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PHYSIOLOGICAL AND OPTICAL PROPERTIES OF *RHIZOSOLENIA FORMOSA* (BACILLARIOPHYCEAE) IN THE CONTEXT OF OPEN-OCEAN VERTICAL MIGRATION[1]

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ABSTRACT

Cultures of Rhizosolenia formosa *H. Peragallo were studied to assess whether or not physiological and optical characteristics of this large diatom were consistent with the ability to migrate vertically in the open ocean. Time-course experiments examined changes in chemical composition and buoyancy of* R. formosa *during nitrate (N)–replete growth, N starvation, and recovery. Cells could maintain unbalanced growth for at least 53 h after depletion of ambient nitrate. Increases in C:N and carbohydrate : protein ratios observed during N starvation reversed within 24 h of reintroduction of nitrate to culture medium. Buoyancy was related to nutrition: Upon N depletion, the percentage of positively buoyant cells decreased to 4% from 11% but reverted to 9% within 12 h of nitrate readdition. Cells took up nitrate in the dark. Nitrogen-specific uptake rates averaged 0.48 d^{-1}; these rates were higher than N-specific growth rates (0.15 d^{-1}), indicating the potential for luxury consumption of nitrate, which can be stored for later use. Measurements of photosynthesis vs. irradiance, chlorophyll-specific absorption ($a_{ph}*(\lambda)$), and pigment composition showed that cells may be adapted for growth under a wide range of irradiances. Values of $a_{ph}*(\lambda)$ were lower for N-depleted cells than for N-replete cells, and N-depleted cells had higher ratios of total carotenoids to chlorophyll* a. *Aggregation of chloroplasts was more pronounced in N-depleted cells. These are possibly photoprotective mechanisms that would be an advantage to N-depleted cells in surface waters. Compounds that absorb in the ultraviolet region were detected in N-replete cells but were absent in N-depleted cultures. Overall, these results have important implications for migrations of* Rhizosolenia *in nature. Cells may survive fairly long periods in N-depleted surface waters and will continue to take up carbon; then they can resume nitrate uptake and revert to positive buoyancy upon returning to deep, N-rich water. Uncoupled uptake of carbon and nitrogen during migrations of* Rhizosolenia *is a form of new production that may result in the net removal of carbon from oceanic surface waters.*

Key index words: Bacillariophyceae; biogeochemical cycling; chemical composition; chlorophyll-specific absorption; nitrate uptake; photosynthesis vs. irradiance; Rhizosolenia formosa; *vertical migration*

Large diatoms of the genus *Rhizosolenia* H. Peragallo (cell volumes up to 10^7 μm^3) are commonly found in open-ocean ecosystems such as the Sargasso Sea (Carpenter et al. 1977, Villareal 1988, Villareal and Lipschultz 1995), the Caribbean (Carpenter et al. 1977, Villareal 1988), the Eastern Pacific Ocean (Alldredge and Silver 1982, Martinez et al. 1983), and the Central North Pacific Gyre (Villareal and Carpenter 1989, Villareal et al. 1993). *Rhizosolenia* cells can be solitary or can exist in the form of chains, mats, or rafts (Venrick 1974, Villareal and Carpenter 1989) and can be both positively and negatively buoyant (Villareal 1988, Villareal and Carpenter 1989). The buoyancy state of *Rhizosolenia* is determined in large part by its intracellular density (Smayda 1970, see also Richardson and Cullen 1995), which may be affected by changes in internal ion concentration (Gross and Zeuthen 1948, Beklemishev et al. 1961, Anderson and Sweeney 1978, Kahn and Swift 1978) and internal carbohydrate concentration (Richardson and Cullen 1995, Moore and Villareal 1996). Cell buoyancy is also affected by the light and nutrient status of the organism (Steele and Yentsch 1960, Bienfang et al. 1982, 1983, Bienfang and Harrison 1984, Waite et al. 1992a, b). Observations of pronounced changes in its buoyancy state first led to the hypothesis that *Rhizosolenia* may undergo vertical migrations in nature (Villareal and Carpenter 1989) similar to migrations of dinoflagellates (Heaney and Eppley 1981, Cullen 1985, Watanabe et al. 1991). Later evidence strongly supported this hypothesis: Particulate nitrogen of *Rhizosolenia* collected in nitrate-depleted surface waters of the Central North Pacific Gyre contained $\delta^{15}N$ signatures characteristic of cells that had exploited deep nitrate pools (Villareal et al. 1993). Positively buoyant *Rhizosolenia* had significantly higher internal nitrate concentrations than sinking cells, consis-

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tent with vertical movements between surface and deep (> 100 m) waters (Villareal et al. 1993, Villareal and Lipschultz 1995). Vertical migrations to deep nutrient pools give cells a distinct ecological advantage in areas such as the Central North Pacific Gyre, where nitrate does not become available until well below the euphotic zone (e.g. Hayward 1991). Access to both saturating irradiance at the surface and high concentrations of nutrients at depth may result in greater overall levels of primary productivity and growth than if migrations were not possible (Gran 1929) and may explain the relative success of large phytoplankton in areas otherwise dominated by picoplanktonic organisms and highly regenerative food webs. The importance of large phytoplankton in predominantly oligotrophic areas is becoming better recognized (Sancetta et al. 1991, Goldman et al. 1992, Goldman 1993, Yoder et al. 1994) and has led to hypotheses about their potential roles in biogeochemical cycling (Goldman et al. 1992, Villareal et al. 1993).

Migrating phytoplankton may have significant effects on water column chemistry and thus can influence the cycling of nutrients like carbon, nitrogen, phosphorus, and oxygen (Fraga et al. 1992). When nitrate is taken up at depth and transported to the euphotic zone by vertical movements of phytoplankton, the resulting growth represents a form of new production as defined by Dugdale and Goering (1967, see also Richardson and Cullen 1995, Moore and Villareal 1996). Furthermore, in many oceanic regimes the top of the nutricline is below the euphotic zone (see Hayward 1991), so uptake of nitrate will occur in the dark without the accompanying uptake of carbon (see Terry 1982). Cells will instead combine nitrogen acquired at depth with carbon acquired in surface waters; that is, the processes of nitrogen uptake and carbon uptake become uncoupled in space and time (Cullen 1985, Fraga et al. 1992). Thus, the input of nitrogen through the vertical movements of cells is not coupled to the input of carbon in the Redfield ratio (Redfield 1958) as is the case for physically transported dissolved nutrients (see Eppley and Peterson 1979). Because photosynthesis occurring in surface waters requires a carbon source, and because stoichiometric equivalents of carbon are not brought to the surface in coupled transport with nitrogen, new production resulting from vertical movements of phytoplankton could result in the net removal of carbon from oceanic surface waters. This, in turn, could affect the air–sea exchange of carbon dioxide (Richardson and Cullen 1995).

The objective of our study was to examine changes in physiological and optical properties of *Rhizosolenia formosa* in response to variations in nutrient regime, with a view toward determining whether or not changes were consistent with vertical migrations in the open ocean. Time-course experiments examined changes in chemical composition and buoyancy of cultures of *R. formosa* during N-replete growth and N starvation and after reintroduction of nitrate to culture medium in order to simulate conditions that would be experienced by these cells during migrations to N-depleted surface waters from N-rich deep waters in nature. Nitrate uptake rates were determined for batch cultures under varied conditions of light and nutrients. Photosynthesis vs. irradiance (P-I), in vivo and in vitro absorption, and determinations of pigment composition of *R. formosa* under N-replete and N-depleted conditions were used to determine whether or not the photosynthetic capabilities of *R. formosa* show adaptations consistent with wide variations in irradiance associated with vertical migrations in nature. Determination of rates of carbon and nitrate uptake are also necessary first steps toward examining potential larger scale effects of *Rhizosolenia* on biogeochemical cycling.

MATERIALS AND METHODS

Unialgal cultures of the marine diatom *Rhizosolenia formosa* (Clone B8, isolated from the Sargasso Sea near Bermuda in August 1992 by T. A. Villareal) were grown in 4-L glass Erlenmeyer flasks containing 3 L of MET-44 medium (Schöne and Schöne 1982) with trace metals and vitamins adjusted to K/20 and K medium concentrations, respectively (Keller et al. 1987). The medium was filter-sterilized using a 0.2-μm Culture Capsule filter (Gelman Sciences, Ann Arbor, Michigan). Cells were grown at 20° C, on a 14:10 h LD cycle with illumination provided from the side by five cool-white fluorescent lamps. Two layers of blue LEE gel filters (#061) were between the lamps and culture containers, which resulted in a quantum scalar irradiance of approximately 50 μmol quanta$\cdot m^{-2} \cdot s^{-1}$ (photosynthetically active radiation [PAR]) as measured with a Biospherical Instruments QSL-100 4π sensor placed just outside the culture containers.

Large diatoms are notoriously difficult to culture (Goldman 1993). *Rhizosolenia,* in particular, is fragile and very sensitive to disturbance. Growth rates are reduced significantly and often cultures do not survive when routine (i.e. frequent) sampling procedures are used. Therefore, all experiments in this study were constrained by the frequency with which cells could be sampled (every 3 d or so at best). Ambient nitrate could be measured more frequently, as cultures could be sampled gently and thorough mixing was not required. Growth irradiance was not manipulated during this study. We used an irradiance of 50 μmol quanta$\cdot m^{-2} \cdot s^{-1}$ because previous studies showed that cultures of *Rhizosolenia formosa* grew well at this relatively low irradiance (Moore and Villareal 1996).

Chemical composition time-course experiment. A time-course experiment was conducted to examine changes in chemical composition of *Rhizosolenia formosa* during nitrate (N)–replete growth and N starvation. Six 3-L cultures of *R. formosa* were grown as already described, but nitrate was adjusted to an initial concentration of 10 μM instead of 40 μM. Samples for chlorophyll, cell counts, particulate carbon and nitrogen, carbohydrate, protein, and ambient nitrate were taken initially, at one time point before N depletion and at one time point after N depletion. Ambient nitrate was monitored more frequently to determine approximate time of N depletion. Once ambient nitrate ran out, the six cultures were divided into three groups of two. Duplicate cultures were left for 24, 30, or 53 h without nitrate, after which nitrate was reintroduced to the culture medium to a final concentration of approximately 15 μM. Cells were left for an additional 24 h to allow for incorporation of nitrate. Then final samples were taken. Chlorophyll *a*, corrected for phaeopigment, was determined fluo-

rometrically using a Turner Designs model 005R fluorometer calibrated with pure chlorophyll *a* (Sigma Chemical Co., St. Louis, Missouri). Samples were collected in triplicate on Whatman GF/C filters and were extracted in 10 mL of 90% acetone in the dark for at least 24 h at −10° C. Cells preserved with acid Lugol's solution (Throndsen 1978) were counted with an inverted microscope. Ambient nitrate was determined with a Technicon Autoanalyzer II on GF/C-filtered samples. Particulate carbon and nitrogen were determined using a Perkin-Elmer 2400 CHN Analyzer on material concentrated onto precombusted GF/C filters. Carbohydrate was determined as glucose equivalents by the phenol-sulfuric acid method of Dubois et al. (1956), and protein was analyzed by the heated biuret-folin method of Dorsey et al. (1978) using bovine serum albumin (BSA) as a standard. Because protein measurements were inconsistent with measurements of particulate nitrogen and uptake of inorganic nitrogen, BSA standard curves were scaled by measurements of particulate nitrogen using the assumptions of Dorsey et al. (1978) assuming that protein is 16% nitrogen by weight (West and Todd 1963). Growth rate of *Rhizosolenia formosa* was determined by nonlinear least-squares regression analysis of a negative exponential curve fit to changes in ambient nitrate with time. Similarly, growth rates based on increases in particulate nitrogen, particulate carbon, and chlorophyll were estimated from data points before depletion of ambient nitrate from culture medium.

