Development of Baltic Sea zooplankton in the presence of a toxic cyanobacterium: a mesocosm approach

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INTRODUCTION

Cyanobacteria blooms are a characteristic of many freshwater systems as well as some brackish and marine environments (Peregrine et al., 1996). Generally, zooplankton are considered to have little grazing impact on such blooms (Heiskanen and Kononen, 1994; Sellner, 1997), but rather to suffer from difficult handling, poor assimilation, low nutritional value and toxicity of cyanobacteria (Lampert, 1987). However, responses of zooplankton to cyanobacteria can be subtle. On one hand, the nutritional value of cyanobacteria varies with the physiological status of the cells (Repka et al., 1999), and the biochemical composition of food items which colonize them (Hoppe, 1981; Klein Bertele et al., 1999) or co-occur with them (Rothhaupt, 1991; Schmidt and Janasdiotti, 1997). On the other hand, zooplankton vulnerability to cyanobacteria is species-specific, depending upon factors such as body size, feeding behaviour, metabolism and sensitivity to toxins (DeMott et al., 1991; Kirk and Gilbert, 1992; Alldredge, 1995). Therefore, cyanobacteria blooms have the potential to induce shifts in the composition of zooplankton communities (Gilbert, 1990; Gloico and Lampert, 1990).

Our knowledge of the effects of cyanobacteria on zooplankton has been derived mainly from short-term laboratory experiments. These have been either with pure cyanobacteria or with simple mixtures, and give basic insights into species feeding behaviour and immediate responses to toxins in terms of egg production and mortality (DeMott and Moxter, 1991; Keski et al., 1999a; Engstrom et al., 2000). Longer-term experiments have rarely been conducted, but they suggest how a prolonged exposure to cyanobacteria can affect population success (Rothhaupt, 1991; Repka, 1997; Twombly et al., 1998).

Cyanobacteria blooms are common in the Baltic Sea and are considered to be a poor food source and sometimes toxic to zooplankton. Most experiments demonstrating harmful effects have been short-term incubations with monocultures or simple mixtures of food. In this study, a mesocosm approach was used to examine zooplankton responses over generation timescales. A toxic strain of the cyanobacterium Nodularia spumigena was added to bag enclosures of ambient water. The initial mesozooplankton concentration was either reduced by prescreening the water or enriched with locally caught zooplankton. Experiments ran for 15 days, long enough to monitor reproductive success and development of the next mesozooplankton generations. There was no major harmful effect on the zooplankton assemblage, even though the concentration of the toxin nodularin was in the upper range of field observations. The copepod Eurytemora affinis, rotifers Synchaeta spp. and the cladoceran Bosmina longispina maritima were able to develop and reproduce successfully in the presence of N. spumigena. The only species showing impaired recruitment was the copepod Acartia bifilosa. The general lack of population level effects from N. spumigena in this study can be reconciled with previous observations of adverse effects. Cyanobacteria alone may be poor food and toxic to zooplankton, but in the mesocosms a rich assemblage of microbiota developed, similar to that associated with blooms in the field. We suggest that, in the context of otherwise food-depleted summer situations in the open Baltic Sea, zooplankton can derive benefit from cyanobacteria bloom assemblages.
These results are not necessarily consistent with those from short-term experiments. For example, Twombly et al. (Twombly et al., 1998) found that cyanobacteria slowed the growth and development of copepods but increased their reproductive output, with response on the population level thus compensating for individual shortcomings. Conversely, algal toxins do not always have immediate lethal effects but can accumulate in zooplankton (Tegarden and Cembella, 1996; Thostrup and Christoffersen, 1995), depressing growth and fecundity even though feeding continues (Dutz, 1998; Fertão-Filho et al., 2000).

Both long- and short-term laboratory studies often cannot account for the diversity of natural food assemblages associated with cyanobacteria blooms. Field observations provide useful 'snapshots' of ingested material or production rates, but these can be hard to project to population development. Clearly, studies of cyanobacteria–zooplankton interactions should aim to reflect the full spectrum of available food, encompassing both individual- and population-based responses.

In the Baltic Sea, blooms of nitrogen-fixing filamentous cyanobacteria occur regularly in late summer (Wasmund, 1997), but recently have increased in frequency, biomass and extent (Kahru et al., 1994; Bianchi et al., 2000; Kahru et al., 2000). Most conspicuous are the blooms of the hepatotoxic *Nodularia spumigena* (Sivonen et al., 1989; Kononen et al., 1993), which are known to cause vertebrate mortality (Kononen and Elbrächter, 1996). Zooplankton abundance often peaks during cyanobacteria bloom periods (Viitasalo, 1992; Anonymous, 1996), but there is still uncertainty over the response of the grazers. Studies in the Baltic Sea have been restricted to short-term laboratory and field measurements of ingestion and egg production, which have found generally harmful effects to females and later developmental stages of common copepod species (Sellner et al., 1994, 1996; Schmidt et al., 1998; Meyer-Harms et al., 1999; Koski et al., 1999a; Engström-Öst et al., 2000).

The present study aimed to extend this work by mimicking as closely as possible the natural food assemblage available during a bloom of toxic cyanobacteria, and by following zooplankton development over more than one generation. A mesocosm approach was used, incubating natural zooplankton in ambient water with additions of toxic *N. spumigena* from culture. In this design, animals are exposed to toxins over a long period of time, and early larvae, possibly the most sensitive development stages, are included. To distinguish between adverse effects of cyanobacteria toxicity or experimental manipulation on zooplankton abundance, we also run bags with ambient water but no addition of *N. spumigena*.

