Photosynthesis and nitrogen fixation in a cyanobacterial bloom in the Baltic Sea

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Daily integrals of photosynthesis by a cyanobacterial bloom in the Baltic Sea, during the summer of 1993, were calculated from the vertical distributions of light, temperature and the organisms in the water column and from photosynthesis/irradiance curves of picoplanktonic and diazotrophic cyanobacteria isolated from the community. The distribution of chlorophyll *a* in size-classes < 20 μm and > 20 μm was monitored over 9 days that included a deep mixing event followed by calm. Picocyanobacteria formed 70% of the cyanobacterial biomass and contributed 56% of the total primary production. Of the filamentous diazotrophs that formed the other 30%, *Aphanizomenon* contributed 28% and a *Nodularia*-containing fraction 16% of the primary production. For the whole population there was little change in standardized photosynthetic O_2 production, which remained at about 31 mmol m^{-2} before and after the mixing event. There were differences, however, between the classes of cyanobacteria: in picocyanobacteria primary production hardly changed, while in *Aphanizomenon* it increased by 2·6 and in *Nodularia* it fell below zero. Total phytoplankton photosynthesis was strongly dependent on total daily insolation with the compensation point at a photon insolation of 22·7 mol m^{-2} d^{-1}. Similar analyses of N_2 fixation showed much less dependence on depth distribution of light and biomass: *Aphanizomenon* fixed about twice as much N_2 as *Nodularia* their; their fixation exceeded their own N demand by about 12%. Together, these species contributed 49% of the total N demand of the phytoplankton population. Computer models based on the measured light attenuation and photosynthetic coefficients indicate that growth of the cyanobacterial population could occur only in the summer months when the critical depth of the cyanobacteria exceeds the depth of mixing.

Key words: Baltic Sea, bloom, cyanobacteria, nitrogen fixation, primary production

Introduction

During summer months, the Baltic Sea contains populations of both filamentous and picoplanktonic cyanobacteria (Kononen *et al.*, 1996). Some of the filamentous forms produce aggregates or colonies that float to the surface forming surface scums (Walsby *et al.*, 1995), readily visible to satellites (Horstmann, 1983; Kahru *et al.*, 1994). Blooms of cyanobacteria were recorded in the nineteenth century, but they may have increased in recent years (Kahru *et al.*, 1994). In other seas and oceans of northern latitudes, picoplanktonic cyanobacteria are abundant but the filamentous diazotrophs are absent. The absence from the northern oceans of the particular forms of *Anabaena*, *Aphanizomenon* and *Nodularia* that inhabit the Baltic Sea (Albertano *et al.*, 1996) can be explained by their inability to tolerate salinities exceeding 15, but this does not explain why other species have not developed there: in tropical oceans, there are species of *Trichodesmium* and *Katagnymene* that are adapted to fully marine salinities.

Various factors may favour the growth of cyanobacteria in the Baltic Sea: their growth in the summer period has been interpreted as indicating a requirement for elevated temperatures (Kuosa, 1991; Kononen *et al.*, 1996); they may be avoided by selectively grazing invertebrates (Sellner, 1997); and they may be differently affected by photo-oxidation and viral attack (Coulombe & Robinson, 1981). Variations in eddy diffusivity and other physical processes may cause locally elevated concentrations of nutrients that may stimulate their development (Williams & Follows, 1998). Many of these factors, however, would apply to both the picocyanobacteria and the diazotrophic cyanobacteria.

Two particular properties of the filamentous diazotrophs may explain their success in the Baltic Sea. The first is their ability to fix N_2, which renders them independent of exogenous supplies of combined nitrogen and favours their growth under low N:P ratios (Kononen *et al.*, 1996). The second is the formation of colonies or aggregates of filaments rendered buoyant with gas vesicles, which are able to float up in the euphotic layer during periods of calm weather. Walsby *et al.* (1997) calculated that the upward movement of *Aphanizomenon* colonies could lead to a 3-fold increase in photosynthesis during calm days and a doubling over a period of alternating turbulent and calm days.

A feature of the Baltic Sea that impinges on both these

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properties is that its stability is affected not only by gradients of temperature but also gradients of salinity (Kullenberg, 1981). The saline water that enters from the North Sea sinks underneath the surface layers of the Baltic Sea creating a halocline at a depth of 45 m or more. In summer, the Baltic Sea also has a pronounced thermocline at depths between 15 and 20 m but, even at these depths, salinity gradients influence the density structure, stabilize the system and reduce the depth of the surface mixed layer. This increases the value of buoyancy in the filamentous cyanobacteria. Another consequence of the increased stability is that, in calm periods, the water of the euphotic zone may become more effectively separated from the deeper, nutrient-rich layers. The depletion of nitrogen will favour the growth of diazotrophic filamentous cyanobacteria. During mixing events, however, there may be significant injections of nutrients from the deeper layers into the euphotic zone and on these occasions the picoplankton, which are more effective in absorbing nutrients and have higher intrinsic growth rates, may respond more rapidly than the larger colonial forms.

We investigated the interrelations of water-column stability and phytoplankton productivity in 1993 by following a population of *Aphanizomenon flos-aquae* in the Bornholm Sea region of the Baltic for 9 days and studying the effects of a mixing event and subsequent calm on its vertical distribution and biomass development. From measurements of irradiance, vertical light attenuation and the relationship between photosynthesis and irradiance (P/I) of the *Aphanizomenon* colonies, we were able to calculate the daily integral of its photosynthesis and show that this could account for its observed growth (Walsby *et al.*, 1997). We performed similar calculations on N_2-fixing activity, which was also sufficient to supply the nitrogen required for growth (Stal & Walsby, 1998).

We also collected information at the drift station on the distribution of other cyanobacteria present: species of *Nodularia*, which form an approximately similar proportion of the population (Walsby *et al.*, 1995; Barker *et al.*, 1999), and *Synechococcus* sp., which can be quantitatively more important (Albertano *et al.*, 1996; Kuparinen & Kuosa, 1993). With cell concentrations as high as 10^5–10^6 ml^{-1} (Jochem, 1988; Kuosa, 1991; Albertano *et al.*, 1997), the picoplankton may contribute more than half of the cyanobacterial biomass and primary production (Kuparinen & Kuosa, 1993). We were not able, at the time of the drift station, to measure the photosynthetic and N_2-fixation coefficients of these other members of the cyanobacterial population but we have subsequently obtained such measurements from cultures of *Nodularia* and *Synechococcus* isolated from the Baltic Sea. We report here calculations and comparisons of the daily integrals of photosynthesis and N_2 fixation for the three groups of cyanobacteria, which responded differently during calm periods that preceded and followed the deep mixing event.

We have incorporated in our calculations, which utilize the integration spreadsheets of Walsby (1997*a*), new corrections for the effect of the measured temperature of the water column. While these produce little change in the results for the depths investigated (because the measurements of P/I were made at the average temperature of the water column), they permit a quantitative analysis of the suggested requirement for high temperatures by the summer population of cyanobacteria. Our analyses indicate that the contribution of temperature is an indirect one, in stabilizing the water column: the development of cyanobacterial blooms in summer is dependent on the combined increase in the daily insolation and the reduction of the depth of the mixed layer brought about by the temperature increase.

Materials and methods

Sampling and measurement of chlorophyll a

The 9 day study was performed from 27 July to 6 August 1993 (days 208–216) in the Bornholm Sea at 55° 23′ N, 15° 04′ E during a cruise on the research vessel *F.S. Alkor*. The ship followed a drifting drogue that permitted sampling of the same water mass. Water was sampled at 3 m depth intervals using 12 litre bottles on a rosette array (General Oceanics) carrying CTD instruments for measuring changes in temperature and salinity with depth. The bottles were emptied into buckets, to ensure homogeneous mixing of the samples. One litre samples were filtered through a 20 μm plankton net (under gravity) and 0·5-litre samples were filtered through a glass fibre filter (GF-C; Whatman). Chlorophyll was extracted from the filters overnight at room temperature in 90% ethanol (v/v) in the dark. The extracts were kept at −20 °C in the dark and measured on return to the laboratory. Absorbance was measured at 665 nm and the chlorophyll concentration was calculated using an absorption coefficient of 72·3 ml mg^{-1} cm^{-1}. No loss of chlorophyll *a* occurred during storage.

