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Journal
Journal of Plankton Research, 18(7)

ISSN
0142-7873

Authors
Villareal, Tracy A
Woods, Samantha
Moore, J Keith
et al.

Publication Date
1996

DOI
10.1093/plankt/18.7.1103

Journal of Plankton Research Vol.18 no.7 pp.1103–1121, 1996

Vertical migration of *Rhizosolenia* mats and their significance to NO_3^- fluxes in the central North Pacific gyre

Tracy A.Villareal, Samantha Woods, J.Keith Moore[2] and Karen Culver-Rymsza[1]

Environmental Sciences Program, University of Massachusetts, 100 Morrissey Blvd, Boston, MA 02125-3393 and [1]Graduate School of Oceanography, University of Rhode Island, Kingston, RI 02881, USA

[2]Present address: College of Oceanic and Atmospheric Sciences, Oregon State University, 104 Ocean Administration Building, Corvallis, OR 97331-5503, USA

Abstract. *Rhizosolenia* mat abundance, distribution and chemical composition were studied on two cruises in the central North Pacific gyre in order to determine large-scale distribution patterns and contribution to upward nitrogen (N) flux. These macroscopic diatom mats are composed of multiple species of *Rhizosolenia* that exploit subsurface nitrate pools by vertically migrating below the euphotic zone. Although numerically dominated by the small-diameter species, *R.fallax* (73–95% of total numbers), mat biovolume was dominated by large-diameter (>50 μm diameter) *Rhizosolenia* spp. (85–99% of total volume). Integrated mat abundance was substantially higher when mats accumulated at the surface during calm weather (≤80 mats m^{-2}) than during windy periods (≤23.1 mats m^{-2}), suggesting that many mats are found below diver-accessible depths. Chemical composition data indicated that negatively buoyant mats were physiologically stressed compared to positively buoyant mats; negatively buoyant mats had significantly higher carbon (C):N ratios and carbohydrate per mat, and lower protein:carbohydrate ratios and internal NO_3^- pools than positively buoyant mats. These ratios suggest that N is a key determinant of buoyancy behavior, and are consistent with vertical migration by mats to exploit deep N pools. The maximum ascent rate of mats was 6.4 m h^{-1} with no relationship to mat size or biovolume. Short-term O_2 evolution revealed no significant photoinhibition; conversion to C fixation yielded assimilation numbers of 4.7 and 7.3 μg C μg^{-1} chl h^{-1} in negatively buoyant and positively buoyant mats, respectively, although photosynthetic parameters were not statistically different between the two buoyancy classes. Based on photosynthetic rates, ascent rates and estimated N uptake rates, we calculate that a complete migration cycle requires 3.6–5.4 days. When combined with two different estimates of average abundance, we estimate that mats could transport 3.9–40 μmol N m^{-2} day^{-1} into the euphotic zone. Using the wide range of literature values for vertical diffusive transport, this represents <1–2000% of the NO_3^- flux into the euphotic zone and the average equivalent of 3–35% of the new NO_3^- consumed in the surface mixed layer.

Introduction

The phytoplankton flora of the open sea contains very large (up to 10^9 μm^3), rare, non-motile cells capable of positive buoyancy at rates of several meters per hour. This buoyant flora contains representatives of the diatoms *Ethmodiscus* (Villareal, 1992) and *Rhizosolenia* (Villareal, 1988; Moore, 1994), the non-motile dinoflagellate *Pyrocystis* (Kahn and Swift, 1978) and the prasinophyte *Halosphaera* (see Jenkinson, 1986). *Rhizosolenia* mats, macroscopic assemblages of buoyant and non-buoyant *Rhizosolenia* spp. reaching up to 30 cm in size (Carpenter *et al.*, 1977), can also ascend at rates of meters per hour (Villareal and Carpenter, 1989). Although noted from the warmer waters of all oceans, mats appear to be particularly abundant in the central North Pacific gyre (Villareal and Carpenter, 1989).

Positive buoyancy by diatoms can only be achieved via density regulation and provides a clear advantage to cells in stratified, stable water columns where turbu-

lence may be insufficient to keep negatively buoyant cells suspended (Smayda, 1970). However, the size requirements for positive buoyancy created by surface-to-volume relationships between the vacuole, cytoplasm and cell wall (Villareal, 1988) introduce restrictions on physiological rates such that large cells should be unable to compete directly for nutrients with the numerically dominant nano- and picophytoplankton (Chisholm, 1992). These restrictions compound an already severe problem of nutrient uptake for *Rhizosolenia* mats since their biomass is orders of magnitude more concentrated than that of ambient phytoplankton (Alldredge and Silver, 1982).

Direct nutrient competition between *Rhizosolenia* mats and dispersed phytoplankton does not seem to occur. A $\delta^{15}N$ characteristic of the subeuphotic zone NO_3^- pool and positive buoyancy correlated with high internal NO_3^- pools suggest that *Rhizosolenia* mats vertically migrate below the euphotic zone to acquire NO_3^- and return to the surface for photosynthesis (Villareal *et al.*, 1993). In *Pyrocystis*, nutrient depletion leads to migration to the nutricline, where nitrogen is acquired, and the cell then returns to shallower depths for photosynthesis in a pattern related to cellular division (Rivkin *et al.*, 1984; Ballek and Swift, 1986). Solitary *Rhizosolenia*, *Ethmodiscus* and *Halosphaera* also have highly variable millimolar internal NO_3^- pools, and, like mats, negatively buoyant cells of these three taxa are significantly depleted in NO_3^- compared to positively buoyant cells (Villareal and Lipschultz, 1995). The similarity in chemical and buoyancy characteristics between *Rhizosolenia* mats and *Pyrocystis*, and the presence of buoyancy relationships to internal nitrate pools in the other taxa, is evidence that vertical migration is a widespread feature of the largest phytoplankton in the open sea (Villareal and Lipschultz, 1995).

Vertical migration is also common in coastal dinoflagellates (Eppley *et al.*, 1968; Cullen, 1985) and is a life history strategy selected for in spatially separated light and nutrient fields (Ganf and Oliver, 1982). In the open sea, nitrogen (N) acquisition for the largest phytoplankton is accomplished by utilizing N in deep water, rather than near-surface, N pools in an analogous fashion. The outstanding feature is the distance traversed (>100 m?), not the strategy itself. However, the mechanisms by which N metabolism interacts with buoyancy are unclear, as are the key factors inducing buoyancy reversals.

