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Effects of toxic cyanobacteria on a plankton assemblage: community development during decay of *Nodularia spumigena*

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ABSTRACT: We studied the development of the plankton community in an artificially created toxic *Nodularia spumigena* bloom during a 2 wk enclosure study at the SW coast of Finland in the Baltic Sea. We measured bacterial abundance, dominant phytoplankton groups and ciliates, as well as concentrations of phytoplankton pigments, fatty acids, nodularin, protein and nutrients. A high POC:chl *a* (<10 µm) ratio (427 ± 185), a decrease in the polyunsaturated:total fatty acid ratio (from 0.4 to 0.2), and a reduction in cyanobacteria filament length indicated decay of *N. spumigena* during the course of the experiment. Along with cyanobacterial decay, high concentrations of ammonium (last day: 2.7 ± 2.0 µmol l^{-1}), nitrate (0.1 ± 0.01 µmol l^{-1}), and organic nutrients were released into the water, whereas chl *a* and the cyanobacterial pigments, echinenone and zeaxanthin, decreased. Nodularin was found in the mesocosms during the whole experiment. A strong increase in filamentous bacteria was detected by the middle of the experiment, most likely indicating a response to grazing pressure. Two ciliate species, *Mesodinium rubrum* and *Urotricha* sp., decreased dramatically during the experiment, probably due to predation by the increasing mesozooplankton community. The ciliate *Euplotes* sp. flourished in the bags and was best suited to escape predation due to its protecting lorica and its surface affinity. No direct harmful effects of the cyano-bacteria on the microorganisms could be documented. We conclude that these blooms provide a potential food source for the heterotrophic food chain, from bacteria, flagellates and ciliates to crustacean zooplankton, and possibly fish.

KEY WORDS: *Nodularia spumigena* · Decay · Bacteria · Fatty acids · Nodularin · Ciliates

INTRODUCTION

Fossil records show that the first cyanobacterial blooms appeared ca. 7000 years ago in the Baltic Sea (Bianchi et al. 2000). Resistance of zooplankton to algal toxins has been documented several times (Hanazato & Yasuno 1987, Fulton 1988) and could have evolved also in the Baltic ecosystem. Besides producing toxins, cyanobacteria also show allelopathic and antibiotic activities (Østensvik et al. 1998, Pushparaj et al. 1999) that may be harmful to phytoplankton and bacteria.

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The carbon flow in the planktonic food webs of the Baltic Sea is well studied (Lignell et al. 1993, Uitto et al. 1997), as are the spatial and temporal dynamics of the cyanobacterial mass-occurrences (Kononen et al. 1999, Bianchi et al. 2000) that are common phenomena especially during warm summers. In contrast, few attempts have been made to study the fate of a decaying cyanobacterial bloom and its effects on other organisms of the pelagic ecosystem. A large part of the diatom spring bloom at higher latitudes settles to the bottom (Wassmann & Slagstad 1993), whereas knowledge about the fate of the cyanobacterial bloom is contradictory. Some studies have concluded that sedimentation and grazing are negligible in the Baltic Sea (Heiskanen & Kononen 1994, Sellner 1997), whereas in other studies, grazing is considered important (Meyer-Harms et al. 1999, Rolff 2000).

One of the most important bloom-forming cyanobacteria species in the Baltic Sea is *Nodularia spumigena* (Kahru et al. 1994, 2000). Several characteristics of *N. spumigena* make it an interesting study object. Cyanobacteria are strong competitors for e.g. phosphorus, nitrogen (Mur et al. 1999), and light (Ibelings & Maberly 1998). Further, *N. spumigena* is toxic and has gas vacuoles (Mur et al. 1999), which operate even in dead cells (reviewed by Sellner 1997). Therefore, an ageing *N. spumigena* bloom has been suggested to resist sedimentation and to decay within the water column (Heiskanen & Kononen 1994, Sellner 1997). Heinänen et al. (1995) found that an ageing cyanobacterial bloom provided elemental substrates for bacteria, whereas the young and growing *Nodularia* filaments seldom were colonised by any organisms. Further, it has been shown that decaying cyanobacterial blooms are diverse biotopes colonised by bacteria, protozooplankton, flagellates and crustaceans (Hoppe 1981).

The purpose of this study was to investigate the plankton community development in an artificial cyanobacteria bloom, created by adding a high concentration of cultured toxic *Nodularia spumigena* to a <100 µm filtered natural plankton community. The study was performed in mesocosm bags in July 1999. Over a period of 2 wk, we monitored the mesocosm bags by measuring organism abundances, chl *a*, toxin, nutrient, protein, fatty acid and phytoplankton pigment concentrations. In this paper, we analyse and discuss the effects of the decaying bloom on the plankton community and the interactions between different organism groups and the factors controlling them.

MATERIALS AND METHODS

***Nodularia spumigena* culture, experimental set-up and sampling.** The toxic *Nodularia spumigena* strain (AV1) was obtained from the Division of Microbiology, University of Helsinki (Lehtimäki et al. 1994, 2000) and grown in a modified Z8 medium at ~6.8 µE m^{-2} s^{-1} (Hughes et al. 1958, Kotai 1972). The light was measured with the LICOR-1000 irradiance meter. Five litre batch cultures were grown at 18°C in a 16 h light: 8 h dark cycle and supplied with air. The cyanobacteria concentration was determined spectrophotometrically using a calibration curve of extinction versus carbon concentration, which was derived from chemical oxygen demand measurements (Gulati et al. 1991).

We set up five 120 l mesocosm enclosures in a sheltered bay, characterised by upwelling (Niemi 1975). The transparent polyethylene enclosures (thickness: 150 µm) were double and their collars mounted on a wooden rack (Kivi et al. 1993). The bags were filled with 100 µm filtered seawater collected from a nearby pelagic area (Tvärminne Zoological Station, Baltic Sea, 59° 51′ N, 23° 15′ E). The monoculture of *Nodularia spumigena* was filtered using a 20 µm mesh size net, and added to 4 bags at a concentration of 460 µg C l^{-1}. *N. spumigena* culture was not added to one of the bags which was kept as a control. All bags were kept in normal daylight and covered with transparent polyethylene. During the 2 wk experiment (2 to 14 July 1999), temperature was measured daily, samples for bacteria, phytoplankton, ciliate and chl *a* measurements were collected daily; and samples for pigment, fatty acid (from 7 July 1999 onwards), total nodularin, protein, and particulate and dissolved nutrient measurements were collected every second or third day. The bags were mixed before sampling.

Analyses. Bacterial cell counts were made according to Hobbie et al. (1977) and Autio (1998): 1 ml was stained with acridine orange. At least 20 fields and 200 cells were counted with a Diaplan microscope using 12.5× oculars and 100× magnification. The cells were divided into 5 morphotypes according to average sizes and morphology: cocci (no size limit), vibroid-like bacteria (no size limit), short rods (1.5 to 10 µm), medium-sized rods (10 to 50 µm) and long rods (50 to 130 µm). A total of 100 cells, randomly selected, were measured at the beginning and the end of the experiment. The cell volumes (µm^3) were calculated according to the formula $(\pi/4)W^2(L - W/3)$ where L is length and W is width (Fuhrman 1981).