Buoyancy time-course experiment. A time-course experiment was conducted to determine whether changes in chemical composition might affect cell buoyancy. Triplicate 3-L cultures of *Rhizosolenia formosa* were grown as already described, with initial nitrate adjusted to 10 μM. Samples for particulate carbon and nitrogen analysis and ambient nitrate were taken initially, at one time point before N depletion and at two time points after N depletion (at least 48 h after cells ran out of ambient nitrate). Cell buoyancy was measured before and after N depletion (described later). Just before the end of the light period, nitrate (40 μM) was added back to the culture medium, and cells were placed immediately into the dark and were left overnight to allow for incorporation of nitrate. Cell buoyancy was measured, and samples for particulate carbon and nitrogen analyses were collected the next day, once before lights-on of the normal photoperiod (0700) and once near the end of the photoperiod (1900).

Cell buoyancy was determined by placing an evenly mixed sample from each culture into one of three sinking columns (each with a total volume of 420 mL) as described by Bienfang (1981). Each column was surrounded by a water jacket through which 20° C water was circulated from a water bath (see Bienfang 1981). The buoyancy of N-replete and N-depleted cells was measured with columns illuminated from the side by two cool-white fluorescent lamps. One blue LEE gel filter (#061) separated the lamps from the columns, resulting in an irradiance of 50 μmol quanta·$m^{-2}·s^{-1}$ PAR. Buoyancy measurements on cells after readdition of nitrate were done either in the dark (for measurements prior to the photoperiod) or with columns illuminated as described earlier (for measurements at the end of the photoperiod). Cells were allowed to settle for 1 h, during which time they separated into three arbitrarily defined fractions: the top 70 mL, the middle 265 mL, and the bottom 85 mL of each column. The percentage of cells in the top, middle, and bottom fractions of each sinking column was expressed as a percentage of the total population by dividing the number of cells in each fraction by the total number of cells in the column, multiplied by 100. The concentration of cells in each fraction was determined from cell count data; the average concentration of cells from triplicate determinations was multiplied by the total volume of water in that fraction.

The sinking velocity of negatively buoyant cells was calculated from average changes in the percentage of cells in the top fraction of the columns over the 1-h incubation. Because the top fraction represented 17% of the total volume of the column, and assuming that cells are uniformly distributed after being mixed and placed into each column, 17% of the cells should be found initially in the top fraction. The change in the percentage of cells, that is, the initial percentage minus the percentage in the top fraction after the 1-h incubation, was expressed as a fraction of the initial 17%, resulting in a specific loss rate (h^{-1}). The specific loss rate was then multiplied by the height of the top fraction of the column (0.1 m) giving an estimate of sinking velocity for the negatively buoyant portion of the population. This sinking velocity is a minimum estimate, as it is a direct function of the height of the column for which the calculation is performed.

Nitrate uptake experiments. Nitrate uptake rates were determined for triplicate cultures of N-replete and N-depleted cells, both in the dark and in the light. Nitrate concentrations were determined as already described. For dark nitrate uptake experiments, disappearance of nitrate was calculated for an overnight incubation of triplicate cultures of both N-replete and N-depleted *Rhizosolenia formosa.* For N-replete cells (nitrate ≥30 μM), samples for ambient nitrate, cell counts, and particulate carbon and nitrogen were taken from exponential phase cultures at the beginning of the dark period of the normal LD cycle. From the same cultures sampled initially, identical postincubation samples were taken at the end of the dark cycle (before lights-on), approximately 10 h later. Exact incubation time was calculated as the time from lights-off in the culture room to the time of filtration of each culture. All culture containers were covered with black plastic bags to exclude light until samples were filtered. Cell counts and particulate carbon and nitrogen analyses were done as already described.

For N-depleted cells (nitrate <0.5 μM for 24 h before the experiment), samples for ambient nitrate, cell counts, and particulate carbon and nitrogen were taken from exponential phase cultures at the beginning of the dark period of the normal LD cycle. Nitrate (5 μM) was added to each culture, a sample for ambient nitrate was taken, and cells were incubated overnight (approximately 10 h). Postincubation samples for ambient nitrate, cell counts, and particulate carbon and nitrogen were taken just before the end of the dark period, and incubation time was calculated as described earlier for N-replete cells. Nitrate was never fully depleted by the end of the incubation period. Cell counts and particulate carbon and nitrogen analyses were done as already described.

For determination of nitrate uptake rates in the light, disappearance of nitrate was calculated for incubations of N-replete and N-depleted cells during the light phase of the normal LD cycle. Initial samples for ambient nitrate, chlorophyll, cell counts, and particulate carbon and nitrogen were taken just before lights-on; then identical postincubation samples were taken just before lights-off (approximately 14 h later). Nitrate was added to N-depleted cells as already described. Statistical analysis of all nitrate uptake data was done by a two-way analysis of variance (ANOVA) using Systat for Macintosh version 5.2.1.

P-I. A modified method of Lewis and Smith (1983) was used to measure photosynthesis as a function of irradiance (P-I) in cultures of exponential phase *Rhizosolenia formosa* grown as already described. Samples were inoculated with ^{14}C-bicarbonate (final activity approximately 0.01 μCi·mL^{-1}) and were dispensed into 20-mL-capacity glass scintillation vials. A range of irradiances was provided from below with four 250-Watt ENH tungsten-halogen projection lamps directed through a heat filter of circulating water and attenuated with a range of neutral density screens and a blue LEE #061 gel filter. Quantum scalar irradiance in each position was measured with a Biospherical Instruments QSL-100 4π sensor that was inserted into an empty scintillation vial for measurements. Temperature during the incubations was 20° ± 0.5° C and was kept constant with a circulating water bath. Dissolved inorganic carbon was driven off by adding 1 mL of 50% HCl and agitating the open vials for at least 4 h (usually overnight) in a fume hood. Ten milliliters of Ecolume fluor was added to each vial, each vial was mixed vigorously, and vials were allowed to sit overnight before counting with a Beckman LS 3801 Liquid Scintillation Counter. The H# method was used to correct

TABLE 1. *Chemical composition of N-replete and N-depleted* Rhizosolenia formosa *used for P-I experiments, absorption measurements, and determination of pigment composition. Values are averages ± SEs of triplicate cultures.*

Culture	Nitrate (μM)	Chlorophyll ($mg \cdot m^{-3}$)	Cell counts ($cells \cdot L^{-1}$)	POC ($mg \cdot m^{-3}$)	PON ($mg \cdot m^{-3}$)	C:N (g:g)	C:Chl (g:g)	Cell volume ($\times 10^6$ μm^3)
N-replete	28.4 ± 0.5	12.3 ± 0.2	24,300 ± 1400	1170 ± 23	200 ± 1	5.8 ± 0.1	95 ± 3.0	3.9 ± 0.1
N-depleted	0.17 ± 0	8.0 ± 1	22,105 ± 520	1200 ± 77	110 ± 4	11 ± 1	150 ± 8	4.5 ± 0.2

counts for quenching. Total CO_2 was assumed to be 2.2 mM, and no correction for isotope discrimination was made.

Triplicate P-I curves were determined for each of the triplicate cultures of both N-replete and N-depleted cells, with the exception of one culture of N-replete cells, for which only two P-I curves were determined. Nitrate-replete cultures had approximately 30 μM ambient nitrate, whereas N-depleted cultures had less than 0.5 μM nitrate for at least 48 h prior to the experiment (determined by frequent monitoring of ambient nitrate near to N depletion). The chemical composition of cultures used for P-I experiments is summarized in Table 1. Ambient nitrate, particulate carbon and nitrogen, and cell counts were determined as described earlier. Chlorophyll was determined spectrophotometrically, as described under Absorbance Measurements, later.

Results were modeled using the equation of Platt et al. (1980):

$$P^B = P_s^B(1 - e^{-\alpha I/P_s^B})(e^{(-\beta I/P_s^B)}), \quad (1)$$

where P^B is the rate of photosynthesis normalized to chlorophyll ($g\ C \cdot g\ Chl^{-1} \cdot h^{-1}$), P_s^B is the maximum rate of photosynthesis in the absence of photoinhibition ($g\ C \cdot g\ Chl^{-1} \cdot h^{-1}$), I is irradiance ($\mu mol\ quanta \cdot m^{-2} \cdot s^{-1}$), α is the initial slope of the P-I curve ($g\ C \cdot g\ Chl^{-1} \cdot h^{-1}\ (\mu mol\ quanta \cdot m^{-2} \cdot s^{-1})^{-1}$), and β is a parameter that characterizes photoinhibition ($g\ C \cdot g\ Chl^{-1} \cdot h^{-1}\ (\mu mol\ quanta \cdot m^{-2} \cdot s^{-1})^{-1}$). Curves were fit to P-I data using a least-squares nonlinear curve-fitting routine in Kaleidagraph for Macintosh version 3.0.1. Values for P_{max}^B, the realized maximum rate of photosynthesis, and I_k, the conventional index of light saturation (Talling 1957), were calculated using the method of Platt et al. (1980) from values of P_s^B, α, and β determined by curve fits. Statistical analyses of differences between N-replete and N-depleted cultures were done by Student's *t*-test for difference of means according to Sokal and Rohlf (1981).

Pigment composition. Pigment composition was determined for triplicate cultures of N-replete and N-depleted *Rhizosolenia formosa.* Cultures used were the same as those used for P-I determinations. Samples were filtered onto precombusted GF/F glass-fiber filters and were immediately placed into liquid nitrogen until analysis with a Beckman C18 reverse-phase high-performance liquid chromatograph according to Head and Horne (1993). The eluting peaks were identified by a Beckman 167 Scanning UV-Vis detector and a Beckman 157 fluorescence detector (excitation 430–450 nm emission 585–650 nm). The limit of detection of individual peaks was set to 1% of the total peak area. Pigment quantities were estimated by factors derived from calibration curves (see Head and Horne 1993). Calibration factors of carotenoids and chlorophyll *c* and standards used for verification were kindly provided by Dr. E. J. H. Head. Factors for chlorophyll *a* were determined using standards obtained from Sigma Chemical Co.

