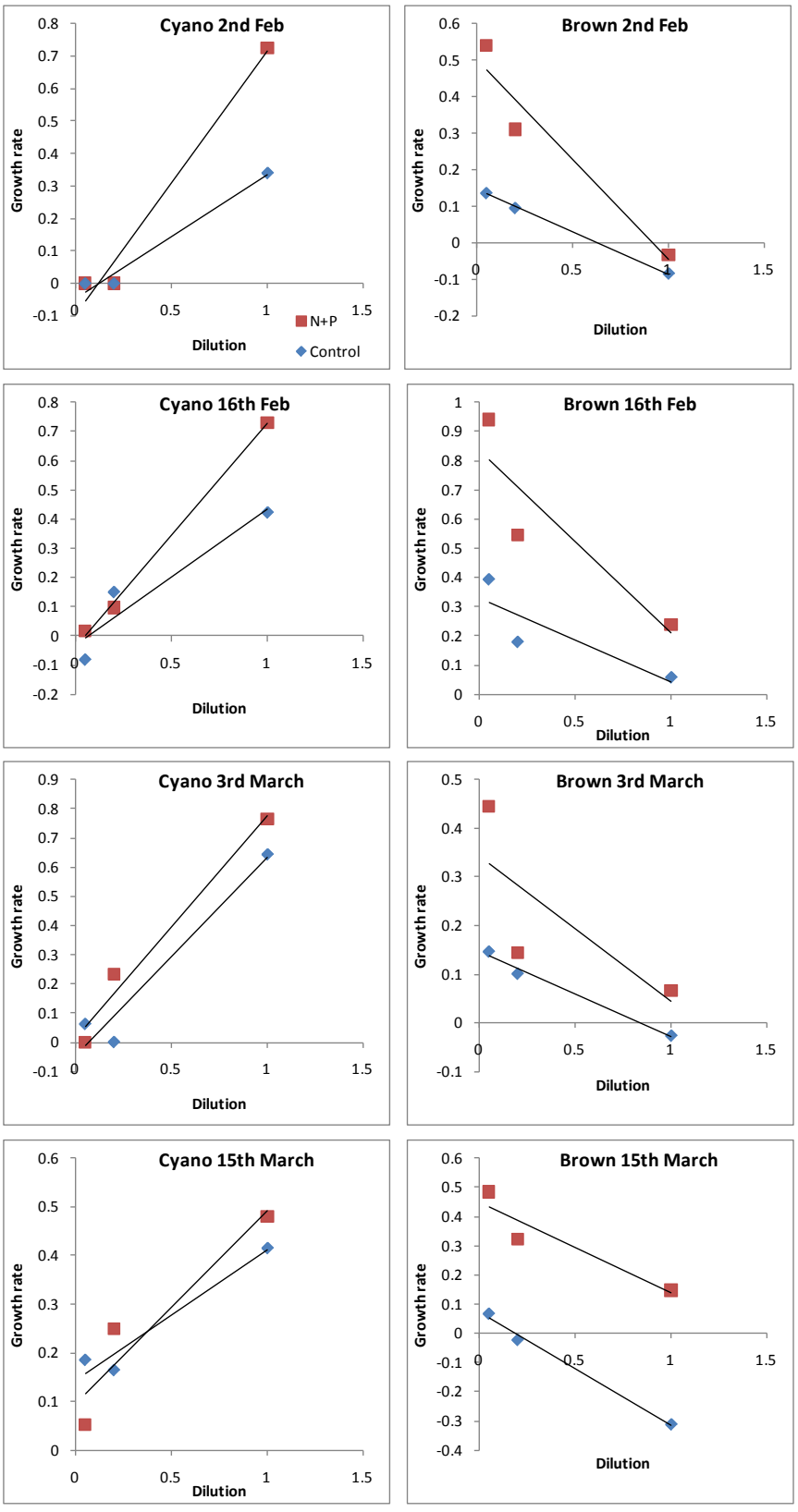

Figure 9. Growth rate of *Nodularia* (left column) and diatoms plus dinoflagellates (right column) after dilution to reduce grazing pressure. Typical dilution experiments follow the trends on the right, where growth is enhanced by a reduction in grazers. Blue symbols represent the control treatment, and red symbols represent the treatment with added phosphorus and nitrogen.

