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Small-scale shear effects on heterocystous cyanobacteria

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Abstract

Planktonic, filamentous, heterocystous cyanobacteria form blooms in certain nitrogen-limited ecosystems but are absent or rare in others that seem to have suitable environmental conditions. We tested the hypothesis that small-scale shear affects physiological activities and morphology of heterocystous cyanobacteria in high turbulence environments. Using Taylor-Couette flow to generate small-scale shear, we conducted one set of experiments on cultures of two strains of Baltic Sea *Nodularia* and a complementary set of experiments using natural Baltic Sea phytoplankton assemblages. Experiments were run at various shear durations (1–72 h) and levels (2.2–18 s^{-1}), corresponding to energy dissipation rates in the upper mixed layer from moderate to strong winds. The effect of shear on nitrogenase activity (NA), CO_2 fixation, pH, dissolved inorganic carbon, and cyanobacterial filament length was tested. Results from the culture experiments showed that shear had a negative effect on NA and CO_2 fixation for both *Nodularia* strains and that filament length decreased for one of them. The lower limit for shear effects on NA and CO_2 fixation appeared to be less than 2.2 s^{-1}. Results from the experiments on natural phytoplankton assemblages from the Baltic Sea showed that both CO_2 fixation (reflecting cyanobacterial photosynthetic activity) and NA decreased in response to shear. However, shear did not affect CO_2 fixation in the <2-μm size fraction. *Aphanizomenon* and *Anabaena* filaments fragmented under shear rates (2.2 s^{-1}, 12 h) that did not affect *Nodularia* filament length. These results suggest that small-scale shear imposes a control on cyanobacterial activity and morphology and that this control appears to be genus and species specific.

Cyanobacterial blooms are often observed during calm, vertically stratified conditions (Reynolds and Walsby 1975; Paerl 1988; Steinberg and Hartmann 1988). Several factors have been proposed to explain the onset of these surface blooms. These include positive cell buoyancy, enhanced growth due to higher light and water temperature at the surface, and decreased inorganic nitrogen levels during stratified conditions, which permit certain N_2 fixing cyanobacteria to outcompete other phytoplankton. Cyanobacterial blooms are often terminated by mixing, which can change light and nutrient availability due to vertical transport of organisms in large scale eddies. Small-scale shear has also been proposed as a control on phytoplankton blooms, and several previous studies have addressed this question. Experiments on dinoflagellates (Thomas and Gibson 1990; Juhl et al. 2000) and a green alga (Hondzo and Lyn 1999) have shown decreased growth rates under natural shear levels. However, work on diatoms has suggested that shear may have beneficial effects due to increased nutrient availability (Pasciak and Gavis 1975). Effects of shear on filamentous cyanobacteria have been estimated within mixed phytoplankton communities in seasonal field studies and enclosure experiments. In these experiments, shear has either increased (Steinberg and Tille-Backhaus 1990) or decreased/arrested (Reynolds et al. 1983; Petersen et al. 1998) cyanobacterial abundance or activity. Genus-specific shear effects have also been reported for cyanobacteria; the nonheterocystous filamentous *Oscillatoria* (*Limnothrix*) appears to be more shear tolerant than the het-

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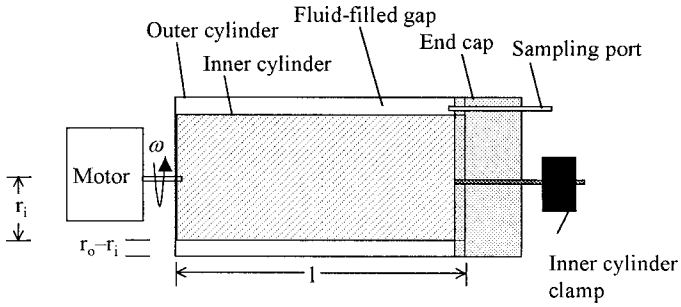

Fig. 1. Schematic diagram of Couette chamber used in the shear experiments. Couette dimensions are l = 26.5 cm, r_i = 4.52 cm, $r_o - r_i$ = 1.41 cm, and the outer cylinder rotation rate is ω.

erocystous *Anabaena* (Reynolds et al. 1983; Steinberg and Tille-Backhaus 1990).

The effect of shear on heterocystous cyanobacteria is unclear (Paerl et al. 1995; Howarth et al. 1995). The results from previous studies are divergent, with Howarth et al. (1993) suggesting no effect and Kucera (1996) indicating a negative effect. Resolving this question is important because these phytoplankton form toxic blooms and can be a significant water quality concern (Edler et al. 1985; Codd et al. 1994; Harding et al. 1995). Moreover, heterocystous cyanobacteria include genera that are adapted to a wide range of salinities and therefore have the potential to substantially expand their present geographic range (Moisander and Paerl 2000). One explanation for their limited geographic range is that shear is controlling their growth in high shear environments that are otherwise suitable.

In this study we systematically tested the shear response of heterocystous cyanobacteria with experiments on cultures and performed a complementary set of experiments on natural phytoplankton assemblages composed primarily of heterocystous cyanobacteria. The culture experiments tested the shear response in physiological activities and filament length of *Nodularia,* the dominant bloom-forming heterocystous cyanobacterium in the Baltic Sea. Two strains were examined to determine whether shear responses are species specific. We also carried out shipboard experiments to investigate shear effects on phytoplankton communities during summer blooms in the Baltic Sea. These blooms were primarily composed of the heterocystous cyanobacteria *Nodularia, Anabaena,* and *Aphanizomenon.* These experiments were designed to address the question of whether shear conditions might select against or favor different cyanobacteria and whether natural cyanobacterial populations behave similarly to laboratory strains in terms of shear response. Collectively these experiments were used to determine the shear responses of heterocystous cyanobacteria.

Materials and methods

Experimental apparatus—A controlled shear environment was generated using a set of three narrow gap Couette chambers (Pasciak and Gavis 1975; Thomas and Gibson 1990) constructed of clear Plexiglas (Fig. 1, *see* Kucera 1996 for details). Each chamber consisted of two horizontally aligned (van Duuren 1968) concentric cylinders, where the inner cylinder was fixed and the outer cylinder was rotated with an electric motor. Two of the chambers were rotated, while the remaining chamber served as a control (exceptions are noted). Each chamber had a gap volume of 600 ml. The end cap was stainless steel (rotated cylinders) or gray PVC (control). Each end cap had a port through which a narrow tube could be temporarily inserted into the chamber to obtain subsamples. In later experiments, the whole chamber was opened for subsampling to avoid the possibility that flow inside the sampling tube would harm the cells. During the shear experiments, constant cool-white light was applied to the chambers at ca. 45 μmol m^{-2} s^{-1} (cultures), or 50–90 μmol m^{-2} s^{-1} (field experiments). Culture experiments were carried out at room temperature, and the shipboard experiments were conducted in a climate-controlled room onboard, kept at the local surface water temperature.