Light measurements

Irradiance was measured with a Kipp & Zonen CM-11 Pyranometer solarimeter (spectral range 305–2800 nm) on the research vessel. These measurements (W m^{-2}) were converted to photon irradiances (μmol m^{-2} s^{-1}) by reference to simultaneous readings made in steady sunlight: 1 W m^{-2} corresponded to 3·746 μmol m^{-2} s^{-1}, measured on the quantum sensor used in P/I measurements (Walsby, 1997a). The light attenuation coefficient of the water column was determined from measurements of the photon irradiance, I_z, at 1 m depth intervals down the water column, made with a LiCor spherical quantum sensor (spectral range 400–700 nm) each day at noon.

Calculation of photosynthesis and nitrogen fixation

Photosynthesis was calculated using the Excel computer spreadsheet programs Integral.xls, and the more detailed

version Complex.xls, of Walsby (1997*a*), which are available at *http://www.bio.bris.ac.uk/research/walsby/integral.htm*. Briefly, the spreadsheet programs each comprise five sheets. In sheet 1 the photosynthetic coefficients are calculated from the P/I curve fitted to the equation

$$P = P_m(1-\exp(-\alpha I/P_m)) + R_d + \beta I \quad (1)$$

where P is the rate of net photosynthetic O_2 production, P_m the maximum gross photosynthetic rate at light saturating irradiance, R_d the rate of respiratory O_2 production in darkness (a negative value), α the photosynthetic affinity coefficient (P/I) and β the photoinhibition coefficient at high irradiance. In sheet 2 the depth distribution of chlorophyll concentration (N, in mg m^{-3}) is calculated at 1 m depth intervals, by interpolating the measured concentrations at the sampling depths (3 m intervals). The vertical profiles of relative photon irradiance, I_z/I_0 (where I_z is the irradiance at the depth z and I_0 is the irradiance immediately under the water surface), and temperature, Θ are calculated in sheet 3. In sheet 4 the underwater photon irradiance (I_0) is calculated from shipboard measurements of energy irradiance and wind speed by making corrections for reflectance at the water surface. In sheet 5 the calculations of the daily integral of *net* photosynthesis through depth and time ($\Sigma\Sigma(NP)$) are performed. The same procedure is used for the integrals of N_2 fixation. The version of the spreadsheet used, Complex.xls, was that used previously for the calculation of the daily integrals of photosynthesis (Walsby *et al*., 1997) and N_2 fixation (Stal & Walsby, 1998) by *Aphanizomenon*, in which corrections were made for the changing position of the population, calculated by interpolation between readings on adjacent days. In addition, we here incorporated the effect of temperature in the calculation: the integral was multiplied by $Q_{10}^{[(\Theta-\Theta')/10]}$, where Θ is the temperature in the water column and Θ' is the temperature at which the P/I measurement was made; the value of Q_{10} was assumed to be 2·0 for photosynthesis and respiration (Reynolds, 1984); for N_2 fixation Q_{10} was calculated to be 1·5, from measurements made at temperatures between 10 and 20 °C (Staal and Stal, unpublished). These modifications are described by Walsby (1997*a*). Computer models of the light field for different days of the year were made with sheet 4 of Complex.xls in which the photon irradiance at the water surface for each 5 min interval is calculated from information on the date and geographical latitude (55·23° N at the drift station).

Photosynthesis

Photosynthesis versus irradiance (P/I) curves were measured as described below for two strains of *Synechococcus*, a phycoerythrin-containing (red) and a phycocyanin-rich (green) strain, and *Nodularia spumigena* strain S34d and strain SN15a, isolated from the Baltic Sea by Dr Paul Hayes, University of Bristol. The photosynthetic characteristics of *Aphanizomenon flos-aquae* were those determined by Walsby (1997*a*).

The P/I curves were recorded by the method of Dubinsky *et al*. (1987). The oxygen cell had a volume of 15 ml. A range of irradiances was obtained using a slide projector with a 150 W halogen lamp and a series of neutral density filters. The culture was illuminated for 3 min at each irradiance. Prior to the recording of a P/I curve, the culture was kept in the dark for 15 min. Measurement of respiration lasted 9 min. P/I curves were corrected for the consumption of the oxygen electrode. The cultures were diluted to OD_{750} 0·1 (1 cm light path) in order to minimize self-shading in the incubation chamber. The cultures were grown in a controlled climate room at 15 °C as batch cultures, under continuous light (of photon irradiance 20–30 μmol m^{-2} s^{-1}, except for the red strain of *Synechococcus*, which was grown at 5–10 μmol m^{-2} s^{-1}).

Nitrogen fixation

Nitrogenase activity versus irradiance curves, determined in cultures of *Nodularia* and *Aphanizomenon* (Evans *et al*. and Stal *et al*., unpublished reports), were fitted to equation (1) by substituting N for P, N_m for P_m and N_d for R_d (Stal & Walsby, 1998). N_2 fixation was determined using the acetylene reduction method (Stal, 1988). The N_2-fixation rate was calculated as 0·25 of the rate of acetylene reduction (Jensen & Cox, 1983). Biomass-specific nitrogenase activities were obtained by dividing the N_2-fixation rate by the chlorophyll content of the culture. Cultures of the picocyanobacteria were tested and found to be incapable of N_2 fixation.

Results

Light and temperature

The phytoplankton community was followed by measuring the depth distribution of chlorophyll during a 9 day drift station in the Baltic Sea. A drogue was used to track a water mass. Temperature profiles on the first day indicated a water column mixed to a depth of 15 m and a steep thermocline at 17 m (Fig. 1). Over the first 4 days there were periods of weak stratification but on the evening of day 211 there was deep mixing, giving a uniform temperature (of 15 °C) down to a depth of 20 m. Over the following 5 days of calm conditions the surface waters were stabilized by temperature gradients rising to 16·5 °C at the surface (Fig. 1).

Over the 9 day period, the average daylength was 15·8 h, with dawn at 0406 hours and dusk at 1954 hours (Walsby *et al*., 1997). The total daily photon insolation, ΣI_0, varied from only 18·7 mol m^{-2} on the cloudiest day (215), to 50·8 mol m^{-2} on the sunniest day (213); the average for the 9 days was 33·5 mol m^{-2}; the average attenuation coefficient was 0·353 m^{-1} (Walsby *et al*., 1997). Further details are given in table 1 of Walsby *et al*. (1997).

