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Bloom formation in heterocystic nitrogen-fixing cyanobacteria: The dependence on colony size and zooplankton grazing

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Abstract

The success of filamentous nitrogen (N)–fixing cyanobacteria in productive, transiently N-limited freshwaters reflects, in large part, their ability to produce and sustain the activities of specialized N-fixing heterocyst cells. Heterocyst production is variable, and the responses of planktonic cyanobacterial blooms to N limitation differ markedly among systems. Temporal variations in cyanobacteria colony size may determine both heterocyst production and sensitivity to top-down control by zooplankton grazers. We promoted the development of cyanobacterial blooms through phosphorus additions and trophic manipulations in freshwater ponds, to test the role of colony size structure in regulating N-fixer bloom development. The in situ growth and heterocyst production of *Anabaena* spp. were strongly linked to variations in colonial filament size. *Anabaena* spp. initially recruited to the water column as short and poorly heterocysted filaments, exhibiting low (mean = 0.09 d^{-1}) rates of population growth. The growth rate increased by more than fourfold (mean = 0.39 d^{-1}) with the onset of colony elongation and heterocyst production, which resulted in rapid seasonal build-ups of cyanobacterial cells ($>10^5$ cells ml^{-1}). Size-dependent growth was also important in determining the outcome of zooplankton-cyanobacteria interactions. In microcosm experiments, zooplankton consumers directly grazed on *Anabaena* spp. colonies, reduced the mean filament size, and reduced the efficiency with which heterocysts fixed nitrogen. These results suggest that colony size is a fundamental mechanism that link cellular physiological constraints to variations in trophic controls and the responses of aquatic systems to N limitation.

The feedbacks between nitrogen (N) availability and planktonic diazotrophic cyanobacteria blooms represent an important pathway for alleviating N limitation in aquatic systems (Redfield 1958; Schindler 1977). Blooms by N-fixing cyanobacteria, however, are not a universal response to N limitation (Vitousek and Howarth 1991). Nitrogen-fixing cyanobacteria blooms vary greatly in occurrence and timing in freshwaters and are paradoxically absent from the vast majority of N-limited estuaries and coastal seas (Howarth et al. 1988; National Research Council 2000). Experiments in freshwater and estuarine systems have indicated that grazing by large daphnids and calanoid copepods can be instrumental in inhibiting the success of N-fixing cyanobacteria (Elser et al. 2000; Marino et al. 2002). However, colonial cyanobacteria taxa are also well known to possess chemical, structural, and nutritional defenses that reduce grazing mortality and can act to facilitate cyanobacterial dominance (Porter and Orcutt 1980; Lampert 1987). The contrasting outcomes are striking, but our understanding of why consumer control of heterocystic cyanobacteria blooms is effective in some instances but not others remains limited.

Heterocystic cyanobacteria populations are known to exhibit wide seasonal variations in both colony size and the prevalence of heterocysts (Horne and Goldman 1972). Recent modeling efforts have predicted that the initial population size structure (the number of cells per colonial filaments and the prevalence of heterocystic N-fixing cells) is a central mechanism mediating the onset of N-fixation capacity and the subsequent ability to resist or overcome grazing mortality (Howarth et al. 1999). Because N fixation is restricted to heterocysts, cyanobacteria growth is predicted to proceed slowly before their development. A slow growth rate leaves cyanobacteria populations highly vulnerable to mortality from zooplankton grazing and competitive exclusion by other phytoplankton taxa. Only when filaments are

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able to increase sufficiently in length does heterocyst differentiation and N fixation proceed.

Although temporal variations in population size structure may provide a mechanistic link between organism-level constraints to N fixation and the variable responses of cyanobacteria blooms to trophic controls and N limitation among aquatic ecosystems, the extent to which the population size structure regulates the ability of heterocystic cyanobacteria to fix N and form biogeochemically significant blooms in situ is unclear. We characterized the seasonal phenology of heterocystic cyanobacteria bloom formation in phosphorus (P)–fertilized experiment ponds. This allowed us to test whether population growth, from initial recruitment to bloom formation, is dependent on thresholds in population size structure and N-fixation capacity. We further assessed the ability of zooplankton grazing to affect cyanobacterial population size structure and, in turn, mediate the rates of N fixation and population blooms.

