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Disruption of the microbial food web and inhibition of metazooplankton development in the presence of iron- and DOM-stimulated Baltic Sea cyanobacteria

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ABSTRACT: Summer N_2-fixing cyanobacterial blooms are a common feature in the Baltic Sea, and the occurrence of *Nodularia spumigena* toxic blooms is of particular concern. Cyanobacterial blooms can be favoured by certain conditions including high concentrations of dissolved organic matter, which may increase the availability of iron critical for N_2 fixation. Cyanobacteria may negatively affect grazers because many species produce toxins and generally lack fatty acids essential for zooplankton reproduction. In this study we investigated the effect of riverine high-molecular weight dissolved organic matter (DOM-)/iron-stimulated cyanobacteria on the development of proto- and metazooplankton, and evaluated the role of DOM in stimulating the zooplankton part of the microbial food web. A plankton community was incubated in cylinders with either nitrate (NO_3) or DOM alone or combined with iron (Fe) or zooplankton >100 µm (G). The development of proto- and metazooplankton was followed for 10 d. Trophic relationships between metazooplankton taxa and their potential food items were assessed by ordination analysis and by feeding and reproduction bottle incubations with the calanoid copepod *Acartia bifilosa*. Contrary to our expectations, DOM did not stimulate the microbial food web, and proto- and metazooplankton developed similarly in all treatments until the middle of the experiment. However, by the end of the experiment, the biomass of proto- and metazooplankton as well as the biomass of diatoms and dinoflagellates was greatly depressed in all DOM and NO_3Fe treatments. In these treatments, cyanobacterial and bacterial biomasses were highest leading up to phosphate depletion. Plankton development seemed to be bottom-up controlled and to be affected by extracellular compound(s) produced by the dominant cyanobacteria, possibly triggered by phosphate limitation. Diatoms, dinoflagellates, protozoans and metazooplankton were instead stimulated in the NO_3 and NO_3G treatments, where cyanobacterial biomass was low. Accordingly, *A. bifilosa* reproduction and survival were sustained in NO_3 bottles. Deleterious effects of cyanobacteria on metazooplankton were diminished in NO_3 and NO_3G tanks where other food resources were available. Overall, the results suggest that increases in the input of DOM to the Baltic Sea can potentially stimulate cyanobacterial blooms that may disrupt the microbial food web and inhibit metazooplankton development.

KEY WORDS: Riverine high-molecular weight dissolved organic matter (DOM) · Iron · Microbial food web · *Acartia bifilosa* · Zooplankton · Cyanobacteria · Extracellular compounds

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INTRODUCTION

Summer cyanobacterial blooms, mostly dominated by the N_2-fixing cyanobacteria *Aphanizomenon flos-aquae* and *Nodularia spumigena*, are a common feature in the Baltic Sea. Of great health and environmental concern is the occurrence of hepatotoxic blooms of the latter species. In the Baltic Sea, cyanobacterial blooms are favoured by certain conditions such as N limitation, low N:P ratios, negligible grazing pressure and large amounts of dissolved organic matter (Howarth et al. 1988, Hagström et al. 2001).

Cyanobacteria may directly use compounds from the low-molecular weight fraction of dissolved organic matter (e.g. Balode et al. 1998), and their success may be facilitated by high concentrations of dissolved organic matter in 2 other ways. Dissolved organic matter might increase the availability of trace metals, such as molybdenum and iron, and through chelation may enable planktonic cyanobacterial N_2 fixation (Howarth et al. 1988). Because dissolved organic matter can be assimilated by bacteria and thereby stimulate the microbial food web (Carlsson et al. 1995), which in turn sustains metazooplankton even in the absence of recent algal production (Daniel et al. 2005), high availability of dissolved organic matter might also lead to relaxation of grazing pressure on cyanobacteria. Thus, even if toxic filamentous cyanobacteria from the Baltic Sea can be consumed by calanoid copepods (Koski et al. 2002, Kozlowsky-Suzuki et al. 2003), dissolved organic matter-stimulated protists plus metazooplankton may collectively graze on smaller-sized and/or on more edible food particles and regenerate nutrients that contribute to cyanobacteria dominance.

In the Baltic Sea, at the onset of summer cyanobacterial blooms, riverine loads of dissolved organic nutrients can be as important as the input of inorganic nutrients (Stepanauskas et al. 2002). This coincides with the increase of heterotrophic bacterial production and the dominance of small (<10 µm) phytoplankton, which in turn stimulates the highest levels of protozoan predation in the annual cycle (Hagström et al. 2001). Under such conditions, even if most of the primary production is lost through respiration in the microbial food web, protozoans may become the main trophic link to metazooplankton. In addition, cyanobacteria may negatively affect grazers. For instance, they generally lack polyunsaturated fatty acids such as 20:5ω3 and 22:6ω3 (Brett & Müller-Navarra 1997) essential for reproduction in crustaceans, and many produce potent toxins that impair direct grazers (Lampert 1987). In spite of this, grazers may consume toxic cyanobacteria in the Baltic Sea (Koski et al. 2002, Kozlowsky-Suzuki et al. 2003) with the deleterious effects possibly being diminished by the presence of other food sources (Reinikainen et al. 1994).

This study is part of a mesocosms experiment (Stolte et al. 2006) in which the central hypothesis was that riverine high-molecular weight dissolved organic matter (referred to as DOM herein) would stimulate the growth of N_2-fixing cyanobacteria (1) directly, by increasing the availability of iron and (2) indirectly, by stimulating a heterotrophic link from bacteria to metazooplankton via heterotrophic flagellates and ciliates. Thus, because cyanobacterial growth and N_2 fixation can be limited by iron in the Baltic Sea (Stal et al. 1999), inputs of riverine organic matter likely stimulate the development of these microorganisms. In addition, by stimulating the microbial food web and metazooplankton development, extra inputs of riverine DOM may release cyanobacteria from strong grazing pressure.

Stolte et al. (2006) reported the development of bacteria and phytoplankton, especially cyanobacteria, following manipulations of DOM/nutrients and grazers. In the present study we evaluated the role of DOM in stimulating the zooplankton part of the microbial food web and the effect of DOM-/iron-stimulated cyanobacteria on the development of proto- and metazooplankton. We expected that DOM would stimulate the microbial food web, which would provide an additional food resource and thus help to counterbalance the possible negative effects of toxic and/or low nutritional quality cyanobacteria on potential grazers (metazooplankton). By using ordination analysis we further assessed the relationships between the metazooplankton taxa and their major food types, and in turn related these to feeding and reproduction of the calanoid copepod *Acartia bifilosa* in bottle incubations.