The study was made in conjunction with two others, Engström-Ost et al. (Engström-Ost et al., 2002) described the development of the microbial assemblage associated with the simulated cyanobacteria bloom within the mesocosm bags, while Koski et al. (Koski et al., 2002) studied copepod feeding and egg production rates in short-term laboratory experiments. As feeding is a crucial life process, the experiments of Koski et al. help towards a mechanistic understanding of copepod net response to toxic *N. spumigena* (Koski et al., 2002). However, population development depends not only on feeding behaviour, but also on longer-term effects of toxicity and food quality on survival, egg production, hatching success and larval growth. In the present study we quantified this net response for several zooplankton species by following changes in their abundance in a realistic environment and time frame.

The short-term adverse effects seen in numerous laboratory studies would suggest a major impact on zooplankton over timescales of population growth. Therefore the central hypothesis of this study is that 2 weeks of exposure to high concentrations of toxic cyanobacteria will reduce zooplankton abundance significantly. Surprisingly, this hypothesis had to be rejected. We have then integrated our results with those of Engström-Ost et al. (Engström-Ost et al., 2002) and Koski et al. (Koski et al., 2002) to suggest why the zooplankton could survive.

**METHOD**

**Experimental details**

The mesocosm studies were carried out at the Tvärminne Zoological Station (south-west coast of Finland, Baltic Sea), from 1–16 July 1999. To set up the experiments, eight clear plastic bags of 120 l were filled with <100 µm filtered ambient water from a nearby open archipelago area (Storfjärden) and placed to float in a rack. In order to homogenize the initial water across bags, 10 l were sequentially added to each. The mesocosms were covered with clear plastic to exclude gnats from resting births, but to allow light penetration.

To address the study hypothesis, two types of treatments were conducted (Table I). In the first (treatment A) the larger zooplankton was removed by 100 µm pre-screening, whereas in the second (treatment B), the bags were enriched with zooplankton. Both treatments A and B comprised bags with added *N. spumigena* from culture (toxic strain AV1, University of Helsinki, Finland), and those without. The initial concentration of *N. spumigena* in the bags was ~1000 µg C l⁻¹. Bags without *N. spumigena* were used to check for adverse effects on zooplankton abundance due to experimental manipulation, which might wrongly be attributed to cyanobacteria toxicity.
However, it should be noted that these ambient bags were not intended as true controls representing optimal conditions for zooplankton development, because within 2 weeks the dynamics of the mesocosm assemblage might lead to large departures from the starting conditions. Treatment A followed a cyanobacteria bloom with reduced mesozooplankton grazing. In this treatment the development of nutrients, cyanobacteria and the associated assemblage of microbiota was analysed in detail (Engström-Öst et al., 2002). In the other supporting study (Koski et al., 2002), the bloom assemblage of treatment A was used in separate, short-term, laboratory experiments on feeding and egg production of field-collected copepods. At the end of the mesocosm experiment the contents of each bag were collected on a 100 µm gauze to check whether mesozooplankton could develop despite the toxins. The samples were fixed with unbuffered 4% formaldehyde for enumeration.

For treatment B, zooplankton were collected from Storfjärden using a 100 µm mesh plankton net. Two hauls were taken from the entire water column (0–30 m) and placed in a tank of 30 l containing water from below the thermocline. After careful mixing, the content of the tank was divided into four parts: three parts were released into the bags, whereas the fourth was formaldehyde preserved for initial species abundance. After 5 and 10 days, the mesocosms were gently mixed with a plunger and two 5 l subsamples were sieved onto a 100 µm gauze. The final samples were taken after 15 days, sieving the remaining water through 100 µm gauze and preserving the zooplankton in acid Lugol’s solution. Samples for chlorophyll a (Chl a) analysis (two of 500 ml) as well as for phytoplankton and ciliate counts (each 100 ml) were taken at the end of the experiment. All bags were then checked for punctures, none of which were found.

**Analyses**

Zooplankton composition was analyzed according to Hernroth and Viljamaa (Hernroth and Viljamaa, 1979). Aliquots of the sample were taken using a stemple pipette. At least three subsamples were counted, comprising a total of 30–50 individuals of the dominant species and development stage. The egg production rate of the sac-spawning *Eurytemora affinis* was calculated from the same preserved zooplankton samples as:

$$P = \frac{N_e}{N_f D}$$

where $P$ is the egg production rate (eggs ind$^{-1}$ day$^{-1}$), $N_e$ is the number of eggs, $N_f$ is the number of females and $D$ is the development time of eggs. Individual egg production rates could not be determined as egg sacs were partly broken and often no longer attached to the females. The development time of *E. affinis* eggs at ambient temperatures (mean 15.6°C, range 13–18.5°C) (Engström-Öst et al., 2002) was calculated to be 1.8–2.2 days, according to Andersen and Nielsen (Andersen and Nielsen, 1997). For each calculation, ~60 females were counted out of at least four subsamples.

