Invited paper

Do toxic cyanobacteria blooms pose a threat to the Baltic ecosystem?*

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Abstract

Cyanobacteria, otherwise known as blue-green algae, are oxygenic, photosynthetic prokaryotes. They occur naturally in many fresh, marine and brackish waters worldwide and play an important role in global carbon and nitrogen cycles. In their long history, cyanobacteria have developed structures and mechanisms that enable them to survive and proliferate under different environmental conditions. In the Baltic Sea, the mass development of cyanobacteria is compounded by a high level of eutrophication. The dominant species in the Baltic, the filamentous *Aphanizomenon flos-aquae* and *Nodularia spumigena*, can fix dissolved atmospheric N_2, as a result of which they can outcompete other phytoplankton organisms. Heterocystous, filamentous cyanobacteria also make a significant contribution

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to the internal nutrient loading in the Baltic. The blooms of *N. spumigena* are of particular concern, as this cyanobacterium produces nodularin (NOD), a hepatotoxic peptide. The concentration of the toxin in the sea is regulated mainly by dilution with uncontaminated water, photolysis, sorption to sediments and microbial degradation. The transfer of the toxin in the Baltic trophic chain through zooplankton, mussels, fish and birds has been reported, but biodilution rather than bioconcentration has been observed. Cyanobacterial blooms are thought to pose a serious threat to the ecosystem. Their harmful effects are related to the occurrence of a high biomass, oxygen depletion, a reduction in biodiversity, and the production of toxic metabolites.

1. Introduction

Cyanobacteria, also called blue-green algae, are photosynthetic prokaryotic organisms. Ecologically, most species of cyanobacteria are characterised by well-developed adaptive strategies. They have the ability to utilise a wide light spectrum; moreover, they can survive poor oxygen conditions and withstand high pH values. Filamentous and colony-forming cyanobacteria are generally of considerable size, which makes them inedible by zooplankton. Furthermore, because of their ability to fix atmospheric nitrogen, these organisms are able to grow in water deficient in dissolved salts of nitrate and ammonium. In this way they can outcompete other phytoplankton organisms. Cyanobacteria can also store nitrates and phosphates in their cells. A characteristic feature of cyanobacteria is the presence of gas vacuoles, which enable them to alter their specific gravity and move vertically in the water column. As a result, they can take advantage of optimal light and nutrient conditions (Paerl & Fulton 2006).

Cyanobacterial blooms frequently occur in eutrophic water bodies, such as lakes, ponds and slow-flowing rivers, and also the lagoons, bays and open waters of the Baltic Sea (Sellner 1997, Pliński et al. 1998, Paerl & Fulton 2006,). The blooms colour the water green, blue-green or yellow, and they can also change the consistency of the water to resemble that of thick paint. Furthermore, during these mass occurrences, cyanobacteria may form a scum on the water surface. This material is usually windblown towards the shore of the water body. A cyanobacterial bloom, and the scum in particular, may pose a threat to human and animal health, as many cyanobacterial species are capable of producing toxic substances (Carmichael 1992, Sivonen & Jones 1999).

2. Ecology of bloom-forming cyanobacteria in the Baltic

Based on the results of palaeolimnological studies, Bianchi et al. (2000) concluded that blooms of nitrogen fixing cyanobacteria occurred in the present Baltic proper as early as 7000 BC, when the Ancylus Lake was

transformed into the saltwater Litorina Sea. As this event took place long before anthropogenic water eutrophication, cyanobacterial blooms in the Baltic Sea can be considered a natural phenomenon. The blooms are dominated by *Aphanizomenon flos-aquae* (L.) Ralfs and *Nodularia spumigena* Mertens; *Anabaena* species are less abundant (Figure 1) (Pliński & Jóźwiak 1995, Stal et al. 2003, Pliński et al. 2007). According to Finni et al. (2001), *Nodularia* originally occurred in the Baltic Sea in the demersal zones of slightly saline bays and estuaries. The presence of *Nodularia* as the prevailing species in the open sea was reported for the first time at the end of July and the beginning of August 1939. In the 1960s, mass blooms of this cyanobacterial species occurred regularly in the central and southern parts of the Baltic, primarily as a result of eutrophication. It is estimated that between 1950 and 1980 there was a four-fold increase in the amount of nitrogen discharged into the Baltic and an eight-fold increase in phosphorus.

