Effects of cyanobacteria on plankton and planktivores

JONNA ENGSTRÖM-ÖST

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This thesis is based on the following papers, which are referred to by their Roman numerals:


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CONTRIBUTIONS

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JONNA ENGSTRÖM-ÖST


Mass-occurrences of cyanobacteria have been reported to increase in frequency, as well as in intensity, due to eutrophication. It is therefore important to investigate how cyanobacteria influence other organisms and the structure and functioning of the aquatic ecosystem.

The aim of this thesis was to study effects of toxic and non-toxic filamentous cyanobacteria on two calanoid copepods, and two mysid shrimps and one fish species in the northern Baltic Sea. Feeding, survival, reproduction and toxin accumulation of these animals, during direct or indirect exposures to cyanobacteria, were measured in different experimental set-ups. We also monitored the plankton community (<100 µm) during the decay of a bloom of the toxic cyanobacteria *Nodularia spumigena*.

The grazing experiments showed that juvenile and adult mysid shrimps as well as the copepod *Eurytemora affinis* fed more on the non-toxic *Nodularia sphaerocarpa* or *Aphanizomenon flos-aquae* than on the toxic *N. spumigena*, whereas the copepod *Acartia bifilosa* did not graze on either of these strains. However, when *A. bifilosa* was provided with mesocosm water containing toxic *N. spumigena*, active grazing on the cyanobacteria was detected.

We monitored the plankton community during the decay of a bloom of the toxic cyanobacteria *Nodularia spumigena* during a 2-week enclosure experiment. An increase in the ratio of particulate organic carbon to chlorophyll *a* (<10 µm), a decrease in the ratio of the polyunsaturated to total fatty acids, and a reduction in cyanobacterial filament length, indicated decay of *N. spumigena*. Total nodularin concentrations remained high during the whole experiment. Several ciliate species and filamentous bacteria flourished among the filaments, indicating that a decaying bloom is a nutrient-rich substrate to live in.

*Acartia bifilosa* incubated in mesocosm water produced more eggs at the beginning and during the middle of the experiment, when copepods were feeding on actively growing cyanobacteria and ciliates, than at the end of the experiment, when the bloom was visibly decaying. Therefore, our results did not fully support the hypothesis that the food quality of blooms increases during decay of cyanobacteria. However, actively growing cyanobacteria combined with increasing ciliate abundances seemed to have provided the best food for *A. bifilosa*.

Accumulation of cyanobacterial toxin in planktivores via copepods was measured by two different toxin detection assays. Our results suggested that accumulation had potentially taken place in mysid shrimps, measured by enzyme-linked immunosorbent assay.

To conclude, the organisms did not generally seem to be adversely affected by filamentous cyanobacteria during our short-term trials. The long-term effects of cyanobacteria on feeding, survival and reproduction of Baltic Sea biota remain to be studied, however.

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INTRODUCTION

Cyanobacteria have a worldwide distribution, living in the sea, in brackish and freshwater areas, in damp soil, glaciers and deserts as well as in hot springs (van den Hoek et al. 1993). They belong to the most archaic organisms on the Earth. The Proterozoic Era is sometimes called the ‘Age of Cyanobacteria’, because, at that time, between 2.5 and 0.5 billion years ago, cyanobacteria dominated the biota (van den Hoek et al. 1993).

Paleolimnological studies indicate that cyanobacterial blooms occurred during ancient conditions. These studies suggest that blooms are natural phenomena, which may be increased by anthropogenic eutrophication, but which are not dependent on this process (e.g. McGowan et al. 1999). In the Baltic Sea, the cyanobacterial blooms are as old as today’s brackish-water period, the ‘Littorina Sea’, which started about 7000 BP. The ancient blooms were most likely similar in size and magnitude to the present ones (Bianchi et al. 2000, Poutanen & Nikkilä 2001).

Mass-occurrences of cyanobacteria have, however, been reported to increase in frequency, as well as in intensity, due to eutrophication (Kahru et al. 1994, Finni et al. 2001, Poutanen & Nikkilä 2001). In the Baltic Sea, annually recurrent blooms usually peak in July and August, at times of calm, warm weather. Different environmental conditions have been suggested to trigger cyanobacterial mass-occurrences. Increasing light and temperature, and decreasing turbulence, obviously stimulate the growth of Nodularia spumigena, though it can be difficult to identify which of these factors is
the most significant in the field. Mixing of the water column, in association with upwelling of essential nutrients, has been shown to initiate the bloom of *Aphanizomenon flos-aquae*. Shallowing of the upper mixed layer due to solar heating has been suggested to initiate the bloom of *Nodularia spumigena* (Kononen et al. 1996). Recently, Kahru et al. (2000) discussed that the frequency and magnitude of saltwater inflows into the Baltic Sea may have a crucial role in the onset of *Nodularia spumigena* blooms, due to their effect on phosphorus availability in the surface water layers.

A phytoplankton species can be considered harmful for several reasons. A harmful phytoplankton bloom may be an aesthetic nuisance, due to its dense occurrence in open water or on beaches. Decaying algal blooms may result in anoxia, due to high biological activity, and lead to fish mortality. Phytoplankton species with long spines, e.g. diatoms, may interfere mechanically with fish gills and cause damage. However, the most common reason for classifying algae as harmful is probably due to their toxin production (Richardson 1997 and references therein). Cyanobacteria commonly produce toxins, and may also have other adverse effects on different species. It is therefore important to investigate how cyanobacteria influence other organisms and the structure and functioning of the aquatic ecosystem.