Nitrate use by vertical migrators defines it as new production (Dugdale and Goering, 1967) and this N source has biogeochemical implications for nutrient transport into the euphotic zone. Although they occur at low concentrations, *Rhizosolenia* mats transport significant amounts of NO_3^- into the upper euphotic zone (Villareal *et al.*, 1993), thus avoiding the NO_3^- sink at the deep chlorophyll maximum (Banse, 1987). Such biological transport has not been widely considered for oceanic nutrient cycles, although the sources supporting new production both in the surface mixed layer as well as the subsurface oxygen maximum are presently unknown (Hayward, 1994). Possible non-Redfield stoichiometry in surface waters may reduce the N requirement and adds further complexity (Sambrotto *et al.*, 1993). A zone of negative pre-formed NO_3^- occurs at the top of the nitricline in the eastern North Pacific Ocean; *Rhizosolenia* mat migration and subnitricline N uptake is one of several possible explanations suggested for its occurrence (see

Emerson and Hayward, 1995). Limited abundance, distribution and rate information has made it difficult to determine how, or if, vertical migrators are related to these widespread features, but the autecology of this class of migrating phytoplankton is clearly relevant to fundamental questions of oceanic biogeochemistry.

We present here the results of two cruises in the North Pacific gyre between Hawaii and the west coast of North America documenting the broad spatial distribution of mats during late spring and summer. Chemical composition and oxygen evolution data provide insights into mat ecology and buoyancy reversal mechanisms, and permit a first calculation of the migration time. This new information is used to revise and extend previous estimates of new N flux in this region by vertically migrating diatom mats.

Method

Rhizosolenia mats were hand collected by SCUBA divers in August 1992 (RV 'Wecoma', W9208C) and May/June 1993 (RV 'New Horizon', PACMAT) along transect lines extending from Hawaii to the western US coast (Figure 1). Mats were collected in 500–1000 ml wide-mouth polycarbonate or polymethylpentane jars and returned to the ship in darkened, insulated chests. They were then sorted into rising and sinking mats by allowing them to sit undisturbed for 5–10 min on the laboratory benches. Mats rising to the top or sinking to the bottom were operationally defined as positively or negatively buoyant populations. In most cases, the mats were pressed against either the top or bottom of the container. However, results from the August 1992 cruise indicated that there was a third category: neutrally buoyant mats. These mats were suspended and not visibly deformed against the container top or bottom.

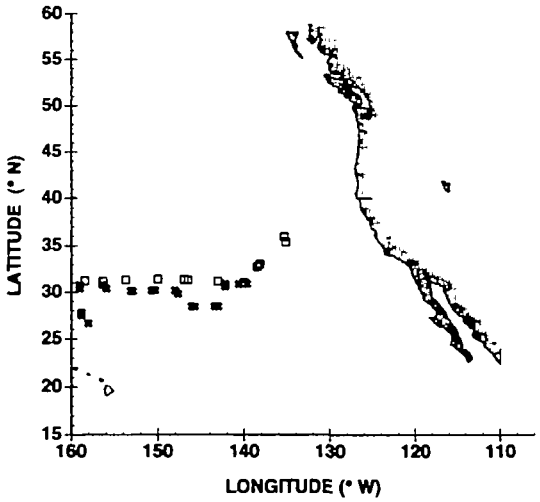

Fig. 1. Cruise track for *Rhizosolenia* mat collections in 1992 and 1993. Station locations are sites where SCUBA collections and/or enumerations were made. W9208C, □; PACMAT,*.

After buoyancy evaluation, mats were removed from containers with wide-mouth pipets, suspended in a known volume and gently shaken to disperse them (Villareal and Carpenter, 1989). Individual aliquots were then taken for the determination of CHN (Sharp, 1974), chlorophyll by fluorometry (Strickland and Parsons, 1972), internal nitrate pools (Villareal *et al.*, 1993), anthrone-reactive carbohydrate (Strickland and Parsons, 1972), protein (Smith *et al.*, 1985) and cell size/numbers. Results were corrected for dilution and normalized to the original mat volume. Not all analyses could be run on one mat, and pooled samples of floating or sinking mats were frequently used. Phase-contrast microscopy for cell size and abundance was performed on aliquots in a Sedgwick–Rafter counting chamber. Nitrate reductase (NR) activity was measured using the Eppley (1978) technique as modified by Culver-Rymsza (1988). *In situ* abundance was measured with a 1 m^2 PVC frame using a General Oceanics flowmeter equipped with a slow-speed rotor (Villareal and Carpenter, 1989). Diving limitations prevented sampling or abundance measurements below 20 m on a routine basis; however, mats were regularly visible below this depth. Areal abundance values (trapezoidal integration) are based on the deepest depth sampled on that day (usually 10–20 m). Surface mat abundance during calm periods was estimated from the ship using a 15 l bucket while divers were in the water. Mat ascent rates were determined *in situ* using an 8 cm diameter polycarbonate tube with ruled lines at 5 cm intervals (Shanks and Trent, 1980). Mats were placed in the tubes and the times recorded when they passed individual lines as the diver remained neutrally buoyant. The slope of the distance versus time plot was the ascent rate ($r^2 \geq 0.95$). Further details of ascent rate determinations may be found in Villareal and Carpenter (1989).

Nutrient samples were collected from discrete depths and frozen for later analysis on a Braun and Lubbe TrAAcs 800 nutrient-analysis system. *Rhizosolenia* mat cell sap was extracted in 10 ml of boiling deionized water, analyzed on the same system and normalized to cell biovolume to quantify intracellular NO_3^- pools. Photosynthesis–irradiance (P–I) curves were generated in June 1993 using a Hansatech oxygen microelectrode system and a slide projector equipped with neutral-density filters to provide a graded irradiance series (Roenneberg and Carpenter, 1993; Villareal and Carpenter, 1994). Mats were placed in the incubation chamber using a wide-bore pipet, and the entire contents of the 1 ml chamber were filtered for chlorophyll (chl) determination after the P–I series (0.5–2 μg chl per incubation). Oxygen evolution was normalized to chlorophyll, and converted to carbon (C) fixation using a photosynthetic quotient of 1.4 for NO_3^--based growth (Laws, 1991). Results were fitted to the Jassby–Platt (1976) hyperbolic tangent curve and the photoinhibition model of Platt *et al.* (1980) modified to include a respiration term using the curve-fitting routine in Deltagraph 3.0 (DeltaPoint, Inc). StatView 2.0 (Abacus Concepts) was used for all statistical determinations. Unless otherwise noted, two-sided *t*-tests were used to detect differences.

