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Release of available nitrogen from river-discharged dissolved organic matter by heterotrophic bacteria associated with the cyanobacterium *Microcystis aeruginosa*

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Abstract. The use of riverine dissolved organic matter by the heterotrophic bacteria associated with a culture of the cyanobacterium *Microcystis aeruginosa* and release of simple nitrogen compounds were studied in an experimental series. Bacteria reduced the bulk of dissolved organic nitrogen (DON) by half, but when associated with *M. aeruginosa*, DON was excreted and its concentration rose by 13%. During the stationary growth phase bacteria released ammonium, doubling the concentration of ammonia as well as of nitrates. Bacteria associated with *M. aeruginosa* consumed riverine DON and joined the ammonification and nitrification process, supplying cyanobacteria with simple nitrogen compounds.

Key words: *Microcystis aeruginosa*, heterotrophic bacteria, DOM, DON, nitrates, ammonia.

INTRODUCTION

The cyanobacterium *Microcystis aeruginosa* is known to thrive mostly in eutrophic fresh waters. However, it is also frequently observed in brackish coastal waters and embayments (Kononen & Sellner, 1995). High biomass concentrations of *M. aeruginosa* occur also in estuarine areas transported there via rivers. In the coastal areas of the Gulf of Riga maximum growth of *M. aeruginosa* continues from late June till September. Although generally the development of *M. aeruginosa* proceeds sporadically, it often initiates blooms in coastal zones (Balode & Purina, 1996; Seppälä & Balode, 1999), including the Gulf of Riga where the culture of the cyanobacterium *Microcystis aeruginosa* (MAGR-2) was isolated. High abundances of *M. aeruginosa* are related to high nutrient concentrations. Studies on this species have been intensified in the last decades mainly due to the discovery of potent hepatotoxic heptapeptides, called microcystins, shown to be detrimental to wild and domestic animals and humans (Lambert et al., 1994; Van Dolah, 2000; Ojaveer et al., 2003).

Wetland-derived dissolved organic matter (DOM) may be an important nitrogen source for coastal ecosystems in summertime when concentrations of inorganic nitrogen are low and the bioavailability of dissolved organic nitrogen (DON) is high (Stepanauskas et al., 1999). For instance, the cyanobacterium *Nodularia spumigena* reaches the highest cell density when cultured in the presence of DOM >1000 D originating from a river, and it was concluded that DOM could substitute dissolved inorganic nitrogen (DIN) as a nutrient source (Panosso & Granéli, 2000). Although it is verified that the compounds of DON sustain the growth of cyanobacteria, including *Microcystis aeruginosa* (Purvina et al., 2008), heterotrophic bacteria are the primary contributors in the mineralization of organic matter. Therefore probably one of the main pathways for DOM utilization by phytoplankton in aquatic ecosystems is bacterially mediated enzymatic breakdown resulting in release of inorganic compounds (Berman et al., 1999; Grossart, 1999). According to investigations of Worm & Søndergaard (1998), *Microcystis* colonies are bacterial incubators for the surrounding water as well as the 'hotspot' for bacterial activity. Because of the particulate nature of *Microcystis* and the influence exerted on the physico-chemical environment of colonies by products of its metabolism at the micro-scale, bacteria associated with *Microcystis* colonies live in a microhabitat distinct from the surrounding water. This indicates close interaction between cyanobacteria and bacteria.

A number of literature sources contain information regarding the consumption of allochtonic organic matter by marine bacteria (Søndergaard & Milddelboe, 1995; Young et al., 2005), the usage of organic matter produced or excreted by phytoplankton (Lignell, 1990; Baines & Pace, 1991; Obernosterer & Herndl, 1995), as well as the degradation of toxins produced by *M. aeruginosa* and other cyanobacteria (Jones et al., 1994; Christoffersen et al., 2002; Bourne et al., 2006). Less is known on how associated heterotrophic bacteria contribute to the biodegradation of allochtonic DOM brought by rivers and whether they supply cyanobacteria with these compounds. Therefore, we performed an experiment to verify the hypothesis that bacteria associated with *M. aeruginosa* are involved in the process of allochtonic DOM biodegradation and production of bioavailable inorganic N compounds. In this study we report the metabolic pathway of DON, ammonia, nitrate, and nitrite in experimental treatments with the cyanobacterium *Microcystis aeruginosa*, associated bacteria, and land-derived DOM as the only source of nitrogen and phosphorus.