The aim of this thesis was to contribute to the present knowledge by studying the effects of filamentous cyanobacteria on a plankton assemblage, copepods, mysid shrimps and a planktivorous fish.

### Competitiveness of cyanobacteria

Due to their early evolutionary history (Lazcano & Miller 1994), cyanobacteria have developed an array of qualities that may favour them in competition with other organisms (reviewed in Sommer et al. 1986, Sterner 1989, Shapiro 1990, Hyenstrand et al. 1998). Concerning temperature, it was stated in a review by Robarts & Zohary (1987), that cyanobacterial dominance generally occurs during temperatures >20º C, but that temperature alone does not determine the occurrence of a species. Other factors include, among others, buoyancy (Reynolds et al. 1987), allelopathic compounds (Keating 1977), grazing resistance (Haney 1987), low total P : total N ratio (Smith 1983), phosphorus and nitrogen storage capacity (Pettersson et al. 1993), effective competition for nitrogen via N₂-fixation (Blomqvist et al. 1994) and ability to grow at low light (Smith 1986), as well as tolerance of high pH or low carbon dioxide concentration (Shapiro 1990).

Buoyancy regulation allows the cyanobacteria to position themselves favourably within gradients of physical and chemical factors (Mur et al. 1999, Mitrovic et al. 2001). Buoyancy regulation can be an important advantage in competition with other phytoplankton organisms, especially during non-turbulent conditions (Mur et al. 1999). Buoyancy of the two most common Baltic cyanobacteria, *Nodularia spumigena* and *Aphanizomenon flos-aquae*, is regulated by gas vesicles and changes in cellular concentrations of carbohydrates. The gas vesicles of these species survive mixing down to 60 m depth (Walsby et al. 1997). *N. spumigena* seems to be more strongly buoyant than *A. flos-aquae* (Niemistö et al. 1989). Some spe-
cies can migrate several metres per hour, and therefore, blooms may be formed in a short time (Walsby et al. 1992). Furthermore, light and nitrogen are important factors controlling the buoyancy regulation (Heiskanen & Olli 1996).

Cyanobacteria produce a variety of secondary metabolites. Secondary metabolites are not used in the primary metabolism of an organism and include compounds that can operate as allelochemicals, antibiotics, hormones or toxins (Carmichael 1992). Allelochemicals are substances produced by one organism that are toxic or inhibitory to the growth of another (Begon et al. 1996). Allelochemicals can suppress or stimulate the growth of other phytoplankton and consequently play an important role in the competition between species in the pelagic ecosystem (Lewis 1986). Furthermore, Kurmayeur & Jüttner (1999) have suggested that chemical defences are more important than morphological characteristics in deterring grazers. Secondary metabolites of cyanobacteria have been studied extensively, and different allelopathic characteristics, such as antialgal, antibacterial, anticyanobacterial, and antifungal properties have been identified (Keating 1977, von Elert & Jüttner 1997, Inderjit & Dakshini 1997, Østensvik et al. 1998, Pushparaj et al. 1999, Casamatta & Wickstrom 2000). Both dominant genera in the Baltic Sea, *Aphanizomenon* and *Nodularia*, have revealed antibacterial properties (Østensvik et al. 1998, Pushparaj et al. 1999). In addition, *Nodularia* sp. showed antifungal activity and allelopathic effects towards other cyanobacteria and green algae (Pushparaj et al. 1999).

Due to their ability to fix nitrogen, cyanobacteria are known to be favoured over other phytoplankton in nitrogen-poor waters (Sellner 1997). N$_2$-fixing genera were shown to be highly dependent on phosphorus input, and consequently on the N : P ratio of the water (Paerl 1990). N$_2$-fixation is directly related to phosphorus loading, especially in eutrophic aquatic ecosystems, but it may also be stimulated by iron (Rueter & Petersen 1987). Furthermore, Lehtimäki et al. (1997) have shown that nitrogen fixation rates were highest during the exponential growth phase, and decreased in the stationary growth phase of the cyanobacteria. However, although N$_2$-fixation indicates an advantage to cyanobacteria, especially in nitrogen-poor waters, other sources of nitrogen are considered more important (Sellner 1997).

Cyanobacteria are superior competitors under low light conditions (Scheffer et al. 1997). Many species are also sensitive to long time periods of high light intensities (Mur et al. 1999) and it has been shown that cyanobacterial blooms may be photo-inhibited (Ibelings & Maberly 1998). Species that form surface blooms, however, seem to possess a greater tolerance for light (Mur et al. 1999). Various mechanisms, such as elevated carotenoid concentration, can offer photo-protection for the cells in surface blooms (Paerl et al. 1983, Ibelings and Maberly 1998 and references therein). In the Baltic Sea, *Aphanizomenon* has been considered more susceptible to light than *Nodularia*, due to its avoidance of the surface (Niemistö et al. 1989, Heiskanen & Olli 1996).

Several of the hypothesised mechanisms probably contribute to the success of cyanobacteria simultaneously. This may explain why single relationships have failed to explain their dominance in various aquatic systems (Burns 1987, Hyenstrand et al. 1998).
Why do cyanobacteria produce toxins?

Several hypotheses have been put forward to explain algal toxin production (Turner & Tester 1997 and references therein). Grazer repellence is probably the most studied hypothesis (Lampert 1987, DeMott & Moxter 1991, Kirk & Gilbert 1992, Carlsson et al. 1995). Other hypotheses suggest that toxins are responsible for allelopathic activity (Windust et al. 1996, Pushparaj et al. 1999), functioning as a nitrogen source (Dale & Yentsch 1978), or that toxins are the result of different metabolic processes (Turner & Tester 1997 and references therein).

