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Buoyancy Regulation and the Potential for Vertical Migration in the Oceanic Cyanobacterium *Trichodesmium*

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Abstract

Diel protein and carbohydrate content in *Trichodesmium thiebautii* was measured to evaluate the relationship to buoyancy status. Carbohydrate:protein ratio was the best predictor of buoyancy and fit a cosine curve with increasing values during the day and decreasing values at night in cycles that paralleled observed diel buoyancy patterns. This ratio also increased in short-term experiments as a function of light and increased in parallel with decreasing positive buoyancy. We used changes in this ratio to estimate the potential for vertical migration. Whereas limited vertical excursions in the upper 70 m are possible, deeper migrations appear unlikely unless respiration rates decrease significantly. N:P ratios in sinking and floating colonies were used to test for the P acquisition at depth (vertical migration). We noted that pooled N:P ratios were not significantly different between sinking and ascending colonies (N:P = 65.6 and 66.3, respectively) collected along the northern Australian coast, much like published results from north of Hawaii. Highly significant differences ($p < 0.0001$) were observed in the western Gulf of Mexico between sinking and ascending colonies (N:P = 87.0 and 43.5, respectively) and provide the best direct evidence to date of vertical migration for P acquisition. Our physiological data on compositional changes during buoyancy reversals suggest a complex relationship between light and nutrients. It appears likely that light and P metabolism interact to regulate the vertical extent of migrations, with deep vertical migration regulated by P metabolism superimposed on a mixed-layer light-driven migration. The variability in N:P ratios suggests that care should be taken in assuming buoyancy reversals always result in P acquisition in this oceanic cyanobacterium.

Introduction

Trichodesmium is a widespread tropical–temperate cyanobacterium genus that accounts for the majority of nitrogen fixation in oceanic systems [3, 4, 24] and at times dominates the phytoplankton community [7, 8]. In most waters of the Sargasso Sea, the Caribbean Sea, and the North Pacific gyre, T. *thiebautii* is the most abundant species with lesser amounts of *T. erythraeum* [3, 6]. There are at least five species in the genus, but field identification

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is often difficult when using gross colony morphology [11].

Like many limnetic cyanobacteria, *Trichodesmium* possesses gas vesicles and forms strongly positively buoyant colonies capable of ascending at several m hr^{-1} [42]. As a result, *Trichodesmium* can accumulate in pronounced surface populations, some extending over thousands of kilometers [2, 5]. Subsurface maxima of *T. thiebautii* also occur at 15–25 m in the Caribbean during stratified periods [7], indicating that the colonies are exercising some form of buoyancy control sufficient to overcome mixed-layer turbulence in that region. In *T. thiebautii*, the general population becomes more negatively buoyant during the day and more positively buoyant at night in diel cycles clearly related to light availability [37]. Models indicate that the observed subsurface maxima are highly dynamic and probably result from numerous individual colonies moving in and out the maximum region rather than the static adoption of a preferred depth [20].

Carbohydrate ballasting is explicit in the Kromkamp and Walsby [19, 20] model and is accepted both as the general mechanism of buoyancy regulation in cyanobacteria [27] and specifically for *Trichodesmium* [30, 37]. *Trichodesmium* gas vesicles can withstand pressures of 12–37 bar depending on the species, and turgor pressure collapse is not possible as a buoyancy regulation mechanism in this genus [42].

Ballast-mediated buoyancy reversals have long been associated with limited vertical migration in limnetic species [13, 19, 29], a possibility that would have important implications if *Trichodesmium* were capable of it as well. Karl et al. [12] inferred *Trichodesmium* vertical migration from phosphorus budgets in the North Pacific gyre. In this model, *Trichodesmium* migrates to the phosphocline, stores P, and returns to the surface for photosynthesis and N_2 fixation. Unless N is being taken up stoichiometrically with P at depth, one would expect N:P ratios in sinking and ascending *Trichodesmium* to be significantly different.

This hypothesis has been difficult to verify. The existing N:P data [22] are ambiguous and lack statistical significance while the reported physiological evidence is not migration-specific. Unlike vertically migrating diatoms [36] where $\delta^{15}N$ signature served as a marker for deep N acquisition, there is no convenient isotopic tracer that can be used as a marker for deep P pools. An integrated approach using chemical composition data, internal nutrient pools, and enzyme activity used to validate deep vertical migration in the diatoms *Rhizosolenia* and *Ethmodiscus* [38, 39] has not been applied to *Trichodesmium*. However, models suggest excursions to 150–200 m may be possible for *Trichodesmium*, but are based on rate constants from limnetic species and may be limited in utility [20]. The question of vertical migration is a fundamental one for *Trichodesmium*, both for its autecology and for understanding global nutrient cycles. Geochemical markers suggest rates of N_2 fixation much higher than directly measured rates [10, 26]. P concentrations in the surface ocean are often at or below the detection limits, and the source of P for supporting N_2 fixation is unclear [25].