Absorbance measurements. In vivo ($a_{ph}(\lambda)$) and *in vitro* ($a_{meth}(\lambda)$) spectral absorbance was measured on triplicate cultures of N-replete and N-depleted *Rhizosolenia formosa.* Measurements were done on the same cultures used for P-I measurements and pigment determinations. Three aliquots of 250 mL were taken from each culture, and cells from each aliquot were concentrated onto GF/F glass-fiber filters. Filters were put immediately into liquid nitrogen after each filtration. Absorbance of particulate material was determined by the method of Mitchell and Kiefer (1988) using a Cary 3 Dual-Beam spectrophotometer. All scans were performed with 1 nm data acquisition from 250 to 800 nm. Filters were scanned against a blank filter that was wetted with 250 μL of 0.2 μm filtered culture medium. All GF/F filters used for this experiment were from the same lot of filters; however, variation among individual filters of the same lot made it necessary to perform an individual blank correction for each scan. Immediately after the scans, each filter was transferred to a scintillation vial containing 10 mL of cold, pure methanol and was extracted overnight at −10° C. The methanol extracts were refiltered, and the resulting extracts were then scanned against pure methanol using a 1-cm pathlength cuvette. Extracted filters were kept at room temperature and were rescanned against a blank filter that was also extracted in pure methanol. Each scan was smoothed by fitting a curve through the center of the data using a locally weighted least-squares error method in Kaleidagraph for Macintosh version 3.0.1. After being smoothed, each curve was corrected for scattering offset by subtracting from all optical densities the average optical density between 745 and 750 nm.

Optical densities were converted to *in vivo* absorbance according to Mitchell and Kiefer (1988). A pathlength amplification correction (also known as a β correction) was determined previously for the spectrophotometer used in this study (A. M. Ciotti, unpubl. data). It was verified using a separate culture of *Rhizosolenia formosa* with a range of cell concentrations comparable to those used in this study and was able to reproduce the optical density of the cells in suspension to within 5–10% (results not shown) of the area of the main absorbance peaks.

The degree of pigment packaging in N-replete and N-depleted cells (or "package effect," see Duysens 1956) was estimated by comparing the ratio of the absorbance of the methanol extracts ($a_{meth}(\lambda)$) to the *in vivo* absorbance ($a_{ph}(\lambda)$) of chlorophyll *a* (using peaks at 440 and 665 nm for the methanol extracts and 436 and 673 nm for *in vivo* absorbance) (see Kirk 1994). Higher ratios were indicative of higher pigment packaging (Kirk 1994).

Chlorophyll *a* concentrations used for P-I calculations were determined spectrophotometrically using triplicate scans from the methanol extracts and applying a specific absorption coefficient of 75 $L \cdot g^{-1} \cdot cm^{-1}$ (for chlorophyll *a* in pure methanol at 665 nm) (Marker et al. 1980).

RESULTS

Chemical composition time-course experiment. During N-replete growth, the six cultures of *Rhizosolenia formosa* reached chlorophyll concentrations of 6.7–9.1 $mg \cdot m^{-3}$ (Fig. 1A) and cell concentrations of 15,000–20,000 $cells \cdot L^{-1}$ after 11 days (Fig. 1B). Cells divided at least once after the sampling on Day 11, reaching concentrations of 25,000–30,000 $cells \cdot L^{-1}$ by Day 18 (Fig. 1B). Growth rate calculated from disappearance of nitrate from culture medium was 0.14 d^{-1}. The estimated rate of increase of *R. formosa* based on chlorophyll measurements during the N-replete phase of growth was 0.10 d^{-1}, whereas similar rates of increase of 0.15 d^{-1} and 0.10 d^{-1} were estimated from increases in particulate nitro-

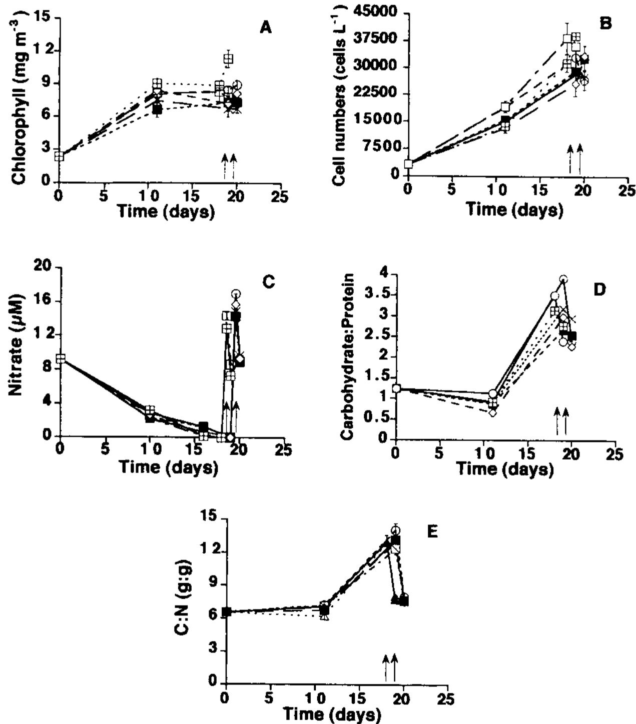

FIG. 1. Time-course measurements of A) chlorophyll, B) cell numbers, C) nitrate, D) carbohydrate : protein, and E) C:N ratio for cultures of *Rhizosolenia formosa*. Different symbols represent replicate cultures. Error bars represent SEs of triplicate measurements for each culture. Arrows indicate time at which nitrate was reintroduced into the medium.

gen and particulate carbon, respectively, over the N-replete portion of the experiment.

Depending on the culture, nitrate was depleted from the medium between Days 16 and 18 (Fig. 1C). After depletion of nitrate, cellular chemical composition changed noticeably. The ratio of C : Chl increased from an average value of 120 (g:g), whereas cells were still N-replete to approximately 200 after depletion of ambient nitrate (Table 2). Carbohydrate·mL^{-1} of culture increased and protein, on average, stayed constant during the N-depleted phase of the experiment (Table 2). Thus, carbohydrate : protein ratios increased from predepletion values of 1.0 to values close to 4.0 (Fig. 1D), whereas C:N changed from values of just above 6 (g:g) to values close to 13 (Fig. 1E).

Cultures were allowed to go between 24 and 53 h without nitrate. After each nitrate-depleted phase, cultures were reenriched with nitrate to concentrations of approximately 15 μM. Within 24 h, ambient nitrate had once again decreased by between 5.2 and 7.7 μM (Fig. 1C). In most cultures, carbohydrate·mL^{-1} culture decreased and protein·mL^{-1} culture increased after reintroduction of nitrate to the medium. As a result, the observed increases in carbohydrate : protein, C : Chl, and C:N during N-depleted growth reversed, with average values tending toward predepletion values independent of the amount of time that cells were left without nitrate (Fig. 1D, E, Table 2).

Buoyancy time-course experiment. For N-replete cells, an average of 11 ± 2% of cells were found in the top fraction (Fig. 2A), 44 ± 1% of cells were found in the middle fraction (Fig. 2B), and 44 ± 3% of cells were found in the bottom fraction of the settling column (Fig. 2C) after the 1-h incubation. After N depletion, the percentage of cells found in the top fraction decreased to 4 ± 0.3% (Fig. 2A), and the percentage cells found in the middle fraction decreased to 17 ± 4% (Fig. 2B) whereas the percentage of cells in the bottom fraction increased to 79 ± 4%. Twelve hours after readdition of N to culture medium, the percentage of cells in the top fraction of the column increased (compared to values for N-depleted cells) to 9 ± 0.6% (Fig. 2A); there were correspondingly small (but detectable) decreases in the percentage of cells in the bottom fraction to values of 73 ± 4% (Fig. 2C). Twenty-four hours after N readdition, at the end of the photoperiod, the percentage of cells in the top fraction (8 ± 1%) was still greater than for N-depleted cells, whereas the percentage of cells in the bottom fraction had decreased further to average values of 70 ± 3%. Regardless of nitrate status, visual inspection showed that essentially all cells found in the top fraction of the settling column after the 1-h incubation were found in the top 2.5 mL of the 70 mL total volume.

TABLE 2. *Chemical composition of* Rhizosolenia formosa *before and after depletion of nitrate and after reintroduction of nitrate to culture medium. N-replete cells are those sampled at the predepletion point (Day 11) in the time-course experiment. N-depleted cells were sampled after 24, 30, or 53 h without ambient nitrate. Values presented are averages ± SEs for duplicate cultures.*

	Time without nitrate	24 h	30 h	53 h	Overall average
N-replete	C : Chl (g:g)	154 ± 8	112 ± 3	93 ± 16	120 ± 17
	Carbohydrate (μg·mL^{-1})	0.6 ± 0	0.6 ± 0	0.7 ± 0.1	0.6 ± 0.1
	Protein (μg·mL^{-1})	0.7 ± 0.02	0.8 ± 0.05	0.7 ± 0.03	0.7 ± 0.03
N-depleted	C : Chl (g:g)	190 ± 11	224 ± 2.5	185 ± 14	200 ± 13
	Carbohydrate (μg·mL^{-1})	2.1 ± 0	2.4 ± 0.1	2.3 ± 0.1	2.3 ± 0.1
	Protein (μg·mL^{-1})	0.6 ± 0.1	0.8 ± 0	0.7 ± 0.02	0.7 ± 0.05
After N readdition	C : Chl (g:g)	182 ± 15	202 ± 7	168 ± 22	184 ± 13
	Carbohydrate (μg·mL^{-1})	1.8 ± 0.1	1.8 ± 0.1	2.2 ± 0.2	2 ± 0.1
	Protein (μg·mL^{-1})	0.8 ± 0.2	0.7 ± 0.1	0.9 ± 0.03	0.8 ± 0.07