According to Pajdak-Stós et al. (2001), cyanobacteria are highly resistant to grazing by protozoans, copepods and cladocerans, due to millions of years of co-evolution with these herbivores. It has, however, been pointed out that the occurrence of co-evolution is hard to assess in the marine environment (cf. Hay 1991). Notably, very few grazers are specialised on cyanobacteria, with the exception of several ciliate strains, e.g. Nassula sp. and Pseudomicrothorax sp. (Canter et al. 1990, Fialkowska & Pajdak-Stós 1997 and references therein), due to the grazing-decreasing characteristics of cyanobacteria, other than toxins (Canter et al. 1990). Furthermore, to the best of my knowledge, it is not known when toxin production of cyanobacteria evolved. Steneck (1992) argues that the fossil record does not support the hypothesis that herbivory would generate strong selective pressures on the algal food species, although grazing is usually intense and universal in the marine environment. To sum up, the ecological purpose of the secondary metabolites produced by planktonic organisms, e.g. phytoplankton, is not known (Car-michael 1992, Verity and Smetacek 1996). Although a certain compound may affect copepod grazing adversely, it does not necessarily imply that it has evolved as a feeding deterrent (Hay 1996).

Why are cyanobacteria considered harmful to zooplankton and fish?

Cyanobacteria have commonly been considered as low quality food for zooplankton, due to their morphology, low nutritional value and toxin content (Porter and Orcutt 1980, Lampert 1987). Many cyanobacteria have a filamentous or colonial morphology, and form aggregates, which could reduce feeding rates, or clog the feeding appendages of suspension feeding zooplankton (Webster & Peters 1978). Unpalatability of a food species, i.e. bad taste or bad odour (DeMott 1986, van den Hoek et al. 1993), is also considered to be a characteristic of poor food quality.

The low nutritional content of cyanobacteria, demonstrated as a low reproductive response, may be due to slow assimilation (reviewed by Lampert 1987) or lack of essential compounds (Holm & Shapiro 1984, Brett & Müller-Navarra 1997, Müller-Navarra et al. 2000), such as highly unsaturated fatty acids (HUFA) (DeMott & Müller-Navarra 1997). Saturated fatty acids (SAFA) are important due to their high calorific content and are mainly utilised for energy, whereas polyunsaturated fatty acids (PUFA) affect the production of eicosanoids, which are crucial for numerous physiological functions related to reproduction in invertebrates, e.g. egg production and egg hatching (Brett & Müller-Navarra 1997 and references there-
in). Previously, the egg production and egg hatching success of copepods, provided with both toxic and non-toxic cyanobacterial monocultures, have been shown to be low. Deformed egg sacs have also been reported (Koski et al. 1999). Finally, zooplankton feeding may be inhibited, or zooplankton mortality increased, due to algal toxins or other secondary metabolites (Nizan et al. 1986, DeMott et al. 1991).

The hepatotoxins of cyanobacteria are considered extremely harmful to vertebrates (Sivonen & Jones 1999). In the marine environment, cyanobacteria may affect fish physiologically (Bury et al. 1995) or cyanobacterial secondary metabolites may deter juvenile fish from feeding. On the other hand, fish may learn to avoid harmful metabolites, though hunger limits this avoidance (Thacker et al. 1997, Nagle & Paul 1998). Grazing studies with herbivorous fish have shown that feeding decreases when the percentage of toxic cyanobacteria cells increases (Keshavanath et al. 1994). Cyanobacterial toxins have caused fish kills (Peñaloza et al. 1990), been detected in fish livers and have been found to accumulate in fish tissues (Sipiä et al. 2001a, b). Contrary to the accumulation hypothesis, Sahin et al. (1996) have shown that cyanobacterial toxins are excreted into the bile of the fish, rather than accumulating in the tissues. Experimental trials have also shown that some fish may be able to detoxicate cyanobacterial toxins (Wiegand et al. 1999).

Hansson (1997) suggested that herring larvae tend to avoid water containing dense *Nodularia spumigena* blooms in the Baltic Sea. Therefore the larvae are forced to stay in colder and deeper water, possibly reducing their rate of growth and increasing mortality. The mechanisms by which toxic cyanobacterial mass-occurrences affect fish behaviour and recruitment are still largely unknown.

**Can cyanobacteria have positive effects on the pelagic community?**

Despite the above stated negative effects, evidence has recently started to accumulate that cyanobacteria may also have neutral or even positive effects on grazers.

Meyer-Harms et al. (1999) studied grazing on cyanobacteria in the Baltic Sea and showed that copepods fed more actively on cyanobacteria when the blooms were dense and also during later growth stages. Rolff (2000) recognised strong signals of cyanobacteria in samples of stable isotopes dominated by rotifers and cladocerans in the Baltic proper. O’Neil & Roman (1994) suggested that pelagic harpacticoid copepods may be able to affect the distribution and species composition of *Trichodesmium* sp. by grazing.