Our report identifies diel changes in particulate composition that are consistent with carbohydrate ballasting in *Trichodesmium* as a means of buoyancy regulation and that covary with buoyancy status. We use observed changes in carbohydrate: protein ratios from surface populations to constraint the extent of vertical excursions and use N:P ratios to evaluate the potential for deep vertical migration to exploit P pools in several oceanic regions.

Methods

Trichodesmium spp. were collected over a period of several years from distinct projects in the Caribbean Sea, the western Gulf of Mexico, and the Indo-Pacific area north of Australia. While *T. thiebautii* dominated in all areas, the difficulty in distinguishing other *Trichodesmium* spp. from *T. thiebautii* based solely on colonial morphology makes it unlikely it was a pure sample. *Trichodesmium erythraeum* is distinctive and was readily sorted out of the sample. We characterize the responses as belonging to *T. thiebautii*, but acknowledge that other species may have been present. Caribbean *Trichodesmium* were collected with a 1 m diameter (254 μm mesh) net towed for 10–20 min at 0.8–1.5 knots in Sept., 1991 (cruise CI-9111, R/V *Columbus Iselin*). Collection depth was usually 15 m, but occasionally was 5–10 m. Several diel samplings were conducted while on station within 2 km of a given location or were collected at various times as the ship was in transit (Fig. 1). Collections were made in the Indian Ocean northeast of Broome, the Timor Sea, the Arafura Sea, and the Coral Sea north of Townsville, Australia, in Nov. 1999 (Fig. 1) using opening/closing 50-cm nets (153 μm mesh) sampling strata from 0 to 100 m. The R. V. *Longhorn* cruise in July 2000 began and terminated in Pt. Aransas, Texas (Fig. 1) and used the same nets.

At each collection site, tuft-shaped *Trichodesmium thiebautii* colonies were immediately picked from the net tow and placed into GFF glass-fiber-filtered seawater. Buoyancy determinations

