Ecological impacts of a *Nodularia* bloom on nitrogen dynamics in food webs and seagrass beds

A report prepared for the Gippsland Lakes Ministerial Advisory Committee

June 2013

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Executive Summary

The seagrass beds in the Gippsland Lakes are an important habitat for the health of the Lakes and for maintaining commercially important fisheries. Blue-green algae blooms are known to negatively impact seagrasses by stopping light from reaching them, but there is little known about the potential impacts of these blooms on seagrasses. In particular, the toxic blue-green algae, *Nodularia spumigena*, introduces large amounts of new nitrogen – an important and often hard-to-get nutrient for seagrasses – to the lakes by fixing atmospheric nitrogen when it blooms.

Atmospheric nitrogen has a particular isotopic signature which differentiates it from other sources of nitrogen, and can therefore be tracked through the food web.

We measured the nitrogen isotope composition of seagrass, phytoplankton, zooplankton grazers and small fish monthly at three seagrass sites (Barrier Landing, Raymond Island North and Gergon Bank) from November 2012 until March 2013. Crested tern *Thalasseus bergii* blood samples were collected near Ocean Grange in December, January and February. Large fish from the estuary and small fish from the coastal ocean were provided by commercial fisherman from the Lakes Entrance Fishermen's Co-operative Society Limited throughout this period.

There was a *Nodularia* bloom in December 2012, although it was mostly contained within Bunga Arm. An extra site was added at the 1st Blowhole from January 2013 to capture the epicentre of the bloom.

Large fish and crested terns had no discernible *Nodularia*-derived nitrogen content. The absence of a signal in these species probably results from avoidance of bloom areas. These are highly mobile animals and are capable of shifting their foraging grounds to avoid feeding in waters contaminated with *Nodularia*.

We saw no assimilation of *Nodularia*-derived nitrogen by any species at our control site at Barrier Landing, a site heavily influenced by the ocean. We detected *Nodularia* in December at Raymond Island, but it was low in abundance and nitrogen from this species was not measureable in other organisms. This site was > 6km from the epicentre of the bloom.

At the 1st Blowhole there was a clear signal of *Nodularia*-derived nitrogen in the phytoplankton, grazers and small fish; there was no seagrass at this site because of very high turbidity. Nitrogen from *Nodularia* was undetectable in plankton, grazers or fish at Gergon Bank; however, there was a clear transfer of nitrogen from *Nodularia* to the seagrass.

We believe that the change in nitrogen signature in the seagrass at Gergon Bank was caused by *Nodularia* particles becoming trapped in the sand, where they broke down and released their nutrients to the seagrass. The fertilization effect of the *Nodularia* bloom nitrogen appears to be localised, at least in this case, to the epicentre of the bloom and the areas close by. Seagrass beds therefore, act as an important sink for the new nitrogen introduced into the Gippsland Lakes during *Nodularia* blooms.

Background

Seagrass and blue-green algae in the Gippsland Lakes

Seagrass meadows are important ecosystems in coastal regions worldwide. The Gippsland Lakes has extensive seagrass meadows in the shallow (<2m depth) regions of Lake King. Seagrass distribution in the Lakes changes seasonally and in response to the impacts of floods, droughts and algal blooms. Seagrasses provide habitat for fish and invertebrates and help to stabilize sediments. Seagrasses are also an important component of nutrient cycles and food webs in estuaries.

The Gippsland Lakes were affected by a large and persistent bloom of the toxic blue-green alga *Nodularia spumigena* from late November 2011 until February 2012. In December 2012 another bloom occurred, although this bloom was of shorter duration and was largely restricted to Bunga Arm.

Algal blooms shade seagrasses by reducing the penetration depth of light which can lead to dieback, or poor growth. Researchers from the Arthur Rylah Institute have been monitoring seagrass in the Gippsland Lakes annually since 2008. Following the collapse of the 2011-2012 *Nodularia* bloom, an ARI survey in April 2012 found that seagrass condition had declined at 42.7% of sites compared to the previous year (Warry and Hindell 2012).

There is one aspect of *Nodularia* blooms that could potentially benefit seagrasses and their associated animal communities. *Nodularia* blooms introduce 'new' nitrogen to the system through the fixation of atmospheric nitrogen, a process that *Nodularia* are capable of supporting at high rates (Woodland et al. 2013). Given that nitrogen is the nutrient that limits primary production in the Gippsland Lakes (Cook and Holland 2012), it is possible that this new nitrogen will stimulate productivity of other primary producers, including seagrass. This addition of nitrogen could also lead to a shift in the pathways through which food webs are supported.

Seagrass can take up nutrients from the sediment via their roots or from the water column through their leaves (Carignan and Kalff 1980). In the Gippsland Lakes, large areas of seagrass grows in sandy sediments. These are permeable to water, allowing a constant supply of nutrients to the roots. Small settling particles such as dead *Nodularia* filaments are likely to be trapped in these permeable sediments (Ehrenhauss and Huettel 2004). Particles (called particulate organic matter or POM) trapped in the sediment are broken down (remineralised) by bacteria and the nutrients that are released may then be taken up through the seagrass roots.

The pathway of nitrogen uptake will therefore be determined by where the *Nodularia* bloom material is remineralised. If it is remineralised in the water column, the nitrogen may be taken up by other algae and move into the food web via grazing by zooplankton, while if remineralisation occurs in the sediment, seagrass may be the primary entry point into the food web.

Micro- and macroalgae that are associated with seagrass beds (often growing on the seagrasses as epiphytes) may also assimilate large amounts of the *Nodularia*-associated nitrogen if the nitrogen is released into the water column. Algae typically have higher rates of nutrient uptake and lower light requirements than seagrasses – characteristics that allow algae to outcompete seagrasses in certain situations. Rapid and excessive growth of algae due to nitrogen enrichment of the water column

could potentially shade seagrasses, compromising their photosynthetic capacity and reducing their ability to support food webs.

How do we trace the fate of the new nitrogen?

