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The microbial and metazoan community associated with colonies of *Trichodesmium* spp.: a quantitative survey

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Association with resource-rich particles may benefit a number of planktonic species in oligotrophic, open-ocean regimes. This study examined communities of microbes and zooplankton associated with colonies of the cyanobacterium Trichodesmium spp. in the Sargasso Sea. Trichodesmium colonies and seawater controls were collected near Bermuda using SCUBA during September 1995, and June, July and August 1996. Organisms associated with the colonies and those in the surrounding seawater were enumerated using light and fluorescence microscopy. We found that 85% of the Trichodesmium puff and tuft colonies examined harbored associated organisms. Associated organisms included bacteria (rod and coccoid), fungi, pennate diatoms, centric diatoms, heterotrophic and autotrophic dinoflagellates, chrysophytes, hypotrich ciliates, amoebae, hydroids, juveniles and nauplii of harpacticoid copepods, and juvenile decapods. The most common associates (in addition to bacteria) were dinoflagellates (present in 74% of the colonies examined), amoebae (50%), ciliates (24%), and diatoms (24%). Numbers of bacteria per colony volume averaged 8.2×10^8 bacteria ml^{-1} (range = $8.1 \times 10^7 - 3.5 \times 10^9$ bacteria ml^{-1}), and the density of associated microzooplankton and metazoans averaged 6.8×10^4 organisms ml^{-1} (range = $0 - 3.6 \times 10^6$ organisms ml^{-1}). Associates of Trichodesmium colonies were enriched by two to five orders of magnitude over plankton in the surrounding water. This unique habitat allows for the association of primarily benthic ciliate, diatom and copepod species and could contribute significantly to plankton heterogeneity in the open-ocean. The distribution of associated organisms was affected by sample characteristics such as colony morphology, mucoid matrix structure and colony integrity. The influence of these factors indicates that succession or competition between heterotrophic microorganisms ultimately determines Trichodesmium microcommunity structure. Similar processes could regulate microbial and metazoan communities associated with other resource-rich microenvironments, such as marine snow particles.

INTRODUCTION

Organisms living in the open ocean must survive in a challenging environment often characterized by low nutrient levels. Subtropical open-ocean gyres, such as the Sargasso Sea, are examples of such extremely oligotrophic marine systems. Subtropical gyres are characterized by chronic (nitrogenous) nutrient depletion and low standing stocks of organisms (Pinet, 1992; Karl, 1999). Plankton can survive in these 'marine deserts' through a number of mechanisms, including acquisition of limiting nutrients from atmospheric sources (Capone *et al.*, 1997; Zehr *et al.*, 2001), symbiosis with intracellular autotrophs (Villareal, 1992; Gordon *et al.*, 1994), and association with resource-rich microenvironments (Swanberg and Harbison, 1980; Caron *et al.*, 1986; Alldredge and Silver, 1988; Steinberg *et al.*, 1994).

Although the occurrence of *Trichodesmium* spp. can be ephemeral in nature (Capone *et al.*, 1997), these cyanobacterial colonies provide a stable substratum and food resource for associated plankton in oligotrophic tropical and subtropical waters. *Trichodesmium* colonies can be abundant in tropical oceans, at times accounting for over 60% of the total chlorophyll *a* (Carpenter and Price, 1977; Capone *et al.*, 1997; Dupouy *et al.*, 2000). These cyanobacteria occur in two different colony morphologies: a

spherical ('puff') form and a parallel ('tuft') form (Janson *et al.*, 1995). The tightly compacted inner core of *Trichodesmium* puffs and tufts adds structural integrity to the colonies. *Trichodesmium* spp. provide associated heterotrophic organisms with nutrition in the form of dissolved nitrogen and carbon compounds in the form of DON and DOC (Capone *et al.*, 1994; Glibert and Bronk, 1994; Sellner, 1997). The structural integrity and labile organic substances provided by *Trichodesmium* colonies encourage the association of bacteria, ciliates and other protozoa, hydroids, and harpacticoid copepods (Borstad and Borstad, 1977; O'Neil and Roman, 1992; Sellner, 1992).

Despite advances in our understanding of *Trichodesmium* colonies, there is still very little fundamental description of the biological structure of these communities, which is the first step in determining the functional role of associated organisms. Previous investigations of *Trichodesmium* associates have enumerated only bacteria (Paerl *et al.*, 1989; Nausch, 1996) and harpacticoid copepods (Roman, 1978; O'Neil and Roman, 1994; O'Neil, 1998). In this study, counts were made of all organisms associated with *Trichodesmium* puff and tuft colonies and of plankton in the surrounding water column. The distribution of *Trichodesmium* associates is related to the morphology, mucoid matrix structure and integrity of *Trichodesmium* colonies. Mechanisms that may regulate *Trichodesmium* associate community structure, including succession within the microcommunity and interactions between associated organisms, are discussed.