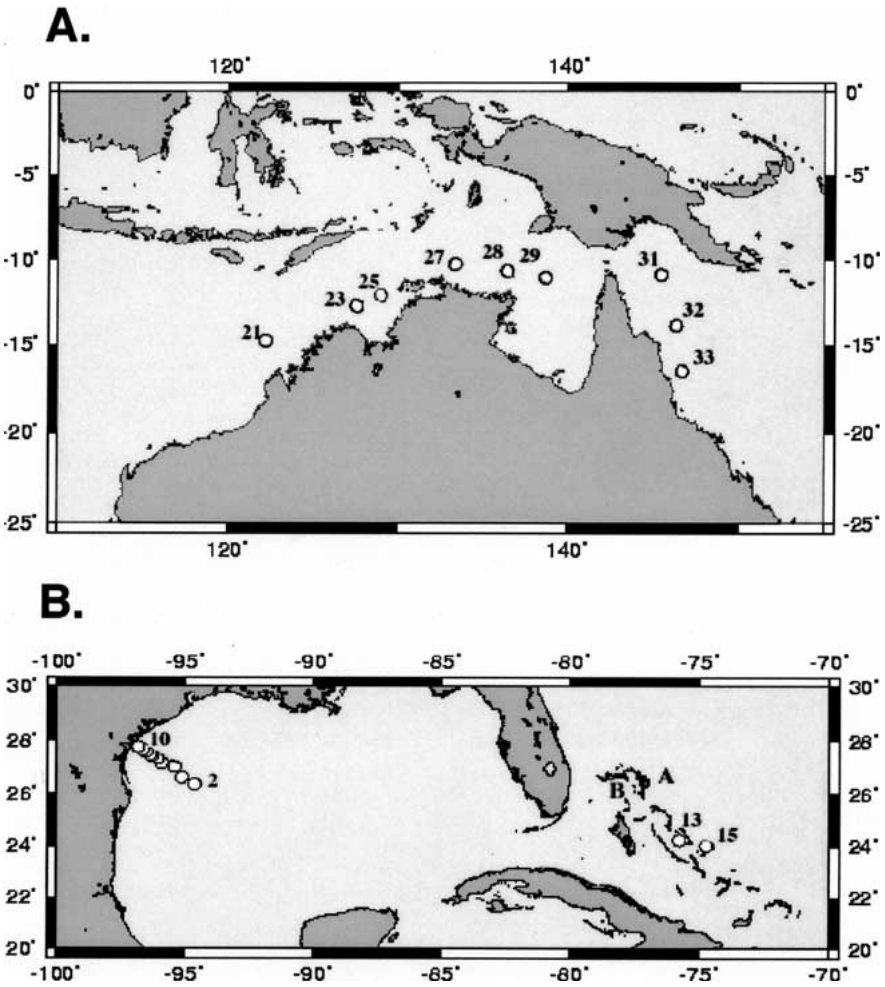

Fig. 1. Station locations for material collected during this study. (**A**) The Australia stations (n = 9) from EW99-12 (R.V. *Maurice Ewing*), Oct–Nov. 1999. (**B**) The Bahamas stations (n = 2) were from cruise CI 91111, (R.V. *Columbus Iselin*) in Sept. 1991, and the Gulf of Mexico stations(n = 10) were from the R.V. *Longhorn* in July 2001. The Bahamas collections (A, B, 13, 14) include two time series collected at regular intervals as the ship steamed off of San Salvador (A, 1 Sept. 1991) and in the Northeast Providence Channel (B, 14 Sept. 1991). Two fixed stations were sampled on 4 Sept. 1991 (13) and 9 Sept. 1991 (15).

were based on a modification of the Walsby and Booker [41] technique for individual trichomes. Approximately 20–30 colonies were reisolated into GFF glass-fiber filtered seawater and gently poured into three small (5 cm diameter, approximately 1.5 cm height) glass petri dishes. The inverted lid was then placed over the top to exclude air bubbles. After 5 min, colonies floating to the top and bottom of the dish were defined as positively buoyant and negatively buoyant, respectively. Gentle ship motion had no discernible effect on colony movement once all air bubbles had been excluded, and the colonies usually sorted within 5 min. This method produced similar results to the SETCOL method used previously [37], although care had to be taken when removing the top to avoid remixing the sample. After the initial buoyancy determination, the lid was gently removed and colonies were sorted by buoyancy into GFF-filtered seawater.

Bulk carbohydrate and protein content was determined by the anthrone method scaled to 5-mL volumes [33] and by the bicinchoninic acid method [32] using bovine serum albumin standards (Sigma kit BCA-1), respectively. All glassware was soaked in 10% HCl, and reagent blanks assayed with each sample set showed no evidence of residual organic contamination on the glassware. Colony number per filter was based on preliminary assays that yielded absorbance values 5–50 times the blank values. Colonies were quantitatively removed from the filters by vortexing them in two successive 1.0-mL rinses of 0.1 N NaOH for 60 s. Two 0.5-mL carbohydrate subsamples were taken at this time and the remaining aliquot digested in the 0.1 N NaOH for 1 hr at 100°C, then subsampled twice for protein. Protein samples were assayed in disposable spectrophotometer cuvettes as recommended by the Sigma kit protocols. The 95% confidence interval on available duplicate measurements from net collections varied from 0.1 to 7.0% of the duplicate samples with an average of 2.9% (n = 24). The 95% confidence interval for duplicate readings (i.e., from the same tube) was 5% of the mean for carbohydrate absorbance and 3% of the mean for protein absorbance (n = 155). Individual measurements were then corrected for dilutions and normalized to colonies. NaOH (0.1 N) does not affect the protein assay [32], and direct comparisons indicated that the NaOH did not influence the carbohydrate assay.

Two laboratory experiments were conducted on board ship using *Trichodesmium* collected from net tows in the Caribbean. On 4 Sept. 1991, a set of 30 colonies in acid-washed 250-mL polycarbonate bottles were placed in light (754 μmol m^{-2} s^{-1}) and dark bottles, and sampled at 0, 3, and 4.75 hr (experiment 1). On 14 Sept. 1991, acid-washed 250-mL polycarbonate bottles

Table 1. *Trichodesmium* carbohydrate synthesis (light) and loss rates (dark)[a]

Date	Light rate	r^2	Dark rate	r^2
Net collections				
1 Sept 91	0.09	0.75	—	—
4 Sept 91	0.02	0.83	−0.04	0.99
9 Sept 91	0.09	0.69	—	—
14 Sept 91	—	—	−0.01	0.73
Bottle experiments				
1 Sept 91	0.02	0.83	−0.01	0.45
14 Sept 91	0.06	0.93	−0.05	—
Average	0.06 ± .02		−0.03 ± 0.01	

[a] Rates are in μg carbohydrate μg protein^{-1} hr^{-1}. The r^2 is from the slope used to generate the rate; all slopes were significant at $p = 0.05$ or better. Averages are ± standard error. The bottle experiment on 14 Sept. 1991 had only two time points.

containing 30 colonies were incubated in flowing seawater at 0.0, 6, 14, 20, 29, and 51% of surface sunlight using neutral-density screening (PAR determined with a QSL-100 quantum sensor) for 2 hr (experiment 2). After both experiments, samples were sorted for buoyancy and the carbohydrate/protein content determined as noted above.