A lack of evident effects on zooplankton, feeding on toxic algae, may be more general than potential adverse effects (review by Turner et al. 1998). Zooplankton can avoid harmful algae by selective feeding (Turriff et al. 1995, Turner et al. 1998) and vertical migration (Forsyth et al. 1990). Laboratory trials show that copepods may benefit from cyanobacteria when provided with appropriate mixtures of cyanobacteria and good quality food (Schmidt & Jónasdóttir 1997). Furthermore, the quality of cyanobacteria as food for zooplankton may change or even improve when the bloom is starting to age and decay. This may take place because the toxin concentration of blooms decreas-
es during the senescent phase (Kankaanpää et al. 2001), and because a decaying *Nodularia spumigena* bloom is known to attract a diverse community of bacteria, flagellates, microzooplankton and crustaceans (Hoppe 1981). Finally, Repka et al. (1998) have shown that detritus derived from cyanobacteria is good quality food.

**OBJECTIVES OF THE THESIS**

The aim of my thesis was to study the effects of cyanobacteria on different heterotrophic organisms, mainly copepods and mysid shrimps. The succession in a plankton community (<100 µm), as well as the potential accumulation of cyanobacterial toxin, in small planktivorous fish and mysid shrimps were also studied. The main study questions were: 1) do copepods and mysid shrimps feed on cyanobacteria, 2) do cyanobacteria interfere with feeding on high quality food, 3) are copepods able to reproduce during different phases of a cyanobacterial bloom, and 4) is cyanobacterial toxin accumulated in planktivores via copepods.

**METHODS**

The data for my thesis was collected at the Tvärminne Zoological Station on the SW coast of Finland, at the entrance to the Gulf of Finland, in the northern Baltic Sea. The experiments were conducted in the laboratory, and in enclosures located in the nearby sea area. The samples needed for the experiments were collected from Storfjärden, a pelagial area in the inner archipelago, and from Längden, an offshore area (Fig. 1).

The study organisms were chosen according to their occurrence simultaneously with cyanobacterial blooms. The mesocosm experiment, to study community development during the decay of *Nodularia spumigena*, was performed in July 1999. In the other experiments, we used animals that potentially may encounter cyanobacterial blooms during their life cycle. *Acartia bifilosa* and *Eurytemora affinis* are known to be abundant throughout the summer (Vitasalo 1994). Mysid shrimps are important planktivores and fish food in the Baltic Sea (Rudstam et al. 1989), which together with planktivorous fish, consume more than half of the autumnal zooplankton production (Hansson et al. 1990; Rudstam et al. 1992). Mysid shrimps are known to feed in small amounts on cyanobacteria in their natural habitat (Viherluoto et al. 2000). They also migrate vertically in the water column between day and night (Rudstam et al. 1989), which may expose them to potential blooms. If herbivorous zooplankton act as a vector for cyanobacterial toxins to higher trophic levels during vigorous blooms, then mysid shrimps and planktivorous fish may potentially accumulate the toxins. The three-spined stickleback, *Gasterosteus aculeatus*, lives both in the open sea and in the littoral zone (Leinikki 1995), where the effect of cyanobacteria may be large, due to shore-drifting blooms.

The experiments presented in this thesis are summarised in Table 1 and the measurements in Table 2.

In the grazing experiments, cyanobacteria were fed to copepods and mysid shrimps in order to observe whether cyanobacteria are utilised as food, and if there are differences between grazing rates on toxic...
Figure 1. Map of the study area. Experimental organisms were obtained from Storfjärden (I, II, III, IV, V), Längden (II, V) and from the littoral area in the vicinity of the Tvärminne Zoological Station (TZS) (V). Map drawn by Markku Viitasalo.

vs. non-toxic strains, and in different concentrations (I, II). We performed two different ‘interference’ experiments in order to study whether feeding on high quality food is reduced in the presence of filamentous cyanobacteria (I, II). Food quality of decaying cyanobacteria was studied in two of the papers (III, IV). We were interested in whether copepods derive sufficient high quality food in different phases of the bloom, and whether food quality improves with time, i.e. when the bloom starts to decay.
Table 1. Summary of experimental designs presented in this thesis.

<table>
<thead>
<tr>
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<th>Study organism</th>
<th>Experiment</th>
<th># replicates</th>
<th>Study</th>
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</table>
| bottle incubation (1.8 l) | *Acartia bifilosa*,
|                         | *Eurytemora affinis*          | grazing                         | 12           | I     |
|                         |                                 | ‘interference’ (A. bifilosa)    | 8            |       |
| bottle incubation (1.8 l),
| aquarium incubation (2.2 l) | *Mysis mixta*                  | grazing                         | 6            | II    |
|                         |                                 | ‘interference’                  | 5            |       |
|                         |                                 | survival                        | 6            |       |
| enclosures (120 l)      | plankton community (<100 µm)   | monitoring                      | 4            | III   |
| bottle incubation (1.8 l) | *Acartia bifilosa*,
|                         | *Eurytemora affinis*          | grazing                         | 3-4          | IV    |
|                         |                                 | egg production (A. bifilosa)    | 3-4          |       |
| aquarium incubation (2.2 l) | *Gasterosteus aculeatus*,
|                         | *Mysis relicta*                | toxin accumulation            | 5            | V     |

(IV). In connection with the food quality study, we monitored the decaying bloom for a number of parameters in order to investigate the pigment, nutrient, fatty acid and toxin dynamics, as well as the dynamics of the heterotrophic organisms (III). We measured survival in three of our studies (II, IV, V). Accumulation of nodularin in higher trophic levels was investigated with two different toxin detection methods, enzyme-linked immunosorbent assay (ELISA) and protein phosphatase (PPase) inhibition assay (V).