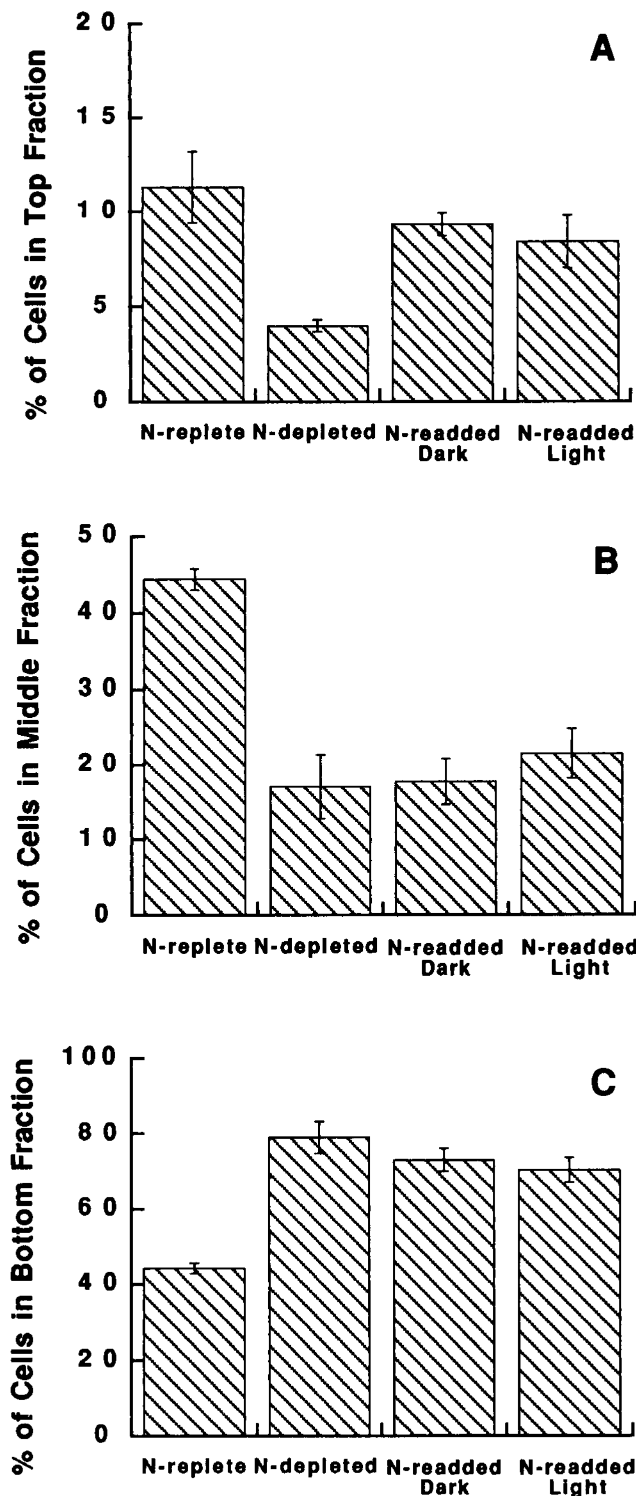

FIG. 2. Average percentages of N-replete, N-depleted, and N-resupplied (N-readded) cells found in the A) top, B) middle, and C) bottom fractions of settling columns. Note that the axes are scaled differently to reflect differences among treatments. Error bars represent SEs of triplicate measurements.

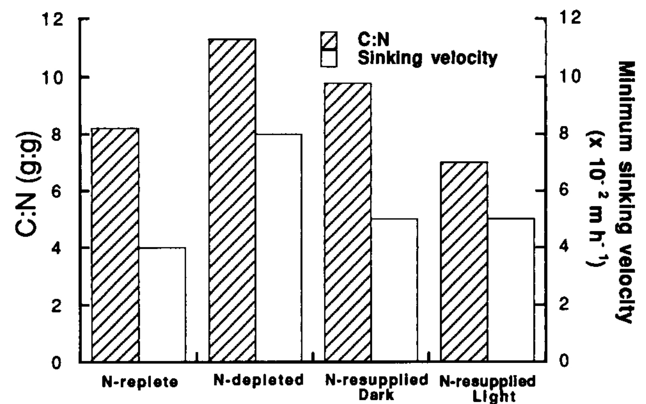

FIG. 3. Calculated minimum sinking velocities and C:N ratios of negatively buoyant *Rhizosolenia formosa* with variations in N status.

That is, the cells were concentrated against the top surface of the settling column rather than being dispersed throughout the entire top fraction.

The average minimum sinking velocity of cells sinking from the top fraction of the column was 0.04 $m \cdot h^{-1}$ when cells were N-replete, but this increased to 0.08 $m \cdot h^{-1}$ when cells became N-depleted (Fig. 3). After N resupply, sinking velocities slowed to 0.05 $m \cdot h^{-1}$ for measurements done both in the dark and in the light. Increased and decreased sinking velocities were consistent with increased and decreased C:N ratios (Fig. 3).

Nitrate uptake experiments. Biomass concentrations, chemical composition, and absolute and specific nitrate uptake rates were determined for N-replete and N-depleted *Rhizosolenia formosa* in the light and in the dark (Table 3). Average rates of nitrate uptake were always higher for N-depleted cells, and rates were slightly higher in the light than in the dark. According to a two-way ANOVA, however, there were no significant differences between any of the treatments (calculated P value for irradiance treatment = 0.367, for nutrients P = 0.308; P values >0.05 were not considered significant). This reflects the fact that variation within a group (e.g. within triplicate N-replete cultures) was greater than variation between groups (e.g. N-replete vs. N-depleted cells). The two-way ANOVA also indicated no significant interaction between nutrient and irradiance treatments (P = 0.86).

P-I experiments. Measurements of P-I were done for both N-replete (Fig. 4A) and N-depleted (Fig. 4B) cultures. Nitrate-replete cultures of *Rhizosolenia formosa* had average P^B_{max} values of 2.6 $g\ C \cdot g\ Chl^{-1} \cdot h^{-1}$ (Table 4), whereas values of P^B_{max} for N-depleted cells were significantly lower (Table 4) with an average P^B_{max} of 1.0 $g\ C \cdot g\ Chl^{-1} \cdot h^{-1}$. Values of α and I_k were both significantly lower for N-depleted cells than for N-replete cells (Table 4). Values of α averaged 0.03 $g\ C \cdot g\ Chl^{-1} \cdot h^{-1}$ ($\mu mol\ quanta \cdot m^{-2} \cdot$

TABLE 3. *Biomass concentrations, chemical composition, and rates of nitrate uptake in the light and the dark by N-replete and N-depleted* Rhizosolenia formosa. *NRLT and NDLT indicate N-replete and N-depleted cultures, respectively, where measurements were done in the light. NRDK and NDDK indicate N-replete and N-depleted cultures, respectively, where measurements were done in the dark. Values presented are averages ± SEs of measurements on triplicate cultures.*

Culture	Average cell concentration ($cells \cdot L^{-1}$)	Average POC ($mg \cdot m^{-3}$)	Average PON ($mg \cdot m^{-3}$)	C:N (g:g)	Uptake rate ($nmol \cdot cell^{-1} \cdot h^{-1}$)	Specific uptake rate (h^{-1})
NRLT	4060 ± 360	270 ± 9	44 ± 4	6.3 ± 0.3	0.013 ± 0.002	0.017 ± 0.003
NDLT	8050 ± 1700	640 ± 14	72 ± 2	8.9 ± 0.1	0.022 ± 0.012	0.03 ± 0.01
NRDK	16,900 ± 1600	730 ± 85	119 ± 16	6.1 ± 0.1	0.006 ± 0.002	0.012 ± 0.004
NDDK	6440 ± 600	580 ± 25	75 ± 2	7.7 ± 0.2	0.015 ± 0.005	0.018 ± 0.007

$s^{-1})^{-1}$ for N-replete cells and 0.016 $g\ C \cdot g\ Chl^{-1} \cdot h^{-1}$ ($\mu mol\ quanta \cdot m^{-2} \cdot s^{-1})^{-1}$ for N-depleted cells. Average values of I_k were 370 $\mu mol\ quanta \cdot m^{-2} \cdot s^{-1}$ for N-replete cells and 298 $\mu mol\ quanta \cdot m^{-2} \cdot s^{-1}$ for N-depleted cultures. The parameter of photoinhibition, β, for N-depleted cells was significantly less than that for N-replete cells, with values of 0.0002 $g\ C \cdot g\ Chl^{-1} \cdot h^{-1}$ ($\mu mol\ quanta \cdot m^{-2} \cdot s^{-1})^{-1}$ and 0.0007 $g\ C \cdot g\ Chl^{-1} \cdot h^{-1}$ ($\mu mol\ quanta \cdot m^{-2} \cdot s^{-1})^{-1}$, respectively (Table 4).

Pigment composition. The main light-harvesting pigments were chlorophyll *a*, fucoxanthin, chlorophylls $c_1 + c_2$ and c_3, and diadinoxanthin in both N-replete and N-depleted cells (Table 5). β-carotene and an unidentified xanthophyll with a single absorbance peak centered at 456 nm were also present. Diatoxanthin was detectable in two of the three N-depleted cultures (Table 5). Small amounts of phaeophorbide-like pigment and significant peaks of chlorophyllide were also detected (see Discussion). For our analyses, total concentration of chlorophyll *a* was considered to be the sum of both the chlorophyllide and the chlorophyll *a* peaks.

Concentration of chlorophyll *a* per cell was about 60% less in N-depleted cultures than in N-replete cultures (Table 5). Overall, the concentration of accessory pigments decreased in N-depleted cells, though the degree of reduction of each individual pigment varied with accessory pigment type. Chlorophyll $c_1 + c_2$, chlorophyll c_3, diadinoxanthin, and diatoxanthin showed increased concentrations relative to chlorophyll *a* in N-depleted cells, whereas fucoxanthin, β-carotene, and the unidentified xanthophyll decreased. Overall, total carotenoids : chlorophyll *a* was higher in N-depleted cells (Table 5).

Absorbance measurements. Spectral *in vivo* absorption ($a_{ph}(\lambda)$, m^{-1}) was determined for N-replete and N-depleted cultures. Chlorophyll *a*–specific spectral absorption ($a_{ph}^*(\lambda)$, $m^2 \cdot mg\ Chl^{-1}$) was lower in N-depleted cultures than in N-replete cultures (Fig. 5) and showed a clear spectral flattening in the blue region consistent with increased package effect (Duysens 1956). Also consistent with increased package effect, the ratio of $a_{meth}(\lambda)$ to $a_{ph}(\lambda)$ was 20% higher in the red and 30% higher in the blue for N-depleted cells (Table 6). In the near-ultraviolet (near-UV) region (300–400 nm), a peak in absorption centered around 330 nm in N-replete cultures was reduced noticeably in N-depleted cultures (Fig. 5).

