Harmful Algae 10 (2010) 30–38

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Production of the cyanotoxin nodularin—A multifactorial approach

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ARTICLE INFO

Article history:
Received 9 October 2009
Received in revised form 7 June 2010
Accepted 7 June 2010

Keywords:
Aphanizomenon sp.
Baltic Sea
Nodularia spumigena
Nitrogen
Phosphorus
UV-radiation

ABSTRACT

Summer blooms in the Baltic Sea are dominated by the diazotrophic cyanobacteria *Nodularia spumigena* and *Aphanizomenon* sp. During the blooms, *N. spumigena* is concentrated to the water surface and exposed to high irradiances of both photosynthetic active radiation (PAR, 400–700 nm) and ultraviolet radiation (UVR, 280–400 nm), in addition, this organism is exposed to seasonal changes in nutrient conditions. *N. spumigena* produces nodularin, a hepatotoxin lethal to wild and domestic animals. It has been suggested that the accumulation of nodularin within the cell and the release from the cell are affected by different environmental factors. One laboratory experiment and two outdoor experiments were performed to investigate the interaction of two radiation treatments, PAR and PAR + UV-A + UV-B (PAB); three nutrient treatments, nutrient replete (NP), nitrogen limited (–N), and phosphorus limited (–P) and the presence and absence of *Aphanizomenon* sp. on intracellular as well as extracellular nodularin concentration in *N. spumigena*. In this study, we hypothesised that the interaction of ambient radiation, nutrient limitation, and the presence or absence of *Aphanizomenon* sp. would affect the accumulation and release of nodularin. We further hypothesised that the presence of *Aphanizomenon* sp. would increase the production and release of nodularin and that this increase would have a negative effect on the specific growth rate of this co-existing species. Significant interaction effects were found between the factors investigated. In all three experiments, the lowest intracellular nodularin concentrations were found under phosphorus limitation. The highest intra- and extracellular nodularin concentrations were observed under nitrogen limitation when shielded from UVR. In our opinion, further increase of nitrogen removal in e.g. sewage treatment, should consider a possible increased toxicity of the *N. spumigena* blooms. The presence of *N. spumigena* had no significant effect on the specific growth rate of *Aphanizomenon* sp. under different radiation and nutrient treatments. Thus, we conclude that although nodularin accumulation and release were dependent on different environmental conditions, it did not affect the co-existing species *Aphanizomenon* sp.

1. Introduction

Cyanobacterial blooms in the Baltic Sea are a natural phenomenon that has occurred for a long time but their frequency and intensity have increased during recent decades (Bianchi et al., 2000; Finni et al., 2001). There is a long-standing debate whether the primary production in the Baltic Sea is limited by dissolved inorganic nitrogen (DIN) or dissolved inorganic phosphorus (DIP), but the most limiting nutrient vary between area and season (Moisander et al., 2003; Rahm and Danielsson, 2007). For example, periods with a molar DIN:DIP of 50, well above the Redfield ratio of 16, suggests phosphorus limitation, but occasional phosphorus release from oxygen depleted sediments reduce this ratio and leads to nitrogen limitation (Elmgren, 2001; Pitkänen and Tamminen, 1995). The diatom spring bloom remove nitrogen from the surface water but leave sufficient phosphorus, resulting in a low DIN:DIP. A reduction in the ratio gives nitrogen-fixing cyanobacteria a competitive advantage over other primary producers during the following summer bloom (Rydin et al., 2002; Kangro et al., 2007; Rolff et al., 2007).