MATERIALS AND METHODS

Mesocosm experiment set-up, sampling and analyses. A short description of the experimental set-up and procedures is given below; for further information see Stolte et al. (2006). A summer plankton community (<100 µm) collected from the Baltic Proper (56° 55′ 85″ N, 17° 01′ 82″ E) was incubated in triplicate in polyethylene cylinders (300 l) with either nitrate (NO_3) or DOM (riverine >1 kDa) alone or in combination with iron (NO_3Fe and DOMFe) or zooplankton >100 µm (NO_3G and DOMG). Nutrients were added daily as follows: nitrogen sources (NO_3^- or DOM): 0.1 µM; PO_4^{3-}: 0.2 µM; and $FeCl_2$: 0.1 µM.

Zooplankton samples were taken with a 100 µm net by vertical hauls (20 m to the surface) at the same time that water for the mesocosm experiments was collected. In the laboratory, the zooplankton >100 µm size fraction was added (simultaneously with the first nutri-

ent addition; see above) to some of the cylinders (NO_3G and DOMG) in the same numbers as observed *in situ*. All cylinders were placed at 18°C and exposed to 250 µmol photons m^{-2} s^{-1} in an 18:6 h light:dark cycle.

Sampling in the cylinders was carried out after careful mixing of the water column. Samples for biological analyses were collected as follows: phytoplankton at Days 0, 3, 5, 7, 10; nanoflagellates every second day from initial sampling (Day 0) onwards; and ciliates at Days 0, 5, 7 and 10. Metazooplankton samples from the pre-screened cylinders were taken at Days 5 and 10, while those from the cylinders containing grazers were collected at Days 0, 4 and 10.

Samples (250 ml) for phytoplankton and ciliates analyses were fixed with acid Lugol's solution. Phytoplankton samples were settled in sedimentation chambers using the Utermöhl (1958) method and analysed under an inverted microscope (DM IL, LEICA). The biomass (wet wt) of each phytoplankton taxon was calculated from cell biovolume according to the Baltic Marine Biologists' recommendations (Edler 1979). At least 20 cells were measured in order to estimate the cell volume. Ciliates were counted in 10 ml sedimentation chambers. Samples (20 ml) for the analysis of nanoflagellates were fixed with 10 µl alkaline Lugol's solution, immediately followed by the addition of 0.4 to 0.5 ml of 1% borate-buffered formalin and 20 µl of 3% sodiumthiosulphate (to bleach the lugol) (Sherr & Sherr 1993). An aliquot was then stained with DAPI (final concentration 0.1 µg ml^{-1}) and filtered through 0.8 µm black polycarbonate filters, which were then mounted on microscope glass slides before cells were enumerated by microscopy (Sherr et al. 1993). For the metazooplankton analysis, 5 l aliquots from each cylinder were concentrated using a 100 µm net and preserved in 4% formaldehyde. Entire samples were counted in sedimentation chambers. The biomass of each metazooplankton taxon was calculated using individual wet wt determinations according to the Baltic Marine Biologists' recommendations (Hernroth 1985).

Bottle incubations: feeding and reproduction experiments. *Acartia bifilosa* feeding and egg production experiments were conducted simultaneously with the mesocosm experiment. Feeding experiments were run on 2 occasions (Expts 1 and 2, corresponding to Days 0 and 4 of the mesocosm experiment), using the treatments that received NO_3 and DOM. Reproduction experiments were conducted on 3 occasions (Expts 1, 2 and 3, the latter corresponding to Day 10 of the mesocosm experiment), using water from the NO_3, NO_3Fe, DOM and DOMFe tanks. *Acartia bifilosa* adult females were collected at sea on the day preceding each of these experiments.

Females were individually isolated and kept overnight in seawater filtered using Whatman GF/C filters. After the starvation period, ca. 20 to 25 females per bottle were incubated in triplicate 1.3 l plastic bottles containing water from the different mesocosm treatments. Control bottles (without copepods) were also incubated in triplicate. All bottles were placed on a plankton wheel under the same temperature and light conditions as the cylinders.

Feeding experiments lasted 24 h, after which the females were gently collected on 100 µm nets and reincubated with water from the same mesocosm treatments for 2 consecutive 24 h periods, in order to determine the egg production rate (EPR). After each 24 h period, females and eggs were gently collected on 100 and 25 µm nets, respectively, and counted. EPR was corrected for the number of eggs in the natural community water at Day 0 and for the potential number of eggs produced by the few adult females that subsequently appeared in the tanks. A number of the eggs (usually 30, but in some cases all) were placed in Petri dishes with filtered seawater in order to estimate egg-hatching success (EH). These Petri dishes were placed under the same temperature and light conditions as the cylinders for 48 h. EH was only estimated from the eggs produced during the first 24 h EPR incubation.

Samples (50 to 100 ml) for the enumeration of phytoplankton and ciliates were taken at the start (0 h) and completion (24 h) of the feeding experiment and preserved in acid Lugol's solution. Entire samples were counted in sedimentation chambers of different volumes, depending on the density of the filaments/cells, and at least 20 cells of the initial samples were measured in order to estimate the cell volume. In the case of filamentous cyanobacteria, cell concentration was estimated by dividing the measured length (in µm) of individual filaments by the average length of a single cell.

Clearance and ingestion rates were estimated according to Frost (1972). Ingestion rates of the different food types were converted to carbon by employing a conversion factor of 0.11 pg $µm^3$ for phytoplankton and ciliates, and 0.13 pg $µm^3$ for armoured dinoflagellates (Edler 1979). Total ingestion rates (TIR) of the 3 major groups (cyanobacteria, ciliates and dinoflagellates) were calculated as the sum of the ingestion rates of all food types in the respective group. Food selection was determined by the selectivity coefficient α, which relates the ingestion rates of the different food types to their availability (Chesson 1978). No selection occurs when $\alpha = m^{-1}$ (where m = no. of food types available) and food items are fed upon in the same proportion as their availability; in contrast, if $\alpha > m^{-1}$ then selection is positive, and when $\alpha < m^{-1}$ then selection is negative.

Data analysis. All data were tested for homogeneity of variances and normality. If those assumptions were not met, the data were log- or square root-transformed. The effect of the different treatments on the abundance of ciliates and metazooplankton was tested with 1-way multivariate analysis of variance (MANOVA), where treatments were independent variables and the abundance estimated on each sampling day was the dependent variable. In case of a significant response, 1-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) *a posteriori* test was used for the different days. Friedman ANOVA was used to test differences in *Acartia bifilosa* clearance and ingestion rates on different food items within each treatment; differences between treatments were tested with *t*-tests for independent samples. Two-way MANOVAs were used to test the effect of nutrient manipulation and experimental time on the following dependent variables: ingestion rates of the 3 major groups (cyanobacteria, ciliates and dinoflagellates) and survival at 24 and 72 h. If significant results were detected, 2-way ANOVAs followed by Tukey's HSD test were used, and *t*-tests for dependent samples were used to test whether EPR (24 and 48 h) and survival (24 and 72 h) at consecutive incubation times differed for each treatment and experiment. The effect of nutrient manipulation and experiment time on TIR, EPR and EH was tested with 2-way ANOVA. Relationships between cyanobacterial biomass and EPR, EH and survival were assessed with the Spearman rank correlation, and *t*-tests followed by the sequential Bonferroni method (applied to adjust α-values) were used to assess whether the selectivity coefficients of the different food types were significantly different from the non-selection value.