METHOD

Spherical and cylindrical *Trichodesmium* colonies were collected four miles southeast of Bermuda on four dates: September 22, 1995, June 19, 1996, July 17, 1996 and August 20, 1996. Colonies were collected at ~0900–1000 local time under calm and sunny conditions. On each sampling date, ~50–100 colonies and separate seawater samples (controls) were individually hand-collected using SCUBA at a depth of 10 m with 10 ml (colonies) and 25 ml (controls) syringe samplers. Colonies, in 3 ml of seawater, and ambient seawater controls (25 ml seawater) were preserved in 2.5% glutaraldehyde.

Organisms associated with *Trichodesmium* colonies and in the surrounding water column were enumerated using microscopy. Measurements of colony diameter and length were made with a dissecting microscope and were converted to volume using the equation for a cylinder (tuft form) or a sphere (puff form). Colonies were gently teased apart on slides and wet mounted. The entire colony was examined using light and epifluorescence microscopy. The remaining seawater was examined under a dissecting microscope for macrozooplankton or associated detritus. Numbers of organisms per colony and per colony volume were calculated. Plankton in ambient seawater were examined using fluorescence microscopy. Surrounding seawater controls (25 ml seawater) were gently filtered onto 0.2 μm Nuclepore filters and mounted with mineral oil. The filters were examined at 400× and the numbers of organisms per volume of seawater control were calculated and compared with organisms per volume of colony.

Bacteria associated with *Trichodesmium* spp. were counted only for colonies collected on August 20, 1996. Colonies and control seawater were individually collected and preserved as above. After measurement using a dissecting microscope, colonies were sonicated at 50 W until fully dispersed (time required for puffs: mean = 76 s, range = 25–180 s; for tufts: mean = 50 s, range = 20–150s), filtered onto blackened 0.2 μm Nuclepore filters, stained with DAPI (4,6-diamidino-2-phenylindole) (Porter and Feig, 1980), and mounted immediately on microscope slides. Bacteria were then enumerated using epifluorescence microscopy. Control treatments (surrounding seawater) and blank treatments (0.2 μm filtered seawater) were filtered, stained with DAPI, and enumerated as were the sonicated colonies.

RESULTS AND DISCUSSSION

Eighty-five percent of the *Trichodesmium* colonies examined in this study harbored associated microzooplankton and metazoans. Organisms associated with *Trichodesmium* colonies included bacteria (rod and coccoid), microflagellates, fungi, cyanobacteria, pennate diatoms, centric diatoms, heterotrophic and autotrophic dinoflagellates, chrysophytes, hypotrich ciliates, amoebae, hydroids, juveniles and nauplii of harpacticoid copepods, and a juvenile decapod (Table I). The most common associates (in addition to bacteria) were dinoflagellates (present in 74% of the colonies examined), amoebae (50%), ciliates (24%) and diatoms (24%). High variability in density and in species composition characterized the communities described in this study (Figure 1, CV% = 40–714). Densities of bacteria (per colony volume, ~0.002 ml for tufts and ~0.010 ml for puffs) averaged 8.2×10^8 bacteria ml^{-1} (range = 8.1×10^7–3.5×10^9 bacteria ml^{-1}). Densities of microzooplankton and metazoans averaged 6.8×10^4 organisms ml^{-1} (range = 0–3.6×10^6 organisms ml^{-1}). Communities associated with *Trichodesmium* puff colonies were significantly more species-rich (mean = 3.4, range = 1–19 spp./colony, $n = 51$) than those associated with tuft colonies (mean= 1.5, range = 1–5 spp./colony, $n = 49$; ANOVA, $P < 0.01$), and puff colonies were more likely to contain associated microorganisms than tuft colonies (94% of puffs were occupied versus 77% of tufts).