Nitrogen has two stable forms (isotopes), a heavy and a light form and the relative ratio of these is an important tool for determining the source of nitrogen. The nitrogen isotope ratio of ambient air is used as a standard during isotope analysis; therefore, organisms that use atmospheric nitrogen gas to satisfy their nitrogen requirements will have nitrogen isotope values near 0 ‰ (the ‰ symbol is used to denote 'per mil' or parts per thousand). Conversely, organisms that rely on dissolved forms of nitrogen such as nitrate or ammonium often have nitrogen isotope values ≥ 8 ‰ in estuaries. *Nodularia* is capable of fixing atmospheric nitrogen gas (i.e., N_2-fixation) when dissolved sources of nitrogen are scarce, making the nitrogen derived from *Nodularia* blooms relatively easy to identify and trace through a food web (Bauersachs et al. 2009; Woodland et al. 2013).

Nutrients in the Gippsland Lakes move through the food web from autotrophs (algae and seagrasses) to small invertebrate grazers (zooplankton) and then to larger consumers such as fish and birds. By measuring the nitrogen isotope ratios in the tissues of these organisms, we can potentially follow the nitrogen that was fixed by *Nodularia* through the food web and into higher consumers such as the economically important finfish black bream *Acanthopagrus butcheri*.

Over the summer of 2012-2013 we investigated the extent to which nitrogen from *Nodularia* was measurable in the water column, and the passage of this nitrogen through the food webs associated with the pelagic (water column) and the demersal (bottom) environments.

Phosphorus can be stored in estuary sediments for years, but any nitrogen in the sediment that is not assimilated by plants will be "denitrified". This means that it is turned back into nitrogen gas by microbes and is then lost from the system. Denitrification is one of the primary reasons why the Gippsland Lakes are typically nitrogen limited. For this reason, measurable nitrogen signals do not carry over from *Nodularia* blooms in previous years; any evidence of *Nodularia*-derived nitrogen in the Gipppsland Lakes would be from more recent activity.

Where does the bloom go?

We know from previous work that *Nodularia* is not grazed by zooplankton (Holland et al. 2011), and so the most likely fate is bacterial breakdown in the water column and settling of the detritus(Woodland et al. 2013). It is now well known that seagrass beds are hotspots of material deposition, and we hypothesised that seagrass beds would act as depositional hotspots for the bloom detritus.

Pre-study conditions

Conditions in the Gippsland Lakes in October 2012 appeared conducive to a summer *Nodularia* bloom and *Nodularia* did appear at the beginning of November. The only factor likely to limit a *Nodularia* bloom was the salinity of the Lakes, which was approaching the threshold (= 20) above which blooms are unlikely to occur.

Methods

Sites

Three seagrass sites were initially selected based on recent seagrass mapping (Warry et al. 2012), the location of the 2011-2012 *Nodularia* bloom, and an initial site-selection survey in November 2012. These sites were Raymond Island North (RI), Gergon Bank (GB, off the N.E. tip of Rotamah Island) and Barrier Landing (Figure 1). We hypothesized that the first two sites were likely to be impacted by the forecast *Nodularia* bloom; whereas, the last site was chosen as a control because the strong ocean influence meant that it was unlikely to be affected by the bloom. When the bloom occurred in December, it was most intense at the 1st Blowhole in Bunga Arm (Figure 1). We therefore added this site despite the absence of seagrass at that location.

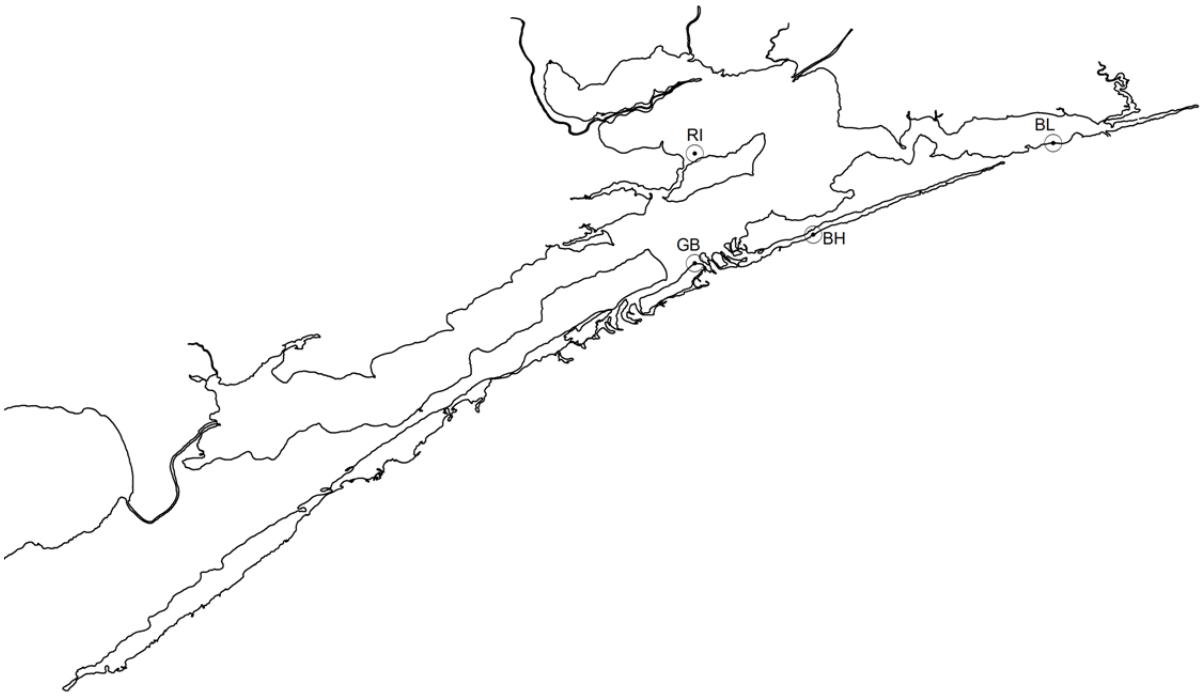

Figure 1. Map of the Gippsland Lakes with the location of the four main sampling sites: RI, Raymond Island Nth; GB, Gergon Bank; BH, 1st Blowhole; BL, Barrier Landing.