Methods

To test the influence of cyanobacteria population size structure on heterocyst differentiation and population growth, we promoted blooms by P additions to four equal-sized ponds (36×36 m; mean depth = 2 m) at the Cornell Experimental Ponds Facility in Ithaca, New York (a full description of these facilities can be found in Hall et al. 1970). Previous studies have indicated that *Anabaena spiroides and Anabaena verrucosa* were responsive to P loading when herbivorous zooplankton densities were suppressed in these ponds (Schaffner et al 1994; Hairston et al. unpubl. data). Two rates of P loading were used. P was added as diluted H_3PO_4 twice weekly at rates of 18.7 mmol P m^{-3} yr^{-1} to two high-P ponds and 4.7 mmol P m^{-3} yr^{-1} to one medium-P pond. Our aim was to characterize population size structure and growth rate from the earliest point of appearance in the water column to bloom development without the confounding influence of zooplankton grazing. Therefore, we eliminated piscivorous fishes by Rotenone applications and restocked each pond with 1.8 kg of the zooplanktivore *Pimephales promela*, to suppress the abundance of large cladoceran and copepod grazers.

Phytoplankton and water chemistry samples were collected by lowering, capping, and withdrawing a 5.1 cm $\times$ 1.5 m PVC tube through the water column in the center of each pond. The depths of the ponds varied between 1.5 and 2 m through the summer, and care was taken to prevent the sampling tube from contacting the benthos. Phytoplankton samples (1 liter) were collected every other day and preserved in acid Lugol's solution (Wetzel and Likens 1991). Each pond was sampled for chlorophyll *a*, total P (TP), and soluble reactive P (SRP) on a weekly basis. Samples for Chl *a* were collected on pre-ashed (500°C for 4 h) 47-mm Whatman GF/F filters and stored frozen until analysis (within 1 week). The filtrate from the chlorophyll sample was collected in acid-washed 125-ml polyethylene bottles and refrigerated until analysis for SRP. Unfiltered water samples for TP analyses were stored frozen in acid-washed 125-ml polyethylene bottles. Water temperature at 0.5 and 1.5 m depths was measured every other day (between 0900 and 1100 h) using a YSI model 57 O_2/temperature meter. A portable open-diaphragm bilge pump was used to collect macrozooplankton samples from the center of each pond once per week (Pace 1984). To sample the water column effectively, a rigid 2-m (2.5 cm diameter) PVC intake pipe was raised and lowered from the surface to ~0.33 m from the bottom. Sixty liters of water were pumped and filtered through a 25-cm diameter funnel fitted with 70 μm mesh side panels. Collected macrozooplankton were preserved in 75% ethanol.

To characterize N-fixer populations at low, prebloom densities, each 1-liter phytoplankton sample was concentrated by gravitational settlement for 72 h in specially constructed 75×5 cm cylinders consisting of clear PVC tubing and a PVC gate valve at the 165-ml position. This allowed the large volume of overlying cell-free water to be discarded without disturbing the settled cells. Cyanobacteria filaments can become buoyant through the development of gas vacuoles (Walsby et al. 1991). Therefore, the overlying water (surface film and bulk volume) was periodically scanned for cyanobacteria and other phytoplankton cells. The phytoplankton cell density was extremely low in the overlying water (<10 cells ml^{-1}), and no cyanobacteria cells were observed. Comparisons of settled water with raw water samples further indicated that phytoplankton cells were concentrated conservatively (data not shown). Depending on the N-fixer cell density, samples were either further concentrated by settlement in 100-ml graduated cylinders or diluted to facilitate enumeration in a 5-ml counting chamber. The contents of the entire 5-ml counting chamber were counted. Thus, samples were concentrated by up to 182-fold, to facilitate counts of *Anabaena* population size and structure when their densities were at their lowest. All phytoplankton samples were counted at $\times$100 or $\times$400 magnifications on a Wild M-40 inverted microscope. Macrozooplankton samples were counted via stereomicroscopy. Chl *a* was determined fluorometrically following methanol extraction with a Turner Designs model 10 series fluorometer (Holm-Hansen and Rieman 1978). SRP was determined via the molybdate-blue method on a Beckman DU-50 spectrophotometer (Murphy and Riley 1962). TP samples were measured as SRP after persulfate oxidation via a microwave digestion method (Marino 2001; Marino and Kolberg unpubl. data).