MATERIALS AND METHODS

Cultures and experimental design

The experiment was carried out in the subsequent series: (1) *Microcystis aeruginosa* culture (Control), (2) *M. aeruginosa* culture + DOM (AB + DOM), and (3) bacterial filtrate + DOM (B + DOM). Cultures were grown in filter-sterilized (0.22 μm) seawater distributed (4 L) into 5-litre polycarbonate pre-autoclaved bottles. The experiment lasted for 15 days. The numbers of bacteria and cyanobacteria,

bacterial production, and dissolved and particulate nutrient concentrations were monitored according to the growth curve of *M. aeruginosa* on days 0, 6, 7, 9, 12, and 15. Bacteria samples were additionally taken on Day 3. The in vivo fluorescence and *M. aeruginosa* cell number concentration were monitored daily till Day 15. The sampling volumes were calculated so that at least 1 L of experimental solution should remain for the last day of sampling. Daily DOM additions to treatments AB + DOM and B + DOM lasted till Day 6 with DON totalling 20 μM and 22 mL concentrate per bottle (the normal in situ concentration of the summer waters from where *M. aeruginosa* MAGR-2 was isolated). Each treatment had three replicates.

The clonal culture of *Microcystis aeruginosa* Kützing, MAGR-2, was isolated from the Gulf of Riga, Baltic Sea. The stock culture was grown in a medium based on the F medium of Guillard & Ryther (1962). Two-week-old *M. aeruginosa* inocula cultures were centrifuged in sterile conic flasks. The supernatant was removed; packed cells were re-suspended in nutrient-free sterile sea water and left for 24 h in the culture room. Centrifugation and re-suspension were repeated, providing partial nutrient depletion of *M. aeruginosa* cells and removal of dissolved nutrients from the mother suspension. Cell suspension of *M. aeruginosa* (13 mL) was inoculated into two treatment series: Control and AB + DOM, providing an initial concentration of 18.7×10^6 cells L^{-1}. Enrichments of Fe–EDTA, metal, and vitamin mixtures were added.

For the preparation of bacterial inoculum, a fraction of *M. aeruginosa* mother culture was centrifuged in sterile conic flasks; then the supernatant was filtered through a Nucleopore membrane filter with 2 μm pore size to remove algae cells. As the bacterial mother suspension 300 mL of filtrate, containing 1.4×10^9 bacteria L^{-1}, was used. It was inoculated in filter sterilized sea water. The inoculum cell counting was done under epifluorescence illumination. Neither bacterial grazers nor autofluorescent algae were detected in the filtrate.

The Control and AB + DOM treatments were incubated at 18 ± 1 °C with the photoperiod of 16 h light and 8 h dark at 53 μM m^{-2} s^{-1} irradiance from cool white fluorescent lamps. The B + DOM treatment was incubated in the dark.

Bacteria and algae counting

Samples (5 mL) for *M. aeruginosa* cell counting (Neubaeur cell) were fixed with acid Lugol solution and counted using inverted Leitz microscope. For counting bacteria 15 mL of samples were preserved by using 0.2 μm filtered formaldehyde (final concentration 2.5%). Aliquots of 5 mL were taken from each preserved sample and filtered onto black polycarbonate filters (pore size 0.2 μm) and stained with DAPI (4',6-diamidino-2-phenylindole) to get a final concentration of 0.1 mg mL^{-1}. Bacteria were counted using epifluorescence microscope Leitz Dialux fitted with a 100 × 1.25 objective and UV excitation light (Hobbie et al., 1977; Porter & Freig, 1980). Bacterial production was measured by uptake of tritiated thymidine (Fuhrman & Azam, 1980, modified by Smith & Azam, 1992).

Dissolved organic matter

Water for DOM extraction was collected in the Pärnu River, upstream from the town of Pärnu. A tangential flow ultrafilter was used for the extraction process (Guo et al., 1995; Benner et al., 1997). Water was pre-filtered applying 1.2 µm and 0.2 µm Opticap filter units (Millipore). Then DOM was concentrated with a tangential device, the Prep/scale^{TM} TFF 6 ft^2 cartridge (Millipore). Total dissolved nitrogen (DN) and phosphorus concentrations were measured after the persulphate digestion procedure at 120 °C (Pujo-Pay & Raimbault, 1994). The concentration of DON was calculated as the difference between total DN and DIN ($NO_3^- + NO_2^- + NH_4^+$), and dissolved organic phosphorus (DOP) as the difference between total dissolved phosphorus (DP) and phosphate–P concentrations. The concentration of DON in the DOM extract was 639 µM, and the concentration of DOP was 5.14 µM. The DOM extract also contained 49 µM NO_3^- and 3.37 µM PO_4^{3-}.

