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Nitrogen inputs into the euphotic zone by vertically migrating *Rhizosolenia* mats

HEATHER R. SINGLER† AND TRACY A. VILLAREAL*

MARINE SCIENCE INSTITUTE, THE UNIVERSITY OF TEXAS AT AUSTIN, 750 CHANNELVIEW DRIVE, PORT ARANSAS, TX 78373, USA

†PRESENT ADDRESS: ENVIRONMENTAL STUDIES, FLORIDA INTERNATIONAL UNIVERSITY, 11200 S. W. EIGHTH STREET, MIAMI, FL 33199, USA

*CORRESPONDING AUTHOR: tracy@utmsi.utexas.edu

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Rhizosolenia mats conduct extensive vertical migrations in the oligotrophic central North Pacific (cNP) gyre that permit these diatoms to acquire nitrate at depth and return to the surface for photosynthesis. The ultimate fate of this N within the ecosystem is unknown, but may include remineralization by grazing, loss to depth by sinking biomass, or N excretion by Rhizosolenia mats. Direct release of N by mats into the mixed layer would represent an upward biological pump that circumvents the diffusion barriers and nutrient sinks at the base of the oceanic euphotic zone. We examined Rhizosolenia mat N release along a transect (28–31° N) in the summer of 2002 (Hawaii to California) and 2003 (Hawaii to west of Midway Island) using sensitive fluorometric and chemiluminescence methods. Nitrate, NO_2^- and NH_4^+ release was determined. Nitrate and NH_4^+ release by the mats occurred in both 2002 (22.84 ± 6.04 and 3.69 ± 1.74 nmol N μg^{-1} Chl a h^{-1}, respectively) and 2003 (23.74 ± 3.54 and 3.60 ± 0.74 nmol N μg^{-1} Chl a h^{-1}, respectively). Nitrite release only occurred in the 2003 summer period but occurred in both years when Fe chelators were added. Fv/Fm values decreased westward in 2003 suggesting a gradient of increasing physiological stress towards the west. The various physiological measures are consistent with concurrent Fe stress; however, other possibilities exist. Nitrate excretion was the dominant form of N release in both years and provided a substantial addition to the ambient nitrate pool in the mixed layer. Rhizosolenia mat nitrate release supplies at least 4–7% of the nitrate pool on daily basis, and possibly as much as 27%. Rhizosolenia mats are part of a large phytoplankton community that appears to migrate, and rates could be significantly higher. Literature reports suggest little or no nitrification in the upper euphotic zone, and thus biological transport and release of nitrate may be a major source to this region. This N release is uncoupled from upward CO_2 transport and, like N_2 fixation, provides a component of the N pool available for net carbon removal.

INTRODUCTION

Rhizosolenia mats are macroscopic assemblages of up to seven different *Rhizosolenia* species (Villareal and Carpenter, 1989) that undergo vertical migrations to exploit deep nitrate pools. These large phytoplankton taxa are found in pico- and nanoplankton-dominated oligotrophic gyres despite their apparent disadvantage (low surface-area-to-volume ratios) in competition for scarce nutrients (Chisholm, 1992). To overcome their size disadvantage, *Rhizosolenia* mats, *Rhizosolenia* spp., *Pyrocystis* spp., *Ethmodiscus* spp. and possibly *Halosphaera* spp. undergo vertical migrations to exploit deep nutrient pools and then return to the surface to photosynthesize (Villareal and Lipschultz, 1995; Villareal *et al.*, 1996, 1999a). This behavioral adaptation prevents direct competition with the dominant nanoplankton size classes for nutrients and allows their survival despite the disadvantages of large size. While several diatoms species possess diazotrophic heterocytous cyanobacteria as symbionts (Sundström, 1984; Ferrario *et al.*, 1995), *Rhizosolenia* mats do not (Villareal *et al.*, 1996). The evidence to support vertical migration for nutrient acquisition comes from high internal nitrate pools, high 15N values, the presence of nitrate reductase (NR) activity, buoyancy reversals, changes in chemical composition between positively and negatively buoyant mats and lack of nitrogen (N_2) fixation (Villareal, 1987; Villareal *et al.*, 1993, 1996,

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1999b; Joseph *et al.*, 1997). Some or all of these proxies have also been used to infer vertical migration in the cyanobacterium *Trichodesmium* (Romans *et al.*, 1994; Villareal and Carpenter, 2003), the dinoflagellates *Pyrocystis noctiluca* and *P. fusiformis* (Rivkin *et al.*, 1984; Ballek and Swift, 1986) and the prasinophyte *Halosphaera* (Wiebe, 1974; Villareal and Lipschultz, 1995). In *Rhizosolenia* mats, buoyancy reversals are associated with changes in nutrient quotas. Positively buoyant mats are higher in internal nitrate pools and have lower C : N, carbohydrate : N and C : Chl ratios than negatively buoyant mats (Villareal *et al.*, 1996). Culture results suggest that carbohydrate ballasting occurs in *Rhizosolenia* and is sufficient to overcome the lift provided by ion exclusion and regulation of the cell sap (Moore and Villareal, 1996). The carbohydrate is consumed in the dark to support respiration and presumably nitrate uptake and results in a deballasting of the cell. These are processes analogous to well documented cycles in limnetic cyanobacteria (Klemer *et al.*, 1996).

Nitrate uptake and subsequent upward vertical migration by *Rhizosolenia* mats is a form of new nitrogen input to surface waters and meets the definition of new production (Dugdale and Goering, 1967). It is conceptually identical to the vertical migration noted in coastal flagellates but is remarkable for the distances involved (~100+ m) and the use of buoyancy regulation rather than flagella. Models suggest that *Rhizosolenia* mats contribute up to 17% (Richardson *et al.*, 1998) of the new production from nitrate into the euphotic zone, and mats can, at times, transport NO_3^- at the equivalent of 59% of export production measured at Hawaii Ocean Time Series (HOT) (Villareal *et al.*, 1999b). However, the fate of this mat nitrogen is still unknown. It may be utilized solely by mats, released into surrounding waters, remineralized by macro- or microzooplankton or, most likely, some combination of all of these.