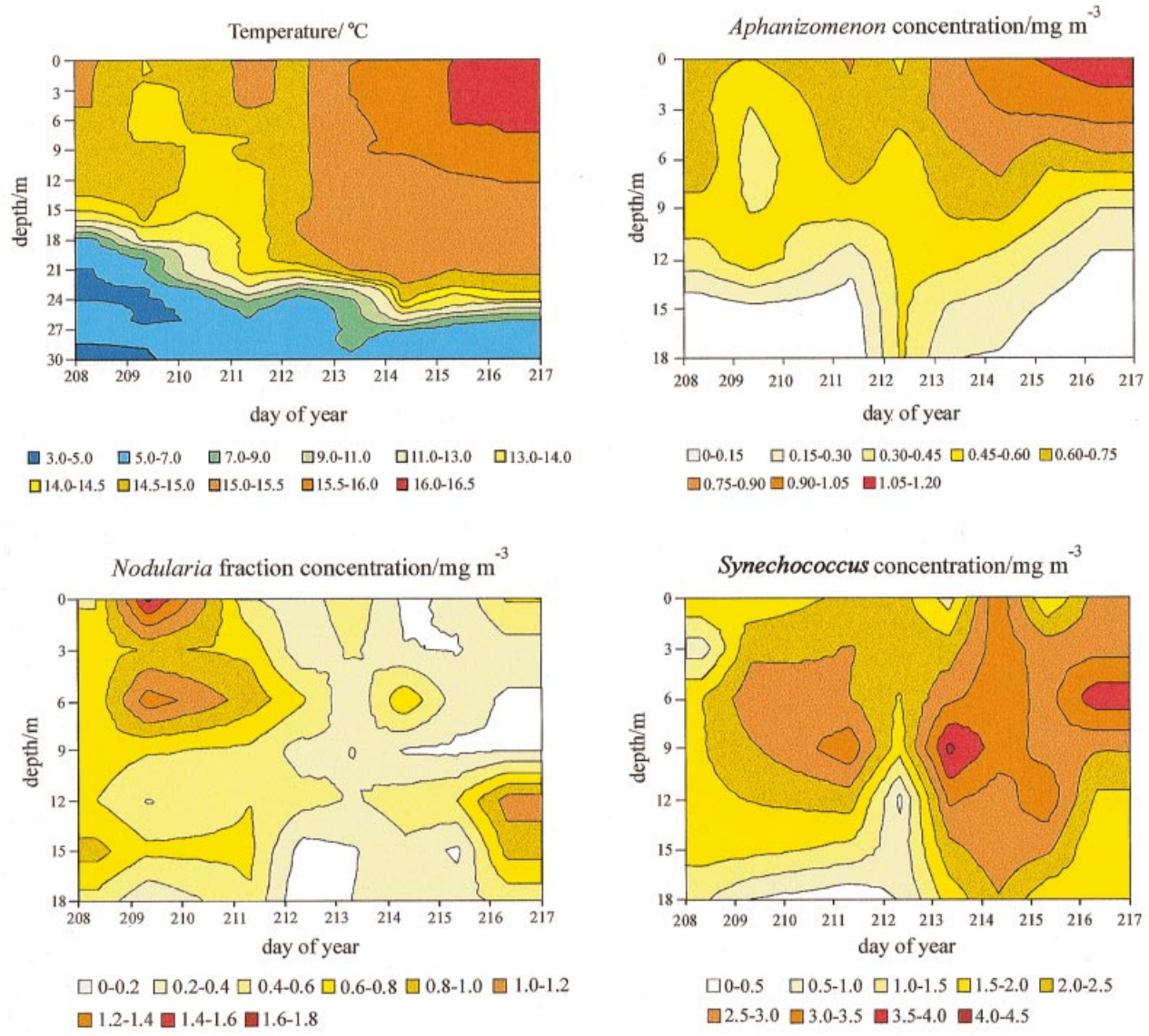

Fig. 1. Depth distribution of temperature and of chlorophyll *a* concentrations during the 9 day study in *Aphanizomenon*, *Nodularia* and *Synechococcus*. Note that the scales for *Aphanizomenon* and *Nodularia* are different from that for *Synechococcus*.

Phytoplankton

The phytoplankton community was composed predominantly of the diazotrophic cyanobacteria *Aphanizomenon* sp. and *Nodularia* spp. and the small picoplanktonic species *Synechococcus* spp. Size fractionation of phytoplankton in > 20 μm (plankton net) and glass fibre filter (GF-C) has shown that the former contained *Aphanizomenon* and *Nodularia*, while the GF-C filter retained all remaining chlorophyll: when the filtrate of the GF-C filter was passed through a 0·2 μm membrane filter, no further chlorophyll was detected. The chlorophyll retained on the GF-C filter belonged predominantly to the fraction smaller than 1 μm and represented picocyanobacteria (Stal *et al.*, 1999). The contribution of *Aphanizomenon* to the chlorophyll in the > 20 μm fraction was calculated from the number of colonies and their average chlorophyll content (1·1 ng per colony; Walsby *et al.*, 1997); the balance was largely composed of the three types of *Nodularia* (straight, coiled and lightly coiled) filaments described by Walsby *et al.* (1995), though in some samples large diatoms of *Chaetoceros* sp. were also present. We refer to this balance (total > 20 μm minus *Aphanizomenon*) as the '*Nodularia* fraction'.

Fig. 1 shows the depth distribution of temperature and of the chlorophyll *a* concentration in the three cyanobacteria during the 9 days in the three different cyanobacterial populations. The *Aphanizomenon* population was mixed down the water column on day 212 but became concentrated towards the surface layers during the following days. This was not the case with the *Nodularia* fraction, which became concentrated at lower depths. The *Synechococcus* increased most at a depth of 9 m following the mixing event and subsequently at a depth of 6 m.

Differences also occurred in the biomass of the three fractions. The picoplankton population dominated; over the 9 day period it accounted for about 70% of the total chlorophyll *a* and 74% over the last 4 days (Table 1). The total *Synechococcus* biomass showed a large increase after the mixing event and then declined (Fig. 2*A*). The biomass

Table 1. Total areal concentrations of chlorophyll *a* in the top 18 m of the water column partitioned in the three components of the cyanobacterial community

	Chlorophyll concentration						
	Days 208–216		Days 208–211		Days 212–216		Change
	mg m^{-2}	(%)	mg m^{-2}	(%)	mg m^{-2}	(%)	(%)
Aphanizomenon	8·43	(14)	7·3	(13)	9·3	(15)	28
Nodularia	9·35	(16)	12·5	(23)	6·8	(11)	−46
Synechococcus	40·74	(70)	35·5	(64)	44·9	(74)	26
Total	58·5	(100)	55·4	(100)	61·0	(100)	10

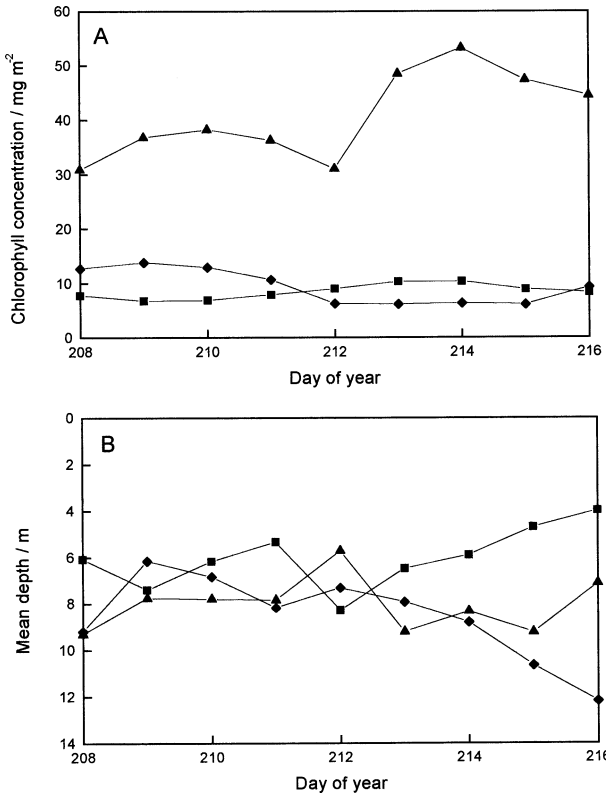

Fig. 2. (*A*). The variation in the total chlorophyll concentration, integrated over the upper 18 m of the water column, during the 9 day study. (*B*). The median depth of chlorophyll *a* distribution (the depth above which 50% of the total chlorophyll *a* is present). Triangles, *Synechococcus*; diamonds, *Nodularia*; squares, *Aphanizomenon*.

of *Aphanizomenon* increased by 28%, after the storm-mixing event, while the *Nodularia* fraction lost 46% (Table 1). The total chlorophyll *a* biomass increased by 10%. The changes in each of the three cyanobacterial components, integrated over the upper 18 m of the water column, during the 9 days drift station are shown in Fig. 2*A*. The biomass of *Aphanizomenon* and the *Nodularia* fraction showed opposite trends. From the median depth of the chlorophyll *a* distribution in the three fractions it was evident that *Aphanizomenon* had floated up while the *Nodularia* fraction had sunk; the distribution of *Synechococcus* appeared to be due to growth because the increases in concentration that occurred at intermediate depths over days 212–213 were not accompanied by corresponding decreases in the upper or lower layers (Figs 1, 2).