Nutrients

Concentrations of inorganic nutrients were measured with a Skalar autoanalyser, following the standard procedures for seawater analyses (Valderrama, 1995). Samples for the analyses of particulate organic carbon (POC) and particulate organic nitrogen (PON) were collected using precombusted glass microfibre filters Whatman GF/F, decarbonated (HCl fumes, overnight), and analysed by applying a CHN analyser (model Carlo Erba 1500). Samples for particulate organic phosphorus (POP) analysis were collected using similar methods as for POC and PON. POP concentration was determined as PO_4^{3-} concentration using the persulphate digestion procedure at 120 °C (Pujo-Pay & Raimbault, 1994). The initial nutrient concentrations in Control and AB + DOM were 8 µM $NO_3 + NO_2$ and 0.4 µM PO_4, in B + DOM treatment 10 µM $NO_3 + NO_2$ and 0.4 µM PO_4.

Statistical analyses

Analysis of variance (ANOVA) was used to test the significance of differences in *M. aeruginosa* and bacterial growth, biomass increase, and nutrient dynamics between the treatments under different experimental conditions.

RESULTS

The growth of *M. aeruginosa* started without a lag phase. A certain number of *M. aeruginosa* were in the exponential growth phase till Day 6 in both treatments

with cyanobacteria added, reaching higher values in the treatment with DOM addition (AB + DOM, 0.74×10^9 cells L^{-1}) than in Control (0.54×10^9 cells L^{-1}). On Day 6 *M. aeruginosa* entered the stationary phase in Control, whereas in the AB + DOM treatment, DOM additions facilitated a continuous growth of cell concentration, which reached a maximum on Day 13 (1.02×10^9 cells L^{-1}) (Fig. 1). Contrary to the instantaneous growth of *M. aeruginosa* cell numbers, in vivo fluorescence started to increase after a 2 days long pause. In Control the in vivo fluorescence increased until Day 10, but in AB + DOM fluorescence continued a gradual increase until the end of the experiment. The cell numbers of *M. aeruginosa* and in vivo fluorescence had higher values in the AB + DOM treatment than in Control ($p < 0.05$) (Fig. 1).

The **Heterotrophic bacterial community** grew quickly in all treatments, reaching maximal values of bacterial production on days 3 and 5, whereas cell

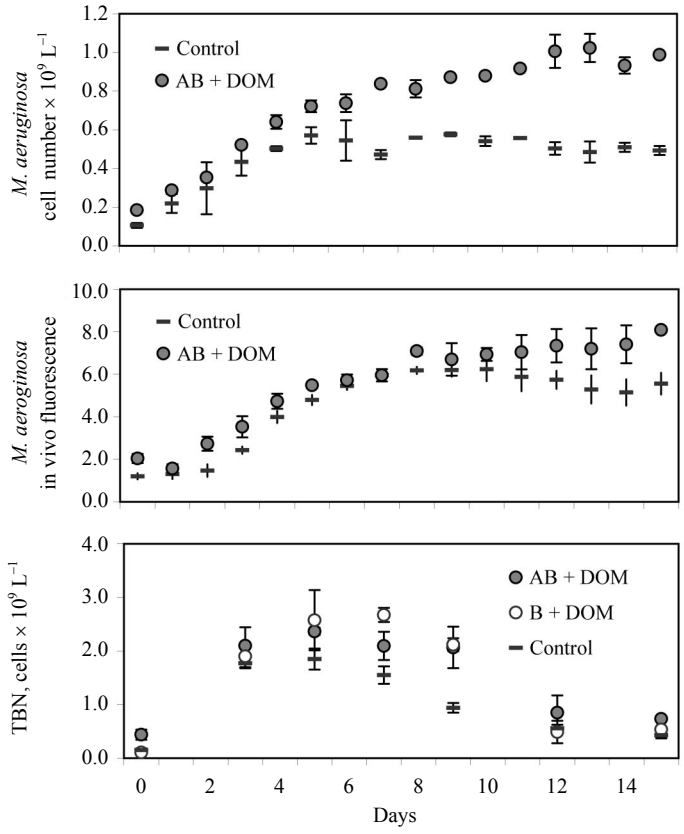

Fig. 1. *Microcystis aeruginosa* cell numbers, in vivo fluorescence, and total bacterial number (TBN) versus time in Control, AB + DOM, and B + DOM treatments. Vertical bars indicate the standard deviation among the triplicates.

numbers reached the peak 2 to 3 days later, on days 5 and 7 (Fig. 1). After five incubation days similar values of total bacterial numbers (TBN) and bacterial biomass (BB) were registered in both treatments with DOM addition irrespective of the presence or absence of *M. aeruginosa* (2.37×10^9 and 2.58×10^9 bacteria cells L^{-1}; 0.37 and 0.38 mgC L^{-1} in AB + DOM and B + DOM, respectively). The heterotrophic bacterial community in the AB + DOM and B + DOM treatments remained in the stationary phase until Day 9, followed by a decline. The dynamics of heterotrophic bacteria was similar in the AB + DOM and B + DOM treatments (ANOVA, $p = 0.98$, $n = 21$). Similarity in the fluctuation of the bacterial number curves both in the presence and absence of cyanobacteria indicates that the growth of associated bacteria was not suppressed owing to *M. aeruginosa* absence in the B + DOM treatment. Bacterial production (BP) demonstrated similar shapes ($r = 0.81$, $n = 63$) (data not presented) to the fluctuation of TBN.