The high-energy requirement for nitrate reduction suggests a close linkage to iron utilization in *Rhizosolenia* mats. Flynn and Hipkin (Flynn and Hipkin, 1999) modeled Fe effects on N assimilation and noted a substantial decrease in growth rate when Fe-stressed cells relied on NO_3^-. The decrease was linked to how Fe was partitioned in the cell when limiting and resulted in lower nitrate and nitrite reductase activity. Raven (Raven, 1988, 1990) predicted, based on theoretical calculations, that phytoplankton growing on NO_3^-, such as *Rhizosolenia* mats, require 60% more iron than phytoplankton growing on NH_4^+. Iron concentrations in the central North Pacific (cNP) gyre at the VERTEX-IV station (28° N and 155° W, ~200 km North of Hawaii) have been found to be as low as 0.02 nM between 70 m and 100 m in depth (Bruland *et al.*, 1994), a level that is not capable of supporting high phytoplankton biomass.

McKay *et al.* (McKay *et al.*, 2000) found *Rhizosolenia* mats collected in the cNP gyre expressed the protein flavodoxin but found no indication for accumulation of ferredoxin, a combination considered to be a diagnostic indicator of iron stress in diatoms. The standard interpretation is that *Rhizosolenia* mats are iron stressed; however, accumulation of flavodoxin in laboratory cultures of *Rhizosolenia formosa* grown in up to 10,000 nM iron led McKay *et al.* (McKay *et al.*, 2000) to suggest flavodoxin expression may be constitutive. Thus, NO_3^- reduction with its high Fe requirement and the extremely low iron concentrations in the cNP gyre seem an unlikely combination. However, nitrate may the primary N source due to ammonium levels so low that NO_3^- assimiliation is not repressed (Flynn and Hipkin, 1999).

Nitrogen release in phytoplankton in both organic and inorganic forms has been well documented (Collos, 1998). Lomas *et al.* (Lomas *et al.*, 2000) found that rapid changes in environmental conditions led to NH_4^+ and NO_2^- release in the diatoms *Chaetoceros* sp., *Skeletonema costatum* and *Thalassiosira weissflogii*. They suggested that nitrogen release can act as a means to dissipate excess photosynthetic energy. Recent studies have suggested the NO_3^- release is a fundamental process that ultimately determines the degree of isotope fractionation during growth on NO_3^- (Needoba *et al.*, 2004).

Nitrite release has been reported in numerous marine phytoplankton taxa as a result of stress, including but not limited to, the diatoms *Chaetoceros curvisetus* (Anderson and Roels, 1981), *S. costatum* and *Phaeodactylum tricornutum* (Collos, 1998) and the dinoflagellates *Scrippsiella trochoidea* and *Alexandrium minutum* (Flynn and Flynn, 1998). Milligan and Harrison (Milligan and Harrison, 2000) have found that during NO_3^-replete, iron-limited conditions, the marine diatom *T. weissflogii* (Grunow) Fryxell et Hasle will increase NO_2^- release to a maximum of 100 fmol cell^{-1} day^{-1} (6% day^{-1}). Throughout their experiment, both NR and nitrite reductase activities remained above nitrogen incorporation rates, suggesting supply of photosynthetically derived reductants (either ferredoxin or flavodoxin) to nitrite reductase limited assimilation of NO_2^-. Reductant supply to nitrite reductase is dependent upon efficiency of electron transfer between PSII and PSI, which can be assessed by fluorescence measurements. Depressed variable fluorescence yield (Fv : Fm) in iron-depleted cells is consistent with decreased electron transfer efficiency between PSII and PSI (Milligan and Harrison, 2000). Given low dissolved iron concentrations within the cNP gyre and lack of ferredoxin expression in mats, it is reasonable to predict that *Rhizosolenia* are iron stressed. Based on this analysis, *Rhizosolenia* mats should have depressed fluorescence values and increased NO_2^- release rates. The actual

mechanisms driving the release may be more complex (Flynn and Flynn, 1998; Flynn and Hipkin, 1999), but the possible release of N acquired below the nitricline into surface waters is the significant issue for our work.

We present the results of two cruises in the cNP gyre documenting nitrogen (NO_3^-, NO_2^- and NH_4^+) release in the euphotic zone by vertically migrating *Rhizosolenia* mats. We hypothesized that *Rhizosolenia* mats release dissolved inorganic nitrogen *in situ* and that these rates increase during periods of iron stress. Our results indicate their N release rates are substantial, vary in time and space and can be a significant input to the nitrate pool in the upper mixed layer of the cNP gyre.

METHOD

Rhizosolenia mats were collected in the cNP gyre in June/July 2002 (*R/V Melville*) and in August/September 2003 (*R/V New Horizon*). Sampling extended along an E–W transect line from Oahu, HI to San Diego, CA, USA in 2002 and along an E–W transect line (28° N) from north of Oahu, HI to ~200 km west of the International Date Line (Fig. 1). Mats were hand-collected between 0 and 10 m depth by scuba divers using wide-mouth polymethylpentane jars ranging in size from 125 to 1000 mL. Collection jars were acid cleaned, rinsed twice with deionized water and filled from the ship's uncontaminated seawater system prior to each use. During return to the ship, collection jars were stored in a dark, insulated container to prevent light and temperature shock. Upon returning to the ship, mats were sorted into floaters, sinkers and neutrals (Villareal *et al.*, 1996).

Mats for incubation were transferred to 250-mL square Nalgene bottles containing seawater collected at time of mat collection. *Rhizosolenia* mats are fragile, and care was taken to maintain mat integrity and to prevent cell lysis. Mat integrity was checked by light microscopy several times during the cruises. Two to three (depending on size) positively buoyant mats were pooled together for incubations to increase biomass for possible nitrogen release. Experimental treatments focused on positively buoyant mats since negatively buoyant mats contain little or no free nitrate and nitrite in the cell sap (Villareal *et al.*, 1996). In addition, positively buoyant mats typically outnumber negatively buoyant mats in our daytime collections by 2–10 : 1. During 2003, comparisons between buoyancy conditions were conducted when sufficient negatively buoyant mats were available. Two to four replicate bottles were used for each treatment. Colonial radiolarians, amphipods, epiphytes and marine snow commonly found in the mat matrix (Villareal and Carpenter, 1989) were removed by pipette prior to mat transfer. Control incubations with no mats were set up using seawater collected at the same time as mat collection ($n = 2$ per station). For induced iron limitation incubations, deferoxamine mesylate (DFB, a iron chelator) was added at a concentration of 100 nM. DFB affects only iron availability and not the availability of other trace metal elements (Timmermans *et al.*, 2001). Mats were incubated onboard for a period of 7 h at 50% surface irradiance. This time interval was chosen to provide a substantial signal yet was short enough to minimize mat deterioration. Time series sampling within the same bottle was not possible. Repetitive sampling of the same incubation bottle created air bubbles that led to turbulence sufficient to disrupt the mat. Since the mats contain large (mM) internal nitrate pools (Villareal *et al.*, 1996), these efforts were likely to lead to significant nitrate increases independent of mat physiology and were discontinued after the first several stations. Running seawater supplied to the onboard incubator maintained the temperature at the ambient surface water temperature.