DISCUSSION

Growth rate and chemical composition. The growth rate of *Rhizosolenia formosa* calculated from nitrate disappearance during the time-course experiment was approximately 0.14 d^{-1}, a generation time of nearly 5 d. This is slow compared to smaller diatoms (see Geider 1984, Goldman et al. 1992), yet it is near

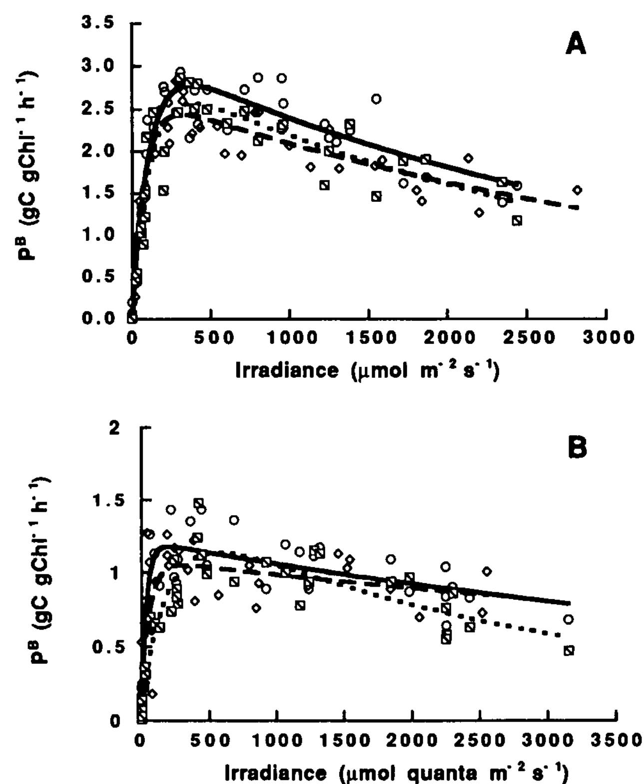

FIG. 4. Representative P-I curves for one culture each of A) N-replete and B) N-depleted *Rhizosolenia formosa.* Triplicate P-I curves were determined for each culture (shown by solid, dashed, and dotted lines). These were essentially pseudoreplicates (see Hurlbert 1984) but were indicative of the within-culture variability encountered in these experiments.

TABLE 4. *Summary of parameters determined from P-I experiments done with N-replete (NR) and N-depleted (ND)* Rhizosolenia formosa. *Values reported are averages of triplicate measurements from each culture ± SE of the mean, except for the second set of NR cultures, for which duplicate P-I curves were determined. Values for the overall averages of N-replete and N-depleted parameters are compared using a Student's t-test for comparison of means. Calculated values of the* t*-statistic are compared with the critical value of* t *(2.1318) at the 0.05 significance level and 4 degrees of freedom.*

Culture	P^B_{max} (g C·g Chl^{-1}·h^{-1})	α (g C·g Chl^{-1}·h^{-1} (μmol·m^{-2}·s^{-1})$^{-1}$)	β (g C·g Chl^{-1}·h^{-1} (μmol·m^{-2}·s^{-1})$^{-1}$)	I_k (μmol·m^{-2}·s^{-1})
NR	2.6 ± 0.08	0.029 ± 0.001	0.0009 ± 0.00007	361 ± 21
NR	2.5 ± 0.07	0.028 ± 0.007	0.0008 ± 0.0001	368 ± 65
NR	2.6 ± 0.02	0.032 ± 0.004	0.0005 ± 0.0001	378 ± 44
Overall average ± SE	2.6 ± 0.08	0.03 ± 0.002	0.0007 ± 0.0001	370 ± 20
ND	1.0 ± 0.09	0.017 ± 0.006	0.0002 ± 0.0001	259 ± 57
ND	1.2 ± 0.05	0.016 ± 0.0002	0.0002 ± 0.00002	334 ± 19
ND	0.8 ± 0.04	0.015 ± 0.003	0.00014 ± 0.0001	301 ± 51
Overall average ± SE	1.0 ± 0.06	0.016 ± 0.002	0.0002 ± 0.00004	298 ± 13
t-statistic	15.2	10.1	4.6	3.2
Significant difference	Yes	Yes	Yes	Yes

rates predicted by allometric relationships (Geider et al. 1986) and those determined previously for this diatom (Moore and Villareal 1996). These slow growth rates are also consistent with relatively high C : Chl ratios determined during this study (90–150 (g:g) for N-replete cells and near 200 (g:g) for N-depleted cells) (see Geider 1987). Cells with high C : Chl ratios tend to grow slowly because cells have to photosynthesize more at a given irradiance (than cells with lower C : Chl) to increase their relatively large amount of carbon.

In this study, C:N ratios for N-replete cells ranged from a minimum of 5.5 to a maximum of 7.2 (g:g), whereas values for N-depleted cells ranged from 9.1 to 14.0 (g:g). Both C:N and C : Chl ratios agree well with values for *Rhizosolenia* mats collected in the field. Villareal and Carpenter (1989) found C:N ratios of 7.4 ± 2.8 (by atoms) and C : Chl ratios of 150 ± 100 (g:g) in mats from the Central North Pacific Gyre. Villareal et al. (1996) divided *Rhizosolenia* mats into ascending and sinking assemblages during two cruises in the Central North Pacific Gyre and analyzed C : Chl, C:N, and carbohydrate : protein ratios. Values of C:N (mol:mol) for ascending (and presumably N-replete) mats were 7.4 and 8.1 (during two different cruises), whereas values for sinking (and presumably N-depleted) mats were between 9.6 and 11.3. Ratios of C : Chl for ascending mats were 115 ± 21 and 146 ± 24, whereas sinking mats had higher ratios (187 ± 43 and 174 ± 47).

Carbohydrate : protein ratios determined for *Rhizosolenia formosa* differ somewhat from ratios determined for field samples. Villareal et al. (1996) found that ascending mats of *Rhizosolenia* had carbohydrate : protein ratios of 0.87 and 0.62 during two different cruises. Sinking mats had ratios of 1.5 and 1.6. These values are slightly different than those of the present study (1.0 for N-replete cells and between 3 and 4 for N-depleted cells). This may be because protocols used for analysis of carbohydrate and protein differed between our study and that of Villareal et al. (1996), but the extent to which this may have influenced the overall carbohydrate : protein ratios is not known. It should be noted, however, that even though the absolute values for carbohydrate : protein ratios differ, the trend of higher ratios in sinking mats is the same as the higher values observed for N-depleted cells.

Nitrate uptake, changes in chemical composition, and cell buoyancy. The chemical composition time-course

TABLE 5. *Pigment composition and pigment ratios (Pig/Chl* a *= pigment concentration divided by estimated chlorophyll* a*) as determined by HPLC analysis. Concentrations in ng·cell^{-1}. Values are averages of triplicate measurements for N-replete and N-depleted cultures ± SE (except for diatoxanthin, where n = 2 for N-depleted). nd means not detected. Est-Chl* a *is estimated chlorophyll* a *and is equal to the sum of both chlorophyll* a *and chlorophyllide* a *in the chromatogram (see text); Unknown xanth. = unidentified xanthophyll (see text); Fucoxan. = fucoxanthin; Diadinox. = diadinoxanthin; Phaeophor. = phaeophorbide* a *plus phaeophorbide* a *–like; Total caroten. = total carotenoids.*

	Chlorophyll c_3	Chlorophyll $c_1 + c_2$	Unknown xanth.	Phaeophor.	Fucoxan.	Diadinox.	Diatoxan.
ng·cell^{-1}							
N-replete	0.046 ± 0.002	0.353 ± 0.040	0.071 ± 0.030	0.057 ± 0.003	0.127 ± 0.021	0.045 ± 0.004	nd
N-depleted	0.031 ± 0.007	0.253 ± 0.026	0.034 ± 0.022	0.017 ± 0.005	0.051 ± 0.006	0.031 ± 0.002	0.002 ± 0.000
Pig/Chl *a*							
N-replete	0.087 ± 0.002	0.274 ± 0.009	0.072 ± 0.009	0.070 ± 0.005	0.244 ± 0.045	0.086 ± 0.005	nd
N-depleted	0.092 ± 0.008	0.293 ± 0.011	0.034 ± 0.003	0.024 ± 0.014	0.160 ± 0.029	0.095 ± 0.001	0.007 ± 0.001

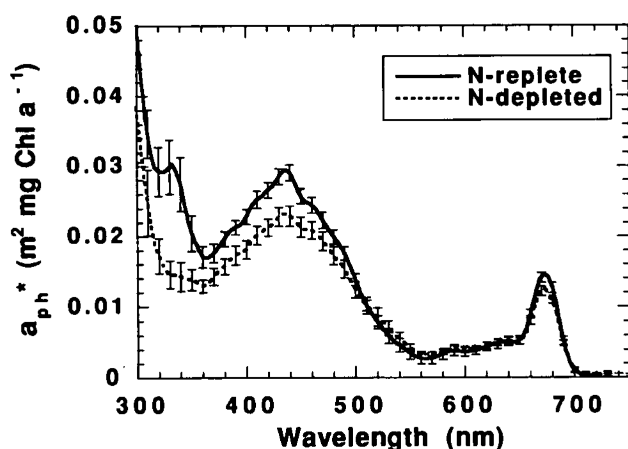

FIG. 5. Chlorophyll-specific absorption spectra for N-replete and N-depleted cultures of *Rhizosolenia formosa*. Curves are averages of nine determinations. Error bars are SDs.

experiment showed that C:N, C:Chl, and carbohydrate : protein increased noticeably upon depletion of ambient nitrate. Similarly, cell buoyancy (as indicated by percentage of cells in the top fraction of the settling column) decreased, and the sinking velocity of cells leaving the top fraction of the column increased. Changes in chemical composition indicate clearly that cells were in a state of unbalanced growth (Eppley 1981); that is, photosynthesis became uncoupled from nutrient acquisition when nitrate was depleted. As a consequence of N depletion, there can be large deviations from Redfield stoichiometry (Sakshaug and Holm-Hansen 1977, Goldman et al. 1992) and large increases in carbohydrate : protein (Richardson and Cullen 1995).

After reintroduction of nitrate to culture medium, the chemical composition of *Rhizosolenia formosa* reversed, tending toward predepletion values. All cultures took up nitrate after the period of N depletion, and most cultures showed concomitant reductions in carbohydrate and increases in protein. This is consistent with the mobilization of carbohydrate combined with the use of newly acquired nitrogen for the production of protein (Cuhel et al.

