SHORT COMMUNICATION

Feeding interactions of the copepods Eurytemora affinis and Acartia bifilosa with the cyanobacteria Nodularia sp.

J. Engström1,2,5, M. Koski1,2, M. Viitasalo1,2, M. Reinikainen2,3, S. Repka4 and K. Sivonen4

1 Department of Ecology and Systematics, Division of Hydrobiology, P.O. Box 17, FIN-00014 University of Helsinki, Finland, 2 Tvärminne Zoological Station, FIN-10900 Hanko, Finland, 3 Department of Animal Ecology, University of Umeå, S-90187 Umeå, Sweden and 4 Department of Applied Chemistry and Microbiology, Division of Microbiology, P.O. Box 56, FIN-00014 University of Helsinki, Finland

1,5 To whom correspondence should be addressed

Abstract. We measured ingestion and clearance rates of two Baltic Sea calanoid copepods, Eurytemora affinis and Acartia bifilosa, on toxic and non-toxic cyanobacteria Nodularia sp. using the isotope technique. Eurytemora affinis fed actively on the non-toxic strain and moderately actively on the toxic strain, whereas A. bifilosa totally avoided feeding on both strains. This suggests that A. bifilosa rejected cyanobacterial filaments due to their nutritional inadequacy or difficult manageability. The different response of E. affinis to the non-toxic and toxic strains, in turn, shows that this copepod species was able to sense the presence of the toxin in cyanobacterial filaments and therefore fed less on the toxic strain. The interaction between A. bifilosa and Nodularia sp. was further examined (with the particle counting method) by measuring the clearance rates of A. bifilosa on edible green flagellates in the presence of cyanobacteria. The presence or concentration of toxic Nodularia sp. did not affect grazing rates of A. bifilosa on Brachiomonas submarina. Since earlier studies have shown that ingestion of Nodularia sp. decreases egg production and increases mortality in E. affinis, we suggest that the occurrence of Nodularia sp. blooms in the Baltic Sea may favour individuals of copepod species capable of selective feeding, such as A. bifilosa.

In the pelagic environment, low quality algal species frequently dominate the phytoplankton community during a bloom event (Richardson, 1997). Low quality may be due to low nutritional value, poor assimilability, difficult handling or toxicity (Infante and Abella, 1985; Gilbert, 1990; Gulati and DeMott, 1997). Thus, pelagic herbivores with different feeding mechanisms and toxin resistances may respond in very different ways to harmful algae (Nielsen et al., 1990; Hansen, 1995). A potential method for revealing why a certain algal species is harmful to a grazer is to compare the response of the grazers to algae with similar morphology but differing in toxicity. However, only a few papers have considered the utilization of non-toxic and toxic algal strains by copepod species (DeMott and Moxter, 1991; Turner et al., 1998; Turriff et al., 1995).

In the Baltic Sea, toxic blooms of the cyanobacteria Nodularia spumigena occur frequently. The toxicity level and factors affecting the formation and decay of N. spumigena blooms are now relatively well known [e.g. (Kononen et al., 1996)], but less is known about their consequence on higher trophic levels. Only Sellner et al. (Sellner et al., 1994, 1996) have shown that grazing of Baltic copepods is
reduced in the presence of cyanobacteria in the Gulf of Finland. Our objective was to investigate experimentally whether two abundant copepods differ in their utilization of toxic and non-toxic *Nodularia* sp. strains. We hypothesized that the calanoid copepods, *Eurytemora affinis* and *Acartia bifilosa*, would respond differently to these strains because they differ in their predominant feeding modes (Jonsson and Tiselius, 1990; Gasparini and Castel, 1997). Further, we tested whether the presence of toxic *Nodularia* sp. would interfere with the feeding of *A. bifilosa* on a good food species (the green flagellate, *Brachiomonas submarina*).

The non-toxic *Nodularia* sp. ‘HKVV’ and nodularin-producing *Nodularia* sp. ‘HEM’ strains were obtained from continuous cultures maintained in the University of Helsinki, Division of Microbiology (Lehtimäki et al., 1994). Both filamentous strains were grown in a modified Z8 medium (Hughes et al., 1958; Kotai, 1972). The length of the filaments was 192 ± 73 µm and 133 ± 41 µm for the non-toxic and the toxic strain, respectively (mean ± s.d., n = 20). The cyanobacterial concentration was determined spectrophotometrically using a calibration curve of extinction versus carbon concentration, which was derived from chemical oxygen demand measurements (Gulati et al., 1991). *Brachiomonas submarina* was obtained from the culture collection of Tvärminne Zoological Station, University of Helsinki, and grown in a modified Erd-Schreiber medium (Hällfors and Hällfors, 1992). The size of the cell is 7.5 ± 0.5 µm (Koski et al., 1999b). The concentration of *B. submarina* was measured with an ELZONE particle counter (Particle Data Inc.). All cultures were grown at 18°C in a 16 h light:8 h dark cycle and supplied with air continuously. The light intensity in the culture room was 37 µE m⁻² s⁻¹. Previous egg production experiments had shown that *B. submarina* is a moderately good food for copepods; *A. bifilosa*, for example, produces 21.6 ± 2.0 eggs female⁻¹ day⁻¹ at a *B. submarina* concentration of 15 µg Chl a l⁻¹ (Sellner et al., 1996).