Phytoplankton species were counted and measured following the recommendations of Edler (Edler, 1979). Two subsamples from each *N. spumigena* bag were settled in 3 ml chambers, whereas a 10 ml chamber was used for the bags without *N. spumigena*. For at least 100 filaments of *N. spumigena*, the length was measured and the condition of the cells was noted. Parts of filaments which showed deformed cell walls, shrunken plasma content and paler colouration were classified as ‘unhealthy’. Carbon contents of ciliates was calculated using a conversion factor of 0.19 pg µm$^{-3}$ (Putt and Stoecker, 1989). Chl a measurements

**Table I: Experimental set up and zooplankton sampling**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bag label</th>
<th>N. spumigena</th>
<th>Zooplankton sampling in treatment A and B</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Nod 1</td>
<td>+</td>
<td>Final (Day 15)</td>
</tr>
<tr>
<td></td>
<td>Nod 2</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nod 3</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nod 4</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ambient 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Nod 5</td>
<td></td>
<td>Initial, Day 5, 10, 15</td>
</tr>
<tr>
<td></td>
<td>Nod 6</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ambient 2</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

* indicates presence.
and HPLC pigment analyses are described in detail in Engström-Öst et al. (Engström-Öst et al., 2002).

Statistics

The zooplankton communities in bags with added N. spumigena in treatment A were analyzed with a 1-way analysis of variance (ANOVA). For treatment B, trends in zooplankton population development within different bags (subsequent sampling at day 5, 10 and 15) were tested with the Wilcoxon matched pairs signed rank test. The Mann–Whitney \( U \) test was used to determine whether the variance in medians of pigment concentration in feeding experiments and proportion of adult copepods in treatment B was significant. Means of N. spumigena filament lengths in treatments A and B were analyzed with a multiple range test (Student–Newman–Keuls test).

RESULTS

In the mesocosm bags, cyanobacteria flourished for most of the experiment and started to decay only during the last few days [Tables II and III and (Engström-Öst et al., 2002)]. However, the concentration of the toxin nodularin remained high over the whole period (Table II). Contrary to our hypothesis that the zooplankton community would be reduced significantly by sustained exposure to toxic N. spumigena, the major result of this study was that most species were able to survive or even to develop. After 2 weeks, the total abundance of zooplankters (>100 µm) was no lower in treatments with cyanobacteria than in those without, and was comparable to in situ values. This was observed both in treatment A and in B. The main features of the experiments are described below, first for the zooplankton and then for the food assemblages within the bags.

Treatment A: zooplankton development in bags with reduced initial zooplankton

The ambient water used for the mesocosm experiments was pre-screened at 100 µm, which let through only eggs and small stages of some mesozooplankters (~10 nauplii and rotifers l–1). After 15 days, new zooplankton assemblages had developed (Figure 1). In the N. spumigena bags (Nod 1–4) the final concentration of zooplankton >100 µm was between 37 and 167 ind l–1 (mean 76 ind l–1), which matches or even exceeds summer zooplankton abundances at the collection site (20–60 ind l–1 at Storfjärden during July 1992) (Koski et al., 1999b). Thus, the prescreened assemblage had recovered their population size within only 2 weeks. This recovery was also seen in the bag without added N. spumigena (final zooplankton concentration 70 ind l–1). Further, the presence of E. affinis adults and the high numbers of nauplii suggests that, within the 15 day experiment, the cyanobacteria-dominated assemblage allowed not only growth from nauplii to adults but also successful reproduction.

Despite this general lack of severe harmful effects of N. spumigena in treatment A, zooplankton abundance differed between bags (1-way ANOVA, \( F_{4,15} = 4.3, P < 0.05\)). Some zooplankters were generally abundant (~10 ind l–1, E. affinis copepodids and adults) or scarce (<1 ind l–1, Acartia bifilosa copepodids and adults) in all bags, while the abundance of others varied (the rotifers Synchaeta spp. and Keratella spp., E. affinis nauplii, the cladoceran Bosmina longispina maritima). The assemblages which developed were either dominated by E. affinis or by Synchaeta spp. A. bifilosa and B. longispina maritima were uncommon in both

<table>
<thead>
<tr>
<th>Day</th>
<th>Ambient 1</th>
<th>Nod 1–4</th>
<th>Ambient 1</th>
<th>Nod 1–4</th>
<th>Ambient 1</th>
<th>Nod 1–4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>1175 ± 165</td>
<td>0.2</td>
<td>1.1 ± 0.1</td>
<td>nd</td>
<td>9.2 ± 0.8</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>3116 ± 546</td>
<td>nd</td>
<td>3.0 ± 0.4</td>
<td>nd</td>
<td>10.3 ± 2.5</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>4658 ± 989</td>
<td>nd</td>
<td>2.9 ± 0.7</td>
<td>0.3</td>
<td>12.7 ± 2.2</td>
</tr>
<tr>
<td>7</td>
<td>26</td>
<td>2394 ± 452</td>
<td>nd</td>
<td>2.0 ± 0.6</td>
<td>nd</td>
<td>13.3 ± 4.4</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>2182 ± 574</td>
<td>nd</td>
<td>2.1 ± 1.0</td>
<td>0.4</td>
<td>13.3 ± 4.4</td>
</tr>
<tr>
<td>12</td>
<td>61</td>
<td>2864 ± 959</td>
<td>nd</td>
<td>1.4 ± 1.0</td>
<td>nd</td>
<td>11.5 ± 3.8</td>
</tr>
</tbody>
</table>

