# Influence of *Acartia* cf. *bifilosa* (Copepoda) on morphology and toxicity of *Nodularia spumigena* (Cyanophyceae)

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## A B S T R A C T

*Nodularia spumigena* was exposed directly and indirectly (grazer cages) to increasing densities of *Acartia* cf. *bifilosa* to investigate if the presence of copepods influenced the morphology and/or the toxicity of the cyanobacterium. Monocultures with only *N. spumigena* and mixed cultures, containing *N. spumigena* and the non-toxic *Dunaliella tertiolecta*, were included in each experiment. Following 6 days of incubation, the morphology and toxicity in grazer treatments were compared with grazer-free controls. We observed no effects of *A.* cf. *bifilosa* on either morphology or toxicity of *N. spumigena*. The lack of grazer induced nodularin production and morphological alterations suggest that these two potential defense strategies either has evolved as constitutive defenses or never evolved as grazer defenses. The mortality of copepods was higher in the monoculture than in the mixed culture treatments. Gut content observations indicated a low level of grazing in monoculture treatments and a higher level of grazing in mixed culture treatments. This higher level of grazing most likely occurred on the alternative food *D. tertiolecta*. Given the indications of low grazing and the concentrations of dissolved nodularin observed, we postulate that the higher mortality was not related to toxic effects, but to starvation. This in turn may have resulted from bad taste, production of unknown grazer deterrents or morphological constraints; although the size of the filaments would not have imposed an absolute limit for ingestion by *A.* cf. *bifilosa*. The higher copepod mortality observed on monocultures of *N. spumigena* may contribute to the success and maintenance of *N. spumigena* blooms.

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## 1. Introduction

Blooms of the cyanobacterium *Nodularia spumigena* (Mertens) occur regularly in the Baltic Sea (Sivonen et al., 1989; Finni et al., 2001). Summer blooms are favored by the onset of high irradiance and warm and calm weather, leading to a stratified water column. Nitrogen depletion, which occurs after the spring bloom, also triggers the growth of heterocystous cyanobacteria, such as *N. spumigena* (Granéli et al., 1990; Pliński and Jóźwiak, 1999).

*N. spumigena* is harmful, producing the toxin nodularin which may cause liver damage if consumed by animals via inhibition of protein phosphatases 1 and 2A (Honkanen et al., 1991). Toxic blooms of *N. spumigena* may result in domestic animal poisoning (Nehring, 1993), harm to fisheries (Karjalainen et al., 2005), and may pose a health risk to humans and interfere with the recreational use of waters (Kuiper-Goodman et al., 1999).

The influence of abiotic factors such as temperature, light, salinity and phosphorus on nodularin concentration is well documented (e.g. Lehtimäki et al., 1997; Hobson and Fallowfield, 2003); generally the highest nodularin concentrations were found with conditions that promoted growth. Orr and Jones (1998) concluded that there is a direct linear relationship between cell division and production rates of cyanotoxins regardless of which environmental factor that is limiting growth. However, the toxin content of *N. spumigena* has been shown to accumulate in the stationary phase (Blackburn et al., 1996). Stolte et al. (2002) also observed an increase in nodularin content under conditions of growth reduction due to phosphate limitation.

Several studies have been carried out regarding the interaction between copepods and *N. spumigena*, and most of these have studied the effects of *N. spumigena* on the copepods. While some authors report high grazing on *N. spumigena* with no apparent harmful effects (Koski et al., 2002), other authors find that there are detrimental effects on copepod feeding and/or fecundity (Sellner et al., 1996; Koski et al., 1999; Schmidt and Jónasdóttir, 1997; Engström et al., 2000; Kozlowsky-Suzuki et al., 2003), and that even lethal effects may occur (Sellner et al., 1994). It is vigorously debated concerning which properties of *N. spumigena*

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are responsible for such negative effects. These could be toxicity, morphological constraints, low nutritional value and/or production of unknown grazer deterrents (reviewed in Lampert, 1987; Tillmans et al., 2008). The differing and contradictory responses of copepods to *N. spumigena* could be due to variation in one or several of these properties but also to development of both physiological and behavioral adaptations of copepods, to the various properties of cyanobacteria. Such adaptations may include feeding avoidance, food selectivity (Schmidt and Jónas-dóttir, 1997; DeMott and Moxter, 1991), and toxin tolerance (e.g. DeMott et al., 1991; Reinikainen et al., 2002; Kozlowsky-Suzuki et al., 2003).

The ecological role of many toxins still remains to be clarified, and they might have evolved for reasons other than grazer deterrence. For example, toxins might be precursors for subcellular organelles, nucleic acid synthesis, cell-wall degradation products and nitrogen storage (Turner and Tester, 1997 and references therein). Toxins could also serve to inhibit the growth of competing phytoplankton species, enabling toxic species to become dominant (Rengefors and Legrand, 2001; Granéli and Hansen, 2006). However, nodularin does not seem to be involved in the allelopathic effects of *N. spumigena* although some other products are (Suikkanen et al., 2004, 2006).

In addition to being toxic, *N. spumigena* shows a large variability in its morphology (Congestrini et al., 2003), with curved, coiled or straight filaments (Mazur-Marzec et al., 2006a). The length of the filaments also varies considerably; from a dozen µm to cm length filaments (Sopanen et al., 2009). This variation in length may affect the feeding behavior of predators. For instance, the copepod *Eurytemora affinis* preferred cultured small filaments instead of natural *N. spumigena*, characterized by larger filaments (Sopanen et al., 2009).