Carbohydrate production or consumption rates normalized to protein were estimated by a linear regression of either the day (production) or night (consumption) carbohydrate–protein ratios versus time using at least four points in the respective L:D cycle. Bottle rates were calculated by the same procedure utilizing the points available (however, see 14 Sept. 1991 data in Table 1). Error estimates (±) represent standard error unless noted otherwise in the text. Curves were fit using the curve-fitting routine in Deltagraph 4.5 (SPSS, Inc.).

Simultaneous N:P determinations [28] were made on groups of 5–15 colonies collected in Oct.–Nov. 1999 (Australia) and July 2000 (Gulf of Mexico). Colonies were picked into 5 mL of distilled water using plastic inoculation loops rinsed in methanol and deionized water between uses to remove material adhering to them from the net-collected sample. Preliminary tests indicated this step was essential to prevent N or P blank values that could reach 40% of the sample value. The blank values only partially covaried and were apparently related to particulate or dissolved material adhering to the transfer loop that desorbed when placed in deionized water. Samples were digested for 30 min at 1 atm (potassium persulfate as per Ref. 28; EM Science PX-1560-1) using an electric pressure cooker, and then analyzed for N and P using a Lachat Quikchem 8000 flow injection ion analyzer. Concentration versus colony number in preliminary experiments using picked colonies of similar size indicated the digestion results were linear in the 5–20 colonies per tube range ($r^2 = 0.99$). Statistical analyses were performed using StatView 5.0 (SAS Institute, Inc.).

Results

Carbohydrate content per colony ranged from 0.8 to 6.5 μg col^{-1} in net-collected *Trichodesmium* (average = 2.9 ± 1.2). The pooled data from the diel sampling increased through the day to a maximum at dusk and decreased during the night (Fig. 2A; cosine fit $r^2 = 0.40$). However, this general pattern was not evident on some days. For example, on 4 and 14 Sept., carbohydrate col^{-1} was actually higher at night than during the day. The average carbohydrate value using the NaOH resuspension (average = 1.9 ± 0.7 μg col^{-1}) was not significantly different from colonies filtered and placed directly into the reagent. Protein content per colony ranged from 3.6 to 26.4 μg col^{-1} (Fig. 2B) with a mean of 15.7 ± 4.1 μg col^{-1} and showed no evidence of any diel periodicity in the pooled data (cosine fit $r^2 = 0.19$). From visual inspection of the samples during collection, it was obvious on several occasions that colony size varied over time in an unpredictable manner. During periods of low wind (<3 m sec^{-1}), colonies became noticeably larger during the day. On other occasions, night-collected *Trichodesmium* were so small and rare that we could no longer collect sufficient material for this study.

In contrast to the colony-specific data, weight:weight carbohydrate:protein (carbo:prot) ratios underwent a clear diel pattern (cosine fit $r^2 = 0.70$) that paralleled the pooled carbohydrate data (Fig. 2C), suggesting that the observed changes were due to carbohydrate, not protein, variation. Ratios were minimal at dawn, increased throughout the day, and reached a maximum at dusk with a diel range of 0.10–0.36. The average value of all measurements was 0.18 ± 0.05. This pattern followed the general diel variation of colony buoyancy (Fig. 3) with a minimum percentage of floating colonies occurring at the time of maximum carbo:prot ratio. The buoyancy patterns paralleled previous observations [37]; however, in the present study colonies did not necessarily become negatively buoyant in the afternoon. For example, on 14 Sept. 1991,