For chl *a* analysis, 2 parallel 100 ml samples were filtered on glass-fibre filters (Whatman GF/F), sonicated and extracted in 96% ethanol for 24 h in darkness. Chl *a* was analysed for 3 size fractions (total, <20 µm, <10 µm) and measured with a Shimadzu spectrofluorometer.

Subsamples (200 to 500 ml) were filtered on Whatman GF/F filters and stored at –20°C for pigment analyses with HPLC. The frozen filters were extracted in

3 ml 100% methanol buffered with 2% ammonium acetate by sonication and centrifugation. An aliquot (300 µl) of the extract was injected into an RSil C_{18} column (150 × 4.6 mm, Bio-Rad RSL). The photosynthetic pigments of different phytoplankton groups were separated at a flow rate of 1 ml min^{-1} by a linear gradient programmed as follows (minutes, %solvent A, %solvent B, %solvent C): (0, 100, 0, 0), (4, 0, 100, 0), (18, 0, 20, 80), (21, 0, 100, 0), (24, 100, 0, 0) and (29, 100, 0, 0). The instrument used was a Merck-Hitachi liquid chromatograph equipped with an L6200A gradient pump with system controller (interface module D-600), a photodiode array detector (L4500), and an F-1050 fluorescence spectrophotometer. Pigment detection was done at 436 nm for all chlorophylls and carotenoids. The HPLC system was calibrated with pigment standards from the International Agency for ^{14}C Determination, Denmark. Pigments were identified by retention time and comparison of on-line collected absorption spectra (between 300 and 700 nm) with those of the pigment standards in the spectral library.

Phytoplankton and ciliates were determined from a 100 ml sample preserved with acid Lugol's solution. Cells were counted in cuvettes with a Leitz Labovert microscope using 10× oculars and 25× objectives for ciliates, and 40× objectives for phytoplankton (Utermöhl 1958). At least 500 flagellates (*Chrysochromulina* sp., *Pyramimonas* sp., cryptophytes), and 300 filaments of *Nodularia spumigena* were counted per cuvette. Less abundant diatoms, dinoflagellates and other cyanobacteria were counted in 20 to 60 eye-fields, depending on the cell density.

Fatty acids were measured to get an estimate of the chemical composition of the bloom during decay. For analysis, 1 sample of 200 to 500 ml was filtered on combusted GF/F (Whatman) filters, placed into Eppendorf tubes, flushed with nitrogen gas and stored at −80°C. Lipids were extracted from the filters for 24 h in CH_2Cl_2-methanol (2:1, v/v) with a known amount of C_{17} fatty acid added to the sample. The fatty acids were transmethylated with BF_3-methanol to form fatty acid methyl esters (FAME). The fatty acids were analysed by gas chromatography on a capillary column. The FAME sample was injected into a gas chromatograph (Hewlett Packard 5890A) with splitless injection using helium as a carrier gas at 1.8 ml min^{-1}. The injection temperature was +200°C. The temperature program was in 2 steps: first at +80°C with a 40°C min^{-1} increase to +160°C where it stayed isothermal for 1 min, and then with an increase from +160°C to +220°C at 3°C min^{-1} where it remained isothermal for 17 min. Peaks from chromatograms were compared with Sigma and Larodan FAME standards for specific fatty acid identification and the integrated peaks compared with the peak area of the C_{17} standard.

Nodularin, the hepatotoxin produced by *Nodularia spumigena*, was measured in order to get an estimate of cyanobacteria biomass and to observe its potential harmful effects on the plankton. Nodularin is known to correlate strongly with *N. spumigena* cell numbers (Heresztyn & Nicholson 1997). The total concentration in mesocosms was determined by extracting the nodularin directly from 1 l of water by sonicating twice for 15 min (Braun Labsonic-U) and freezing and thawing twice. The remaining cell material was removed by filtering with GF 52 glass-fibre filters (Schleicher & Schuell). The filtrate was concentrated on C-18 cartridges (Oasis, Waters). The cartridges were washed with 5 ml of 20% methanol prior to elution with 2 ml of 100% methanol. The samples were dried in an air stream and dissolved in 0.5 ml of 20% methanol. Nodularin was analysed with a Hewlett-Packard HP1090 liquid chromatograph equipped with a Hewlett-Packard UV/VIS diode array detector and Hewlett-Packard ODS Hypersil column (100 mm × 4.6 mm). The mobile phase was a 77:23 mixture of 10 mM ammoniumacetate buffer and acetonitrile. Flow rate was 1 ml min^{-1}, injection volume 25 µl, and detection at 238 nm. Nodularin was identified by its retention time and UV-spectrum. Purified nodularin was used as a reference.

Ammonium (NH_4^+), nitrate (NO_3^-), total nitrogen, phosphate (PO_4^-) and total phosphorus were analysed according to Koroleff (1979). DON was analysed similarly as total nitrogen (Koroleff 1979), after the sample had been filtered through acid-washed and precombusted (450°C) Whatman (GF/F) glass fibre filters. POP was analysed according to Solórzano & Sharp (1980). For POC, PON and POP, all glassware and glass fibre filters (Whatman GF/F) were acid-washed, and filters precombusted (450°C). POC and PON analyses were measured with a mass spectrometer (Europa Scientific Roboprep and Tracermass).

Protein analysis was measured to get an estimate of the chemical composition of the bloom according to Herbert et al. (1971).

Statistical analysis. We used principal component analysis (PCA) in order to find out which variables correlated best with cyanobacteria added to the mesocosms. The reason for using PCA was to obtain the interrelationship between the high number of variables included in this study. PCA identifies principal components of which the first one consists of a linear combination of the original variables and reflects the correlation between them, whereas the second one consists of the remaining information, unrelated to the first one. PCA does not make any *a priori* assumptions about the relationships among variables. A score higher than 0.6 on a component indicates moderate significance, whereas scores higher than 0.7 indicate high significance (Meglen