The development of the plankton community in the different treatments and the relationships between metazooplankton taxa and their potential food items (entered as environmental variables) were assessed by Redundancy Analysis (RDA). The average biomass of each metazooplankton taxa and the main food items (cyanobacteria, diatoms, dinoflagellates, ciliates < 20 µm and ciliates > 20 µm) in each treatment on Days 5 and 10 were used in the analysis. The biomasses of the different food items were square root-transformed before entry into the analysis. The significance of the RDA axes was tested by running 1999 unrestricted permutations in the Monte Carlo test. Because no samples for metazooplankton analysis were taken from the pre-screened tanks on Day 0, the initial sampling was not used in the analysis; furthermore, major responses to the experimental manipulations only began to diverge at the middle of the experiment (Day 5) and continued to do so until the end.

RESULTS

Mesocosm experiment

Protozooplankton development

The abundance of <5 µm nanoflagellates (which comprised at least 60% heterotrophic flagellates) increased in all treatments until Day 8, after which it decreased considerably (DOM treatments) or stabilised (NO_3 treatments) (Fig. 1A). Numbers of small (<20 µm) and large (>20 µm) ciliates increased from the start to the middle (Day 5) of the experiment (Fig. 1B,C). Thereafter, small ciliates decreased in numbers, whereas larger ciliates continued to increase until the end of the experiment by which time large ciliates were dominated by *Euplotes* sp.

Both small and large ciliates were affected by nutrient manipulation (1-way MANOVA; $p < 0.001$), with the addition of grazers having no clear effect (Tukey's HSD; $p > 0.05$). Ciliates reached higher numbers in the treatments receiving NO_3, contrasting with the lower numbers in the DOM treatments. However, complete

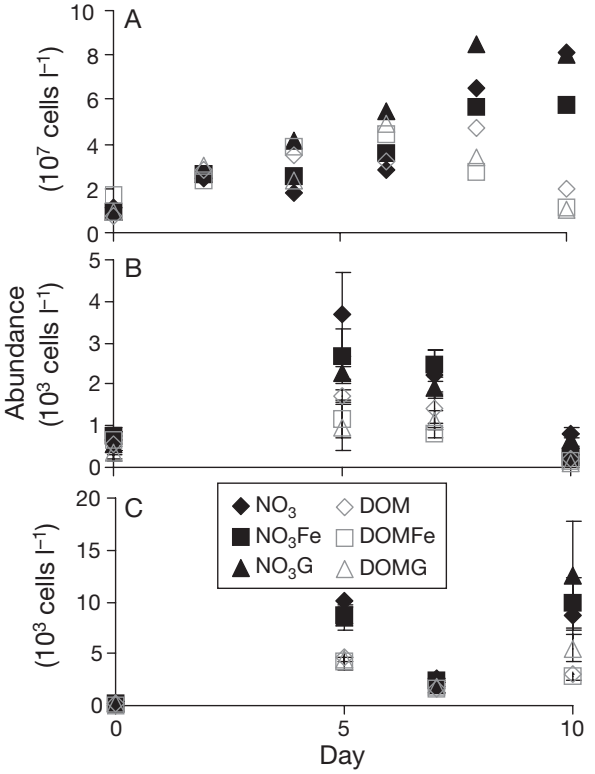

Fig. 1. Protozooplankton abundance in different treatments: (A) nanoflagellates <5 µm, (B) small ciliates (<20 µm) and (C) large ciliates (>20 µm). Nitrate (NO_3) and DOM were added to mesocosms alone or in combination with iron (NO_3Fe and DOMFe, respectively) or zooplankton >100 µm (NO_3G and DOMG, respectively). Data are mean ± SD (NB: SD not given in A). DOM: riverine hight-molecular weight dissolved organic matter

separation of these 2 major treatments was only significant (Tukey's HSD; $p < 0.05$) on Day 5 for large ciliates, when their abundance was on average 2-fold higher in all NO_3 treatments (Fig. 1C).

Metazooplankton development in cylinders with added grazers

The number of grazers (>100 µm) in the tanks receiving NO_3G (mean ± SD: 18 ± 6 ind. l^{-1}) and DOMG (16 ± 9 ind. l^{-1}) at the start of the experiment was in the same range as the *in situ* zooplankton abundance (17 ± 1 ind. l^{-1}). The total metazooplankton abundance ranged from 5 to 268 ind. l^{-1} throughout the experiment and was affected by the nutrient manipulations (Fig. 2A,B; 1-way MANOVA; $p < 0.05$). From the start (Day 0) to the middle (Day 4) of the experiment, metazooplankton community structure developed similarly in both treatments. However, by the end of the experiment (Day 10), the abundance of most taxa, especially copepods (nauplii, copepodites and adults), had decreased considerably in the DOMG treatment (Fig. 2A,B). At that time, total zooplankton abundance was significantly lower in the DOMG treatment (Tukey's HSD; $p < 0.01$) and the community was also less diverse (*Synchaeta* spp. comprised >90% of the total metazooplankton abundance) than that in the NO_3G tanks (Fig. 2A,B).

Cladocerans and copepods dominated at the start of the experiment (Fig. 2B). Cladocerans were represented by *Evadne nordmanni* (average abundance 85%), *Pleopsis polyphemoides* (14%) and *Bosmina longispina maritima* (<1%), whereas nauplii (65% of *Acartia bifilosa*), copepodites (79% of *A. bifilosa*) and adults of the calanoids *A. bifilosa*, *Centropages hamatus* and *Temora longicornis* represented the copepods.

Numbers of *Evadne nordmanni* and nauplii, copepodites and adults of *Centropages hamatus* and *Temora longicornis* all declined over the course of the experiment, and some were even absent from both treatments (NO_3G and DOMG) on the last day. The contribution of rotifers (*Synchaeta* spp.) progressively increased and became considerably higher in the NO_3G treatment on the last day of experiment, whereas the population in the DOMG tanks was unable to develop after Day 4 (Fig. 2A). By the end of the experiment, *Pleopsis polyphemoides* and *Bosmina longispina maritima* numbers increased slightly in the NO_3G treatment, whereas the abundance of nauplii and adults of *Acartia bifilosa* (Figs. 2B & 3) were significantly higher (Tukey's HSD; $p < 0.01$) than in the DOMG tanks.