nd, not detected. Original data and methods are given in Engström-Öst et al. (Engström-Öst et al., 2002).
<table>
<thead>
<tr>
<th>Nano- and microplankton groups</th>
<th>Initial</th>
<th>Treatment A/Final</th>
<th>Treatment B/Final</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nod 1–4</td>
<td>Ambient 1</td>
<td>Nod 1–4</td>
</tr>
<tr>
<td>Chlorophyll a (total, mg m⁻³)</td>
<td>58 ± 6</td>
<td>3</td>
<td>36 ± 13</td>
</tr>
<tr>
<td>Chlorophyll a (&lt;10 µm, mg m⁻³)</td>
<td>3 ± 0.1</td>
<td>2</td>
<td>3 ± 0.8</td>
</tr>
<tr>
<td>Chlorophyll a (&lt;20 µm, mg m⁻³)</td>
<td>6 ± 0.8</td>
<td>2.5</td>
<td>5 ± 1.2</td>
</tr>
<tr>
<td>Chlorophyll a (total, mg C m⁻³)</td>
<td>1100 ± 160</td>
<td>–</td>
<td>2800 ± 2300</td>
</tr>
<tr>
<td>N. spumigena (mg C m⁻³)</td>
<td>1050 ± 160</td>
<td>–</td>
<td>2300 ± 2000</td>
</tr>
<tr>
<td>N. spumigena: average filament length (µm ± SD)</td>
<td>266 ± 266</td>
<td>–</td>
<td>160 ± 154</td>
</tr>
<tr>
<td>Small filamentous cyanobacteria (mg C m⁻³)</td>
<td>100 ± 0</td>
<td>–</td>
<td>14 ± 6</td>
</tr>
<tr>
<td>Dinoflagellates (mg C m⁻³)</td>
<td>0 ± 0</td>
<td>–</td>
<td>300 ± 220</td>
</tr>
<tr>
<td>Prymnesiophytes (mg C m⁻³)</td>
<td>7 ± 1</td>
<td>–</td>
<td>63 ± 29</td>
</tr>
<tr>
<td>Diatoms (mg C m⁻³)</td>
<td>5 ± 1</td>
<td>–</td>
<td>14 ± 8</td>
</tr>
<tr>
<td>Chlorophytes (mg C m⁻³)</td>
<td>0.5 ± 0.1</td>
<td>–</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>Ciliates (total, number ml⁻¹)</td>
<td>1 ± 0.2</td>
<td>–</td>
<td>3 ± 1.5</td>
</tr>
<tr>
<td>Ciliates (&lt;20 µm, number ml⁻¹)</td>
<td>39 ± 8</td>
<td>–</td>
<td>37 ± 15</td>
</tr>
<tr>
<td>Ciliates (&lt;20 µm, number ml⁻¹)</td>
<td>31 ± 7</td>
<td>–</td>
<td>14 ± 8</td>
</tr>
<tr>
<td>Ciliates (&gt;20 µm, number ml⁻¹)</td>
<td>9 ± 9</td>
<td>–</td>
<td>23 ± 10</td>
</tr>
<tr>
<td>Ciliates total, mg C m⁻³</td>
<td>13 ± 5</td>
<td>–</td>
<td>49 ± 15</td>
</tr>
<tr>
<td>Ciliates (&lt;20 µm, mg C m⁻³)</td>
<td>1.3 ± 0.3</td>
<td>–</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Ciliates (&gt;20 µm, mg C m⁻³)</td>
<td>12 ± 4.5</td>
<td>–</td>
<td>48 ± 15</td>
</tr>
</tbody>
</table>

The equivalent spherical diameter (ESD) was 10–20 µm for dinoflagellates, diatoms, chlorophytes, and >5 µm for prymnesiophytes. The initial concentrations of phytoplankton and ciliates in the bags Nod 5 and Nod 6 are expected to be within the range of those in Nod 1–4/Initial, likewise in the two Ambient bags except for the content of N. spumigena, –, missing data.
the *N. spumigena* and the ambient bags, suggesting that there were few of their eggs or nauplii to start with.

**Treatment B: zooplankton development in bags with enriched initial zooplankton**

Two bags with toxic *N. spumigena* and one without were enriched with >100 µm zooplankton to ~500 ind l⁻¹, which is ~10 times higher than at the nearby sampling site in summer (Koski et al., 1999b). At the first subsampling point, zooplankton abundance was reduced in bags with *N. spumigena* (~150 ind l⁻¹) as well as in the bag with ambient water (200 ind l⁻¹). Later on, zooplankton concentration was constant in one of the two *N. spumigena* bags (Nod 5, final abundance 150 ind l⁻¹), increased in the other (Nod 6, final abundance 250 ind l⁻¹), and became finally reduced in the bag with ambient water (~150 ind l⁻¹).

In the initial zooplankton, the rotifers *Synchaeta* spp. accounted for >70% of the mesozooplankton numbers, whereas the two copepod species *A. bifilosa* and *E. affinis* comprised 8 and 3%, respectively (Figure 2). Cladocerans were also of minor importance: *Evadne nordmanni* 7%, *Pleopsis polyphemoides* 4%, *B. longispina maritima* 2%. The rest of the initial assemblage comprised Cirripedia nauplii (3%) and cyclopoids (1%).

**Fig. 1.** Treatment A. Abundance of major zooplankton species after 15 days of development in mesocosms with reduced initial zooplankton. Grey columns, bags 1–4 with added *N. spumigena*; black columns, ambient bag without *N. spumigena*. 

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**A)** *Synchaeta* spp. 

**B)** *Boernina longispina maritima* 

**C)** *Eurytemora affinis* 

**D)** *Acartia bifilosa*
Synchaeta spp., E. nordmannii and cyclopoids disappeared from all bags, both with and without toxic cyanobacteria, at an early stage of the experiment (Figure 2A, F and H). This contrasts with the results at reduced initial zooplankton concentration (treatment A), which showed that Synchaeta spp. have the ability to develop in the bags (Figure 1). Cirripedia nauplii and P. polyphemoides had constant or even increased abundances after the first 10 days, but high mortality thereafter (Figure 2E, G). Again, these reductions also occurred in the bag of ambient water, so a negative effect of the cyanobacteria, such as an accumulation of toxins, cannot be the reason.