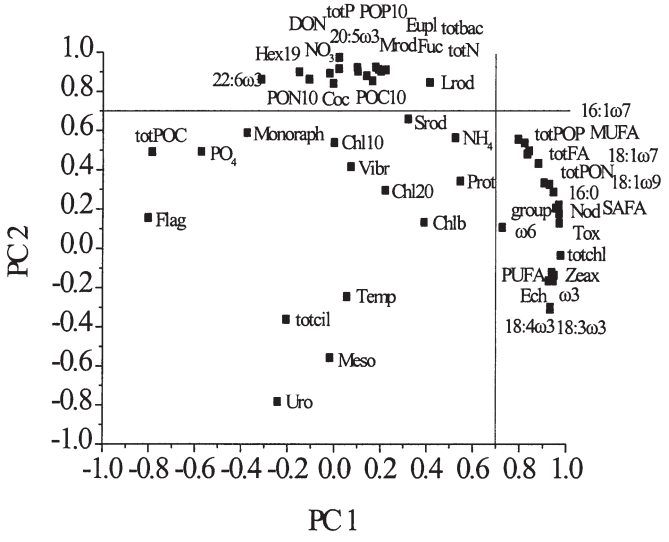

Fig. 1. Biplot of variables analysed with principal component analysis (PCA) for the total data. Loadings above ±0.7, shown with a line on both axes, indicate substantial to high relationships. See 'Results' for interpretations of components. Group: control vs treatment, Temp: temperature, Flag: flagellates, Nod: *Nodularia spumigena*, Monoraph: *Monoraphidium contortum*, Uro: *Urotricha* sp., Eupl: *Euplotes* sp., Meso: *Mesodinium rubrum*, Totcil: total ciliates, Coc: cocci, Srod: short rods, Mrod: medium-sized rods), Lrod: long rods, Vibr: vibroid-like bacteria, totbac: total bacteria, totchl: total chlorophyll *a*, Chl10: chlorophyll *a* <10 µm, Chl20: chlorophyll *a* <20 µm, Tox: nodularin, NH4: ammonium, NO_3: nitrate, totN: total nitrogen, PO_4: phosphate, totP: total phosphorus, totPON: total particulate organic nitrogen, PON10: PON <10 µm, totPOP: total particulate organic phosphorus, POP10: POP <10 µm, totPOC: total particulate organic carbon, POC10: POC <10 µm, DON: dissolved organic nitrogen, Prot: protein, Ech: echinenone, Chlb: chlorophyll *b*, Zeax: zeaxanthin, hex19: 19′-hexanoyloxyfucoxanthin, Fuc: fucoxanthin, SAFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, totFA: total fatty acids, ω3: ω3-series of PUFA, ω6: ω6-series of PUFA

1992). Data were log transformed in order to homogenise variance. All data were not normally distributed, but PCA should be robust concerning slight deviations from normality (Mayzaud et al. 1989). The PCA was run with Varimax rotation, indicating that the sum of variances is maximised of loadings in the factor matrix (Hair et al. 1998). Conventional statistical methods such as correlation analysis or repeated-measures ANOVA were not applicable in this case, due to the unreplicated control and the large number of variables.

RESULTS

We interpreted that the first principal component (PC 1) was strongly associated with the added *Nodularia spumigena* and associated characteristics: total chl *a*, nodularin, total PON and POP, the cyanobacterial pigments echinenone and zeaxanthin, saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), ω3 PUFA-series and total fatty acids (FA), and 16:0, 16:1ω7, 18:1ω7, 18:1ω9, 18:3ω3 and 18:4ω3. The second component (PC 2) was closely related to processes that showed similar dynamics in both the control and the treatment, including the ciliate *Euplotes* sp., cocci, medium-sized rods, long rods and total bacteria, NO_3^-, total N and P, PON, POP and POC (<10 µm), DON, the pigments 19′-hexanoyloxyfucoxanthin, fucoxanthin and 2 FAs: 20:5ω3 and 22:6ω3 (Fig. 1, Tables 1 & 2). The first 2 components ex-plained 74% of the total variance; hence no other components are discussed. All dynamics described below are based on the PCA.

Bloom decay

Cyanobacteria started to decay already by the middle of the experiment. The filaments were observed under the microscope and they were in poor condition; the cells were transparent and covered by epifauna and flora. Several of the measured parameters indicated bloom decay. The filament length of *Nodularia spumigena* decreased significantly (Kruskal-Wallis 1-way ANOVA, $H_{2,299} = 9.9$, $p < 0.01$) between the first and the last day of the experiment, from 288 ± 267 to 158 ± 89 µm (mean ± SD, n = 100). The POC:chl *a* (<10 µm) ratio was 427 by the end of our study (Fig. 2A). The PUFA:total FA ratio was significantly lower in the end than in the middle of the study (Mann-Whitney *U*-test, $U = 2.2$, $n_1 = 4$, $n_2 = 4$, $p < 0.05$; Fig. 2B). Total chl *a* and the cyanobacterial pigments, echinenone and zeaxanthin decreased strongly towards the end of the experiment (Fig. 2C,D,E). NH_4^+ increased towards the end of the experiment (Fig. 2F). On the other hand, the development of total nodularin and protein concentrations did not indicate bloom decay because total nodularin was quite stable throughout the experiment (Fig. 2G), whereas protein varied a lot (data not shown).

Succession in the enclosures

The average temperature fluctuated at 15.6 ± 1.6°C during the experiment due to upwelling of cold water in the bay where the enclosures were situated (data not shown).

The abundance of different bacteria, total bacteria, cocci, vibroid-like bacteria and short rods was lowest during the middle of the experiment (Fig. 3A to D). Filamentous bacteria such as medium-sized and long rods became more abundant from 10 July onwards

Table 1. Diagnostic pigments for characterisation of the different algal groups (Millie et al. 1993, Meyer-Harms & von Bodungen 1997) and the main species recorded in the mesocosm bags

Phytoplankton marker pigment	Autotrophic groups and main species
Zeaxanthin and echinenone	Cyanobacteria: *Nodularia spumigena*, *Anabaena* sp., *Aphanizomenon flos-aquae*, *Limnothrix* sp.
Fucoxanthin	Chrysophytes: *Pseudopedinella* sp. *Spiniferomonas* sp., *Uroglena* sp. Diatoms Dinoflagellates Prymnesiophytes: *Chrysochromulina* sp.
19′-hexanoyloxyfucoxanthin	Dinoflagellates Prymnesiophytes: *Chrysochromulina* sp.
Chl *b*	Chlorophytes: *Monoraphidium contortum* Euglenophytes: *Pyramimonas* sp. Prasinophytes
Alloxanthin[a]	Cryptophytes

[a]Pigment not recorded in HPLC, whereas cryptophytes found in microscopic countings

especially in the cyanobacteria bags (Fig. 3E,F). The average cell volumes of the different bacterial morphotypes at the end of the experiment were 1.4 ± 2.1 μm^3 (cocci + vibroid-like bacteria), 2.7 ± 1.3 μm^3 (short rods), 9.1 ± 4.3 μm^3 (medium-sized rods), and 21.0 ± 12.0 μm^3 (long rods). Bacterial volume in the treatment enclosures was significantly higher at the end than at the beginning of the experiment (Mann-Whitney U, $z = 10.5$, $n_1 = 100$, $n_2 = 100$, $p < 0.0001$).

Among the different measured pigments, there was no trend to be found in chl *a* (<10 and <20 µm) and chl *b* (Fig. 4A,B,C), whereas fucoxanthin and 19′-hexanoyloxyfucoxanthin showed high scores on PC 2, indicating that these pigments developed in the same manner in all enclosures (Fig. 1). Fucoxanthin and 19′-hexanoyloxyfucoxanthin concentrations increased in all bags towards the end of the experiment (Fig. 4D,E).

The number of *Nodularia spumigena* filaments in the enclosures showed a varying pattern, but a slight decrease was to be detected at the end of the study (Fig. 5A). Flagellates were significantly less abundant in the cyanobacteria bags than in the control (Fig. 5B), and were strongly negatively associated with *N. spumigena* (Fig. 1). Heterotrophic flagellates were not counted during the study. The green alga *Monoraphidium contortum* did not show any clear pattern during the study (Fig. 5C, Table 1). Among the dominant groups of ciliates, 2 patterns could be distinguished: *Euplotes* sp. increased fast in number from 10 July onwards in the cyanobacteria bags (Fig. 5D), whereas *Mesodinium rubrum* and *Urotricha* sp. (10 to 40 µm) declined and almost disappeared from all en-closures by the end of the experiment (Fig. 5E,F). By the end of the experiment, *Urotricha* sp. increased slightly again. No particular trend was ob-served for *Strombidium* sp. and *Strombilidium* sp. (data not shown). Total ciliates showed a varying pattern and were not associated closely with any component (Fig. 1). In the end, the total number of ciliates increased rapidly (Fig. 5G).