Results

Hydrographic conditions noted on the cruise tracts were typical of the central North Pacific Ocean during the cruise periods. Surface NO_3^- and NH_4^+ concentrations were <0.1 μM with the nutricline starting at ~110–120 m (Figure 2). A

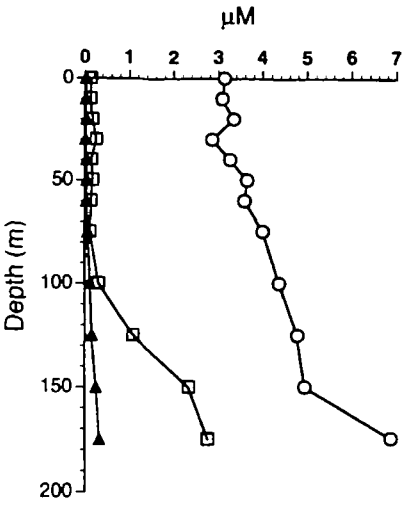

Fig. 2. Nutrient profiles at 32°43.81'N, 138°23.01'W, 2 September 1992. $NO_3 + NO_2$ (□); PO_4^{3-} (▲); $Si(OH)_4$ (○).

shallow surface mixed layer (30–40 m) and deep chlorophyll maximum near the top of the nutricline occurred as noted previously (Eppley *et al.*, 1973; Venrick *et al.*, 1973; Venrick, 1979, 1990; Hayward and McGowan, 1985; Hayward, 1987). The diatom *Hemiaulus hauckii* was particularly abundant at some stations (up to 6000 cells l^{-1}) and was noted *in situ* by divers as spiral chain aggregates up to 1 mm in size. *Hemiaulus hauckii* blooms sporadically during the summer in the central North Pacific (Venrick, 1974) so our observations are not atypical.

As noted on previous cruises, mats were composed of multiple *Rhizosolenia* spp. in two distinct size classes. The small-diameter *R.fallax* Sundström (diameter <20 µm) was the most abundant species in mats (3500–9500 cells per mat) and comprised the matrix of the mat. Total mat biovolume ranged from 1.00×10^{11} to 2.42×10^{11} μm^3 ($n = 34$) with the larger cells in the mat (volume > 10^6 μm^3, diameter = 50–250 µm) accounting for 84–99% of the total mat biovolume, but only 5–27% of the total cell numbers (580–25 000 cells per mat). There was no significant correlation between mat biovolume, biovolume ratios and buoyancy status. Other diatoms, although rare, were noted in the mats. *Hemiaulus hauckii* appeared to be incidental particles scavenged by the mats, while the *Nitzschia* spp. were epiphytic on the mats. Two types of protozoans were noted. The first was seen only within the frustules of dead, large-diameter *Rhizosolenia*. The other, similar to the parasitic dinoflagellate noted by Villareal and Carpenter (1989), was seen attached to the external surface. In a brief survey of all the mats collected from two dives in June 1993, it was found that 20–30% of the mats were parasitized either by the internal or external protozoans.

Rhizosolenia mats were found at every SCUBA station listed in Figure 1. In August 1992, snorkel observations east of the Figure 1 transect line found that mats were not present until the surface temperature exceeded ~19°C. At the western terminus of this transect (north of Hawaii), equipment failures prevented further SCUBA collections; however, snorkellers observed mats from the side of the

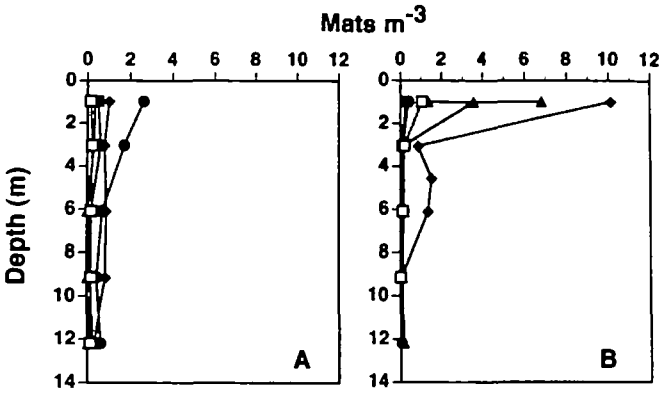

Fig. 3. Summary of diver-collected *Rhizosolenia* mat abundance from June 1993. (**A**) Morning dives (09.00 h). (**B**) Afternoon dives (15.00 h). The three triangles are replicate measurements collected at 1–1.5 m by a diver during a low-wind event.

ship south of this point (25°19.34′N 156°47.50′W). In June 1993, mats were present at the eastern end of the transect, but below the level of detection of our surveys (<0.02–0.03 mats m^{-3}, temperature = 20.3°C). Diver-measured abundance ranged from below detection limits in both cruises to 6.0 mats m^{-3} in August 1992 and 11.5 mats m^{-3} in June 1993. Vertical profiles indicated a general decline in mat abundance with depth (Figure 3); however, mats were usually visible to much greater