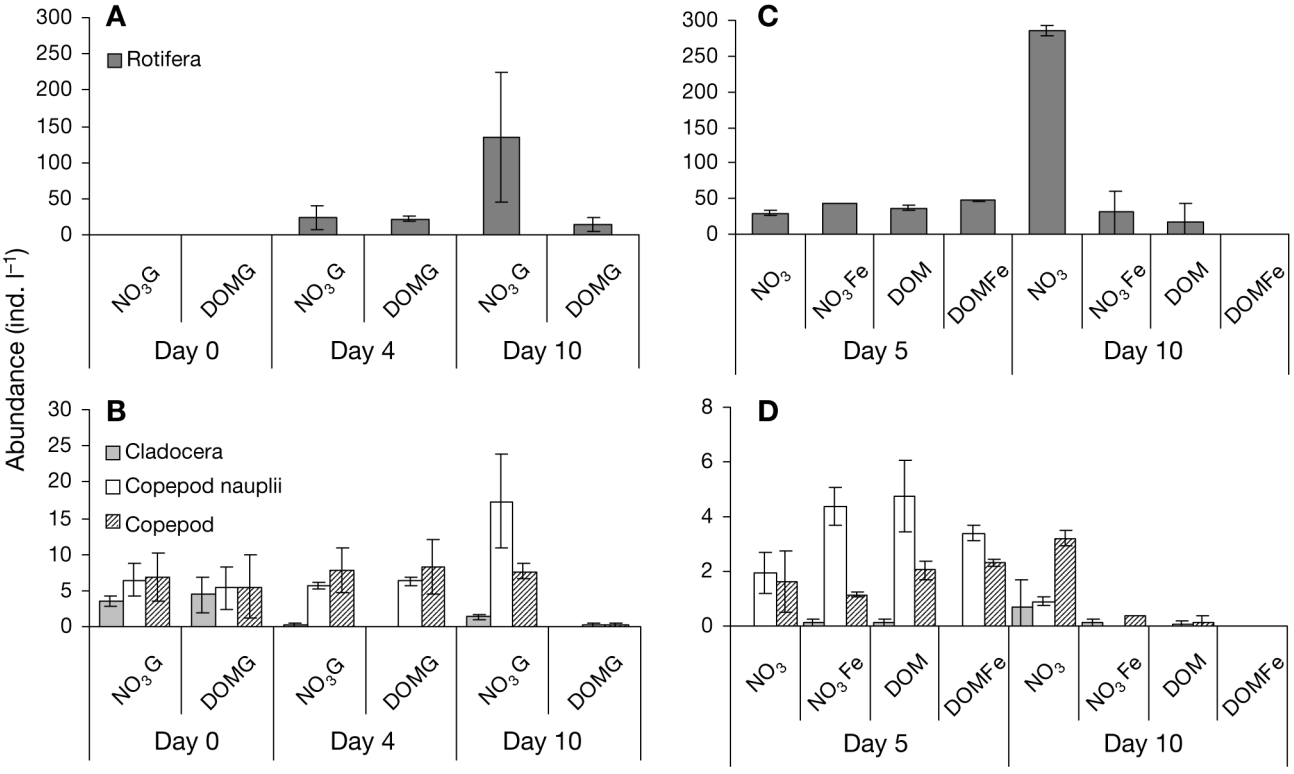

Fig. 2. Metazooplankton abundance (mean ± SD). Abundance of (A) Rotifera and (B) Cladocera, copepod nauplii and copepods in tanks with >100 µm grazers; abundance of (C) Rotifera and (D) Cladocera, copepod nauplii and copepods in pre-screened tanks. Abbreviations as in Fig. 1

Metazooplankton development in pre-screened cylinders

After 5 d of experiment, some metazooplankton taxa (i.e. *Synchaeta* spp. and copepod nauplii) in the pre-screened (<100 µm) treatments had reached numbers comparable with those observed in the tanks where grazers were added (Figs. 2C,D & 3). The community in the NO_3 treatment tended to develop similarly to those in the NO_3G tanks, even if a population of adult copepods was not yet established (Fig. 3). By the end of the experiment, the abundance of most taxa had decreased markedly in the NO_3Fe, DOM and DOMFe treatments and exhibited similar trends to that observed for the DOMG treatment (Fig. 2). At that time, the abundances of the total metazooplankton and *Synchaeta* spp. were significantly lower (Tukey's HSD; $p < 0.01$) in the NO_3Fe, DOM and DOMFe treatments than in the NO_3 tanks.

Plankton development and relationships between metazooplankton and food items

The development of the plankton community following experimental manipulations (addition of nutrient and grazers) and the relationships between metazooplankton and potential food items are summarised in the ordination diagram (Fig. 4). The proximity of the different treatments (numbers 1 to 6) in the ordination diagram by the middle of the experiment (Day 5) indicates that the plankton structure and development were fairly similar in the different tanks at this time. However, by Day 10, plankton responses in the different treatments had diverged considerably.

The first 2 axes of Fig. 4 have eigenvalues of 0.67 and 0.07, and explain 75% of the metazooplankton distribution and 97% of the species-environment (metazooplankton-food item) relationship. Both the first and all canonical axes together were significant ($p = 0.02$) in the Monte Carlo test. The correlations

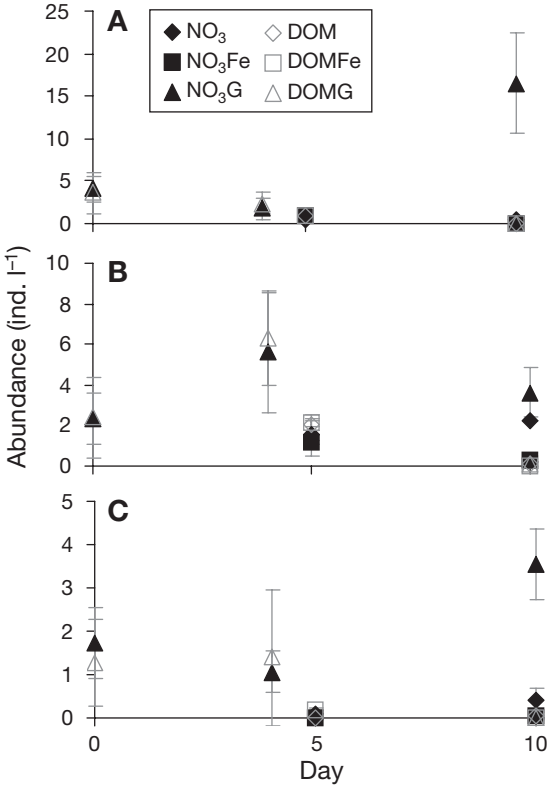

Fig. 3. *Acartia bifilosa*. Mean abundance (±SD) of (A) nauplii, (B) copepodites and (C) adults in different treatments. Abbreviations as in Fig. 1