Dissolved nitrogen (NH_4^+, NO_3^- and NO_2^-) samples for excretion measurements were collected immediately after mat collection from the ambient water and at the end of incubations from the experimental bottles. Ammonium was sampled first to minimize contamination and determined using a fluorometric method (Protocol A, Holmes *et al.*, 1999) scaled to 50% of the original volume. Nitrate and NO_2^- concentrations were measured by chemiluminescent techniques using a continuous sparging

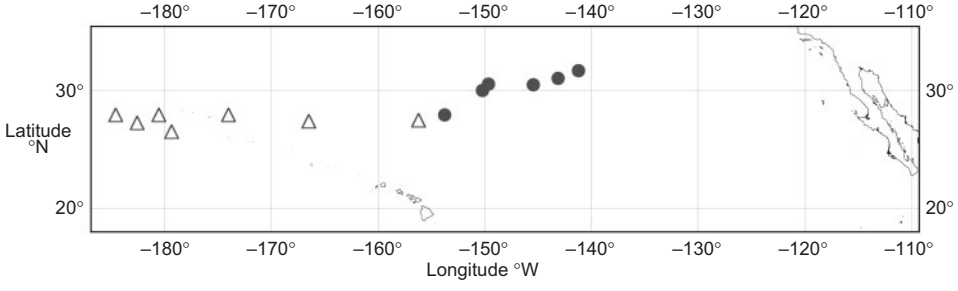

Fig. 1. Cruise track for *Rhizosolenia* mat collections in 2002 (●) and 2003 (△).

method (Joseph *et al.*, 1997). Samples that were not immediately read were syringed-filtered with 5.0 μm Millipore Isopore Membrane filters and frozen at –20°C in 125 mL polyethylene bottles. Dore and Karl (Dore and Karl, 1996) found no significant differences in NO_2^- concentrations between fresh samples and samples that were slow frozen to –20°C in polyethylene bottles. Initial nutrient concentration was assumed to be the same between control and experimental bottle (verified by spot checks). Release rates were calculated by the equation:

$$\text{N release}\left(\text{nmol N } \mu\text{g Chl}^{-1}\ \text{h}^{-1}\right) = \frac{(N_f - B_F)(V)}{T \cdot \text{Chl}}$$

where N_f = final concentration (nM), B_f = blank final concentration (nM), V = bottle volume (0.25 L), T = time (7 h) and Chl = mat Chlorophyll (μg). In this formulation, uptake will be negative. Conversion to mat N content can be made using a 1.65 μmol N : μg Chl conversion (Villareal *et al.*, 1996), and C : N ratios given below.

For chlorophyll measures, 10-mL samples were removed and filtered with 5-μm pore size Osmonics MAGNA Nylon 25-mm filters. Chlorophylls were extracted in the dark, overnight in MeOH at –20°C and determined fluorometrically using a nonacidification technique (Welschmeyer, 1994). Particulate carbon and nitrogen samples were filtered onto precombusted 25-mm Whatman GF/C glass fiber filter, wrapped in precombusted aluminum foil and kept frozen at –20°C until analysis. *Rhizosolenia* mat abundance was determined by scuba divers using a frame with a calibrated flowmeter (Villareal *et al.*, 1996). Fluorescence yield (Fv/Fm) was measured using a Xe-pulse amplitude modulation (Xe-PAM) fluorometer (Schreiber, 1994) zeroed with filtered seawater (Cullen and Davis, 2003) after a dark adaptation period of 10 min. Statistical analyses of data used SPSS 10.0.7 for Windows (SPSS).

RESULTS

Surface NO_3^- and NO_2^- concentrations in the cNP gyre were 2 nM for both 2002 and 2003. Surface NH_4^+ concentrations were <30 nM during the 2002 period and 2003 period except for an area between 172° W and 180° W (Fig. 1). Within this area, divers noticed the presence of a *Hemiaulus hauckii* diatom bloom. The chains had a typical helical appearance; identification was confirmed using shipboard microscopy. Within this area, there was an increase in surface NH_4^+ concentrations (<200 nM) that probably was associated with N fixation by its *Hemiaulus* symbiont *Richelia*. Surface irradiance at time of incubations ranged from 840 to 1090 μmol (photon) m^{-2} s^{-1} during 2002 and from 1060 to 1650 μmol (photon) m^{-2} s^{-1} during 2003.

Rhizosolenia mats were abundant along both the 2002 and 2003 transects (average 0.16 ± 0.05 and 0.27 ± 0.08 mats m^{-3}, respectively). *Rhizosolenia fallax* Sundström was the small diameter species found in mats as previously noted by Villareal and Carpenter (Villareal and Carpenter, 1989) and Villareal *et al.* (Villareal *et al.*, 1996). In summer 2003, there was a notable shift in dominant large-diameter species from *R. castracanei* H. Peragallo to *R. acuminata* (H. Peragallo) H. Peragallo west of 175° W, a transition also noted in 1995 during a cruise in the same area (Shipe *et al.*, 1999). Chl *a* content of incubated mats from both sampling periods averaged 0.93 ± 0.05 μg Chl *a* mat^{-1} (n = 61) and did not significantly vary after incubation between the two sampling periods (P = 0.46, Table I). Mats sampled immediately after collection averaged 1.29 ± 0.15 μg Chl *a* mat^{-1} (n = 30), significantly

Table I: Rhizosolenia mat characteristics and N release rates (nmol N μg^{-1} Chl a h^{-1}) for all unamended mats during 2002 and 2003 sampling periods

