There are two other, although less well-defined, microtektite occurrences present in upper Eocene marine sediments at about 38.5 million years (sites 292, 242, and E67-128) and near the Eocene-Oligocene boundary at about 36.0 million years [sites 242 and 94 and St. Stephen quarry (Fig. 2)]. The stratigaphic occurrences of these microtektites indicate events separate from the North American tektite-strewn field of 37.5 to 38.0 million years ago; no major reworking or bioturbation is evident at these intervals.

In addition, rare microtektites have been found at stratigraphic levels estimated to have an age of 31 to 32 million years [sites 292, 242, and St. Stephen quarry (Table 1; Fig. 2)]. Chemical analyses will be necessary to determine whether these glassy microspheres are indeed microtektites and to differentiate these from the microtektite horizon at 37.5 million years ago.

Sediments in which microtektites were found are affected by $CaCO_3$ dissolution, and in many sections a short hiatus is present (Fig. 2). Microtektites are most abundant in the radiolarian ooze of sites 149 and RC9-58 where, incidentally, the iridium anomalies were found (*4, 5*). Most of the $CaCO_3$ in these sediments has been dissolved. This suggests that microtektites, and perhaps iridium, are selectively concentrated at dissolution intervals.

The extinction of five radiolarian species at the microtektite horizon appears to be due to the hiatus that is commonly present at this interval. The terrestrial mammal extinctions which Alvarez and his colleagues refer to as "mass extinctions" occur in the mid-Oligocene at 32.4 million years (*13*), that is, 5 million years after the supposed bolide impact. Berggren *et al.* have pointed out (*10*) that this faunal turnover is not interpreted by vertebrate paleontologists in terms of catastrophism.

Quantitative population analyses of nannofossils (*14*) and planktic and benthic foraminifers (*15, 16*) reveal no major extinctions in the marine records. Five distinct faunal assemblage changes do occur, however, in the marine plankton record between middle Eocene and middle Oligocene time at 43 to 42, 41 to 40, 38 to 37, 36 to 35, and 31 to 30 million years ago. These faunal changes coincide with paleoclimatic cooling episodes as determined by $\delta^{18}O$ studies (*15*). During these cooling episodes, faunal turnover occurred gradually; as a result, there was a reduction in the relative abundance of some species and increased dominance of others (*15*). There is no evidence, however, for a catastrophic event at any of the faunal assemblage changes. Climatic cooling in the interval from the middle Eocene to the middle Oligocene appears to have been induced by the growth of a major Antarctic ice sheet. These climatic changes were presumably triggered by the separation and northward movement of India and Australia from Antarctica and the subsequent development of the circum-Antarctic current (*17*).

The conclusions of Glass and his coworkers (*1–3*) should be reevaluated since (i) all middle Eocene to middle Oligocene microtektite horizons are not coeval and (ii) no faunal extinctions can be correlated with any of the microtektite horizons or with the late Eocene bolide impact (*4, 5*) that occurred in the late Eocene about 37.5 to 38.0 million years ago.

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Nitrogen Fixation by Floating Diatom Mats: A Source of New Nitrogen to Oligotrophic Ocean Waters

Abstract. *Nitrogen fixation, apparently by bacterial endosymbionts, is associated with intertwining chains of two species of the diatom* Rhizosolenia. *In situ fixation rates were enhanced by incubation in the dark, whereas concurrent shipboard experiments either underestimated or did not detect nitrogen fixation. This is the first example of nitrogen fixation associated with a bacteria-diatom symbiosis in the pelagic zone, and it indicates that these systems may contribute a significant amount of "new" nitrogen to oligotrophic waters.*

Although oligotrophic central oceanic regions such as the North Pacific Gyre and the Sargasso Sea are traditionally viewed as biological deserts, more recent evidence indicates that they may in fact be highly productive (*1, 2*). Researchers seeking explanations for high productivity in waters that often lack measurable amounts of nitrogenous nutrients have hypothesized processes ranging from salt-fingering (*3*) and small-scale nutrient pulses produced as a result of zooplankton excretion (*2*) to remineralization (*4*) and nitrogen fixation (*5*). Although current models describing inputs of "new" nitrogen to oligotrophic waters consider nitrogen fixation unimportant (*6*), our work suggests that the fixation rates may have been underestimated and that this process may introduce significant amounts of nitrogenous nutrients into these waters.