Nutrients

Dissolved inorganic nitrogen (DIN) and filterable reactive phosphorus (FRP) concentrations were analysed by passing surface water through 0.4 μm filters. These filters were then stored on ice and frozen at -20°C at Monash University.

Samples for total nitrogen (TN) and total phosphorus (TP) were collected by bottling ambient surface water. Bottles were stored on ice in the field and frozen at -20°C at Monash University.

Analysis was performed in the Water Studies Centre analytical laboratory using standard flow injection analysis techniques.

Water quality parameters

A Hydrolab multi-probe water quality analyser was used to measure temperature, dissolved oxygen, salinity, chlorophyll *a*, pH and turbidity at each site.

Seagrass

Five plants, including roots, were collected from each site where possible. They were transported in zip-lock bags on ice and then stored frozen at -20°C at Monash University.

Plankton community

Ambient sub-surface water and 63 µm pre-filtered water was filtered through 0.7 µm (nominal pore size) Whatman GF/F filters until they clogged. The filters were stored on ice and then frozen at -20°C at Monash University.

Zooplankton

An 80 µm plankton net was towed horizontally just below the surface for three minutes at ~3 km/hr. The plankton was stored on ice and then frozen at -20°C at Monash University.

Fish

A 15 x 1.5 m beach seine (2 mm mesh) was used to catch small fish (<80 mm). Up to three attempts were made in an effort to collect up to 10 pelagic (associated with the water column) and 10 demersal (bottom dwelling) specimens from each site. Fish were put in zip-lock bags and stored on ice before being frozen at -20°C at Monash University.

Large fish from the estuary (black bream *Acanthopagrus butcheri* and tailor *Pomatous saltatrix*) and small fish from the coastal ocean (sandy sprat *Hyperlophus vittatus* and pilchard *Sardinops neopilchardus*) were collected and frozen by commercial fishermen associated with the Lakes Entrance Fishermen's Co-Operative Society Limited.

Birds

Flocks of crested terns were captured on the ground using canon nets whilst the birds were roosting on sandbanks. Birds were extracted from the net and placed in holding cages constructed from shade cloth prior to blood sampling. Blood sampling involved piercing a brachial vein with a 26 or 27.5 G needle and placing the tip of a capillary tube at the point of where the blood began to pool. The blood was immediately fractionated using a generator-powered centrifuge such that plasma and cellular fractions were separated and available for separate stable isotope analysis.

$\delta^{15}N$

Stable isotope composition of seagrass, plankton, fish and birds followed standard drying and pulverizing methods. These methods are outlined below. All samples were analysed for carbon and nitrogen stable isotope composition on an isotope ratio mass spectrometer located at the Water Studies Centre, School of Chemistry (Monash University).

Sediment samples were dried at 60°C until completely dried (minimum two days), pulverized using a mortar and pestle, then analysed for carbon and nitrogen content. Where necessary, sediment samples were acidified with weak hydrochloric acid to remove inorganic carbonates prior to stable isotope analysis.

Plankton samples were thawed in the laboratory and prepared for stable isotope analysis. Ambient plankton community samples that were collected on glass fibre filters were dried at 60°C for 24 hr. Filters were then packed in tin sample capsules and analysed for stable isotope composition. Plankton samples that were collected with the plankton net were thawed, rinsed thoroughly, then

size-fractionated into two size-classes: 80-149 µm and >150 µm. *Nodularia* was removed from these size-class samples by adding de-ionized water to the samples and allowing the sample to settle briefly. The buoyant *Nodularia* was then removed from the sample and the sinking plankton was collected for isotope analysis. This procedure was repeated until the sample was free of *Nodularia*. In some cases, a centrifuge was used to separate the *Nodularia* cells from the plankton if gravity settling proved ineffective. Both size-classes of plankton and an isolated sample of the pure *Nodularia* (where available) were dried at 60°C for two days and then analysed for stable isotope composition.

Seagrass samples were thawed and a scalpel blade used to gently scrape the epiphyte material from the seagrass blades. The cleaned seagrass blades were dried at 60°C for two days, pulverized and then analysed for stable isotope content. If sufficient material was present, we also analysed the stable isotope composition of the epiphytes removed from the seagrass.

In the laboratory, we removed a small (~10 mg) fillet of white muscle tissue from each fish. We also collected a similarly sized sample of liver tissue from the black bream, tailor, sandy sprat and pilchard for isotopic analysis. The stomach of larger fish was removed and fixed in a formalin solution in the event that stomach-content analysis is required at a future date. Muscle and liver samples were dried for two days at 60°C in an oven, pulverized, then analysed separately for stable isotope composition.

Red blood cells and plasma from the crested terns were thawed, transferred to clean glass microscope slides and dried at 60°C for 24 hr. Blood and plasma samples were pulverized and analysed separately for stable isotope composition.

Results

Nodularia

Jonathan Smith (First Alert Algae Monitoring: FAAM) monitored *Nodularia* on a weekly basis throughout the study period, and his results are summarised here.

Nodularia first appeared on November 6 at Nungurner, Chinamans Creek, Progress Jetty and Newlands Arm. By November 20 it was present at most of the FAAM sites that were sampled with the highest concentrations occurring at Bunga Arm and Steamer Landing, although even these concentrations were relatively low at <0.02 mm^3/L. Although the bloom persisted throughout December, *Nodularia* concentrations were below the bloom threshold at all sites except Ocean Grange (maximum concentration = 7.96 mm^3/L) and the 1st Blowhole (maximum concentration = 23.15 mm^3/L, see Figure 2).

On the 8th of January *Nodularia* was detected in low concentrations at three out of 20 FAAM sites (Ocean Grange, 1st Blowhole and Paynesville Marina) and the following week only at Ocean Grange and 1st Blowhole. By the 29th of January *Nodularia* was no longer detected at any site.