Photosynthesis

The photosynthetic coefficients used for the calculations were derived from P/I curves measured in cultures of cyanobacteria that were isolated from the Baltic Sea. An example of a P/I curve fitted to equation (1) is depicted in Fig. 3*A*. The photosynthetic coefficients used are summarized in Table 2. The coefficients of the two strains of *Nodularia* were averaged as were those of the two strains of *Synechococcus*. A number of differences between the three groups of cyanobacteria were noted. *Synechococcus* showed high rates of dark respiration, resulting in a low ratio P_m/R_d of 3·8 compared with values of 9·6 in *Aphanizomenon* and 6·8 in *Nodularia*. The values of P_m differed less. The chlorophyll-specific photosynthetic affinity coefficient, α, was lowest for *Aphanizomenon*, intermediate for *Nodularia* and highest for *Synechococcus*. Consequently, photosynthesis in *Synechococcus* saturated at the lowest irradiance, in *Nodularia* at an intermediate value and in *Aphanizomenon* at the highest irradiance.

Daily integrals of photosynthetic O_2 production were calculated from the vertical distributions of biomass, light and temperature through the water column. Comparisons were made with previous calculations on *Aphanizomenon* in which no correction was made for temperature. The equivalent daily integrals through the top 18 m differed by only 3%; the reason for the small difference is that the photosynthetic coefficients were measured at a temperature (Θ') of 15 °C that is very close to the mean temperature (Θ = 14·95 °C) of the water in the top 12 m where most of the *Aphanizomenon* colonies were distributed.

The total phytoplankton production (*Synechococcus*, *Aphanizomenon* and the *Nodularia* fraction) on day 212 and day 216 is shown in Fig. 4. On day 216 photosynthetic rates were higher than on day 212. However, due to the increased biomass, particularly of the picoplankton, which manifested high rates of respiration, the losses during the night and below the euphotic depth were higher, leading

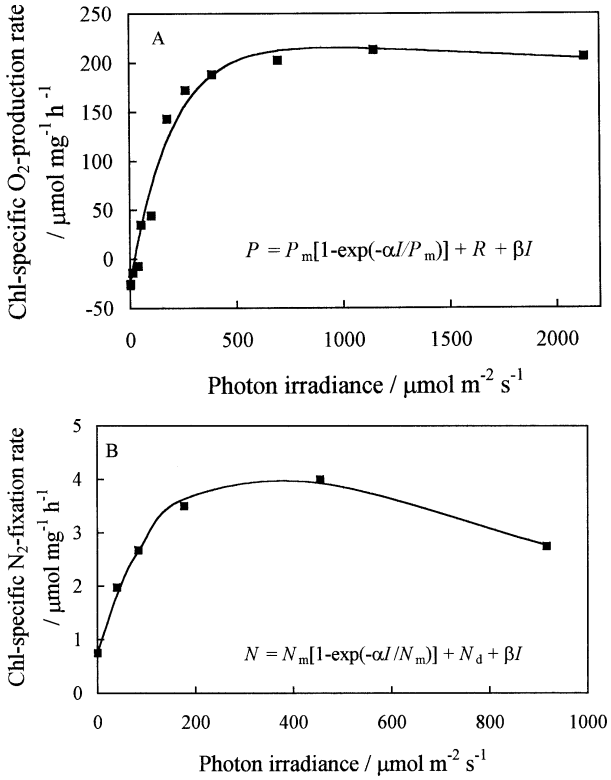

Fig. 3. (*A*) Photosynthesis versus irradiance curve for *Aphanizomenon flos-aquae*. The line is the curve generated by equation (1). (*B*) Curve of N_2-fixation rate versus photon irradiance for *Nodularia* SN15A.

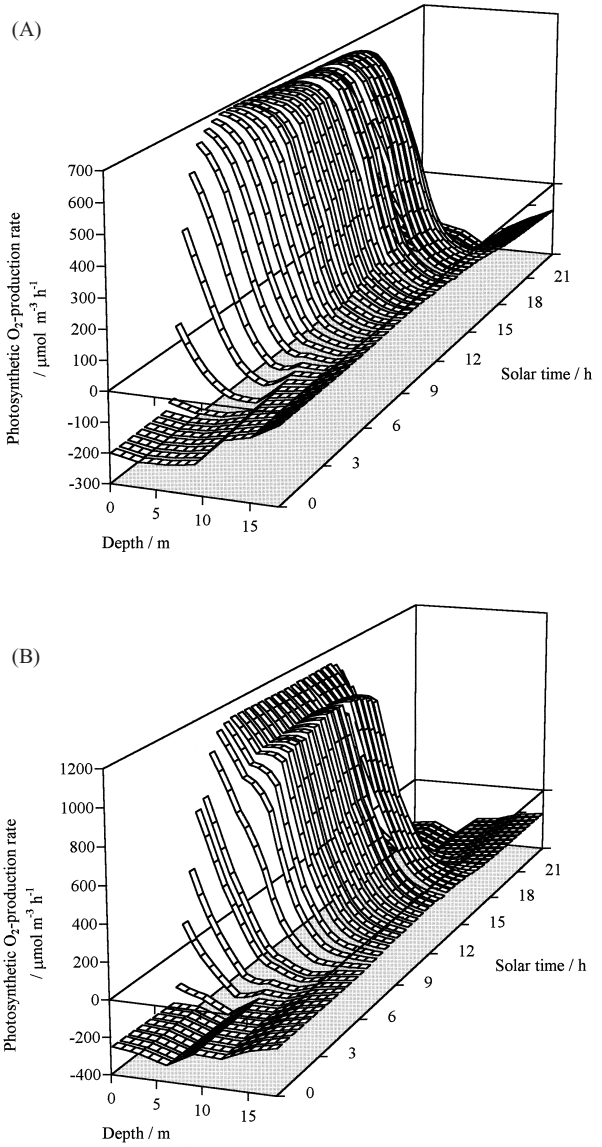

Fig. 4. The rates of photosynthetic O_2 production with depth and time by *Synechococcus*, *Aphanizomenon* and *Nodularia* during (*A*) day 212 and (*B*) day 216.

to a decreased daily integral of photosynthesis (Table 3). This occurred due to a relatively small difference in photon insolation between day 212 (47·5 mol m^{-2}) and day 216 (41·6 mol m^{-2}). When differences in insolation were disregarded by considering the standardized photosynthetic integral (Walsby *et al.*, 1997), using the 9 day average of insolation (33·5 mol m^{-2}), little difference in total photosynthesis between day 212 and 216 was found (Table 3). Between the different components of the

Table 2. Photosynthetic coefficients of Baltic Sea cyanobacteria in culture (see equation 1)