The toxin concentration of the two *Nodularia* sp. strains was analysed twice during the experimental period. Cyanobacterial culture (30 ml) was freeze-dried and stored at −20°C until analysis. The material was weighed (5–10 mg) and the toxins were extracted in water by sonicating (Braun Labsonic U) for 5 min. The remaining cell material was removed by filtering with GF52 glass-fibre filters (Schleicher & Schuell). The supernatant fluid was concentrated on Octyldecyl Silane cartridges (Bond-Elut C18:Varian). The cartridges were washed with 15 ml 20% methanol prior to elution with 7 ml 90% methanol. The samples were then dried in an airstream and dissolved in 1 ml 20% methanol. Nodularin concentration was determined with a Hewlett-Packard HP1090 liquid chromatograph. The toxic ‘HEM’ strain contained 0.58 ± 0.566 µg nodularin (mg DW)⁻¹ (mean ± s.d., n = 2), whereas the ‘HKVV’ strain was non-toxic (n = 2).

Adults of *A. bifilosa* and *E. affinis* [0.7 mm in prosome length (Viitasalo et al., 1995)] were collected from an open archipelago area (Storfjärden) near the Hanko Peninsula, southwest coast of Finland. The samples were taken with a 200 µm mesh-sized Hensen-type plankton net, with three to five hauls from 25 m depth to the surface. The copepods were carefully poured into 30 l containers with water collected from near the bottom, and transported to the laboratory. A adult females were picked and placed into 250 ml Erlenmeyer flasks with filtered
seawater to adapt to the experimental temperature for ~24 h and to assure that the guts were empty at the start of the experiment.

Grazing on cyanobacteria was measured with the ¹⁴C-method (Steemann-Nielsen, 1952). Approximately 100 ml of each Nodularia sp. strain were labelled with 20 µCi ¹⁴C-NaHCO₃ two to four days before the experiment, depending on the culture density. Flasks were then placed at 18°C in a 16 h light:8 h dark cycle. On the day of experiment, the non-toxic strain (benthic) was centrifuged at 4000 g, and the toxic strain (containing gas-vacuoles) at 7000 g, for 5 min at 18°C. The supernatant fluid was discarded and the cyanobacteria were added, at 400 µg C l⁻¹, in 1.18 l bottles filled with 0.2 µm-filtered seawater. A approximately 30 female copepods were added to each bottle and incubated in a plankton wheel (~1 rev min⁻¹) for 15 min under dim light at 13°C. There were 12 replicates and four controls in the experiment. Similarly prepared control bottles were not incubated but processed immediately; any radioactivity in these controls originated from labelled algae stuck on the surface of the copepod. The experiments with A.bifilosa and E.affinis were conducted on different days, due to lack of individuals of both species simultaneously in the water.

After the incubation, 10 ml water from every bottle was filtered twice through Whatman GF/F glass fibre filters in order to separate activity in the food algae and the filtrate. Copepods were sieved onto a 200 µm net and transferred onto Petri dishes, after which they were narcotized with a small amount of carbonated water. Copepods were rinsed three times in filtered seawater, counted, and transferred onto GF/F filters. All filters were placed in separate scintillation vials and 100 µl of 0.1 N HCl were added. Vials were placed uncapped for 1 h in a ventilation cupboard, and 250 µl of tissue solubilizer (ICN Pharm.) were added to break down the chitinous carapaces. After 4 h, 10 ml liquid scintillation cocktail (Ecolite, ICN Pharm.) were added. Vials were shaken thoroughly and cleaned before measurement in a liquid scintillation counter (Wallac 1219 Rack Beta) the next day. Clearance rates on Nodularia sp. were calculated according to Lampert and Taylor (Lampert and Taylor, 1985).

The second experiment was carried out to determine whether cyanobacteria interfere with A.bifilosa grazing on B.submarina. The copepods were provided with the green algae as a single diet and in mixtures with toxic Nodularia sp. (at 50, 100, 200 and 400 µg C l⁻¹). The concentration of B.submarina was 250 µg C l⁻¹ in all treatments. Thirty adult female copepods were placed into each bottle and incubated for 24 h in a plankton wheel at 1 rev min⁻¹ in dim light at 13°C. There were eight replicates and six controls in the experiment. Before and after incubation, 100 ml samples were taken from every bottle. Samples were sieved through a 50 µm mesh-sized net to remove Nodularia sp. filaments, preserved with 2 ml acid Lugol’s solution, and B.submarina concentrations measured by particle counting. Clearance rates on B.submarina were calculated according to Frost (Frost, 1972).