One notable example of high primary production under nutrient-limited conditions is demonstrated by free-floating diatom mats composed of *Rhizosolenia castracanei* and *R. imbricata* var. *shrubsolei* (*7*) (Fig. 1A). These mats have been noted in surface waters of the Sargasso Sea (*8*), the North Pacific Gyre, and the California Current (*7*). On a recent cruise to the eastern Pacific (*9*), we found *Rhizosolenia* mats in great abundance (up to 4.4 m^{-3}). The aggregations are large (average length, 7 cm) and conspicuous to scuba divers, yet they are often not

visible from shipboard. These fragile mats are also easily fragmented by net tows and disrupted or missed by standard bottle casts. As a result, those using conventional techniques may overlook the existence and importance of these mats.

Unlike another closely related diatom *R. styliformis*, whose occurrence in oligotrophic water is attributed in part to its nitrogen-fixing symbiont *Richelia intracellularis* (*10*), our specimens did not harbor heterocyst-bearing cyanobacteria. They did, however, possess high densities of bacteria within cytoplasmic vacuoles (*7*) (Fig. 1B). Because of their high biomass and high rates of primary productivity, we had hypothesized that our *Rhizosolenia*-bacteria complexes fix nitrogen (*7*). We present here the results of a test of this hypothesis. We used a modified version of the standard acetylene reduction method in which the nitrogenase enzyme responsible for nitrogen fixation reduces acetylene to ethylene (*11, 12*).

In view of the fragility of *Rhizosolenia* mats and the irreversible inhibition of the nitrogenase enzyme by high oxygen concentrations (*13*), our experiments were designed to minimize manipulation and consequent disruption of the mats. All samples were hand-collected in the incubation chambers by scuba divers and either brought back to the shipboard incubator or inoculated and suspended in situ. For each experiment we used six light and six dark replicate incubation chambers, each containing a single mat suspended in seawater, and 12 similar control chambers containing ambient seawater only. We used "clean techniques" to minimize potential metal toxicity, which is known to reduce primary production (*14*).

Our results show that *Rhizosolenia* mats can fix nitrogen (Table 1). The highest rates of acetylene reduction occurred when mats were incubated in the dark. These rates are significantly higher than corresponding values for mats incubated in the light. Ethylene values for both light and dark treatments of chambers containing ambient seawater showed no significant difference from values for the initial acetylene mixture (blanks) ($P = .01$) (*15*). Although many organisms seem to show light-dependent nitrogen fixation, low rates of fixation can continue throughout the night (*16*). Dark enhancement in our system suggests that here, where physical isolation of the nitrogen-fixing "machinery" may be minimal, there may be a temporal separation (that is, diel alternation) between oxygen-evolving photosynthesis and oxygen-inhibited nitrogen fixation (*17*). Dark enhancement also supports the hypothesis that the nonphotosynthetic intracellular bacteria are responsible for the nitrogen fixation (*18*).

Deckboard experiments consistently underestimated or did not detect nitrogen fixation (Fig. 1E). At our westernmost station, the in situ experiment yielded maximum values while the simultaneous deckboard experiment did not show fixation. After deckboard incubation the *Rhizosolenia* showed signs of cellular damage, whereas *Rhizosolenia*