Lake Surveys

On the 3rd and 15th of March 2011, lake water from a number of different sites was included in the bioassay experiments – although with no added nutrients (Figure 11). *Nodularia* concentrations increased exponentially throughout the incubations, while the dominant diatoms and dinoflagellates declined (e.g. Figure 10). Growth of *Nodularia* was highest in Eagle Bay water, and was generally higher in the northern half of Lake King than in the southern half (Figure 11).

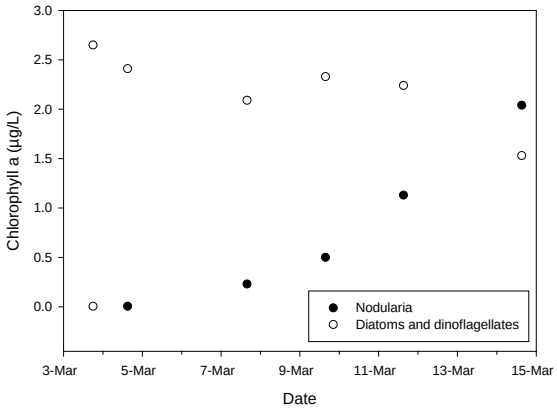

Figure 10. Growth of *Nodularia* versus the diatoms and dinoflagellates in water taken from Eagle Bay. *Nodularia* fluorescence was undetectable at the start of the incubation, but overtook the dominant diatom/dinoflagellate population after 12 days.

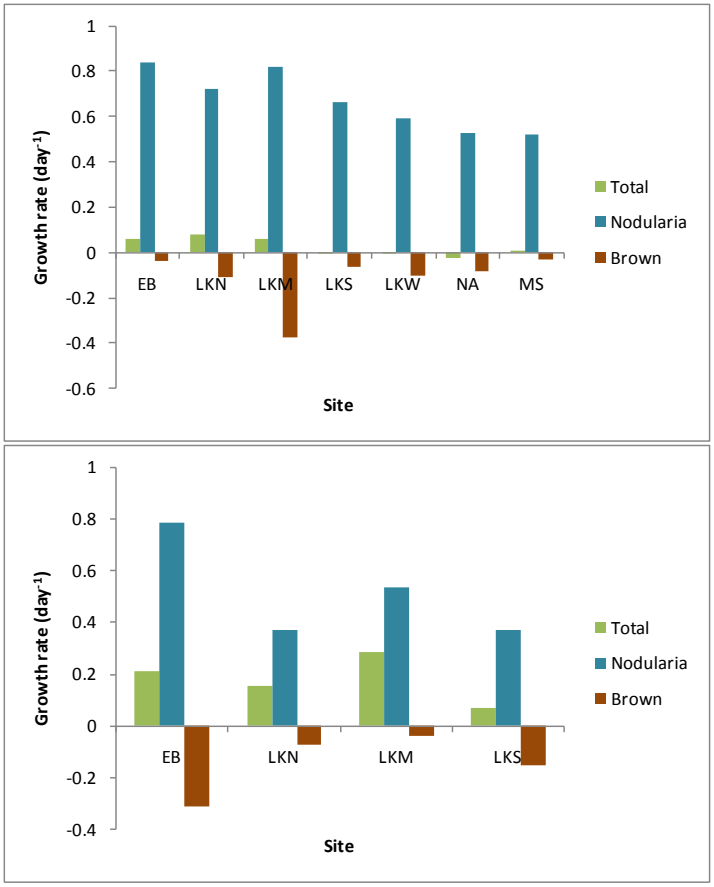

Figure 11. Growth rates of the total phytoplankton population, *Nodularia* and combined diatoms and dinoflagellates (brown), from lake water taken on the 3rd of March (top) and the 15th of March (bottom) from various sites throughout Lake King.

Nitrogen fixation

The excess nitrogen produced in the bioassay containers could have no other source other than nitrogen fixation by *Nodularia*. There was a clear positive relationship between the final *Nodularia* concentration and the amount of excess nitrogen produced in the C and P treatments, but there was no relationship between these factors in the N treatment or the A treatment Figure 12.

The relationship between *Nodularia* concentration and nitrogen fixation is particularly strong when just the control treatments from the eight sites sampled on the 3rd of March are compared (R^2 = 0.99, Figure 12).