The density of organisms associated with *Trichodesmium*

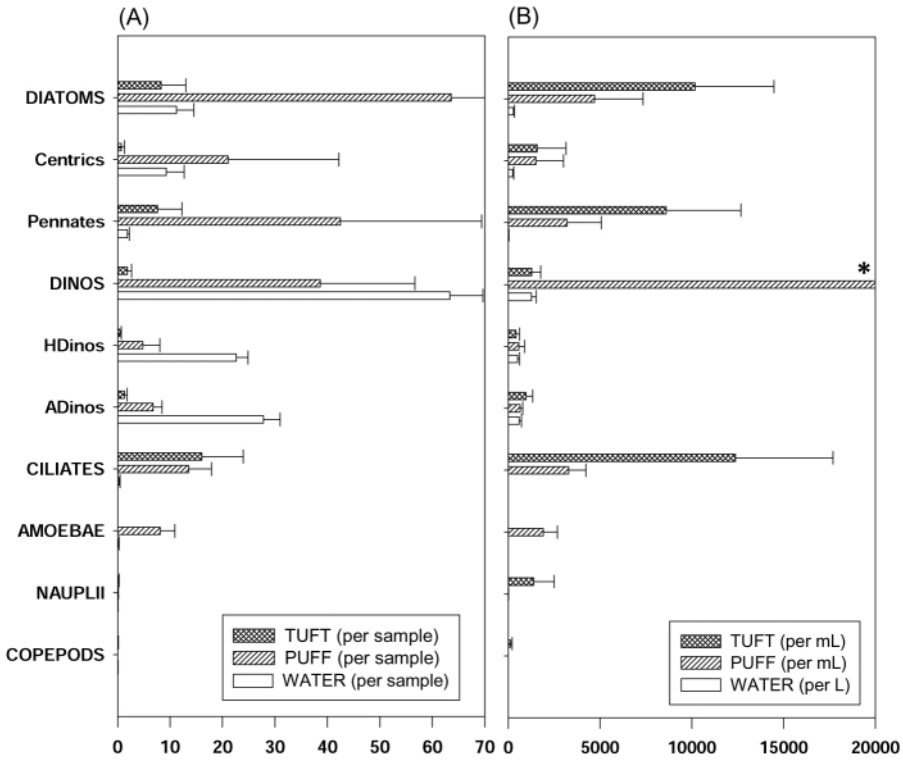

Fig. 1. Counts (**A**) and density (**B**) of organisms associated with *Trichodesmium* puff ($n = 51$) and tuft ($n = 49$) colonies in the Sargasso Sea, and plankton in the surrounding water column ($n = 24$). Counts are number of organisms per individual colony or per 25 ml seawater sample. Densities are number of organisms per ml colony volume or per liter seawater. Error bars are SE of the mean. *Dinoflagellate: mean = 35 592 ml colony^{-1}; SE = 32 833 ml colony^{-1}.

Table I: Organisms associated with Trichodesmium colonies in the Sargasso Sea

Cyanobacterium	*Synedra* sp.	**Amoebae**
Phormidium sp.	*Navicula* sp.	**Hydroid**
Synechococcus sp.	*Amphiprora* sp.	*Pelagiana trichodesmiae*
Centric diatoms	**Dinoflagellates**	**Copepods**
Guinardia cylindrus	*Peridinium trochoideum*	*Macrosetella gracilis*
Dactyliosolen fragilissimus	*Gonyaulux* sp.	*Miracia efferata*
Rhizosolenia alata	*Amphidinium* sp.	**Decapod**
Bacteriastrum sp.	*Gymnioid* sp.	*Lysmata* sp.
Skeletonema sp.	Unidentified sp.	**Other**
Thalassiothrix sp.	**Chrysophytes**	Bacteria (rod and coccoid)
Pennate diatoms	*Dictyocha fibula*	Microflagellates
Cylindrotheca closterium	Unidentified unicell	
Nitzschia longissima	**Hypotrich ciliates**	
Pseudonitzschia seriata	*Euplotes* sp.	

Colonies were collected in September 1995, and June, July and August 1996 by SCUBA at 10 m depth four miles southeast of Bermuda.

colonies was, in most cases, significantly higher than plankton density in the surrounding water column (Table II, sample type, ANOVA, $P < 0.05$). Differences between colony and water column abundance of taxa are represented by enrichment factors (Table III, Puff and Tuft EF). [EF = (number of organisms per ml colony) × (number in equal volume of surrounding water)$^{-1}$]. Organisms associated with *Trichodesmium* colonies were

Table II: Factors influencing the density of organisms associated with Trichodesmium colonies