TABLE 5. *Extended.*

Diadinox. + Diatoxan.	β-carotene	Total caroten.	Est-Chl *a*
0.045 ± 0.004	0.012 ± 0.002	0.222 ± 0.028	0.524 ± 0.031
0.032 ± 0.002	0.007 ± 0.003	0.101 ± 0.003	0.330 ± 0.023
0.086 ± 0.005	0.023 ± 0.002	0.034 ± 0.004	
0.099 ± 0.003	0.021 ± 0.008	0.044 ± 0.006	

TABLE 6. *Estimation of the effect of pigment packaging in N-replete and N-depleted* Rhizosolenia formosa *sing the blue and red peaks of* in vivo *particulate absorption* (a_{ph}) *and of the absorption of methanol extracts* (a_{meth}). *Blue peaks are at 436 and 440 nm for* a_{ph} *and* a_{meth}, *respectively. Red peaks are at 673 and 665 nm for* a_{ph} *and* a_{meth}, *respectively (Rowan 1989). Values for* a_{ph} *and* a_{meth} *are averages of nine samples. A higher ratio of* a_{meth}/a_{ph} *indicates higher pigment packaging.*

	N-replete				N-depleted			
Peak	a_{ph}* (m^2·mg Chl a^{-1})	a_{ph} (m^{-1})	a_{meth} (m^{-1})	a_{meth}/a_{ph}	a_{ph}* (m^2·mg Chl a^{-1})	a_{ph} (m^{-1})	a_{meth} (m^{-1})	a_{meth}/a_{ph}
Blue	0.029	0.366	0.709	1.9	0.023	0.156	0.419	2.7
Red	0.014	0.179	0.217	1.2	0.012	0.084	0.124	1.5

1984, Cullen 1985). Two cultures, however, showed no increase in protein to account for the disappearance of nitrate from the culture medium, although there were (somewhat unexplained) decreases in cellular carbohydrate. Analytical variability is one explanation, but it is possible that nitrate was still being stored in the vacuole as intracellular nitrate and that protein synthesis had not yet "caught up" at the time of sampling.

Overall, reversion of chemical composition occurred whether cells were without nitrate for 24, 30, or 53 h. As shown by the resumption of nitrate uptake by all cultures, no significant mortality of cells was observed even though two cultures were without detectable nitrate for over 2 d. These reversions have important ecological implications, as will be discussed later. The recovery of cells from close to 3 d of nitrate depletion was also observed by Richardson and Cullen (1995) using *Thalassiosira weissflogii,* and Strickland et al. (1968) noted similar reversions in chemical composition when cultures of the dinoflagellate *Cachonina niei* grown in a deep tank were depleted of nitrogen for 5 d and then reinoculated with nitrate.

Changes in cell buoyancy were noted when nitrate was reintroduced to culture medium. The percentage of cells in the top fraction of the settling column increased and the percentage of cells in the bottom fraction decreased in response to nitrate readdition, although shifts were small percentages in either direction. The minimum sinking velocity of cells leaving the top fraction also decreased after reintroduction of nitrate to culture medium. Although they represented only a small percentage of the total population (4–11%), cells were always present in the top fraction of the sinking column after the 1-h incubation. Their presence could be explained in at least three ways. First, assuming that the entire population of cells was negatively buoyant, these cells could represent the most slowly sinking portion of the population, that is, cells that were still present because they did not sink out of the top fraction during the 1-h incubation time. It could be argued that if the experiment was left longer than 1 h then the number of cells in the top fraction would decrease

TABLE 7. *Calculation of positively, neutrally, and negatively buoyant fractions of* Rhizosolenia formosa *during buoyancy experiments using volumes of arbitrarily defined fractions (= measured) and volumes that account for the concentration of cells in the top and bottom 2.5 mL of the settling column (= adjusted). The fractions of positively and negatively buoyant cells were calculated using the following equations:* $N_{top} = [C_o \times AV] + [Fl(C_o \times BV)] - [Si(C_o \times AV)]$, $N_{mid} = [C_o \times DV] - [Si(C_o \times DV)] - [Fl(C_o \times DV)]$, *and* $N_{bott} = [C_o \times EV] - [Fl(C_o \times EV)] + [Si(C_o \times FV)]$, *where the coefficients A, B, D, E, and F represent the fraction of the total volume of the column occupied by cells in the top, middle + bottom, middle, bottom, and top + middle fractions, respectively (dimensionless values).* N_{top}, N_{mid}, *and* N_{bott} *represent the number of cells observed in each fraction of the column after the 1-h incubation,* C_o *(cells·mL*$^{-1}$*) is the initial concentration of cells in the column, V is the total volume of the column (mL), and Si and Fl are the fractions of sinking and floating cells, respectively (dimensionless numbers with values less than 1). The fraction of neutrally buoyant cells is 1 − (Fl + Si). The calculation assumes that sinking and floating cells reach the very bottom and top of the column, respectively, during the 1-h incubation. To solve for Si and Fl, only two of the preceding equations were needed.*

Parameters	A	B	D	E	F	N_{top} (cells)	N_{mid} (cells)	N_{bott} (cells)	V (mL)	C_o (cells·mL^{-1})	Fl	Si	Neutral
Measured volumes	0.17	0.83	0.63	0.2	0.8	238	772	624	420	4.0	0.017	0.25	0.73
Adjusted volumes	0.006	0.994	0.988	0.006	0.994	238	772	624	420	4.0	0.14	0.4	0.46

with time. Second, these could have been neutrally buoyant cells that had no detectable sinking rate and thus were there when the experiment started. Third, cells in the top fraction could have been positively buoyant cells that rose to the top during the 1-h incubation. Using the settling column method alone it is not possible to prove that any one or combination of the preceding reasons is correct. However, visual observations of cells in the upper layer support the contention that cells found in the top fraction of the columns after the incubation period were positively buoyant, because cells were not found dispersed through the entire fraction but were instead concentrated within the top 2.5 mL of the 70-mL upper fraction. *Rhizosolenia formosa* in culture also shows similar behavior: the majority of cells are found on the bottom of the culture vessel, but a layer of cells is always found floating at the surface (T. L. Richardson, pers. observ.). The contention that cells in the upper fraction were concentrated near the surface, and thus were positively buoyant, is also supported by an algebraic calculation of the positively, neutrally, and negatively buoyant fractions (for equations and parameters see Table 7). Using measured volumes of the top, middle, and bottom fractions of the sinking columns (70, 265, and 85 mL, respectively) and the number of cells found in each of these fractions after the 1-h incubation, two of the equations shown in Table 7 were solved simultaneously for the fraction of cells positively and negatively buoyant. This resulted in values of 1.7% for positively buoyant cells and 25% for negatively buoyant cells. By subtraction, the proportion of neutrally buoyant cells would then be 73.3%. These values are substantially different from those obtained during buoyancy experiments. However, calculations based on measured volumes of each fraction assume that cells are distributed evenly throughout the entire volume of the top and bottom fractions. Because visual examination showed that the cells found in the top and bottom fractions were concentrated against the top and bottom of the column in volumes of approximately 2.5 mL rather than the arbitrarily defined fractions of the experimental apparatus, the calculation was repeated with the volumes of each fraction adjusted accordingly. Values for the positively buoyant and negatively buoyant fractions were 14 and 40%, respectively, making the neutrally buoyant fraction 46%. These values are very similar to the proportions of cells found in the three layers during buoyancy experiments on N-replete cells (11, 44, and 44% for cells in the top, middle, and bottom layers; thus, from hereafter, the fraction of cells in the top, middle, and bottom fractions of the column will be referred to as positively, neutrally, and negatively buoyant, respectively.

In general, the percentage of positively buoyant cells (for either N-replete or N-depleted cultures) determined during this study is lower than values determined previously for *Rhizosolenia* (Moore and Villareal 1996). This may be due to decreases in cell size. The extent to which a diatom can control its buoyancy is partly determined by the ratio of total cell volume to internal cell volume. Assuming equal thickness of cells walls, smaller cells will have smaller capacities for changing internal cell density. Moore and Villareal (1996) found that 40% of cells 129 μm in diameter were positively buoyant at an irradiance of 50 μmol quanta·m^{-2}·s^{-1} (used for experiments in the present study), but this number decreased to 0% for cells 117 μm in diameter. The cultures used for the present study are the same strain as that of Moore and Villareal (1996); however, the cultures are now older and have decreased in size as the culture has aged and attempts at inducing auxospore formation have not been successful. Measurements done on cells during P-I experiments showed that these cells had a maximum diameter of 90 μm, consistent with the expected decrease in percentage of positively buoyant cells compared to 129-μm-diameter cells, although 11% of the N-replete cells was still positively buoyant even at this relatively small cell size. The percentage of positively buoyant cells in natural populations may be much higher (as high as 40%), as *Rhizosolenia* will be reproducing naturally

and may not experience reductions in size with successive divisions. High concentrations of positively buoyant cells in nature have been observed by Barber et al. (1994) and Yoder et al. (1994), who described concentrations of cells in a convergence region in the Equatorial Pacific; concentrations of cells in convergence regions requires cells to be positively buoyant. Use of a larger species of *Rhizosolenia* (e.g. *R. castracanei, R. acuminata*) may also have resulted in larger percentages of positively buoyant cells (see Moore and Villareal 1996); however, cultures of these organisms were not available at the time this work was initiated. Even though the percentages of positively buoyant cells were lower than what would be expected in a natural population, consideration of the trends in the data show that both the direction of buoyancy changes in response to nutrient deprivation and resupply, and the time scales of these buoyancy changes, are consistent with vertical migrations: the percentage of negatively buoyant cells increased when cells ran out of nitrate, and more cells became positively buoyant when reenriched with nitrate even within 12 h of nitrate readdition. It is these relative changes in buoyancy that are important within the context of vertical migration.

The results of time-course experiments showed clearly that the physiology of *Rhizosolenia formosa* is extremely flexible, an attribute that is consistent with vertical migrations in nature. *Rhizosolenia formosa* could maintain unbalanced growth for at least 53 h without nitrate. Because cells are expected to spend some portion of their migration cycle in N-depleted waters and because internal nitrogen stores will eventually run out, *Rhizosolenia* must be capable of accumulating large amounts (perhaps one full cell quota or more) of carbon during this N-depleted, but illuminated, phase of migration. Cellular capacity for changes in chemical composition will be determined by the extent to which photosynthate can be stored for later use after nutrient acquisition. As shown by buoyancy experiments, the nutritional status of the cell also affects cell buoyancy. The percentage of positively buoyant cells decreased markedly upon N depletion, possibly due, in part, to increases in cellular carbohydrate. Carbohydrate has been shown to play a significant role in the regulation of buoyancy of blue-green algae such as *Trichodesmium* (Romans et al. 1994, see also review by Oliver 1994) because of its fairly high density (1600 $kg \cdot m^{-3}$). Presumably, there is an upper limit to how much carbohydrate the cell can accumulate before its buoyancy reverts from a state of positive or neutral buoyancy to one of negative buoyancy (see also Moore and Villareal 1996), although the exact mechanism for buoyancy control in *Rhizosolenia* has not yet been identified.