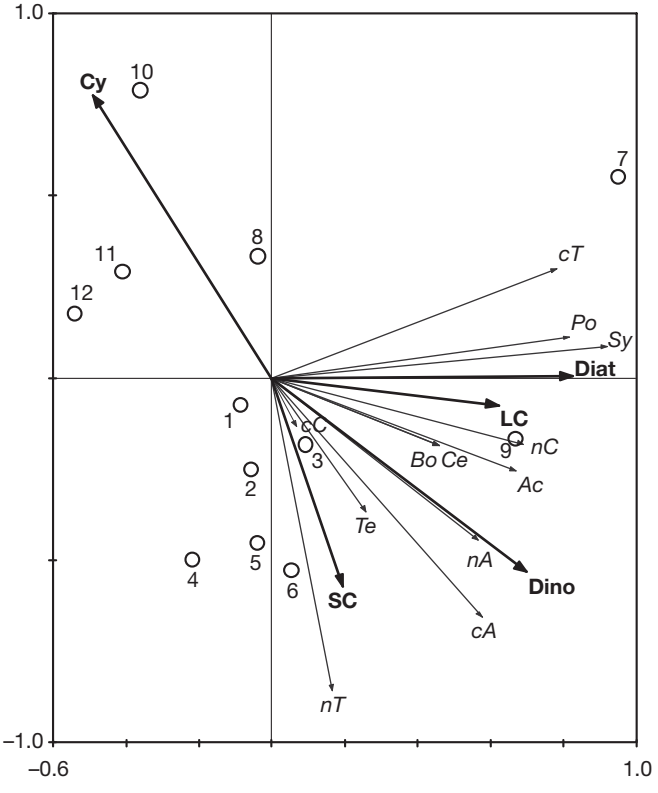

Fig. 4. Ordination diagram showing the first (horizontal) and second (vertical) axes of Redundancy Analysis (RDA). Numbers represent different treatments on Day 5 (1 = NO_3; 2 = NO_3Fe; 3 = NO_3G; 4 = DOM; 5 = DOMFe; 6 = DOMG) and Day 10 (7 = NO_3; 8 = NO_3Fe; 9 = NO_3G; 10 = DOM; 11 = DOMFe; 12 = DOMG) (Abbreviations as in Fig. 1). Potential food items (environmental variables) represented by bold arrows: Cy = cyanobacteria; Diat = diatoms; SC = ciliates < 20 µm; LC = ciliates >20 µm; Dino = dinoflagellates. Metazooplankton taxa represented by thin arrows: *nT*, *cT* & *Te* = nauplii, copepodites and adults of *Temora longicornis*; *nC*, *cC* & *Ce* = nauplii, copepodites and adults of *Centropages hamatus*; *nA*, *cA* & *Ac* = nauplii, copepodites and adults of *Acartia bifilosa*; *Po* = *Pleopsis polyphemoides*; *Sy* = *Synchaeta* spp.; *Bo* = *Bosmina longispina maritima*

between the ordination axes and food types (environmental variables) indicated that the first axis was related to the availability of diatoms, dinoflagellates and large ciliates, whereas the second axis was mainly related to the development of cyanobacteria. The first axis alone explained 87% of variation in the metazooplankton-food items relation, whereas the second axis accounted for only 10% of this variation. This means that most metazooplankton biomass was associated with the more diverse plankton community (represented by the first axis), which by the end of the experiment (Day 10) had developed in the NO_3 and NO_3G tanks (numbers 7 and 9). In those treatments, even if cyanobacteria became the dominant food type, the importance of the other food items was as great or had become greater since the start of the experiment. At the same time, cyanobacteria biomass had considerably increased (Stolte et al. 2006) in all DOM treatments in addition to that receiving NO_3Fe (numbers 8, 10, 11 and 12); in contrast, the biomass of all the other food types and metazooplankton was generally much lower.

Bottle incubations

Feeding rates and food selectivity

Acartia bifilosa TIR ranged from 28 to 95 ng C female^{-1} h^{-1} in the NO_3 treatment, and from 29 to 34 ng C female^{-1} h^{-1} in the DOM treatment (Table 1). This variable increased from Expt 1 to Expt 2 (performed on Days 0 and 4 respectively; 2-way ANOVA; $p < 0.05$) but did not differ between treatments (2-way ANOVA; $p > 0.05$).

When the ingestion rates of the different food items were combined for taxonomic groups (cyanobacteria, ciliates and dinoflagellates) they differed between treatments (2-way MANOVA; $p < 0.05$). The interaction between treatment and experiment was also significant (2-way MANOVA; $p < 0.05$). In Expt 1, ciliates were the food type ingested most by *Acartia bifilosa* (13 ng C female^{-1} h^{-1}) irrespective of treatment; in contrast, in Expt 2, ciliates were ingested at higher rates (57 ng C female^{-1} h^{-1}) in the NO_3 treatment (2-way ANOVA; $p < 0.05$, Tukey's HSD; $p < 0.01$). The ingestion rate of cyanobacteria was higher in Expt 2 (2-way ANOVA, Tukey's HSD; $p < 0.05$; up to 33 ng C female^{-1} h^{-1}) irrespective of treatment. The ingestion rate of dinoflagellates ranged from 4.7 to 8.7 ng C female^{-1} h^{-1} and was neither affected by nutrient manipulation nor did it differ between experiments (2-way ANOVA; $p > 0.05$).

Clearance and ingestion rates of the different food items were highly variable and ranged from negative values to 5.8 ml female^{-1} h^{-1} and 55 ng C female^{-1} h^{-1}, respectively (Table 1), and did not differ significantly between treatments and experiments. However, in Expt 1, large ciliates (>20 µm) were the most ingested food item (up to 12 ng C female^{-1} h^{-1}) in both treatments. In Expt 2, large ciliates were still the most ingested food item in the NO_3 treatment (55 ng C female^{-1} h^{-1}), followed by *Anabaena inaequalis* (29 ng C female^{-1} h^{-1}). Although the ingestion rate of *A. inaequalis* in the DOM treatment was highly variable, this cyanobacterium was always fed upon to some extent (19 ng C female^{-1} h^{-1}). *Acartia bifilosa* fed non-selectively: the selectivity coefficient for all food types did not differ statistically from the non-selection value (Fig. 5).

Table 1. *Acartia bifilosa*. Mean (SD) clearance (ml female^{-1} h^{-1}) and ingestion rate (IR, ng C female^{-1} h^{-1}) of food items cyanobacteria, ciliates and dinoflagellates and total ingestion rates (TIR) in NO_3 and DOM treatments during Expts 1 (Days 0 to 1) and 2 (Days 4 to 5). For treatment abbreviations see Fig. 1

