Nitrogen fixation by *Nodularia spumigena* Mertens (Cyanobacteriaceae). 1: Field studies and the contribution of blooms to the nitrogen budget of the Peel-Harvey Estuary, Western Australia

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Abstract

Variations in nitrogen fixation (acetylene reduction) by *Nodularia spumigena* blooms in the Peel-Harvey estuarine system were examined with respect to spatial (sampling station location, and depth) and temporal (seasonal and diurnal) distribution. The annual contributions of nitrogen fixation by the blooms to the nitrogen budget of the estuary were estimated to range from 309 to 713t. Contributions by nitrogen fixation were similar to the riverine inputs in the Harvey Estuary, but lower in the Peel Inlet.

The Harvey Estuary had higher biomass and total fixation rates (to 0.4 nmol $C_2H_2 \cdot ml^{-1} \cdot h^{-1}$), but the heterocyst nitrogen fixation rates were greater in the Peel Inlet (to 9×10^{-4} nmol $C_2H_2 \cdot$ heterocyst$^{-1} \cdot h^{-1}$). Nitrogen fixation decreased with depth in response to light, though other factors also appeared to be involved. The rates of fixation decreased concurrently with increasing bloom age, total soluble inorganic nitrogen and salinities. Maximum daily fixation rates occurred in the early morning.

Introduction

Estuaries are not generally considered to be suitable environments for large populations of planktonic cyanobacteria. In fact, Horne (1977) stated that no examples of 'algal' nitrogen-fixation in open estuarine waters were known. However, in the Peel-Harvey Estuarine system in southwest Western Australia, massive phytoplankton blooms of the nitrogen-fixing cyanobacterium *Nodualria spumigina* Mertens occur in the spring and early summer. *Nodularia* also plays a very significant role in the nitrogen economy of the Baltic Sea (Oström, 1976; Lindahl *et al.*, 1978, 1980; Hübel & Hübel, 1980), which may be considered a very large estuary.

In the Peel-Harvey Estuary, phosphorus is considered to be the primary limiting nutrient (Hodgkin & Birch, 1982). However, in the summer months, following winter rains and subsequent diatom blooms, soluble nitrogen is usually in low concentrations (Lukatelich & McComb, 1983). The low nitrogen to phosphorus ratios of riverine inputs, particularly into the Harvey Estuary (mean ratio of 4.5 from 1977 to 1983 (Birch & Bott, pers. commun.)) favour nitrogen-fixing cyanobacteria. In this situation, *Nodularia spumigena* rapidly assumes bloom proportions.

The estimated contributions to the nitrogen budgets of various lakes by nitrogen-fixing cyanobacteria range from less than 0.2% to approximately 80% (Horne, 1977). Oström (1976) estimated the annual contribution by *Nodularia* to the nitrogen budget of the Baltic Sea to be roughly 17.4% of the annual river input of 11 500t. No other estimates of nitrogen-fixation as a proportion of nitrogen budgets of estuarine systems have been found in the published literature.

In the present study, seasonal, diurnal, and spatial changes in nitrogen fixation (as measured by acetylene reduction), heterocyst frequency, and physical and nutritional parameters were measured

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for *Nodularia* blooms in the Peel-Harvey estuarine system. The contributions by the annual blooms to the nitrogen budget of the estuary were estimated and the significance of these contributions is discussed.

Methods

1. Sampling locations. The Peel-Harvey estuarine system is a large (131 km^2), shallow (mean depth 1 m) body of water located in southwestern Western Australia. The salinity varies seasonally from nearly fresh (2‰ (parts per thousand)) to hypersaline (47‰). Generally, nutrient input is confined to the winter months. The locations of the sampling sites are presented in Figure 1.

2. Temperature and dissolved oxygen were monitored with a Delta Scientific Model 211 – multirange temperature and dissolved oxygen analyzer. The pH of the water was measured with a Metroholm Herisau E588 pH meter. Salinity was monitored with an Auto-lab salinity meter (Model 602).

Nitrogen (nitrate+nitrite, ammonia, organic and total) concentrations were determined as described by Gabrielson *et al.* (1983).

3. Biomass was usually estimated by cell counts, but sometimes by chlorophyll concentrations (Huber & Hamel, 1984). Heterocyst frequencies were determined by counting the number of heterocysts per unit length of filament.

4. An acetylene reduction technique, based on that of Hardy *et al.* (1973), was used to estimate the nitrogen fixation capacity of the *Nodularia.* In laboratory studies, 10 or 100 ml of bloom water were placed in 32.4 ml vials (Filtrona®) or 268 ml tissue culture flasks (Sterilin®) and sealed with caps containing rubber septa. Ten per cent of the atmosphere was replaced with acetylene (instrument grade) and the containers were incubated at room temperature under fluorescent lights. Gas samples were either analyzed immediately or subsampled with 5 or 15 ml Vacutainers® for later analysis. The concentration of ethylene in the gas samples was measured with a Varian Model 3700 gas chromatograph fitted with a 6′×1/8″ stainless steel Poropak N column.

Results have been expressed in terms of acetylene reduced per hour, normalized to sample volume, biomass or heterocyst concentrations. The ratio of 100μg chlorophyll to $5.03 \pm 0.44 \times 10^7 \mu m^3$ *Nodularia* cell volume can by used as a guide for conversions. However, since this ratio is somewhat variable depending on nutritional and physico-chemical concentrations, the data presented have not been converted.

In situ acetylene reduction rate determinations were conducted using 700 ml serum bottles clipped onto an iron rod at the shallow site used for the diurnal study (see Fig. 1). At deeper water sites, the bottles were clipped onto heavy chains which were kept vertical by a combination of weights and buoys. For the grid study, samples were incubated onboard the sampling boat. After the appropriate incubation times, gas samples were taken with Vacutainers, and later analyzed in the laboratory as previously described.