Experimental design—The culture experiments used two *Nodularia* strains isolated from a Baltic Sea bloom (*see* Moisander and Paerl 2000 for details). Strains UP16a and FL2f used in the culture experiments were identified as *N. sphaerocarpa* (Lehtimäki et al. 2000) and *N. spumigena,* respectively. *Nodularia* strains were grown in Z8 medium with combined nitrogen omitted and salinity adjusted to 10 psu. Cultures were grown under constant air bubbling and light of ca. 50 μmol m^{-2} s^{-1} until late exponential growth phase. The day prior to each shear experiment the culture was diluted with fresh medium (ca. 1 : 1). Throughout each of the experiments, subsamples were collected from the chambers every few hours. Experiments were usually continued for 24 h (up to 72 h). Triplicate aliquots of each subsample were assayed for nitrogenase activity (NA), CO_2 fixation, and chlorophyll *a* (Chl *a*). In the culture experiments, dissolved inorganic carbon (DIC) and pH were also measured. Cyanobacterial filament lengths were microscopically determined from subsamples preserved at the end of the experiment. A culture flask kept under the light bank with the chambers served as an additional unmanipulated control for filament length calculations for strain FL2f. Shear exposures in culture experiments are presented in Table 1. Shipboard experiments (hereafter referred to as field experiments) used natural phytoplankton assemblages collected during July–August of 1997, 1998, 1999, and 2000 in the Baltic Sea, onboard the RV *Aranda* (Finnish Institute of Marine Research). The study area varied slightly from year to year, but was located within the entrance to the Gulf of Finland and the Northern Baltic Proper. Table 2 shows the water sampling methods, shear exposures, and number of experiments in the field studies.

Analytical methods—Nitrogenase activity (nitrogen fixation) was measured using the acetylene reduction assay (ARA) (Burris 1972). Samples were incubated from 1 to 1.5 h (cultures) or 4 h (field) under the same light bank used for the chambers. Ethylene production was measured as previously described (Moisander and Paerl 2000) and normalized to the Chl *a* concentration. CO_2 fixation was measured using a ^{14}C incorporation method as described by Parsons et al. (1984), using the same incubation conditions as for NA. Radioactivity in the culture experiments was measured as in

Table 1. Computed Couette flow parameters, shear level grouping, and number of culture experiments with *Nodularia* strains UP16a and FL2f. Shear level groups were chosen as shown below to have a close to similar number of observations in each group for the ANOVAs.

Rotation rate N(rpm)	Re_{gap}(-)	Shear rate (s^{-1})	Dissipation rate ($m^2\ s^{-3}$)	Shear level	Number of experiments UP16a/FL2f
3	116	2.2	4.66×10^{-6}	Low	3/2
5	193	3.6	1.29×10^{-5}	Low	3
7	270	5.0	2.53×10^{-5}	Medium	3/1
10	385	7.2	5.17×10^{-5}	Medium	2
12	462	8.6	7.45×10^{-5}	High	1
13	501	9.4	8.74×10^{-5}	High	1
15	578	10.8	1.16×10^{-4}	High	1/1
17	655	12.2	1.49×10^{-4}	High	1
19	732	13.7	1.87×10^{-4}	High	1
20	771	14.4	2.07×10^{-4}	High	1
25	963	18.0	3.23×10^{-4}	High	1

Moisander and Paerl (2000) and in the field experiments by following guidelines for the Baltic Monitoring Programme (BMP) (Baltic Sea Environ. Proc. 27D 1988). Dark incorporation was subtracted from light treatments, and CO_2 fixation per ml was normalized to Chl *a*. DIC was measured using a LI-COR infrared analyzer. DIC in the field samples was measured (in 1998) from samples stored with mercuric chloride (50 μM final concentration), or calculated from pH, salinity, and temperature (in 1997, 1999, and 2000). Photosynthetic activity was also measured during the field studies by conducting the ^{14}C incubation inside the chambers (hereafter referred to as ''hot experiments''). $NaH^{14}CO_3^-$ was added to the sample in each chamber (final concentration 0.066 to 0.133 μCi ml^{-1}), and subsamples were filtered over time. The samples in the ''hot experiments'' were size fractionated by first filtering through 20-μm mesh plankton net, next through a 2-μm polycarbonate filter, and finally through a GF/F filter to examine which fractions of the community were responsible for incorporating the ^{14}C. The radioactivity on these filters was measured as described above. For Chl *a* measurement from cultures, a 5-ml sample from the ARA was analyzed as in Moisander and Paerl (2000), and for the field experiments, a BMP protocol was followed (Baltic Sea Environ. Proc. 27D 1988). Samples for microscopy were preserved in formalin (cultures) or Lugol solution (field samples). Filament lengths were determined using a Nikon Eclipse 800 phase contrast microscope, at 200× magnification. For each sample, a minimum of 50 filaments of each species (an individual cell or several cells together in a filament) was measured. In the field experiments the phytoplankton community (cells of ca. >10 μm in size) was also determined. The Utermöhl (1958) technique, Sedgewick-Rafter counting cell, or an ordinary microscope slide was used for counts, depending on the cell density. Cell numbers were converted to carbon according to Edler (1979).

Statistical analyses—The effect of shear on NA, CO_2 fixation, DIC, pH, and filament length was tested using two-tailed, paired *t*-tests. The values used in the *t*-test (one value for shear treatment and control treatment in each experiment) were calculated as follows. First, the mean in each chamber was calculated for all the values measured during the experimental time period (included all time point measurements). Second, the mean for the replicate chambers (kept in the same shear) was calculated. The *t*-test was then run separately for each variable and strain. Field data for NA, CO_2 fixation, and filament length were analyzed in the same way, separately for each study year (1999–2000 filament length data were combined because identical shear conditions were used).

To determine whether the level of shear influenced the magnitude of the difference between shear and control conditions, further analysis was done for those variables, which according to the *t*-tests proved to be significantly different. To compare the effect of different shear levels, the data were grouped into low, medium, and high shear treatments (Table 1). The magnitude of the shear effect among groups was compared using a one-way analysis of variance (ANOVA). The dependent variable in the ANOVA was the difference between control and shear. The effect of different shear lev-

Table 2. Experimental design of the field experiments. Net samples were diluted with GF/F-filtered seawater from the same site.

Year	Shear rate (s^{-1})	Duration (h)	Sampling net (μm)	Number of experiments with measurements of				
				CO_2 fix.	Fil. length	Community	NA	Total
1997	7.2–14.4	24	10	4 + 3 × ''hot''	0	3 × ''hot''	*	7
1998	10.8	24	10	5	4	5	*	5
1999	2.2	12–24	500	5	5	5	*	5
2000	2.2	12	100	3	8	5	4	9

* NA values were measured but below detection.

els was also compared using a repeated measures ANOVA (proportion of control as the dependent variable). In this test, the variability from the time-course measurements was incorporated. Prior to ANOVAs and *t*-tests, homogeneity of variances were tested using a Cochran's C-critical test ($\alpha = 0.05$). When heterogeneous, data were square root or $\log_{10}$ (constant + value) transformed to satisfy the homogeneity assumption. We used $\alpha < 0.05$ to indicate statistically significant differences.