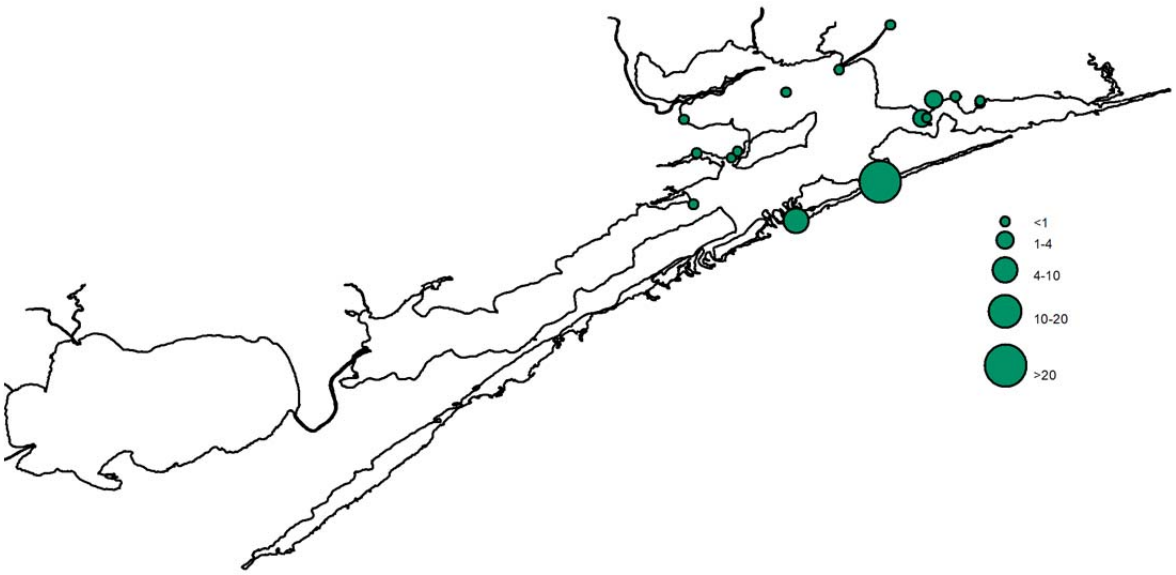

Figure 2. Maximum *Nodularia* concentration at the 12 sites in the Gippsland Lakes where it was detected. Units are mm^3/L.

Salinity

Salinity appears to be the factor that limited the extent of the bloom. Salinity at Raymond Island North and Gergon Bank was about 22 in December and increased consistently over the summer to the high 20s (Figure 3). Salinity at the 1st Blowhole was similar to these sites when it was sampled in January. Barrier Landing had consistently high salinity due to its marine influence.

Salinity was not measured in November due to a faulty probe.

Chlorophyll a

Chlorophyll a, which is a measure of the amount of algae in the water, was highest at Gergon Bank in December during the height of the *Nodularia* bloom (Figure 3). The 1st Blowhole had consistently high chlorophyll a and its waters were noticeably green during the three occasions that it was sampled. This greenish coloration was not due to *Nodularia*; rather, it was caused by other species, including the blue-green algae *Synechococcus* which was responsible for a Lakes-wide bloom in 2008.

Nutrients

Dissolved inorganic nitrogen (DIN, the nitrogen that is available for algal or seagrass growth) concentrations were higher than expected at Raymond Island North and at Gergon bank. These concentrations were very high (>4 µM) at the 1st Blowhole in January (Figure 3).

Nitrogen limitation generally occurs when the ratio of DIN to dissolved phosphorus (filterable reactive phosphorus or FRP) is less than about 10:1, and we have observed that *Nodularia* blooms usually only occur when this ratio is <5:1. During the 2012-2013 *Nodularia* bloom, the Lakes were nitrogen limited at Gergon Bank, but the water was generally not nitrogen limited at the other sites (Figure 3).

Total nitrogen (which includes nitrogen locked up in phytoplankton) was particularly high at the 1st Blowhole (121 µM in February, Figure 3). The ratio of total nitrogen to total phosphorus was

consistently above 20:1, further indicating that nitrogen was not a limiting nutrient during this study (Figure 3).