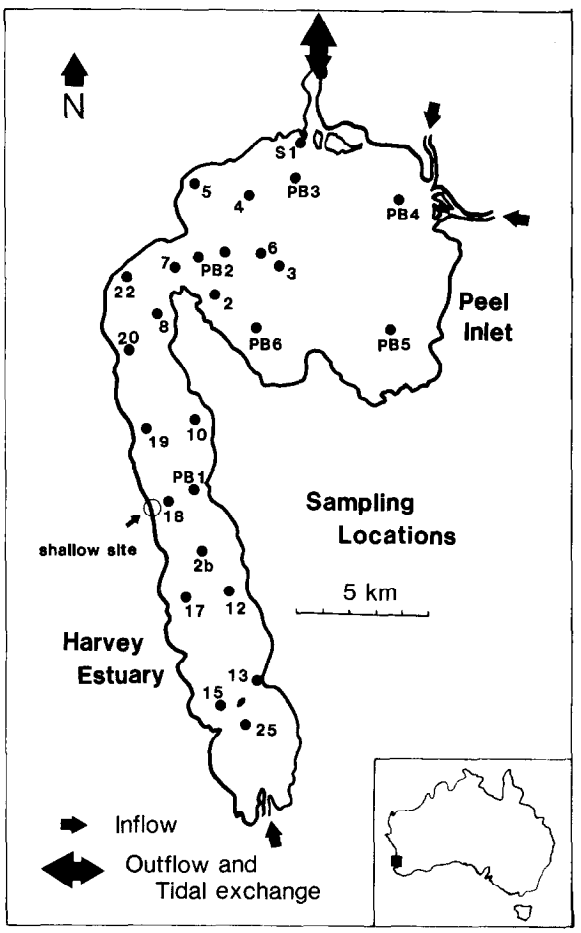

Fig. 1. Locations of sampling stations. Inset shows position of the Peel-Harvey Estuary in Australia.

Results and discussion

1. Spatial distribution

a. Grid study

On November 17, 1978, the *Nodularia* bloom was examined for cell and heterocyst concentrations, heterocyst frequencies (heterocysts per unit filament length), and acetylene reduction rates (Fig. 2a, b). Acetylene reduction rates ranged from 0 to 3.3 μmoles C_2H_2 reduced $\cdot l^{-1} \cdot h^{-1}$ and, like cell and heterocyst concentrations, were greatest in the Harvey Estuary. However, the reduction rates per heterocyst were generally higher in the eastern Peel Inlet, where the heterocyst frequencies were lower.

The most comprehensive studies of the spatial distribution of nitrogen fixation by phytoplankton are those of Horne *et al.* (1972, 1979) who examined nitrogen fixation, biomass, heterocyst frequencies, and physical and nutritional parameters during an autumn *Anabaena* and a spring *Aphanizomenon* bloom at 32 sites on Clear Lake, California. Horne *et al.* (1972) found positive correlations between nitrogen fixation and *Anabaena* heterocyst number, biomass, and phosphorus, but a negative correlation with nitrate concentration. Similar results were obtained for the *Aphanizomenon* bloom (Horne *et al.*, 1979) with an additional negative correlation between nitrogen fixation and ammonia concentrations. These results are similar to those obtained in the present

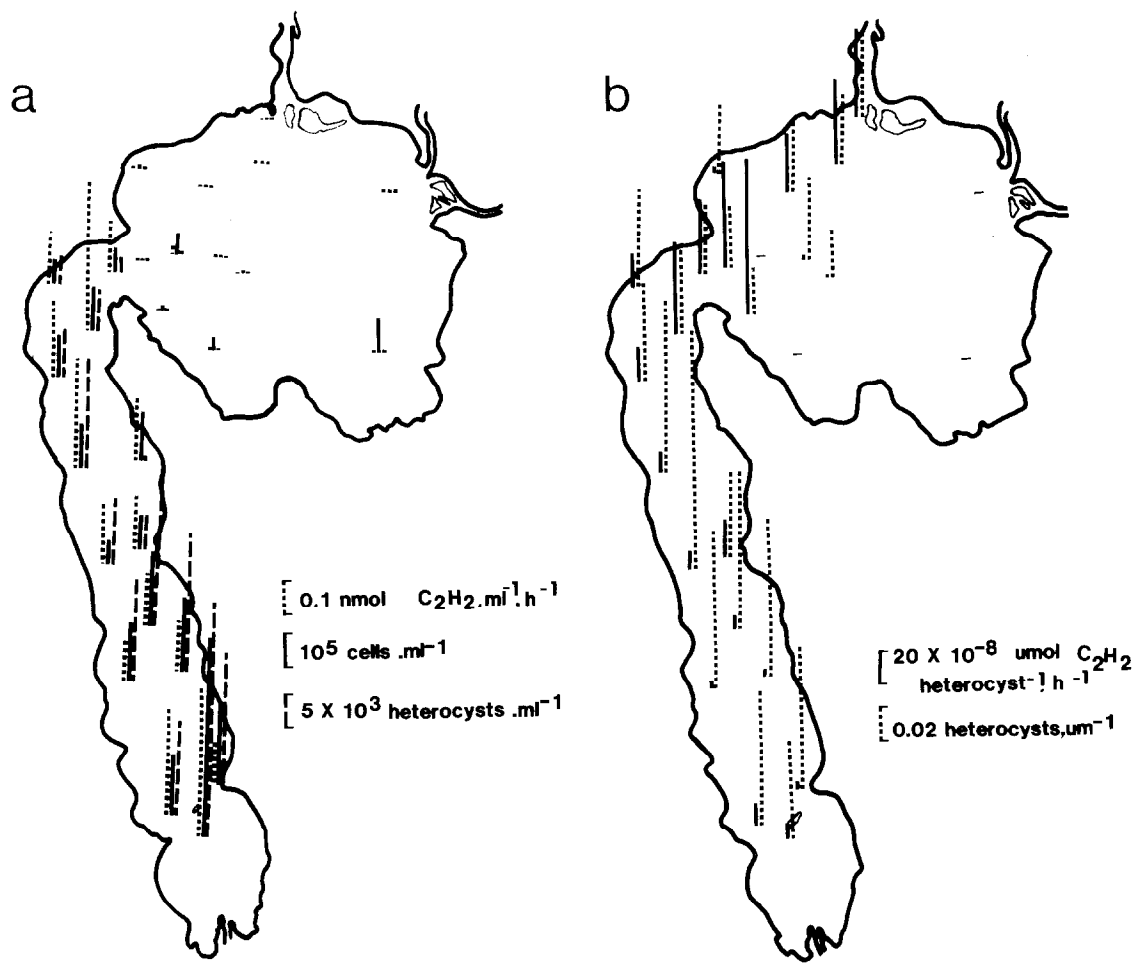

Fig. 2a. Acetylene reduction rates, and cell and heterocyst concentrations in surface water, 17.11.78.
2b: Acetylene reduction rates per heterocyst, and heterocyst frequencies (heterocysts per μm filament).