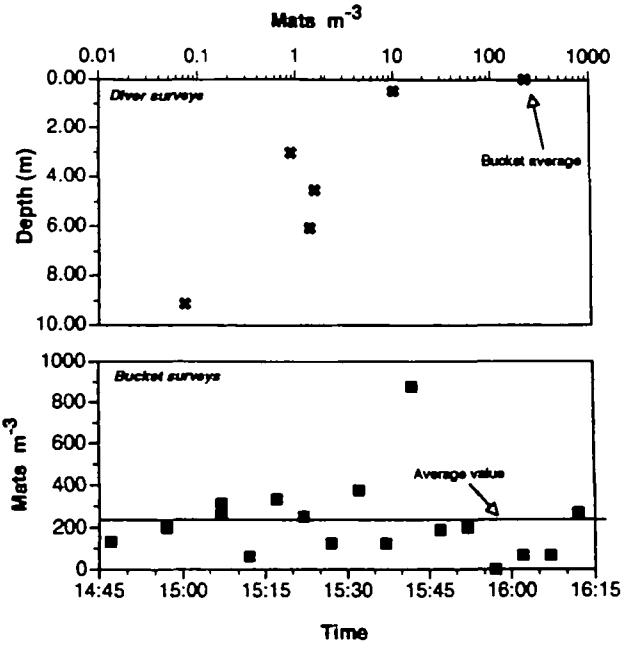

Fig. 4. *Rhizosolenia* mat abundance under low wind speeds (<3 m s^{-1}), 30°45.41′N, 159°00.90′W, 29 June 1993. (**A**) Diver-collected abundance, also shown as diamonds in Figure 3. (**B**) Surface bucket abundance at the same time.

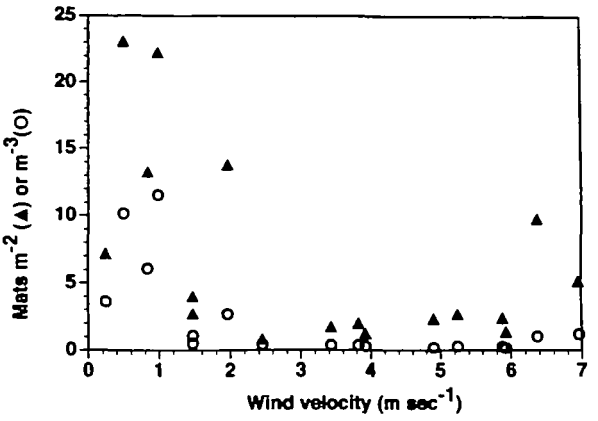

Fig. 5. Relationship between diver-determined mat abundance and wind speed from both cruises. Surface (m^{-3}) samples are from 1–2 m

depths than we could sample. Under low wind speeds (<2–3 m s^{-1}), a layer ~0.25–0.50 m thick formed rapidly at the surface (overnight or less). The mats were visible as dense silver–white patches (see also Venrick, 1969) with small-scale patchiness in these surface samples on the order of 6- to 7-fold over a 1–2 h period (Figure 4). These events were noted several times during both cruises, and during the June 1993 cruise they lasted several days with no obvious diurnal pattern. Average abundance based on surface bucket samples was 250 mats m^{-3} with a maximum of 875 mats m^{-3}. Wind effects were particularly evident in the relationship between surface mat abundance, areal mat abundance and wind speed (Figure 5). Meteorological effects created highly patchy areal abundance in both cruises (0.8–23.1 mats m^{-2}; Figure 6). Based on diver surveys alone, abundance over the upper 15 m averaged 0.4 ± 0.2 mats m^{-3} in 1992 and 0.9 ± 0.3 mats m^{-3} in 1993. Volume and areal

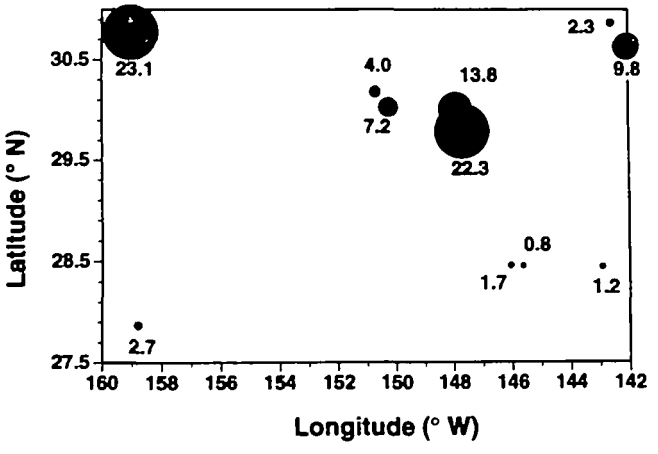

Fig. 6. Areal mat abundance during the June 1993 cruise. Circle diameter is proportional to the areal abundance indicated by the adjacent number.

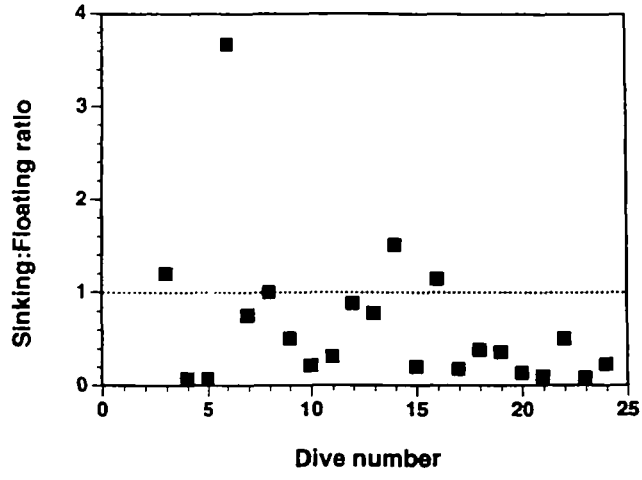

Fig. 7. Ratio of sinking to floating mats from the June 1993 cruise Dive number is approximately in a west to east sequence.

abundance were higher, but not significantly different, in June 1993 than in August 1992.

Positively and negatively buoyant mats were present at all stations. The ratio of positively to negatively buoyant mats varied considerably (Figure 7), and showed no correlation with the time of day or station location. Ascent rates ranged from 1.2 to 6.4 m h^{-1} with an average of 2.8 ± 0.6 m h^{-1}. Frequency histograms of both our data and those of Villareal and Carpenter (1989) indicate that mats have a wide range of ascent rates, and suggest that ascent rate is not a normally distributed property of the mat population (Figure 8).