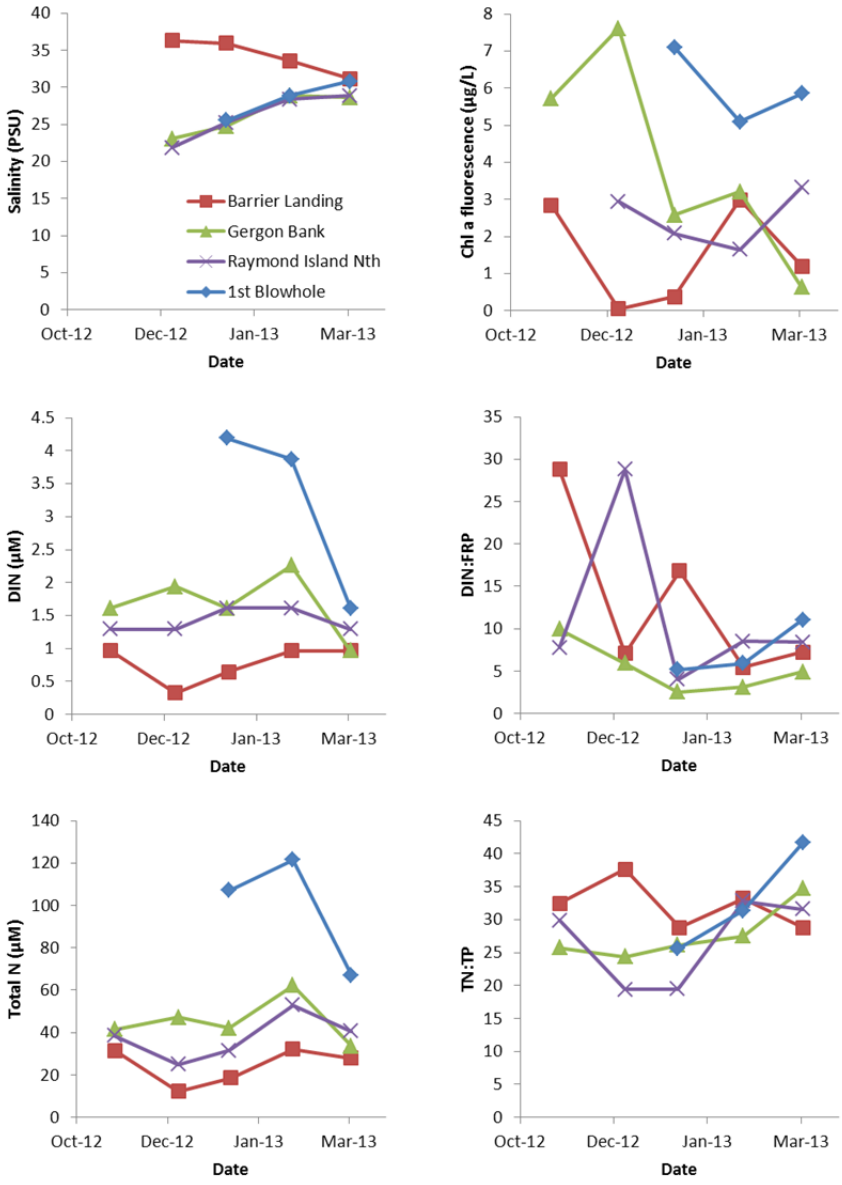

Figure 3. Salinity, chlorophyll a, dissolved inorganic nitrogen (DIN), DIN:FRP (filterable reactive phosphorus), total N (TN), total P (TP), TN:TP at four sites in the Gippsland Lakes, November 2012 until March 2013.

Plankton

The nitrogen isotope composition (reported as $\delta^{15}N$; which represents the ratio of the heavy to light nitrogen isotope concentrations in the sample relative to the ratio of the heavy to light nitrogen isotope concentrations in a standard) of the ambient plankton community was lowest at the 1st Blowhole, as expected from the location of the *Nodularia* bloom (Figure 4). At Raymond Island North and Gergon Bank in December, the plankton sample that was not prefiltered through a 63 µm mesh was lighter than the prefiltered sample. This is because there was *Nodularia* at both sites, and the majority of the isotopically light *Nodularia* is removed by prefiltering. The $\delta^{15}N$ values of these samples were not as low as would be expected from a sample dominated by *Nodularia*. This

indicates that *Nodularia* was preferentially using DIN rather than N_2 for the majority of its nitrogen needs and provides further evidence that the system was not severely nitrogen limited.

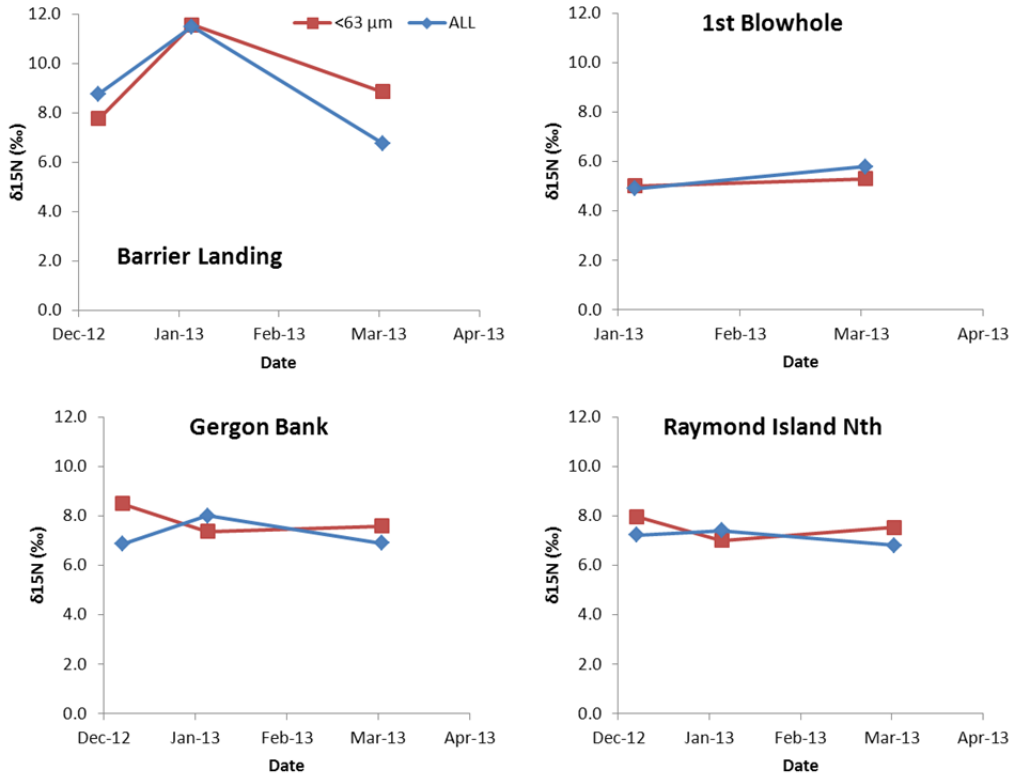

Figure 4. $\delta^{15}N$ of plankton at four sites in the Gippsland Lakes, November 2012 until March 2013.

Seagrass and epiphytes

Seagrass was abundant at Barrier Landing for the duration of the study. It was sparse at Raymond Island in November, but steadily increased in abundance throughout the study. At Gergon Bank, seagrass was sparse and difficult to find due to high turbidity/low visibility throughout most of the study period. During the final sampling period in March, water clarity had improved and there was evidence of large amounts of fresh growth.

At Barrier Landing, the seagrass $\delta^{15}N$ rose slightly between December and March, and there was also a slight rise at the Raymond Island North site (Figure 5). At Gergon Bank (the closest seagrass site to the *Nodularia* bloom epicentre), there was a steady and dramatic decline in seagrass $\delta^{15}N$ from 6.7 ‰ to 3.0 ‰ from November to March. There was little change in the $\delta^{15}N$ of the epiphytes at any site.