Mesozooplankton abundance and composition are discussed in detail in K.S. et al. (unpubl.). In short, copepodites and adults of the copepod *Eurytemora affinis* were on average found at 10 ind. l^{-1} at the end of the experiment, whereas the number of copepodites and adults of *Acartia bifilosa* was negligible (<1 ind. l^{-1}). The abundances of the rotifers *Synchaeta* spp. (5 to 90 ind. l^{-1}) and *Keratella* spp. (<10 ind. l^{-1}), *E. affinis* nauplii (10 to 50 ind. l^{-1}), the cladoceran *Bosmina longispina maritima* (<10 ind. l^{-1}) were variable.

NO_3^- and total N increased strongly towards the end of the experiment (Fig. 6A,B). Nitrate increased simultaneously with ammonium in all enclosures. PO_4^- did not

Table 2. Fatty acid composition (µg l^{-1}) of phytoplankton in mesocosm enclosures in the middle of the experiment (7 July). SAFA: saturated, MUFA: monounsaturated, PUFA: polyunsaturated fatty acids

Fatty acids	Treatment (*Nodularia spumigena* added) mean ± SD	Control (no *N. spumigena* added)
14:0	11.2 ± 1.3	15.1
16:0	119.8 ± 12.3	14.2
16:1ω7	37.9 ± 4.0	6.9
18:0	9.1 ± 0.6	2.6
18:1ω9	12.1 ± 0.8	3.2
18:1ω7	13.7 ± 0.7	2.3
18:2ω6	12.7 ± 2.7	4.2
18:3ω3	50.5 ± 8.4	5.4
18:4ω3	52.6 ± 11.5	4.3
20:3ω3	3.0 ± 0.2	5.7
20:5ω3	6.7 ± 0.3	6.2
22:6ω3	11.1 ± 1.4	12.0
SAFA	141.8 ± 12.0	34.9
MUFA	68.7 ± 3.6	15.4
PUFA	141.0 ± 22.1	41.0
ω3	126.2 ± 19.4	35.1
ω6	13.2 ± 2.7	4.6
Total	384.2 ± 40.5	98.6

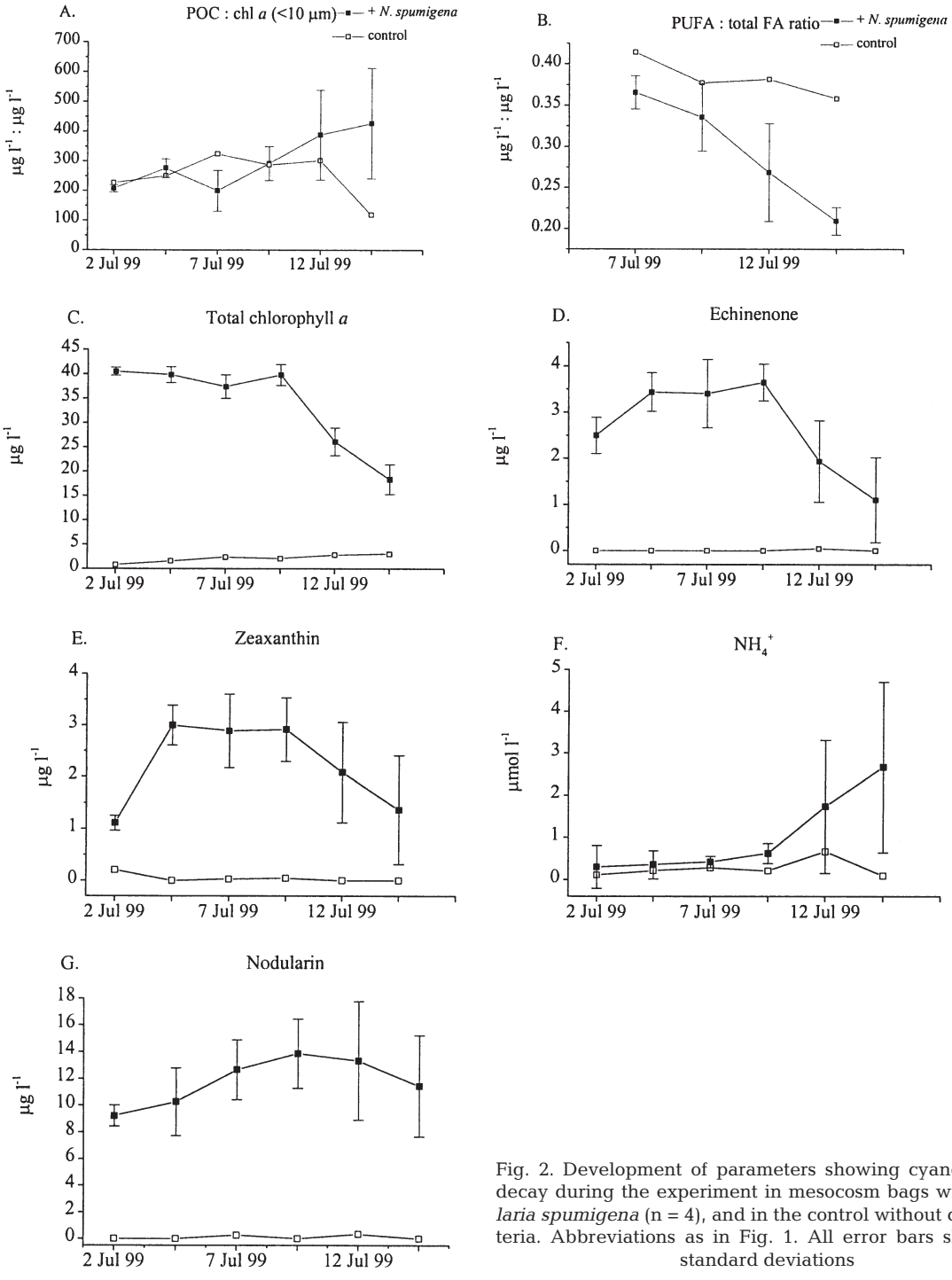

Fig. 2. Development of parameters showing cyanobacterial decay during the experiment in mesocosm bags with *Nodularia spumigena* (n = 4), and in the control without cyanobacteria. Abbreviations as in Fig. 1. All error bars shown are standard deviations

show any clear pattern (Fig. 6C), whereas total P was high in the treatment bags in the beginning of the experiment (53 ± 0.4 µg l^{-1}), but continued to decrease until the end of the monitoring period (Fig. 6D). Total PON and POP were strongly correlated with *Nodularia spumigena* (PC 1) and showed a clear decrease at the end of the experiment (Fig. 6E,G). Total POC showed the same pattern (Fig. 6H). POC, PON and POP (<10 µm) were closely associated with processes similar in all bags (PC 2), indicating that they developed in a similar pattern in all bags (data not shown). DON increased in all bags from the middle of the experiment onwards, up to 43 ± 7 µmol l^{-1} during the last measurement (Fig. 6F). The total N:P ratio increased continuously in all bags and was