	Clearance rate		Ingestion rate	
	NO_3	DOM	NO_3	DOM
Expt 1				
Pseudoanabaena sp.	0.6 (1.9)	0.4 (2.1)	2.1 (3.8)	1.1 (4.6)
Aphanizomenon flos-aquae	1.5 (3.9)	5.4 (2.9)	4.4 (9.3)	5.5 (1.8)
IR cyanobacteria			7.8 (9.2)	7.6 (5.4)
Ciliates < 20 µm	1.4 (1.8)	1.5 (1.7)	1.2 (1)	1.1 (1.1)
Ciliates > 20 µm	5.8 (5.4)	3.1 (2.8)	12 (4.2)	11 (7.6)
IR ciliates			13 (4.5)	12 (8.8)
Gymnodinium spp. < 20 µm	4.0 (3.8)	2.7 (1.7)	2.1 (1.1)	2.0 (1.2)
Gymnodinium spp. > 20 µm	5.8 (1.9)	3.1 (2.2)	2.2 (0.3)	1.9 (1.3)
Peridinales < 20 µm	1.3 (3.4)	2.3 (1.7)	0.6 (0.9)	1.3 (0.7)
Dinophysis acuminata	0.3 (1.1)	0.6 (0.9)	1.5 (4.4)	3.1 (3.8)
IR dinoflagellates			7.5 (3.7)	8.7 (3.7)
TIR			28 (9)	29 (9)
Expt 2				
Anabaena inaequalis	1.8 (0.4)	1.1 (1.8)	29 (8)	19 (27)
Pseudoanabaena sp.	0.5 (0.6)	0.4 (0.5)	1.6 (1.5)	2.2 (2.4)
Nodularia spumigena	−0.2 (0.9)	−0.2 (3)	−0.2 (5)	−5.6 (13.6)
IR cyanobacteria			33 (8)	25 (21)
Ciliates < 20 µm	0.2 (1.5)	−3 (1.6)	1.3 (2.9)	−1.9 (1)
Ciliates > 20 µm	2.3 (1.1)	0.1 (0.8)	55 (27)	1.6 (10.5)
IR ciliates			57 (27)	4.5 (7.8)
Gymnodinium spp. < 20µm	3.2 (2)	0.6 (0.6)	1 (0.8)	0.2 (0.2)
Peridinales > 20µm	1.3 (1.4)	0.3 (2)	2.7 (3)	0.6 (3)
Dinophysis acuminata	0.02 (1)	0.5 (1.3)	0.4 (3.6)	2.1 (5.1)
IR dinoflagellates			5.1 (4.7)	4.7 (5.9)
TIR			95 (38)	34 (35)

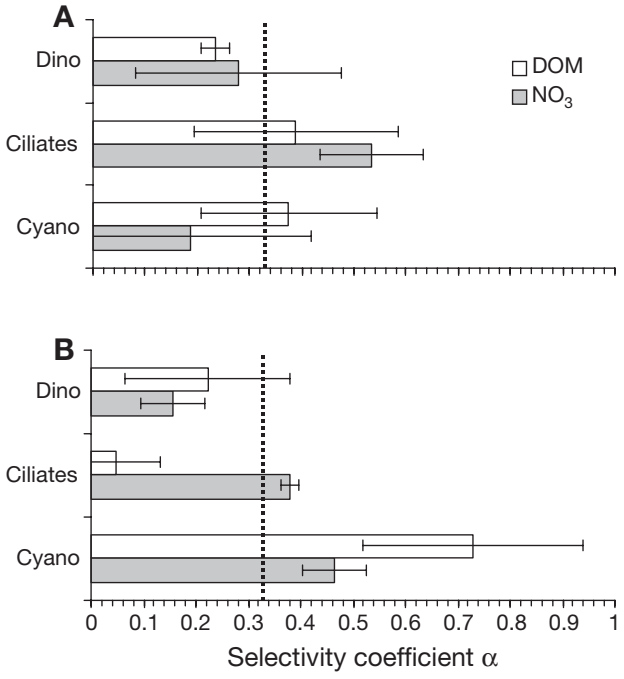

Fig. 5. *Acartia bifilosa*. Mean (±SD) selectivity coefficient (α) when feeding on dinoflagellates (Dino), ciliates and cyanobacteria (Cyano) in the NO_3 and DOM treatments during (A) Expt 1 and (B) Expt 2. Dotted line represents the α-value when no selection occurs (0.33)

Survival and reproduction

Acartia bifilosa survival was highly variable, ranging from 0 to 92% (Table 2), and differed among treatments and experiments (2-way MANOVA; $p < 0.05$). In addition, the interaction between these factors was significant (2-way MANOVA; $p < 0.05$). Survival tended to decrease with increasing incubation time (24 and 72 h) even if not significantly (*t*-test; $p > 0.005$). It was lowest in Expt 3 (corresponding to Day 10) both at 24 h (2-way MANOVA; $p < 0.05$) and 72 h (2-way MANOVA; $p < 0.01$), except for the NO_3 treatment in which no difference was detected (Tukey's HSD; $p > 0.05$). In this last experiment, survival was highest in the NO_3 treatment (Tukey's HSD; $p < 0.01$; 71%), whereas in the DOM and DOMFe treatments all females were dead after 72 h and very few were still alive in the NO_3Fe treatment (6%). Survival was negatively related to cyanobacterial biomass (Spearman correlation, $r = -0.53$; $p < 0.001$) when considering the data from all treatments (NO_3, NO_3Fe, DOM and DOMFe) and experiments (1, 2 and 3).

EPR did not differ between consecutive days (24 and 48 h) for any of the treatments or experiments (*t*-test; $p > 0.005$). Therefore, average values for each treatment were calculated using both incubation days

Table 2. *Acartia bifilosa*. Mean (SD) egg production rate (EPR, egg female^{-1} d^{-1}), egg-hatching success (EH, %) and survival (SUR, %) at 24 and 72 h for different treatments in Expts 1 (Days 1 to 3), 2 (Days 5 to 7) and 3 (Days 9 to 11). Results from Tukey's HSD test show treatments/experiments in increasing order. =: no significant difference at $p = 0.05$; <: significant difference at $p = 0.05$; groups that overlap are underlined. For treatment abbreviations see Fig. 1

	NO_3	NO_3Fe	DOM	DOMFe	Tukey's HSD
Expt 1					
EPR	10 (4)	7 (3)	7 (3)	6 (3)	DOMFe = NO_3Fe = DOM = NO_3
EH	62 (25)	50 (0)	56 (18)	34 (9)	DOMFe = NO_3Fe = DOM = NO_3
SUR 24 h	77 (25)	82 (3)	72 (9)	79 (9)	DOM = DOMFe = NO_3 = NO_3Fe
SUR 72 h	55 (48)	64 (12)	54 (6)	61 (18)	DOM = NO_3 = DOMFe = NO_3Fe
Expt 2					
EPR	7 (3)	5 (2)	5 (2)	3 (1)	DOMFe DOM NO_3Fe NO_3
EH	51 (5)	56 (4)	50 (12)	60 (12)	DOM = NO_3 = NO_3Fe = DOMFe
SUR 24 h	74 (9)	92 (13)	75 (3)	69 (5)	DOMFe = NO_3 = DOM = NO_3Fe
SUR 72 h	69 (15)	86 (16)	63 (23)	42 (6)	DOMFe = DOM = NO_3 = NO_3Fe
Expt 3					
EPR	6 (1)	0	0	0	DOMFe = DOM = NO_3Fe < NO_3
EH	26 (9)	0	0	0	DOMFe = DOM = NO_3Fe = NO_3
SUR 24 h	73 (13)	55 (9)	40 (32)	24 (27)	DOMFe DOM NO_3Fe NO_3
SUR 72 h	71 (5)	6(6)	0	0	DOMFe = DOM = NO_3Fe < NO_3
Tukey's HSD					
EPR	3 = 2 = 1	3 < 2 = 1	3 < 2 = 1	3 < 2 = 1	
EH	3 = 2 = 1	3 < 1 = 2	3 < 2 = 1	3 < 2 = 1	
SUR 24 h	3 = 2 = 1	3 = 1 = 2	3 = 1 = 2	3 2 1	
SUR 72 h	1 = 2 = 3	3 < 1 = 2	3 < 2 = 1	3 < 2 = 1	