There was no significant difference between average C, N or chl per mat values from the two cruises (P = 0.95, 0.47 and 0.23, respectively; Table I). However, both average carbohydrate and protein values were significantly higher in the August 1992 cruise (P = 0.015 and 0.0006, respectively). Significant compositional differences were evident between rising and sinking mats (Figure 9, Table I). C:N ratio was significantly ($P \leq 0.05$) higher in sinking mats than in ascending mats in both cruises. Internal nitrate pools (INP) were higher in ascending mats than sinking mats ($P \leq 0.04$) and ranged from 0.1 to 27.1 mM, while protein:carbohydrate ratios were lower in sinking mats ($P \leq 0.001$) in both cruises. The magnitude of this difference is particularly noteworthy given the uncertainties introduced due to the multiple separations and analyses involved. Nitrate reductase activity was not significantly different between rising and sinking mats. However, the numbers of mats required per assay (8–10) reduced our ability to examine patterns in activity. Activity averaged 0.153 ± 0.055 nmol nitrite μg^{-1} chl h^{-1} (n = 3) in sinking mats and 0.278 ± 0.181 nmol nitrite μg^{-1} chl h^{-1} in floating mats (n = 5).

P–I curves were generated on the June 1993 cruise from both rising and sinking mats (Figure 10). Approximately one-quarter of the mats failed to evolve oxygen. Results presented here are only for mats that had positive oxygen evolution. Correlation coefficients to both photosynthesis models ranged from 0.93 to 0.99. No significant difference between the photosynthetic parameters of rising and sinking

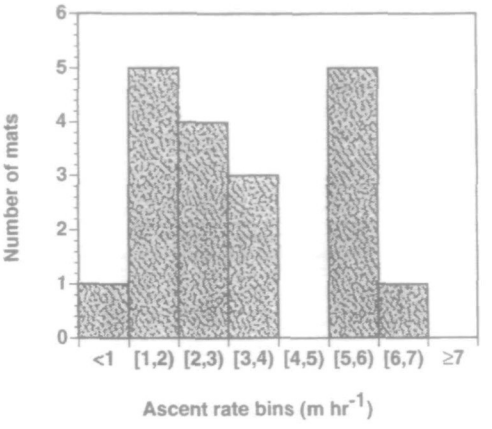

Fig. 8. Frequency histrogram of *Rhizosolenia* mat ascent rates from the June 1993 cruise and data from Villareal and Carpenter (1989).

mats was found (Table II). A slight degree of photoinhibition was observed in some mats; however, β (photoinhibition term) rarely exceeded 0.004. Conversion to C-fixation rates using a PQ of 1.4 (Laws, 1991) and the appropriate C:chl ratio yielded assimilation numbers of 4.7 and 7.3 μg C μg^{-1} chl h^{-1}, and C-specific doubling rates of 0.4 and 1.1 doublings day^{-1} in sinking and floating mats, respectively. Estimates are comparable to rates derived from short-term ^{14}C measurements on mixed *Rhizosolenia* mat populations (Alldredge and Silver, 1982; Martinez *et al.*, 1983).

Discussion

Previous reports have noted *Rhizosolenia* mats in the central North Pacific gyre in April, May, September and October (Alldredge and Silver, 1982; Martinez *et al.*, 1983; Villareal and Carpenter, 1989). Quantitative data for the period of August 1992 and June 1993 provide evidence that extends this distribution across much of the eastern North Pacific gyre, and anecdotal evidence suggests that mats are probably present throughout the year (K.L.Smith, Jr, personal communication). Available data suggest (Table III) that *Rhizosolenia* mats may be predicted to occur at 0.3–0.9 m^{-3} throughout much of the year in the region near 30–31°N. No data are available for the region west of 159°W, although mats have been reported from the Coral Sea north of Australia (Carpenter *et al.*, 1977). Station-to-station variation is great and is strongly influenced by weather. Much like the buoyant cyanobacteria *Trichodesmium* (Carpenter, 1983), local increases cannot be attributed solely to growth, but may be due to populations floating to the surface under low winds. This complicates abundance estimates, but does provide potential for remote sensing during quiescent periods (Yoder *et al.*, 1994).

As noted previously, mats are highly diverse in species composition, size and numbers of cells per mat (Carpenter *et al.*, 1977; Villareal and Carpenter, 1989). Large cells dominated mat biovolume (up to 99%) and presumably the chemical composition. It is unlikely that the small cells provide much, if any, lift for the mat; previous observations suggest that *R.fallax* Sundström is negatively buoyant

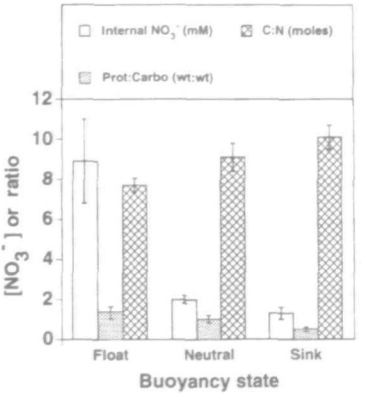

Fig. 9. Chemical composition differences between rising and sinking mats using pooled data from the two cruises, n = 12–35. All results are significant ($P \leq 0.05$).

(Villareal and Carpenter, 1989) and the similarly sized species *R.imbricata* var. *shurbsolei* (Cl.) Schröd. from waters north of Hawaii has a sinking rate of 6.3 m day^{-1} (Smayda and Bienfang, 1983). Size–density models also argue against positive buoyancy in this size class (Villareal, 1988). Since solitary *Rhizosolenia* can apparently migrate (Villareal and Lipschultz, 1995) and have similar ascent rates to mats (Moore and Villareal, 1995), it is not clear what advantages mat formation has for the large *Rhizosolenia* spp. capable of positive buoyancy. The small-diameter *R.fallax* is the only species found in all mats and forms the matrix in which the larger cells are embedded (Villareal and Carpenter, 1989). It is possible that *R.fallax* creates mats by passively capturing buoyant cells and subsequent growth of the species assemblage creates mats. *Rhizosolenia fallax* can then exploit the migration capabilities of the large *Rhizosolenia* spp. to utilize nutrient pools otherwise unavailable to it. Previous studies have noted mat-like aggregations in small-diameter *Rhizosolenia*, suggesting that large cells are not required for their formation (Villareal, 1987).