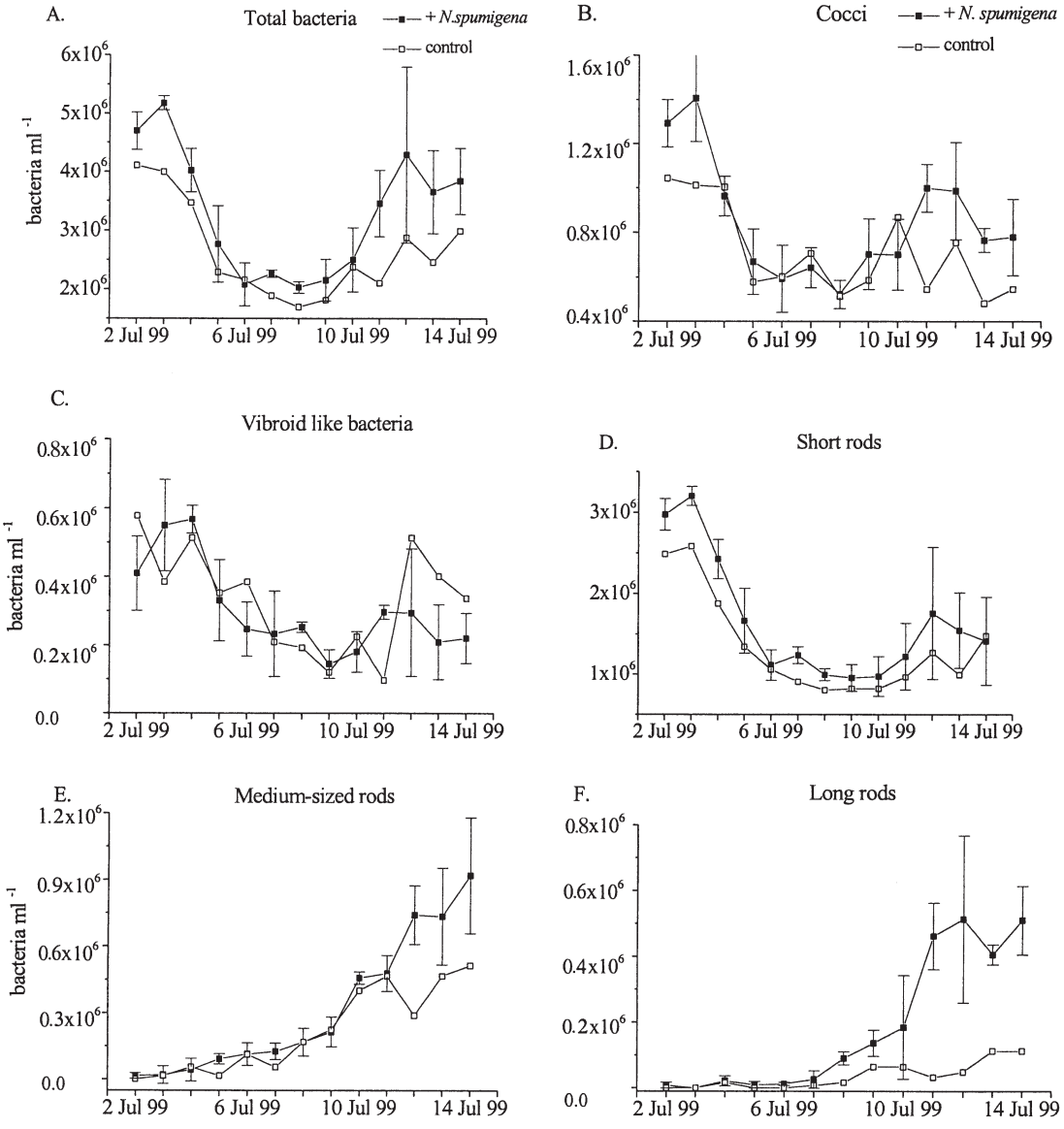

Fig. 3. Abundance of bacteria (ind. ml^{-1}). Symbols as in Fig. 2

already above the Redfield ratio in the control enclosure in the beginning of the experiment. In the cyanobacteria bags, the total N:P ratio reached the Redfield ratio on the fifth day of the experiment (Fig. 6I).

DISCUSSION

Bloom condition

Hoppe (1981) observed that the decay of *Nodularia spumigena* was a long process and that the filaments remained suspended in the upper water column for weeks in a stage of progressive decay. By the end of our study, the POC:chl *a* (<10 µm) ratio indicated that the bloom consisted of detritus and was well into the decaying process (Fig. 2A). A POC:chl *a* ratio below 100 indicates healthy and growing cells (Granéli et al. 1999). A high PUFA:total FA ratio indicates high growth rates (Ahlgren et al. 1992). In our study, the PUFA:total FA ratio as well as total chl *a* decreased with time, which suggests low growth rates and decay of the cyanobacteria (Fig. 2B,C). Further, a low protein concentration of the seston, among other factors, also indicates bloom decline (Jónasdóttir et al. 1995). In our study, the variation within the protein measurements was too large to find this relationship.