	Sample type $F_{2,121}$	Matrix structure $F_{2,97}$	Integrity $F_{1,98}$	Collection date $F_{3,120}$
Bacteria	(T > P, P > W, T > W)**	na	(U > H)**	na
Diatoms	(T > W)*	(T > L, T > S)**	–	–
Centric diatoms	–	–	–	–
Pennate diatoms	(T > W)*	(T > L, T > S)**	–	–
Dinoflagellates	(P > T, P > W)**	(T > L)*	–	–
Heterotrophic dinoflagellates	–	–	–	–
Autotrophic dinoflagellates	(P > T, P > W)**	–	–	–
Ciliates	(P > W, T > W)**	–	(H > U)*	–
Amoebae	(P > T, P > W)**	–	–	(Jun > Sep)*
Harpacticoid copepod nauplii	(P > W, T > W)*	–	–	–
Harpacticoid copepods	(T > W)*	–	–	(Sep > Jun, Jul, Aug)*
Number of species	(P > T)**	(T > L, T > S)**	–	–

Densities of organisms (#/ml) were transformed [ln(x + 1)] and the influence of different categories within each factor compared using one-way ANOVA. Multiple comparisons using the Scheffe method precede significant ANOVA results. Abbreviations as follows: sample type, P = puff colony (n = 51), T = tuft colon, (n = 49), W = surrounding seawater (n = 24); mucoid matrix structure, L = little or none (n = 44), S = some structure (n = 39), T = thick structure (n = 17); colony integrity, H = young, healthy (n = 81), U = unhealthy, senescing (n = 19); collection date, Sep = 22 Sep 1995 (n = 46), Jun = 19 Jun 1996 (n = 26), Jul = 17 Jul 1996 (n = 26), Aug = 20 Aug 1996 (n = 26). Asterisks indicate significance levels: *P < 0.05, **P < 0.01. na, not applicable.

Table III: Enrichment factors calculated for organisms associated with Trichodesmium colonies, and published for organisms associated with marine snow particles

	Puff	Tuft	Marine snow
Bacteria	401	1709	83–298[a], 2–100[b]
Nanoplankton (2–20 μm)	–	–	5–370[b]
Diatoms	16 751	36 211	167–3882[c], 0–27[d]
Centric diatoms	6139	6418	–
Pennate diatoms	97 463	262 118	–
Dinoflagellates	27 975	1003	116–12 076[c], 0–2100[d]
Heterotrophic dinoflagellates	1117	810	–
Autotrophic dinoflagellates	1095	1610	–
Ciliates	569 548	2 149 926	316–2131[a], >8000[d], 9080–77 400[e]
Amoebae	462 548	0	413–6190[e]
Copepod nauplii	31 546	1 983 817	–
Copepods	–	–	197 ± 141[f]

Enrichment indicates increased abundance of associated organisms versus organisms in the surrounding water column. Enrichment factor = (number of organisms per ml colony) × (number in equal volume of surrounding water)$^{-1}$. [a]Davoll and Silver, 1986; [b]Caron *et al.*, 1982; [c]Alldredge *et al.*, 1998; [d]Silver *et al.*, 1978; [e]Caron *et al.*, 1986; [f]Steinberg *et al.*, 1994. Dash indicates data not available.

enriched between two to six orders of magnitude over organisms in the surrounding water column. Although the exact methods used to enumerate plankton in the water column and on *Trichodesmium* colonies did differ, large enrichment factors indicate that association with colonies of *Trichodesmium* spp. can be advantageous for planktonic organisms in oligotrophic, open ocean regimes. The accumulation of associate biomass would

also potentially enrich *Trichodesmium* particles consumed by large grazers. We note, however, that many organisms are thought to find certain species of *Trichodesmium* toxic (Sellner, 1997).

Distribution of associated organisms and colony characteristics

Variation in microcommunity composition suggests that the distribution of associated organisms was influenced by colony characteristics such as mucoid matrix structure, colony integrity, or colony morphology (Table II). Many of the colonies examined (65% of puffs, 50% of tufts) contained a mucilaginous matrix. Matrix abundance was categorized as 'little or none' (a thin, gluey substance scattered throughout the colony), 'some' (dense mucus bundles in the center of the colony), or 'thick' (an almost impenetrable mucus structure strung throughout the colony) (Table II). Additionally, a few of the colonies examined in this study (14% of puffs, 24% of tufts) appeared unhealthy or senescent. The integrity of *Trichodesmium* puff and tuft colonies was therefore described as either 'healthy' (comprised whole, brown–green trichomes) or 'unhealthy' (comprised light green, broken and senescent trichomes) (Table II).

Matrix composition differed with colony type. The matrix on puff colonies included bacteria and microflagellates, whereas the matrix on tuft colonies comprised bacteria and cyanobacterial filaments (e.g. *Phormidium* sp.), or fungal filaments. The fungal filaments formed very dense bundles concentrated around a few trichomes in the colony, and seemed to preclude the growth of dense bacterial populations. Bacteria and fungi may compete for nutrients from the polysaccharide matrix, algal exudates, or dead cells (O'Neil and Roman, 1992).