Previous studies of *Ethmodiscus* have noted progressive decreases in both P_{max} and α as cells underwent depletion of internal NO_3 pools and became increasingly negatively buoyant (Villareal and Lipschultz, 1995). Mats did not show this response, although it was expected. *Ethmodiscus* INP frequency histograms showed a continuum from recently ascended, high-INP cells to depleted, sinking cells (Villareal and Lipschultz, 1995). Since we were unable to track individual mats over time, it is possible that the simple separations used here (sinkers versus floaters) obscured photosynthetic patterns that were present. The apparent physiological collapse of one-quarter of the mats in the electrode system further complicates analysis since we found no easy way to include this in average photosynthetic parameters. More recent analyses (August 1995) suggest that this response represents a real physiological condition, and is not an artifact of collection or incubation (T.A.Villareal, unpublished observations).

Compositional changes noted in mats correlate with nutritional deficiencies as a mechanism leading to buoyancy changes. Based on the sinker/floater differences, carbohydrate accumulates in the mat concurrent with INP depletion. In laboratory

Table I. Chemical composition of *Rhizosolenia* mats. Results from the two cruises are shown separately with the *P* value for differences between floating and sinking mats. Bold, italicized values are significant differences between rising and sinking mats. Average values in the June 1993 cruise include values from neutrally buoyant mats. Bulk composition was calculated only from individual mats; ratios were derived from samples including multiple mats

Component	August 1992				June 1993			
	Average value	Sinking mats	Ascending mats	*P*	Average value	Sinking mats	Ascending mats	*P*
C (μmol)	19.1 ± 2.7	20.9 ± 6.6	17.4 ± 3.0	0.52	27.1 ± 3.8	41.3 ± 9.3	19.6 ± 6.0	0.06
N (μmol)	2.4 ± 0.3	2.3 ± 0.5	2.5 ± 0.5	0.76	2.8 ± 0.4	3.7 ± 0.9	2.5 ± 0.7	0.31
Chl *a* (μg)	1.4 ± 0.2	1.0 ± 0.3	1.5 ± 0.3	0.34	***2.5 ± 0.3***	***1.7 ± 0.4***	***3.6 ± 1.0***	***0.03***
Protein (μg)	292 ± 23	284 ± 34	298 ± 32	0.77	162 ± 14	161 ± 43	158 ± 89	0.9
Carbohydrate (μg)	***334 ± 43***	***434 ± 80***	***261 ± 42***	***0.04***	180 ± 21	260 ± 15	98 ± 15	0.0006
Internal NO_3 (mM)	***7.1 ± 1.5***	***2.0 ± 1.3***	***9.7 ± 1.2***	***0.005***	***3.0 ± 1.4***	***0.6 ± 0.1***	***8.2 ± 3.9***	***0.04***
C:chl (wt)	141 ± 22	187 ± 43	115 ± 21	0.11	139 ± 23	174 ± 47	146 ± 24	0.55
N:chl (μmol:μg)	1.6 ± 0.2	1.9 ± 0.4	1.4 ± 0.3	0.33	1.7 ± 0.1	2.0 ± 0.3	1.8 ± 0.5	0.63
Protein:carbohydrate (wt)	***0.9 ± 0.2***	***0.4 ± 0.2***	***1.1 ± 0.2***	***0.05***	1.0 ± 0.1	0.6 ± 0.1	1.6 ± 0.4	<0.0001
C:N (mol)	***8.4 ± 0.4***	***9.6 ± 0.7***	***7.4 ± 0.4***	***0.01***	9.4 ± 0.3	11.3 ± 0.5	8.1 ± 0.3	<0.0001

batch culture, N depletion leads to a diversion of carbon to storage in diatoms (Myklestad, 1989) and can easily vary 2-fold over the diel cycle (Vårum *et al.*, 1986). In this study, a 2.7-fold increase in carbohydrate was seen in sinking mats and was the only particulate component consistently statistically higher in sinking mats. Protein:carbohydrate ratios range from 0.4 to 5.5 in cultures (Myklestad, 1974), with values <1 considered to indicate nutrient deficiency (Sakshaug *et al.*, 1983). In this study, positively buoyant mats had a protein:carbohydrate ratio of 1.1–1.6, a value which decreased to 0.4–0.6 in negatively buoyant mats concurrent with decreased INP. The effect of a third buoyancy class, neutrally buoyant, is particularly evident in this ratio as the significance level is much greater in the June 1993 cruise than the August 1992 cruise. The increase in C:N ratio (7.4–8.4 to 9.6–11.3) is also consistent with nutrient limitation (Laws and Bannister, 1980). P limitation does not generally lead to rapid decreases in protein:carbohydrate ratio, although it can lead to enhanced extracellular polysaccharide production (Myklestad, 1972; Myklestad and Haug, 1977).

We suggest that N limitation is a key factor regulating *Rhizosolenia* mat vertical migration via its role in carbohydrate metabolism. Carbohydrate accumulation leads to increased density in cyanobacteria (Kromkamp and Walsby, 1990, 1992), and culture studies note that *Rhizosolenia* buoyancy is inversely related to carbohydrate content (Moore and Villareal, 1995). Dark carbohydrate consumption at depth during NO_3^- uptake would reduce cellular density and provide a mechanism enabling mats to return to the surface for photosynthesis. Night NO_3^- uptake has been observed in *R.formosa* H.Peragallo, a species found in mats, and calculations indicate that carbohydrate production and consumption is adequate to account for buoyancy reversals (Moore and Villareal, 1995). In dinoflagellates, carbohydrate serves an essential role as an energy reserve for dark NO_3^- uptake and reduction (Cullen, 1985), and can also support dark protein synthesis in natural populations (Cuhel *et al.*, 1984; Lancelot and Mathot, 1985). These observations suggest that carbohydrate plays an important role in the life history cycles of taxonomically diverse vertical migrators.

Internal NO_3 is an important marker of N status and may be the major storage pool. Nitrate reductase activity confirms NO_3^- as an N source for mats. Our observed rates are well below those required to support C fixation, but the Eppley assay as performed (Culver-Rymsza, 1988) was not optimized for diatoms (Berges and Harrison, 1995), and our more recent modifications yield nitrate pool reduction rates that are similar to C doubling times (L.Joseph and T.A.Villareal, in preparation). Using the average internal NO_3^- value for ascending mats in June 1993 (8.2 mM), an average mat volume (2×10^{11} μm^3) and average value of 2.8 μmol N mat^{-1}, we calculate that internal NO_3^- represents 57% of the total N present in ascending mats. Since maximum internal NO_3^- concentrations reach up to 27–28 mM in both field samples and *Rhizosolenia* cultures (Moore and Villareal, 1995; Villareal and Lipschultz, 1995), NO_3^- may be the dominant storage pool similar to what has been noted in *Ethmodiscus* (Villareal and Lipschultz, 1995). However, since amino acids or proteins can be synthesized in the dark (Cuhel *et al.*, 1984), they may also be important storage compounds.