RESULTS AND DISCUSSION

Grazing on cyanobacteria

Toxic vs. non-toxic strains

Grazing and foraging behaviour of two calanoid copepods, *Acartia bifilosa* and *Eurytemora affinis*, and the common mysid shrimp, *Mysis mixta*, in the presence of cyanobacteria were studied (I, II). We used cultures of different cyanobacteria strains in the experiments. Both juvenile and adult mysid shrimps, as well as the copepod *E. affinis*, fed more on the non-toxic *Nodularia sphaerocarpa* or *Aphanizomenon flos-aquae*, than on the toxic *N. spumigena*,...
Table 2. Summary of methods presented in this thesis.

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<th>Method</th>
<th>Study</th>
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<td>PO₄³⁻, tot P, POP</td>
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<td>high performance liquid</td>
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<tr>
<td>Toxins</td>
<td>phosphatase (PPase) inhibition assay</td>
<td>V</td>
<td>Ward et al. (1997)</td>
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whereas grazing by A. bifilosa was non-detectable.

A herbivore can distinguish a toxic cell, either by recognising the toxin prior to ingestion of the cell, which would indicate the presence of an extracellular toxin in the water, or by learning, which would indicate the prior ingestion of a toxic cell, and subsequent avoidance due to its unpleasant taste or odour (Carlsson et al. 1995). According to DeMott et al. (1991), feeding inhibition is “either an adaptive behaviour to avoid eating toxic cells or a direct consequence of a weakened condition due to poisoning”.


Eurytemora affinis is a suspension feeder and considered less selective than, e.g., species of the genus Acartia (Jonsson & Tiselius 1990, Kiorboe et al. 1996, Gasparini & Castel 1997), which could be the reason for its feeding on the toxic strain. Cyanobacterial toxins are known to remain inside the cells during the exponential growth stage of the cyanobacteria, i.e., only 10-20% of the toxin in a log phase culture is extracellular (reviewed by Sivonen & Jones 1999). Extracellular toxin release is affected by several factors: temperature, light, salinity, growth stage and phosphorus concentration (Lehtimäki et al. 1997 and references therein). Consequently, our experimental conditions should have promoted low nodularin release from the cyanobacteria, because we used growing cultures (I, II).

We conducted a 2-week enclosure study in which we monitored a decaying cyanobacteria bloom for different parameters, organism abundances, toxins, fatty acids, protein and pigments. In the beginning of the study, when Nodularia was still in the growth phase, Acartia bifilosa fed mainly on ciliates and cyanobacteria (IV). Also, during the middle and at the end of the experiment, when N. spumigena was in its decay stage, A. bifilosa mainly selected ciliates. The fact that A. bifilosa fed on cyanobacteria is in contrast to our previous results, where A. bifilosa did not feed on any of the *Nodularia* strains, which were provided as sole food (I). Our results suggest that grazing rates seem to differ considerably, depending on whether a food source is provided alone, or in a mixture with other food. Schmidt & Jónasdóttir (1997) made similar observations, where A. tonsa did not feed on *Nodularia* sp. when provided as sole food. In contrast, Meyer-Harms et al. (1999) found moderate grazing in situ by the copepod *Acartia* sp., on cyanobacteria, during the late phases of the bloom.

In the grazing experiments with mysid shrimps, adults fed less on the toxic strain of *Nodularia spumigena* than on the non-toxic strains of *N. sphaerocarpa* and *Aphanizomenon flos-aquae* (II). The juveniles also fed less on the toxic strain than on the non-toxic *A. flos-aquae*. Mysid shrimps are known to be omnivorous (Viherluoto 2001), and in the present study they fed on cyanobacteria, probably because nothing else was available. In the field, the phytoplankton fraction of the stomach contents of *Mysis mixta* was dominated in September by dinoflagellates and also cyanobacteria to some degree (Viherluoto et al. 2000). The results of the present study suggest that, in nature, mysid shrimps ingest cyanobacteria, probably by suspension feeding, and in mixtures with other food.

**Interference with feeding by cyanobacteria**

The mechanical interference of cyanobacterial filaments with feeding has been studied mostly in freshwater lakes dominated by *Daphnia* and other filter-feeding cladocerans (e.g. Kirk & Gilbert 1992). We studied the possible mechanical interference of filaments with copepods and mysid shrimps feeding on high quality food. One experiment was conducted with the calanoid copepod, *Acartia bifilosa*, grazing on the flagellate, *Brachionus submarina* (I), whereas in the other experiment, we provided mysid shrimps with *A. bifilosa* (II). In the study with *A. bifilosa* as a grazer (I), no interfer-
ence could be detected, i.e. no reduction in clearance rates was observed with increasing concentrations of cyanobacteria. The reason for this may be, however, that the concentration of high quality food was too high in this experiment. Consequently, the feeding may have been satiated and the potential interference effect of the cyanobacteria would have remained undetected. Another interpretation of our result is simply that cyanobacterial filaments did not interfere mechanically with the feeding of *Acartia bifilosa*. This is quite possible, because *Acartia* spp. are capable of feeding selectively, by suspension feeding on phytoplankton, as well as raptorially, on motile prey (e.g. Kiorboe et al. 1996).

In the study with mysid shrimps, clearance rates on copepods were lower in the presence of filamentous non-toxic *Nodularia sphaerocarpa* (II). We believe that the reduction in clearance rates was due to clogging, because filaments were observed in the feeding appendages of all mysid shrimps after the experiment. The response pattern, seen in Figure 2 (II), is similar to the type IV functional response (Wootton 1999), where the response is initially identical to type II (Holling 1959), but decreases at the highest food concentrations. This kind of response could be expected in feeding planktivores when their ingestion is hampered due to clogging by cyanobacterial aggregates (Viherluoto 2001).