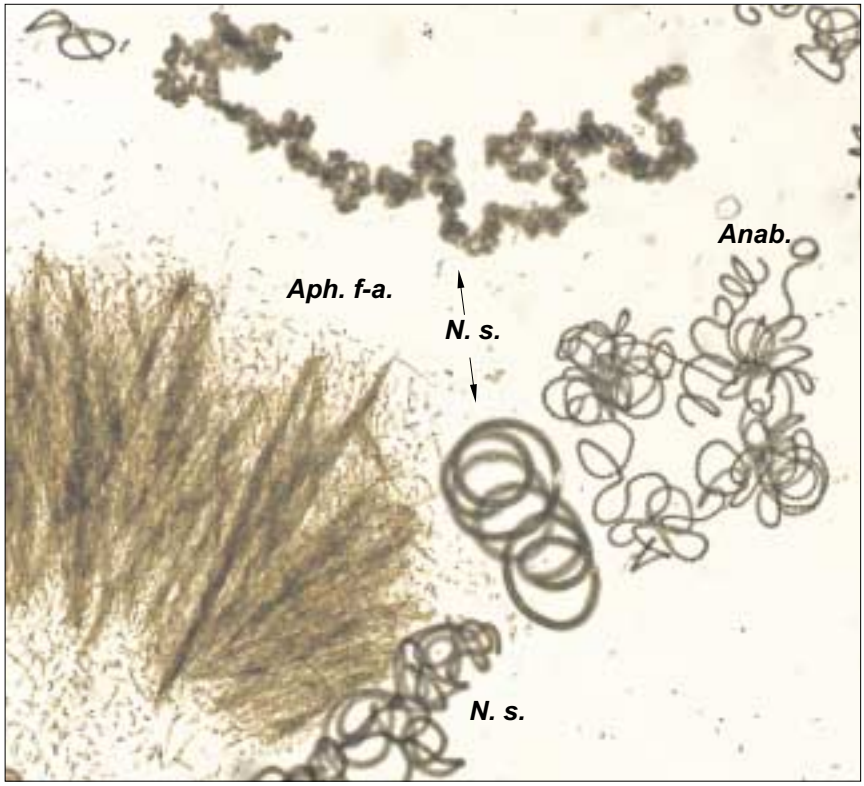

Figure 1. Cyanobacterial species dominating in the Baltic Sea: *Aphanizomenon flos-aquae* (*Aph. f-a.*), *Nodularia spumigena* (*N. s.*), *Anabaena* spp. (*Anab.*)

Additionally, at the end of the 1960s and in the 1970s, several considerable inflows of salt water from the North Sea occurred, which enriched the Baltic water in macronutrients. Studies demonstrated that a significant increase in the concentrations of pigments characteristic of cyanobacteria (myxoxanthophyll, echinenone and zeaxanthin) occurred in the sediments during that period (Poutanen & Nikkilä 2001). Whereas in the 1980s the toxic species *N. spumigena* constituted ca 17% of the total cyanobacterial biomass, between 1997 and 2003, its fraction was estimated to be > 50% (HELCOM). Thus, eutrophication has contributed not only to a change in the phytoplankton biomass but has also had a tremendous impact on community structure; it has also caused a significant reduction in biodiversity. Currently, blooms of *Aph. flos-aquae* and *N. spumigena* are typical occurrences in the waters of the Baltic proper, whereas *Anabaena* species, mainly *A. lemmermannii*, occur primarily in coastal waters. Though *Aph. flos-aquae* and *N. spumigena* differ slightly in their temperature and salinity preferences, they can co-dominate in the same ecological niche. In the Baltic, *N. spumigena* is the primary phytoplanktonic organism producing toxins.

Satellite data and surveys conducted during monitoring cruises have confirmed the continual increase in cyanobacterial biomass in the Baltic. This process is intensifying even though concentrations of macronutrients are not increasing; indeed, they are even decreasing in some regions in comparison with the 1980s (Łysiak-Pastuszak et al. 2004). Large blooms of cyanobacteria are a manifestation not only of intensified eutrophication, but also of a disturbance to the equilibrium of the aquatic ecosystem. According to the Declaration signed in 1988, the states bordering the Baltic Sea undertook to reduce the riverine discharge of phosphorus and nitrogen compounds into the sea by 50%. Over a relatively short period (ca 7 years), the discharge of harmful compounds was indeed reduced by 35% (Neumann & Schernewski 2005), but further reduction in N and P discharges has proved much harder to accomplish. The reduction in N discharges during that short period contributed to a proportional reduction in the dissolved inorganic nitrogen (DIN) in the water (attributed to the process of denitrification, especially in oxygenated sediments). However, the reduction in dissolved inorganic phosphorus (DIP) is proceeding much more slowly. According to Łysiak-Pastuszak & Drgas (2001a,b), the phosphate concentration in the Gulf of Gdańsk has decreased by only 5% since 1990, even though the concurrent reduction in nitrate concentration has been much greater. Under oxygen deficient conditions P is released from sediments. This in turn increases DIP and consequently decreases the N/P ratio (Vahtera et al. 2007). A low N/P value creates unfavourable growth conditions for algae and at the

same time makes the N_2 fixing cyanobacteria more competitive. Neumann & Schernewski (2005) maintain that the upward trend in cyanobacterial blooms is a temporary phenomenon (following the 35% reduction in macronutrient discharges) and will last until the Baltic ecosystem reaches a new state of equilibrium. It is believed that it may take 20–30 years for the DIP level to fall to that of DIN.

In filamentous cyanobacteria from the orders *Nostocales* and *Stigonematales*, the important structures enabling them to adapt to environmental nitrogen deficiency are the heterocysts. These are specialised cells within which the process of molecular nitrogen fixation (N_2) occurs. In *N. spumigena*, heterocysts make up approximately 5% of all cells in a filament. Because of the smaller numbers of heterocysts (1%) it contains, *Aph. flos-aquae* fixes N_2 less effectively. It is estimated that in the Baltic proper, cyanobacteria alone yield from 30 to 430×10^3 t of N annually (Rahm et al. 2000, Larsson et al. 2001). This quantity of nitrogen is comparable to that discharged into the Baltic Sea by rivers. In this situation, the primary element limiting the growth of N_2 fixing cyanobacteria is phosphorus (e.g. Moisander et al. 2002). Based on measurements of P concentration in water – in fact the excess of P over N, considering that the demand of phytoplankton for these elements is N:P 16:1 – it has been possible to determine the probability of a cyanobacterial bloom as early as February. The authors of the most recent prognostic models have paid special attention to the DIP content around 15 June, that is, after the spring algal blooms and before the summer cyanobacterial blooms (Laanemets et al. 2006). However, a cyanobacterial bloom may occur even if there is no excess DIP in the second half of June. These compounds may appear in the surface water as a result of the intensive mixing of water masses or of upwelling events. It is also known that cyanobacteria accumulate reserves of P, which allow them to survive and proliferate in the case of a temporary deficiency of this element in the water.