Flow characterization—We investigated the flow conditions in our experiments both theoretically and using flow visualization. Assuming laminar flow, the velocity profile for circular Couette flow (with the inner cylinder fixed and the outer cylinder rotating) can be determined by integrating the Navier-Stokes equations (e.g., Kundu 1990):

$$u(r) = \frac{\omega_o r_o^2 (r^2 - r_i^2)}{(r_o^2 - r_i^2) r} \tag{1}$$

where the rotation rate of the outer cylinder $\omega_o = 2\pi N/60$, N is the revolutions per minute, and r_i and r_o are the inner and outer radii of the Couette chamber. At high enough rotation rates, this type of Couette flow can become turbulent and Eq. 1 would not apply. The flow stability can be estimated using a Reynolds number appropriate for Couette chambers (Taylor 1936b):

$$\mathrm{Re}_{\mathrm{gap}} = \frac{\omega_o r_o (r_o - r_i)}{\nu} \tag{2}$$

where ν is the kinematic viscosity. Taylor (1936a) showed that the onset of turbulence in circular Couette flow occurs at a critical Reynolds number, $\mathrm{Re}_{\mathrm{gap\text{-}critical}}$, and is strongly dependent on the ratio of the gap thickness to the outer radius $(r_o - r_i)/r_o$. We estimated $\mathrm{Re}_{\mathrm{gap\text{-}critical}}$ for our Couette chambers using Taylor's (1936*a*) fig. 11. With $(r_o - r_i)/r_o = 0.135$ for our Couette chambers, $\mathrm{Re}_{\mathrm{gap\text{-}critical}} \approx 12{,}500$. This value is more than an order of magnitude greater than the maximum $\mathrm{Re}_{\mathrm{gap}}$ used in our experiments (Table 1), indicating that our experiments were well within the laminar flow regime.

A series of flow visualization experiments were conducted to confirm that our Couette flows were laminar and to check for end wall effects (described in detail by Coles 1965). We used a solution of reflective rheoscopic fluid (Kalliroscope) (Matisse and Gorman 1984) at 2% concentration in water. The reflective particles in the solution orient parallel to the shear, and flow patterns can be readily visualized (Savas 1985). To study the effects of aggregates on the flow, we conducted a second set of dye experiments, but this time instead of pure water we added the same concentration of *Nodularia* in growth medium as in the experiments. Flow patterns were documented using a Nikon Coolpix 950 digital camera. At all tested flow speeds (2.2–18 s^{-1}), the flow with water only was clearly laminar. End wall effects were minor and apparent only at the highest flow speeds. With the laminar assumption verified, the shear stress in cylindrical coordinates can be defined as

$$\tau(r) = \mu\left[r\frac{\partial}{\partial r}\left(\frac{u}{r}\right)\right] \tag{3}$$

where μ is the dynamic viscosity. Substituting Eq. 1 into Eq. 3 and simplifying gives an expression for Couette flow shear stress

$$\tau(r) = \frac{2\mu\omega_o r_o^2 r_i^2}{(r_o^2 - r_i^2) r^2} \tag{4}$$

The mean shear stress $\bar{\tau}$ is found by integrating Eq. 4 over the gap distance and dividing by the gap width

$$\bar{\tau} = \frac{2\mu\omega_o r_o r_i}{(r_o^2 - r_i^2)} \tag{5}$$

A number of useful fluid mechanical quantities, needed to relate Couette flows to natural flows, follow from Eq. 5. These include the mean shear rate $\bar{\gamma} = \bar{\tau}/\mu$ and the dissipation rate $\varepsilon = \nu(\bar{\gamma})^2$. These parameters were computed for each of the Couette experiments and are shown in Table 1.

Comparison of Couette flows with natural flows—We estimated natural turbulence dissipation rates to compare with dissipation rates in our Couette experiments. Assuming a homogeneous fluid and that the only forcing is surface wind stress, a simple expression can be obtained for the dissipation rate (e.g., Csanady 1984; Soloviev et al. 1988; MacKenzie and Leggett 1993; Anis and Moum 1995):

$$\varepsilon = u_*^3/\kappa z \tag{6}$$

where u_* is the friction velocity, $\kappa = 0.4$ is von Karman's constant, and z is depth. The assumptions in this approach are reasonable here since wind forcing often dominates the upper mixed layer (~1–15 m) where *Nodularia* are found, and astronomical tides are negligible in the Baltic (Kullenberg and Jacobsen 1981), so bottom boundary layer turbulence is relatively weak in the surface layers. However, recent observations (Agrawal et al. 1992; Anis and Moum 1995; Terray et al. 1996) have shown that breaking waves can increase ε by an order of magnitude over the theoretical predictions from Eq. 6. With this in mind, we view Eq. 6 as a reasonable estimate of what is found in nature, although actual values can be substantially higher or lower. For our estimates, the friction velocity $u_* = \sqrt{\tau_o/\rho_w}$ was computed using a water density $\rho_w = 1{,}003$ kg m^{-3}, which is representative of Baltic Sea surface waters during the summer bloom season (Kullenberg and Jacobsen 1981). A quadratic law was used for the surface wind stress $\tau_o = \rho_a C_D W_{10}^2$, where C_D is a drag coefficient and W_{10} is the wind speed 10 m above the sea surface. An air density of $\rho_a = 1.2$ kg m^{-3} was used, and C_D values were specified using Yelland and Taylor (1996). Dissipation rate profiles corresponding to various wind speeds were compared to dissipation rates in our Couette experiments (Fig. 2).

Meteorological and physical measurements—Temperature and wind data were analyzed from the beginning of July until the end of the cruise each year (until 26 July to 18 August) (Table 3). Wind speeds were measured every 3 h at Bogskär (59°30′North, 20°21′East) weather station (Finnish Meteorological Institute), and water temperatures were recorded every 1.5 h at a wave buoy (FIMR) in the Northern Baltic Proper (59°15′North, 21°00′East). Cruises were con-

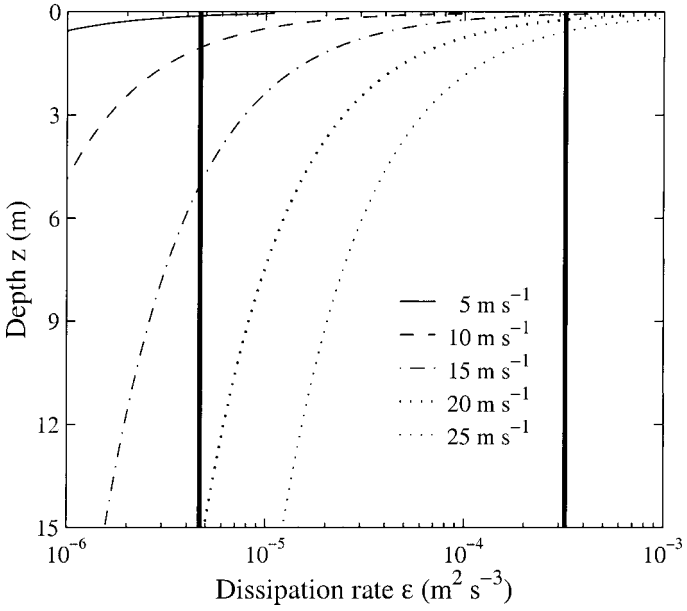

Fig. 2. Dissipation rate profiles computed for various wind speeds in the 15-m surface layer. Bold vertical lines bracket the range of dissipation rates from the Couette chamber experiments (*see Table 1*). The dissipation rate is a strong function of depth.

ducted within 200 km from both of these sites. Wind conditions were relatively similar during the years 1997–2000, with 1998 having the highest mean recorded wind speeds. Mean surface water temperatures were approximately 3°C lower in 1998 and 2000 than in 1997 and 1999. Upper mixed layer depths were determined from temperature and salinity profiles of the water column during cruises in July–August (Table 3). The years with highest water temperatures (1997 and 1999) had the shallowest mixed layer depths.