Effects of cyanobacteria on survival

Resistance to cyanobacterial toxins in zooplankton has been demonstrated a few times, mainly in freshwater systems or estuaries (Starkweather & Kellar 1983, Fulton 1988). Calanoid copepods from the Baltic Sea, *Acartia bifilosa* and *Eurytemora affinis*, seem to be very tolerant to cyanobacterial toxins as well (M. Karjalainen pers. comm., Reinikainen et al. 2001). These observations were partially confirmed in the present study. Survival of *A. bifilosa* was high during the middle and at the end of the mesocosm experiment but was somewhat lower at the beginning of the experiment (IV). This pattern cannot be due to nodularin-related toxicity, since nodularin concentrations remained high in the enclosures during the whole experiment.

Tolerance to nodularin may include advantages, e.g. the ability to derive nutrition from the filaments, or from the epifauna or flora associated with the filaments, during mass-occurrences of cyanobacteria. In the present study, *Daphnia*-fed mysid shrimps showed high survival when exposed to toxic cyanobacteria (II). The mysid shrimps either avoided the filaments or fed on them together with *Daphnia*, which, in the latter case, suggests tolerance to nodularin. In any case, cyanobacteria probably have little short and medium-term (days to weeks) effects on mysids, whereas the true long-term (seasonal) effects on, e.g. reproduction could not be assessed in this study.

Effects of cyanobacteria on the heterotrophic community

**Dynamics of decaying blooms**

Several hypotheses have been put forward concerning the fate of decaying cyanobacterial blooms. Grazing and grazing control
have been considered of minor importance (Sellner 1997), due to the characteristics of cyanobacteria discussed above. Cyanobacterial blooms are commonly thought to decay within the water column (Hoppe 1981, Sellner 1997). The cells are buoyant and remain on the surface as long as the gas vesicles remain intact (Horstmann 1975). Sedimentation of both *Aphanizomenon flos-aquae* and *Nodularia spumigena* was concluded to be insignificant in the Baltic Sea and the Gulf of Finland, due to buoyancy and high mineralisation of senescent populations (Heiskanen & Kononen 1994, Heiskanen & Olli 1996, Sellner 1997), whereas sedimentation is considered important by Kankaanpää et al. (2001).

In the present study, *Nodularia* had proceeded well into the decay process by the middle of the 2-week experiment. Decay was indicated by the increasing particulate organic carbon (POC):chlorophyll *a* ratio, the decreasing polyunsaturated fatty acid (PUFA):total fatty acid ratio and decreasing filament length (III). These parameters give an estimate of growth conditions, presence of healthy cells and approximate growth rates (Ahlgren et al. 1992, Granéli et al. 1999 and references therein). A further indication of decay was that total chlorophyll *a* and the two cyanobacterial pigments, echinenone and zeaxanthin, decreased towards the end of the experiment (III).

Hoppe (1981) reported high species diversity of bacteria and microzooplankton within decaying *Nodularia* aggregates, although he did not detect any feeding on the filaments themselves. Bacteria may also be strongly associated with actively growing blooms. Some bacteria are chemotactically attracted to the nitrogen-rich heterocysts (Paerl 1990 and references therein). Bacterial epiphytisation, i.e. attachment, is associated with metabolically active, rather than senescent *N*₂-fixing species (Paerl 1990).

In the present study, the number of filamentous bacteria (rods) increased towards the end of the mesocosm experiment (III). The observed size structure of the bacterial community can be a response to grazing pressure by bacterivores, since longer cells are probably more resistant to grazing than short ones (Jürgens & Güde 1994). Also, the increasing mesozooplankton community probably predated strongly on the ciliates and thus, indirectly diminished the predation pressure on the bacterivores (i.e. heterotrophic flagellates). A similar increase in the number of filamentous bacteria, due to strong grazing pressure, has previously been shown in a reservoir system (Bouvy et al. 2001) and in freshwater enclosures (Jürgens & Güde 1994). Furthermore, Christoffersen et al. (1990) showed that bacterial populations peaked shortly after the collapse of a cyanobacterial bloom, indicating that the bacteria were stimulated by lysis products released from the cyanobacteria (Hansen et al. 1986). In contrast, the abundance of heterotrophic nanoflagellates decreased during a strong toxic *Microcystis* bloom (reviewed by Christoffersen 1996).

Few studies have been performed to investigate the interactions between zooplankton and cyanobacteria in mesocosms. The disadvantages of enclosures increase with decreasing bag size. Different effects including ‘wall effects’ may result in low water circulation and unnatural mixing in the enclosures. In addition, species that thrive on substrates and surfaces may predominate (Burns 1987). However, *in situ* enclosures
can be considered more realistic than any "bottle experiments" performed in the laboratory and are thus appropriate for studying community scale processes. In the present study, the ciliate *Euplotes* sp. increased with time in the enclosures containing cyanobacteria (III). Although the "wall effect" probably affected the abundance of the thigmotactic *Euplotes* sp. positively, it seemingly thrived better among the cyanobacterial filaments than in the control.

We were not able to detect any direct harmful effects attributable to the stable and high concentrations of nodularin on any of the studied organism groups in our short-term experiment (III). The results show that the plankton community can exist in the presence of nodularin.