A. bifilosa, E. affinis and B. longispina maritima maintained high abundances until the end of the experiment (Figure 2B, C and D). The most successful species was E. affinis, whose abundance increased by a factor of 6 in both bags with toxic N. spumigena. However, for the first 10 days, E. affinis and particularly A. bifilosa were less abundant in the N. spumigena bags than in the one without, suggesting at least some harmful effect from N. spumigena. In contrast to E. affinis, A. bifilosa developed differently in the two N. spumigena bags (Wilcoxon test, n = 3, P = 0.11), having an almost constant abundance in Nod 5 and a reduction in Nod 6. B. longispina maritima showed the opposite trend with an increase in Nod 6 and little change in Nod 5 (Wilcoxon test, n = 3, P = 0.11).

The development stage structures confirmed that E. affinis was able to thrive in N. spumigena treatments, whereas recruitment of A. bifilosa might have been affected. In both N. spumigena bags, E. affinis nauplii represented about one third of the increasing populations throughout the experiment (Figure 3A), inferring successful reproduction. This is supported by the egg production rates, which remained at high levels (Table IV). In contrast, A. bifilosa showed stable or decreasing populations, but a clear decline of younger stages (Figure 3B). E. affinis had a higher proportion of nauplii and copepods than A. bifilosa at days 5, 10 and 15 of the experiment but not in the seeding population (Mann–Whitney U test, n = 6, P < 0.05). However, in both species the proportion of adults increased, reaching ~ 20% of the E. affinis population and ~ 50% for A. bifilosa. This accumulation of adults probably reflects the lack of predation.

Fig. 2. Treatment B. Abundance of major zooplankton species during development in mesocosms with enriched initial zooplankton. Species are presented in decreasing order of maximum abundance.
Zooplankton development in relation to the food assemblages

In the *N. spumigena* bags, a rich assemblage of alternative food developed (Table III). This spanned a large size range, including ciliates (>30 ciliates ml⁻¹, *Euplotes* spp., *Strombidium* spp. or *Strombilidium* spp.) and a variety of phytoplankters (dinoflagellates, small prymnesiophytes and small unidentified filamentous cyanobacteria). The only exception was Nod 6 of treatment B, where the autotrophs consisted almost exclusively of *N. spumigena* and the number of large ciliates was reduced. *B. longispina maritima* thrived in this poorer assemblage dominated by healthy cyanobacteria filaments (Figure 2B). In contrast, *A. bifilosa* was more successful in Nod 5, where alternative food sources for nauplii as well as for large copepods developed (Figure 2D).

There is evidence that the zooplankton ran out of food in the ambient bag towards the end of treatment B. Abundances of *A. bifilosa* and *E. affinis* (Figure 2C, D) and egg production rate of the latter (Table IV) decreased in this bag over the last subsampling interval. Although its final Chl a concentration was high (5 mg m⁻³), 80% was in phytoplankton <20 µm and alternative food items, such as ciliates, were less abundant than in the other bags (Table III). This was not the case in the ambient bag of treatment A, where the autotrophs were larger and more abundant (5 mg Chl a m⁻³ in cells >20 µm) and the number of ciliates higher.

In all bags with added *N. spumigena*, the filament biomass roughly doubled over a period of 2 weeks (Table III), which suggests that they were not under grazing control, even at the high zooplankton concentration used in treatment B. However, in bags with reduced initial zooplankton abundance the filaments were shorter (Student–Newman–Keuls multiple range test, *n* = 250, *P* < 0.05) and less healthy than in bags with enriched zooplankton, suggesting that zooplankton might have influenced the progress of the cyanobacteria bloom, for example by selective feeding or nutrient release.

**DISCUSSION**

In this study, both the concentration of *N. spumigena* (1–3 g C m⁻³) and its nodularin content (2–4 µg mg⁻¹ dry wt⁻¹) were very high, and in the upper range of values for cyanobacteria blooms in the Baltic Sea (Sivonen *et al*., 1989; Wasmund, 1997). Our hypothesis was that *N. spumigena* bags, a rich assemblage of alternative food developed (Table III). This spanned a large size range, including ciliates (>30 ciliates ml⁻¹, *Euplotes* spp., *Strombidium* spp. or *Strombilidium* spp.) and a variety of phytoplankters (dinoflagellates, small prymnesiophytes and small unidentified filamentous cyanobacteria). The only exception was Nod 6 of treatment B, where the autotrophs consisted almost exclusively of *N. spumigena* and the number of large ciliates was reduced. *B. longispina maritima* thrived in this poorer assemblage dominated by healthy cyanobacteria filaments (Figure 2B). In contrast, *A. bifilosa* was more successful in Nod 5, where alternative food sources for nauplii as well as for large copepods developed (Figure 2D).

There is evidence that the zooplankton ran out of food in the ambient bag towards the end of treatment B. Abundances of *A. bifilosa* and *E. affinis* (Figure 2C, D) and egg production rate of the latter (Table IV) decreased in this bag over the last subsampling interval. Although its final Chl a concentration was high (5 mg m⁻³), 80% was in phytoplankton <20 µm and alternative food items, such as ciliates, were less abundant than in the other bags (Table III). This was not the case in the ambient bag of treatment A, where the autotrophs were larger and more abundant (5 mg Chl a m⁻³ in cells >20 µm) and the number of ciliates higher.