We estimated mean net growth rates for *Anabaena* populations in each pond in relation to their population density and structure (i.e., mean filament size and heterocyst frequency). The net growth rate is the difference between the gross (cell growth and immigration) growth rate and the rate of mortality and export losses from a population (Tilzer 2000) and was calculated as

$$r = \ln(N_2/N_1)/t \quad (1)$$

where r is the net growth rate, N_2 is population size at time 2, N_1 is the population size at time 1, and t is the time, in days, between population size measurements. To control for the effects of temperature changes on population growth along the time series, net growth rates for each time interval were normalized to 20°C. We used temperature data collected concurrently with each phytoplankton samples and an

assumed Q_{10} value of 2 (Cole and Christian 2000) in the following equation:

$$r_{20} = r \times e^{0.0693\times(20-T)} \quad (2)$$

where r_{20} is net growth rate normalized to 20°C and T is the mean depth-integrated water temperature for the corresponding time period.

One phytoplankton sample from high-P pond A was also intensively counted, to characterize the relationship between colony size and heterocyst frequency (the number of heterocyst produced per colony) and the gross rate of growth (i.e., the growth rate in the absence of mortality). The gross growth rate of a population can be directly calculated if rates of net growth, mortality (due to consumption, pathogens, and natural cell death), immigration, and emigration are known. Quantifying all of these rates is difficult in natural phytoplankton populations (Tilzer 2000). We assessed relative gross growth rates by quantifying the proportion of cells in a colonial filament that are undergoing one specific stage of cell division at ×400 magnification. One stage in the division of cells in heterocystic cyanobacteria can be clearly identified by the initiation of cleavage in the inner cell membrane but before cleavage of the outer cell membrane (Fogg et al. 1973). A total of 245 individual filaments composed of 11,005 cells were randomly chosen and enumerated for the frequency of dividing cells and heterocysts. The absolute gross growth rate can be estimated from the frequency of dividing cells if the duration of specific cell-division stages is known (McDuff and Chisholm 1982). We did not attempt to measure the relative durations of the cell cycle. We used the frequency of cells observed at a specific stage of cell division as a relative index of the gross growth rate and assessed its variations across filaments of differing lengths and heterocyst prevalence.

We tested for the direct effects of macrozooplankton grazing on cyanobacteria colony morphology and N-fixation potential. Twelve 8-liter polycarbonate containers were filled with a 1 : 10 ratio mixture of 70 μm filtered water from high-P pond A, where *A. spiroides* was present at countable densities, and an adjacent unmanipulated pond, where heterocystic cyanobacteria were absent. Water from the two ponds was used to achieve an initial target density of 10 filaments ml^{-1}. A mixed assemblage of macrozooplankton (consisting of *Daphnia pulex, Diaptomus pallidus,* and *Bosmina* sp., in ratios of 8 : 1 : 3 individuals, respectively) was added to half of the vessels at a total density of 20 individuals l^{-1}. Zooplankton consumers can affect N-fixation rates indirectly by altering the absolute and relative supplies of N and P via remineralization (Roth and Horne 1981; MacKay and Elser 1998). To isolate the direct effects of grazing on cyanobacteria morphology and N fixation, 50 μmol L^{-1} NH_4^+ (NH_4Cl) and 100 μmol L^{-1} of PO_4^{3-} (H_3PO_4) were added to each container on the first and third day of the experiment, to saturate the nutrient needs of the cyanobacteria and therefore ensure that zooplankton excretion did not stimulate growth. Although N and P uptake kinetics can be quite variable among cyanobacteria taxa, the concentrations of NH_4^+ and PO_4^{3-} that we used exceed those associated with reported cyanobacteria V_{max} values for either nutrient (Smith and Kalff 1982; Reynolds 1987) and were thus considered to be above saturating concentrations. In addition, 2.4 mmol L^{-1} of $NaHCO_3$ was added to prevent potential CO_2 limitation of production in the closed containers (Marino 2001; Marino et al. 2003). The containers were covered with 30% shade cloth, to minimize possible photoinhibition effects, and were placed in floating frames in the surface waters of an adjacent pond. Each container was inverted every 10–12 h, to minimize phytoplankton sedimentation. The experiment ran for 5 d, to allow cyanobacteria populations to undergo approximately two generations of growth.

Table 1. Mean (SE) TP, Chl *a*, SRP, and macrozooplankton densities in the experimental ponds for the weekly samples taken between 24 Jun and 5 Aug 1999.