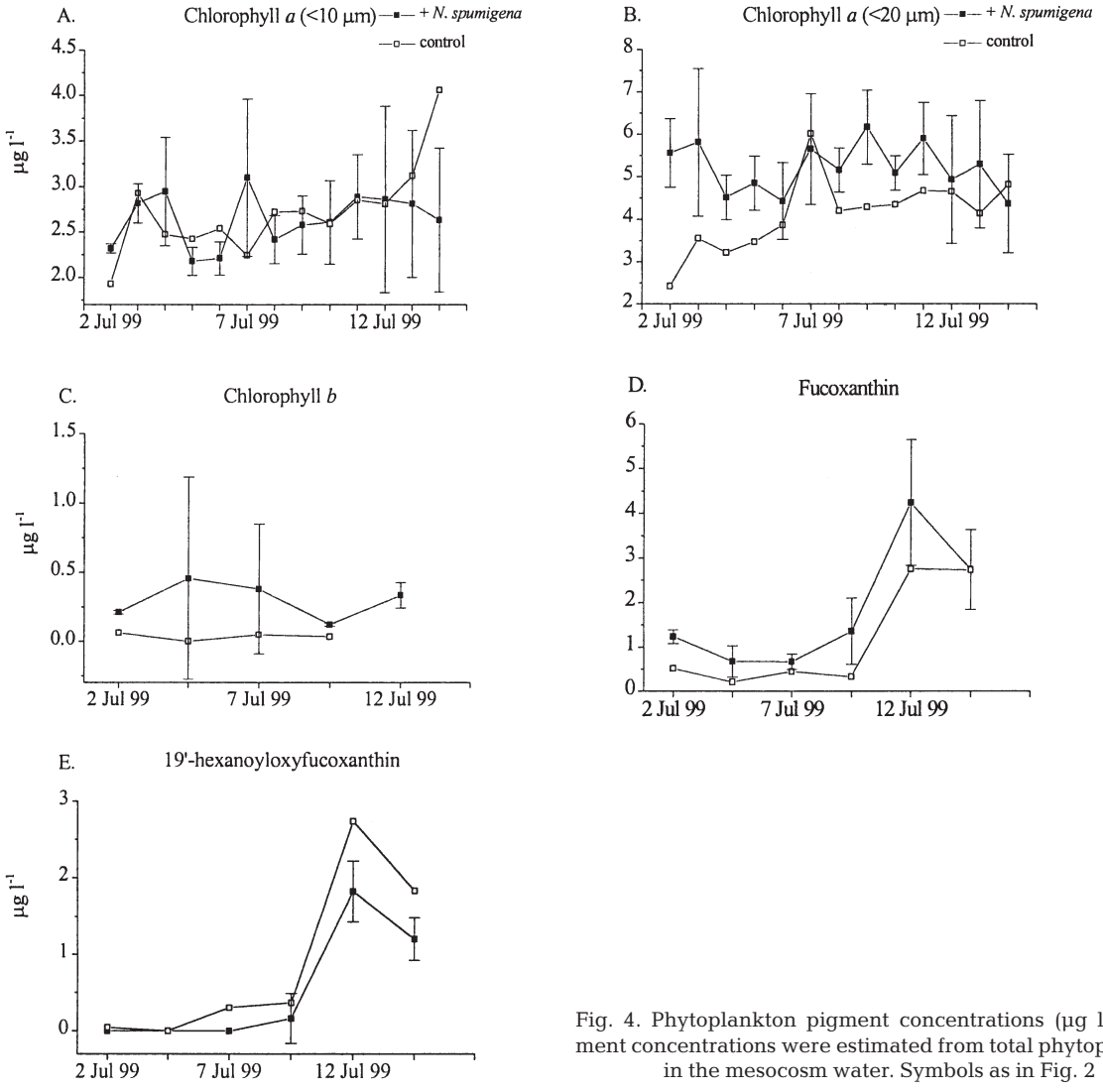

Fig. 4. Phytoplankton pigment concentrations (µg l^{-1}). Pigment concentrations were estimated from total phytoplankton in the mesocosm water. Symbols as in Fig. 2

Nutrient concentrations and ratios

By the end of the experiment, there was almost 3 µmol ammonium (NH_4^+) l^{-1} in the cyanobacteria bags, indicating that organic nitrogen was leaking into the water from the dying filaments and was decomposed by heterotrophic organisms to ammonium, as suggested by Heinänen et al. (1995). Although ammonium is a major excretory product of aquatic organisms (e.g. crustaceans, ciliates, heterotrohic nanoflagellates), this source is generally minor compared to the one generated by bacteria (Wetzel 1983). The increase in nitrate suggests that nitrification in the enclosures was active. In the Baltic Sea, it has been shown that nitrification occurs during summer down to the chemocline (Enoksson 1986, Rheinheimer et al. 1989).

The high initial total P concentration in the treatment originated most likely from the growth medium of *Nodularia spumigena*, although the cyanobacteria were carefully filtered in advance in order to remove as much of the medium as possible. Consequently, after the addition of cyanobacteria into the enclosures (Fig. 6I), the total N:P ratio was lower than the ratio recorded during the same time in a nearby pelagic area (17 to 19, Tvärminne Zoological Station unpubl. data). Heiskanen & Tallberg (1999) showed that the decay of the cyanobacterial bloom resulted in an increased N:P ratio, which was also observed in our study. Sahlsten & Sörensson (1989) showed that DON increased simultaneously with the decline of a cyanobacterial bloom. The authors suggested that DON was gradually released from the cyanobacteria and formed a new nitrogen source for planktonic organisms. In our study, DON increased in all enclosures. Generally, DON is mineralised or taken up by bacteria and appears in the water column due to e.g. cell rup-

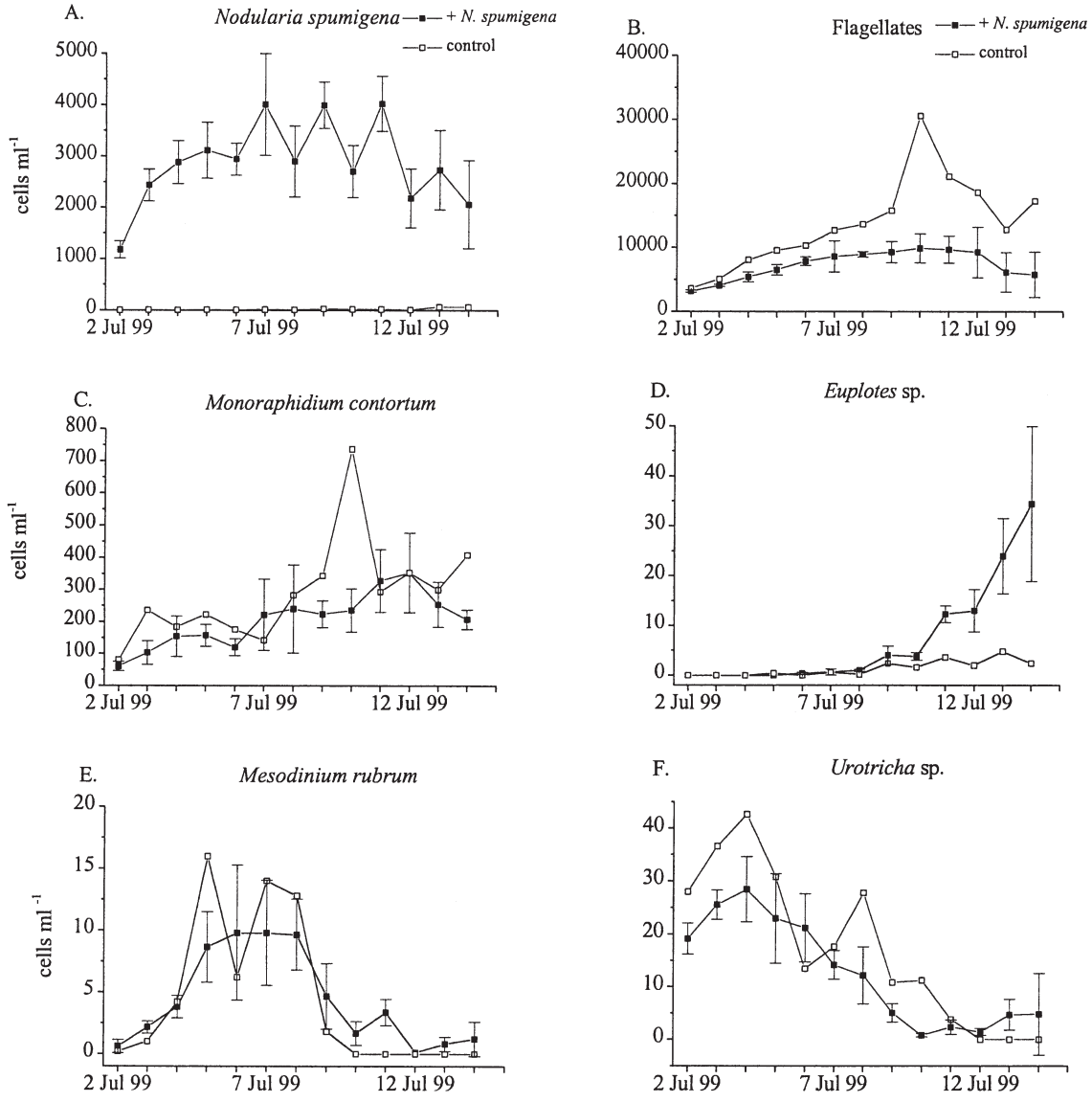

Fig. 5. Phytoplankton and ciliate abundance (cells ml^{-1}). Symbols as in Fig. 2

ture caused by grazing (Bronk & Glibert 1993) or cell lysis caused by viral infections (Procter & Fuhrman 1990).