study of *Nodularia* blooms. However, unlike the present study where the nitrogen fixation rate per heterocyst was lower when the heterocyst number was very high, in the *Aphanizomenon* bloom inactive or less active heterocysts were present when the heterocyst numbers were low (Horne *et al.*, 1979).

The only study of estuarine nitrogen fixation in which the spatial distribution was determined appears to be that of Hübel & Hübel (1980) who examined *Nodularia* blooms at 12 locations in the back waters of the Baltic Sea. A high correlation was shown between nitrogen fixation and *Nodularia* biomass and heterocyst number and, on any given sampling day, the heterocyst nitrogen fixing activity was lower when there were high heterocyst frequencies and/or very large concentrations of heterocysts.

b. Depth

In situ acetylene reduction activities through the water column during the 1978–79 and 1980–81 *Nodularia* blooms are presented in Figures 3a-c and 4a-c. In both years the acetylene reduction activity decreased with depth. This reduction was due to a combination of decreased cell and heterocyst numbers and decreased heterocyst activity (Fig. 3a-c). Most of the decrease in heterocyst activity would have been due to light limitation since secchi depth measurements were less than 0.5 m at each of the sampling times. The heterocyst activities of bottom and mid-depth *Nodularia* were improved when incubated at the surface, but did not equal the heterocyst activities of surface *Nodularia*. The *Nodularia* at the bottom and mid-depths may have been older or less healthy and, therefore, not fixing at a high rate, or there could have been a lag in fixation rate due to the changed light conditions.

Similar depth related variations of nitrogen fixation *in situ* by *Nodularia* were reported by Hübel & Hübel (1980). Both the heterocyst concentration and heterocyst activity decreased with depth, 0 to 6 m, in coastal Baltic Sea waters. Horne (1979) reported variations in nitrogen fixation with depth in Clear Lake during the daytime, but not at night when fixation was reduced but still significant. Generally, maximum fixation occurred on the surface during *Anabaena* blooms. However, for one of three blooms, a mid-afternoon subsurface maximum was shown. Dugdale & Dugdale (1962) also reported a decline in nitrogen fixation with depth for an *Anabaena* bloom in Sanctuary Lake, and ascribed the decline to decreasing light, as did Torrey & Lee (1976) for Lake Mendota.

From the present and cited studies, it appears that light is the dominant factor in controlling changes in nitrogen fixation with water column depth. Cox & Fay (1969) attributed this to limitation of photosynthetic energy supply, but other factors may be involved since fixation does occur in the absence of light, as shown in the present study

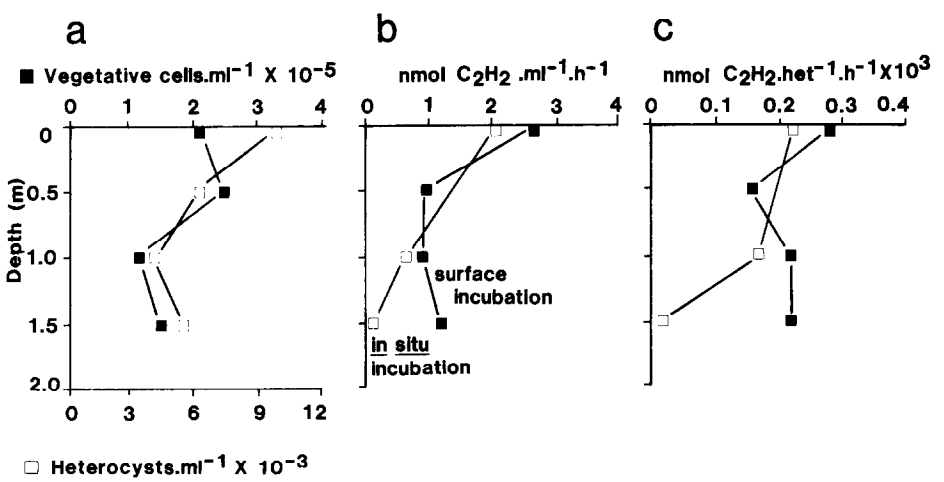

Fig. 3a. Vegetative cell and heterocyst concentrations over depth.
3b, c: Acetylene reduction rates per ml (b), and per heterocyst (c) under *in situ* (□) and surface incubation (■) at sampling location 20, 30/11/78.

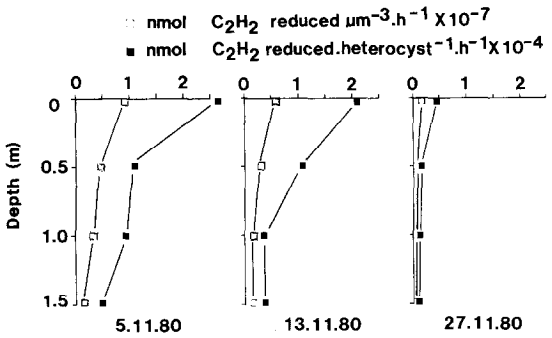

Fig. 4. Change in acetylene reduction rates per volume of *Nodularia* (□) and per heterocyst (■ with depth; 5.11.80, 13.11.80, 27.11.80.

and by Horne (1975). It was also observed that *Nodularia* taken from below the photic zone failed to completely recover their activity when incubated in surface light.