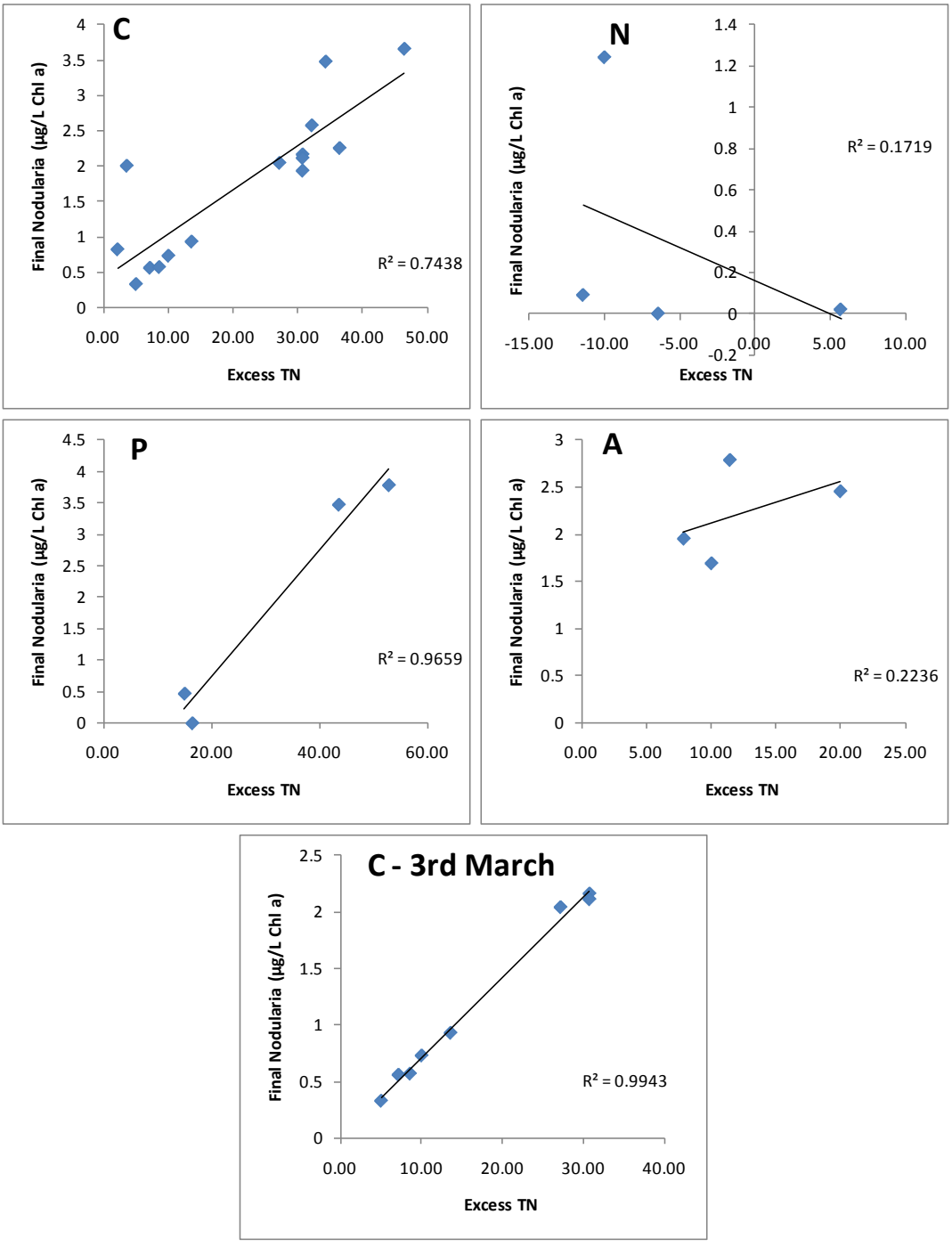

Figure 12. Excess total nitrogen plotted against the final *Nodularia* concentration. Each nutrient treatment is plotted separately, as is the data from the lake-wide sampling of the 3rd of March.

Discussion

What stopped a major *Nodularia* bloom?

Nodularia persisted in relatively low concentrations throughout February and into March 2011 but a significant bloom did not eventuate. The physico-chemical conditions were ideal for a bloom to occur, and we were able to induce a bloom in laboratory incubations with no manipulation apart from the provision of consistent temperature and light.