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**Table IV: Mean egg production rates (eggs female⁻¹ day⁻¹) of *E. affinis* from the field (Storfjärden) and after 10 and 15 days of incubation in treatment B**

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Storfjärden</th>
<th>Nod 5</th>
<th>Nod 6</th>
<th>Ambient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>5.6</td>
<td></td>
<td>5.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Day 10</td>
<td>5.1</td>
<td>5.3</td>
<td>5.3</td>
<td>2.0</td>
</tr>
</tbody>
</table>

In the preserved samples, egg sacs were partly broken and often no longer attached to the females, so individual egg production rates could not be calculated. –, Missing data.
prolonged exposure to the N. spumigena dominated assemblage would reduce zooplankton abundance significantly. However, the lack of such an effect, seen in all six bags of concentrated N. spumigena and in two separate treatments, means that we have to reject this hypothesis. This result was unexpected because short-term studies have shown increased copepod mortality or inhibition of feeding and egg production in the presence of cyanobacteria in the Baltic Sea (Sellner et al., 1994, 1996; Schmidt et al., 1998; Koski et al., 1999a; Engström et al., 2000). Therefore we will discuss, first, some features of the mesocosm approach which influence zooplankton abundance; second, how our results can be reconciled with former experiments; and third, the possible field implications for Baltic Sea zooplankton.

The mesocosm approach

The objective of this study was to examine the effect of a simulated cyanobacteria bloom on population growth of zooplankton. For such timescales (~2 weeks), mesocosms are a realistic compromise between bottle incubations and field sampling (Gamble and Davies, 1982). They allow us to follow complex trophic interactions over generation timescales, yet avoid problems of repeat field sampling (Gamble and Davies, 1982). They allow us to follow complex trophic interactions over generation timescales, yet avoid problems of repeat field sampling caused by advection, patchiness and recruitment from benthic stages.

However, mesocosms also have disadvantages. First, some zooplankters are sensitive to net capture and handling, which probably explains why the cladoceran E. nordmanni disappeared from all bags of treatment B within the first 5 days. Second, larger predators are often excluded from mesocosms. However, predation control within the first 5 days. Second, larger predators are often excluded from mesocosms. However, predation control can be important, but is hard to quantify (Kiørboe, 1998). Third, replication can be poor (Gamble and Davies, 1982). Variable and sometimes large differences between replicate bags appear to be a feature of mesocosms (DeMott and Kerfoot, 1982; Hunt and Smith, 1982; Carlsson et al., 1995; Turner et al., 1999) and were also found in the present study. These differences were genuine rather than from experimental error, as only some species differed in their abundances, while others showed good replication. Further, successive subsampling of bags in treatment B revealed discernible trends. Thus the differences between replicates probably reflect slight variability in the seedling populations and the complexity of subsequent ‘food web’ interactions.

These factors undoubtedly influenced the abundance of zooplankton in our experiments. Nevertheless, whatever the in situ predation rates and whatever caused the differences between replicates, our major result still holds. Zooplankton were capable of surviving and developing despite prolonged exposure to very high concentrations of N. spumigena. Variation between replicates does not negate this basic ability. Rather it points to additional interactions with other food items or zooplankton species.

Species responses to a simulated N. spumigena bloom

In addition to the generally low food quality of cyanobacteria (Ahlgren, 1993; Müller-Navarra et al., 2000), several N. spumigena strains produce a cyclic pentapeptide, nodularin, which inhibits eukaryotic protein phosphatase activity in the liver of mammals but also in the hepatic diverticula of crustaceans (Rinehart et al., 1988; Carmichael et al., 1990; Honkanen et al., 1990). The effects of the hepatotoxins nodularin and microcystin are mainly from ingested cells, as those released to the surrounding water lose most of their potential (Reinikainen et al., 1994) and are rapidly degraded (Herszyn and Nicholls, 1997). Therefore, zooplankton survival in the presence of toxic N. spumigena depends on their feeding behaviour, physiological sensitivity and nutritional requirements.

In this study, E. affinis, Synchaeta spp. and B. longispina nanus were able to develop and to reproduce successfully in the simulated N. spumigena bloom. A. híthoa showed impaired recruitment, even though the survival of individuals was not affected drastically. These findings contrast with previous laboratory and field experiments on Baltic Sea copepods, which showed low rates of feeding and egg production and increased mortality in the presence of high cyanobacteria concentrations (Sellner et al., 1994, 1996; Schmidt et al., 1998; Koski et al., 1999a). This discrepancy was not due to low nodularin concentrations in the present study as they were high (8–20 mg m−3; Table II), and comparable with those during an exceptionally heavy bloom in the field (Koönne et al., 1993). The animals were exposed to this concentration for 2 weeks and were unable to avoid it by vertical migration as is possible in the field (Burns et al., 1987). However, in the bags with N. spumigena, a rich assemblage of nano- and microplankton was associated with the different stages of the initiated bloom (Engström-Öst et al., 2002). Therefore we suggest that in the Baltic Sea, abundance and quality of alternative food sources are crucial for zooplankton success during cyanobacteria blooms. This argument is developed below.