(Table 2). Overall, EPR was highest in the NO_3 treatment (2-way ANOVA, Tukey's HSD; $p < 0.001$; up to 10 eggs female^{-1} d^{-1}). Until the middle of the experiment (data from NO_3 and DOM treatments, Expts 1 and 2), EPR was neither related to the ingestion rate of the different food groups nor to their proportions in TIR. However, there was a negative relationship between EPR and cyanobacterial biomass (Spearman correlation, $r = -0.77$; $p < 0.001$) when all treatments and the entire experimental time-course were considered. EPR was thus lowest in Expt 3 (2-way ANOVA; $p < 0.001$,Tukey's HSD; $p < 0.005$), except for the NO_3 treatment in which no difference was found among experiments (Tukey's HSD; $p > 0.05$). In this last experiment, no eggs were produced in any other treatment (NO_3Fe, DOM and DOMFe).

EH was lowest (2-way ANOVA, Tukey's HSD; $p < 0.001$) in Expt 3 (26%), and was in general highest in the NO_3 treatment and lowest in the DOMFe treatment (2-way ANOVA, Tukey's HSD; $p < 0.05$). As observed for the survival and EPR, cyanobacterial biomass was negatively associated with EH (Spearman correlation, $r = -0.53$; $p < 0.001$).

DISCUSSION

Development of the microbial food web in DOM treatments: link to metazooplankton?

Contrary to previous observations (e.g. Carlsson et al. 1995), DOM did not directly stimulate bacterial growth. Instead, bacterial development was coupled with phytoplankton biomass (linear regression; $p < 0.001$, irrespective of treatment), and cell-specific bacterial production did not differ among treatments (Stolte et al. 2006).

Marine bacteria seem to be better adapted to use dissolved organic nitrogen, DON (including high molecular weight DON) than are freshwater bacterioplankton (Stepanauskas et al. 1999). Stepanauskas et al. (1999) not only detected higher DON bioavailability, but also higher aminopeptidase activity by marine bacteria. Thus, we suggest that the low salinity (ca. 6 to 7‰) bacterial community in our study might have been unable to use DOM efficiently. Alternatively, DOM used in our experiments may have been mostly refractory to bacterial degradation. For instance, the high bioavailability of DON and DOP observed in the Baltic Sea by Stepanauskas et al. (2002) was due to a large contribution of organic matter from riverine algal-derived dissolved organic matter; in contrast, DOM seemed more resistant to bacterial degradation.

By the end of the experiment, heterotrophic nanoflagellates and ciliates were generally less numerous in all DOM treatments in which bacterial and cyanobacterial biomass accumulated. Metazooplankton was also much less abundant in the DOM treatments, even in the cylinders (DOMG) where >100 µm grazers were stocked. Therefore, our results could not confirm the hypothesis that a heterotrophic DOM-stimulated microbial food web would account for most metazooplankton development. In addition, DOM- and iron-stimulated cyanobacteria dominated regardless of whether grazers were added and subsequently depressed proto- and metazooplankton development (see below).

Bottom-up control and allelopathic/inhibitory effect by cyanobacteria in DOM and NO_3Fe cylinders

Phytoplankton development

By the end of the experiment, cyanobacteria numerically dominated in all DOM tanks, and also dominated in tanks incubated with NO_3Fe (which followed DOM tanks in terms of abundance) (Stolte et al. 2006). These were the treatments in which the biomass of other phytoplankton groups such as diatoms and dinoflagellates decreased greatly. We suggest that some negative allelopathic effect of cyanobacteria, possibly triggered by phosphate limitation (von Elert & Jüttner 1997), may account for this response in our study. Phosphate depletion in the NO_3Fe treatment and all DOM tanks after Day 8 (see Stolte et al. 2006) might have led to competition among phytoplankton. Under such circumstances, the production of allelopathic compounds by cyanobacteria may be a mechanism that improves competitive ability (von Elert & Jüttner 1997).

Proto- and metazooplankton development

Low biomass of protozoans and metazooplankton in all DOM tanks and the NO_3Fe treatment by the end of the experiment also seemed related to deleterious effects of the dominant cyanobacteria. In addition, the survival, EPR and EH of *Acartia bifilosa* measured in the bottle incubations were not only negatively related to cyanobacterial biomass, but were also extremely low in the NO_3Fe, DOM and DOMFe treatments by the end of the experiment, which supported the results obtained from the tanks.

We suggest that some inhibitory effect of cyanobacteria caused the high mortality of *Acartia bifilosa* recorded during the bottle incubations. Food-deprived females (kept in filtered seawater) had much higher survival (>70%, data not shown) than those incubated

with water from the NO_3Fe, DOM and DOMFe tanks. Nevertheless, during the first half of the experiment, *A. bifilosa* fed upon cyanobacteria, particularly the dominant *Anabaena inaequalis*, with no negative effect on survival and reproduction. Similarly, *A. bifilosa* can ingest the nodularin-producing *Nodularia spumigena* without any obvious negative effects on survival, even at a higher concentration of this cyanobacterium (~460 µg C l^{-1}, Koski et al. 2002; ~1000 µg C l^{-1}, Kozlowsky-Suzuki et al. 2003) than used in the present study (~200 µg C l^{-1}). Therefore, we suggest that the negative effect was likely due to extracellular compound(s) produced by the dominant cyanobacteria, rather than being a result of the ingestion of cyanobacterial cells containing intracellular toxins (e.g. nodularin produced by *N. spumigena* or some other cyanotoxin[s] produced by *A. inaequalis*). In addition, intracellular toxins might only negatively affect organisms capable of feeding on filamentous cyanobacteria, which are most likely to be larger metazooplankton (e.g. calanoid copepods), seldom protozoans. Decaying blooms of nodularin-producing *N. spumigena* may actually constitute a nutrient-rich substrate for the microbial food web (Engström-Öst et al. 2002) that sustains metazooplankton development (Koski et al. 2002).

Negative allelopathic activity is dependent on the concentration of the inhibitor (e.g. von Elert & Jüttner 1997). Thus, the higher cyanobacterial biomass in all DOM tanks relative to the NO_3Fe treatment may explain the lower biomass of proto- and metazooplankton in the former treatments. Such an effect was even more diluted in the other NO_3 treatments, where cyanobacteria biomass was considerably lower and phosphate never became depleted (Stolte et al. 2006). Metazooplankton reproduced successfully and developed in those tanks. Accordingly, *Acartia bifilosa* reproduction and survival were sustained in the NO_3 bottle incubations.