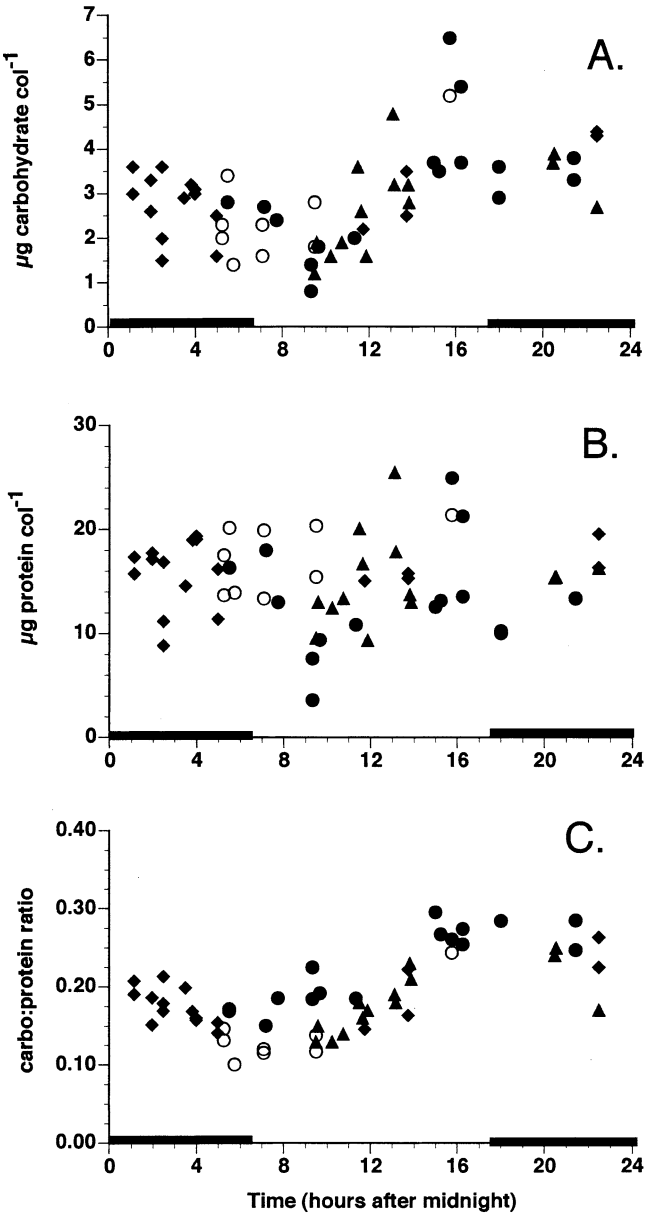

Fig. 2. Chemical composition of *Trichodesmium* colonies during diel sampling. Collection sites noted in Fig. 1. (○) 1 Sept. 1991, (●) 4 Sept. 1991, (▲) 9 Sept. 1991, (◆) 14 Sept. 1991. Dark bars represent night. (**A**) *Trichodesmium* carbohydrate content (μg glucose equivalents colony^{-1}). (**B**) *Trichodesmium* protein content (μg protein col^{-1}). (**C**) Carbohydrate:protein ratio (μg:μg) from the net collections.

floating colonies exceeded 75% of all colonies collected at all times, whereas on 4 and 9 Sept. 1991, they decreased to 40%. Pooled data from all the stations showed a broad general relationship between buoyancy and carbo:prot ratio (Fig. 4) with more negatively buoyant populations having a higher carbo:prot ratio. The correlation from the pooled data was not strong, but was highly significant

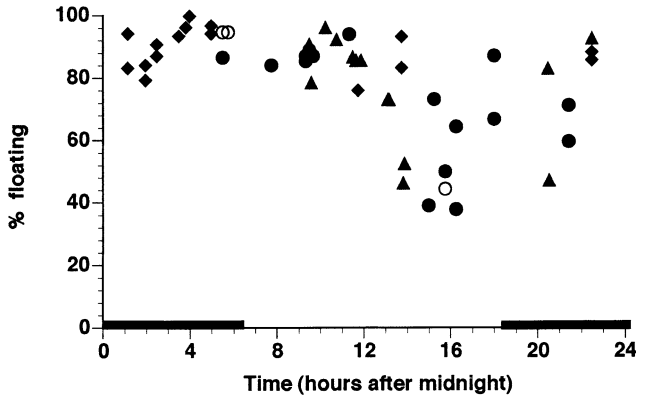

Fig. 3. Diel buoyancy patterns in *Trichodesmium* from Sept. 1991. Symbols are the same as Fig. 1.

(r^2 = 0.48, p = <0.0001). When the individual days were treated separately, the r^2 improved to 0.50–0.76 except for 14 Sept. 1991 (r^2 = 0.03). The 14 Sept. 1991 sampling was conducted while steaming over a broad area and may have been influenced by multiple populations of *Trichodesmium* with different histories.

Laboratory samples incubated at 754 μmol quanta m^{-2} s^{-1} (experiment 1) indicated a progressive decrease in buoyancy accompanied by an increase in the carbo:prot ratio (Fig. 5). This did not occur in the dark samples. Additional experiments revealed that the time required to sort sufficient colonies for replicates was a sizeable fraction of the time required for significant buoyancy changes. Thus, we lack sufficient replicates for statistical tests in Fig. 5; however, the 95% confidence interval as a percentage of the mean (7% based on precision of duplicate net tow collections) suggests that the differences were real. In both light and dark experiments, the buoyancy changes in response to light are similar to those noted previously [37]. Two-hour incubations under different irradiance levels (experiment 2, Fig. 6) produced a saturation response in protein-specific carbohydrate production with a maximum rate of 0.06 μg carbohydrate μg protein^{-1} hr^{-1}. The dark consumption rate was 0.05 μg carbohydrate μg protein^{-1} hr^{-1}. The highest light level (706 μmol m^{-2} s^{-1}) appeared to suppress increases in the carbo:prot ratio and was reduced to <1/3 the maximum value.