No or an insignificant amount of grazers of heterotrophic bacteria were found in all treatments until Day 12. Due to its suppressing influence on heterotrophic flagellates, *M. aeruginosa* manifested itself during the exponential and stationary growth phases. In the bacterial treatment, flagellates started their rapid development after Day 12.

A few cells of *M. aeruginosa* were observed in the bacterial treatment, but their numbers as well as fluorescence did not increase because of the lack of light. We considered that there was no influence of algae and grazers in the B + DOM treatment until Day 12. Therefore the consumption or release of nutrients was calculated from the first day after the interruption of DOM addition, between Day 7 and Day 12, when grazers of bacteria started to develop.

Nitrates and nitrites were used fast in both treatments where *M. aeruginosa* was present (Fig. 2). In Control and AB + DOM the concentration of NO_3^- was very low (0.7 μM) on Day 7 and fell below detection limits at the end of the experiment. In the bacterial treatment B + DOM the consumption of nitrates was observed during the exponential growth phase. At the end of the stationary phase nitrites and nitrates were released (Fig. 2). At first, the concentration of nitrites increased reaching a maximum on Day 9 (0.35 μM), a peak of nitrates followed on Day 12 (4.45 μM). Altogether 2.46 μM of nitrates was released until Day 12 (B + DOM) (Table 1).

Till the end of the experiment a slow and gradual accumulation of **ammonia** took place in Control, indicating a gradual decay of organisms without additional nutrient supply (Table 1, Fig. 2). In the AB + DOM treatment a slight increase in the ammonia concentration occurred on Day 12. In the bacterial treatment (B + DOM) both the release and consumption of ammonia occurred, with the concentration of ammonia doubling on Day 9, followed by partial depletion on Day 12.

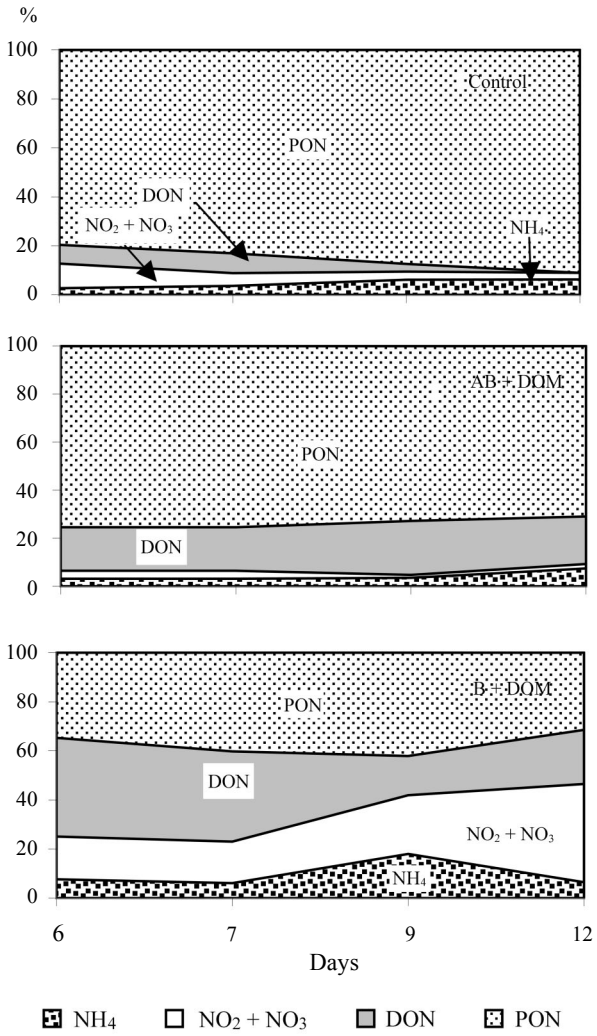

Fig. 2. Changes in particulate organic nitrogen (PON), dissolved organic nitrogen (DON), nitrites + nitrates ($NO_2 + NO_3$), and ammonia (NH_4) in experimental treatments from Day 6 till Day 12, %.