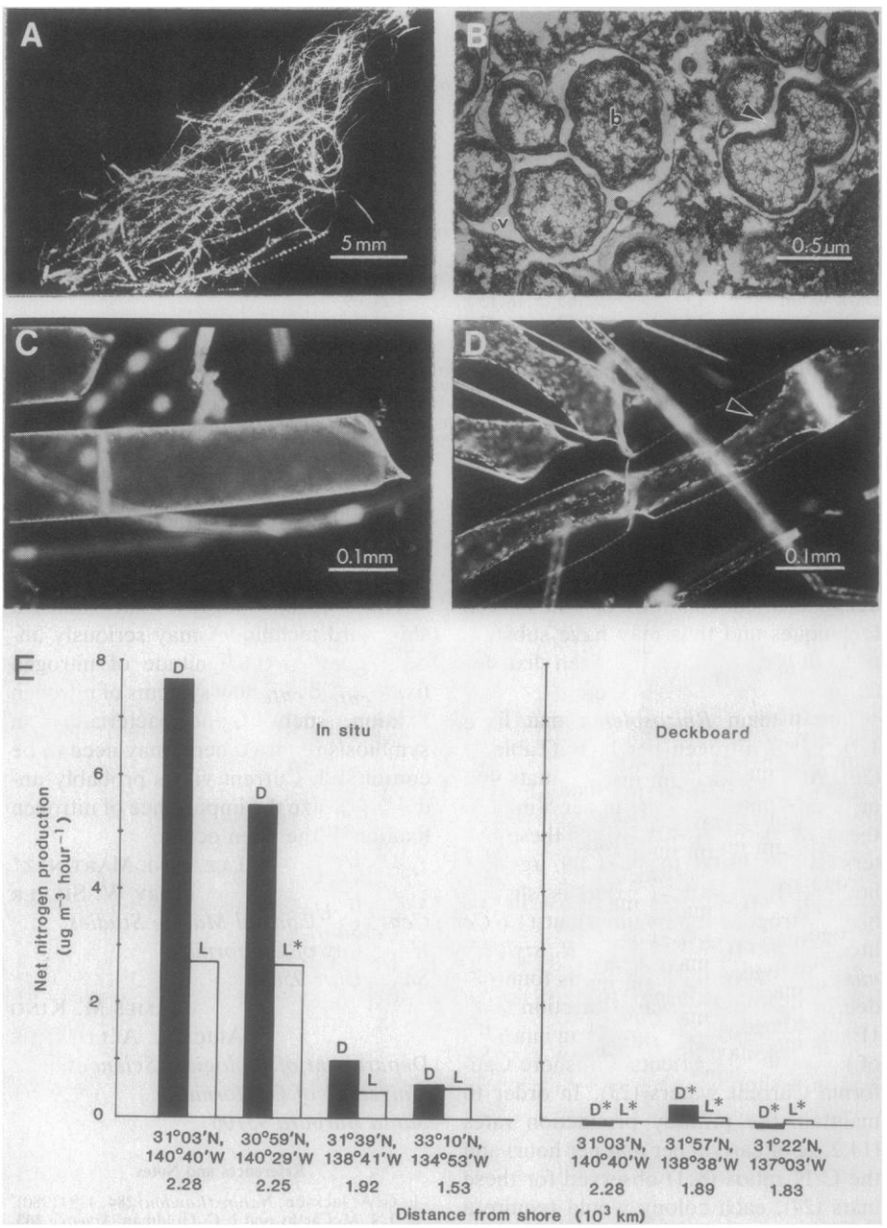

Fig. 1. (A) In situ photograph of a typical *Rhizosolenia* mat composed of intertwining chains of *R. castracanei* (wider cells) and *R. imbricata* var. *shrubsolei* (narrower cells). This mat is approximately 5 cm long. (B) Transmission electron micrograph showing a cross section of *R. imbricata* var. *shrubsolei* containing large numbers of intracellular bacteria (*b*) just outside the nucleus. Bacteria are located within membrane-bound vacuoles (*v*). Note the dividing bacterial cells (arrow). Material preserved in situ. (C) Light micrograph of *R. castracanei* cells preserved immediately after an in situ incubation period, showing an apparently healthy cell with protoplasm extended to the siliceous cell wall (frustule). (D) Light micrograph of *R. castracanei* cells preserved immediately after a deckboard incubation period, showing the collapse of the protoplast. The bright vertical band across the cell is the nucleus and surrounding cytoplasm in which the highest densities of bacteria are found. The arrow shows the cell membrane withdrawn from the frustule. (E) Net nitrogen produced by nitrogen (acetylene reduction) fixation. Numbers are calculated by subtracting ambient seawater values from mat values, converting to equivalent nitrogen fixation (see text), and scaling the fixation according to station mat densities. The values plotted are the means for each station: shaded bars (*D*), dark incubation; unshaded bars (*L*), light incubation; asterisks indicate nonsignificant values (Duncan's multiple range test, $P > .10$), that is, mat values not different from corresponding seawater values.