Since there was no direct investigation of feeding on animals in the mesocosm bags, we use observations from concomitant short-term laboratory experiments (Koski et al., 2002). In these laboratory experiments, freshly caught copepod females were incubated in mesocosm water at days 1, 7 and 14. Throughout the experiment, A. híthoa and E. affinis were preferentially feeding on the ciliate community, which comprised different species at different stages of the bloom (Koski et al., 2002). However, the
contributions of ciliates to the total ingested carbon varied with ciliate abundance in the mesocosm, and did not exceed 50% (Koski et al., 2002). Even though ciliates became very abundant in some of the mesocosm bags, they remained within the range of concentrations observed in the Baltic Sea (McKellar and Hobbs, 1976; Tiselius, 1989).

The rest of the ingested carbon derived mainly from phytoplankton (Koski et al., 2002). Table V illustrates copepods grazing on phytoplankton in the bottle incubations at day 1. This experiment might be representative for copepod initial behaviour in the mesocosm bags, as those animals have been sampled from the same field population and were not yet adapted to the cyanobacteria. Using the reduction of Chl a (Table V), we calculated ingestion rates on phytoplankton of ~700 and ~200% body C day⁻¹ for A. bifilosa and E. affinis, respectively (the C:Chl a ratio was 20 according to Table III, and the body C content was 1.5 µg for A. bifilosa and 2.1 µg for E. affinis according to Koski, unpublished). The concomitant reduction of cyanobacteria marker pigments (Table V), as well as the overwhelming dominance of healthy filaments of N. spumigena in the phytoplankton (Table III), imply that copepods were feeding on this toxic species in significant amounts. Thus, being part of a semi-natural food assemblage, N. spumigena was not generally rejected but included in the diet of copepods. This result is in clear contrast to previous laboratory studies, where both A. bifilosa and E. affinis avoided feeding on toxic N. spumigena when offered as sole food or in simple mixtures (Koski et al., 1999a; Engström et al., 2000).

An uptake of toxins might explain why copepod populations in bags with N. spumigena were slightly less successful than those in ambient bags over the first 10 days of the experiment (Figure 2), even though the negative effect was not overwhelming. Laboratory studies on cladocerans and rotifers have shown that high concentrations of other food might reduce their sensitivity to toxic cyanobacteria (Reinikainen et al., 1994; Gilbert, 1996; Ferrão-Filho et al., 2000). Likewise, Turner et al. (Turner et al., 2001) observed that the negative effect of a unialgal diatom on copepod egg viability was 'diluted' in a mixed diet. Reinikainen et al. (Reinikainen et al., 1994) provided two plausible explanations. A greater proportion of non-toxic food reduces the probability that toxic food will be ingested. Also, well-fed zooplankton are in better condition to tolerate the toxins.

Field observations on copepod ingestion of blooming cyanobacteria in the Baltic Sea are inconsistent, showing high uptake (Meyer-Harms et al., 1999) and low uptake (Sellner et al., 1994, 1996; Meyer-Harms et al., 1999). The latter authors noticed that cyanobacteria become more attractive to copepods when filaments aged and became colonized by heterotrophs. In contrast, Sellner et al. (Sellner et al., 1994) found few cyanobacteria pigments in the guts of A. bifilosa and E. affinis, even though an active microheterotrophic community was associated with N. spumigena filaments. Moreover, A. bifilosa had high mortality and generally appeared unhealthy. However, during their study, the co-occurring cyanobacterium Aphanizomenon flos-aquae was present in ~10 times the concentration of N. spumigena, and this species may produce harmful compounds other than hepatotoxins (Sellner, 1997). The authors’ unpublished data). For a tropical bloom of an unidentified filamentous cyanobacterium, Turner et al. (Turner et al., 1998) reported that zooplankton, including the copepod Acanthella bifilosa, fed on cyanobacteria and co-occurring phytoplankton without any apparent adverse effect. These contrasting results suggest that in the field, copepod survival in a cyanobacteria bloom is influenced by the actual toxicity of the dominant species, as well as by the presence of additional food items.

In our mesocosm, the differing development of the zooplankton reflects their responses to the cyanobacteria colonized assemblages. E. affinis copepod better in N. spumigena treatments than did A. bifilosa, whose success might be more dependent on suitable alternative food. In the parallel bottle incubations, A. bifilosa females fed preferentially on large ciliates and had higher egg production rates when ciliates were abundant (Koski et al., 2002). Likewise, in treatment B, A. bifilosa developed better in the N. spumigena bag with the richest protozoan and phytoplankton assemblage (Table III). The reasons for species differences can be that A. bifilosa is more sensitive to high particle concentration and low food quality than E. affinis (Jeffries et al.,

### Table V: Copepod grazing on autotrophs in the mesocosm center

<table>
<thead>
<tr>
<th>Pigment (µg l⁻¹)</th>
<th>Control</th>
<th>E. affinis</th>
<th>A. bifilosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a</td>
<td>40.14 ± 1.05</td>
<td>36.89 ± 0.34*</td>
<td>32.82 ± 1.31*</td>
</tr>
<tr>
<td>Fucoxanthin</td>
<td>1.14 ± 0.07</td>
<td>1.08 ± 0.04</td>
<td>0.90 ± 0.03</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>0.25 ± 0.07</td>
<td>0.28 ± 0.02</td>
<td>0.34 ± 0.04</td>
</tr>
<tr>
<td>Echinonene</td>
<td>2.48 ± 0.06</td>
<td>2.06 ± 0.23*</td>
<td>1.53 ± 0.46*</td>
</tr>
</tbody>
</table>