Reversion to negative buoyancy would remove cells from illuminated, N-depleted waters and would allow them to sink to deep nutrient pools. We have shown that *Rhizosolenia formosa* is capable of resuming uptake of nitrate upon returning to N-replete waters (also see discussion of nitrate uptake experiments, later). In the open ocean, nitrate often does not become detectable until well below the euphotic zone (see Hayward 1991), so it is necessary for *R. formosa* to have the ability to take up nitrate in the dark if migrations are to be ecologically useful. Buoyancy experiments also showed that N-depleted cells can revert to positive buoyancy when reenriched with nitrate in the dark. The resumption of nitrate uptake after prolonged unbalanced growth, the ability to take up nitrate in the dark, and the reversal of buoyancy upon N repletion are all consistent with, and critical to, the ability of *Rhizosolenia* to migrate vertically in nature.

Nitrate uptake measurements verified that both N-replete and N-depleted cells can take up nitrate in the dark and determined that rates of both light and dark uptake were comparable. Calculation of an overall average from nitrate uptake data yielded an N-specific uptake rate of 0.02 h^{-1}. Previous work has shown that prior N starvation often increases the rate of (or induces) nitrate uptake in phytoplankton (e.g. Harrison 1976, see also Syrett 1981). However, the variability in our results made it impossible to detect the effects of treatments such as N depletion on uptake rate.

Moore and Villareal (1996) provided the first evidence that *Rhizosolenia formosa* could take up nitrate in the dark. During the dark period of a 24-h time-course experiment, intracellular nitrate of *R. formosa* increased by at least 6 mM over a 6-h time period. Dark nitrate uptake has also been observed in other diatoms and in other vertically migrating phytoplankton (Harrison 1976, Bhovichitra and Swift 1977, Syrett 1981, Cullen 1985). Nocturnal uptake of nitrate is a well-known phenomenon in coastal dinoflagellates and may contribute to the formation of dinoflagellate blooms (Eppley and Harrison 1975, Harrison 1976, Cullen 1985).

Nitrogen-specific uptake rates (0.02 h^{-1}, which converts to 0.48 d^{-1}) were higher than estimated N-specific growth rates (0.15 d^{-1}). This indicates that over short time scales cells have the ability to take up nitrate in excess of what is necessary for immediate cell division. Nitrate can be stored in the relatively large vacuole of *Rhizosolenia* (see Villareal and Lipschultz 1995, Moore and Villareal 1996); from there it can be mobilized and combined with carbon acquired during photosynthesis in surface waters to make proteins (see also Cullen 1985). The enhanced ability of a cell to take up nitrogen in excess of its immediate requirements has been demonstrated for other marine phytoplankton (McCarthy and Goldman 1979, Glibert and Goldman 1981, Horrigan and McCarthy 1981, 1982). Horrigan and McCarthy (1982) showed that *Thalassiosira pseudonana* and *Skeletonema costatum* had enhanced uptake of ammonium and urea even for nutrient-saturated cultures. Enhanced uptake allows phytoplankton to

exploit the episodic pulses of nutrients often encountered in the marine environment, thereby satisfying their daily quota of nitrogen (see also Glover et al. 1988). For the case of migrating *Rhizosolenia*, the "patchiness" of its nutrient environment is in the form of intermittent exposures to deep pools of nutrients.

Photosynthesis and absorption. The photosynthetic physiology of *Rhizosolenia formosa* and possible adaptations to vertical migration were examined through measurements of P-I and *in vivo* and *in vitro* absorption. Overall, values for P_{max} determined here for N-replete *R. formosa* (average = 2.6 g C·g Chl^{-1}·h^{-1}) are in good agreement with production rates calculated previously for cultures of *R. formosa* in the laboratory and with ^{14}C determinations of primary productivity of mats collected in the field. Moore and Villareal (1996) calculated an apparent production rate for nutrient-replete *R. formosa* of 2.9 g C·g Chl^{-1}·h^{-1} from experimentally determined growth rates and C : Chl ratios. In the Equatorial Pacific, Yoder et al. (1994) determined a daily production rate of 68 g C·g Chl^{-1}·d^{-1}, which converts to approximately 5.7 g C·g Chl^{-1}·h^{-1} assuming a 12-h light period. Alldredge and Silver (1982) measured the primary productivity of mats of *Rhizosolenia castracanei* and *R. imbricata* var. *shrubsolei* in the boundary waters of the Central North Pacific Gyre and found average maximal photosynthetic rates of 5.8 g C·g Chl^{-1}·h^{-1}. More recent estimates of the primary productivity of *Rhizosolenia* have been done by measuring O_2 evolution. Villareal et al. (1996) converted O_2 evolution rates of mats of *Rhizosolenia* to C fixation rates of 4.7 g C·g Chl^{-1}·h^{-1} for sinking mats and 7.3 g C·g Chl^{-1}·h^{-1} for floating mats.

Photosynthetic rates determined for N-replete cells generally agree with those determined in the field, but rates for N-depleted cells from this study are generally lower than field values. It is possible that the growth temperature of *Rhizosolenia formosa* in culture has affected its maximal photosynthetic rate when compared to natural populations. Li and Morris (1982) looked at the effects of growth temperature on P_{max} of *Phaeodactylum tricornutum* and found that P_{max} increased as growth temperature increased with a Q_{10} of about 2.6. Thus, we would not expect *R. formosa* grown in the laboratory at 20° C to have exactly the same P_{max} as mats of *Rhizosolenia* collected from 28°–30° C surface waters in the open ocean. Also, *Rhizosolenia* mats collected in the field are assemblages of various species and therefore should not necessarily reflect the P_{max} of any particular species. Finally, *R. formosa* was grown in the laboratory at 50 μmol quanta·m^{-2}·s^{-1}; thus, we would expect P_{max} to be lower because the cells have adapted to this relatively low irradiance (see Falkowski 1980).

Values of P^B_{max} and α measured during this study for N-depleted *Rhizosolenia formosa* were significantly lower than those determined for N-replete cells. Similarly, Villareal and Lipschultz (1995) found decreases in both P_{max} and α during progressive depletion of internal nutrient stores of the vertically migrating diatom *Ethmodiscus*. Decreases in P_{max} and α in N-depleted cells were likely due to the dependence of cellular physiology on available nitrogen; reduction in available nitrogen will decrease the production of chlorophyll and accessory pigments (Zevenboom 1986, Sosik and Mitchell 1991, Latasa and Berdalet 1994). The effects of nutrient depletion on phytoplankton photosynthesis have been examined previously (e.g. Yentsch and Lee 1966, Welschmeyer and Lorenzen 1981, Cleveland and Perry 1987). Welschmeyer and Lorenzen (1981) found that both α and the quantum yield of photosynthesis decreased as cultures of *Thalassiosira pseudonana* entered stationary phase (and thus N-depletion). Cleveland and Perry (1987) found that decreases in the cell nitrogen quota of *Chaetoceros gracilis* resulted in decreased quantum yield and increased $a_{ph}*$ (although this increase in $a_{ph}*$ is not consistent with our results, as is discussed later). Because α (mg C·mg Chl^{-1}·h^{-1} (μmol quanta·m^{-2}·s^{-1})$^{-1}$) is the product of quantum yield (mg C·μmol $quanta^{-1}$) and $a_{ph}*$ (m^2·mg Chl^{-1}), we can approximate a value for quantum yield based on our observed values of α and of $a_{ph}*$ integrated over the visible range (400–700 nm). Using this approximation, we calculated quantum yield for N-replete cells to be 0.00016 mg C·μmol $photons^{-1}$ whereas the value of this parameter for N-depleted cells was 0.00012 mg C·μmol $photons^{-1}$. Based on these calculations, the decreases in α observed in this study may be due to decreases in both $a_{ph}*$ and in quantum yield.

Specific chlorophyll *a* absorption ($a_{ph}*(\lambda)$) decreased in N-depleted cells. This result contradicted the expected increase in $a_{ph}*(\lambda)$, as previous studies have shown that the decrease in pigmentation induced by N depletion often leads to reduced packing of cell pigments, which would result in an increase in $a_{ph}*(\lambda)$ (Cleveland and Perry 1987, Sosik and Mitchell 1991). However, both the spectral shape of the $a_{ph}*(\lambda)$ and the ratio between *in vitro* and *in vivo* absorption observed in this study indicated that N-depleted cells showed a higher degree of pigment packaging than N-replete cells. The reason for this discrepancy cannot be resolved conclusively, but it is possible that increased packaging in N-depleted cells could be a result of internal rearrangement of chloroplasts, often referred to as systrophe (Jenkin 1937). We examined N-replete and N-depleted cells under a light microscope and found that chloroplasts in N-replete cells were present as individual units that flowed "single file" during vigorous cytoplasmic streaming. In contrast, chloroplasts of N-depleted cells existed as clusters that moved in packets during slow, but detectable, cytoplasmic streaming. Stephens (1995) also showed rearrangements in the large dinoflagellate *Pyrocystis lunula*

upon exposure to high irradiance. Stress by N depletion can be considered equivalent to stress by exposure to high irradiance using the source/sink argument outlined by Cullen (1985).

Average values for the saturation parameter, I_k, determined during P-I experiments were 370 μmol quanta·m^{-2}·s^{-1} for N-replete cells and 298 μmol quanta·m^{-2}·s^{-1} for N-depleted cells. Because there is a direct dependence of I_k on P_{max} (Henley 1993) and the rate of the dark reactions of photosynthesis decrease during N depletion, it was not surprising that I_k shifted to lower irradiances (see Yentsch and Lee 1966). It is likely that I_k has been influenced by growth irradiance (50 μmol quanta·m^{-2}·s^{-1}) (see Kana and Glibert 1987a); however, values of I_k determined by Villareal et al. (1996) for field samples of *Rhizosolenia* generally agree well with these data: sinking mats had $I_k = 140 \pm 22$ μmol quanta·m^{-2}·s^{-1} whereas I_k for floating mats was 241 ± 36 μmol quanta·m^{-2}·s^{-1}.

The parameter of photoinhibition, β, determined during this study ranged from an average of 0.0007 g C·g Chl^{-1}·h^{-1} (μmol quanta·m^{-2}·s^{-1})$^{-1}$ for N-replete cells to 0.0002 g C·g Chl^{-1}·h^{-1} (μmol quanta·m^{-2}·s^{-1})$^{-1}$ for N-depleted cultures. Photoinhibition is defined as the depression of photosynthesis at supra-optimal light (Neale 1987). It is tempting to conclude, therefore, that a low value of β is indicative of a low degree of photoinhibition. However, photoinhibition is a truly complicated phenomenon. This makes it difficult to use values for β generated in the laboratory to make any concrete conclusions as to the potential for photoinhibition of cells in nature. The parameter of photoinhibition for N-depleted cells was significantly lower than that for N-replete cells. One possible reason for this is because N-depleted cells may lack the photoprotective mechanism that deactivates photosystem II reaction centers, thereby reducing short-term photosynthesis at high irradiance but promoting survival (see Neale 1987, Henley 1993). This would be manifested as a lower β, but the lack of photoprotection means that cells would not survive indefinitely.