Eurytemora affinis fed actively on both the non-toxic and the toxic Nodularia sp. strains in our experiments (Figure 1). Ingestion of the non-toxic Nodularia sp. was also confirmed by the strong bluish coloration of E.affinis guts after separate 35 h incubations. With the toxic strain, a similar increase in gut coloration was
not obvious. In contrast, *A*. *bifilosa* did not graze much on either of the two strains; clearance and ingestion rates of *A*. *bifilosa* on both toxic and non-toxic *Nodularia* sp. were close to zero.

There were strong significant differences between the treatments of *E*. *affinis* and *A*. *bifilosa* exposed to toxic and non-toxic cyanobacteria (Scheirer-Ray-Hare two-way ANOVA, $H_{1,44} = 35.3$, $P < 0.00001$). *Eurytemora affinis* grazed significantly faster on non-toxic and toxic *Nodularia* sp. than did *A*. *bifilosa* on the nontoxic or the toxic strain (Tukey HSD, $P < 0.001$). There was also a significant difference between clearance rates of *E*. *affinis* on the non-toxic *Nodularia* sp. strain and the toxic strain (Tukey HSD, $P < 0.001$).

Ingestion rates of *B*. *submarina* were $1429 \pm 1503$ cells ind.$^{-1}$ h$^{-1}$ with *B*. *submarina* alone, and varied from $3376 \pm 1036$ (50 µg C l$^{-1}$ *Nodularia* sp. added) to $4124 \pm 3029$ cells ind.$^{-1}$ h$^{-1}$ (400 µg C l$^{-1}$ *Nodularia* sp. added) in mixtures. The concentration of toxic *Nodularia* sp. did not significantly affect clearance rates of *A*. *bifilosa* grazing on *B*. *submarina* (Kruskal–Wallis one-way ANOVA, $H_{4,39} = 2.5$, $P > 0.05$) (Figure 2).

*Eurytemora affinis* and *A*. *bifilosa* showed different feeding rates in our experiments; *E*. *affinis* grazed most actively on the non-toxic *Nodularia* sp. and to some extent on the toxic strain, whereas *A*. *bifilosa* totally avoided feeding on the two strains. This difference may be due to the different feeding behaviours of the copepods. Both species are omnivorous (*Stoecker* and *Egloff*, 1987; *Tiselius* and *Jonsson*, 1990; *Revis* et al., 1991), but *Acartia* spp. are especially adapted to raptorial feeding on larger moving prey (*Jonsson* and *Tiselius*, 1990; *Kjørboe* et al., 1996). In the Gironde estuary in France, *E*. *affinis* maintained ingestion rates, whereas *A*. *bifilosa* reduced ingestion rates, when low quality food became abundant (*Gasparini* and *Castel*, 1997). Also, in a study by *Schmidt* and *Jónasdóttir* (*Schmidt* and *Jónasdóttir*, 1997) *A*. *tonsia* did not ingest *Nodularia spumigena*, which was thought to be a reaction to the taste of cyanobacteria, or due to filament
handling difficulties. The fact that E. affinis grazed more actively on the non-toxic strain than on the toxic one suggests that E. affinis was able to sense the toxin in the algal strain and adjust its ingestion accordingly. Acartia bifilosa, in contrast, did not graze on either of the strains, which suggests that it mainly selected against Nodularia sp. filaments due to their bad taste or difficult manageability.

In our second experiment, in which A. bifilosa was provided with edible algae in mixtures with different amounts of cyanobacteria, clearance rates on B. submarina did not differ with and without Nodularia sp. We therefore suggest that the cyanobacteria did not significantly interfere with A. bifilosa feeding on B. submarina.

We hypothesize that in a strong bloom situation where other food items are scarce, A. bifilosa might starve [e.g. (Sellner et al., 1994)] while E. affinis would continue feeding at a low rate. On the other hand, the population response of the copepods depends on the longer-term effects of cyanobacteria on their egg production and mortality. Koski et al. found increased mortality, and declining egg production and hatching success in E. affinis when fed toxic Nodularia sp. for 3–5 days (Koski et al., 1999a). Furthermore, Repka et al. (Repka et al., 1998) have shown that decaying cyanobacterial filaments are more nutritious than live filaments for cladocerans, most probably because of attached bacteria. This suggests that despite the better initial food gain of E. affinis, the longer term feeding success and survival of A. bifilosa may be better, especially if this copepod species is able to feed on organisms associated with the late phase of the bloom.

Acknowledgements

We wish to thank U. Sjölund for laboratory analyses, S. and G. Hällfors for algal cultures, R. Lignell and H. Kuosa for advice in isotope techniques and K. Lindström for statistical advice. We further thank two anonymous referees for
constructive comments. J.E. and M.K. were financed by Maj and Tor Nessling Foundation and Walter and Andrée de Nottbeck Foundation, M.V., M.R. and K.S. by the Academy of Finland and S.R. by the EU research programme 'Preserving the Ecosystem' under BASIC project #ENV4-CT97-0571. This is ELOISE publication no. 123.

References


J. Engström et al.


Received on October 11, 1999; accepted on January 6, 2000