This then raises the question: why was there no bloom in Lake King? The reason appears to be a fortuitous period of inclement weather during the growing period. The rainfall in January was below average (31.2 mm compared to 49.3 mm) and inflows were low (Figure 7). These conditions probably allowed the *Nodularia* to germinate, float to the surface and begin proliferating. The rainfall in February, however, was 50% above average (76.6 mm compared with 50.6 mm), and in March almost twice the average (76.8 mm compared with 41.5), while inflows were very high for this time of the year. More than 10 mm of rain fell every week during February and March, and it appears that this disturbance halted the proliferation of the *Nodularia*. We hypothesise that an extended period of calm, warm weather (which the bioassay incubations replicated) would have resulted in a significant bloom.

What role do grazers play in *Nodularia* growth?

Grazing is often seen to have a mitigating effect on cyanobacterial dominance, especially in estuarine systems. For example, in a series of mesocosm experiments, wherein grazers and nutrients were manipulated, Chan et al. (2006) found that the relative abundance of heterocystous cyanobacteria was suppressed by grazers, although the total chlorophyll-*a* was not affected. The implication of this is that the heterocystous cyanobacteria (in this case *Anabaena* sp.) were either being preferentially grazed or were less able to cope with grazing than competing phytoplankton taxa. Grazing reduced the size of the filaments, often below the length able to support heterocyst production, thus removing the organism's ability to fix nitrogen.

The results of the current study contradict the above paradigm. In our experiments, grazers were able to suppress the growth of the total phytoplankton population, and in particular, the dominant diatom and dinoflagellate population, but they apparently allowed the *Nodularia* to proliferate. We believe that the most likely explanation for this is that the grazers were preferentially consuming the diatoms and dinoflagellates, whilst giving the *Nodularia* a "free ride". Once the grazers were removed, the diatoms and dinoflagellates were then able to outcompete the *Nodularia*.

Mussel and copepod larvae were the dominant grazers when the *Nodularia* was present. *Nodularia* filaments are too large to be consumed by mussel larvae (Widdows 1991), and even if they could consume *Nodularia*, it would be a poor quality food, as it lacks long-chain fatty acids which are essential to the diets of these organisms (Vanderploeg et al. 1996). Likewise, copepods have been shown to be poor grazers of *Nodularia* (Sellner 1997). More research specific to the zooplankton and phytoplankton communities of the Gippsland Lakes is needed to fully understand what is going on, but we hypothesise that because *Nodularia* is a rare and sporadic occurrence in the Gippsland Lakes, the grazing community is not well adapted to it and cannot adapt quickly enough to stop a bloom forming. Instead, grazing may facilitate a bloom by removing competitors. Whether the grazers affect the persistence or breakdown of a bloom is a separate question that we are not able to answer at this time.

What is the limiting nutrient?

The waters of Lake King were low in DIN and had an exceedingly low DIN:FRP ratio throughout the summer of 2010-2011, indicating strong nitrogen limitation. Prior to February, the bioassays and the *in situ* nutrient concentrations agreed on the limiting nutrient, with the exception of the 8th of December 2011, where the bioassays suggested nitrogen and phosphorus co-limitation. When *Nodularia* appeared, however, the situation changed. The 16th of February and the 15th of March 2011 were clearly not nitrogen limited in the bioassays, even though DIN:FRP ratio was less than one. The phytoplankton nitrogen requirements must therefore have been met by the fixation of N_2 by *Nodularia*, with the total nitrogen from the start to the end of the bioassay more than doubling in some cases. On three occasions the addition of nitrogen actually inhibited *Nodularia* growth relative to the controls. Once again this finding is consistent with what we would expect – faster growing, but non-N_2 fixing species are able to outcompete *Nodularia* when N is available in the water column. This further confirms that *Nodularia* is more likely to bloom when DIN concentrations are very low, and is consistent with our previous explanation for the occurrence of the *Synechococcus* bloom following the 2007 floods which brought unprecedented loads of nitrogen into the Gippsland Lakes (Cook and Holland 2011).

Given that *Nodularia* was not nitrogen limited, it might be assumed that it was therefore phosphorus limited, and the addition of phosphorus would stimulate *Nodularia* growth. This was not the case, as growth of *Nodularia* was just as fast in the control treatments. We have two explanations for this. First, filterable reactive phosphorus concentrations in the water column were high throughout the summer (~1 μM), and second, *Nodularia* may have been scavenging phosphorus from other phytoplankton species as those populations declined (Figure 10), and therefore had no need for additional phosphorus.