Linear regression of carbo:prot ratio on time during the period 0800–1800 hrs for three net collection data sets and two bottle sets indicated that the protein-specific carbohydrate accumulation rate ranged from 0.02 to 0.09 μg carbohydrate μg protein^{-1} hr^{-1} (r^2 = 0.69–0.83; Table 1), with an average (± std. err.) of 0.06 ± 0.03 μg carbohy-

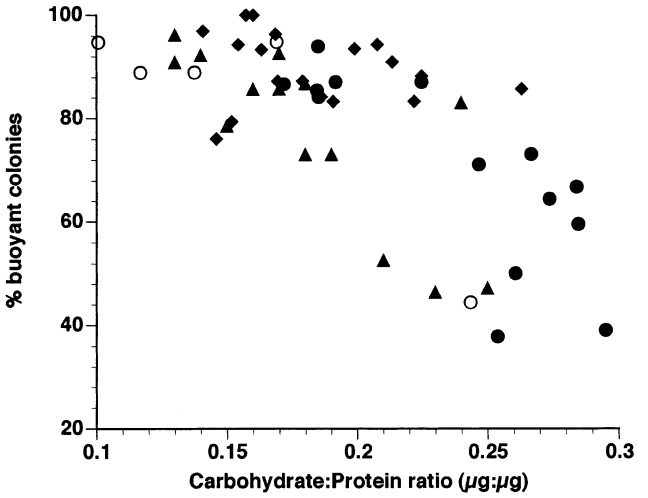

Fig. 4. Scatter plot of buoyancy and carbo:prot ratio in the diel sampling. Data include only samples taken directly from the net tow. Correlation coefficients are given in the text. Symbols are given in Fig. 1.

drate μg protein^{-1} hr^{-1}. Average of all dark loss estimates was 0.03 ± 0.01 (range = −0.01 to −0.05).

N:P ratios for *Trichodesmium* averaged 66.1 ± 4.0 (95% C.I., n = 144) over all samples from Australian waters. Pooled sinker and floaters from all depths were not statistically distinct (sinkers = 66.6 ± 9.6; floaters = 66.1 ± 4.6; p = 0.89). In the Gulf of Mexico samples, N:P ratios were significantly higher in sinkers than in floaters (p < 0.0001). Sinking *Trichodesmium* had N:P ratios of 87.4 ± 3.5 while floating *Trichodesmium* had N:P ratios of 43.5 ± 2.0. Results are summarized in Table 2.

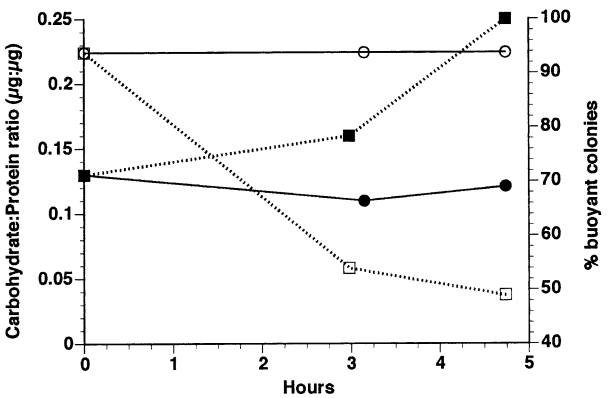

Fig. 5. Time series light and dark bottle incubations on 1 Sept. 1991 (exp. 1) at 754 μE m^{-2} sec^{-1}. *Trichodesmium* buoyancy (□) light, (■) dark; *Trichodesmium* protein: carbohydrate ratios (○) light (●) dark. Based on net collected duplicates, the 95% confidence interval was <7% of the mean.

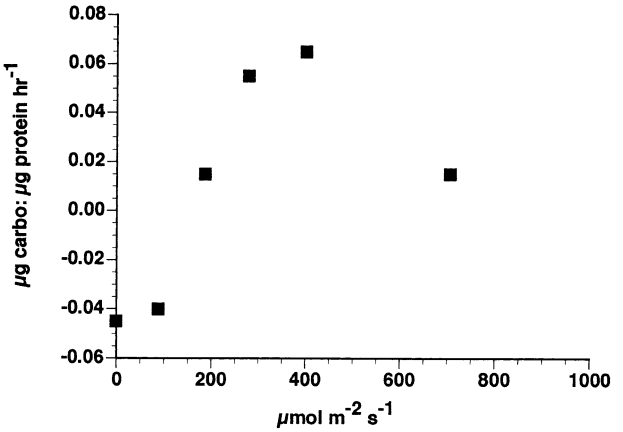

Fig. 6. Carbohydrate:protein ratio variation as a function of light intensity (exp. 2; 14 Sept. 1991). Rate of change is expressed relative to an initial control measured in duplicate. Based on netcollected duplicates, the 95% confidence interval was <7% of the mean.