Results

Culture experiments—Measurements of NA, CO_2 fixation, and pH from each of the experiments were pooled and plotted as proportion of control in Fig. 3. In the vast majority of cases, NA rates decreased under shear relative to control. The difference in NA between shear and control treatments was significant for both *Nodularia* strains (Table 4). NA decreased significantly more under high shear than under medium and low shear (one-way ANOVA, Fisher's test for pairwise comparisons, Table 5). The effect of shear duration on NA is shown in Fig. 4. After 1–2 h of shear, there was either very little effect or an increase in NA. However, at all times greater than 4 h, shear had a clear negative effect on NA. There were differences between the shear levels as well, with the higher shear levels having a stronger negative effect on NA than lower levels. The differences between shear durations and interaction between shear levels and durations were both statistically significant (Fig. 4, Table 6).

CO_2 fixation rates also generally decreased under shear, and this effect was significant (Fig. 3, Table 4). Occasional increases in CO_2 fixation were seen throughout the experimental time period, but these increases were mostly during the first 10 h. Interaction between shear duration and shear level had a significant effect on CO_2 fixation (Table 6); this relationship was driven by an initial increase in CO_2 fixation under high shear.

Table 3. Wind speed, water temperature, and upper mixed layer (UML) depth in the Baltic Sea during the bloom season of the study years. Data from 1 July until the end of the cruise for each year are included in the temperature and wind speed data. Wind speeds were recorded every 3 h; temperature was measured every 1.5 h. Cruises ended in between 26 July and 18 August. UML depths were determined from CTD casts on the cruises in July–August each year (*n* = number of CTD profiles analyzed).

	Wind speed ($m\ s^{-1}$)			Water temp (°C)		UML depth (m)		
	Mean	Max	Sdev	Mean	Sdev	Mean	Sdev	*n*
1997	4.8	13.7	3.0	17.9	1.7	11.1	4.7	23
1998	6.6	16.3	3.6	14.8	1.0	14.3	2.0	99
1999	5.8	12.3	2.8	18.4	1.5	9.2	3.1	130
2000	6.1	19.0	3.1	15.5	1.3	17.8	3.5	37

The measured responses in NA and CO_2 fixation were compared with changes in pH and DIC, which are both dependent on photosynthetic activity under conditions of restricted gas exchange in the culture vessel. During the experiments, DIC concentrations generally decreased, while pH increased. pH under shear was consistently lower than in control, and the difference between shear and control increased with time (Fig. 3). Differences in pH between shear and control treatments were significant (Table 4), but there were no significant differences between different shear levels (low, medium, high) (Tables 5–6). DIC concentrations were not significantly different between shear and control (Table 4).

Morphological damage was assessed by comparing filament lengths in control and shear treatments. Shear significantly decreased filament lengths for strain UP16a, but those for strain FL2f were not significantly different between shear and control (Table 4).

Both UP16a and FL2f formed aggregates within several minutes of applying shear. Aggregate sizes were up to ca. ⌀0.5 cm for UP16a and several cm for FL2f but decreased in size with increased rotation rate. The experiments with Kalliroscope dye revealed that there was a region of accelerated flow around the perimeter of the aggregates (Fig. 5). In our observations of flow acceleration around large aggregates, flow disturbances were quickly damped by viscosity within a half Couette chamber circumference distance ''downstream'' from the aggregates. This phenomenon was observed for both *Nodularia* strains and within the entire range of shear rates used.

Field experiments—The species composition of the phytoplankton community was determined for each year. Cell counts were carried out for the ca. >10-μm size fraction, and proportions of each species are shown in Fig. 6. In 1997 and 1999 *Nodularia* dominated the biomass while in 1998 *Heterocapsa triquetra* was the dominant species. In 2000, *Aphanizomenon* dominated three of the experiments while *Nodularia* dominated one of them.