Nodularin concentrations

Survival of crustaceans was high in our experiments (Koski et al. in press), although the highest total nodularin concentration was 19.7 µg l^{-1}. No direct harmful effects of nodularin could be detected. Total nodularin concentrations, measured from unfiltered water, remained high during the whole experiment in the cyanobacteria bags. Cyanobacteria toxins have been shown to be very persistent, e.g. Kiviranta et al. (1991) did not detect biodegradation of microcystin in an experiment where toxin was leaking from the cells into the water and remained at high concentrations for 5 wk. On the other hand, degradation of hepatotoxins has been detected in other studies (Lahti et al. 1997). We have no data on the dissolved toxins, measured from filtered water. In a recent investigation (S.R. et al. unpubl. data), dissolved nodularin was below the detection limit (0.1 µg l^{-1}) even though toxic *Nodularia spumigena* was present in the Baltic Sea. In our mesocosm bags, dissolved toxins could have been present due to the small volume of the bags and zero water exchange. Reinikainen et al. (in press) measured low

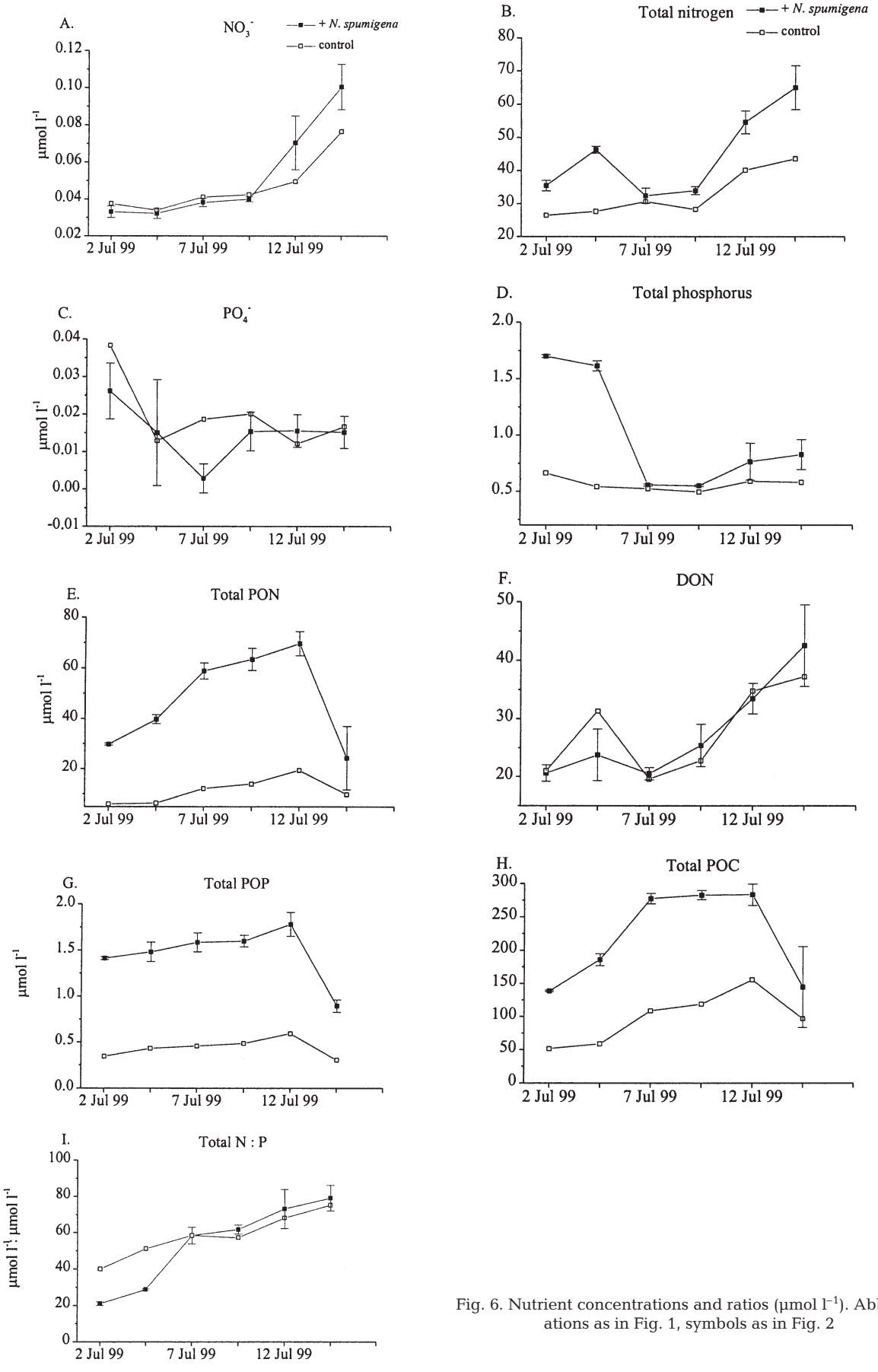

Fig. 6. Nutrient concentrations and ratios (µmol l^{-1}). Abbreviations as in Fig. 1, symbols as in Fig. 2

mortality of *Acartia bifilosa*, *Bosmina longispina maritima* and *Eurytemora affinis* to dissolved nodularin (max. 200 µg l^{-1}); and DeMott et al. (1991) to microcystin.

Fatty acids

The major cyanobacterial fatty acids, 16:0, 16:1ω7 and 18:3ω3 (Ahlgren et al. 1992, Vargas et al. 1998), and the cyanobacterial pigments, echinenone and zeaxanthin (Kabata et al. 1992, Piippola & Kononen 1995), correlated strongly with *Nodularia spumigena* and associated characteristics (Fig. 1). Few filaments of other cyanobacteria (*Anabaena* sp., *Aphanizomenon flos-aquae*, *Limnothrix* sp.) were observed in the bags. This suggests that the pigments and fatty acids mainly originated from *N. spumigena*. The fatty acid 18:1ω7 is common in bacteria, but uncommon in cyanobacteria and phytoplankton (Mayzaud et al. 1989, Volkman et al. 1989). Its concentrations were higher in the cyanobacteria bags than in the control, and therefore it most likely originated from the filamentous bacteria. The main food quality indicators, 20:5ω3 and 22:6ω3 (Brett & Müller-Navarra 1997, Müller-Navarra et al. 2000), showed high relationships with PC 2, associated with processes similar in all enclosures. This result demonstrates that *N. spumigena* is low in fatty acids that are important for grazers.

The fatty acid concentration was considerably higher than the total chl *a* concentration in the control. The reason for this was probably that all organisms contain fatty acids (e.g. bacteria, phytoplankton and animals), and were included in the measurement (Fraser et al. 1989, Mayzaud et al. 1989, Volkman et al. 1989, Işik et al. 1999).