In general, measurements I_k suggest that this diatom is moderately high-light–adapted even when grown at 50 μmol quanta·m^{-2}·s^{-1}. Some degree of high-light adaptation would be useful to cells migrating to high-irradiance surface waters, but because cells move vertically throughout the euphotic zone and are not confined to a particular depth stratum maximal photosynthetic performance at high irradiance is not necessary for their survival. Both the shape of the P-I curve and the high ratio of I_k to growth irradiance suggest that the photosynthetic physiology of *Rhizosolenia formosa* is flexible and that cells may be able to adapt to changes in irradiance as they move vertically through the water column (Sakshaug et al. 1987). Cells also show evidence of "excess photosynthetic capacity" (Kana and Glibert 1987b). Using measurements of particulate carbon taken during P-I experiments, values of P^B_{max} normalized to carbon were calculated to be 0.026 h^{-1} or 0.3 d^{-1}. This value is 3 times the estimated C-specific growth rate of this diatom, indicating that the photosynthetic capabilities of this organism are flexible enough to respond to changing irradiance and have the capability to "shift up" its photosynthetic rate in response to episodic exposures to high irradiance. The assimilation of carbon in excess of immediate metabolic requirements in high irradiance surface waters was observed also by Rivkin et al. (1984) in a study of migrations of the open-ocean dinoflagellates *Pyrocystis noctiluca* and *Pyrocystis fusiformis.*

Pigment composition. Characterization of the photosynthetic physiology of *Rhizosolenia formosa* from P-I experiments and absorbance measurements was supplemented with measurements of pigment composition by high-performance liquid chromatography (HPLC). Although filters used for HPLC analysis were placed into liquid nitrogen immediately after filtration, hydrolysis of chlorophyll *a* could not be avoided, as shown by the large peak of chlorophyllide *a* in the chromatograms. We believe that this is an artifact of the analysis. Jeffrey and Hallegraeff (1987) showed that the process of filtration can promote various degrees of chlorophyll *a* hydrolysis via the enzyme chlorophyllase, which results in the production of chlorophyllide *a*. Because chlorophyll *a* and chlorophyllide *a* have the same absorption characteristics (Rowan 1989), it was assumed that total chlorophyll *a* is the sum of the chlorophyll *a* and chlorophyllide *a* peaks. There was good agreement between total chlorophyll *a* estimated in this way and total chlorophyll *a* as determined by spectrophotometric analysis of methanol extracts.

Pigment composition was normalized to cell numbers. It is important to note, however, that measurements of cell volumes of N-depleted cultures were significantly larger (by 14%) than those of N-replete cells. In this study, N depletion decreased both chlorophyll *a* and accessory pigments. As well, the ratios between individual accessory pigments and total chlorophyll *a* varied depending on the pigment. The relationship between individual accessory pigments and total chlorophyll *a* during N depletion has been shown previously to be variable (Geider et al. 1993 and references therein). The overall ratio of carotenoids to chlorophyll *a* was higher in N-depleted cultures, mostly due to a proportional increase in diadinoxanthin and diatoxanthin. Diadinoxanthin and diatoxanthin are usually involved in photoprotection rather than for light harvesting (Hager 1980). Interconversions between diadinoxanthin and diatoxanthin are known as the xanthophyll cycle (Rowan 1989). These interconversions can be rapid and have been associated with fast and reversible changes in *in vivo* fluorescence in both dinoflagellates (Demers et al. 1991) and dia-

toms (Sakshaug et al. 1987). Latasa and Berdalet (1994) found that diatoxanthin accumulation can occur under both nitrogen and phosphorus depletion and suggested that an increase in diatoxanthin could contribute to increased shading within the chloroplast. Geider et al. (1993) found that accumulation of these two xanthophylls in *Phaeodactylum tricornutum* under phosphate starvation offered extra protection from photoinhibition. Because our samples were kept for approximately 2 h in the dark before filtration, and because diatoxanthin rapidly converts to diadinoxanthin in the dark, we will consider that their role in photoprotection is coupled. Thus, it seems that the incremental increase in the ratio of carotenoids to chlorophyll *a*, due to increases in diatoxanthin and diadinoxanthin, found for N-depleted *Rhizosolenia formosa* could be a physiological response that would be advantageous as protection against photoinhibition.

Particulate *in vivo* absorption measurements showed a peak in absorbance at 332 nm in N-replete cells that was missing from N-depleted cultures. No pigment identified by the HPLC method had absorbance peaks in that range. It is possible that this compound is a mycosporine-like amino acid (MAA), which has been shown to occur at similar wavelengths (336 nm) in several algal species including mat-forming diatoms (Karentz et al. 1991). The possible presence of MAAs in N-replete cells might afford direct protection against photoinhibition by UV irradiance; however, N-replete cells are more often found at depths where UV irradiance is minimal. Though the MAA peak was absent in N-depleted cells, possible photoprotective mechanisms include 1) a high ratio of xanthophylls to chlorophyll *a* and 2) higher packaging of chlorophyll *a*. Both these mechanisms would afford some degree of protection from photoinhibition.

Potential contributions to new production and biogeochemical cycling. We have shown that the physiological and optical characteristics of *Rhizosolenia formosa* are consistent with, and sometimes advantageous to, the migration of this diatom in nature. Their capacity for unbalanced growth and for return to balanced growth as shown by changes in chemical composition is compatible with spending prolonged periods of time in N-depleted waters and with the return to N-rich deep water. They can exploit deep sources of nutrients and can take up nitrate in excess of their daily requirements. Finally, their photosynthetic physiology indicates that they are well adapted to the continually changing irradiances encountered during vertical migrations, and their pigment composition affords some degree of protection against photoinhibition in high irradiance surface waters. Let us now consider the possible contribution of these migrations to new and primary production.

It is becoming better recognized that large diatoms like *Rhizosolenia* are ubiquitous in oligotrophic regions of the world's oceans (Goldman et al. 1992, Goldman 1993) and that they may make substantial contributions to global primary production and new production and, hence, to the export of carbon from the euphotic zone (Michaels and Silver 1988, Sancetta et al. 1991, Goldman et al. 1992, Goldman 1993). These contributions have been largely overlooked because the numerical abundance of large phytoplankton has likely been underestimated by traditional sampling with small-volume bottles (Alldredge and Silver 1982, Goldman 1988, 1993, Carpenter and Romans 1991, Sancetta et al. 1991) and by traditional sampling strategies that miss episodic blooms of large diatoms (Goldman 1993).

If cells are sufficiently abundant, the ability of *Rhizosolenia* to migrate vertically (Villareal et al. 1993) may have important implications for estimates of global primary productivity and global new production. Biological transport of nitrogen to the euphotic zone by migrations of *Rhizosolenia* represents a novel form of new production (Richardson and Cullen 1995, Moore and Villareal 1996). We have shown that *Rhizosolenia formosa* can take up nitrate in the dark, and, because this dark uptake will occur without concurrent uptake of carbon, cells will instead combine nitrate acquired at depth with carbon acquired in surface waters. That is, the processes of nitrate uptake and carbon uptake become uncoupled in space and time (Cullen 1985, Fraga et al. 1992). Fraga et al. (1992) showed uncoupled movements of carbon and nitrogen by their measurements of Broecker's parameters (Broecker 1974) during a red tide of the dinoflagellate *Gymnodinium catenatum.* Broecker's parameters ("NO," "CO," and "PO") are intended to be conservative properties that characterize water masses. If biochemical reactions in the water mass are occurring in Redfield proportions, each of Broecker's parameters will be constant with depth. Fraga et al. (1992) found distinct vertical differences in Broecker's parameters during the red tide, indicating that biochemical reactions were not occurring in Redfield proportions. They attributed these departures from Redfield stoichiometry to vertical migrations by the dinoflagellates whereby carbohydrate synthesis at the surface and nutrient uptake at depth resulted in a carbon deficit near the surface and a nitrogen deficit below. Nitrogen deficits below the euphotic zone may also be exhibited as negative preformed nitrate signals. Emerson and Hayward (1995) cited nitrate uptake by migrating diatom mats as a possible explanation for the layer of negative preformed nitrate observed in the subtropical North Pacific Ocean.

Input of nitrogen to the euphotic zone during migrations of *Rhizosolenia* is not coupled to the input of carbon, as is the case for physically transported dissolved nutrients (Eppley and Peterson 1979). Because photosynthesis occurring in surface waters requires a carbon source, and because stoichiometric equivalents of carbon are not brought to the surface by cells in coupled transport with nitrogen, new pro-

duction resulting from vertical migrations could result in the net removal of carbon from oceanic surface waters that, in turn, would affect the air–sea exchange of carbon dioxide (see also Richardson and Cullen 1995).

Calculation of the potential contribution of *Rhizosolenia* to new and primary production relies on having robust estimates of nitrate and carbon uptake; thus, differences in photosynthetic rates between N-replete and N-depleted cells should be considered in estimates of total carbon consumption during migrations. Because *Rhizosolenia* may be N-depleted during part of the migration cycle, carbon uptake would be overestimated if only N-replete rates are used. Determination of rates of carbon and nitrate uptake also help determine the time scale of the migration cycle, which will in part determine the time scale of the flux of nitrogen to the surface. The exact contribution of *Rhizosolenia* to new and primary production relies on robust estimates of cell abundance, but the geographical coverage of abundance estimates is not yet sufficient to determine the overall biogeochemical importance of *Rhizosolenia*.

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MACROALGAL RESPONSES TO NITROGEN SOURCE AND AVAILABILITY: AMINO ACID METABOLIC PROFILING AS A BIOINDICATOR USING *GRACILARIA EDULIS* (RHODOPHYTA)[1]

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ABSTRACT

The use of macroalgae as biological indicators of dissolved nutrient source and availability in the water column was investigated. Total tissue nitrogen (N) content, pigments, and amino acids of the red alga Gracilaria edulis *(Gmelin) Silva were compared to N source and availability in laboratory and field incubations to identify responses that would serve as bioindicators of N. Field-collected algae were preincubated (6–8 wk) in low-nutrient seawater to deplete their luxury reserves of N. Incubations were then conducted for periods of 3 d in laboratory aquaria (N-spiked seawater) and in the field using macroalgal incubation chambers. After incubation in different N sources (NH_4^+, NO_3^-, and urea) in laboratory aquaria, photosynthetic pigments (phycoerythrin and chlorophyll* a*) and total tissue N increased, in response to increasing [NH_4^+] but not to [NO_3^-] or [urea]. Incubation in two ranges of [NH_4^+], one from 0 to 80 μM and the other from 0 to 800 μM, in laboratory aquaria increased the total amino acid pool. Citrulline concentrations were the most responsive to [NH_4^+] (r^2 = 0.84). NH_4^+ source treatments produced increases in citrulline, phenylala-*

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