Diazotrophic cyanobacterial blooms in the Baltic Sea are dominated by *N. spumigena* and *Aphanizomenon* sp., but different niches for the two species have been proposed (Niemistö et al., 1989; Kononen et al., 1996; Lehtimäki et al., 1997; Vintala and El-Shehawy, 2007). For *N. spumigena*, it has been suggested that due to this species' ability to fix molecular nitrogen, tolerate phosphorus starvation and to store phosphorus, *N. spumigena* is better adapted to the environmental conditions prevailing in the

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doi:10.1016/j.hal.2010.06.001

surface waters during summer (Lehtimäki et al., 1997; Staal et al., 2003; Degerholm et al., 2006). Moreover, *N. spumigena* can utilize dissolved organic matter as a nutrient source (Panosso and Granéli, 2000; Põder et al., 2003) and is tolerant to wide ranges of variations in temperature (7–30 °C) and salinity (0–35 PSU) (Lehtimäki et al., 1994, 1997; Mazur-Marzec et al., 2005). It has been observed that *Aphanizomenon flos-aquae* are better adapted to areas and periods of hydrodynamic activity with weaker stratification, and have a deeper biomass maximum than *N. spumigena* (Niemistö et al., 1989; Vahtera et al., 2005). During the late summer bloom, *N. spumigena* cells are often concentrated to the upper water layers where they are exposed to high irradiances of both photosynthetic active radiation (PAR, 400–700 nm) and ultraviolet radiation (UVR, 280–400 nm). High PAR and UVR are likely to favour phytoplankton with photoprotective strategies, such as the production of mycosporine-like amino acids (MAAs) in *N. spumigena* (Wulff et al., 2007; Mohlin and Wulff, 2009). Because MAAs contain nitrogen, nitrogen deficiency might lead to a decreased production, thereby giving nitrogen-fixing cyanobacteria like *N. spumigena* an additional competitive advantage over other non-nitrogen-fixing and MAA-producing phytoplankton.

N. spumigena is capable of producing significant amounts of nodularin, a pentapeptide hepatotoxin that acts as a tumour promoter and have been reported to harm wild and domestic animals (Nehring, 1993). Fish kill in Gulf of Finland in 1999 were suggested to be linked to the *N. spumigena* bloom that occurred during the same period (Kankaanpaa et al., 2001). Changes in nodularin concentrations as a response to various environmental factors including temperature, salinity, radiation and nutrient concentrations have been studied in laboratory experiments (Lehtimäki et al., 1994; Granéli et al., 1998; Repka et al., 2001; Mazur-Marzec et al., 2005). It has been suggested that cyanobacterial toxins accumulate within the cells and are only passively released into the surrounding water due to cell lyses (Heresztyn and Nicholson, 1997). On the other hand, Hobson and Fallowfield (2003) predicted that high temperature and high irradiances could increase an active exudation of nodularin during natural blooms. It has been suggested that the release of hepatotoxin from Baltic cyanobacteria may have an allelopathic effect on other organisms (Sellner, 1997; Engström-Öst et al., 2002). In studies by Suikkanen et al. (2004, 2005), the results indicated that the release of cyanobacterial toxins may play an ecological role in the interspecific competition via stimulating the abundance of the same or other cyanobacterial species in the community rather than inhibiting the abundance of competitors to cyanobacteria.

To improve our understanding of factors controlling production and release of the toxin nodularin, we performed one laboratory experiment and two outdoor experiments to test the interactive effects between radiation, nutrient limitation, and species composition on the accumulation and release of nodularin. In addition, we hypothesized that the presence of *N. spumigena* would have an allelopathic effect on the specific growth rate of the co-existing *Aphanizomenon* sp.

2. Material and methods

2.1. Culture material

The cyanobacteria *N. spumigena* Mertens (KAC71) and *Aphanizomenon* sp. Morren ex Bornet et Flahault (KAC 61) isolated from the Baltic Sea were obtained from the Kalmar Algal Collection (KAC), Kalmar University, Sweden. The stock culture was inoculated in f/2 medium (Guillard, 1975) in several 500 ml NUNC bottles, and with continuous addition of f/2 to obtain enough biomass for the different experiments performed. Cultures were maintained inside a temperature-controlled room under 18 °C and 16:8 h light:dark period with 75 μmol photons m^{-2} s^{-1} of PAR.