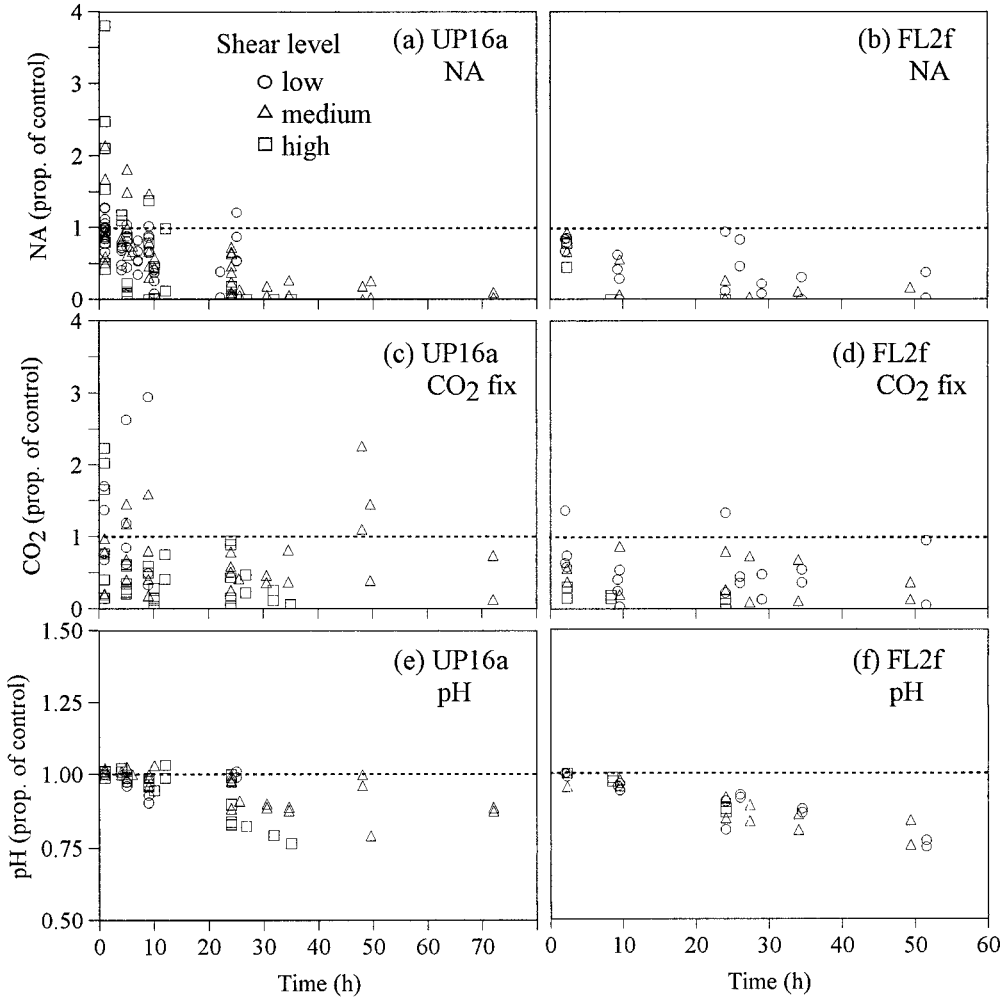

Fig. 3. Nitrogenase activity (NA), CO_2 fixation, and pH from the culture experiments (*Nodularia* strains UP16a and FL2f) expressed as proportion of control. Broken line indicates the value 1 (no difference between shear and control). Data from all the culture experiments (shear rates 2.2–18 s^{-1}) are shown. Data are grouped into low, medium, and high shear (*see Table 1*).

Table 4. Physiological and morphological responses in shear and control. Data from all experiments (shear rates 2.2–18 s^{-1}) are included. Control and shear values include all time points (n = number of time points). NA (nmol C_2H_4 μg Chl *a* h^{-1}), CO_2 fixation (mgC mgChl a^{-1} h^{-1}), pH, and DIC (mg L^{-1}) data include both *Nodularia* strains. Filament length (μm) data are shown for each strain separately (Fil UP 16a and Fil FL2f). For statistical comparison of control and shear, data were converted to proportions of control. Means of all measurements during the course of each experiment were calculated, replicate chambers averaged, and P values computed using paired, two-tailed t-tests (n = number of experiments). Tests were run separately for strains UP16a and FL2f. NS = nonsignificant difference.

	Control			Shear			UP16a	FL2f
	Mean	Sdev	n	Mean	Sdev	n	$P(n)$	$P(n)$
NA	3.02	3.68	81	1.10	1.50	162	0.035 (16)	0.000 (4)
CO_2 fix	2.01	1.98	56	1.44	2.29	112	0.022 (8)	0.034 (4)
pH	8.71	0.75	65	8.18	0.90	130	0.006 (13)	0.018 (4)
DIC	5.9	2.6	56	6.7	2.6	110	NS (9)	NS (4)
Fil UP16a	216	65.0	13	185	46.2	25	0.016 (13)	
Fil FL2f	2,425	1,689	7	1,899	651.1	13		NS (7)

Table 5. Results of one-way ANOVAs comparing the effect of low, medium, and high shear (*see Table 1*) on nitrogenase activity (NA), CO_2 fixation, and pH. Data included culture experiments with both strains. Dependent variable was the difference between control and shear.

Variable	Source of variation	*df*	MS	*F*	*P*
NA	Shear level	2	1.314	3.870	0.041
	Residual	17	0.340		
CO_2 fixation	Shear level	2	0.336	0.530	0.606
	Residual	9	0.634		
pH	Shear level	2	0.017	0.109	0.897
	Residual	14	0.153		

Table 6. Results of repeated measures ANOVAs, testing whether time (i.e., shear duration) had an effect on the magnitude of the shear response in nitrogenase activity (NA), CO_2 fixation, or pH under different shear levels. Data included culture experiments with both strains and were grouped into low, medium, and high shear (*see Table 1*). Dependent variable was the proportion of shear of control.

Variable	Source of variation	*df*	MS	*F*	*P*
NA	Time	3	2.336	39.38	<0.001
	Time × shear level	6	0.741	12.50	0.000
CO_2 fixation	Time	3	0.311	3.24	0.074
	Time × shear level	6	0.385	4.02	0.031
pH	Time	3	0.005	1.51	0.253
	Time × shear level	3	4.2×10^{-5}	0.012	0.998

NA was measured in Couette experiments with natural phytoplankton assemblages during each study year, but detectable values were only found in 2000 (Fig. 7). These results indicated that shear reduced NA; this result was close to significant ($P = 0.056$, $n = 4$).

CO_2 fixation in shear during the field studies was computed as proportion of control (Fig. 7). CO_2 fixation consistently decreased in shear, and this effect was significant, except for 1997. There was a remarkable consistency between experiments in 1999 and 2000, although the community in 1999 consisted mostly of *Nodularia* and in 2000 of *Aphanizomenon.* Greatest reduction under shear was observed in 1998, but this may be a consequence of higher shear level and longer duration of the experiments. CO_2 fixation in the picoplanktonic size fraction (<2 μm) appeared unaffected by shear (Fig. 8), while in the 2–20-μm and >20-μm size fractions CO_2 fixation showed a stronger negative response with longer shear exposure. An initial increase in CO_2 fixation in response to shear was also observed (Fig. 8).

Filament length data from the field experiments are shown in Fig. 9. A significant difference in filament length between shear and control treatments was observed for *Anabaena* and *Aphanizomenon* (Fig. 9 a–d). *Anabaena* and *Aphanizomenon* filaments in the control treatment were longer in 1998 than in 1999–2000, possibly due to different sampling methods. In contrast, *Nodularia* filament length was not reduced under shear in any of the study years (Fig. 9 e,f).

Discussion

Comparison of Couette flow with flows in the Baltic Sea—Our estimates of wind-produced dissipation rates indicated that the experiments with the lowest shear (2.2 s^{-1}) correspond to moderate wind forcing conditions (10 to 15 m s^{-1}) in the upper approximately 2 m of the water column (Fig. 2). These winds are stronger than observed mean wind speeds measured in July–August of 1997–1999 in the Baltic Sea but are comparable to some of the stronger wind events observed during the same time period (Table 3). The highest experimental shear value (18 s^{-1}) corresponds to wind forcing of 20 to 25 m s^{-1} or higher (Fig. 2), which would be at

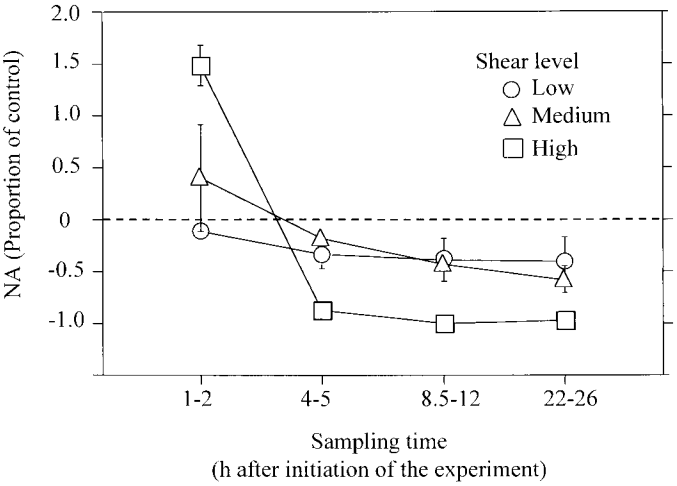

Fig. 4. Effect of shear duration on nitrogenase activity (NA) expressed as proportion of control in low, medium, and high shear rates (*see Table 1*). Data were pooled from 20 culture experiments and both *Nodularia* strains were included. Each point is a mean with SE of values from different experiments. Where error bars are not visible, they are smaller than the data marker.