2. *Temporal changes*

a. Seasonal changes

Acetylene reduction activities per ml of water and per heterocyst, heterocyst frequencies, and soluble nitrogen concentrations in surface waters in the Harvey Estuary (Sites 1 and 59) during the 1980–81 bloom are shown in Figure 5a-c. The first sampling date was at the beginning of the bloom, the latter three were in the stationary phase. Acetylene reduction rates per ml of water remained stable during the first week and then decreased. However, the rates per heterocyst, and heterocyst frequencies decreased most rapidly during the first week (Fig. 5a). Conversely, the total soluble inorganic nitrogen ($NH_4^+ + NO_3^- + NO_2^-$) increased over the month examined (Fig. 5c). Salinity levels, which have been reported to affect nitrogen fixation (Telor 1980), have also been plotted in Figure 5c. The decrease in acetylene reduction rates is also shown in Figures 4a-c.

During the course of the 1981–82 *Nodularia* bloom, cell numbers and heterocyst frequencies were monitored at Sites 1, in the Harvey Estuary, and 4, in the Peel Inlet. The results are presented in Figures 6a, b. The bloom was more dense and protracted in the Harvey Estuary (Fig. 6a). Heterocyst frequencies were variable, but were high during periods of rapid growth, i.e. the beginning of

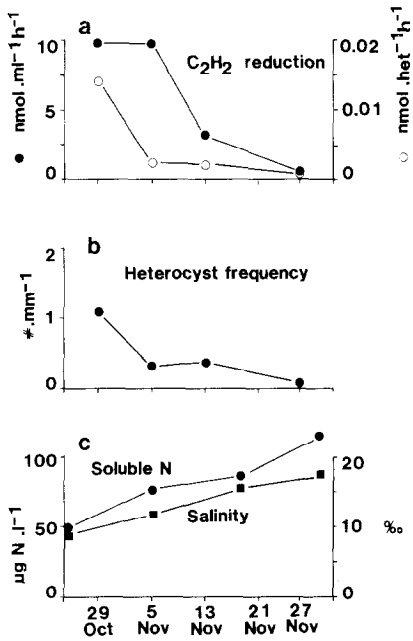

Fig. 5a. Acetylene reduction rates per water volume (●) and per heterocyst (○); 29.10.80–27.11.80.
5b: Changes in heterocyst frequency (heterocysts per µm *Nodularia* filament); 29.10.80–27.11.80.
5c: Changes in soluble nitrogen (●) and salinity (■) from 23.10.80 to 2.12.80 (taken from Lukatelich & McComb, 1983).

the bloom, the beginning of December, and the end of January. The regrowth at the end of January was due to a heavy summer rainfall which resulted in an input of freshwater and nutrients (in particular phosphorus) into the estuary. The soluble nitrogen concentrations at Site 1 (Harvey Estuary) have been plotted (Fig. 6c). Changes in heterocyst frequencies were inversely correlated with changes in soluble nitrogen concentrations.

Seasonal changes in nitrogen fixation by phytoplankton have been extensively examined and related to physical and chemical parameters. As in the present study, Findley *et al.* (1973) and Rother & Fay (1979) found that fixation capacities were greatest at the beginning of blooms. Studies by Ahluwalia & Kumar (1982), Jewell & Kulasooriya (1970), and Stewart (1977) have verified that maximum fixation occurs in the log phase of growth. Soluble inorganic nitrogen supresses nitrogen fixation. Negative correlations between bloom heterocyst numbers and/or nitrogen fixation, and nitrate or ammonia-nitrogen have been

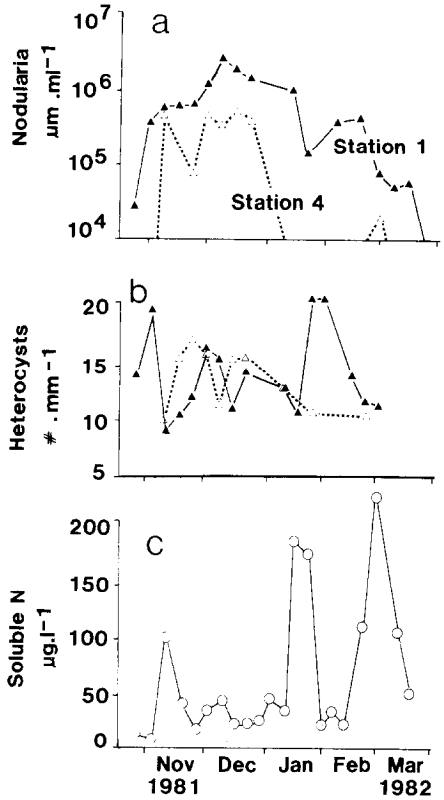

Fig. 6. Changes in surface waters a) *Nodularia* biomass at Stations 1 (— ▲ —) and 4 (... △ ...), b) heterocyst frequencies at Stations 1 (— ▲ —) and 4 (... △ ...), and c) soluble nitrogen concentrations at Station 1 (○) (taken from Lukatelich & McComb, 1983).

shown by Rother & Fay (1979), Horne *et al.* (1972, 1979), and in the present study. Laboratory studies have confirmed the inhibition of nitrogenase activity by soluble inorganic nitrogen (Ahluwalia & Kumar, 1982; Bottomley *et al.*, 1979, 1980; Meeks *et al.*, 1983). Sensitivity of nitrogenase to salt has been demonstrated by Tel-or (1980), and a negative correlation existed between *Nodularia* nitrogen fixation and ambient salinity. As well, phosphate has been shown to be required for fixation (cf. Stewart & Alexander, 1971). In the present study, ambient phosphate concentrations were always low during blooms (less than 20 $\mu g \cdot l^{-1}$, Lukatelich & McComb, 1983). However, the water column was mixed and phosphorus was supplied from sediments (Huber & Hamel, 1984) so the degree of phosphorus limitation during the earlier blooms is not known.