The experiments of Vuorio et al. (2005), which measured the impact of low N/P values with increasing concentrations of P, did not confirm the decisive role of these factors. Cole et al. (1993) argued that in the case of cyanobacteria that fix molecular nitrogen, molybdenum, the element that is an integral part of nitrogenases, might also be a factor limiting growth. Molybdenum occurs in seawater in the form of molybdate, which is a structural analogue of sulphate. In the Baltic Sea, Mn does not limit the growth of cyanobacteria. However, high concentrations of SO_4^{2-} in the environment may lead to the poorer assimilation of MnO_4^{2-} and, in turn, hinder the activity of nitrogenase – an enzymatic complex that takes part in nitrogen fixation (Stal et al. 2003). A significant reduction of nitrogenase

activity in *N. spumigena* was observed at a SO_4^{2-} concentration exceeding 9 mmol dm^{-3}, which corresponds to a salinity of ca 9 PSU (Stal et al. 1999). Fe is another element that could limit cyanobacterial growth. The biological concentration of accessible forms of Fe in seawater is low, and the demand for this element in N_2 fixing cyanobacteria such as *Nodularia* is considerable (Stal et al. 1999, Paczuska & Kosakowska 2003). Iron is a component of both nitrogenase and ferredoxin, which is an electron donor for nitrogenase.

The slow exchange of water in the Baltic has resulted in a salinity gradient from 9 PSU in the southern part of the Sea to 1–2 PSU in the northern part of the Gulf of Bothnia. The salinity of deep water is greater than that of surface water and may be as high as 10–13 PSU in the Baltic proper. After a period of intensive water exchange with the North Sea through the Kattegat, the salinity of the near-bottom water in the Bornholm Basin may even reach 20 PSU. It is believed that salinity is one of the most important abiotic factors determining the structure of phytoplankton communities and the development of cyanobacterial blooms. It has been demonstrated that the salinity level optimal for the growth and nitrogen fixation of *N. spumigena* is 5–13 PSU (Sivonen et al. 1989). Blooms of this cyanobacterium do not occur in the Kattegat or in the northern part of the Gulf of Bothnia, that is, in waters of salinities >20 PSU or <3 PSU. The growth of *Aph. flos-aquae* is significantly hindered in waters of salinities >10 PSU; optimal growth for this species was observed at salinities of 0–5 PSU (Lehtimäki et al. 1997). It is believed that salinity affects cyanobacterial growth directly by curbing physiological processes, and indirectly by altering the impact of other environmental factors (Moisander et al. 2002, Mazur-Marzec et al. 2005). The freshwater species *Microcystis aeruginosa* shows slight growth and decelerated CO_2 fixation in an environment where the salinity is >2 PSU. Numerous species of cyanobacteria are capable of adapting to salinity fluctuations due to the production of osmotically active organic substances, active transport of ions into and out of the cell, or production of stress proteins. Higher levels of salinity decrease the activity of nitrogenase, the enzyme that plays a key role in N_2 fixation.

Global climate warming and the increase in water temperature is another important factor stimulating the mass development of cyanobacteria (Paerl & Huisman 2008). Generally, the optimal temperature for cyanobacterial growth is higher than for other phytoplanktonic organisms. When the water temperature reaches 16°C, germination of *N. spumigena* akinetes, the endospore forms, begins. Although upwelling enriches surface water with nutrients, it does not have a positive impact on cyanobacterial growth because of the temperature requirements of this process (Vahtera et al.

2005). The water temperature within an upwelling area is approximately 4–10°C lower than that of the surrounding water and does not usually exceed 16°C. Furthermore, temperature indirectly affects the blooms by contributing to water column stratification, which has a stabilising effect and restricts water mixing. In stratified waters, cyanobacteria may accumulate on the surface. Wasmund (1997) noted the role of temperature in the acceleration of phosphorus remineralisation in sediments. This factor is of essential importance, especially in shallow waters. The optimal temperature for *Aph. flos-aquae* is slightly lower than for *N. spumigena* and ranges from 16 to 22°C. *Aph. flos-aquae* can be found in the Baltic Sea throughout the year, even in winter.

Cyanobacteria have developed a range of mechanisms enabling them to adapt to fluctuations in light intensity. The impact of light on cyanobacterial growth is significant, especially in the case of nitrogen fixing organisms, which require an extra portion of energy for this process. Blooms were observed to disappear when the intensity of photosynthetically active radiation was reduced by 50% and the wind velocity exceeded 5 m s^{-1}. The respective optimal ranges of light intensity for the growth of *N. spumigena* and *Aph. flos-aquae* are 105–155 and 25–45 μmol photons m^{-2} s^{-1} (Konoshina et al. 2003). When solar radiation and the rate of photosynthesis are both low, cyanobacteria accumulate extra material in the form of polysaccharides, which reduces a cell's osmotic potential. This state is responsible for the increased production of gas vesicles, which cause the cells to rise in the water column. At wind velocities < 4 m s^{-1} and poor mixing of water masses, *N. spumigena* forms aggregates that may move towards the surface at a speed of 36 m per day (Walsby et al. 1995). An increase in wind velocity above 6–8 m s^{-1} results in the dispersion of these aggregates; cells are transported to greater depths, where the lower light intensity reduces photosynthesis and nitrogen fixation rates. Under such conditions the growth of cyanobacteria is limited.