	2002				2003			
	n	Minimum	Maximum	Mean ± SE	*n*	Minimum	Maximum	Mean ± SE
Abundance	11	0.03	0.65	0.16 ± 0.05	14	0.04	1.27	0.27 ± 0.08
Chl *a*	19	0.03	1.84	0.93 ± 0.13	42	0.35	1.57	0.92 ± 0.04
Fv/Fm	19	0.54	0.69	**0.61 ± 0.01**	42	0.26	0.66	**0.50 ± 0.02**
C : N ratio	15	5.35	9.46	**7.3 ± 0.3**	40	3.1	7.8	**5.6 ± 0.2**
NO_2^-	19	0	0	**0 ± 0**	32	0.11	2.02	**0.77 ± 0.18**
NO_3^-	19	0	90	22.84 ± 6.04	35	4.18	86.27	31.32 ± 5.76
NH_4^+	14	–7.8	19.66	3.69 ± 1.74	24	–1.22	10.65	3.60 ± 0.74

Abundance in mats^{-3} and Chl *a* in units of μg mat^{-1}. Mat abundance measurements are from scuba enumerations. Negative values indicate N uptake by mats. Bold text indicates significant differences (P < 0.05) between sampling periods.

higher ($P < 0.001$) than Chl *a* content of incubated mats. The difference represents the small, but unavoidable, loss of material during transfer. Quantum yield of fluorescence (Fv : Fm) of incubated mats during both sampling periods ranged averaged 0.52 ± 0.01 ($n = 61$). Fv : Fm of fresh, nonincubated mats differed significantly between the 2002 and 2003 sampling periods ($P < 0.001$, Table I). There was a notable depression in the Fv : Fm of *Rhizosolenia* mats west of longitude 165° W during the summer 2003 period (Fig. 2). Fv : Fm was significantly correlated with longitude ($r^2 = 0.60$, $P < 0.001$) with decreasing Fv : Fm values to the west. Post-incubation C : N ratios of mats from both 2002 and 2003 averaged 5.9 ± 0.1 ($n = 55$). C : N ratios were significantly higher (mean = 7.2 ± 0.2) in the 2002 period ($P < 0.001$, Table I).

Nitrogen release occurred during both sampling periods in unamended mat incubations (Table I) although at different rates between years and N species. Final concentrations of NO_2^- averaged 0 ± 0 nM ($n = 19$) in 2002 (NO_2^- release occurred only in +DFB incubations) and 26.95 ± 3.57 nM ($n = 32$) in the 2003 sampling period. Nitrite in blank incubations, when detectable, was found to increase on average to 7.96 ± 2.29 nM ($n = 10$). Nitrite release rates (normalized to Chl *a*) averaged 0 ± 0 ($n = 19$) and 0.68 ± 0.09 nmol N μg^{-1} Chl *a* h^{-1} ($n = 32$) in 2002 and 2003, respectively. Final NO_3^- concentrations averaged 915.01 ± 241.97 ($n = 19$) and 940.83 ± 140.29 nM ($n = 35$) for 2002 and 2003 sampling periods, respectively. Final concentrations of NO_3^- showed an average increase in blank bottles of 315.60 ± 41.60 nM ($n = 10$). Nitrate release rates averaged 22.84 ± 6.04 ($n = 19$) and 23.74 ± 3.54 nmol N μg^{-1} Chl *a* h^{-1} ($n = 35$) in 2002 and 2003 respectively. Though NH_4^+ concentrations varied considerably under different conditions, final concentrations increased on average to 147.83 ± 69.71 ($n = 14$) nM and 142.67 ± 29.33 nM ($n = 24$) for 2002 and 2003 sampling periods, respectively. Average blank values for ammonium were 32.85 ± 26.27 nM ($n = 8$). Ammonium release rates in unamended mats averaged 3.69 ± 1.74 ($n = 14$) and 3.60 ± 0.74 nmol N μg^{-1} Chl *a* h^{-1} ($n = 24$) in 2002 and 2003, respectively. Ammonium uptake was found at several stations in 2003. These stations started with elevated *in situ* ammonium concentrations associated with the *Hemiaulus* bloom.

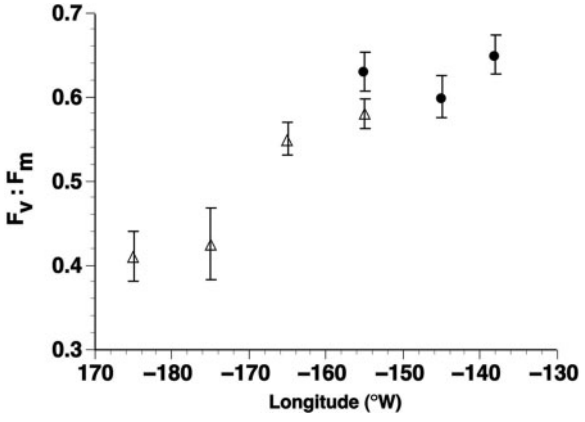

Fig. 2. Average Fv : Fm values for nonincubated *Rhizosolenia* mats across longitude for 2002 (●) and 2003 (△) cruises. Values (±SE) are the average across a 10° longitude and are located at the midpoint of the 10° longitude bin ($n = 11$–47 mats per bin). The point at –138° W was not sampled for N release and does not appear in Fig. 1.

Only induced iron stress (DFB) resulted in NO_2^- release during the summer 2002 period (Fig. 3a). Induced iron limitation resulted in significantly higher NO_2^- release rates relative to controls (2.15 ± 0.78 and 0 nmol N μg^{-1} Chl *a* h^{-1}, respectively; $P < 0.001$). Both control and induced iron-limited incubations had NO_3^- release (22.84 ± 6.04 and 24.21 ± 13.86 nmol N μg Chl a^{-1} h^{-1}, respectively; Fig. 3b). Induced iron-limited incubations did not have significantly different NO_3^- release rates from control incubations ($P = 0.47$). Induced iron-limited incubations showed no decrease in Fv/Fm, Chl *a* and C:N ratios ($P = 0.19$, 0.11 and 0.26, respectively; Table II) relative to control incubations.