Pigment concentration (µg l⁻¹) in bottles without copepods (controls, n = 3) and in bottles with either 20 females of E. affinis or A. bifilosa (n = 3) at the end of 24 h incubation. Significant differences between controls and bottles with copepods are indicated: *P < 0.05, Mann-Whitney U test. Fucoxanthin is typical for dinoflagellates, diatoms, prymnesiophytes and cryptophytes (Jeffries et al., 1997). Chlorophyll a is marker pigment in chlorophytes and Euglenophytes, whereas zeaxanthin and echinenone characterized cyanobacteria (Jeffries et al., 1997). The incubation water was mixed from bags Nod 1–4 of treatment A at Day 1. Copepods were freshly caught from the field. For calculated clearance rates, grazing on heterotrophs and further details on the methods see (Koski et al., 2002). The HPLC pigment analysis is described in (Engström-Öst et al., 2002).
1996; Gasparini and Castel, 1997), but also to toxins (Reimink et al., 2002). Conversely, the similar development of E. affinis in the replicate N. spumigena bags is probably caused by its broad food spectrum and low nutritional requirements (Heinle et al., 1977; Gyllencreutz, 1980; Gasparini and Castel, 1997), combined with a reduced body size and consequent lower maximum egg production rate at the high summer temperatures (Hirche, 1992).

The parallel bottle incubations with A. bifilosa and E. affinis (Koski et al., 2002) provide complementary information on short-term responses of adult females to N. spumigena. While the overall results were similar to those of the mesocosms, showing low mortality and high rates of feeding and egg production, there was one important difference. The short-term incubations did not reveal any inhibitory effect on A. bifilosa females which was evident in the mesocosm population response. Probably other processes apart from feeding or egg production were affected by the toxins. Likewise, a study by Turner et al. (Turner et al., 2001) has shown that components of the diatom Thalassiosira nordica inhibited copepod egg hatching success even though feeding and egg production rates were very high. This underlines the value of monitoring population development as well as the short-term response of single maturity stages and processes.

The present study demonstrates that both the rotifers Synchaeta spp. and the cladoceran B. longispina maritima have the capability of surviving in a N. spumigena bloom. There are few previous data for these taxa in the Baltic Sea. Synchaeta spp. is known only to have a broad food spectrum including filamentous cyanobacteria (Arndt et al., 1990; Sellner, 1997). The cladoceran, B. longispina maritima, might be the zooplankton species of the Baltic Sea with the highest selective pressure to resist cyanobacteria toxins (Gilbert, 1990). Unlike the other major zooplankters, its short, intensive reproductive period often coincides with cyanobacteria blooms (Wasmund, 1997). Gut pigment analyses (Sellner et al., 1994) and stable nitrogen isotope measurements (Struck et al., 1998) have suggested that cyanobacteria are prominent in the diet of B. longispina maritima. Likewise, freshwater Bosmina spp. often co-occur with cyanobacteria blooms, feed on them and resist hepatotoxins (Hanazato and Yasumo, 1987; Fulton, 1988; Watanabe, 1992). However, studies on freshwater Synchaeta spp. as well as Bosmina spp. have also shown that these groups need additional, more nutritious food to coexist with toxic cyanobacteria blooms (Fulton, 1988; Lundstedt and Brett, 1991; Gilbert, 1996).

Field implications

In summer, the Baltic Sea is often characterized by vertical stratification and nutrient depletion in the euphotic zone (Anonymous, 1996). With low nutrient concentrations, phytoplankton biomass decreases and small flagellates tend to dominate (Malone, 1980; Kiørboe, 1993). These flagellates are mostly too small to be captured efficiently by copepods (Berggreen et al., 1988) and feeding on micro-zooplankton often cannot compensate, resulting in food shortage (Klein Breteler et al., 1982; Tielens et al., 1987; Kiørboe and Nielsen, 1994).

These nutrient-limited conditions during summer often coincide with cyanobacteria blooms (Wasmund, 1997). By fixing molecular nitrogen, cyanobacteria build new organic matter and thus transfer energy into the food web at a time of the year when phytoplankton are otherwise scarce. Using a stable isotope approach, Rolff (Rolff, 2000) found that fixed nitrogen from a cyanobacterial bloom was transported to all size classes of the plankton, including rotifers, cladocerans and large copepods. Both the cyanobacteria and the heterotrophs associated with the decaying blooms can be ingested by zooplankton. Therefore, any adverse effects of cyanobacteria blooms on zooplankton should be seen in the context of the alternative—potential food limitation.

In the mesocosm experiments, the ambient bags simulated a non-bloom field situation. The water for these was collected from a coastal site and was more food replete (~5 mg Chl a m \(^{-3}\)) than often found in the open Baltic Sea in summer (~1–2 mg Chl a m \(^{-3}\)) (Anonymous, 1996) (N. Wasmund, personal communication). Nevertheless, food shortage probably arose in the ambient bag towards the end of treatment B, restricting egg production rates of E. affinis (Table IV) and development of both copepod species (Figure 2).

Compared with such food limitation, our results suggest that zooplankton are capable of deriving some benefit from cyanobacteria blooms and their associated assemblages. The spectrum of alternative food might have motivated feeding in the presence of cyanobacteria, supporting the nutritional balance and reducing the toxic effect of ingested cyanobacteria. The major food sources, cyanobacteria or microheterotrophs, needs further investigation, but probably differs with species and stages of the bloom. Nevertheless, our mesocosm approach provides support for the notion that naturally occurring cyanobacteria bloom assemblages need not always be bad for zooplankton communities.

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