3. Harmful compounds produced by Baltic cyanobacteria

Blooms of toxic cyanobacteria in the Baltic Sea are thought to pose a real threat to both the ecosystem and the people using coastal areas for recreation. There have been several cases of the poisoning of terrestrial and aquatic animals following contact with a bloom (Edler et al. 1985, Nehring 1993, Andersen et al. 1993, Kuiper-Goodman et al. 1999): they were attributed to toxic metabolites produced by cyanobacteria. The best known of them is nodularin (NOD) (MW = 824 Da) – a cyclic pentapeptide hepatotoxin derived from *N. spumigena*. The structure of nodularin is cyclo(-MeAsp^1-L-Arg^2-Adda^3-D-Glu^4-Mdhb^5-), where MeAsp is D-

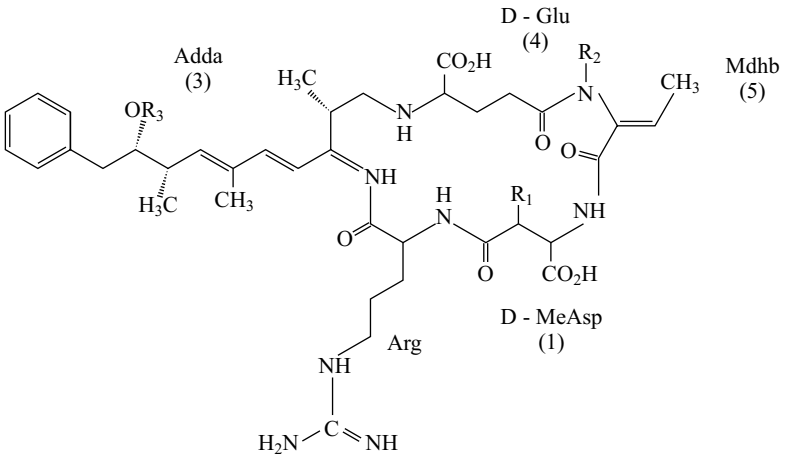

Figure 2. Chemical structure of nodularin (NOD). In demethylated analogues, at positions R_1, R_2 and R_3, hydrogen (H) is replaced by a methyl group (CH_3) (in [Asp^1]NOD, [dhb^5]NOD and [DMAdda^3]NOD respectively)

erythro-β-methyl aspartic acid, Mdhb is *N*-methyldehydrobutyric acid and Adda is (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8,-trimethyl-10-phenyldeca-4(*E*),6(*E*)-dienoic acid (Rinehart et al. 1988) (Figure 2). The Adda amino acid is unique to the group of cyanobacterial peptide hepatotoxins comprising nodularins and the structurally similar microcystins (MCs). In the Baltic Sea, NOD was identified for the first time by Sivonen et al. (1990). Further studies have demonstrated that, apart from unmodified NOD, *N. spumigena* produces linear NOD, geometrical isomers of the toxin, three demethylated variants with the demethylation sites located on aspartic acid [Asp^1]NOD, the Adda residue [DMAdda^3]NOD and dehydrobutyric acid [dhb^5]NOD, as well as three nodularin variants with an additional methyl group located on the Adda [MeAdda^3]NOD, Glu [Glu^4(OMe)]NOD and Asp [MeAsp^1(OMe)]NOD residues (Namikoshi et al. 1994, Mazur-Marzec et al. 2006b). The lethal dose (LD_{50}) of NOD in mouse tests is 50 μg kg^{-1} body weight (b.w.) as administered via intraperitoneal injection (i.p.) (Sivonen & Jones 1999). The configuration of the Adda-Glu part of the toxins is essential for its activity. Studies on the structure-activity relationship showed that the formation of the [6(*Z*)Adda^3] stereoisomer, saturation of the diene in Adda, methylation of glutamic acid or linearisation render the compound non-toxic or significantly decrease toxicity (Choi et al. 1993, An & Carmichael 1994, Rinehart et al. 1994). The Adda-Glu part of the nodularin molecule shows strong interactions with catalytic units of the eukaryotic-type protein phosphatases 1 and 2A (PP) (Yoshizawa et al. 1990). PP play a role in maintaining cellular homeostasis and

take part in many important processes in the cell (e.g. carbohydrate and lipid metabolism, signal transduction, cell division). Inhibition of the enzymes leads to hyperphosphorylation of cytoskeletal proteins, disruption of cytoskeletal structure and massive hepatic haemorrhage. Experiments on animals exposed to sub-lethal doses of nodularin revealed its activity as a direct carcinogen (Ohta et al. 1994, Falconer & Humpage 1996). It has also been shown that in cultured hepatocytes MCs and NOD induced oxidative stress, which was expressed by enhanced production of reactive oxygen species (ROS), lipid peroxidation and depletion of intracellular glutathione (Lankoff et al. 2002, Bouaïcha & Maatouk 2004). As a consequence, oxidative degradation of DNA takes place.

According to Kankaanpää et al. (2009), NOD makes up more than 90% of cyanobacterial hepatotoxins in the Baltic. Occasionally, production of microcystins, mainly by *Microcystis* and *Anabaena* species, can also occur (Mazur et al. 2003, Karlsson et al. 2005a). Microcystin-producing cyanobacteria are more frequently recorded in lagoons of low salinity, which favour the growth of freshwater species (Paldavičienė et al. 2009). The general structure of microcystins is cyclo-(D-Ala^1-X^2-D-MeAsp^3-Z^4-Adda^5-D-Glu^6-Mdha^7) (Figure 3), where **X** and **Z** are variable L-amino acids – leucine (L), arginine (R), alanine (A), tyrosine (Y), methionine (M), tryptophan (W) or phenylalanine (F); Mdha^7 stands for N-methyldehydroalanine (Rinehart et al. 1994). There are over 80 microcystin variants; they are named according to their variable amino acids – for example MC-LR contains leucine (L) and arginine (R) residues (Sivonen & Jones 1999, Spoof et al. 2003). The molecular weight of MCs ranges from 800–1100 Da and the LD_{50} value for most MCs variants is 50–1200 μg kg^{-1}.