As DOM additions ended on Day 6, the cultures evolved as batch cultures. **Dissolved organic nitrogen** (DON) was depleted in Control, but in the AB + DOM treatment its concentration was enriched by cyanobacteria by 13%, whereas bacteria alone managed to reduce the bulk of DON by 53% (Table 1, Fig. 2). A similar pattern was observed for DN: in Control 60% of DN was used up, but in the AB + DOM treatment a 34% increase was observed; heterotrophic bacteria alone used 10% of DN (Table 1).

Table 1. Concentration of dissolved inorganic nitrogen (NH_4, NO_2, NO_3), dissolved organic nitrogen (DON), and dissolved nitrogen (DN) on Day 7 and uptake (indicated by –) or release (+) of nutrients between Day 7 and Day 12

Treatment		NH_4	NO_2	NO_3	DON	DN
Control	Concentr., µM	0.59 ± 0.15	0.07 ± 0.02	0.77 ± 0.3	1.31 ± 0.28	2.73 ± 0.22
	Uptake/release, %	+69	0	–54	–100	–60
AB + DOM	Concentr., µM	0.72 ± 0.05	0.05 ± 0.00	0.73 ± 0.02	4.20 ± 0.48	5.70 ± 0.52
	Uptake/release, %	+145	+212	–62	+13	+34
B + DOM	Concentr., µM	0.91 ± 0.06	0.07 ± 0.00	2.44 ± 0.70	5.47 ± 0.36	8.88 ± 0.51
	Uptake/release, %	–17	+186	+82	–53	–10

Table 2. Mean particulate organic nitrogen (PON), particulate organic phosphorus (POP), and particulate organic carbon (POC) concentrations and POC : PON and PON : POP ratios (atom : atom) recorded on Day 12 in different treatments. Numbers within parentheses show the relative biomass increase due to the addition of DOM (%)

Treatment	PON, µM	POP, µM	POC, µM	POC : PON	PON : POP
Control	14.3 ± 1.3	0.53 ± 0.23	250 ± 23	17.5 ± 0.1	26.6 ± 8.3
AB + DOM	16.9 ± 1.7 (18%)	1.08 ± 0.17 (103%)	340 ± 37 (36%)	20.1 ± 0.3	15.6 ± 1.7
B + DOM	3.7 ± 0.7	0.35 ± 0.1	29.0 ± 4.6	7.8 ± 0.2	10.6 ± 1.6

The addition of DOM increased biomass indices, such as particulate organic carbon (POC), particulate organic nitrogen (PON), and particulate organic phosphate (POP) concentrations in AB + DOM versus un-enriched Control ($p < 0.05$). In the AB + DOM treatment the increase of POC content was 36%, that of PON 18%, but of POP over 100%. The added DOM prompted a decrease of the PON : POP ratio of *M. aeruginosa* by 24%, changing the content of cyanobacteria cells from P-limited (PON : POP = 21.1) to N : P-balanced (PON : POP = 15.6). The POC : PON ratio of *M. aeruginosa* cells rose from 11.9 on Day 6 to 20.1 on Day 12 (Table 2). We therefore conclude that *M. aeruginosa* took up proportionally more C and P than N, and the surplus N was released as DON.

DISCUSSION

Heterotrophic bacteria used in our experiment live freely in water or are attached to aggregates of *M. aeruginosa*, utilizing allochthonous organic matter brought by rivers and/or autochthonous organic matter released by cyanobacteria. The fact

that these heterotrophic bacteria reduced the riverine DON to half of its initial concentration indicates that they are adapted to this kind of nutrient substrate. Our results showed that heterotrophic bacteria associated with *M. aeruginosa* in the presence of riverine DOM in the treatments conducted with and without autotrophic cyanobacteria had similar growth with similar numbers and production curves. This is an evidence of poor advantages of the presence of photosynthetic extracellular products from algae. Hence, bacteria living in eutrophic brackish waters with cyanobacterial aggregates have a high capability to mineralize riverine organic matter.