Organisms	Photosynthetic coefficients						
	P_m [a]	R_d [a]	P_m/R_d	α [b]	β [b]	I_c [c]	I_k [c]
***Aphanizomenon* sp. (strain BC 9401)**	**254·3**	**−26·39**	**−9·64**	**1·279**	**−0·01107**	**27·0**	**198·8**
Nodularia sp. (strain S34d)	522·0	−52·40	−9·96	3·133	0	18·2	166·6
Nodularia sp. (strain SN15a)	229·5	−57·50	−3·99	2·332	0	28·5	98·4
***Nodularia* sp. (average)**	**375·8**	**−54·95**	**−6·84**	**2·733**	**0**	**23·4**	**137·5**
Synechococcus sp. (green strain)	380·6	−100·10	−3·80	6·714	0	25·9	56·7
Synechococcus sp. (red strain)	201·7	−54·20	−3·72	6·311	0	10·6	32·0
***Synechococcus* sp. (average)**	**291·2**	**−77·15**	**−3·77**	**6·513**	**0**	**18·3**	**44·7**
Field sample diazotrophs[d]	449·1	−146·60	−3·064	9·417	0·0655	18·7	47·7

P_m is the maximum achievable rate of net photosynthesis at light saturation, R_d the dark respiration, α the chlorophyll related photosynthetic affinity coefficient (P/I), β the chlorophyll related coefficient describing photoinhibition at high irradiance, I_c the compensation point (the irradiance at which net oxygen exchange is zero) and I_k the saturation irradiance (P_m/α).

[a] μmol O_2 (mg Chl *a*)$^{-1}$ h^{-1}.

[b] μmol O_2 (mg Chl *a*)$^{-1}$ h^{-1} (μmol m^{-2} s^{-1})$^{-1}$.

[c] μmol m^{-2} s^{-1}.

[d] Walsby *et al.* (1997).

Table 3. Photosynthetic integrals in the top 18 m of the water column partitioned in the three components of the cyanobacterial community.

	Photosynthetic integral						
	Days 208–216		Day 212		Day 216		Change
	mmol m^{-2}	(%)	mmol m^{-2}	(%)	mmol m^{-2}	(%)	(%)
Daily integrals							
Aphanizomenon	5·82	(28)	6·46	(16)	10·47	(28)	62
Nodularia fraction	3·27	(16)	6·36	(15)	0·16	(1)	−98
Synechococcus	11·82	(56)	28·92	(69)	26·33	(71)	−9
Total	20·91	(100)	41·75	(100)	36·96	(100)	−11
Standardized integrals[a]							
Aphanizomenon	6·36		3·71		9·63		160
Nodularia fraction	4·10		4·76		−0·63		−113
Synechococcus	15·76		22·99		22·11		−4
Total	26·23		31·46		31·11		−1

[a] Integral, standardized for the mean daily insolation (33·5 mol m^{-2}) and mean attenuance (0·353).

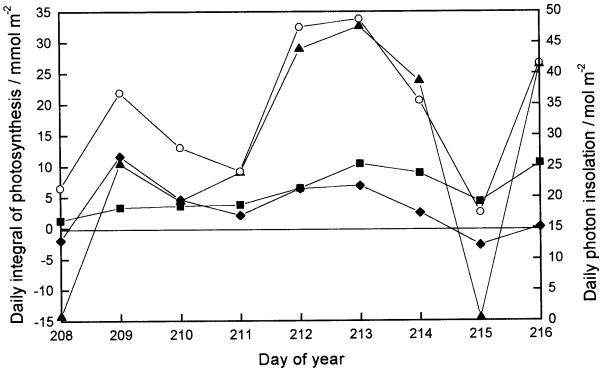

Fig. 5. Total primary production integrated over 24 h and all depths (filled symbols) and daily insolation (open circles) during the 9 day study. Triangles, *Synechococcus*; diamonds, *Nodularia*; squares, *Aphanizomenon*.

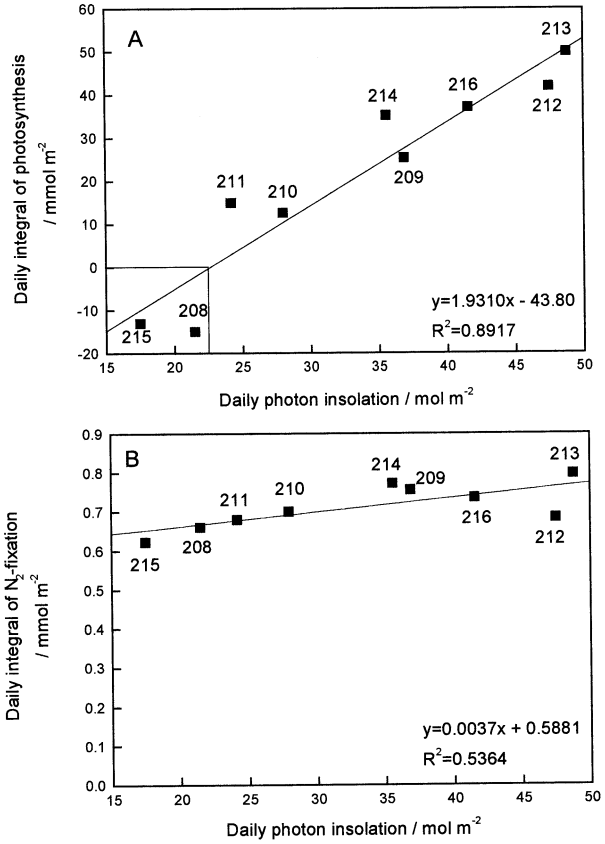

Fig. 6. (*A*) The relationship between the daily integrals of photosynthesis of total phytoplankton and total daily insolation. (*B*) The daily integrals of N_2 fixation by the diazotrophs. The day numbers are marked at the data points.

cyanobacterial community large differences were found (Table 3). The standardized photosynthetic integral of *Synechococcus* showed no difference between day 212 and 216. In *Aphanizomenon*, in contrast, it increased 2·6-fold, and in the *Nodularia* fraction even decreased, from 4·76 to −0·63 mmol O_2 m^{-2} d^{-1}. The average contributions to primary production during the 9 day experiment were about 56% for picoplankton, 28% for *Aphanizomenon* and 16% for the *Nodularia* fraction. Hence, the contribution to primary production by picoplankton was less than suggested by its contribution to biomass.

The daily integral of photosynthetic O_2 production by the cyanobacteria seemed to be mainly a function of the total insolation (Fig. 5). Only in the *Aphanizomenon* population was there an increase in production due to buoyancy. This, and the high P_m/R_d ratio in *Aphanizomenon*, explain why, even at the lowest daily insolation (on day 215), net photosynthesis by this cyanobacterium was positive, whereas that in the *Synechococcus* and *Nodularia* populations was negative (Fig. 5).

The strong dependence of phytoplankton photosynthesis on total daily insolation was clear from the linear relationship (Fig. 6). In Fig. 6*A* the total 24 h integrated photosynthesis is plotted against total daily insolation. It shows that, averaged over the 24 h, the compensation point (I_c) is at a daily insolation of 22·7 mol m^{-2}; below this, the net photosynthetic O_2 production for the day is negative. The average respiration at night was relatively

Table 4. Nitrogen fixation coefficients of Baltic Sea cyanobacteria in culture (see equation 1)

Organisms	Nitrogen fixation coefficients					
	N_m[a]	N_d[a]	N_m/N_d	α[b]	β[b]	I_k[c]
***Aphanizomenon* sp.**[d]	**4·59**	**1·50**	**3·06**	**0·0257**	**−0·00286**	**179**
Nodularia sp.[d] (strain S34d)	1·34	0·44	3·07	0·0209	−0·00004	64
Nodularia sp.[e] (strain SN15a)	4·64	0·75	6·19	0·0343	−0·00286	135
***Nodularia* sp. (average)**	**2·99**	**0·59**	**5·04**	**0·0276**	**−0·00145**	**108**
Field sample 1993[f]	6·50	1·48	4·40	0·0196	−0·0000012	331

N_m is the maximum achievable rate of N_2 fixation at light saturation, N_d the N_2-fixation rate in the dark, α the chlorophyll-specific affinity coefficient (N/I), β the chlorophyll-specific coefficient describing photoinhibition at high irradiance and I_k the saturation irradiance (P_m/α).