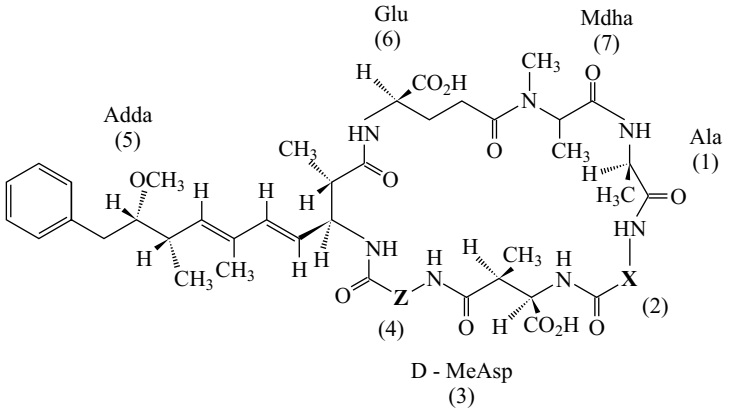

Figure 3. General structure of microcystins (MCs) (**X** and **Z** stand for variable amino acids)

The toxins are synthesised non-ribosomally by large multienzyme complexes consisting of peptide synthetase, polyketide synthase and the tailoring enzymes. The modules are encoded by 9–10 genes (Tillett et al. 2000, Moffitt & Neilan 2000). The nodularin synthetase gene cluster (*nod*) from *Nodularia* and microcystin gene clusters (*mcy)* from several freshwater cyanobacterial genera (*Anabaena*, *Microcystis* and *Planktothrix*) have been sequenced. According to recent studies, cyanobacteria possessed the genes for microcystin biosysthesis before their potential predators appeared on Earth. It is from these genes that the genes encoding nodularin biosynthesis probably evolved (Moffitt & Neilan 2004, Rantala et al. 2004). These findings may be of key importance in resolving the problem of the ecological function of the toxins. In view of this, the role of the toxins as elements of a defence mechanism against predators seems to be less probable.

The nonribosomal pathway of hepatotoxic peptide synthes is reflected in their small size, cyclic structure, occurrence of non-proteinogenic or modified amino acids, and unusual peptide bonds. In contrast to the Baltic *N. spumigena* strains, which always produce nodularin, both microcystin-producing and non-microcystin producing clones can be found among other cyanobacterial species (e.g. belonging to the genera *Microcystis* or *Anabaena*) (Kurmayer & Christiansen 2009).

There are significant temporal and spatial variations in the concentrations of cyanobacterial hepatotoxins in the Baltic. According to Kankaanpää et al. (2009), total NOD concentrations in the surface waters of the northern Baltic in 2006–07 ranged from 74 to 2450 μg m^{-3}. In 2004 and 2005, the maximum NOD concentrations in Polish coastal waters (southern Baltic) were $25\,852 \pm 107$ and 3964 ± 125 mg m^{-3} respectively (Mazur-Marzec et al. 2006a). Henriksen (2005) reported NOD concentrations of up to 565 mg m^{-3} in samples from the Danish Straits. Karlsson et al. (2005a) identified MC-LR in pelagic waters using liquid chromatography-mass spectrometry (LC-MS) coupled with multiple reaction monitoring (MRM). The MC-LR concentrations measured in the phytoplankton samples were 2–4 μg g^{-1}. HPLC analyses with a diode array detector revealed the presence of MC-LR (1.4 μg g^{-1}) and MC-RR (0.9 μg g^{-1}) in cyanobacterial bloom material collected in the coastal waters of the Gulf of Gdańsk (Mazur et al. 2003). Kankaanpää et al. (2009), estimated that toxin production by cyanobacteria in the Baltic is higher than the input of anthropogenic xenobiotics to the sea. The variability in hepatotoxin concentration can be explained by the abundance, of cyanobacteria, the contribution of the *nod* and *mcy* genotypes, and the impact of environmental factors on toxin production. It was demonstrated that temperature, solar radiation and nutrient concentration can change the rate of toxin biosynthesis by cyanobacterial

cells only by a factor of 3–5, on average (Zurawell et al. 2005, Kurmayer & Christiansen 2009). These parameters, however, may significantly increase the toxin concentration in water in an indirect way by increasing the biomass of toxic cyanobacterial strains or favouring the growth of the most efficient NOD and/or MC-producing ones. Depending on the strain, the Baltic *N. spumigena* produces from <1 to 45×10^3 pmol NOD mg^{-1} protein (Moffitt & Neilan 2001).

Studies have shown that nodularin and microcystins are not the only toxins produced by Baltic cyanobacteria. In some isolates of *N. spumigena*, *Aph. flos-aquae* and *Nostoc* 268, a neurotoxic amino acid, β-N-methylamino-L-alanine (BMAA) (Figure 4), was detected (Cox et al. 2005). The toxin is stored in animal tissues by binding with endogenous proteins and is slowly released in the organism when proteins are metabolised (a 'slow toxin'). According to Cox et al. (2005), BMAA is produced by a large number of cyanobacterial genera (95%). These authors also reported biomagnification of the toxin within the Guam terrestrial ecosystem (Cox et al. 2003). BMAA was suggested as being a possible cause of an atypical neurodegenerative disease known as the amyotrophic lateral sclerosis/parkinsonism-dementia complex (ALS/PDC). Opponents of this hypothesis claim that high BMAA concentrations are required to induce behavioural abnormalities and neuronal death. New evidence supporting the association of BMAA with neurological diseases was supplied by Lobner et al. (2007), who postulated that the mechanism of BMAA toxicity is complex; at low concentrations the toxin can accelerate the death of cortical neurons. The action of BMAA is probably potentiated by other environmental toxins.