Bacterial density on *Trichodesmium* was significantly greater on tuft colonies than on puff colonies (Table II, sample type, ANOVA, $P < 0.05$). This difference may reflect the fact that a greater number of senescent tuft colonies were examined than senescent puff colonies, and significantly more bacteria were associated with 'unhealthy' colonies (Table II, colony condition, ANOVA, $P < 0.05$). The composition of associated bacteria was primarily influenced by colony type: 41% tuft colonies contained rod shaped and filamentous bacteria versus 25% of puff colonies. Enrichment of rod shaped bacteria on particulate matter was observed previously for marine snow particles (Caron *et al.*, 1982, 1986), crustacean fecal material (González and Biddanda, 1990), and phytodetritus aggregates (Kunnis, 1998). The enrichment and diversity of bacteria associated with macroaggregates such as *Trichodesmium* colonies indicate that puffs and tufts form a distinct niche for microbial populations in pelagic environments.

The density of total diatom and pennate diatom populations was greater on *Trichodesmium* colonies with a thick mucoid matrix (Table II, ANOVA, $P < 0.05$). This supports Borstad and Borstad's hypothesis that mucoid secretions of pennate diatoms may contribute substantially to the overall matrix structure, increasing the colonies' suitability as a substrate (Borstad and Borstad, 1977). The benthic-like environment afforded by *Trichodesmium* colonies allows for the association of heavily silicified diatoms such as *Amphiprora* sp. Truly benthic diatom species (such as *Amphiprora* sp., *Cocconeis* sp. and *Acnanthes* sp.) were rare, and only observed on *Trichodesmium* puff colonies that exhibited a very thick mucoid matrix structure in this and previous studies (Borstad and Borstad, 1977).

Associated dinoflagellates were relatively species-rich in composition and were commonly present in both puff and tuft colonies. As with diatoms, dinoflagellate density increased significantly on puff colonies with a thick mucoid matrix structure (Table II, ANOVA, $P < 0.05$). Larger dinoflagellate populations may be attributed to the increased protection provided by a puff colony with a thick mucoid matrix. A dense mucoid matrix also would provide mixotrophic and heterotrophic dinoflagellates with access to enhanced food resources (Silver and Alldredge, 1981; Caron *et al.*, 1986).

Hypotrich ciliates of the genus *Euplotes* sp. were common associates of *Trichodesmium* colonies (found in 24% of puff and 50% of tuft colonies). Population density of *Euplotes* sp. was significantly larger on healthy colonies than on unhealthy, senescent colonies (Table II, ANOVA, $P < 0.05$). This distribution suggests that motility or food resources available to the bacterivorous ciliate differ on healthy and senescent *Trichodesmium* colonies. Hypotrich ciliates, including *Euplotes* sp., are common associates of marine snow particles (Silver *et al.*, 1978; Caron *et al.*, 1982; Silver and Alldredge, 1984), and spirotrich ciliates have been observed in association with decaying phytodetritus (Biddanda and Pomeroy, 1988). It is likely that the persistence of these organisms in the pelagic realm is determined largely by their association with benthic-like structures, such as *Trichodesmium* colonies (Caron *et al.*, 1986).

Large populations of amoebae were associated with healthy *Trichodesmium* puff colonies, whereas amoebae were never observed on *Trichodesmium* tuft colonies (Figure 1). Scale-bearing amoebae (probably *Paramoeba* sp.) were previously found on *T. theibautii* puff colonies (Anderson, 1977) and on marine snow particles (Caron *et al.*, 1982). This significant difference in association with puff versus tuft colonies (Table II, ANOVA, $P < 0.05$) may indicate some regulatory characteristic of colony morphology. Observations of bacteria in

amoebic food vacuoles (Anderson, 1977) indicate that the quantity and quality of bacterial food resources on *Trichodesmium* puff colonies could influence the distribution of associated amoebae.

The hydroid *Pelagiana trichodesmiae* was observed on one puff colony examined in this study and has previously been observed on puff colonies containing a thick mucilaginous matrix (Borstad and Brinckmann-Voss, 1979). No *P. trichodesmiae* were associated with tuft colonies. The hydroid is a voracious feeder, and living on the colonies could be advantageous for *P. trichodesmiae*. Not only does association with *Trichodesmium* provide a stable structure for growth, but the number of potential prey (copepods and chaetognaths) the hydroid could encounter is increased. Large numbers of non-motile autotrophic dinoflagellates were present in the same colony in which the hydroid *P. trichodesmiae* was observed. It could be that consumption of metazoan predators (e.g. copepods) by the hydroid provides some degree of protection from predation for dinoflagellates and other microbial members of puff colonies.