Pond	TP (μmol L^{-1})	SRP (μmol L^{-1})	Chl *a* (μg L^{-1})	Macrozooplankton (ind. L^{-1})
Med-P	1.67 (0.19)	0.19 (0.09)	9.3 (1.4)	0.6 (0.2)
High-P A	3.73 (0.69)	0.11 (0.01)	80.0 (43.71)	0.9 (0.2)
High-P B	2.36 (0.43)	0.21 (0.06)	39.9 (20.1)	0.8 (0.4)

After 5 d, a subsample of water (~500 ml) was collected from each container, gently filtered through 70-μm Nitex netting to remove macrozooplankton, and assayed for nitrogenase (the enzyme responsible for N fixation) activity via the acetylene reduction method (Flett et al. 1980, as modified by Marino 2001). Water subsamples were kept in the dark at ambient temperature and were immediately transported to our lab at Cornell University within 30 min of collection. Concurrently, phytoplankton and zooplankton samples were collected and preserved from all experimental containers. At the laboratory, duplicate 90-ml water subsamples of each treatment were added to 125-ml glass serum bottles to which 10 ml of acetylene-saturated deionized water was added. Acetylene was added in the dissolved phase to lower background levels of ethylene present in the acetylene source (Marino 2001). The samples were incubated in water baths in a temperate-controlled greenhouse for 4 h at 21°C (±1°C). Supplemental mercury vapor lighting was used at saturating irradiance of ~600 μ mol quanta, as determined from preliminary studies (Tartowski and Marino unpubl. data). The assay was stopped at 4 h by plunging the serum bottles into a large ice bath (Marino 2001). Ethylene concentrations were determined on a Varian model 3300 gas chromatograph.

Results and discussion

The additions of P resulted in a range of elevated water-column TP and Chl *a* concentrations in the experimental ponds (table 1). The average TP and Chl *a* concentrations encountered are characteristic of eutrophic systems and are similar to values found in other systems where freshwater planktonic heterocystic cyanobacteria blooms are common (Horne and Goldman 1972; Findlay et al. 1994). Concentrations of SRP were maintained at moderate levels (mean among ponds = 0.17 μmol L^{-1}). Such values are at or above reported half-saturation constants for SRP in colonial cyanobacteria (Tilman et al. 1982). The addition of the zooplanktivore *P. promela* resulted in strong suppression of ma-