As a result of DON mineralization by heterotrophic bacteria, inorganic N compounds were released. It is noteworthy that during the elimination of reduced nitrogen (ammonia) oxidized compounds of N were produced. The total concentration of nitrites increased by 186% and that of nitrates by 82% (Table 1). During the stationary phase ammonia was produced by bacteria, with a decline following. Goldman & Dennett (1991) concluded that elimination of NH_4^+ in the marine environment occurs when the nitrogen source is an organic compound (e.g. aminoacids, proteins, etc.). Tupas & Koike (1991) showed that bacteria can mineralize DON to NH_4^+ even when the net NH_4^+ uptake by bacterial assemblages is high and when C:N models predict no net production of ammonium. Our results indicated the capabilities of heterotrophic bacteria separated from *M. aeruginosa* to eliminate ammonia, nitrates, and nitrites when maximal biomass of bacteria cells was reached and the convenient substrate was depleted. Ammonium release by bacteria occurs commonly in marine and freshwater environments while the simultaneous release of nitrites or nitrates is poorly studied. Berman & Chava (1999) expressed an opinion that bacterial degradation of organic nitrogen will lead to the release of NH_4^+ and/or urea and perhaps even NO_3^-, which would be subsequently utilized by phytoplankton. Our findings supported this hypothesis.

Microcystis aeruginosa does not belong to the N-fixing cyanobacteria. It is estimated that the best nitrogen sources for *M. aeruginosa* are nitrate and ammonium; besides, Berman & Chava (1999) reported its good development sustained by urea. Maestrini et al. (1999) found that addition of urea, glycine, hypoxantine, putrescine, and spermine as the single source of nitrogen significantly provokes the growth of the *M. aeruginosa* culture, and dissolved organic matter from the Daugava River sustains the growth of cyanobacterial biomass at a level of 38% of that accrued from nitrate. Since the cultures of autotrophic cyanobacteria used in the foregoing experiments were not axenic, the contribution of heterotrophic bacteria in the mineralization of DON substances is not known. In our experiment the cyanobacteria could obviously benefit from nitrogen mineralization by heterotrophic bacteria or could utilize DON themselves, as utilization of organic nitrogen by cyanobacteria becomes significant during the summer season in brackish waters when DIN resources are depleted

and DON bioavailability increases (Stepanauskas et al., 1999). However, 70% of DN transported by rivers worldwide, as well as to the Gulf of Riga, is DON (Meybeck, 1982; Laznik et al., 1999; Stålnacke et al., 1999). The primary production in marine and fresh waters is often limited because of low bioavailability of nitrogen. The estimated range of bioavailable DON in the Baltic Sea is broad, between 8% and 72% (Stepanauskas et al., 2002). In our case, after the bacterial growth bioassay, 53% of DON from the Pärnu River could be characterized as biologically labile, and bacterially mediated final products were the main nutrients determining the growth of *M. aeruginosa*.

Besides nitrogen uptake by *M. aeruginosa*, the excretion of DON occurred, amounting to 0.54 µM (between Day 7 and Day 12). In some studies the excretion of organic matter by phytoplankton is considered as a continuous process (Larsson & Hagström, 1979), but others suggest that significant excretion is a response to environmental stress, such as changes in the quantity and quality of light (Zlotnik & Dubinsky, 1989) or nutrient limitation (Myklestad & Haug, 1972; Lancelot, 1983; Obernosterer & Herndl, 1995). Obernosterer & Herndl (1995) hypothesized that large amounts of photosynthetic extracellular release (PER) from phytoplankton occur under severely (N : P = 100) P-limited primary production. In our case the release of DON by cyanobacteria can be explained by changing cell PON and POP content from P-limited (PON : POP = 21.5) at the beginning of the experiment to N : P balanced (PON : POP = 15.6) (AB + DOM treatment, Table 2). Altogether the PON : POP ratio of *M. aeruginosa* cells decreased by 27%. In total, DOM sustained the relative increase of the PON concentration by 18%, while POP increased by 103%. Therefore we can conclude that *M. aeruginosa* took up more P in relation to N from DOM with a subsequent release of surplus N.

Our findings support the hypothesis that bacteria associated with the cyanobacteria species *Microcystis aeruginosa* isolated from brackish waters can release ammonia, nitrite, and nitrate as a result of DON mineralization providing *M. aeruginosa* with simple and ready to use nitrogen compounds.

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REFERENCES

Baines, S. P. & Pace, M. L. 1991. The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems. *Limnol. Oceanogr.*, **36**, 1078–1090.

Balode, M. & Purina, I. 1996. Harmful phytoplankton in the Gulf of Riga (the Baltic Sea). In *Harmful and Toxic Algal Blooms* (Yasumoto, T., Oshima, Y. & Fukuyo, Y., eds), pp. 69–72. Intergovernmental Oceanographic Commission of UNESCO.

Benner, R., Biddanda, B., Black, B. & McCarthy, M. 1997. Abundance, size distribution, and stable carbon and nitrogen isotopic compositions of marine organic matter isolated by tangential-flow ultrafiltration. *Mar. Chem.*, **57**, 243–263.

Berman, T. & Chava, S. 1999. Algal growth on organic compounds as nitrogen sources. *J. Plankton Res.*, **21**, 1423–1437.