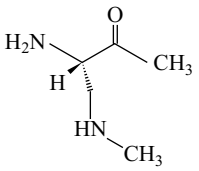

Figure 4. Chemical structure of β-N-methylamino-L-alanine (BMAA)

In most cyanobacteria, as in all Gram-negative bacteria, lipopolysaccharides (LPS) are produced. These components of the bacterial outer cell envelope are complex polymers composed of lipid A, a core oligosaccharide and an O-specific polysaccharide chain consisting of repeating oligosaccharide units. Cyanobacterial LPS, unlike the LPS of other bacteria, do not contain any phosphate in the lipid A core. The compounds have endotoxic activity and are recognised as contact irritants; they can be involved in septic shock syndrome. It has been reported that LPS endotoxins may

reinforce the effects of microcystins by inhibiting the activity of glutathione-S-transferases (GST) – the key enzymes involved in the detoxication of microcystins (Wiegand & Pflugmacher 2005). In animals and humans, they elicit pyrogenic, irritant and allergic responses. LPS produced by cyanobacteria are ten times less toxic in mouse tests than those produced by pathogenic bacteria such as *Salmonella* spp. (Keleti & Sykora 1982). However, high concentrations of toxins were measured in cyanobacterial bloom samples. Presumably, most of the endotoxins were produced by the associated Gram-negative bacteria (Rapala et al. 2002).

In the search for other toxic compounds produced by Baltic cyanobacteria, the activities of numerous benthic isolates were tested. Surakka et al. (2005) showed that some strains of *Anabaena*, *Nostoc* and *N. harveyana* (but not *N. sphaerocarpa*) are highly cytotoxic to human leukaemia cells, while the benthic *Phormidium* strains induce apoptosis in HL-60 cells. Further studies by Herfindal et al. (2005) confirmed the presence of strong apoptogens in a high proportion (50%) of benthic strains of the genus *Anabaena*. So far, neither the structure of the toxic agents nor the mechanism of their activity has been discovered.

4. The fate of cyanobacteria and their toxins

During the bloom, cyanobacterial aggregates become colonised by microorganisms, mainly bacteria, protozoa and diatoms. Salomon et al. (2003) and Tuomainen et al. (2006) discovered that *Nodularia* filaments and aggregates were colonised by many bacteria, including those whose 16S rDNA sequence have not been yet described. It has been postulated that, through their lytic activity, bacteria regulate the abundance of cyanobacteria and terminate the blooms (Rashidan & Bird 2001, Choi et al. 2005). The role of lytic viruses in cyanobacterial bloom termination has also been revealed (Manage et al. 2001, Sullivan et al. 2003, Simis et al. 2005, Yoshida et al. 2006). According to the 'kill the winner' hypothesis (Thingstad & Lignell 1997), the most abundant, bloom-forming cyanobacteria are more susceptible to viral infections. Some of the interactions between microorganisms are strain-specific, while others are characterised by a wide host range. The lytic activity of microorganisms and the decay of cyanobacterial aggregates lead to the release of nitrogen, previously fixed by heterocystous cyanobacteria. This is followed by a small-scale shift in pH and the depletion of oxygen, especially in the inner part of cyanobacterial aggregates (Ploug 2008). The changes in environmental conditions in the aggregates have a significant effect on the structure and abundance of associated bacteria and other microorganisms (Tuomainen et al. 2006).

Due to the positive buoyancy of cyanobacteria, most of the bloom decomposes and is remineralised in the upper layers of the water column. In actively growing cyanobacteria, the major fraction of nodularins and microcystins are retained within the cells. When cells undergo lysis, the toxins are passively released into the surrounding water. Being stable compounds, they can persist in the aquatic environment for some time after the bloom. Natural processes reducing toxin concentrations include dilution by surrounding water masses, photolysis, adsorption to particles and sediments, bioaccumulation and biotransformation by aquatic organisms. Extracellular NOD, as well as other bioactive compounds produced by cyanobacteria, can take part in interactions with Baltic organisms. A role for the toxin in cyanobacterial defence against potential consumers and bacteria has been postulated. The activity of cyanobacterial peptide toxins as antibacterial agent was tested in a few studies (Valdor and Aboal 2007, Mazur-Marzec et al. 2009). In an experiment with pure NOD, Gram-negative bacterial isolates from the Baltic were more sensitive to the toxin (Mazur-Marzec et al. 2009). When extracts of other marine cyanobacteria (*Synechocystis* and *Synechococcus*) were used, it was mostly the growth of Gram-positive bacteria that was inhibited (Martins et al. 2008). As only 1% of the bacteria living in the marine environment are cultivable, laboratory experiments may not reveal the real character of bacteria-cyanobacteria interactions in the sea.

Cyanobacterial metabolites released into the surrounding water can also take part in allelopathic interactions that affect the structure and dynamics of the phytoplankton community. Suikkanen et al. (2004) showed that the exudates of *N. spumigena*, *Aph. flos-aquae* and *A. lemmermannii* were allelopathic to at least one of the algal species used in the experiments. The growth of the cryptophyte *Rhodomonas* sp., which co-occur with cyanobacteria in summer, was inhibited by all the cyanobacteria used in the study. The results, however, did not indicate any correlation between the presence of known cyanobacterial hepatotoxins and the allelopathic effects.