2.2. Experimental design

2.2.1. Radiation conditions

Artificial radiation in the laboratory (Experiment A) was provided by daylight Osram L36W/72-965 Biolux and Philips ultraviolet-B TL 20W/12 RSF 20T 12/UV-B. The quartz bottles (50 ml) were covered with a 395-nm cut-off filter foil (Ultraphan URUV, Digefra, Munich, Germany) to eliminate ultraviolet-A radiation (UV-A, 320–400 nm) and ultraviolet-B radiation (UV-B, 280–320 nm) and a 295-nm cut-off filter (Ultraphan UBT, Digefra, Munich, Germany) to allow transmission of wavelengths >295 nm. For spectral properties, see Mohlin and Wulff (2009). For the outdoor experiments (Experiments B and C), aquaria were covered with the same cut-off filter foils to obtain ambient solar radiation treatment consisting of PAR (>395 nm) and PAR + UV-A + UV-B (PAB) (>295 nm).

Artificial PAR was measured with a PMA2100 radiometer equipped with a PAR sensor PMA2132 (Solar Light, Philadelphia, USA) and artificial UVR was measured with a PMA2100 radiometer equipped with UV-A sensor PMA2110 and UV-B sensor PMA2106 (Solar Light, Philadelphia, USA). During Experiment B, ambient PAR and UV-A (air) were logged with a PMA2100 radiometer equipped with a PAR sensor PMA2132 and a UV-A sensor PMA2110 (Solar Light, Philadelphia, USA). Ambient UV-B measurements were interrupted due to a malfunctioning sensor (PMA2106). For Experiment C, ambient PAR was measured by a cosine quantum sensor connected to a LICOR data logger (LI-1400, LICOR Biosciences, Lincoln, Nebraska, USA). Ambient UV-A was logged as for Experiment B. For both outdoor experiments (Experiments B and C), PAR and CIE-weighted UVR were received from the Swedish Meteorological and Hydrological Institute (SMHI, STRÅNG data). In addition, PAR inside the aquaria was measured using a submersible spherical sensor (QSL-2100, Biospherical Instrument Inc. San Diego, USA). Radiation measured in the laboratory (Experiment A) is shown in Table 1 and ambient radiation for the whole experimental period in outdoor experiments is shown in Fig. 1a, b, c, and d. During the 2006 outdoor experimental period (Experiment B), the weather was stable with clear sky (Fig. 1a). During the 2007 experimental period (Experiment C), the weather was more variable with a mixture of sunny, cloudy and rainy days (Fig. 1c). In field, PAR was nearly 10 times higher compared to artificial PAR in the laboratory (Table 1, Fig. 1a and c). Ambient UVR was 8 times and 2 times higher compared to the UV-A and UV-B radiation in the laboratory, respectively (Table 1, Fig. 1b and d).

2.2.2. Nutrient treatment and analysis

The f/2 medium was prepared from filtered (GF/F) and heat-sterilized natural seawater (salinity 32) mixed with Milli-Q water to obtain salinity 7. The different nutrient treatments were established: f/2 medium (NP), f/2 medium without NO_3^- (–N) and f/2 medium without PO_4^{3-} (–P) for respective experiments (Table 1). To mimic a gradual decrease in nutrient concentrations and to prevent the cells to reach stationary growth phase, growth medium corresponding to NP, –N and –P treatments were added semi-continuously (approximately every second day) during the experiments. Nutrient limitation in the medium was based on the Redfield ratio constant (DIN:DIP = 16:1). With the semi-continuous dilution, we slowly decreased the inorganic nutrient concentrations. 20 ml cell suspensions from each aquarium were taken throughout the experiments when the cell suspension were diluted (semi-continuous culturing) and filtered through 0.45 μm syringe filters for analysis of inorganic nitrate and phosphate.