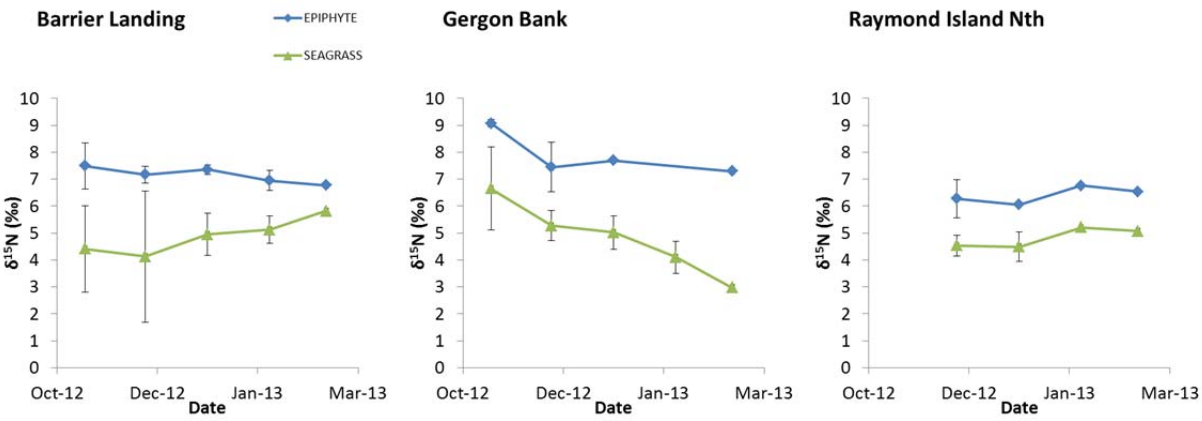

Figure 5. $\delta^{15}N$ of seagrass and associated epiphytes at three sites in the Gippsland Lakes, November 2012 until March 2013. Error bars represent the standard deviation. Points without error bars are based on a single sample.

Grazers

The $\delta^{15}N$ of both the mesozooplankton and the microzooplankton was considerably lower at the 1st Blowhole than at any other site, indicating assimilation of *Nodularia*-derived nitrogen (Figure 6). There is little evidence that grazers assimilated appreciable amounts of *Nodularia*-derived nitrogen at any of the other sites.

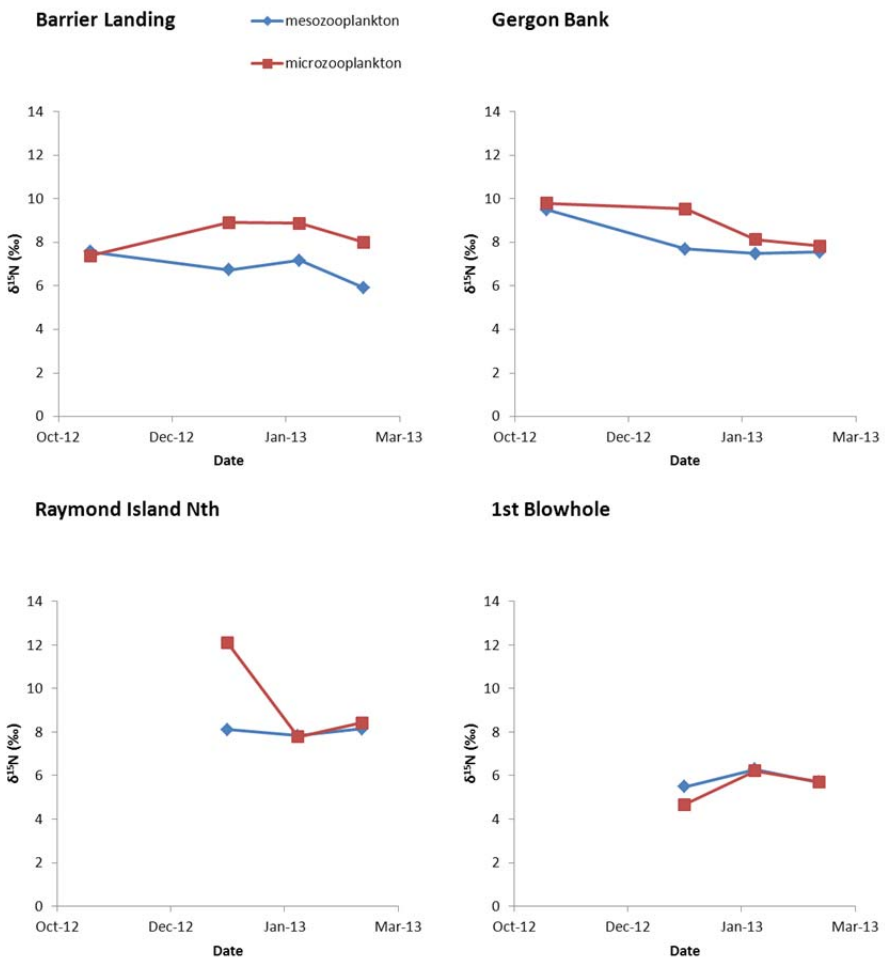

Figure 6. $\delta^{15}N$ of small and large grazers (micro and mesozooplankton) at four sites in the Gippsland Lakes, November 2012 until March 2013

Small fish

Six species of small (<80 mm) fish were collected from the estuary during the study: congoli (*Pseudaphritis urvillii*), glass goby (*Coryphopterus hyalinus*), Port Jackson glassfish (*Ambassis jacksoniensis*), smallmouth hardyhead (*Atherinosoma microstoma*), southern longfin goby (*Favonigobius lateralis*), and Tamar River goby (*Afurcagobius tamarensis*). Across species, the lowest $\delta^{15}N$ values occurred in January at the 1st Blowhole (Error! Reference source not found.). There was no other indication that any of these fish were accumulating the isotopically light *Nodularia*-derived nitrogen.

Two small species of fish were collected from the coastal ocean, pilchard (*Sardinops sagax neopilchardus*) and sandy sprat (*Hyperlophus vittatus*). Neither of these species showed a significant temporal change in the $\delta^{15}N$ composition of their muscle or liver tissues (Figure 8).