Table 1. Nitrogen (acetylene reduction) fixation by *Rhizosolenia* mats in the North Pacific Gyre. Hand-collected specimens of mats and ambient water samples were incubated either in a deckboard incubator or in situ at 5 m in transparent (light) or opaque (dark) incubation chambers. The mean incubation time was 53 minutes, and the blank time 0 minutes. In our statistical analysis of the net acetylene reduction, we tested mat values against water under the same light conditions and water values against blanks; S.D., standard deviation of the mean.

Treatment	*N*	Acetylene reduction (nmole per chamber per hour)*		Nitrogen fixation†	
		Mean ± S.D.	Net	μg per mat per hour	μg per cubic meter per hour
		Deckboard			
Dark mat	14	90.23 ± 26.12	1.15	0.011	0.025
Light mat	17	72.78 ± 23.31	1.27	0.012	0.028
Dark water	9	89.08 ± 10.95	3.46		
Light water	15	71.51 ± 15.41	−14.11‡		
Blank	6	85.62 ± 49.72			
		In situ			
Dark mat	21	217.70 ± 99.74	125.05§	1.167	3.423
Light mat	24	154.11 ± 46.99	65.59§	0.612	1.796
Dark water	13	92.65 ± 18.15	21.09		
Light water	15	88.52 ± 16.43	16.96		
Blank	8	71.56 ± 32.90			

*Median time of incubations (deckboard = 46 minutes, in situ = 42 minutes) used to calculate rates (*12*). †Deckboard values do not differ significantly from blank values but are shown to allow comparison with in situ values. ‡Negative value results from questionable blanks taken several hours after the completion of the experiment (*15*). §Significant (Duncan's multiple range test, $P = .01$).

from in situ incubations did not (Fig. 1, C and D). Other investigators of nitrogen fixation in the open ocean have used deckboard inoculation or incubation techniques and thus may have substantially underestimated nitrogen fixation (*19*).

The median *Rhizosolenia* mat fixes 1.17 μg of nitrogen per hour (Table 1) (*20*). At the average density of mats (2.3 m^{-3}) encountered in the upper 5 m (*21*), the contribution of nitrogen to these waters is estimated to be 2.69 μg m^{-3} $hour^{-1}$. This median value is slightly higher than the maximum input (1.6 ng $liter^{-1}$ $hour^{-1}$) reported for *R. styliformis* (*22*). Nitrogen fixation was found to decrease in a shoreward direction (Fig. 1E), consistent with increasing amounts of nitrogenous nutrients in inshore California Current waters (*23*). In order to maintain the primary production rates (14.2 μg of carbon per mat per hour) and the C:N ratios (8:1) observed for these mats (*24*), each colony would require a nitrogen input of approximately 1.77 μg per mat per hour. The estimated rate of 1.17 μg of nitrogen fixed per mat per hour suggests that these diatoms may supply over half their nitrogen needs through fixation. At our westernmost station, simultaneous in situ ^{14}C and acetylene reduction measurements show that *Rhizosolenia* mats, which account for 12.7 percent of all primary production (mat density, 4.4 m^{-3}; rate, 12.68 μg of carbon per mat per hour) may fill 110 percent of their immediate nitrogen requirement through fixation (1.74 μg of nitrogen per mat per hour). This nitrogen input is 14 percent of the total nitrogen required for primary production in these waters.

These results suggest that standard shipboard techniques may seriously underestimate the magnitude of nitrogen fixation and that new systems of nitrogen fixation, such as the bacteria-diatom symbiosis reported here, may need to be considered. Current views probably underemphasize the importance of nitrogen fixation in the open ocean.

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19. Analysis of data from a study on the blue-green alga *Trichodesmium* in the Sargasso Sea showed similar deckboard and in situ trends, as do ^{14}C fixation rates for *Rhizosolenia* mats (*24*).
20. Since the conversion factor for acetylene reduction to nitrogen fixation is not known for this system, we used the theoretical ratio of 3 moles of acetylene to 1 mole of nitrogen fixed [C. L. Masterson and P. M. Murphy, in *Recent Advances in Biological Nitrogen Fixation*, N. S. Subba Rao, Ed. (Holmes and Meier, New York, 1980), pp. 8–33]. The mean specific nitrogen fixation rate was 5.1 μg of nitrogen per milligram of cell nitrogen per hour for mats incubated in situ. This is high for a symbiotic relationship but within the range reported for *Trichodesmium* and other cyanobacteria (*23*).
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