Although the toxic effects of intracellular phycotoxins on zooplankton have been largely investigated, the effects of extracellular compounds on potential grazers are relatively unknown. Immobilization of heterotrophic dinoflagellates caused by extracellular compound(s), rather than by saxitoxins produced by the dinoflagellate *Alexandrium* spp., was reported by Tillmann & John (2002). Survival of the ciliate *Euplotes affinis* was affected by increasing densities of the haptophyte *Prymnesium parvum* added to a fixed concentration of *Rhodomonas* cf. *baltica*, especially when the former was grown under nutrient-limiting conditions (Granéli & Johansson 2003). The negative effect was presumably due to some extracellular compound, because *P. parvum* was consistently avoided by the ciliate (Granéli & Johansson 2003). *Anabaena* spp. may produce extracellular compounds that negatively affect survival, feeding activity and reproduction of some zooplankton (see Lampert 1987). Extracellular compounds produced by dinoflagellates (Huntley et al. 1986) or by the haptophyte *Chrysochromulina polylepis* (Nielsen et al. 1990) may also affect copepods. *C. polylepis* further inhibited the activity of bacteria and ciliates both in the field and in the laboratory (Nielsen et al. 1990). Nevertheless, filtrates from the cyanobacterium *Anabaena affinis* caused no toxic effect on *Daphnia pulex*, whereas suspensions containing filaments induced strong inhibition (Gilbert 1990). Thus, extracellular compounds produced by phytoplankton evoke species-specific responses in the same way as intracellular phycotoxins, and may affect organisms at different trophic levels (e.g. Nielsen et al. 1990).

Trophic relations between metazooplankton and food types in NO_3 cylinders

Contrary to what was observed in all DOM and NO_3Fe tanks, bacterioplankton had not accumulated in the NO_3 and NO_3G cylinders by the end of the experiment (Stolte et al. 2006). This indicates grazing pressure by bacterivores such as nanoflagellates, ciliates and nauplii (Laybourn-Parry 1992, Roff et al. 1995), which are in turn preyed upon by metazooplankton (Daan et al. 1988, Stoecker & Capuzzo 1990).

Proto- and metazooplankton developed where availability of diatoms and dinoflagellates was high, but were unable to control phytoplankton development under the nutrient-enriched conditions (e.g. Olsson et al. 1992). However, by the end of the experiment, the biomass of *Skeletonema costatum* was significantly lower (*t*-test; $p < 0.05$) in the NO_3G treatments than in the tanks receiving only NO_3, which was indicative of grazing pressure. Species of the genus *Acartia* (Olsson et al. 1992) including *A. clausi*, in addition to *Podon polyphemoides* (Turner & Granéli 1992), may for instance feed upon *S. costatum*.

The association of *Acartia bifilosa* and *Centropages hamatus* with large ciliates (displayed in Fig. 4) is consistent with their size preferences (e.g. Tiselius 1989, Koski et al. 2002) and supported by the bottle incubations, in which large ciliates were the food item most ingested by *A. bifilosa*. However, grazing pressure on large ciliates could not be inferred in the tanks because no significant difference was detected between NO_3 and NO_3G treatments.

Besides feeding on ciliates, *Acartia bifilosa* also fed on cyanobacteria and, to a lesser extent, on dinoflagellates. Unfortunately we could not estimate the feeding rates on *Skeletonema costatum*: by the time that most

of the phytoplankton counts for the feeding experiments were finished, the majority of the diatom cells were no longer recognizable owing to the long preservation time. However, as discussed above, *S. costatum* likely contributed to the diet of *A. bifilosa* and of other metazooplankton because grazing impact on the diatom was detected in the NO_3G tanks. *Acartia* spp. may sometimes also graze upon *Synchaeta* spp. (Stoecker & Egloff 1987), and the higher abundance of rotifers (Mann-Whitney test; $p < 0.05$) in the NO_3 tanks (compared with the NO_3G tanks) by the end of the experiment could suggest either decreased predation (adult *A. bifilosa* population was not yet established in the NO_3 tanks) or decreased competition with other metazooplankton (e.g. Gilbert 1990).

Acartia bifilosa egg production rates were in the range previously reported for the species (Schimdt et al. 1998, Koski et al. 2002), and did not improve with increased food concentration. At least until the middle of the experiment, increased feeding rates on ciliates, cyanobacteria or dinoflagellates did not affect EPR, indicating that the females were not limited by the amount of food. However, because cyanobacteria generally lack polyunsaturated fatty acids such as 20:5ω3 and 22:6ω3 (Brett & Müller-Navarra 1997) that are essential for reproduction in crustaceans, and because ciliates do not necessarily improve copepod EPR (e.g. Ederington et al. 1995, but see Stoecker & Egloff 1987), females could have been limited by the quality of the food. However, by the end of the experiment, EPR tended to decrease in the NO_3 treatment, and no eggs were produced by the females in the NO_3Fe, DOM, DOMFe treatments. A similar trend was observed for egg hatching success, possibly caused by inhibitory compounds released by cyanobacteria (e.g. Koski et al. 1999). Cyanobacteria were the dominant food item in the NO_3 and NO_3G treatments, even though their biomass was much lower than in all DOM and NO_3Fe tanks. Furthermore, other food types were available for zooplankton in the NO_3 and NO_3G tanks. Thus, possible deleterious effects of cyanobacteria were diminished, provided that other resources were available (e.g. Reinikainen et al. 1994). We cannot exclude the possibility of reduced hatching success due to some inhibitory compound released by *Skeletonema costatum* (Miralto et al. 1999), even if this diatom may contribute to positive secondary production by *A. bifilosa* (Schimdt et al. 1998).

CONCLUSIONS

Contrary to our hypothesis, DOM did not stimulate nanoflagellate, ciliate and metazooplankton development; on the contrary, their biomasses decreased greatly. Cyanobacteria dominated in the DOM and NO_3Fe tanks in which other phytoplankton groups and proto- and metazooplankton were negatively affected. Our results suggest that, excluding cyanobacteria, the plankton development in all DOM and NO_3Fe tanks was partly bottom-up controlled and was simultaneously affected by extracellular compounds produced by the dominant cyanobacteria, possibly triggered by phosphate limitation. The development of non-cyanobacterial plankton was stimulated in the NO_3 and NO_3G treatments. In these treatments, the lower cyanobacterial biomass and the higher contribution of other food types likely decreased the negative effects of cyanobacteria on zooplankton. Thus, metazooplankton development and secondary production were sustained. Overall, our results suggest that increases in the input of DOM to the Baltic Sea can potentially stimulate cyanobacterial blooms that may disrupt the microbial food web and inhibit metazooplankton development.

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