Several potential defense mechanisms of phytoplankton may be affected by infochemicals released during grazing activities (reviewed in Van Donk et al., 2011). The recognition of such infochemicals can save the prey costs by only expressing the defense when predators are present (Agrawal, 1998). Induced defenses would therefore be favored over constitutive ones in environments with variable predatory pressure (Tollrian and Harvell, 1999). Some studies have now observed changes in both the morphology (Hessen and Van Donk, 1993; Long et al., 2007; Lundgren and Granéli, 2010) and the toxin production (Jang et al., 2003; Selander et al., 2006; Bergqvist et al., 2008) of various phytoplankton. Such responses may reduce vulnerability to grazing (Hessen and Van Donk, 1993; Selander et al., 2006).

The only study that has studied grazer-induced defense in terms of toxin production in *N. spumigena* is that of Gorokhova and Engström-Öst (2009), in which a decrease in cell-bound nodularin was observed when *N. spumigena* was directly exposed to the copepod *Eurytemora affinis*. To our knowledge, no studies have yet examined if the morphology of *N. spumigena* might be altered in response to grazers.

The purpose of the present study was to investigate if two potential defense mechanisms of *N. spumigena* (morphology and toxin production) could be affected by direct exposure (via for instance selective grazing) as copepods can selectively feed on cells with lower toxin content (c.f. Teegarden, 1999) and/or indirect exposure (via infochemicals) to the copepod *A.* cf. *bifilosa*.

## 2. Materials and methods

This study consisted of two separate experiments to evaluate the influence of grazing copepods on toxin production and filament length of *N. spumigena*: the "*direct exposure experiment*" and the "*indirect exposure experiment*". This experimental design makes it possible to elucidate whether any differences in toxin production

and/or filament length or coiling are due to a direct grazing effect, for instance selective grazing, or if infochemicals released during grazing activities could play a role in such possible responses.

As grazers we chose *A.* cf. *bifilosa* which belongs to a cosmopolitan calanoid copepod genus. *Acartia bifilosa* are also common in the Baltic Sea where they occur together with *N. spumigena* (Sellner et al., 1994).

### 2.1. Organisms and culture conditions

The non-axenic toxic strain of *N. spumigena* (KAC 13) and the chlorophyte *Dunaliella tertiolecta* (Butcher), CCMP 1320, were obtained from the Kalmar Algal Collection, Linnaeus University, Kalmar, Sweden. The *N. spumigena* KAC 13 strain was chosen because it is characterized by short, straight filaments and we wanted to examine whether the presence of *A.* cf. *bifilosa* would result in the appearance of longer and/or more coiled filaments. Although *D. tertiolecta* is nutritionally inadequate (Klein-Breteler et al., 1999), it was chosen as a non-toxic food alternative due to culture availability.

For each experiment, two batch cultures of *N. spumigena* were grown in 10 L Schott–Duran flasks with modified F/2-Si medium (Guillard, 1975). Batch cultures of *D. tertiolecta* were grown in duplicate in 4 L glass culture bottles, also using modified F/2-Si medium. The medium used to grow and maintain the cultures was prepared with filtered (Munktell glass fiber filter, pore size: 1.2 µm) and autoclaved 1 year aged Baltic Sea water. The trace metal solution was adjusted to the L1 media recipe (Guillard and Hargraves, 1993).

The growth of all cultures was monitored by measurements of chlorophyll *a* (Chl *a*) concentrations in ethanol extracts according to Jespersen and Christoffersen (1987). All cultures were grown at 16 °C, with a light intensity of 100 µmol photons $m^{-2}s^{-1}$ in a light:dark regime of 16:8 h. In addition, the *N. spumigena* cultures were supplied with sterile air (Sartorius Minisart® filters, pore size: 0.2 µm) to facilitate mixing. Cultures in their mid-exponential phase were used for all experiments.

Zooplankton was collected with a 45 µm mesh-size plankton nylon net in the Kalmar Sound (Baltic Sea) in August 2010 for the indirect exposure experiment and in September 2010 for the direct exposure experiment. Adult *A.* cf. *bifilosa* individuals were picked out from these samples with a wide-mouth glass Pasteur pipette and transferred to a 100 L glass aquarium containing 25 L filtered and autoclaved Baltic Sea water. The copepods were kept at 16 °C in dim light and daily fed a surplus concentration of *D. tertiolecta* for 7 days before the start of the experiments. Prior to each experiment, adult females of *A.* cf. *bifilosa* were placed in filtered seawater for 12 h to ensure that their guts were empty.

### 2.2. Experimental set-up

The direct and indirect experiments were conducted at the same temperature and light conditions as the phytoplankton cultures were grown. Each experiment consisted of 2 monoculture grazer treatments, with increasing densities of *A.* cf. *bifilosa* and with *N. spumigena* as the sole phytoplankton species. In addition, we had 2 mixed culture grazer treatments in each experiment, again with increasing densities of *A.* cf. *bifilosa*, but in which both *N. spumigena* and *D. tertiolecta* were offered as potential food sources. For each experiment, 2 controls were set up in the same manner as the grazer treatments, but without grazer additions (Figs. 1 and 2). Hereafter, when discussing the two grazer treatments and control together within each diet offered, the monoculture grazer treatments and the monoculture control will be referred to as monocultures while the mixed