Berman, T., Béchemin, C. & Maestrini, S. Y. 1999. Release of ammonium and urea from dissolved organic nitrogen in aquatic ecosystems. *Aquat. Microb. Ecol.*, **16**, 295–302.

Bourne, D. G., Blakeley, R. L, Riddles, P. & Jones, G. J. 2006. Biodegradation of the cyano-bacterial toxin microcystin LR in natural water and biologically active slow sand filters. *Water Res.*, **40**, 1294–1302.

Christoffersen, K., Lyck, S. & Winding, A. 2002. Microbial activity and bacterial community structure during degradation of microcystins. *Aquat. Microb. Ecol.*, **27**, 125–136.

Fuhrman, J. A. & Azam, F. 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. *Appl. Environ. Microbiol.*, **39**, 1085–1095.

Goldman, J. C. & Dennett, M. R. 1991. Ammonium regeneration and carbon utilization by marine bacteria grown on mixed substrates. *Mar. Biol.*, **109**, 369–378.

Grossart, H. P. 1999. Interactions between marine bacteria and axenic diatoms (*Cylindrotheca fusiformis, Nitzschia laevis*, and *Thalassiosira weissflogii*) incubated under various conditions in the lab. *Aquat. Microb. Ecol.*, **19**, 1–11.

Guillard, R. R. L. & Ryther, J. H. 1962. Studies on marine planktonic diatoms I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Microb.*, **8**, 229–239.

Guo, L., Santschi, P. H. & Warnken, K. W. 1995. Dynamics of dissolved organic carbon (DOC) in oceanic environments. *Limnol. Oceanogr.*, **40**, 1392–1403.

Hobbie, J. E., Daley, R. L. & Jasper, S. 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.*, **33**, 1225–1228.

Jones, G. I., Bourne, D. G., Blakeley, R. L. & Doelle, H. 1994. Degradation of the cyanobacterial hepatotoxin microcystin by aquatic bacteria. *Nat. Toxins*, **2**, 228–235.

Kononen, K. & Sellner, K. G. 1995. Toxic cyanobacteria blooms in marine, estuarine and coastal ecosystems. In *Harmful Marine Algal Blooms, 6th Internat. Conf. Toxic Mar. Phytoplankton* (Lassus, P., Arzul, G., Erard-Le Denn, E., Gentien, P. & Marcaillou-Le Baut, C., eds), pp. 858–860. Nantes, France.

Lambert, T. W., Holmes, C. F. B. & Hrudey, S. E. 1994. Microcystin class of toxins: health effects and safety of drinking water supplies. *Environ. Rev.*, **2**, 167–186.

Lancelot, C. 1983. Factors affecting phytoplankton extracellular release in the Southern Bight of the North Sea. *Mar. Ecol. Prog. Ser.*, **12**, 115–121.

Larsson, U. & Hagström, Å. 1979. Phytoplankton exudate release as an energy source for the growth of pelagic bacteria. *Mar. Biol.*, **2**, 199–206.

Laznik, M., Stålnacke, P., Grimvall, A. & Wittgren, H. B. 1999. Riverine inputs of nutrients to the Gulf of Riga – temporal and spatial variations. *J. Marine Syst.*, **23**, 11–25.

Lignell, R. 1990. Excretion of organic carbon by phytoplankton: its relation to algal biomass, primary productivity and bacterial secondary productivity in the Baltic Sea. *Mar. Ecol. Prog. Ser.*, **68**, 85–99.

Maestrini, S. Y., Balode, M., Béchemin, C. & Purina, I. 1999. Nitrogenous organic substances as potential nitrogen sources, for summer phytoplankton in the Gulf of Riga, eastern Baltic Sea. *Plankton Biol. Ecol.*, **46**, 8–17.

Meybeck, M. 1982. Carbon, nitrogen, and phosphorus transport by world rivers. *Am. J. Sc.*, **282**, 401–450.

Myklestad, S. & Haug, A. 1972. Production of carbohydrates by the marine diatom *Chaetoceros affinis* var. *willei* (Gran) Hustedt. I. Effect of concentration of nutrients in the culture medium. *J. Exp. Mar. Biol. Ecol.*, **9**, 125–136.

Obernosterer, I. & Herndl, G. J. 1995. Phytoplankton extracellular release and bacterial growth: dependence on the inorganic N : P ratio. *Mar. Ecol. Prog. Ser.*, **116**, 247–257.

Ojaveer, E., Simm, M., Balode, M. & Purina, I. 2003. Effect of *Microcystis aeruginosa* and *Nodularia spumigena* on survival of *Eurytemora affinis* and embryonic and larval development of the Baltic herring *Clupea harengus membras*. *Environ. Toxicol.*, **19**, 236–242.