Discussion

Colony size variation presents a significant challenge for quantifying either temporal or spatial changes in *Trichodesmium* particulate content. In our study, both protein and carbohydrate exhibited nearly an 8-fold variation during our sampling. By applying ratios of key compositional measures as well as methods that permitted us to sample for two analyses from the same colony aliquot, we reduced much of the variability normally seen in *Trichodesmium* due to colony size variation. For example, our carbo:protein ratio data yielded much cleaner data than either protein or carbohydrate col^{-1}, and it is clear that a diel pattern occurs.

Carbo:protein variations in *Trichodesmium* (0.10–0.38) were smaller than reported in other cyanobacteria species where minimum values range from 0.1 to 0.2, and maximum values range from 0.7 to 1.8 [16, 18, 21, 34, 35]. This ratio can be constrained with simple calculations. Glucose is 40% C by weight and assuming 11.6 ug C col^{-1} [5], the average *Trichodesmium* colony carbohydrate content is 10% of the total C. This is within the range (8–21%) reported for *Oscillatoria agardhii* [35]. Protein is approximately 16% N by weight and using a range of N col^{-1} of 2.17–2.32 μg col^{-1} [5, 6], and we estimate that our measured protein accounts for approximately 108–115% of the average N. This reasonably good concordance with literature data on cyanobacteria for both carbohydrate and protein suggests that there was no systematic bias. It is not

Table 2. *Trichodesmium* N:P ratios (± std. err.) from stations in northern Australian waters and the Gulf of Mexico[a]

	Australia	Gulf of Mexico	Pacific (HOTS)
Floating	66.3 ± 2.3	43.5 ± 2.0	43.8–34.19
Sinking	65.6 ± 4.7	87.4 ± 3.5	42.8–44.8
n =	145	181	Not reported
Phosphocline depth	80–100 m	100–125 m	>100 m
Significance level for difference p =	0.88	<0.0001	Not reported

[a] HOTS data are from [22]. Significance level compares sinker to floater N:P from the same cruise. Phosphocline depths are based on nutrient data provided post cruise or from the reference

clear why the maximum carbo:protein value is lower in *Trichodesmium* than in other cyanobacteria.

Carbohydrate ballasting is considered the only plausible mechanism for rapid buoyancy shifts in cyanobacteria [27], particularly for *Trichodesmium* since it cannot collapse its gas vesicles by increased turgor pressure [42]. Carbohydrate is but one part of the overall density term [15, 30, 31], and mechanistic models for quantifying rates of buoyancy change [i.e., 15] require detailed density budgets that we could not generate. In addition, other factors such as growth rate [14], light limitation [18], and nutrient limitation [16] will modify the long-term (days to weeks) buoyancy response, probably because of changes in gas vesicle content and total cellular ballast. In our study, buoyancy patterns paralleled *Trichodesmium* diel carbo:prot ratios both in both field and experimental incubations as expected (Figs. 4, 5), although at any given carbo:prot ratio there may be a wide range in percentage of floating colonies. Recovery of positive buoyancy in *Trichodesmium* is rapid (hours) whereas gas vesicle synthesis requires up to several generations [17, 40]. Our data are consistent with carbohydrate ballasting as the mechanism for short-term *Trichodesmium* buoyancy responses [30]. The carbo:prot ratio is a general, albeit limited, proxy for this behavior that gives us an indirect means for examining the role of vertical migration in *Trichodesmium* autecology.

A key ecological aspect of buoyancy regulation in *Trichodesmium* is the potential for migration to the phosphocline [12, 37]. We can use the carbo:prot ratios and protein-specific carbohydrate accumulation rates to estimate the range of vertical migration in *Trichodesmium* over time scales of hours, and thereby indirectly address this potential. We can test the utility of this ratio by comparing respiratory O_2 consumption [6] to measured changes based on carbo:prot ratios. Using their measured dark respiration rate in *T. thiebautii* (14.9 mg O_2 mg chl a^{-1} hr^{-1}), chl *a* col^{-1} (48 ng), and N col^{-1} (2.32 μg col^{-1}), we calculate a respiration rate of 0.31 μg O_2 μg N^{-1} hr^{-1}. Assuming all N was due to protein-derived N (N = protein × 0.16), and that 1 mol of glucose consumes 6 mol of O_2 during respiration, then our carbohydrate consumption rates (0.01 to 0.05 μg carbohydrate μg $protein^{-1}$ hr^{-1}) yield O_2 consumption rates solely from carbohydrate of 0.08–0.35 μg O_2 μg N^{-1} hr^{-1}. Thus, the carbohydrate loss rates appear reasonable based on known O_2 consumption rates.