After the bloom, part of the cyanobacterial biomass falls to the bottom of the sea. The average sedimentation rate of *N. spumigena* was estimated to be 8500 filaments $m^{-2}d^{-1}$ (Kankaanpää et al. 2009). *N. spumigena* filaments were found in the Baltic even at a depth of 90 m (Kankaanpää et al. 2001, Mazur-Marzec et al. 2007). The growth cells of *N. spumigena* deposited in sediments change into akinetes. In the following year, recruitment of akinetes to the water column and their germination is induced by increases in water temperature, solar radiation and dissolved oxygen concentration. Cyanobacterial hepatotoxins, and probably also those contained in the akinetes, were detected in sediments of the northern Baltic (0.4–20 μg kg^{-1};

Kankaanpää et al. 2009), Gulf of Finland and Gulf of Gdańsk (1.4–342 and < 0.1 – 75 μg kg^{-1} respectively; Mazur-Marzec et al. 2007). The highest concentrations were measured in the uppermost sediment layers collected during the *N. spumigena* bloom. Sediments with a fine-grained structure tend to accumulate a greater amount of the toxins (Toruńska et al. 2008). LC-MS/MS analyses showed that, apart from NOD, demethylated analogues of nodularin ([DMAdda^3]NOD and [dhb^5]NOD) (Mazur-Marzec et al. 2007), and microcystin-LR (Kankaanpää et al. 2009) were present in sediments. As a result of their transfer from surface waters to sediments, cyanobacteria and their bioactive compounds can contaminate both pelagic and benthic organisms, thereby affecting the whole ecosystem.

Aquatic biota can accumulate cyanotoxins directly from the surrounding water or as a result of feeding on cyanobacteria and other nodularin-contaminated prey organisms. The lack of essential fatty acids in cyanobacterial cells and the size of their filaments reduce their value to consumers. Some authors, however, have reported cyanobacteria uptake by zooplankton, especially in nutrient-poor conditions (Łotocka 2001, Koski et al. 2002, Karjalainen et al. 2003, 2005, Gorokhova 2008). The attractiveness of cyanobacteria as food for mesozooplankton increases in the late stage of the bloom, when the filaments are colonised by heterotrophic bacteria, flagellates and ciliates (Engström-Öst et al. 2002).

Studies by Karjalainen et al. (2005), carried out in the northern Baltic Proper in 2001 and 2002, showed cyanobacterial hepatotoxins to be present in zooplankton tissue at an average concentration of 0.07 ± 0.01 μg g^{-1} w.w. (wet weight) (max $= 0.62$ μg g^{-1} w.w.). In later studies in the same area of the Baltic Sea as much as 2.36 μg g^{-1} w.w. of these toxins were measured in the cladoceran *Pleopis polyphemoides* (Karjalainen et al. 2008). Species-specific variations in NOD content have been observed; they have been attributed to the different feeding preferences and vertical distributions of zooplankton in the water column. Nodularin has been detected in the Baltic copepod *Eurytemora affinis*, which grazes directly on *N. spumigena* and inhabits the surface layers of the water column where cyanobacterial blooms occur. In *Acartia* spp., which can avoid cyanobacteria by reducing ingestion rates and migrating to greater depths, the toxin content was lower, even below the limit of detection (Kozlowsky-Suzuki et al. 2003, Karjalainen et al. 2006). In uptake experiments with radiolabelled dissolved NOD the highest bioconcentration factor was measured in the ciliate *Strombidium sulcatum* (BCF $= 22$); in the copepods *E. affinis* and *A. tonsa* the BCF values were 18 and 12, respectively (Karjalainen et al. 2003). These experiments additionally demonstrated that even if zooplanktonic organisms

avoid feeding on *N. spumigena*, they can still accumulate NOD directly from the water.

Studies on the depuration of nodularin in *E. affinis* revealed significant decreases in the toxin content already after 0.5 h, but even after 24 h, 58–51% of the toxin could still be detected (Karjalainen et al. 2005). The level of nodularin detected in *E. affinis* faecal pellets was low compared to the tissue content, which could suggest that NOD is effectively transformed in the zooplankton organism. However, no enhanced activity of detoxication enzymes was observed, nor were any biotransformation products detected.

Benthic communities are thought to be more exposed to cyanobacterial toxins than pelagic ones. During an *N. spumigena* bloom in the Gulf of Finland, filter-feeding blue mussels *Mytilus edulis* accumulated from 0.095 to 2.1 μg g^{-1} d.w. of nodularin (Sipiä et al. 2001a, Karlsson et al. 2003). In the Gulf of Gdańsk, the highest recorded concentration of the toxin in soft tissue of blue mussels was about 0.14 μg g^{-1} d.w. (Mazur-Marzec et al. 2007). Experiments conducted by Strogyloudi et al. (2006) showed that after 24 h the digestive gland contained 60% of the total amount of toxin accumulated by mussels. After 4 days the proportion of the toxin in the digestive gland increased to 90%. Generally, accumulation of NOD in blue mussels was rapid and proportional to the intensity of the *N. spumigena* bloom (Sipiä et al. 2002, Mazur-Marzec et al. 2007). After the bloom, there was a rapid decrease in NOD content. As NOD was also detected in mussel tissue several months after the *N. spumigena* bloom, it was concluded that depuration was not complete. Incomplete NOD removal was also confirmed in laboratory experiments by Strogyloudi et al. (2006) and Kankaanpää et al. (2007). Using the MALDI-TOF-MS technique, Sipiä et al. (2002) found the GSH-NOD conjugate in *M. edulis* collected from Sundholm Bay. The conjugation of xenobiotics to GSH is a common process in detoxication and the removal of toxins from organisms. But other studies on NOD biotransformation neither confirmed the presence of NOD-GSH in the mussel tissue nor indicated GST involvement in NOD detoxication process (Karlsson et al. 2003, 2005b, Lehtonen et al. 2003, Kankaanpää et al. 2007, Mazur-Marzec et al. 2007).