It is clear that the nitrogen fixation capacity of *Nodularia* blooms changed with bloom age, soluble inorganic nitrogen concentrations and ambient salinities (Fig. 5a-c). However, since all these factors changed simultaneously, it was not possible to determine the relative significance of each in controlling nitrogen fixation. Laboratory studies in which these factors were examined separately are reported in the accompanying paper (Huber, 1986).

b. Diurnal changes

Diurnal changes in acetylene reduction activities were determined on three occasions: 27.11.80, 13.11.81, and 03.02.82. Physical and chemical conditions of light, temperature, salinity, dissolved oxygen, pH, soluble nitrogen and phosphorus were measured as well, though not all were determined in each study (Fig. 7a-c). There were distinct diurnal patterns in acetylene reduction activities in the Nov. '80 (Fig. 7a) and Feb. '82 (Fig. 7c) studies, with the maximum activity occurring after sunrise. Acetylene reduction activities remained high for most of the day in the Nov. '80 study, and minimum activities occurred at night. The same was true for the February study but, because of the very high biomass (to 23.4 mg chl $\cdot l^{-1}$) and water temperatures (to 40 °C), the magnitude of activity during most of the day was very low compared to the peak activity. It appears that the only time suitable for significant nitrogen fixation to occur was just after sunrise before high temperatures were reached. During the Nov. '81 diurnal study, the acetylene reduction activity appeared to continually increase. However, an abrupt decrease in salinity and increase in biomass at 2300 h suggest a movement of the water mass, and this may account for the apparent lack of any diurnal pattern.

In 1962, Dugdale and Dugdale demonstrated a correlation between nitrogen fixation and light. Cox & Fay (1969) then showed that nitrogen fixation depended on photosynthetically produced carbon skeletons. However, peak acetylene reduction activities by phytoplankton do not always correspond to maximum light conditions (cf. Horne, 1985; Millineaux *et al.*, 1981). Nitrogenase generally responds to light, but because it is oxygen sensitive, there is a requirement either to separate nitrogen fixation, spatially or temporally, from the high oxygen concentrations concomitant with maximum photosynthetic activity, or to remove some

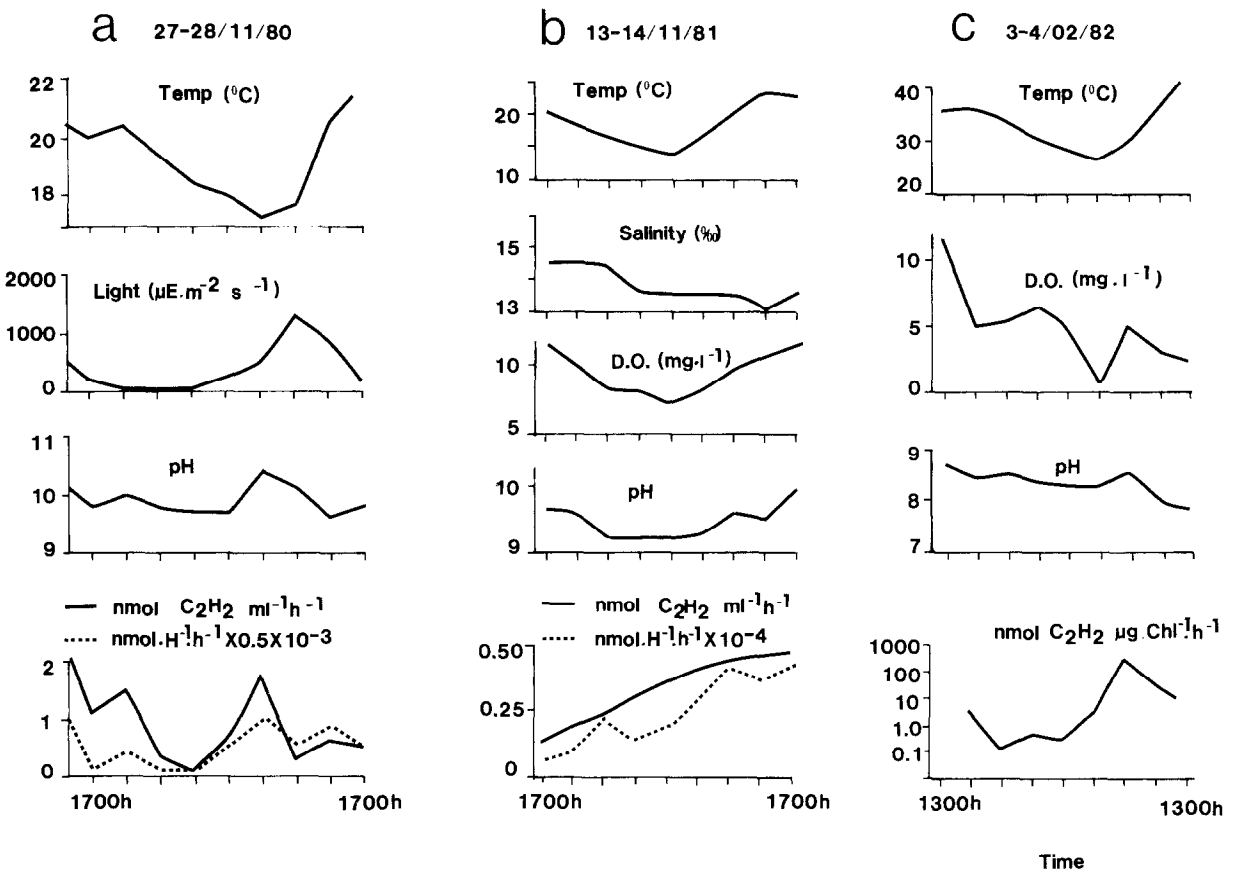

Fig. 7. Diurnal changes in acetylene reduction rates and physico-chemical parameters on a) 27 – 28.11.80, b) 13 – 14.11.81, and c) 3 – 4.01.82.

of the oxygen produced. Paerl (1980) examined *Anabaena* under oxygen supersaturated conditions and concluded that uptake hydrogenase activity increased with the result that O_2 was utilized in the formation of H_2O and ATP. Thus the intracellular oxygen concentration decreased and the energy supply available for nitrogen fixation (ATP) increased. The rate at which such a mechanism could operate, and the resultant balance between oxygen inhibition and requirement for a photosynthetic energy supply would dictate the time of maximum fixation rates. Differences in this balance may explain the variations in the fixation patterns among the many studies conducted.