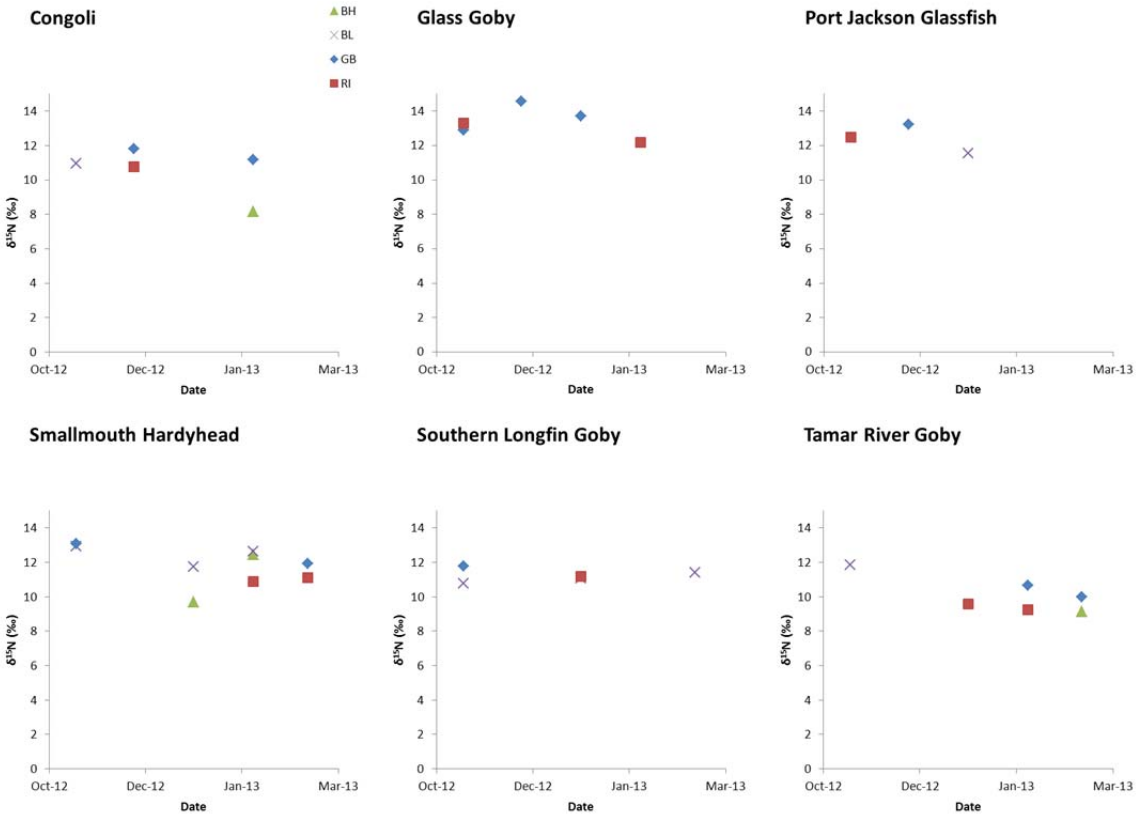

Figure 7. $\delta^{15}N$ of small fish (<80 mm long) at four sites in the Gippsland Lakes, November 2012 until March 2013: BH, 1st Blowhole; BL, Barrier Landing; GB, Gergon Bank; RI, Raymond Island.

Large fish

Stable isotope patterns in the two species of large fish were similar to those of the smaller species (Figure 8). Liver tissue has a higher turnover rate and a change in the isotopic composition of the liver should be measurable if nitrogen from the *Nodularia* bloom was being assimilated by the fish in appreciable amounts. Despite this, the $\delta^{15}N$ composition of the liver did not change. Muscle tissue has a slower turnover rate than liver and there was likewise no change in the muscle $\delta^{15}N$ (Figure 8).

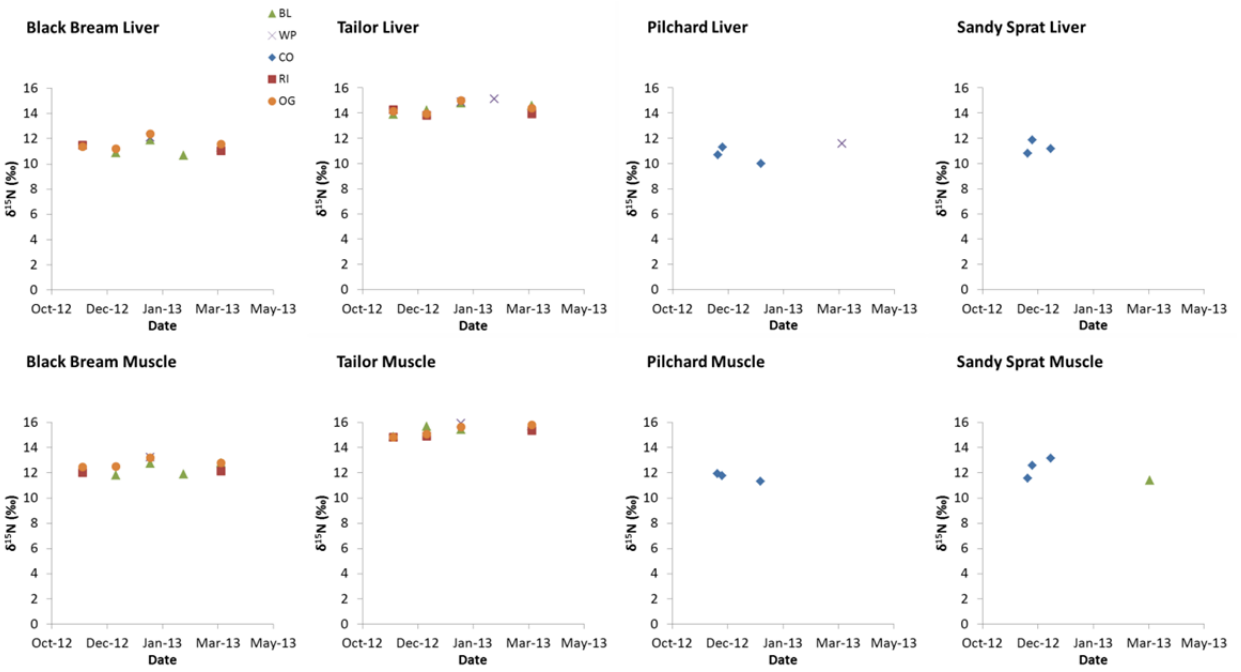

Figure 8. $\delta^{15}N$ of liver and muscle of large fish species collected at four sites in the Gippsland Lakes and in the ocean, between November 2012 and March 2013. CO, coastal ocean; RI, Raymond Island; BL, Barrier Landing; WP, Waddy Point; OG, Ocean Grange.