In 2003, NO_2^- release rates under induced iron limitation averaged 1.59 ± 0.26 nmol N μg^{-1} Chl *a* h^{-1} and were significantly higher than controls (0.77 ± 0.18 nmol N μg^{-1} Chl *a* h^{-1}; $P < 0.01$; Fig. 3a). Induced iron limitation had no effect on NO_3^- release rates relative to controls (average 33.51 ± 5.01 and 31.32 ± 5.76 nmol N μg^{-1} Chl *a* h^{-1}, respectively; $P = 0.39$; Fig. 3b). Induced iron limitation resulted in NH_4^+ uptake (negative release rate; mean = –1.89 ± 3.38 nmol N μg^{-1} Chl *a* h^{-1}) compared to release by unamended controls ($P = 0.16$; Fig. 3c). However, upon further examination, it was found that the NH_4^+ uptake at a single station (175.60° E and 28° N) may have skewed rates with induced iron-limited mats having significantly higher release rates relative to control incubations (–11.39 ± 2.81 and 0.16 ± 0.14 nmol N μg^{-1} Chl *a* h^{-1}, respectively; $P < 0.01$). This station was located west of the region with ~200 nm ambient NH_4^+ concentrations and had still had elevated ammonium concentrations of 20–40 nmol L^{-1}. When this station was excluded from analysis, there is no significant difference found between NH_4^+ release rates from induced iron-limited and control incubations (7.62 ± 2.47 and 6.44 ± 0.96, respectively; $P = 0.33$). Induced iron-limited incubations showed no difference in Fv/Fm, Chl *a* content and C : N ratios from controls ($P = 0.17$, $P = 0.42$ *and* $P = 0.31$, respectively; Table II).

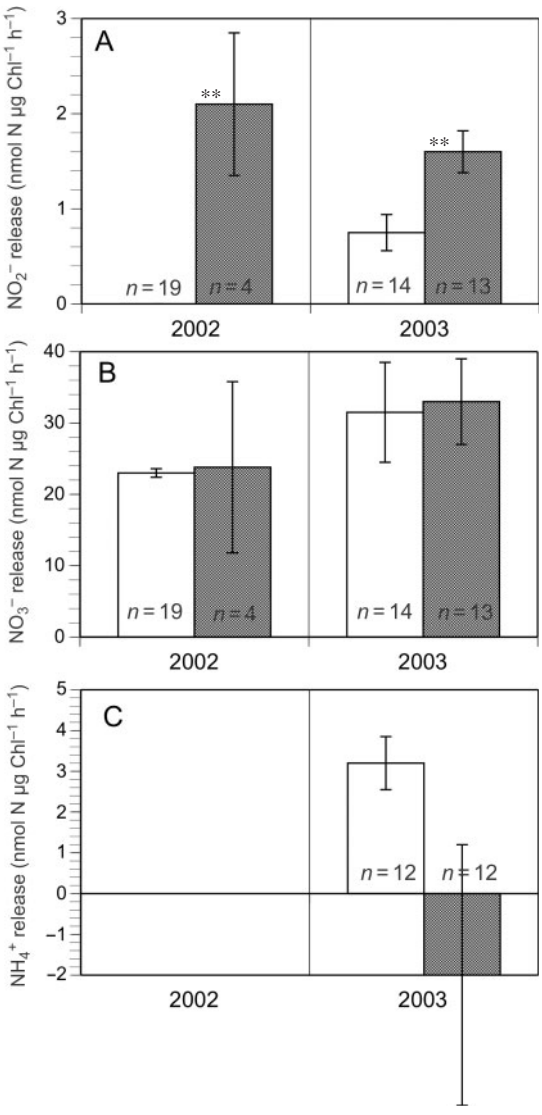

Fig. 3. Effect of DFB addition on N release: (**A**) NO_2^-, (**B**) NO_3^- and (**C**) NH_4^+ release rates (nmol N μg^{-1} Chl *a* h^{-1}) under control (white) and induced iron-limited (grey) conditions from both 2002 and 2003 cruises. Error bars are average ±SE. ** denotes significant difference ($P < 0.05$) between treatments within sampling periods.

Mat buoyancy status was not related to NO_2^- release rates. No NO_2^- release was observed in 2002, and no significant differences were observed between positive and negatively buoyant mats in 2003 ($P = 0.23$; Fig. 4a). Positively buoyant mats had significantly higher NO_3^- release rates relative to negatively buoyant mats in the 2002 sampling period ($P = 0.03$, Fig. 4b). However, in the 2003 sampling period, positively buoyant mats had significantly lower NO_3^- release rates relative to negatively buoyant mats ($P < 0.01$; Fig. 4b). NH_4^+ release rates did not significantly differ between buoyancies in 2003 ($P = 0.07$; Fig. 4c). Chl *a* content did not differ between buoyancies during the 2002 sampling period ($P = 0.49$) but was significantly lower in negatively buoyant mats in the 2003 sampling period ($P = 0.02$; Table II). Fv : Fm and C : N ratios did not significantly differ among buoyancies during both 2002 and 2003 sampling periods ($P = 0.31$, 0.12; 0.32, 0.12, respectively).

DISCUSSION

This study found that *Rhizosolenia* mats release nitrogen in various forms (NO_3^-, NO_2^- and NH_4^+) with release rates that vary with environmental conditions. The dominant form of nitrogen released by *Rhizosolenia* mats was NO_3^- with release rates as high as 90 nmol N μg^{-1} Chl *a* h^{-1} (0.06 nmol N nmol mat N^{-1} h^{-1}). As previous studies have indicated, active release of NO_2^- and NH_4^+ may act as a possible means of relieving burdens placed on the nitrogen assimilation pathway during periods of stress (Lomas *et al.*, 2000; Milligan and Harrison, 2000). Nitrogen release rates varied between the two sampling periods suggesting spatial and/or temporal effects play a major role in nitrogen excretion by *Rhizosolenia* mats.

In any study, artifacts during handling are serious concern. Concentrations for all three nutrients increased intermittently during the blank (no mat) incubations. For ammonium and nitrite, these increases were small and sometimes were not observed; however, for nitrate, the increases were often substantial (300+ nM). We have no explanation for this. Eppley *et al.* (Eppley *et al.*, 1990) noted increases in dissolved NOx when airspaces were present in incubation bottles. In our case, air drying (inverted) the incubation bottles after the acid/deionized water rinse may have allowed NOx to adsorb to the plastic container. In addition, the NO_3^- concentrations were often determined after freezing and shipping. Dore and Karl (Dore and Karl, 1996) noted no effect from doing this, but their samples were transported directly to the laboratory, not shipped to the mainland. Ambient seawater values were run onboard ship, and we have no samples to examine for shipping artifacts. While the blank values are higher than desired, the values are reproducible and substantially lower than the experimental values.