[a] μmol N_2 (mg Chl *a*)$^{-1}$ h^{-1}.

[b] μmol N_2 (mg Chl *a*)$^{-1}$ h^{-1} (μmol m^{-2} s^{-1})$^{-1}$.

[c] μmol m^{-2} s^{-1}.

[d] Data from Evans & Gallon (unpublished report).

[e] Data from Stal & Villbrandt (unpublished report).

[f] Stal & Walsby (1998).

constant and amounted to an O_2-consumption rate of $31{\cdot}7 \pm 5{\cdot}0$ mmol m^{-2} for the whole night (8·2 h), equivalent to 4·0 mmol m^{-2} h^{-1}.

Nitrogen fixation

Nitrogen fixation was calculated using the same integration method (Stal & Walsby, 1998). N_2 fixation versus irradiance (N/I) curves were fitted with equation (1). An example is shown in Fig. 3*B*. The N_2-fixation coefficients are summarized in Table 4. The data from the two strains of *Nodularia* were averaged. The N_2-fixation coefficients of a field sample of a mixed population of *Aphanizomenon* and *Nodularia*, used for the calculation of the daily integral of N_2 fixation in a previous publication (Stal & Walsby, 1998), are shown for comparison. The total N_2-fixation rates at each depth and time were calculated separately from coefficients obtained from the N/I curves of cultures of *Aphanizomenon* and *Nodularia*, and from the vertical distributions of biomass and photon irradiance; the two sets of values were summed to give the distributions of N_2-fixation rates depicted (Fig. 7). In contrast to photosynthesis, rates of N_2 fixation cannot be negative and, as shown by direct measurements (Stal & Walsby, 1998), may have relatively high values at night. N_2 fixation, however, was depressed to a greater extent at high irradiance (Fig. 3*B*) and there is therefore relatively less activity during the middle of the day as well as in the topmost water layers (Fig. 7). However, because *Aphanizomenon* was floating near the surface on day 216 and this organism had a relatively high dark nitrogenase activity, total N_2 fixation was high at night and during dawn and dusk (Fig. 7*B*). The standardized N_2-fixation integral (using the average insolation of 33·5 mol m^{-2}) of day 212, immediately after the storm mixing event, was compared with that on day 216, when the organism floated nearer the surface: N_2 fixation in *Aphanizomenon* showed little benefit from floating nearer the surface (Table 5). The *Nodularia* fraction also showed little difference in N_2 fixation between the two days. Total N_2

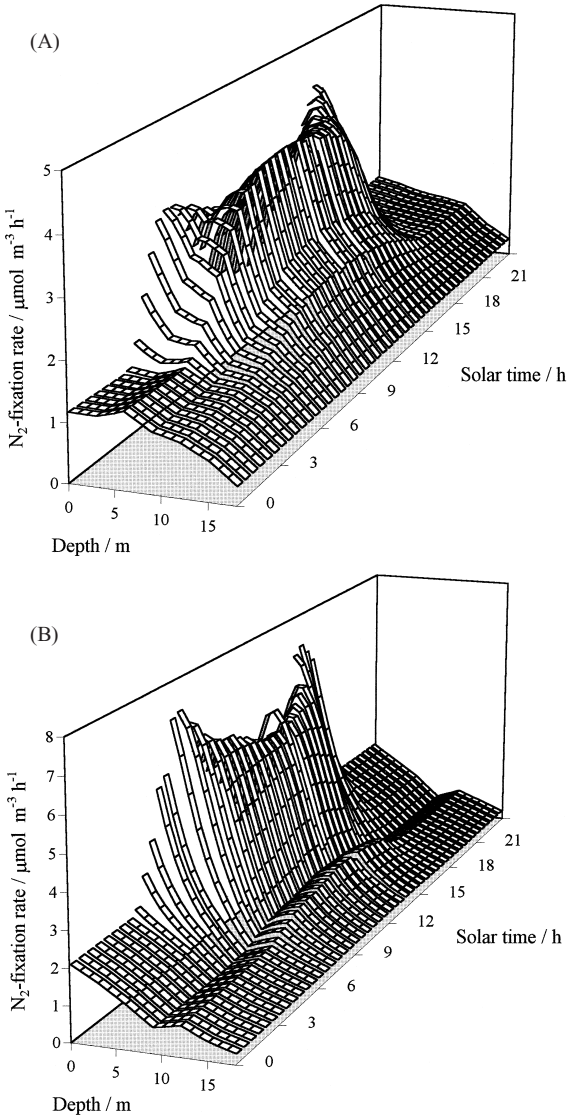

Fig. 7. Rates of N_2 fixation with depth and time by *Aphanizomenon* and *Nodularia* during (*A*) day 212 and (*B*) day 216.

Table 5. Integrals of N_2-fixation in the top 18 m of the water column partitioned in the two components of the cyanobacterial community

	N_2-fixation integral						
	Days 208–216		Day 212		Day 216		Change
	mmol m^{-2}	(%)	mmol m^{-2}	(%)	mmol m^{-2}	(%)	(%)
Daily integrals							
Aphanizomenon	0·46	(65)	0·49	(71)	0·51	(70)	5
Nodularia fraction	0·25	(35)	0·20	(29)	0·22	(30)	14
Total	0·71	(100)	0·68	(100)	0·74	(100)	8
Standardized integral[a]							
Aphanizomenon	0·52		0·44		0·52		19
Nodularia fraction	0·23		0·29		0·23		−23
Total	0·74		0·73		0·74		2

[a] Integral, standardized for the mean daily insolation (33·5 mol m^{-2}) and mean attenuance (0·353).

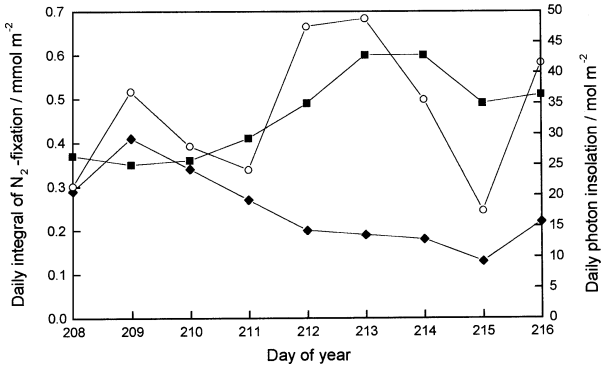

Fig. 8. The daily integral of N_2 fixation (filled symbols) and total daily insolation (open circles). Diamonds, *Nodularia*; squares, *Aphanizomenon*.

fixation increased by a factor of only 1·1 when comparing day 216 with day 212. Averaging N_2 fixation over the whole 9 day period, 65% could be attributed to *Aphanizomenon* and 35% to the *Nodularia* fraction. In contrast with primary productivity, the daily integral of N_2 fixation showed less dependence on insolation (Fig. 6*B*). This was not expected because N_2 fixation itself was clearly dependent on light (Fig. 3*B*). However, the rate of nitrogenase activity was relatively high in the dark whereas it was inhibited at high irradiance. Nitrogen fixation remained more-or-less constant over the whole 9 day period (Fig. 8) but this was the outcome of compensating changes in the two species: N_2 fixation in *Aphanizomenon* increased during the 9 day period, whereas it decreased in *Nodularia* by approximately the same amount (Table 5).

Discussion

Our results provide another example where both the biomass and the primary production of the picoplanktonic cyanobacteria exceed those of the larger, more conspicuous diazotrophic cyanobacteria, *Aphanizomenon flos-aquae* and *Nodularia spumigena* (cf. Jochem, 1988; Kuparinen & Kuosa, 1993): the picoplanktonic species may account for more than 50% of the biomass and as much as 74% of the chlorophyll *a*.