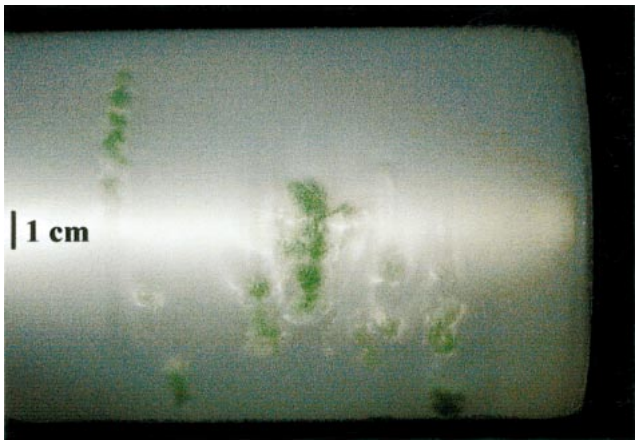

Fig. 5. Side view of Couette chamber flow with reflective fluid Kalliroscope and *Nodularia* strain FL2f at a shear rate of 9.4 s^{-1}. The green patches are aggregates of *Nodularia.* The reflective particles in the fluid orient in the direction of flow; areas of uniform gray indicate uniform flow, while variations in fluid color indicate velocity gradients. The localized vortices and wake patterns around the edges of the aggregates show enhanced localized shear.

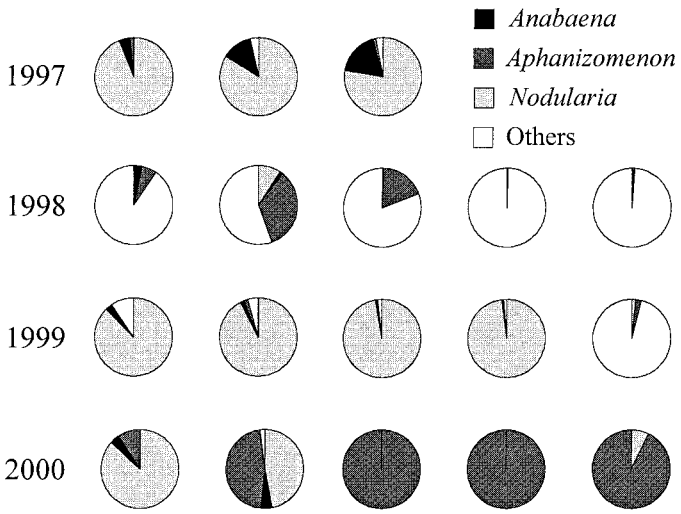

Fig. 6. Phytoplankton community composition in the field experiments (percentage carbon in phytoplankton ca. >10 μm in size). Data are shown for each experiment in which CO_2 fixation or NA was measured. Counts shown for the 1997 experiments were done only for the ''hot experiments'' in which the $^{14}CO_2$ fixation was followed directly.

or beyond the upper limit of what is typically found in the Baltic. Further comparison can be made with Zulicke et al. (1998), who measured near-surface dissipation rates in a Baltic coastal jet that ranged from 10^{-6} to 10^{-5} m^2 s^{-3}. These values are comparable to the dissipation rates for the low shear experiments (2.2 to 3.6 s^{-1}) but are over an order of magnitude smaller than the high shear experiments. In summary, the dissipation rates in our Couette chambers for the low shear experiments were comparable to what is found in nature during moderate wind forcing, but the highest shear rates in our experiments were probably larger than commonly found in nature, particularly on a sustained level over a day.

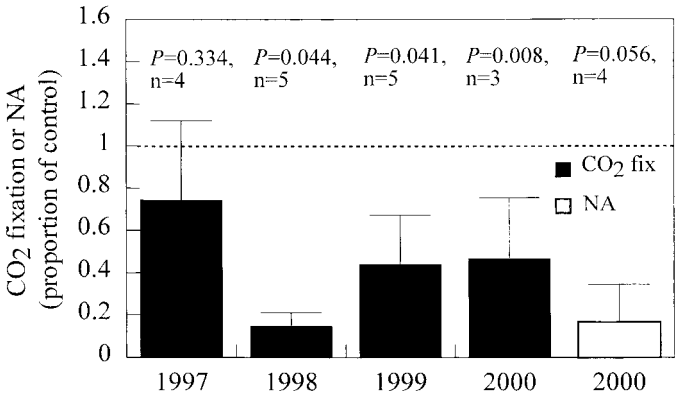

Fig. 7. CO_2 fixation and NA in shear (expressed as proportion of control) from the field experiments. Broken line indicates the level of no difference between shear and control. *P* values are from paired *t*-tests (shear vs. control). *T*-tests were carried out separately for each year, using absolute values. Data for 1997, 1999, and 2000 are from measurements at 12–14 h, and for 1998 at 24 h after the initiation of the experiment. Shear exposure was 7.2–14.4 s^{-1} in 1997, 10.8 s^{-1} in 1998, and 2.2 s^{-1} in 1999 and 2000. Error bars indicate standard deviation.

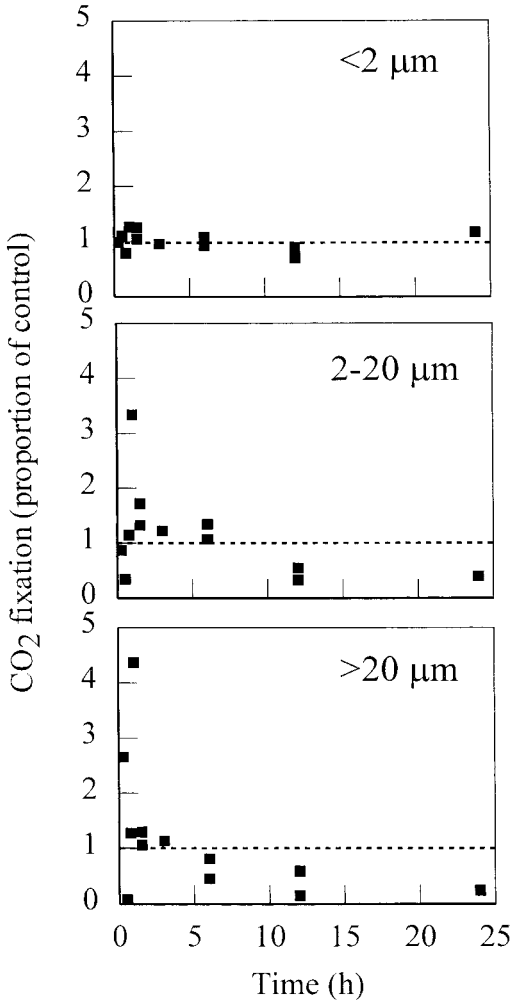

Fig. 8. CO_2 fixation in shear as proportion of control for different size fractions in the field ''hot experiments'' in 1997. ^{14}C label was added at the start of each experiment, subsamples were extracted periodically and size fractionated. Data from three experiments with durations of 3, 12, and 24 h are shown. In each experiment, one chamber was sheared at 18 s^{-1} and one served as a control. Broken line indicates the level of no difference between shear and control.