Martinez *et al.* (Martinez *et al.*, 1983) noted a potential handling artifact in their study of *Rhizosolenia* mats. In their collections, cells within the mats rapidly lysed when returned to the ship. Mats incubated *in situ* did not. If present in our study, this would have created an apparent excretion of nitrate because of the high intracellular

Table II: Rhizosolenia mat characteristics (2002 and 2003): Chlorophyll a content (μg mat^{-1}), quantum yield of fluorescence (Fv/Fm) and C : N ratios of mats incubated under control and deferoxamine mesylate additions and from separate studies of unamended positively (positive) and negatively (negative) buoyant mats

	2002				2003			
	Control	+DFB	Positive	Negative	Control	+DFB	Positive	Negative
Chl *a*	0.93 ± 0.13	0.53 ± 0.28	0.83 ± 0.23	0.84 ± 0.32	1.04 ± 0.07	1.08 ± 0.18	**0.89 ± 0.08**	**0.60 ± 0.10**
Fv/Fm	0.61 ± 0.01	0.63 ± 0.01	0.64 ± 0.03	0.62 ± 0.05	0.48 ± 0.03	0.44 ± 0.03	0.56 ± 0.02	0.52 ± 0.02
C : N ratio	7.3 ± 0.3	6.8 ± 0.4	7.1 ± 0.2	7.6 ± 0.1	5.6 ± 0.2	5.4 ± 0.2	6.0 ± 0.3	6.8 ± 0.6

DFB, deferoxamine mesylate.
Bold text indicates significant differences ($P < 0.05$) between mat buoyancies.

concentration in buoyant mats. However, this artifact has never been seen in the numerous studies by this lab over the years. In our studies, we take care to place collection bottles into insulated thermal coolers as soon as the divers ascend in order to protect the mats from both temperature and light shock. In this study, mats were examined periodically in the microscope for such lysis; none was observed. There is also the possibility that handling may not lyse the cells, but stress them and enhance their release rate. This is difficult to assess in bottle experiments and remains a possibility. However, we note that there are consistent sinker/floater differences that appear to be unrelated to handling, suggesting that N release is a real phenomenon.

Nitrite release

As expected, DFB addition resulted in increased NO_2^- release rates in field incubations of *Rhizosolenia* mats in both years. There were significant differences in N release between the two years suggestive of either regional or temporal differences in *Rhizosolenia* mat nutritional status. In 2002, nitrite release occurred only during incubations with the addition of an iron chelator (DFB) (east of Hawaii). In 2003 (west of Hawaii), both control and DFB addition experiments released nitrite with DFB additions having significantly higher NO_2^- release rates than controls. The elevated nitrite release rates in 2003 data and the westward gradient of decreasing Fv : Fm are consistent with greater physiological stress occurring in the waters west of Hawaii (Fig. 2). Since DFB is a selective chelator of Fe (Timmermans *et al.*, 2001), and enhanced nitrite release is the result of Fe limitation (Milligan and Harrison, 2000), the data is highly suggestive of Fe stress. However, Fv/Fm values in experimental bottles did not decrease with the addition of DFB. In our short incubations (7 h), there may have been insufficient time for a fluorescence starvation response. Laboratory experiments with *R. formosa* (found occasionally in mats, Villareal and Carpenter, 1989) noted 24 h was insufficient to see an increase in Fv : Fm when all the Fe was removed with DFB (R. M. L. McKay *et al.*, in preparation). Parkhill *et al.* (Parkhill *et al.*, 2001) noted that macronutrient stress can be tolerated with little effect on Fv : Fm, although starvation has a pronounced effect. There is little information on similar physiological gradients in the bulk phytoplankton from this region, although similar measures on bulk seawater using a Xe-PAM did not show the same westward decline in Fv : Fm (T. A. Villareal *et al.*, in preparation). Our experimental manipulations indicate that Fe is the probably the nutrient creating these gradients; however, we recognize that Fe additions would have provided more information. Unfortunately, *Rhizosolenia* mats do not tolerate long-term (24–48 h) incubations well, particularly when much handling is required, and these experiments could not be performed.

Iron-stress is one possible explanation for this pattern; however, Si-limitation may need to be considered as well as an interacting factor. Previous studies have indicated that Si may limit overall diatom production within the cNP gyre (Brzezinski *et al.*, 1998). Shipe *et al.* (Shipe *et al.*, 1999) reported that surface Si-concentrations limited Si-production by *Rhizosolenia* mats in similar transects east and west of the Hawaiian Islands, indicating a widespread occurrence of Si-limitation within the cNP gyre. Despite the prevalence of Si-limitation in *Rhizosolenia* mats in the cNP gyre there are indications of regional differences in where mats acquire Si during their migrations. Shipe *et al.* (Shipe *et al.*, 1999) suggested that in the eastern region surface Si concentrations are high enough to permit Si uptake at both the surface and at depth; however, in the western region, Si uptake likely