Greater densities of harpacticoid copepods (nauplii and adults) were associated with *Trichodesmium* tuft colonies than with puff colonies (Figure 1). Although density of copepods did not vary significantly with colony morphology, mucoid matrix structure, or colony integrity, these organisms were present on only a few of the colonies examined. Harpacticoid nauplii may have greater mobility on tuft colonies (O'Neil and Roman, 1992), and, as noted above, puff colonies harbor the carnivorous hydroid *P. trichodesmiae*. It is likely that primarily benthic harpacticoid copepods have adapted to a pelagic existence through association with *Trichodesmium* colonies (O'Neil and Roman, 1992). Other 'benthic-type' structures in the pelagic realm are known to harbor harpacticoid copepods, including *Rhizosolenia* mats (Carpenter *et al.*, 1977), radiolarian colonies (Swanberg and Harbison, 1980), and larvacean houses (Steinberg *et al.*, 1994).

In general, species richness increased with increased matrix thickness and colony stability (Table II, ANOVA, $P < 0.05$). In providing a unique benthic habitat for associated organisms, *Trichodesmium* colonies may contribute to the overall heterogeneity of plankton in open ocean waters.

Processes regulating *Trichodesmium* microcommunity structure

The distribution of organisms associated with *Trichodesmium* spp. indicates that microcommunity structure is regulated in part by interactions between associated organisms. In particular, successional processes, predation, competition between heterotrophic organisms, or temporal changes in organism abundance could regulate associate distribution on *Trichodesmium* colonies.

Succession in *Trichodesmium* microbial communities [similar to colonization and aging of phytodetritus (Biddanda and Pomeroy, 1988), marine snow particles (Caron *et al.*, 1986; Davoll and Silver, 1986), and metazoan fecal pellets (Pomeroy *et al.*, 1984; González and Biddanda, 1990)] would begin with bacterial inoculation of a *Trichodesmium* colony. As bacterial density increased, heterotrophic associates (such as dinoflagellates or ciliates) could successfully colonize *Trichodesmium* spp. Mucoid matrix thickness would increase as a result of bacterial growth, if calm conditions allowed for colony stability. The denser mucoid matrix would support a stable community structure characterized by high species richness and the inclusion of relatively rare species such as dinoflagellates, amoebae, diatoms, or the hydroid *P. trichodesmiae*. Senescence of *Trichodesmium* colonies may be induced by cyanophage infection (Ohki, 1998) or unfavorable environmental conditions. Either would promote bacterial growth and the production of a thick mucoid matrix structure on unhealthy, senescent colonies. It has been postulated that the puff morphology represents a mature form of *Trichodesmium* tufts (E. J. Carpenter, personal communication). Although differences in matrix composition, species richness, and % occupied colonies do support the hypothesis that puff microbial communities are older successional forms of tuft communities, senescent tuft colonies were also observed in this study, indicating that the succession from one form to the other is not deterministic. Infection by cyanophages could interrupt *Trichodesmium* microbial community succession at both tuft and puff stages.

Mature successional stage *Trichodesmium* communities would likely be regulated by predation and competition among heterotrophic associates, such as amoebae, ciliates and dinoflagellates. The increased diversity of organisms contributing to mature stage *Trichodesmium* communities is likely a causal factor in the positive correlation between population densities of several associates (Table IV, Pearson correlation, $P < 0.05$). However, significant negative correlations were observed between densities of ciliates and amoebae associated with *Trichodesmium* puff colonies (Table IV, Pearson correlation, $P < 0.05$), and these protozoa coexisted on only 4% of the puff colonies examined. Amoebae may control ciliate populations on *Trichodesmium* puff colonies through predation (endocytosis). Additionally, ciliates and amoebae may compete for space on the colonies, and thus access to bacterial food resources. If interspecific competition between associated heterotrophs exerts a strong influence on their distribution, it is likely that initial colonization by one protozoan will lead to the preclusion of the other.