Conclusion

The aims of this project were contained in three questions, which we now answer.

Did the flood waters of August 2010 provide a non-limiting amount of nutrients for phytoplankton growth?

No. Phytoplankton growth was always highest in the bioassays provided with both phosphorus and nitrogen, and it was clear that the phytoplankton population was nutrient limited in both their growth rates and the maximum achievable biomass (Figure 8).

Does the observed nutrient limiting growth of phytoplankton agree with the nutrient limitation implied by* in situ *nutrient concentrations?

Yes. On most occasions, the bioassays and the *in situ* nutrient concentrations agreed on the limiting nutrient. The major exception was when *Nodularia* was present, which removed the nitrogen limitation through the fixation of gaseous N_2.

Are phytoplankton blooms controlled solely by nutrient availability, or does grazing play a major role?

In nearly all cases, the control samples were growing at the same rate that they were being grazed. When nutrients were added, however, the growth rate outstripped the grazing rate, at least for the first five days. The most interesting aspect of the grazing, though, is the apparent grazer preference for the dominant diatom/dinoflagellate community over *Nodularia*, to the point that *Nodularia* was only able to dominate under full grazing pressure, and was outcompeted by other phytoplankton when the grazers were removed.

Acknowledgements

This work was undertaken with the assistance of Kerrie Brown, Vera Folkman, Iris van Erp, Todd Scicluna, Michael Lane and Peta Dewar. Funding was provided by the Gippsland Lakes and Catchment Task Force.

References

Adams, D.G. and Duggan, P.S. (1999) Tansley Review No. 107. Heterocyst and akinete differentiation in cyanobacteria. New Phytologist 144, 3-33.

Bolch, C.J.S., Orr, P.T. and Jones, G.J. (1999) Genetic, morphological, and toxicological variation among globally distributed strains of *Nodularia* (cyanobacteria). Journal of Phycology 35, 339-355.

Chan, F., Marino, R.L., Howarth, R.W. and Pace, M.L. (2006) Ecological constraints on planktonic nitrogen fixation in saline estuaries. II. Grazing controls on cyanobacterial population dynamics. Marine Ecology Progress Series 309, 41-53.

Cook, P. and Holland, D. (2011) Long term nutrient loads and chlorophyll dynamics in a large temperate Australian lagoon system affected by recurring blooms of cyanobacteria. Biogeochemistry, 1-14.

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

Cook, P.L.M., Holland, D.P. and Longmore, A.R. (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.

Holland, D., Cook, P. and Longmore, A. (2010) Nutrient cycling and phytoplankton population dynamics in the Gippsland Lakes, Water Studies Centre, Monash University, and Fisheries Research Branch, Department of Primary Industries.

Huber, A.L. (1985) Factors affecting the germination of akinetes of *Nodularia spumigena* (Cyanobacterioacea). Applied and Environmental Microbiology 49(1), 73-78.

Jakob, T., Schreiber, U., Kirchesch, V., Langner, U. and Wilhelm, C. (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(3), 343-361.

Landry, M.R. and Hassett, R.P. (1982) Estimating the grazing impact of marine micro-zooplankton. Marine Biology 67(3), 283-288.

Mazur-Marzec, H., Krezel, A., Kobos, J. and Plinski, M. (2006) Toxic Nodularia spumigena blooms in the coastal waters of the Gulf of Gdansk: a ten-year survey. Oceanologia 48(2), 255-273.

Sellner, K.G. (1997) Physiology, Ecology, and Toxic Properties of Marine Cyanobacteria Blooms. Limnology and Oceanography 42(5), 1089-1104.

Vanderploeg, H.A., Liebig, J.R. and Gluck, A.A. (1996) Evaluation of different phytoplankton for supporting development of Zebra Mussel larvae (*Dreissena polymorphya*): The importance of size and polyunsaturated fatty acid content. Journal of Great Lakes Research 22, 36-45.

Widdows, J. (1991) Physiological ecology of mussel larvae. Aquaculture 94, 147-163.

Yamamoto, Y. (1975) Effect of desiccation on the germination of akinetes of Anabaena cylindrica. Plant and Cell Physiology 16(4), 749-752.