Using the protein-normalized carbohydrate consumption rates (Table 1), we calculate the time required to reduce the maximum carbo:prot ratio (0.38) to the minimum (0.10) to be approximately 9 hr. Since this also represents the ratio range for maximum % positively and negatively buoyant colonies, we can use Walsby's [42] maximum ascent and sinking rate measurements (6.1 m hr^{-1}) to estimate that during this time the colonies would sink or rise no more than approximately 55 m. If the colonies started at the observed abundance maximum at 15 m [7], the effective maximum depth reached would be on the order of 70 m. This is consistent with observations that almost all *Trichodesmium* occurs above 50 m [4, 7]. Seventy meters is also above the phosphocline in most stratified oceanic systems; thus it appears that ballasting alone will not permit a vertical migration to deep nutrient pools in most oligotrophic seas (note that the phosphocline depth for these regions is listed in Table 2). In addition, virtually no time would be spent at the maximum depth since ballast is nearly consumed by the time this depth is reached. However, these rates can permit limited vertical excursions that generate the observed subsurface maxima of *Trichodesmium* that models predict [20]. In addition to oscillating around a preferred depth, such buoyancy reversals would also permit continuous motion through the water and reduce diffusion limitation of nutrients near the colony [9]. Since these calculations are based on extreme values, actual carbohydrate-mediated excursions are probably less. This calculation supports the Kromkamp and Walsby [20] result that high respiration rates seen in limnetic cyanobacteria are not consistent

with deep vertical migration in *Trichodesmium*, as well as the observation that *Trichodesmium* has a shallow compensation depth [22]. Respiration will probably decrease as growth slows as a result of P stress; however, we know of no measurements to test this.

Our N:P ratios are a direct measure of whether vertical migration to the phosphocline is occurring. In Table 2, it is clear that N:P ratios vary between oceanic regions. The highly significant difference in N:P ratio found in the Gulf of Mexico is the best direct evidence to date of vertical migration for P acquisition. The N:P ratios reported at HOTS (Hawaii Ocean Time Series, Station ALOHA) are very similar to that noted in ascending *Trichodesmium* in the Gulf of Mexico. If one assumes that these latter colonies are reasonably P sufficient, then it would imply that at HOTS, *Trichodesmium* is not highly P stressed. The statistically indistinguishable N:P ratios between ascending and descending colonies at HOTS and in Australian waters also suggest that these particular colonies were either acquiring sufficient P in surface waters or were limited by some other nutrient such as Fe [1]. To further complicate the matter, Romans et al. [30] noted that polyphosphate (a P storage product) was more abundant in sinking colonies of *T. tenue* (Caribbean) than in ascending colonies in collections at 15 m. Although this is only a portion of the total cellular P, it does suggest that P dynamics in *Trichodesmium* are complex and variable between regions.

The differing scenarios presented by the two data sets (carbo:protein/buoyancy versus the N:P ratios) lead us to hypothesize that there are two migrations to consider: a light-driven oscillation above the phosphocline, and P-driven migration superimposed on top of it via respiration regulation. Resolving this in mixed field populations of *Trichodesmium* will be a formidable problem. Generic assumptions of vertical migration as a major source of P acquisition require further validation, particularly if there are differences in the depth of vertical excursions that are linked by interactions of light and P status. Species composition is probably important as well. For example, *Trichodesmium erythraeum* has a gas vesicle collapse depth of approximately 120 m [42], a depth well above the phosphocline in regions such as the Sargasso Sea [25]. It would be incapable of migrations in such waters.

Trichodesmium surface accumulations still present an enigma. Ballasting is apparently inadequate to overcome the lift generated by gas vesicles once the population is trapped at the surface. Carbohydrate production is severely photoinhibited at high irradiance [23], suggesting that a production/consumption imbalance occurs. Our carbo:protein ratio changes as a function of light also indicate a differential synthesis at higher light levels (Fig. 6) and support their observation. However, this by itself cannot explain what mechanisms create the lift/ballast imbalance that cause the surface accumulations in the first place. Since many of the blooms are associated with calm weather [5], a certain amount of turbulence may be required to keep the colonies below the carbohydrate synthesis inhibition threshold.

In summary, carbohydrate ballasting is reflected in diel changes of carbo:protein ratios; however, there is not an obligate relationship between carbo:protein ratio and buoyancy status. Respiration rates measured in near-surface samples appear to be too high to allow vertical migration for P acquisition, although oscillations above the phosphocline appear reasonable. This analysis predicts that *Trichodesmium* sinking to the phosphocline must have reduced respiration rates if P acquisition is to be successful. This implies a potential for *Trichodesmium* to have both light- and phosphorus-driven vertical excursions that are superimposed on top of each other, and that these migrations extend to different depths. Direct measurement of migration potential based on N:P ratios suggests that there are regional differences in the expression of vertical migration. In northern Australian waters and at HOTS, P acquisition at depth seems to be limited, or at least is not adequate to cause significant variations in the N:P ratio. P acquisition at depth occurs in the Gulf of Mexico based on a twofold range in N:P ratios between sinking and floating colonies.

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