Table IV: Pearson correlation coefficients (r) for organisms associated with Trichodesmium puff colonies and colony volume

	Bacteria	Centric diatoms	Pennate diatoms	Heterotrophic dinoflagellates	Autotrophic dinoflagellates	Ciliates	Amoebae	Nauplii	Harpacticoid copepods	Volume
Bacteria	1									
Centric diatoms	na	1								
Pennate diatoms	na	0.38**	1							
Heterotrophic dinoflagellates	na	0.49**	0.52**	1						
Autotrophic dinoflagellates	na	0.19	0.14	0.29	1					
Ciliates	na	0.16	–0.12	–0.12	0.09	1				
Amoebae	na	–0.01	0.11	0.39*	0.14	–0.31*	1			
Nauplii	na	0.21	0.09	0.10	0.2	0.10	–0.12	1		
Harpacticoid copepods	na	–0.03	–0.07	–	–	–0.12	–0.08	–0.05	1	
Volume	–0.99**	–0.1	0.15	–0.09	0.1	–0.19	–0.1	0.01	0.02	1

Coefficients are calculated for transformed [ln(x + 1)] densities (#/ml). Asterisks indicate significance levels: *P < 0.05, **P < 0.01. na, not applicable.

The significance of our Pearson correlations (Table IV) were evaluated using a P value plot (Figure 2) (Schweder and Spjøtvoll, 1982). This method can be used to reduce the possibility of a type I error when evaluating a large number (>~20) of cross correlations (Jenkinson and Biddanda, 1995). In theory, non-significant P values should fall along a straight line in the P value plot [e.g. Rank, N_p = T_0 (1 – P)], with significant P values lying above and to the right of this line. Because the large P value line most accurately represents the distribution of non-significant P values, its slope, T_0, is equivalent to the number of true null hypotheses (Schweder and Spjøtvoll, 1982). As expected, our significant correlations (boxed data points) lie above and to the right of the line that best fits large correlation P values. Moreover the regression slope, T_0 = 21.6, indicates that we have rejected the correct number of null hypotheses. Deviation from the regression line is likely due to positive correlation between our P values, as noted for other cross correlation data sets (Schweder and Spjøtvoll, 1982).

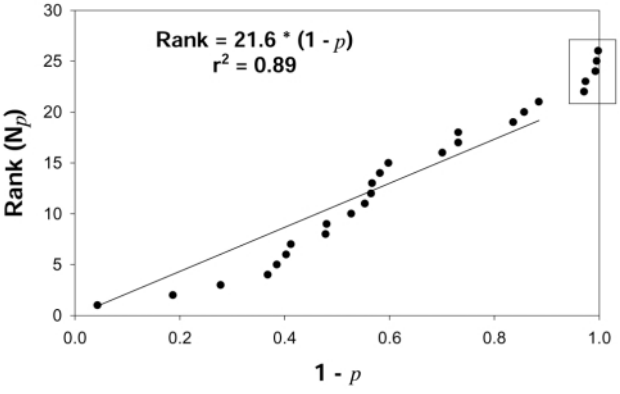

Fig. 2. P value plot for the Pearson correlations of associate density listed in Table IV, after Schweder and Spjøtvoll (Schweder and Spjøtvoll, 1982). Probability values (i.e. 1 – P) are plotted against rank of probability (N_p). This plot demarcates significant P values (upper right boxed area) versus non-significant P values for the correlations. The slope of the regression line best describing large P values is used to estimate the number of true null hypotheses (T_0) in the Pearson correlations.

The distribution of some organisms associated with *Trichodesmium* spp. varied with season. In particular, the percentage of puff colonies with associated ciliates and amoebae varied with collection date (Table II). This may reflect seasonal differences in plankton distribution and numbers of organisms available to inoculate *Trichodesmium* colonies. However, the density of most associates and plankton in the surrounding water column did not vary significantly with collection date (Table II). The distribution of ciliates and amoebae in time (Figure 3) may also be the result of an oscillatory predator–prey cycle, which happened to coincide with a seasonal change in this study. As the fraction of puffs containing amoebae decreased over the summer months, the fraction of puffs containing ciliates increased (Figure 3). This is further illustrated by the significant negative regression between month versus % colonies containing amoebae (regression: y = –18.36x – 74.54, r^2 = 0.995, P < 0.05) and the significant positive regression of month versus % colonies containing ciliates (y = 16.04x – 4.32, r^2 = 0.900, P < 0.05). Future studies