**Food quality effects on zooplankton egg production**

In the present study, we monitored the fatty acid composition of a decaying cyanobacterial bloom in enclosures (III). The main food quality indicators, fatty acids 20:5\(\omega3\) and 22:6\(\omega3\) (Brett & Müller-Navarra 1997, Müller-Navarra et al. 2000), were not associated with *Nodularia spumigena* in the mesocosm experiment (III). This shows that *N. spumigena* is deficient in highly unsaturated fatty acids (HUFA), which are important for grazers (Jónasdóttir et al. 1995, Müller-Navarra 1995). Nevertheless, in the present study, another fatty acid, 18:3\(\omega3\), was strongly associated with *N. spumigena* (III). 18:3\(\omega3\) is one of the main fatty acids in *Nodularia* sp. and other cyanobacteria, in addition to 16:0 and 18:2\(\omega6\) (Ahlgren et al. 1992, Vargas et al. 1998). All herbivores, and probably also all omnivores, seem to be able to elongate 18:3\(\omega3\) to 20:5\(\omega3\) and 22:6\(\omega3\) (Ackman et al. 1968, Brett & Müller-Navarra 1997, Desvillettes et al. 1997). Therefore, cyanobacteria may provide some elements that are useful or even essential for grazers. However, synthesis of HUFA is costly, thus animals grow best when provided with direct sources of 20:5\(\omega3\) and 22:6\(\omega3\) (Brett & Müller-Navarra 1997).

Ageing cyanobacterial blooms attract numerous ciliates and other microzooplankton, due to bacteria attached to the filaments (Hoppe 1981). They may thus serve as a significant food source for crustaceans, which are able to feed selectively. Ederington et al. (1995), however, showed that an algal diet supported higher egg production than a ciliate diet did in copepods, due to the sterols and PUFAs in the algae. On the other hand, bacterial fatty acids can be transferred from ciliates to copepods and their eggs (Ederington et al. 1995). It has also been shown that protozoans may ‘upgrade’ low quality food by producing long-chain polyunsaturated fatty acids (PUFAs). The growth of copepods in turn improves when upgraded food is consumed (Kleppel et al. 1998, Klein Breteler et al. 1999).

In the present study, we measured egg production of an abundant calanoid copepod, *Acartia bifilosa* (IV), during different phases of a cyanobacterial bloom. In contrast to previous findings, our results showed that both copepod species were able to feed, and *A. bifilosa* even produced eggs, during all phases of a *Nodularia* bloom (IV), though our results did not give full support to the hypothesis of improved food quality of the decaying cyanobacterial bloom. However, the cyanobacteria clearly did not have any
harmful effects on copepod egg production. One reason for the relatively high egg production measured in the mesocosm experiment could be the availability of a diverse food environment. Generalist herbivores perform best when provided with a mixture of several plant species, or when their algal food is occasionally supplemented with protein-rich animal tissue (Sommer 1998, Cruz-Rivera & Hay 2000). In conclusion, our results suggest that a cyanobacterial bloom, and its associated organisms, is a diverse and highly useful food source for the dominant copepods of the northern Baltic Sea, *Acartia bifilosa* and *Eurytemora affinis*.

**Accumulation of toxin in higher trophic levels**

An increase of cyanobacterial toxin concentration with time in the study organism, in comparison to the environment, i.e. by accumulation, has been recorded in different bivalves (Prepas et al. 1997, Williams et al. 1997) and crustacean zooplankton (Thostrup & Christoffersen 1999). Crustacean zooplankton may be an important vector for algal toxins from phytoplankton to planktivorous fish (Maneiro et al. 2000, Tester et al. 2000).

In the present study, we conducted experiments with mysid shrimps and three-spined sticklebacks, *Gasterosteus aculeatus*, in order to detect transfer and potential accumulation of nodularin, produced by *Nodularia spumigena*, to the planktivores, via cyanobacteria-fed copepods. Toxin samples were measured by two methods, in order to achieve more reliable results. The enzyme-linked immunosorbent assay, ELISA (Chu et al. 1990), measures the concentration of liver toxin in the sample, whereas protein phosphatase (PPase) inhibition assay (Ward et al. 1997) measures inhibition of protein phosphatases by liver toxins, reflecting their toxicity (Kukkonen 1999). In the present study, we detected accumulation in one trial, in the experiment using mysid shrimps and measured using ELISA. It is difficult to speculate whether a real accumulation had taken place or not, because the increase was detected only by ELISA and not by the other method, protein phosphatase (PPase) inhibition assay. However, it is difficult to imagine any other mechanism but true accumulation to explain the ELISA result.

The comparison between the two methods showed that the PPase inhibition assay gave higher values than ELISA. There may be several reasons for this. The methods can be classified according to their sensitivity and selectivity. In our experiments, ELISA had a lower detection limit, i.e. was more sensitive in detecting toxin, whereas the PPase inhibition assay gave somewhat higher values. Consequently, the PPase inhibition assay was probably less selective than the ELISA, as was also concluded by Harada et al. (1999). One fact that makes interpretation difficult is that the controls in our experiment were also positive. The reason for this is either that the predators had retained toxins in their tissues from the field, or that there was a bias in the measurements. The percentage of methanol in the toxin samples is a crucial factor, because false positives may arise if the concentration of methanol is too high (Metcalf et al. 2000). The final concentration of methanol in our samples was 10% (Sipiä 2001), which should
not be too high. In conclusion, we were able to detect cyanobacterial toxin in the tissues of both of the planktivores used in the experiments and our results thus suggest that accumulation in higher trophic levels is possible.