3. *Contribution of* Nodularia *to the nitrogen budget of the Peel-Harvey estuarine system*

The monthly mean ammonia-N, nitrate and nitrite-N, organic-N and total nitrogen concentrations at Site 1 for the period 1978 to 1983 are shown in Figure 8a-d. These data are summarized from Lukatelich & McComb (1983). No *Nodularia* bloom occurred in 1979 – 80. In general the soluble nitrogen concentrations were high during the winter period (e.g. July), decreased in spring (Sept., Oct.), were very low during the blooms, and increased during bloom decomposition. Organic nitrogen concentrations were very high during the blooms. The sequence of nitrogen conversions was most apparent after the 1978 bloom when a decrease in organic nitrogen was followed by an increase in ammonia and then nitrate and nitrite. This sequence of events is typical of organic matter decomposition and denitrification, and is similar to those events described by Dugdale & Dugdale (1962) and Fallon & Brock (1979). From 1980 to 1982, the trend was less apparent, primarily be-

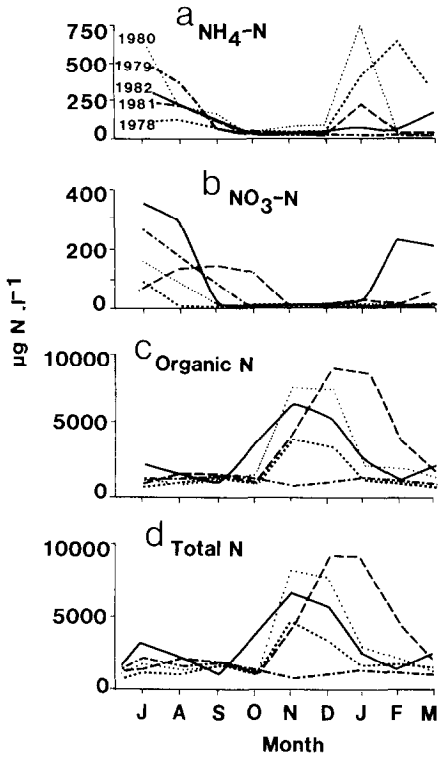

Fig. 8. Mean monthly a) ammonia-N, b) nitrate + nitrite-N, c) organic-N, and d) total nitrogen concentrations in Harvey Estuary surface waters from July to March, 1978 – 1983.
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1982 – 83 ————

cause released nitrogen was immediately taken up by diatom blooms which followed the collapse of the *Nodularia* (Lukatelich & McComb, 1983).

There are several methods by which the contribution to the nitrogen budget of a nitrogen-fixing phytoplankton bloom can be calculated. These include methods based on acetylene reduction or $^{15}N_2$ fixation rates (Ashton, 1981; Horne & Viner, 1971; Torrey & Lee, 1976), heterocyst concentrations (Oström, 1976), and changes in total nitrogen concentrations.

Calculations of the 1978 – 79 *Nodularia* bloom contribution using acetylene reduction rates and heterocyst concentrations were 69.5t and 58.8t over the course of the bloom (Table 1). Though these estimates are in close agreement, the assumptions used for calculations were necessarily broad and, as has been shown, nitrogen fixation rates vary seasonally, diurnally and spatially.

The alternative method is based on the subtraction of any inputs into the system during the bloom and any nitrogen in the system prior to the bloom from the total nitrogen present during the peak of the bloom. These calculations for the 1978, 1980, 1981, and 1982 blooms are presented in Table 2. Since losses of nitrogen to the ocean or estuary shores, or decomposition and dentrification prior to the peak of the bloom are unknown, these will be minimum estimates. These calculations indicate that the contributions of the nitrogen budget by *Nodularia* blooms were equivalent to riverine contributions in the Harvey Estuary. The percentage contribution to the total measured nitrogen budget of the Harvey Estuary (river input plus calculated

Table 1. Calculation of nitrogen fixation capacity of the *Nodularia* during the 1978 – 1979 bloom.

Parameter	Acetylene Reduction Rate Calculation	Heterocyst Conc. Calculation
Depth of light penetration	0.2 m	0.2 m
Area of Harvey Estuary[a]	60 km^2	60 km^2
Volume in which fixation could occur	10^{10} l	10^{10} l
$\bar{X}$ acetylene reduction rate	1.6 μmol$C_2H_2 \cdot l^{-1} \cdot h^{-1}$	–
$\bar{X}$ Heterocyst C_2H_2 reduction rate[b]	–	10^{-5} $\mu g \cdot H^{-1} \cdot day^{-1}$
$\bar{X}$ Heterocyst concentration	–	$1.37 \times 10^4 l^{-1}$
Daily fixation time	7 h	7 h
N_2 fixed/week	11.6 t	9.8 t
N_2 fixed/bloom	69.5 t	58.8 t

[a] In 1978, the *Nodularia* bloom was restricted to the Harvey Estuary.
[b] Based on Oström, 1976.

Table 2. Nitrogen contribution by *Nodularia spumigena* based on nitrogen concentrations: 1978, 1980, 1981, 1982.