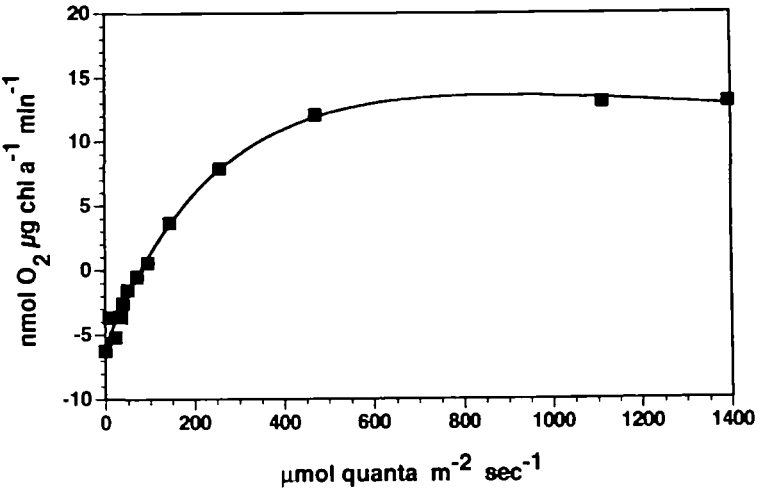

Fig. 10. Typical irradiance O_2 evolution plot in *Rhizosolenia* mats fitted to the Platt *et al.* (1980) model. $r^2 = 0.997$.

Vertical migrating phytoplankton import new N into the euphotic zone. *Rhizosolenia* mat NO_3^- transport has been estimated to be the equivalent of 2–27% of the nitricline diffusive flux (Villareal *et al.*, 1993), based on a C-specific doubling rate of 0.6 doublings day^{-1}. However, a vertically migrating cell must periodically transit the euphotic zone to replenish N reserves, and must therefore grow at a reduced rate relative to light-saturated values (Villareal and Carpenter, 1994). Laboratory studies indicate that *R.castracanei* H.Peragallo, *R.formosa*, and *R. debyana* H.Peragallo, species all found in mats, can grow at 0.4–0.8 divisions day^{-1} and these values serve as a reference point for our calculations (Moore and Villareal, 1995).

We have calculated the time for one complete migration cycle using C and N pool-filling times based on oxygen evolution rates (this study), and N uptake kinetics of *Ditylum brightwelli* (Eppley and Coatsworth, 1968), respectively. This latter assumption is untested, but represents the largest cell with both a K_s and V_{max}. Eppley and Thomas (1969) reported a K_s for NO_3^- from *R.robusta* of 2.5–3.5 µM, higher than values for *D.brightwelli*, but had no corresponding V_{max} values. We have assumed that ascent and descent rates (maximum and average) are the same, that our measured dark respiration rates are valid at night, that C fixation occurs only at the surface and that no N uptake occurs until the mat sinks to the depth of the defined N concentration. The latter two assumptions are oversimplifications and will increase the generation time. The migration time is calculated as follows:

Migration time = C doubling time + descent time + nitrogen doubling time based on nitrate uptake + ascent time

Table IV summarizes the parameters and results for two scenarios: descent to a depth of 1 and 2 µM NO_3^-. We have compared the estimated dark nitrate uptake time with carbohydrate energy reserves using a specific glucanase rate of 0.10 mg

Table II. Photosynthetic parameters of *Rhizosolenia* mats determined from oxygen evolution measurements conducted in June 1993. Values are averages of all measurements ± SE. Average values include results from neutrally buoyant mats

Buoyancy	I_c	α	I_k	P_{max}	Respiration
Sinkers	73 ± 16	0.061 ± 0.015	140 ± 22	9.2 ± 2.8	−3.6 ± 0.5
Floaters	73 ± 20	0.048 ± 0.011	241 ± 36	14.1 ± 1.8	−2.9 ± 0.7
Average (all mats)	70 ± 10	0.055 ± 0.007	189 ± 19	11.5 ± 1.4	−3.2 ± 0.4

I_c, I_k = μmol quanta m^{-2} s^{-1}; α = nmol O_2 μg^{-1} chl min^{-1} (μmol quanta m^{-2} s^{-1})$^{-1}$; P_{max}, respiration = nmol O_2 μg^{-1} chl min^{-1}.

glucose mg^{-1} protein h^{-1} derived from light-limited *Skeletonema costatum* (40% μ_{max}; Vårum and Myklestad, 1984) and our protein:carbohydrate ratios (June 1993; Table I). Average mats have sufficient reserves for 10–16 h below their compensation depth. Since sinking mats would probably be growing at a lower rate than 40% μ_{max}, it appears the calculated N doubling times of 11.6–17.3 h (Table IV) are reasonable based on the carbohydrate reserves in the cells. Culture studies of *R.castracanei* have also noted similar uptake rates to those assumed in Table IV (Richardson *et al.*, submitted).

This simple model suggests that mats migrate on the time scale of 3.6–5.4 days. Faster N uptake at depth due to higher NO_3^- concentrations appear to be offset by increased transit time. This revised time scale is substantially greater than the turnover time of 1.7 days used in Villareal *et al.* (1993) and suggests that upward N fluxes may be less than initially suggested. To evaluate this, we considered two different estimates of *Rhizosolenia* mat abundance based on either the average values reported here, or estimates based on surface accumulations noted under low-wind conditions (Figure 4B). This latter model uses the surface as an inverted sediment trap and assumes that surface abundance increases represent mats ascending from depth rather than increases from growth. We cannot specify the integration depth in this case, but it is unimportant for this calculation.