Panosso, R. & Granéli, E. 2000. Effects of dissolved organic matter on the growth of *Nodularia spumigena* (Cyanophyceae) cultivated under N or P deficiency. *Mar. Biol.*, **136**, 331–336.

Porter, K. & Freig, Y. S. 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.*, **25**, 943–948.

Pujo-Pay, M. & Raimbault, P. 1994. Improvement of the wet-oxidation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters. *Mar. Ecol. Prog. Ser.*, **105**, 203–207.

Purvina, S., Béchemin, C., Balode, M., Grzebyk, D. & Maestrini, S. 2008. The influence of inorganic nutrients and dissolved organic matter on the growth of cyanobacteria *Microcystis aeruginosa* isolated from the Gulf of Riga. *Acta Univ. Latv. Biol.*, **745**, 61–74.

Seppälä, J. & Balode, M. 1999 Spatial distribution of phytoplankton in the Gulf of Riga during spring and summer stages. *J. Marine Syst.*, **23**, 51–67.

Smith, D. C. & Azam, F. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using 3H-leucine. *Mar. Microb. Food Webs.*, **6**, 107–114.

Søndergaard, M. & Milddelboe, M. 1995. A cross-system analysis of labile dissolved organic carbon. *Mar. Ecol. Prog. Ser.*, **118**, 283–294.

Stålnacke, P., Grimvall, A., Sundblad, K. & Tonderski, A. 1999. Estimation of riverine loads of nitrogen and phosphorus to the Baltic Sea, 1970–1993. *Environ. Monit. Assess.*, **58**, 173–200.

Stepanauskas, R., Leonardson, L. & Tranvik, L. J. 1999. Bioavailability of wetland-derived DON of freshwater and marine bacterioplankton. *Limnol. Oceanogr.*, **44**, 1477–1485.

Stepanauskas, R., Jørgensen, N. O. G., Eigaard, O. R., Zvikas, A., Tranvik, L. J. & Leonardson, L. 2002. Summer inputs of riverine nutrients to the Baltic Sea: bioavailability and eutrophication relevance. *Ecol. Monogr.*, **72**, 579–597.

Tupas, L. & Koike, I. 1991. Simultaneous uptake and regeneration of ammonium by mixed assemblages of heterotrophic marine bacteria. *Mar. Ecol. Prog. Ser.*, **70**, 273–282.

Valderrama, J. C. 1995. Methods of nutrient analysis. In *Manual on Harmful Marine Microalgae. IOC Manuals and Guides No. 33* (Hallegraeff, M. G., Anderson, D. M. & Cembella, A. D., eds), pp. 251–282. UNESCO, Paris.

Van Dolah, F. M. 2000. Marine algal toxins: origins, health effects, and their increased occurrence. *Environ. Health Perspect.*, **108**, 133–141.

Worm, J. & Søndergaard, M. 1998. Dynamics of heterotrophic bacteria attached to *Microcystis* spp. (Cyanobacteria). *Aquat. Microb. Ecol.*, **14**, 19–28.

Young, K., Docherty, K., Maurice, P. & Bridgham, S. 2005. Degradation of surface-water dissolved organic matter: influences of DOM chemical characteristics and microbial populations. *Hydrobiologia*, **539**, 1–11.

Zlotnik, I. & Dubinsky, Z. 1989. The effect of light and temperature on DOC excretion and extracellular products of natural phytoplankton. *Limnol. Oceanogr.*, **34**, 831–839.

Mineraalsete lämmastikuühendite eraldamine jõevee lahustunud orgaanilisest ainest tsüanobakteriga *Microcystis aeruginosa* assotsieerunud heterotroofsete bakterite poolt

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Eksperimentides uuriti jõevee lahustunud orgaanilise aine (DOM) kasutamist tsüanobakteriga *Microcystis aeruginosa* assotsieerunud heterotroofsete bakterite poolt ja lihtsate mineraalsete lämmastikuühendite eraldamist DOM-i baasil. Bakterid vähendasid lahustunud orgaanilise lämmastiku (DON) hulka poole võrra, kuid *M. aeruginosa* manulusel suurenes see 13%-ni. Statsionaarses kasvufaasis bakterid eritasid ammooniumit ja nitraati, kahekordistades nende kontsentratsiooni. *M. aeruginosa* manulusel kasutavad bakterid jõevee DON-i ja ammonifitseerimise ning sellele järgneva nitrifitseerimise kaudu varustavad tsüanobaktereid lihtsate lämmastikuühenditega.