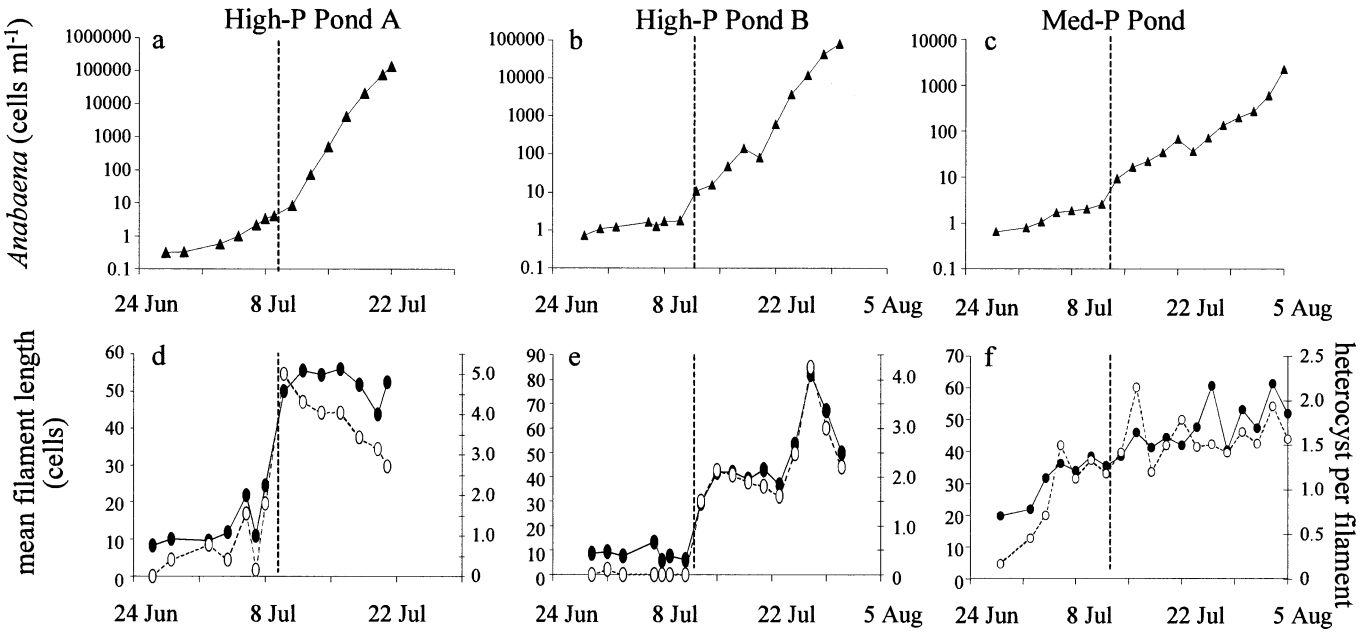

Fig. 1. Time series of *Anabaena* spp. population (a, b, and c) growth and (d, e, and f) structure in ponds subject to P additions and the suppression of macrozooplankton grazing pressure. Mean filament length and heterocyst prevalence are white and black circles, respectively. The vertical dashed line marks the transition point between slow rates (0.19, 0.02, and 0.07 d^{-1} for high-P pond A, high-P pond B, and medium-P pond, respectively) and rapid rates (0.65, 0.33, and 0.20 d^{-1} for high-P pond A, high-P pond B, and medium-P pond, respectively) of population growth and the corresponding transitions in population structure.

crozooplankton abundance. Combined *Daphnia* and *Diaptomous* densities averaged <1 individual L^{-1}.

Colony size structure and population growth—Bloom development in *Anabaena* populations showed strong dependence on colony size. Initial populations were composed of short and heterocyst-deficient filaments. The mean colony size ranged from 15 cells for *A. spiroides* in the two high-P ponds to 30 cells for *A. verrucosa* in the medium-P pond, with all populations exhibiting <1 heterocyst per filament. *Anabaena* populations grew slowly during this prebloom period (defined arbitrarily as <10 cells ml^{-1}), exhibiting time-averaged rates of 0.02, 0.07, and 0.19 d^{-1} (Fig. 1a–c). These low rates represent net increases in population size of 2–21% d^{-1}. Growth rates were not constant over time, however, as evidenced by nonlinear increases in the log population size. Mean net growth rates increased to 0.33, 0.20, and 0.65 d^{-1} during a subsequent phase of rapid population growth and allowed *Anabaena* populations to reach peak bloom densities of >100,000 cells ml^{-1} within 4 weeks of their initial appearance.

For each *Anabaena* population, the increase in the growth rate was abrupt and coincided with marked increases in mean filament length and heterocyst frequency (Fig. 1d–f). For *A. spiroides*, increases in population growth rates were temporally matched with increases in colony size to >50 cells per filament. Filament elongation and increases in growth rate occurred more gradually over the course of the season for *A. verrucosa*. Filament elongation was tightly coupled with heterocyst development, which resulted in increased heterocyst prevalence to >4 and >2 heterocysts per filament by the end of the time series in *A. spiroides* and *A. verrucosa*, respectively (Fig. 1d–f).

Filament elongation and heterocyst acquisition had important consequences for the timing and strength of cyanobacteria blooms. Because the minimum time needed to reach peak biomass is a reciprocal function of growth rates, increases from slow to rapid growth considerably shortens the period of favorable environmental and biotic conditions needed for bloom development. At initially low growth rates, the time to observed peak biomass would range 2–19 months for the three populations in the study. With growth rate increases, the actual time to peak biomass observed decreased to between 25 and 37 d. Seasonal changes in phytoplankton dominance suggest that individual taxa face restricted phenologic windows when conditions such as temperature, stratification, nutrient availability, and grazing pressure are favorable for blooms (Sommer et al. 1986). The benefits of filament elongation, heterocyst production, and increased growth can thus be substantial in mediating cyanobacteria success, particularly in systems where the length of the growing season is short, grazing mortality is important, or seasonal fluctuations in biotic and abiotic conditions are sufficiently great.

Colony morphology and gross growth rate—Anabaena population size structures before and during bloom development indicate that colony size is important in regulating the heterocystic cyanobacteria growth. However, net changes in population growth can reflect changes in the gross growth rate, in addition to variations in immigration, sedimentary losses, and mortality rates. The control of the *Anabaena*