Year	1978–79	1980–81	1981–82	1982–83
Surface organic N conc. ($\mu g \cdot l^{-1}$)				
Harvey (Station 1)	9225	9164	6301	9608
Peel (Station 4)	–	4342	2658	5777
Depth to which bloom occurs at maximum concentrations	0.75 m	0.75 m	0.75 m	0.75 m
Volume through which bloom occurs				
Harvey (X 10^{10} l)	4.2	4.2	4.2	4.2
Peel (X 10^{10} l)	–	4.6	4.6	4.6
Total nitrogen in water at the peak of the bloom:				
Harvey	387.5 t	384.9 t	684.6 t	403.5 t
Peel	–	199.7 t	123.5 t	265.7 t
Total	387.5 t	584.6 t	808.5 t	669.2 t
River input				
November loading	3.7 t	2.3 t	2.0 t	3.6 t
Nitrogen concentration in water prior to blooms				
Harvey	75 t	89 t	54 t	68 t
Peel	–	61 t	39 t	104 t
Total	75 t	150 t	93 t	172 t
Nitrogen contribution by the *Nodularia* blooms	308.8 t	434.6 t	713.4 t	497.0 t
Riverine nitrogen loadings				
Peel	1376 t	254 t	944 t	562 t
Harvey	343 t	379 t	367 t	359 t

nitrogen fixation input) ranged from 43.9% to 57.7%. In the Peel Inlet, the contribution was less, ranging from 0 to 35.5%. They also indicate that calculations based on acetylene reduction rates may underestimate the contribution of nitrogen fixation.

Contributions by nitrogen fixation equivalent to annual river or runoff inputs for freshwater lakes and empoundments have been reported by Ashton (1981), Ganf & Viner (1973), and Horne & Goldman (1972). Using heterocyst fixation rates, Ostrom (1976) estimated the contribution of *Nodularia* to the Baltic Sea to be equivalent to about 1/6th of the riverine inputs. This figure is comparable to the estimates made in the present study based on heterocyst activity. Estimates for the nitrogen fixation contribution to Lake Windermere (less than 0.5%, Horne & Fogg, 1970) or to Lake Mendota (7%, Torrey & Lee, 1976) are much smaller than for *Nodularia* in the Harvey Estuary, but similar to its contribution in the Peel Inlet. The only report of a significantly greater input from nitrogen fixation than has been determined for the Harvey Estuary is that of Granhall & Lundgren, 1971, for Lake Erken in Sweden (approximately 80%, as recalculated by Horne, 1977).

This large input of nitrogen does not appear to have resulted in a permanent increase in the nitrogen status of the system. Sediment total nitrogen concentrations fluctuate very dramatically, but there does not appear to be an overall increase. For example, while the total nitrogen concentrations in Site 4 sediments ranged from 928 to 5 684 $\mu g \cdot g^{-1}$. The concentrations in March 1978 and March 1980 were 928 and 982 $\mu g \cdot g^{-1}$, respectively (J. Gabrielson, pers. commun.). This does not indicate an overall increase in sediment storage. What has increased is the potential for larger biomasses, either phytoplankton or macrophytes, to grow and retain nitrogen (as tissue nitrogen) in the system.

Summary

1. Nitrogen fixation rates, as measured by acetylene reduction, and total *Nodularia* biomass and heterocyst concentrations were highest in the Harvey Estuary. However, the nitrogen fixation rate per heterocyst was higher in the Peel Inlet.

2. Nitrogen fixation decreased with depth,

primarily due to light limitation. However, fixation did occur in the dark and *Nodularia* taken from bottom waters and incubated at the surface did not fix nitrogen at as high a rate as surface *Nodularia*.

3. The rate of nitrogen fixation (both by volume and by biomass) was highest in the early stages of the bloom. Decreasing nitrogen fixation rates were concurrent with increasing soluble inorganic nitrogen concentrations and salinities.

4. Diurnal changes in nitrogen fixation were observed with the maximum occurring early in the morning.

5. Calculations based on acetylene reduction rates *in situ* and on heterocyst concentrations gave estimates of nitrogen contributions which were lower than those based on total nitrogen in the peak standing biomass. The latter calculations indicated the following total nitrogen contributions: 1978 – 79, 308.8t; 1980 – 81, 434.6t; 1981 – 82, 713.4t; 1982 – 83, 497.0t. These estimates for the Harvey Estuary are similar to the annual riverine nitrogen loadings; for the Peel Inlet, they are less.

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References

Ahluwalia, A. S. & H. D. Kumar, 1982. Cellular differentiation and nitrogenase activity in the cyanobacterium *Anabaena*. Biologia Pl. 24: 136 – 141.

Ashton, P. J., 1981. Nitrogen fixation and the nitrogen budget of an eutrophic impoundment. Wat. Res. 15: 823 – 833.

Bottomley, P. J., J. F. Grillo, C. van Baalen & F. R. Tabita, 1979. Synthesis of nitrogenase and heterocysts by *Anabaena* sp. CA in the presence of high levels of ammonia. J. Bacteriol. 140: 938 – 943.

Bottomley, P. J., C. van Baalen & F. R. Tabita, 1980. Heterocyst differentiation and tryptophan metabolism in the cyanobacterium *Anabaena* sp. CA. Arch. Biochem. Biophys. 208: 204 – 213.

Cox, R. M. & P. Fay, 1969. Special aspects of nitrogen fixation by blue-green algae. Proc. r. Soc. Lond. B 172: 357 – 366.

Dugdale, V. A. & R. C. Dugdale, 1962. Nitrogen metabolism in lakes. 2. Role of nitrogen fixation in Sanctuary Lake, Pennsylvania. Limnol. Oceanogr. 7: 170 – 177.

Fallon, R. D. & T. D. Brock, 1979. Decomposition of blue-green algal (cyanobacterial) blooms in Lake Mendota, Wisconsin. Appl. envir. Micrbiol. 37: 820 – 830.

Findley, D. L., D. I. Findley & J. R. Stein, 1973. Surface nitrogen and plankton in Skaha Lake, British Columbia, Canada. Feshwat. Biol. 3: 111 – 122.

Gabrielson, J. O., P. B. Birch & K. S. Hamel, 1983. Decomposition of *Cladophora*, 2. Nitrogen and phosphorus regeneration. Bot. mar. 26: 173 – 179.

Ganf, G. G. & A. J. Horne, 1975. Diurnal stratification, photosynthesis and nitrogen fixation in a shallow equatorial lake (Lake George, Uganda). Freshwat. Biol. 5: 13 – 119.