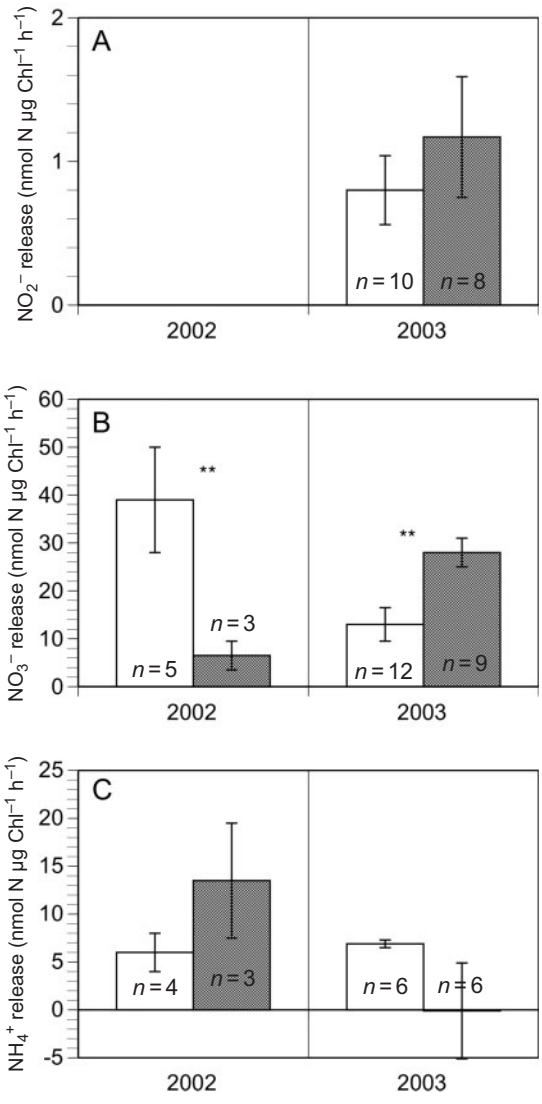

Fig. 4. Effect of buoyancy: (**A**) NO_2^- (2003 only) (**B**) NO_3^- and (**C**) NH_4^+ release rates. Positively buoyant mat samples (white) and negatively buoyant mat samples (grey). Plotted as N release rates (nmol N μg^{-1} Chl *a* h^{-1}). Error bars are average ±SE. ** denotes significant difference ($P < 0.05$) between treatments within sampling periods.

occurred only at depth. Brzezinski *et al.* (Brzezinski *et al.*, 1998) noted a lack of elevated Si-production rates (total diatom community) north of Hawaii (26° N, 159° W) in regions with elevated amounts of lithogenic silica. They suggested that this is evidence against iron-limitation given that aeolian dust, the source of lithogenic Si, would also presumably release iron into the surface waters. Our high Fv : Fm values in *Rhizosolenia* mats at that longitude in the 2003 tend to support this region as not being Fe stressed. In contrast, annual inputs of dust aerosols indicate greater aeolian iron flux rates in the western North Pacific but that summer and winter periods are characterized by low dust concentrations (Duce and Tindale, 1991). The two *Rhizosolenia* data sets may reflect alternative scenarios that depend on varying dust inputs and the results from this study are likely responding to the temporal or spatial variability found in atmospheric iron deposition. Fe and Si limitation can interact (Bruland *et al.*, 2001; Firme *et al.*, 2003) creating an additional complication in these low nutrient waters. Phosphorous (P) limitation seems unlikely based on our data since mat N:P ratios rarely exceeded 18 (Villareal *et al.*, unpublished results). While we cannot exclude all other possibilities, Fe stress is the most consistent explanation.

McKay *et al.* (McKay *et al.*, 2000) addressed the issue of possible iron acquisition at depth by vertically migrating *Rhizosolenia* mats. They noted that it is not possible to fully assess whether mats are acquiring iron at depth because of sampling constraints. Villareal *et al.* (Villareal *et al.*, 1999b) imaged a mat at a depth of 150 m using a video plankton recorder (VPR); however, the ferricline is >200 m in this region (Bruland *et al.*, 1994; Rue and Bruland, 1995; McKay *et al.*, 2000). Towed video systems and remotely operated vehicles on the 2002/2003 cruise noted *Rhizosolenia* mats at these depths (C. H. Pilskaln *et al.*, submitted for publication) so Fe uptake remains a possibility.

Nitrate release

Nitrate was the dominant form of nitrogen release by *Rhizosolenia* mats during both sampling periods. Handling and cell lysis are potential artifacts in these release experiments. Using an average internal NO_3^- concentration of 9 mM and mat volume of 2 × 1011 µm (Villareal *et al.*, 1996), we estimate that 5–10% of the cells would need to lyse to obtain the final NO_3^- concentrations (585 and 988 nM NO_3^-, this study) found after mat incubations. Several observations argue against NO_3^- release as an artifact of handling and cell lysis. Negligible cell lysis was observed during *Rhizosolenia* mat incubations based on microscopic examination. No changes in mat Fv : Fm pre- and postincubations were noted. In addition, NO_3^- release rates varied over time, space and between mat buoyancies at statistically significant levels, further supporting the fact that random cell lysis was not a major contributor to increased NO_3^- concentrations during incubations.

The mechanisms driving mat excretion are not clearly understood. Cellular leakage as result of concentration or electrochemical gradients is an inevitable consequence of the high internal concentration of these constituents (Raven, 1986). Specific cases of nitrite, ammonium and DON release have been documented (Bronk *et al.*, 1994; Collos, 1998; Lomas *et al.*, 2000) and are often related to

energy imbalances or enzyme rate limitation in the cells. Nitrate release is much harder to evaluate, largely due to the observation that nitrate only accumulates intracellularly when external nitrate is present (Dortch *et al.*, 1985). Bjornsen (Bjornsen, 1988) suggests that passive diffusion could be entirely responsible for extracellular release from healthy phytoplankton. With extracellular NO_3^- concentrations in the nanomolar range and internal NO_3^- concentrations (INC) in the millimolar range (Villareal *et al.*, 1996), there is a large concentration difference across the cell plasma membrane of *Rhizosolenia* cells that could easily drive a passive efflux. However, if passive diffusion is the sole mechanism for NO_3^- release, then mats maintaining higher INC should have greater NO_3^- release rates. In this study, we noted no consistent patterns in N release between positively buoyant mats and negatively buoyant mats between years although there are clear differences within years, and significant differences in internal nitrate concentration (Villareal *et al.*, 1996). Mechanisms other than just passive diffusion appear to be active in mats, and may be linked to the spatial variation in physiological condition suggested by the PAM data. Using a mean N value of 2.4 μmol N mat^{-1} and assuming a maximum NO_3^- pool size of 50% of total nitrogen (Villareal *et al.*, 1996) the loss rates in Table III are <3% of the NO_3^- pool per day. This is consistent with the calculation above indicating how much mat material would need to lyse to provide the final nitrate concentration. However, this loss would get proportionally larger as internal the NO_3^- pool was consumed during growth at the surface.