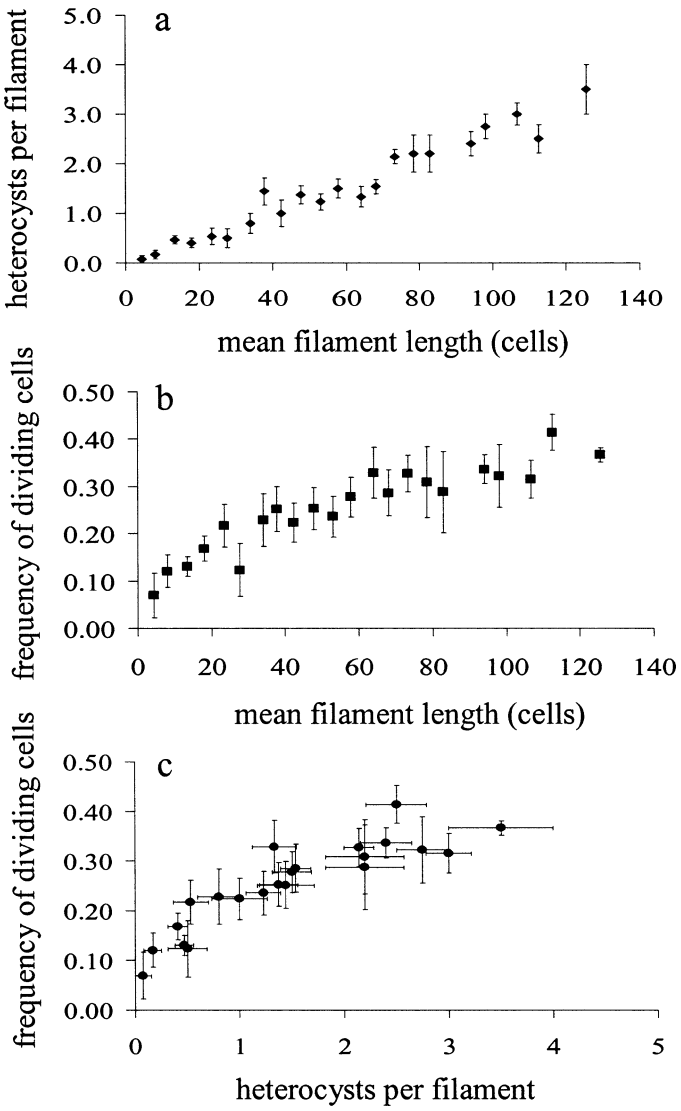

Fig. 2. Results from intensive enumeration of mean filament length and heterocyst prevalence for *A. spiroides* colonies from high-P pond A. Relationship among (a) the frequency of dividing cells, (b) mean filament length, and (c) heterocyst prevalence. Filament size classes were divided at five-cell increments. Means and SEs for each filament size class are shown.

gross growth rate by colony size is most directly supported by the relationships among filament length, heterocyst prevalence, and the frequency of dividing cells. The prevalence of heterocyst(s) in a colony increased linearly with the mean filament length (Fig. 2a), which indicates that the ratio of photosynthetic cells to heterocysts was conserved at ~35 cells per heterocyst for *A. spiroides.* This suggests that *Anabaena* colonies are able to add more heterocysts only by increasing the filament length and not simply by reducing the ratio of photosynthetic to heterocystic cells. The dependence of heterocyst production on filament size also indicates that short filaments are limited in their ability to acquire heterocysts. For example, more than one-half of the colonies that were <20 cells in length actually lacked heterocysts, and heterocyst frequency averaged <1 until filaments were >40 cells in length (Fig. 2a).

The extent to which colonial filaments are able to increase in length and produce heterocysts appears to have direct consequences for gross rates of population growth. Colonies that are short or lack heterocysts have marked reductions in the gross growth rate, as shown by the frequency of dividing cells (Fig. 2b,c). In contrast, colonies that are longer and have produced one or more heterocysts realize more rapid gross rates of growth. Although the frequency of dividing cells only describes relative and not absolute estimates of growth, the consequences of filament size and heterocyst frequency were nevertheless important. For the largest colonies, 37% of the cells were undergoing cell division (Fig. 2b). This rate dropped to only 7% for filaments that averaged five cells in length (Fig. 2b). Similarly, the gross growth rate increased asymptotically with heterocyst frequency (Fig. 2c). Because the ratio of photosynthetic cells to heterocysts is conserved (Fig. 2a), the increased growth rate observed for filaments with multiple heterocysts suggests that the N fixation rate per heterocyst and/or growth efficiency is higher for longer cyanobacteria colonies.