Birds

Red blood cell samples from crested terns showed a small drop in $\delta^{15}N$, but not enough to be considered a likely result of *Nodularia*-derived nitrogen assimilation (Figure 9). There was no evidence of change in the $\delta^{15}N$ composition of the blood plasma.

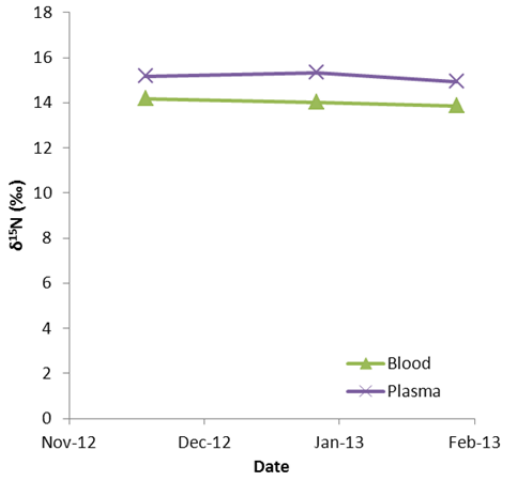

Figure 9. Blood and plasma $\delta^{15}N$ from Crested terns caught near Ocean Grange between November and February 2013.

Discussion

The influence of a *Nodularia* bloom on nitrogen dynamics depends on the magnitude and duration of the bloom. In a study conducted during the intense bloom of 2011-2012, we found clear evidence of a major input of atmospheric nitrogen into the Gippsland Lakes and we documented the transfer of that nitrogen into other phytoplankton, the grazing community and a large fish species (Woodland et al. 2013). We did not measure nitrogen in seagrass, small fish or birds in that study.

In the current study, we again found clear evidence of atmospheric nitrogen entering the Gippsland Lakes food web, but only in and around Bunga Arm. The plankton, including phytoplankton and grazers, and small fish had lower $\delta^{15}N$ values at Bunga Arm than at other sites. The most likely source of this depleted isotope signal is atmospheric nitrogen fixed by *Nodularia*. Larger fish and birds sampled from Bunga Arm and the nearby Ocean Grange did not show any noticeable change in $\delta^{15}N$. We know that these animals are capable of moving throughout the Lakes as well as transitioning between the Lakes and the coastal ocean in the case of crested terns and tailor. Coupled with the localized nature of the 2012-2013 *Nodularia* bloom, this high mobility suggests it would be relatively easy for these consumers to alter their foraging areas to avoid waters contaminated with the toxic *Nodularia*.

The seagrass at Raymond Island North did not show any appreciable change in $\delta^{15}N$ over time, which agrees with the failure of a major bloom to eventuate in that area. Likewise, we did not expect (nor did we see) a decline in $\delta^{15}N$ at Barrier Landing. In fact, there was evidence of a slight rise in the $\delta^{15}N$ value of seagrass at the Barrier Landing site. This could be due to a general decline in the relative availability of isotopically light DIN to seagrass at Barrier Landing over the course of the summer. Plants will preferentially assimilate DIN composed of the light form of nitrogen because it is metabolically favourable to the heavier form. As growth rates increase during the summer, the biological demand for more nitrogen to sustain growth outweighs the metabolic discrimination against isotopically heavy DIN; thus, the plants begin to scavenge the remaining heavy DIN and the overall $\delta^{15}N$ value of the plant's tissues increases. At Gergon Bank, there was a continual drop in seagrass $\delta^{15}N$ over the course of the study, with a lesser drop in epiphyte $\delta^{15}N$.

The most likely pathway by which *Nodularia*-derived nitrogen entered the seagrass is through the sediment. Our conclusion is based on the fact that the epiphytes that grow on the seagrass leaves showed a weaker decline in $\delta^{15}N$ than that of the underlying seagrass. If the *Nodularia*-derived nitrogen had been assimilated from the water column, the epiphytes should have demonstrated a more pronounced decline in their $\delta^{15}N$ composition than the seagrass. This is because epiphytes can assimilate nitrogen faster than the seagrass and given their location on the surface of the seagrass leaves, they are able to access nitrogen in the water column before it reaches the seagrass. Seagrass beds slow water movement and trap particulate material that is in the water column or being transported along the bottom. These particulates are retained and remineralised within the sediment, thereby becoming available as a source of nutrition for the seagrass (Gacia et al. 1999). During this study however the seagrass beds were initially very sparse and we suggest that the most likely mechanism of incorporation was gravitational settling and possibly also trapping of the bloom remnants in the sandy sediments present at the study sites. Sandy sediments are permeable (water can flow through them) and the interaction of currents and waves with sediment topography (ripples) causes water to flow through the sediment, trapping particles. Indeed, it has previously

been observed that the remnants of algal blooms can be trapped and mineralised within sandy sediments (Huettel et al. 2007)

Why was the *Nodularia* bloom smaller than the previous year?

We have previously observed that *Nodularia* only blooms when the water temperature is > 20°C, salinity is between 9 and 20, surface DIN:FRP is < 5:1 and DIN < 0.4 µmol l^{-1}. Temperature regimes > 20°C occur every summer in the Gippsland Lakes and should not be a limiting environmental factor. Salinity in Lake King however, was already above 20 by the start of the bloom, and DIN concentrations at Raymond Island North and Gergon Bank never dropped below 1 µmol l^{-1}. Similarly, the DIN:FRP remained above 5:1 until January. It is not surprising therefore, that the bloom failed to develop in the main body of Lake King. Two other blue-green algae species, *Synechococcus* sp. and *Synechocystis* sp., occurred in high abundances at the same time as the minor *Nodularia* bloom. These other species do not fix atmospheric nitrogen and are an indicator that nitrogen was not a limiting nutrient in the lakes.

Acknowledgements

Funding was provided through the Gippsland Lakes Environment Fund, and this report was prepared for the Gippsland Lakes Ministerial Advisory Committee.

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