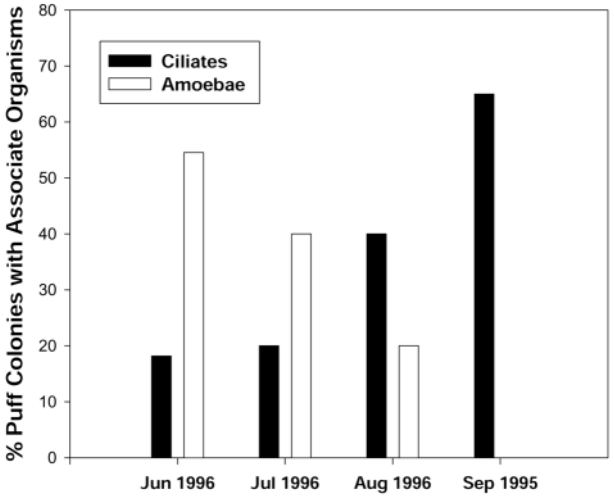

Fig. 3. The relationship between percentage of *Trichodesmium* puff colonies containing protozoan associates and season. No amoebae were found on *Trichodesmium* puff colonies in September 1995.

should examine the possible influence of season on the distribution of associated heterotrophic organisms.

Trichodesmium and other marine microcommunities

The processes structuring *Trichodesmium* microcommunities are likely to operate in communities associated with other resource-rich microenvironments, such as marine snow particles (Silver *et al.*, 1978; Alldredge and Silver, 1988), phytodetritus aggregates (Biddanda and Pomeroy, 1988), and metazoan fecal pellets (Pomeroy *et al.*, 1984; González and Biddanda, 1990). As with *Trichodesmium* associates, microbes and metazoans on marine snow particles are often enriched in number over plankton in the surrounding water column (Silver *et al.*, 1978; Caron *et al.*, 1986; Steinberg *et al.*, 1994). The enrichment factors calculated for *Trichodesmium* colonies are in most cases higher than those published for marine snow aggregates at approximately the same sampling depth and of larger volume, although from different locations (Table III). This study uses different enumeration methodology (epifluorescence microscopy) than that used in two previous investigations [e.g. dilution techniques (Caron *et al.*, 1982, 1986)]. However, it is likely that differences in abundance of *Trichodesmium* versus marine snow associates reflect differences in the quantity and quality of food resources available to the microbes. Release of dissolved nitrogen-rich organic compounds by *Trichodesmium* spp. (Capone *et al.*, 1994; Glibert and Bronk, 1994; Mulholland and Capone, 2001) could support a larger biomass of heterotrophic associates on puff and tuft colonies than would nitrogen-poor marine snow particles [e.g. mucoid marine snow (Caron *et al.*, 1986)].

SUMMARY

Organisms associated with *Trichodesmium* colonies collected from the Sargasso Sea included rod and coccoid bacteria, fungi, pennate and centric diatoms, chrysophytes, heterotrophic and autotrophic dinoflagellates, hypotrich ciliates, amoebae, hydroids, juveniles and nauplii of harpacticoid copepods, and juvenile decapods. Organisms were associated with 85% of the colonies examined. Concentrations of organisms associated with *Trichodesmium* spp. were enriched by two to five orders of magnitude over concentrations in the surrounding water. This enrichment indicates the advantage of associating with colonies of *Trichodesmium* spp. in oligotrophic open ocean environments.

Variation in the density of organisms associated with *Trichodesmium* spp. suggests that associate distribution is influenced by colony morphology, mucoid matrix structure and colony integrity (healthy and intact versus unhealthy and disintegrating). The copious amounts of mucilage observed on some colonies would increase their suitability as a substrate. This unique habitat allows for the association of primarily benthic ciliate, diatom and copepod species, and likely contributes to plankton diversity in open ocean waters. The relationship between organism distribution and colony characteristics is consistent with community structure regulation by processes such as succession, predation, competition between heterotrophic organisms, or temporal changes in organism abundance. These processes may apply to plankton communities associated with other resource-rich microenvironments, such as marine snow macroaggregates.

The microbial and metazoan community associated with *Trichodesmium* colonies was extremely variable in density and species composition. The influence of this variation on the physiology of *Trichodesmium* spp. should be addressed in future studies. Future studies characterizing the *Trichodesmium* microcommunity should also examine the role of abiotic factors, such as oxygen concentration or chemical differences in mucoid matrix structure to help determine the factors controlling distribution of associated organisms. Additionally, the role of bacteria and bactivorous protozoa in nutrient remineralization, organic matter turnover, and growth of *Trichodesmium* colonies should be explored. Tight coupling between dissolved organic matter use and the release of remineralized macro- and micronutrients would support the hypothesis of a mutualistic relationship between *Trichodesmium* and associated microbes (O'Neil and Roman, 1992). These interactions could promote nitrogen fixation by

Trichodesmium colonies, postulated to be extremely important for biogeochemical cycling in subtropical ocean gyre systems (Letelier and Karl, 1996; Capone *et al.*, 1997; Dupouy *et al.*, 2000).

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