On average, the areal concentration of chlorophyll *a* in cells in the < 20 μm fraction was 40·7 mg m^{-2} in the top 18 m of the water column (Table 1). Using a cell C content of 0·29 pg and a C/Chl *a* ratio of 133 for picoplankton (Cuhel & Waterbury, 1984), a cell concentration of $1{\cdot}04 \times 10^6$ ml^{-1} is calculated. Similar cell concentrations have been observed by Jochem (1988), Kuosa (1991) and Albertano *et al.* (1997).

Fractionation with a 20 μm plankton net and a glass-fibre filter effectively separated the larger diazotrophic species from the picoplanktonic species (Stal *et al.*, 1999). Albertano *et al.* (1997) have demonstrated that picoplankton in the Baltic Sea occurs in three size classes. Twenty-seven per cent are approximately 0·4 μm wide, 47% are 0·8 μm wide and the remaining 26% are 1·4 μm wide. However, some of the picoplankton form aggregates that are retained by a wider-mesh plankton net (Albertano *et al.*, 1997). We also found some aggregates of picoplanktonic forms, but the > 20 μm fraction was largely composed of *Nodularia* and *Aphanizomenon* (Stal *et al.*, 1999).

Different behaviour of the three cyanobacteria

Not only were there differences in the biomass of the three fractions, but they changed in different ways after the storm mixing event: the *Synechococcus* biomass increased over the following day, the *Aphanizomenon* biomass increased by 28% over the next 5 days and the *Nodularia* biomass decreased substantially (Table 1). These differences may be explained by different responses of the cyanobacteria to changes in vertical distribution and nutrient availability.

(1) The *Synechococcus* population doubled within the first 24 h after the storm and then decreased (Fig. 2*A*). Much of the increase occurred in the subsurface layers at depths between 4 and 10 m. Since their small cells have a

very low sinking velocity ($< 0{\cdot}1$ m d^{-1}), their vertical distribution would have changed little by sinking in 5 days. The initial increase must therefore have been due to growth rather than redistribution and may have been stimulated by input of nutrients from deeper layers to the nutrient-depleted water of the euphotic zone by the mixing event. Kuosa (1991) also reported a minimum doubling time of 1 day in Baltic Sea populations of picocyanobacteria, though longer doubling times were more usual (Jochem, 1988; Kuosa, 1991), and suggested that the picocyanobacteria are normally nutrient-limited. Stal *et al*. (1999) found that *Synechococcus* was nitrogen-limited. The greater growth of *Synechococcus* in the middle layers rather than at the surface may have been influenced both by differences in nutrient availability and by the P/I response: this organism has a higher photosynthetic affinity coefficient (α), a lower compensation point (I_c) and lower light saturation value (I_k), though its P_m is relatively high (Table 2).

(2) *Aphanizomenon* became uniformly distributed by the mixing event but subsequently moved upwards each day with the median depth decreasing from 8·3 to 4·0 m (Fig. 2*B*). Its increase in biomass can be attributed to the higher photosynthetic activity supported by the higher insolation it received nearer the water surface (Walsby *et al*., 1997); in contrast there was little change in N_2 fixation. The colonial habit of this organism is necessary to provide the required floating velocity; the flake-like colonies were densely packed with parallel, adhering trichomes and were not disrupted by the mixing event. This colonial habit would also make the organism less effective in taking up nutrients introduced by mixing. *Aphanizomenon* would not have been nitrogen-limited, however, as its demands for growth were met by N_2 fixation (see below).

(3) *Nodularia* resembles *Aphanizomenon* in possessing gas vesicles, in forming rapidly floating aggregates (Walsby *et al*., 1995) and in fixing N_2, but it responded differently after the mixing event: its median depth in the water column increased from 7·3 to 12·2 m (Fig. 2*B*), and its biomass decreased by 46%. The aggregates of *Nodularia* filaments were more loosely conformed and may have been more susceptible to disruption by turbulence associated with the mixing event; some of the aggregates appeared bleached. Fragmentation would have resulted in smaller aggregates with a greatly decreased floating velocity (velocity is proportional to the square of diameter) and this would have prevented upward movement of the sort observed in *Aphanizomenon*. In *Trichodesmium*, colony fragmentation is also correlated with loss of nitrogenase activity (Carpenter & Price, 1976), though in that organism nitrogenase is not protected within heterocysts.

The proportion of nitrogen contributed by N_2 fixation

From the daily integrals given in Tables 3 and 5, the contributions of N_2 fixation to the nitrogen requirement of the cyanobacteria can be calculated. If the ratio of O_2 produced to C fixed is 1·2 (Kirk, 1994) then the 9 day mean of the daily integral of photosynthetic O_2 production, 20·9 mmol m^{-2}, is equivalent to a C biomass increase of 17·4 mmol m^{-2}, of which 9·85 mmol m^{-2} is in *Synechococcus* and 7·57 mmol m^{-2} is in the two diazotrophs. From these values are calculated the equivalent N biomass, by dividing by the cellular C:N ratio of 6·0 (Cuhel & Waterbury, 1984), giving 1·64 mmol m^{-2} in *Synechococcus* and 1·26 mmol m^{-2} in *Aphanizomenon*. The daily integral of N_2 fixation by the two diazotrophs is 0·71 mmol m^{-2}, and hence twice this for N, i.e. 1·42 mmol m^{-2}. N_2 fixation therefore produces an excess of 0·16 mmol m^{-2} for the diazotrophs' nitrogen requirement, equivalent to 12% of that fixed. This excess, if excreted, contributes to 10% of the nitrogen requirement of *Synechococcus*. During the same cruise, U. Kumitz (personal communication) has calculated from measurements using $^{15}N_2$ that up to 10% of the N_2 fixed is transferred to the picoplankton.

The total N_2 fixation of 0·71 mmol m^{-2} would support a C increase of 8·52 mmol m^{-2}, which is 49% of the total. The rest of the nitrogen required must have originated from other sources such as wet and dry deposition, run-off, transport of nitrogen from deep water, and from regeneration (Sörensson & Sahlsten, 1987). The total amount of N_2 fixation (0·71 mmol m^{-2} d^{-1}) would allow for 112% of the total production ($9{\cdot}1/1{\cdot}2 = 7{\cdot}58$ mmol C m^{-2}) by the diazotrophic cyanobacteria, partitioned as 110% in *Nodularia* and 114% in *Aphanizomenon*: hence both diazotrophic species support their own growth by N_2 fixation. Sörensson & Sahlsten (1987) also found that the diazotrophs support their growth requirement by N_2 fixation but contributed only 16% of the total nitrogen utilized by the whole phytoplankton community, while in this study N_2 fixation was calculated to contribute 49%.

Responses of the overall cyanobacterial population

By summing the daily integrals of photosynthesis and N_2 fixation of the different genera it is possible to assess the overall contribution of the cyanobacteria to the biological processes in the sea. The relationship of these total integrals to the total insolation for each day (Fig. 6) indicates the strong dependence of photosynthesis on the insolation and demonstrates that, even during this period of the year, the cyanobacterial community may be unproductive on cloudy days. N_2 fixation, in contrast, shows much less dependence on insolation, due to the contributions from cells in low irradiance and darkness. A consequence of these different responses is that imbalances will occur in C and N_2 fixation, which may require dynamic adjustment of stores of the two elements or result in excretion of that in excess.

Analyses of other factors for productivity are better made separately on the different cyanobacteria because in the summed curves the contributions of the more abundant picocyanobacteria dominate, their vertical distribution is more uniform, and the effect of differences in photosynthetic coefficients is lost.