Ganf, G. G. & A. B. Viner, 1973. Ecological stability in a shallow equatorial lake (Lake George, Uganda). Proc. r. Soc. Lond. B 184: 321 – 346.

Hardy, R. W. F., R. C. Burns, & R. D. Holsten, 1973. Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biol. Biochem. 5: 47 – 81.

Hodgkin, E. P. & P. B. Birch, 1982. Eutrophication of a Western Australian estuary. Oceanol. Acta 1982: 313 – 318.

Horne, A. J., 1975. Algal nitrogen fixation in California streams: diel cycles and nocturnal fixation. Freshwat. Biol. 5: 471 – 477.

Horne, A. J., 1977. Nitrogen fixation – a review of this phenomenon as a polluting process. Proc. Wat. Tech. 8: 359 – 372.

Horne, A. J., 1979. Nitrogen fixation in Clear Lake, California. 4: Diel studies on *Aphanizomenon* and *Anabaena* blooms. Limnol. Oceanogr. 24: 329 – 341.

Horne, A. J., J. E. Dillard, D. K. Fujita & C. R. Goldman, 1972. Nitrogen fixation in Clear Lake, California. II. Synoptic studies on the autumn *Anabaena* bloom. Limnol. Oceanogr. 17: 693 – 703.

Horne, A. J. & G. E. Fogg, 1970. Nitrogen fixation in some English lakes. Proc. r. Soc. Lond. B 175: 351 – 366.

Horne, A. J. & C. R. Goldman, 1972. Nitrogen fixation in Clear Lake, California, 1. Seasonal variation and the role of heterocysts. Limnol. Oceanogr. 17: 678 – 692.

Horne, A. J., J. C. Sandusky & C. J. W. Carmigglet, 1979. Nitrogen fixation in Clear Lake, California, 3. Repetative synoptic sampling of the spring *Aphanizomenon* blooms. Limnol. Oceanogr. 24: 316 – 328.

Horne, A. J. & A. B. Viner, 1971. Nitrogen fixation and its significance in tropical Lake George, Uganda. Nature 232: 417 – 418.

Hübel, H. & M. Hübel, 1980. Nitrogen fixation during blooms of *Nodularia* in coastal waters and backwaters of the Arkona Sea (Baltic Sea) in 1974. Int. Revue ges. Hydrobiol. 65: 793 – 808.

Huber, A. L., 1986. Nitrogen fixation by *Nodularia spumigena* Mertens (Cyanobacteriaceae). a: Laboratory studies. Hydrobiologia 132: 193 – 203.

Huber, A. L. & K. S. Hamel, 1984. Phosphatase activities in relation to phosphorus nutrition in *Nodularia spumigena* (Cyanobacteriaceae), 1. Field studies. Hydrobiologia 123: 145 – 152.

Jewell, W. J. & S. A. Kulasooriya, 1970. The relation of acetylene reduction to heterocyst frequency in blue-green algae. J. exp. Bot. 21: 874 – 880.

Lindahl, G., K. Wallström & G. Brattberg, 1978. On the nitrogen fixation in a coastal area of the Northern Baltic. Kieler Meeresforsch. 4: 171 – 177.

Lindahl, G., K. Wallström & G. Brattberg, 1980. Short-term variations in nitrogen fixation in a coastal area of the Northern Baltic. Arch. Hydrobiol. 89: 88 – 100.

Lukatelich, R. J. & A. J. McComb, 1983. Water quality of the Peel-Harvey estuarine system. March 1981 – August 1982 Appendix 2. Waterways Comm. Peel Inlet Mgmt Auth., Perth, West. Aust.

Meeks, J. C., K. L. Wycoff, C. S. Chapman & C. S. Enderlin, 1983. Regulation of expression of nitrate and dinitrogen assimilation by *Anabaena* species. Appl. envir. Microbiol. 45: 1351 – 1359.

Millineaux, P. M., J. R. Gallon, & A. E. Chaplin, 1981. Acetylene reduction (nitrogen fixation) by cyanobacteria grown under alternating light-dark cycles. F.E.M.S. microbiol. Lett. 10: 245 – 247.

Oström, B., 1976. Fertilization of the Baltic by nitrogen fixation in the Blue-green alga *Nodularia spumigena*. Remote Sens. Envir. 4: 305 – 310.

Paerl, H. W., 1978. Light-mediated recovery of N_2-fixation in the blue-green algae *Anabaena* spp. in O_2 supersaturated waters. Oecologia (Berl.) 32: 135 – 139.

Paerl, H. W., 1979. Optimization of carbon dioxide and nitrogen fixation by the blue-green alga *Anabaena* in freshwater blooms. Oecologia (Berl.) 38: 275 – 290.

Paerl, H. W., 1980. Ecological rationale for H_2 metabolism during aquatic blooms of the cyanobacterium *Anabaena*. Oecologia (Berl.) 47: 43 – 45.

Rother, J. A. & P. Fay, 1979. Some physiological-biological characteristics of planktonic blue-green algae during formation in three Salopian meres. Freshwat. Biol. 9: 369 – 379.

Saino, T. & A. Hattori, 1978. Diel variation in nitrogen fixation by a marine blue-green alga, *Trichodesmium thiebautii*. Deep Sea Res. 25: 1259 – 1263.

Stewart, W. D. P. 1977. Blue-green algae. In R. W. F. Hardy & W. S. Silver (eds.), A Treatise on Dinitrogen Fixation. J. Wiley & Sons, N.Y.: 63 – 123.

Stewart, W. D. P. & G. Alexander, 1971. Phosphorus availability and nitrogenase activity in aquatic blue-green algae. Freshwat. Biol. 1: 389 – 404.

Tel-or, E., 1980. Response of N_2-fixing cyanobacteria to salt. Appl. envir. Microbiol. 40: 689 – 693.

Torrey, M. S. & G. F. Lee, 1976. Nitrogen fixation in Lake Mendota, Wisconsin. Limnol. Oceanogr. 21: 365 – 378.

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