**SUMMARY**

Grazing response to filamentous cyanobacteria was measured in three of the studies (I, II, IV). We used different toxic and non-toxic cyanobacterial strains cultured in the laboratory. In study IV, *Acartia bifilosa* and *Eurytemora affinis* fed on toxic cyanobacteria *Nodularia spumigena* during different phases of the bloom. In study I, *Eurytemora affinis* grazed less on toxic *Nodularia spumigena* than on the non-toxic strain; the same was observed both for mysid juveniles and adults: non-toxic cyanobacteria were fed upon more than the toxic strain (II). *Acartia bifilosa* avoided cyanobacteria when provided with cultured, actively growing, toxic and non-toxic strains (I). These results suggest that *Eurytemora affinis* and *Mysis mixta* showed adaptive behaviour by decreasing feeding rates when exposed to the toxic strain.

The aim of the study was to monitor the community development during the decay of toxic filamentous *Nodularia spumigena* (III). We measured organism abundances, chlorophyll *a*, toxin, nutrient, protein, fatty acid and phytoplankton pigment concentrations. The bloom was in its decay stage approximately by the middle of the experiment. Two ciliate species, *Mesodinium rubrum* and *Urotricha* sp. decreased strongly, probably due to predation by the increasing mesozooplankton community, whereas the numbers of filamentous bacteria increased towards the end of the study period. Although nodularin concentrations remained high during the whole experimental period, no direct negative effects were recorded during our short-term experiment. Saturated, monounsaturated and total fatty acids increased, whereas polyunsaturated fatty acids decreased during the experiment, which suggests that the bloom decay was initiated. However, fatty acids that are important to grazers were present during the whole time (III).

The aim of the study was to find out if *Acartia bifilosa* was able to produce eggs during different phases of the toxic *Nodularia spumigena* bloom (IV). The results showed that *A. bifilosa*, incubated in mesocosm water containing cyanobacteria in different phases of the bloom, produced either more, or similar amounts of eggs than in comparison to food of good quality, *Brachionus submarina*. This suggests that *A. bifilosa* was able to utilise ciliates and other heterotrophic organisms, associated with different stages of the bloom, for egg production and that a cyanobacterial bloom has little, if any, negative effects on the copepod community. *A. bifilosa* produced eggs during all phases of the toxic bloom of *N. spumigena* (IV).

The aim of study V was to measure potential accumulation of cyanobacterial toxin in two common planktivores. The mysid shrimp, *Mysis relicta*, and the three-spined stickleback, *Gasterosteus aculeatus*, were provided with cyanobacteria-fed copepods (V) during 10-days time. Both sticklebacks and mysid shrimps are important planktivores and live in habitats that are usually exposed to cyanobacteria during strong
blooms. Samples were measured with two toxin detection methods, enzyme-linked immunosorbent assay (ELISA) and protein phosphatase (PPase) inhibition assay. We detected cyanobacterial toxin in both mysids and fish, whereas no accumulation was observed in sticklebacks or mysid shrimps, except for mysids as measured by ELISA. The results suggest that zooplankton, under certain conditions, may act as a vector for cyanobacterial toxin to higher trophic levels.

CONCLUSIONS

My thesis deals with the effects of cyanobacteria on pelagic and planktonic heterotrophic organisms, including mysid shrimps and three-spined sticklebacks. The main finding was that, generally, we did not record any direct harmful effects of filamentous, cultured strains of toxic and non-toxic cyanobacteria on feeding, survival and reproduction of the study organisms on a short-term basis, in any of the studies. In the grazing experiments, *Eurytemora affinis* and mysid shrimps were able to reduce feeding when exposed to toxic cyanobacteria. This can be interpreted as an adaptive behaviour, because an animal can usually not gain from ingesting toxic food, except in the case of resistance to the toxin.

Survival was high in all experiments where it was recorded, both with direct and indirect exposure to cyanobacteria. In nature, copepods and mysid shrimps are able to avoid dense, mono-species, mass-occurrences of phytoplankton by vertical migration or selective feeding. However, the long-term effects of cyanobacteria on feeding, survival and reproduction of Baltic Sea biota remain to be studied.

Reproduction was measured in one of our studies (IV). *Acartia bifilosa* was able to produce eggs during all different phases of the toxic cyanobacterial bloom. The result shows that the copepod can select nutritious food and reproduce, even though the bloom is dense and toxic. *A. bifilosa* feeds actively on ciliates, which are abundant in decaying cyanobacterial blooms. Copepods may act as a vector for cyanobacterial toxin to planktivores. Results from our laboratory experiment and from a field study on a cruise to the Gulf of Finland, suggest that cyanobacterial toxin is either transferred via copepods to planktivores (V), or to the copepod tissues, or leaves the copepod with faecal pellets (Lehtiniemi et al. submitted). This could have implications for the planktivores, especially fish that may be sensitive to hepatotoxins such as nodularin. In addition, cyanobacterial toxins decay slowly (Kiviranta et al. 1991), which may further increase the potential risks of accumulation in nature.

It must be noted that the effects of cyanobacterial blooms on the experimental organisms may be stronger in nature than in laboratory experiments, due to many factors working simultaneously on the individual in nature. Biotic factors are significant, e.g. predation pressure, competition and parasites, as well as fluctuating abiotic factors, e.g. pH, oxygen, weather conditions, salinity, temperature, light. All these factors are usually either stabilised or excluded in the laboratory. In conclusion, the organisms did not seem to be adversely affected by filamentous cyanobacteria during our short-term trials.
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