We assume that all N in mats is derived from NO_3^- and that only enough N for one doubling is transported per migration. Using an average 2.5 μmol N per mat and our revised migration times, it is calculated that mats could transport from 3.9 to 40 μmol N m^{-2} day^{-1} based on average abundance (0.3 mats m^{-3} over 30 m) and surface accumulation data (250 mats m^{-3} over 0.3 m + 0.3 mats m^{-3} over 29.7 m), respectively. The former estimate (based on average abundance) is an underestimate since mats below 30 m were not included. The latter estimate may be closer to the actual value, but still lacks validation from abundance estimates through the entire water column. These two abundance estimates yield mat N flux rates from <0.01 to 20 times the diffusive flux based on the very large 95% confidence interval for N flux (2.0–890 μmol m^{-2} day^{-1}; Lewis *et al.*, 1986). Mats transport 3–4 and 18–29% of Lewis *et al.*'s (1986) average nitrate diffusive flux rate based on the diver-measured average or surface accumulation counts, respectively. The sediment trap N flux estimates for 110 m (411.6 μmol N m^{-2} day^{-1}) of Martin *et al.* (1987) yield a mat N-flux contribution of <1–10%. Assuming Redfield stoichiometry,

Table III. *Rhizosolenia* mat abundance in the North Pacific gyre. Values are integrated to the deepest records from each study, usually 10–20 m. Volume-specific abundance is taken directly from the literature reference, areal abundance is calculated using a trapezoidal integration. Values do not include surface accumulations from low-wind conditions in this study

Reference	Period	Mean abundance (mats m^{-1})	Areal abundance (mats m^{-2})
Alldredge and Silver (1982)	October 1980	0.7 ± 0.1	11.5
Martinez *et al.* (1983)	September 1981	2.3 (upper 5 m only)	–
Villareal and Carpenter (1989)	April 1989	0.3 ± 0.1	1.3 ± 0.4
This study	August 1992	0.5 ± 0.2	3.3 ± 0.9
	June 1993	0.9 ± 0.3	8.1 ± 2.5

the range of eddy diffusion coefficients considered by Emerson and Haywood (1995) converts to an N flux range of 41.5–414 μmol N m^{-2} day^{-1} with mats representing 0.9–96% of this value. The larger value suggests that mats may contribute substantially to the negative pre-formed nitrate zone at the top of the nutricline observed by Emerson and Hayward (1995) in some areas of the North Pacific. Data presented here cannot resolve whether mats have the required depth distribution or abundance to account for this zone's distribution across other regions of the eastern basin.

Mats can transport a significant quantity of N, but both physical and biological fluxes probably vary over time. Mats appear to have real station-to-station abundance variability, and standard nutrient budgets suggest that steady-state nutrient flux is inadequate to support observed primary production rates (Hayward, 1987, 1991). In addition, diapycnal mixing may not be the principal mechanism responsible for transport across the pycnocline, rather isopycnal processes may dominate (Ledwell *et al.*, 1993; Hayward, 1994). If this is the case, then fundamentally different time scales are being considered and meaningful comparisons can be drawn only using seasonal patterns of mat distribution and abundance.

Characteristics of *Rhizosolenia* mat migration require that much of the primary production associated with this NO_3^- transport occurs in the mixed layer well above the nutricline. Villareal *et al.* (1993) estimated that mats transport an amount equivalent to 50% of the new N demand in this region. Our revised N flux estimates (9–40 μmol N m^{-2} day^{-1}) suggest values of 3–35% using an '*f* ratio of 0.05, a carbon fixation rate of 0.5 mmol C m^{-3} day^{-1} (Marra and Heinemann, 1987) and a surface mixed layer of 30 m. The fate of this mat N is unknown. It may be utilized only by mats, leaked to the surrounding phytoplankton, or be remineralized by either zooplankton or protozoan parasites noted previously (Caron *et al.*, 1982) and in this study. In addition, vertically migrating fish have been found to contain mats in their guts (Robinson, 1984).

The assumption that mats always return to the surface mixed layer is untested. Buoyancy–light responses in *Trichodesmium* can lead to buoyancy reversals occurring at different depths on subsequent days due to time-dependent interactions between carbohydrate metabolism and light history (Kromkamp and Walsby, 1992). Moore and Villareal (1995) noted growth photoinhibition above ~150–200 μmol quanta m^{-2} s^{-1} in batch cultures of several mat-forming

Table IV. Estimated *Rhizosolenia* mat migration times calculated from ascent rates, NO_3 uptake and photosynthetic rates. Sinking rates and O_2 evolution were measured; NO_3 uptake is based on *Ditylum brightwelli* (Eppley and Coatsworth, 1968)

NO_3 concentration (μM)	1		2	
V_{max} (h^{-1})	0.12		0.12	
K_s (μM)	2		2	
NO_3 uptake (h^{-1})	0.04		0.06	
N doubling time (h)	17.3		11.6	
C doubling time (h)	29.5		29.5	
Transit depth (m)	125	125	150	150
Sinking/ascent rate ($m\ h^{-1}$)	6.4 (maximum)	3 (average)	6.4 (maximum)	3 (average)
Transit time to depth (h)	19.5	41.7	23.4	50.0
Total hours for entire migration	85.9	130.2	87.9	141.1
Days	3.6	5.4	3.7	5.9

Rhizosolenia spp., and inverse relationships between positive buoyancy and light. The obvious discrepancy between diver-measured abundance and estimates from surface accumulations also suggests that many mats occur at depth. Thus, there is evidence to suggest that a simple surface-to-depth migration pattern is an oversimplification and that our calculation is only a first approximation. Taxonomic complexities between taxa can be seen in the differing internal NO_3^- pool sizes found in migrating diatoms, dinoflagellates and prasinophytes (Villareal and Lipschultz, 1995). Our considerations have only explicity considered N fluxes by migrating phytoplankton, but the utilization and transport of other nutrients such as P, Si or trace metals appears possible as well.

Acknowledgements

This work was supported by NSF grant OCE-9100888 to T.A.V. We wish to thank the officers and crew of the RV 'Wecoma' and 'New Horizon' for their assistance in our diving operations, F.Hawse, Drs D.Caron and E.Delong for their help with collections, and Dr A.E.Walsby for his careful comments on the manuscript.

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Received on August 2, 1995; accepted on January 16, 1996