Colony size–dependent growth is consistent with basic constraints on the initiation and maintenance of N fixation in heterocystic taxa. Nitrogenase, the enzyme responsible for N fixation, is irreversibly damaged by the in vivo presence of O_2. Heterocysts allow N fixation and oxygenic photosynthesis to be spatially segregated along filamentous colonies (Hill 1988; Gallon 1992). However, because heterocysts lack photosystem II, the high energetic costs involved in N fixation must be met by the translocation of fixed carbon from photosynthetic cells along the colonial filament (Turpin et al. 1985). As a consequence, filaments with only a small number of photosynthetic cells may face deficits in the fixed-carbon supply needed to sufficiently support the energetic demands of N fixation.

Zooplankton: heterocystic cyanobacteria interactions—The results of the zooplankton grazing experiment indicate that the size structure of cyanobacteria populations can also alter by consumers. *A. spiroides* exhibited moderate growth rates during the zooplankton grazing assay when they were incubated under saturating concentrations of NH_4^+ and PO_4^{3-}, increasing in cell density by fivefold in 4.5 d (r = .37 d^{-1}). Net population growth was not affected by the presence of zooplankton grazers, as evidenced by parity in the final cell densities between the treatments with or without zooplankton (Fig. 3a). In contrast, zooplankton grazing resulted in significant reductions in mean filament lengths (Fig. 3b). The reductions in mean filament length appeared to reflect physical filament breakage—*Anabaena* populations were characterized by a greater number of short filaments in the presence of zooplankton grazers (Fig. 3c). Modification of the colony size by zooplankton grazing resulted in direct reductions in N-fixation rates in *A. spiroides* populations (Fig. 3d). The reductions in N-fixation rates were not due to reductions in heterocysts alone, and heterocyst densities did not differ significantly between treatments (Fig. 3e). Instead, zooplankton-mediated reductions in N fixation appear to reflect decreases in heterocyst-specific efficiencies in N fixa-

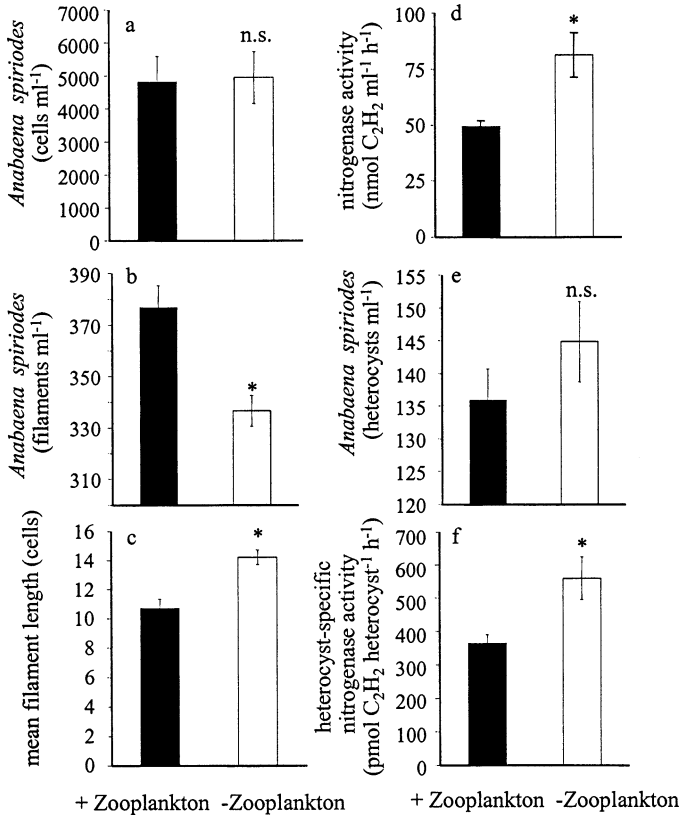

Fig. 3. Effects of zooplankton grazing on *An. spiroides*. (a) Cell density, (b) filament density, (c) mean filament length, (d) volumetric nitrogenase activity, (e) heterocyst density, and (f) heterocyst-specific nitrogenase activity. Means and SEs are depicted for each treatment ($n = 6$). * denotes significance at the $p < .05$ level (Student's *t*-test); ns = not significant

tion. In the presence of grazers, heterocysts that are found on shortened filaments showed a 40% decline in the rate of N fixation (Fig. 3f). By reducing filament length, zooplankton grazing may act to preempt cyanobacteria blooms by suppressing N fixation and cyanobacterial growth.