Both internal and external parasitic protozoans occur within *Rhizosolenia* cells and within the matrix of *Rhizosolenia* mats (Villareal *et al.*, 1996). These parasitic protozoans were present in both cruises during this study and may be causing cell lysis in *Rhizosolenia* resulting in the leakage of NO_3^- from cells. However, the data on the rate of occurrence of parasitic protozoans on *Rhizosolenia* mats is too limited to assess their potential influence on NO_3^- release by *Rhizosolenia*. Such a mechanism would likely have only a weak linkage to nutrient status and be more tightly coupled to the parasite's growth and colonization dynamics. Caron *et al.* (Caron *et al.*, 1982) noted that mats in the Atlantic were often heavily colonized by heterotrophs. DAPI (4′, 6-diamidino-2-phenylindol) staining and microscopic investigations of Pacific mats (Martinez *et al.*, 1983) have shown little evidence of a colonizing community. Our own microscopic observations support this, although there are clearly times when mats have abnormally high C : N ratios and are heavily colonized (Villareal and Carpenter, 1989). Small epiphytic diatoms occur in some mats and likely benefit from the excreted N. In addition, the bulk of the biovolume (84–99%) in the mats is in the larger, buoyant cells (Villareal *et al.*, 1996). The smaller *R. fallax* that make up the mat matrix are likely to have a smaller storage capacity, and may able to take up the released N. Such utilization would recycle the N internally and not appear in our measurements. Thus, depending on the nature of the colonizing community (heterotrophic or autotrophic), much of the nitrogen could be recycled within that mat and not evident in our experiments.

Ecological implications

The release of mat nitrogen into the surface waters provides a source of new nitrate (Dugdale and Goering,

Table III: Nitrate release from Rhizosolenia mats

	2002		2003	
	NO_3^- (Diver)	NO_3^- (VPR)	NO_3^- (Diver)	NO_3^- (VPR)
Avg release rates[a]	22.84	22.84	23.74	23.74
nmol N mat^{-1} d^{-1}[b]	510	510	524.18	524.18
nmol N m^{-3} d^{-1}[c]	82	367	131	590
Ambient NO_3^- (nM)[d]	2	2	2	2
% N addition by mats[e]	4	17	6	26

The 'Diver' column uses direct visual abundance estimates by scuba divers. 'VPR' column uses video plankton recorder corrections of mat abundance from Villareal *et al.* (Villareal *et al.*, 1999b). They estimated that the previously unrecorded small mats increase particulate N in total mats by 4.5 (Villareal *et al.*, 1999b) assuming the mat N : Chl ratio is constant with size.

[a]Average nitrate (dominant form of nitrogen released) release rates (nmol N μg^{-1} Chl *a* h^{-1}) from unamended samples during both sampling periods.

[b]Release rates per mat using average Chl *a* of 0.93 and 0.92 μg mat^{-1} for 2002 and 2003, respectively.

[c]Mat specific release rates × abundance estimates (0.16 and 0.25 mat^{-3} for 2002 and 2003, respectively).

[d]Our measured values were 2 nmol L^{-1}.

[e][ln(NO_3^- + mat release/ NO_3^-)] × 100%.

1967) to surrounding phytoplankton. Our data provide a means of estimating the total contribution. While mats released NO_2^-, NO_3^- and NH_4^+, the dominant source of nitrogen released by vertically migrating *Rhizosolenia* mats was NO_3^-. Surface NO_2^- + NO_3^- concentrations at Station ALOHA rarely exceed 5 nM (Fujieki *et al.*, 2004); our values for both were usually below detection (<2 nM) by our chemiluminescence methods. Using the average NO_3^- release rates of unamended *Rhizosolenia* mats for both 2002 and 2003, we calculate that mats in 2002 and 2003 contributed 4 and 6% day^{-1}, respectively, of the ambient NO_3^- in the upper 20 m using diver-estimated abundances (Table III). However, diver estimates of mat abundance are underestimates compared to VPR estimates (Villareal *et al.*, 1999b) due to the lack of contrast between mats (1 cm) and the surrounding water. Video enumeration was conducted on the 2003 cruise, and while the data is not yet available, there were clearly large numbers of small mats evident (C. H. Pilskaln, personal communication). Inclusion of small mats (using the Villareal *et al.*, 1999b corrections) increases the particulate N present at mats by a factor of 4.5. If we correct diver abundance by this factor then this increases nitrogen addition by mats (via nitrate excretion) to 17–27% of the ambient nitrate in the cNP gyre (Table III).

While mat abundance is patchy in time and space, nitrogen release by vertically migrating *Rhizosolenia* mats is potentially an important source of nitrate in the mixed layer of the cNP gyre. Karl (Karl, 2002) has noted that surface nitrogen dynamics are significantly more complex than understood in the 1960s and 1970s. He argues that the coupling of N, P and C pools via the Redfield ratio leads to little or no export of atmospheric C to deep waters when production is based on upward mixing of deep nutrient pools and that uncoupled inputs are required. N_2 fixation is a notable candidate for inputs of new N uncoupled to remineralized CO_2 pools; however, he also notes the potential for vertical transport of N and P by migrating diatoms and *Trichodesmium*, respectively. In this article, we have observed direct release of nitrate by migrating diatoms and at rates sufficient to perturb the ambient nitrate pools on the time scales of days. While it quantitatively is smaller than N fixation in these waters, both by unicells and *Trichodesmium* (Montoya *et al.*, 2004), the chemical form has the distinctive isotopic signature of the deep nitrate pool (Liu and Kaplan, 1989) and may be traceable into food webs using stable isotopes. Mahaffey *et al.* (Mahaffey *et al.*, 2004) used similar methods to determine that nitrate is a dominant N source in the south Atlantic. High abundance patches (up to 100's mats m^{-3}) noted in Villareal *et al.* (Villareal *et al.*, 1996) could lead to elevated local concentrations of nitrate, particularly if mats are trapped at the surface in low wind conditions and exposed to elevated photosynthetically available radiation and UV.

Rhizosolenia mat nitrate release provides one of the few biological mechanisms for supplying nitrate to the surface mixed layer. This region is likely to have minimal bacterial nitrification relative to deeper parts of the euphotic zone (Ward *et al.*, 1989; Zehr and Ward, 2002). Direct measurements of nitrification at Station ALOHA (HOT) indicate little nitrate production in the upper 80 m (Dore and Karl, 1996). *Rhizosolenia* mats are not the only vertical migrators in the open ocean. *Pyrocystis*, *Halosphaera*, *Ethmodiscus* and free chains of large *Rhizosolenia* spp. possess large internal nitrate pools (Villareal and Lipschultz, 1995) that can only be obtained below the euphotic zone. If the mM to nM concentration gradient found in cells is driving diffusive flux out of the cell, then these species are likely contributors to the surface nitrate pool as well. While there are large error bars associated with our measurements, the results suggest that nitrate released by vertical migrating species is an input that should not be ignored and may well be a dominant input to the surface nitrate pool in this region.

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