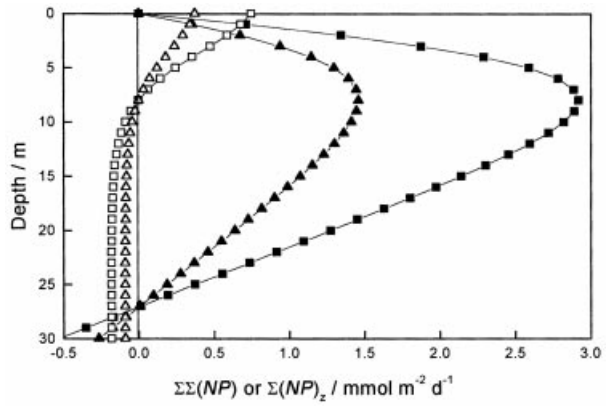

Fig. 9. Changes in the daily integral of photosynthesis ($\Sigma(NP)_z$) at each depth (z) (open symbols) and in the cumulative increase in the integral through the depths ($\Sigma\Sigma(NP)_z$) (filled symbols). The daily integrated euphotic depth (z_d) occurs at 8 m, where $S(NP)_z$ falls to zero, and the critical depth (z_c) occurs at 27·1 m, where $\Sigma\Sigma(NP)_z$ falls to zero. Integrals differ at temperatures of 15 °C (squares) and 5 °C (triangles) but z_d and z_c are not affected.

Simulating effects of insolation, mixed depth and temperature: explaining why growth is limited to summer months

The spreadsheets that were used to calculate the daily integral of photosynthesis from measurements made on the surface irradiance and the vertical distributions of biomass, irradiance and temperature, can also be used to provide quantitative assessments of the effect of changing these factors. Walsby (1997*b*) has already analysed the effects of insolation and mean depth on the photosynthesis of *Aphanizomenon*. The effect of mean colony depth was determined by calculating the potential photosynthesis for the same mean values for the 9 days of daily photon insolation (33·5 mol m^{-2}), attenuance (0·353 m^{-1}), temperature (15 °C) and colony chlorophyll biomass (8·58 mg m^{-2}); these integrals were plotted against the different median colony depth each day. The procedure was then repeated for different multiples of the mean daily insolation (but retaining the same 5 min variation of irradiance), from 10 to 56 mol m^{-2} (full sunshine). The results indicate a strong linear decrease in photosynthesis with depth and a positive curvilinear increase in photosynthesis with insolation. Calculations made with vertical distributions of *Aphanizomenon* on different days indicated the depth at which the daily integral of photosynthesis was compensated by the daily integral of respiration; it increased from about 6 m at the lowest insolation to 11 m at the highest. We have now extended these calculations to analyse the effects of mixed depth and temperature on the *Aphanizomenon* population.

Simulating effects of different mixing depths and temperatures

Over the 9 day study, the mean *Aphanizomenon* chlorophyll biomass was 8·6 mg m^{-2} in the top 30 m. In a completely mixed water column the biomass would have a uniform concentration: the situation is simulated by setting the value of N to 0·287 mg m^{-3} at each depth. Using the same standardized values of attenuation, temperature and insolation given above, the integral $\Sigma(NP)_z$ at each depth, z, is calculated by integrating the NP values throughout the day. The value of $\Sigma(NP)_z$ decreases with the daily insolation reaching each successive depth (Fig. 9) and at a certain depth falls to zero: this is designated the *daily integrated euphotic depth* (z_d) by Walsby (1997*b*). A running total of $\Sigma(NP)_z$, made from the surface to each successive depth, gives a value of $\Sigma\Sigma(NP)_z$, which increases until z_d is reached and then decreases. Eventually a depth is reached at which $\Sigma\Sigma(NP)_z$ also reaches zero: this is designated the critical depth (z_c) by Reynolds (1984). In a water column mixed below z_d the population will still be productive if the net gains above z_d compensate for the losses below it; when the depth of mixing exceeds z_c, however, the losses exceed the net gains and the population will be unable to grow.

The curves of $\Sigma(NP)_z$ and $\Sigma\Sigma(NP)_z$ shown in Fig. 9 are for the standardized insolation following the time course calculated for 1 August (day 212, the middle of the 9 day period). The value calculated for z_d is 8·0 m and for z_d is 27·1 m. Changing the values of the biomass concentration affects the magnitude of the photosynthetic integrals but does not change z_d or z_c. Moreover, if the Q_{10} for photosynthesis and respiration are equal, as assumed here, then the values of z_d and z_c are independent of the temperature.

Simulating effects of daylength and insolation

The seasonal changes in daylength and insolation at the latitude of the Baltic Sea will have a profound effect on z_d and z_c. The maximum photon irradiance (I_t) value at each 5 min interval were calculated for each day of the year at the latitude of the Bornholm Basin (55° 23′ N), using the equations of Kirk (1994) formulated in the spreadsheet Integral.xls (see table 3, column N, of Walsby, 1997*a*). These I_t values, multiplied by 0·60 (the correction factor allowing for average irradiance losses due to cloud and surface reflection calculated for the whole 9 day period), were inserted in the integration spreadsheets together with the uniform values of N and standardized values of K_d and Θ given above. In this way, the values of the daily integrated euphotic depth and critical depth were calculated for each day of the year (Fig. 10). It was calculated that z_d increased from a minimum of 1·4 m at the winter solstice to 8·4 m at the summer solstice, while z_c increased from 2·8 m to 30·8 m. These calculated values of z_d and z_c would vary inversely with K_d but would not vary with temperature (see Fig. 9).

As Reynolds (1984, 1987) has emphasized, growth of a phytoplankton population stops when the mixed depth, z_m, exceeds z_c. In the Bornholm Basin of the Baltic Sea the seasonal thermocline reaches its minimum depth of about 17 m in early August; in the period of November to April the water column becomes isothermal and is completely mixed down to the halocline at depths of 45 m or more.

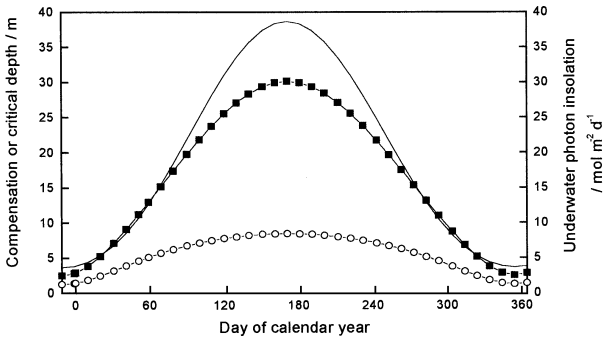

Fig. 10. Changes in the values of the daily integrated euphotic depth (z_d) (open symbols) and critical depth (z_c) (filled symbols) calculated for each day of the year. The changes are related to the calculated insolation: the value plotted is $0{\cdot}6\Sigma I_t$ (line without symbols) (see text).

Since this greatly exceeds z_c, growth of the population would be impossible during these winter months. The tradition that cyanobacterial blooms require warm temperatures for their development is founded on the observation that the blooms occur during summer when the temperatures are highest. A change in temperature from 5 to 15 °C that doubles growth rate will certainly affect the rate of development of a cyanobacterial bloom (as it will other phytoplankton) but it will not have the overriding effect on the z_c/z_m ratio, which determines whether growth occurs or not. We suggest that the controlling effect of the rise in temperature is not mediated principally through growth rate but rather through its effect in stabilizing the water column and reducing the mixed depth to a value that does not exceed z_c. These suggestions can be tested by comparing the z_m values on different dates and locations in the Baltic Sea with the z_c values calculated from measured values of K_d and the photosynthetic coefficients of different phytoplankton. The prediction is that growth of the phytoplankton population does not begin until z_c exceeds z_m (Reynolds, 1984; Talling, 1957).

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