Zooplankton grazing can indirectly suppress cyanobacteria N fixation by increasing the absolute supply of N or by increasing its availability relative to P (Roth and Horne 1981; MacKay and Elser 1998). The addition of NH_4^+ and PO_4^{3-} to the grazing assay nonetheless allowed us to isolate the direct effects of zooplankton grazing on colony size and N fixation. An increased NH_4^+ supply can decouple *Anabaena* growth from rates of N fixation (Layzell et al. 1985). The suppression of N fixation, but not *Anabaena* biomass, by zooplankton grazers may reflect an artificial enhancement of cyanobacteria growth toward N-replete rates. Although reductions in cyanobacteria filament size by zooplankton grazing have been reported elsewhere (Schaffner et al. 1994; Turner et al. 1998), our study appears to be the first to demonstrate that incidental zooplankton grazing can directly inhibit rates of heterocystic cyanobacteria N fixation by reducing colony size. It should be noted that zooplankton-mediated nitrogen remineralization and direct colony size reduction are not mutually exclusive mechanisms and highlight the potential for trophic processes to counter simple biogeochemical feedbacks between N limitation and N fixation.

Ecological implications—High species richness is a common attribute of many phytoplankton communities (Hutchinson 1961). In contrast, seasonal changes in numerical and biomass dominance are often restricted to a much smaller subset of species within the community (Sommer 1989; Sterner 1989). This disparity between species richness and evenness suggests that growth and mortality at low individual population size serve as key bottlenecks for the eventual functional composition of a community. For heterocystic cyanobacteria, our results indicate that bloom initiation can be mediated by fundamental changes in population size structure. Before the development of heterocysts, the slow growth rates exhibited by short initial colonies are consistent with low surface area : volume ratios evident in short colonies of large spherical cells (Smith and Kalff 1982). In contrast to simple allometric predictions, larger colonies did not show reductions in growth rates. The transition into long, fully heterocysted filaments is, thus, a critical step in the ability of cyanobacteria to fix N and to increase in population size relative to other phytoplankton taxa.

Our results also suggest that heterocystic cyanobacteria blooms may, at times, exhibit a disproportionate sensitivity to zooplankton grazing relative to other phytoplankton taxa. On recruitment, short, slow-growing filaments are less likely to overcome even incidental rates of grazing mortality. Grazing losses that reduce or constrain filament elongation can further act to inhibit N-fixation capacity and cyanobacteria growth. In contrast, phytoplankton taxa whose growth are not size structure dependent are not expected to show reductions in the gross growth rate as a direct consequence of grazing. In estuarine mesocosms, we have found a similar sensitivity of heterocystic cyanobacteria size structure and growth to direct control by macrozooplankton grazing (Chan 2001). Grazing by *Acartia tonsa* prevented filament elongation and bloom formation by heterocystic cyanobacteria while exerting little control on the overall phytoplankton biomass.

Such feedbacks between filament size and sensitivity to grazing losses may help explain the disparate responses of cyanobacteria blooms to trophic control. Carpenter (1989) proposed that the ability of heterocystic cyanobacteria to successfully invade and dominate productive systems may be dependent on seasonal asynchronies in the timing of cyanobacteria recruitment and zooplankton growth. Divergent outcomes in cyanobacteria-consumer interactions may be reinforced where cyanobacteria are highly vulnerable to grazing mortality at the time of initial recruitment but are able to fix N and outgrow consumer pressure when a sufficiently large filament size is attained. Working in ponds at the same facility as the present study, Schaffner et al. (1994) noted that the early onset of zooplankton growth appeared to be instrumental in the suppression of N-fixer dominance. In contrast, where zooplankton populations developed later in the growing season, heterocystic cyanobacteria became dominant components of the phytoplankton community.

The seasonal dominance of N-fixing cyanobacteria has been attributed to factors such as grazing resistance, buoy-

ancy, diazotrophy, increased efficiency in dissolved inorganic carbon uptake, and increased competitive ability at high temperatures or low light (Hyenstrand et al. 1998). Accordingly, the strength and timing of cyanobacteria blooms can reflect complex pathways and feedbacks among recruitment, nutrient availability, food-web structure, and physical conditions (Elser 1999). Where the ability to fix nitrogen or grow rapidly relative to competitors and mortality terms is central, temporal changes in the population size structure may be a critical determinant and predictor of cyanobacteria success. Consideration of population size structure may thus provide a basic mechanism for integrating our understanding of physiological, trophic, and biogeochemical constraints to heterocystic cyanobacteria blooms among systems.

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