Development of flagellates

The abundance of flagellates (e.g. *Chrysochromulina* sp., *Pyramimonas* sp. cryptophytes) was negatively correlated to PC 1, associated with different characteristics of *Nodularia spumigena*. In the study by Christoffersen et al. (1990), flagellates decreased due to nutrient limitation. This was not likely in our experiment, considering the high concentrations of inorganic phosphorus and nitrogen available in the cyanobacteria enclosures. Instead, predation by microzooplankton and especially by the ciliate *Euplotes* sp. most likely controlled the flagellates (cf. Vrede et al. 1999). Other factors that may have affected the number of autotrophic flagellates negatively were e.g. low light caused by shading filaments (Christoffersen et al. 1990, Ibelings & Maberly 1998) or potential antibiotic or -algal substances released by cyanobacteria (Østensvik et al. 1998, Pushparaj et al. 1999).

Based on microscopy, pigment and fatty acid analyses, we aimed at grouping autotrophic groups (pigments) with different flagellates and fatty acids (Tables 1 & 2). The pigment 19′-hexanoyloxyfucoxanthin originated most likely from the prymnesiophyte *Chrysochromulina* sp. The major fatty acids of prymnesiophytes are 14:0, 16:0, 16:1ω7 and 20:5ω3, which all were abundant in the bags (Zhukova & Aizdacher 1995). Fucoxanthin originated most likely from different chrysophytes (*Pseudopedinella* sp., *Spiniferomonas* sp., *Uroglena* sp., Table 1), because all the diatoms and dinoflagellates recorded that contained the same pigment were in bad condition. Chrysophytes are characterised by being rich in C_{18} acids (Ackman et al. 1968), which also were abundant in the enclosures. The major fatty acids of the chlorophycean *Monoraphidium* sp., 16:0, 18:0, 18:2ω6 and 18:3ω3 (Işik et al. 1999), and chl *b*, were found throughout the experiment and originated most likely from *Monoraphidium contortum*, a common species in the mesocosms.

Heterotrophic food chain

Bacterial production is mainly controlled by nutrient concentrations, temperature and grazing during summer (Autio 1992). In our study, nutrient concentrations remained high in the experimental units. The lowest temperature recorded in the enclosures was 13°C, which is not limiting for bacterial growth (Autio 1992). Predation was in all likelihood the most important factor structuring the population in the enclosures, partly because filamentous bacteria, most likely grazing-resistant, developed, and partly due to the fact that ciliates and bacteria increased simultaneously towards the end of the experiment. This suggests intensive predation by ciliates on the main bacterivores, heterotrophic nanoflagellates (HNAN). The shift in the bacterial community towards filamentous forms strongly suggests that bacteria were imposed a strong grazing pressure (Jürgens & Güde 1994, Jürgens et al. 1994, 1997).

The abundances of non-filamentous bacteria and short rods can be considered low in the treatment enclosures, especially when taking into account that there probably was plenty of substrate available (Fig. 3B,C,D). In most studies, cyanobacteria have not been found to inhibit bacteria, rather they have been shown to provide good growth conditions for them (Hoppe 1981, Heinänen et al. 1995). Hansen et al. (1986) found that lysis products from dead filamentous

cyanobacteria may sustain the major part of the bacterial production. A stimulation of the bacterial community could also be explained by the exudation of DON from decaying cyanobacteria. On the other hand, the allelopathic effect of cyanobacteria, e.g. antibacterial activities, has been demonstrated (Østensvik et al. 1998).

Despite prefiltration of the mesocosm water, the abundance of copepodites and other zooplankton was relatively high by the end of the experiment in all the bags (K.S. et al. unpubl.). The zooplankton had presumably grown from eggs and nauplii passed through the mesh during prefiltration (cf. Turner et al. 1999). In the studies by Olsson et al. (1992) and Kivi (1996), copepods efficiently eliminated *Urotricha* sp. and *M. rubrum*. In our study, the number of *Mesodinium rubrum* and *Urotricha* sp. decreased in all the bags (Fig. 5E,F), most likely due to increased predation by nauplii and later by copepodites. The findings of Kivi et al. (1993) support our results; they found the highest protozooplankton growth rates in enclosures treated with 100 µm prefiltration and/or with ammonium addition. We suggest that the thigmotactic, i.e. surface-bound, *Euplotes* sp. thrived in the decaying cyanobacteria community due to the physical support of large algal filaments (Ricci 1989) and by feeding on nanoflagellates (4 to 10 µm). *Euplotes* sp. may also have escaped predation due to its surface affinity and its protecting lorica (K. Kivi, University of Helsinki, pers. comm.).

Mesozooplankton biomass was low due to prefiltration of the incubated water in the beginning of our experiment. We suggest that, due to lack of predation, microzooplankton were able to suppress HNAN. Consequently, the grazing pressure on bacteria by HNAN remained low but size-selective on smaller-sized bacteria (cocci, vibroid-like bacteria and short rods decreased). We suggest that predation on microzooplankton by mesozooplankton (76 ind. l^{-1}), grown from eggs and nauplii, was the main reason for the dramatic decrease of 2 ciliates *Mesodinium rubrum* and *Urotricha* sp. at the end of the experiment. Subsequently, HNAN was able to increase slowly, whereas the filamentous bacteria were able to increase rapidly. Although we did not count the HNAN, the POC:chl *a* ratio (<10 µm) increased rapidly towards the end of the experiment, suggesting that heterotrophic organisms peaked by then. Jürgens & Güde (1994) demonstrated that the removal of *Daphnia* spp. from a freshwater system resulted in a peak of protozoans, which grazed on the dominating part of the bacterial community. After 3 d, filamentous bacteria had developed, which subsequently dominated the community. We suggest that the same phenomenon occurred in our enclosures.

CONCLUSIONS

The toxic cyanobacteria *Nodularia spumigena* started to break down after a few days of the initiation of the experiment in the mesocosm bags. The cells were in bad condition and covered by epiflora and fauna. The PUFA:total FA ratio, as well as the POC:chl *a* (<10 µm) ratio, indicated bloom decay. Ammonium increased due to the decomposition of filaments. A diverse system, seemingly top-down controlled, developed simultaneously with the decay. Planktonic organisms colonised the decaying bloom and used it as a substrate. Several ciliate species and bacteria, probably grazing-resistant, flourished upon the filaments, showing that a decaying bloom is a nutrient-rich substrate to live in. We were not able to detect any direct harmful effects of the stable and high concentrations of nodularin on any of the studied organism groups. The results show that decomposer communities can exist in the presence of toxin. The bacterial biomass increased approximately by a factor of 13 during the experiment in comparison to the biomass at the start, due to the steep increase in bacterial volume. We conclude that these blooms, the occurrence of which is highly dependent on unpredictable factors such as weather conditions, provide a food source for the heterotrophic food chain from bacteria, flagellates and ciliates to crustacean zooplankton, and eventually fish.

Future studies should focus on the ability of different zooplankton groups, including protozoans, to resist cyanobacterial toxins and to use cyanobacteria as food. Also, the possible transfer of toxins from one trophic level to another and the effect of different phases of cyanobacteria blooms on the pelagial carbon flow are largely unknown.

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