Shear effects on NA and CO_2 fixation—Nodularia filaments commonly form aggregates in the Baltic Sea and these function as microcosms of heterotrophic bacteria, algae, and zooplankton (Hoppe 1981) and possibly provide a mechanism for grazing avoidance. Shear decreases the diffusive boundary layer around cells and aggregates, theoretically increasing nutrient diffusion to cells (Lazier and Mann 1989). This effect could explain the initial increase we observed in NA and CO_2 fixation in response to shear in both the culture and field experiments.

Because our Couette chambers were isolated from the atmosphere, changes in the gas concentrations in the chambers could be used as a measure of shear response. Dissolved

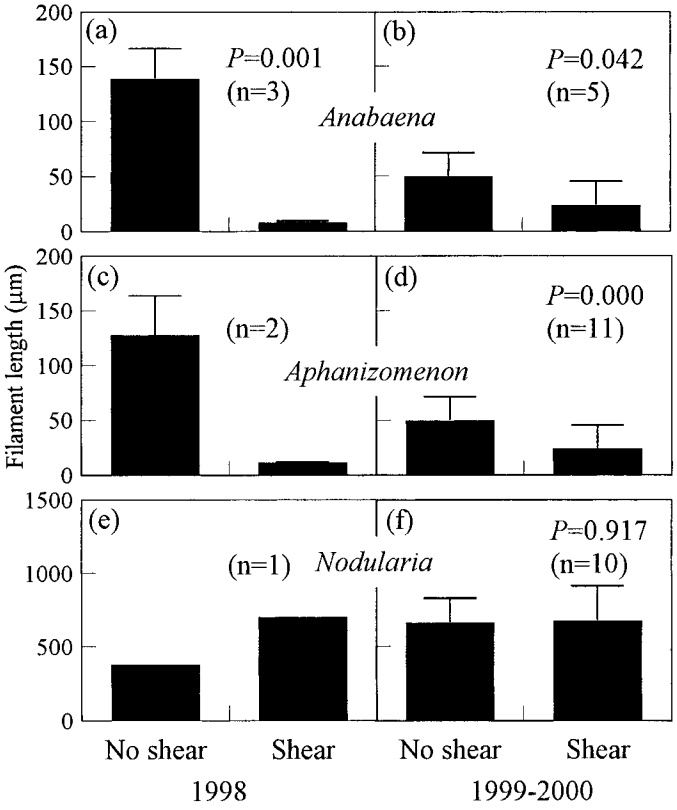

Fig. 9. Filament lengths from the field experiments. (a–b) *Anabaena*; (c–d) *Aphanizomenon*; (e–f) *Nodularia*. Shear exposure: (a, c, e) 10.8 s^{-1}, 24 h (1998); (b, d, f) 2.2 s^{-1}, 12–14 h (1999–2000). *P* values are based on two-tailed, paired *t*-tests. Error bars indicate standard deviation, *n* = number of pairs (number of experiments). Each *n* is the mean length of at least 50 filaments in each treatment.

inorganic carbon concentrations in most cases were lower in the shear treatment than in control. As expected, pH was higher in the control treatment than in the sheared treatment, indicating reduced photosynthetic activity due to shear. Although DIC had a tendency to decrease during the experiments, it remained at levels higher than V_{max} for DIC uptake in 159 cases out of total of 166 culture measurements, assuming DIC demand of *Nodularia* is similar to that of *Anabaena variabilis* (ca. V_{max} 1.8 mg L^{-1}) (Volokita et al. 1984). DIC levels remained equally high in the chambers during the field experiments. Therefore, it is unlikely that DIC limitation affected the CO_2 fixation rates.

Shear consistently reduced NA and CO_2 fixation for *Nodularia*, but there was considerable variability in the magnitude of these responses. This variability may stem from at least three sources: (1) Cells within an experiment experienced different shear levels due to aggregation, evidenced by the localized areas of increased shear around aggregates (*see discussion below*). (2) Heterogeneous distribution of aggregated culture from the chambers to subsamples caused variability in the Chl *a* measurements, which could carry over to the CO_2 fixation results because CO_2 fixation was normalized to the mean Chl *a* determined from the NA subsamples. (3) Cultures may have been in a slightly different initial physiological state when the shear experiments were started. The shear sensitivity of filamentous cyanobacteria may vary depending on the growth stage, which has been demonstrated in the dinoflagellate *L. polyedrum* (Juhl et al. 2000). Other studies have shown that culture stage plays a role in aggregation because cell stickiness changes with growth phase (Waite et al. 1997).

There was a mixed phytoplankton community in the field experiments, but genus-specific shear responses in CO_2 fixation and NA can be evaluated from the parallel phytoplankton counts, size fractionation experiments, and filament length measurements. Dominance of cyanobacteria in cell counts suggested that the reduced CO_2 fixation under shear in 1997, 1999, and 2000 reflected decreases in cyanobacterial activity. The proportion of carbon biomass in the community is by no means a straightforward measure of the proportion of CO_2 fixation. However, generally the experimental community exhibited low species diversity, and the smallest size fractions were excluded during net sampling. Therefore, our biomass calculations should give a reasonable estimate of the contribution to CO_2 fixation by different species/genera. The <10-μm size fraction probably did not account for a significant portion of CO_2 fixation because samples were initially concentrated using 20-μm to 500-μm mesh nets. Moreover, CO_2 fixation in the phytoplankton size fraction <2 μm was not affected by shear. Therefore, any reduction in bulk CO_2 fixation could not be attributed to this size fraction. This is an expected result, since small size keeps these organisms well within the viscous size range. This point is supported by previous work with nonattached bacteria, showing that their nutrient uptake is not affected by extremely high shear rates (<50 s^{-1}) (Logan and Dettmer 1990).

Nitrogenase activity is an ideal indicator of the activity of diazotrophic cyanobacteria, but it proved difficult to detect in the field experiments. We observed a decrease in NA in the field Couette experiments; the decrease was seen both in control and sheared chambers. Only after increasing the density of the sample and the subsample size considerably (in year 2000) could we detect NA. Although the NA data were only available in 2000, the results of reduced NA under shear support the conclusions based on CO_2 fixation, cell counts, and filament length data, suggesting that cyanobacteria were negatively affected by shear.

Shear effects on morphology—Filament breakage is a possible mechanism for negative physiological effects of shear. Mitsuhashi et al. (1995) noted filament breakage of the nonheterocystous *Spirulina*, although their dissipation rates were an order of magnitude greater than the highest rate used here. Our results showed variable morphological damage among laboratory strains as well as between genera in field populations. Filament length of *Nodularia* strain UP16a decreased in response to shear, but strain FL2f was not affected. In the field experiments, filament length of *Nodularia* spp. was not affected (filament length measurements from the Baltic Sea included all morphological types of *Nodularia*). While filament length was not reduced for strain FL2f, NA and CO_2 fixation decreased. Similarly, the filament length of *Nodularia* during the field studies was not affected by shear, but its CO_2 fixation was reduced. These results

suggest that shear negatively affects cyanobacterial physiological activity prior to morphological damage and are consistent with the findings of Kucera (1996). In contrast to *Nodularia,* shear reduced *Anabaena* and *Aphanizomenon* filament lengths, indicating that the filament integrity in these genera is more sensitive to shear than in *Nodularia.* Biomass of *Aphanizomenon* is typically distributed over the entire upper mixed layer, while *Nodularia* accumulates closer to the surface (Lindahl et al. 1980; Walsby et al. 1995). Closer proximity to the pycnocline provides the low light and low temperature adapted *Aphanizomenon* (Lehtimäki et al. 1997) better access to nutrients. Results of this study indicate that sensitivity to shear may be another reason why *Aphanizomenon* has evolved mechanisms to maintain growth at depth. The trichome structure may help explain the better shear tolerance of *Nodularia* filaments than *Aphanizomenon.* Flexible fibers such as filaments of *Nodularia* may bend in fluid motion easier than rigid, straight filaments of *Aphanizomenon,* which would experience higher shear rates, following the theory for diatom chains presented by Karp-Boss and Jumars (1998). These genus-specific differences in shear sensitivity may play a role in determining cyanobacterial community composition in nature.

Nodularia commonly forms aggregates in nature, very similar to those we observed in our laboratory experiments. From our flow visualization experiments it is apparent that cells on the perimeter of these aggregates experience larger shear values than one would predict analytically based on homogenous flow in the Couette chamber. Conceptually, the flow can be considered in terms of a Reynolds number based on the velocity and length scales for a flow around a cell, $\mathrm{Re}_{\mathrm{cell}} = (UL)_{\mathrm{cell}}/\nu$, versus flow around an aggregate, $\mathrm{Re}_{\mathrm{agg}} = (UL)_{\mathrm{agg}}/\nu$, where U and L are appropriate velocity and length scales for a cell or aggregate. Since aggregates are larger than cells, and flow velocities around an aggregate are faster than around cells, we expect $\mathrm{Re}_{\mathrm{agg}} > \mathrm{Re}_{\mathrm{cell}}$. However, the net implications of aggregates on shear and vice versa are unclear. We observed that aggregates are not very cohesive and may stretch and strain with flow, so cells outside of an aggregate may move to the inside at some subsequent time. There may be a protective effect where cells on the outside of an aggregate protect inner cells from shear. However, filaments inside the aggregates will experience lower light levels due to shading. The filaments on the edges of aggregates may experience the highest nutrient fluxes, which could have a beneficial effect. In summary, the variation in the shear, nutrient, and light environment that filaments in the Couette chambers are exposed to may explain some of the variability in the measured NA and CO_2 fixation rates.

Comparison of phytoplankton shear sensitivity—Direct comparisons of shear tolerance between different organisms and ecosystems are complicated by differences in shear levels and duration (total and daily) used in previous experiments. During the summer bloom period in the Baltic, windy periods lasted longer than a day; therefore, the shear duration in our experiment was a reasonable approximation of its duration in nature. Gibson and Thomas (1995) showed the growth of the dinoflagellate *L. polyedrum* to be sensitive to shear exposure of only 15 min a day. However, when exposed to shear for short periods, the organism may tolerate much higher shear rates than the threshold shear for growth (Latz et al. 1994). Thomas and Gibson (1990), Gibson and Thomas (1995), and Juhl et al. (2000) showed that growth inhibition of *L. polyedrum* occurs at shear rates of 2.2–8 s^{-1}, at intermittent or constant shear continued for several days. Growth of the green alga *Scenedesmus quadricauda* decreased at an even lower shear rate of <1.5 s^{-1} (4 d constant shear) (Hondzo and Lyn 1999). We could not establish a lower limit for shear effect in *Nodularia*; it appears to be less than 2.2 s^{-1}, our lowest shear treatment, and appeared prior to apparent morphological damage. We observed an additional decrease for NA at 7.2–8.6 s^{-1} and higher, which may indicate a second mechanism for negative shear effect. Hence, the tolerance of NA in *Nodularia* appears to be close to the tolerance for growth by *L. polyedrum.*

It is possible that the slightly higher wind speeds in 1998 had a negative effect on growth of *Nodularia* in 1998. However, it is likely that the development of *Nodularia* blooms was delayed in 1998 and 2000 by relatively cold temperatures (Table 3), because optimal growth temperature for *Nodularia* is >25°C (Lehtimäki et al. 1997). Shallow upper mixed layer related with high surface water temperature probably promoted the blooms in 1997 and 1999 (Kononen et al. 1996). If shear played a role in the community structure in 1998, one would expect the dinoflagellate *Heterocapsa triquetra,* the dominant species in 1998, to be more shear tolerant than *Nodularia.* Shear (10.8 s^{-1}) drastically decreased CO_2 fixation and fragmented *Anabaena* and *Aphanizomenon* filaments within the community during the 1998 experiments. Most likely, the reduced CO_2 fixation under shear in 1998 reflected reduced activity of the filamentous cyanobacteria. However, CO_2 fixation of *Heterocapsa* in 1998 was probably also reduced in shear because *Heterocapsa* formed 42% to 99% of the community biomass, while CO_2 fixation under shear was only 8% to 20% of that in control.

Implications for cyanobacterial blooms—The establishment and proliferation of planktonic heterocystous cyanobacteria in lakes, estuaries, and coastal waters might be inhibited by their sensitivity to shear. Extensive work on shear tolerance with oceanic red tide dinoflagellates has shown that these organisms are sensitive to shear (Thomas and Gibson 1990; Gibson and Thomas 1995; Juhl et al. 2000). Growth of these dinoflagellates is reduced by shear (at levels capable of reducing NA in *Nodularia*), and these organisms typically form red tides in oceanic and estuarine environments only during calm conditions. Heterocystous cyanobacteria may similarly benefit from periods of low shear. Coupled with the low growth rates typifying heterocystous cyanobacteria (Reynolds and Walsby 1975), shear sensitivity might inhibit establishment in areas with periodically intense shear, such as tidally well-mixed estuaries. Suitable nutrient, temperature, and light conditions are the initial growth requirements, and in certain conditions, shear may be a controlling factor.

In conclusion, we have demonstrated that small-scale shear decreased photosynthetic and nitrogen fixation rates of filamentous Baltic Sea cyanobacteria. Species-specific filament breakage occurred in response to shear and was par-

tially able to explain the negative response to shear. These results suggest that small-scale shear regulates cyanobacterial blooms at natural shear levels and may be a factor controlling geographical distributions. Vertical migration and preference for low light such as in Baltic Sea *Aphanizomenon* may be adaptations allowing shear sensitive organisms to proliferate in the phytoplankton community. Future studies are warranted on careful comparisons of experimental shear tolerance between phytoplankton from high and low turbulence environments, and development of alternative flow generating and measuring systems for use with aggregating species.

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