Mussels are an important food resource for fish and marine birds. Contaminated with nodularin for a couple of months, the blue mussel can be an important source of the toxin for vertebrates, which are thought to be more sensitive to the toxin. Harmful effects of cyanobacterial peptide hepatotoxins on fish were demonstrated by Fladmark et al. (1998) and Andersen et al. (1993). The toxins turned out to be more effective in inducing apoptosis in cultured salmon hepatocytes than in rat cells. Liver tumours and significant histopathological changes in liver cells were reported

in Baltic fish exposed to cyanobacterial toxins (Wiklund & Bylund 1994, Kankaanpää et al. 2002, 2005). The toxic effects of cyanobacteria on the early life stages of fish were studied by Wiegand et al. (1999) and Tymińska et al. (2005), among others. According to Drevs et al. (2007), the abundance of flounder tends to decrease in areas of cyanobacterial blooms.

Studies on NOD accumulation in Baltic fish showed the toxin to be present in flounder, sprat, herring, cod, three-spined stickleback, roach and salmon (Sipiä et al. 2001a,b, 2002, 2007). The NOD content in the planktivorous herring and in salmon was the lowest (2.5–6.5 ng g^{-1} d.w.). Flounders feeding on mussels contained the highest amounts of toxin. The NOD content in the livers of flounders (*Platichthys flesus*) collected in summer from the Gulf of Finland ranged from 0.04 to 0.41 μg g^{-1} d.w. (Sipiä et al. 2002). Comparable NOD concentrations were measured in flounders from the Gulf of Gdańsk (0.49 μg g^{-1} d.w.) (Mazur-Marzec et al. 2007). Toxin concentrations in muscles were much lower and often below the values reported for the reference material (0.7–1.1 ng g^{-1}). These results refer to pooled fish samples. When individual fish were analysed, significant differences in NOD concentration were found (Kankaanpää et al. 2005, Sipiä et al. 2007). Kankaanpää et al. (2005) calculated the average relative standard deviation (RSD%) for NOD concentrations measured in flounder by LC/MS to be 91%. As the maximum toxin content in flounder liver (2.23 μg g^{-1} d.w.) was close to the tolerable daily intake (TDI) for a human being weighing 60 kg (2.4 μg), the authors recommended avoiding the consumption of such liver.

Apart from NOD, two demethylated NOD analogues of the toxin – [d-Asp^1]NOD and [$DMAdda^3$]NOD – were detected in the tissue of Baltic mussels and flounder (Karlsson et al. 2003, Mazur-Marzec et al. 2007).

The presence of nodularin in eiders (*Somateria mollissima*) feeding on blue mussels and flounder was also reported (Sipiä et al. 2003). Concentrations of the toxin in eider liver ranged from 0.003–0.18 μg g^{-1} d.w. and were generally lower than those in mussels and flounders (Sipiä et al. 2003, 2008). Minor NOD concentrations were also found in the feathers and edible parts of these seabirds. Analysis of eider feathers could be a useful non-invasive diagnostic tool for assessing contamination of birds with cyanobacterial toxins. Based on measurements of NOD content in Baltic biota, it was estimated that the amount of NOD transferred to higher trophic levels is $< 1\%$ of the ingested toxin (Karjalainen et al. 2007). This indicates that biodilution rather than bioconcentration of the toxin is taking place in the Baltic ecosystem.

Kankaanpää et al. (2009) estimated the yearly production of nodularin in the Baltic to be 1000–40 000 times larger than the inputs of total

PCB and total DDT, which are characterised by LD_{50} values higher than that of NOD. Given such large amounts of acute cyanobacterial toxin produced in the system, one might expect extremely harmful effects to occur. In actual fact, these effects are better documented in laboratory experiments than in field studies. One of the reasons for this could be the long-term evolution of resistance in Baltic organisms to cyanobacterial toxins. The other possible explanation is the rapid and effective removal of harmful substances by dilution, photolysis and microbial degradation The degrading activity of microcystin and/or nodularin was found in samples of natural water and sediment (e.g. Heresztyn & Nicholson 1997, Christoffersen et al. 2002, Chen et al. 2008, Edwards et al. 2008, Kankaanpää et al. 2009, Mazur-Marzec et al. 2009). Several strains of bacteria capable of degrading microcystins have been isolated from freshwater ecosystems experiencing frequent cyanobacterial blooms. Of these, only the *Sphingomonas* strain B9 and a number of strains of *Paucibacter toxinivorans* gen. nov., sp. nov., were able to degrade nodularin (Imanishi et al. 2005, Rapala et al. 2005, Kato et al. 2007). Most of the active strains belong to the family Sphingomonadaceae. Microbial degradation of the cyclic peptide hepatotoxins causes ring opening in or isomerisation of the Adda moiety, which in turn results in decreased toxicity (Ishii et al. 2004, Imanishi et al. 2005). Further enzymatic activity of bacteria leads to the formation of tetrapeptides, demethylated and decarboxylated forms and Adda residues (Bourne et al. 1996, Amé et al. 2006, Edwards et al. 2008, Mazur-Marzec et al. 2009). The studies showed that various pathways of cyanotoxin biodegradation are possible and provide confirmation of the self-purification capacity of aquatic environment.

In laboratory experiments, microbial degradation of cyanotoxins was often proceeded by a lag phase, the duration of which depended on bacterial number and activity, initial toxin concentration and incubation temperature. After the lag phase, decomposition was usually rapid. In the presence of a natural community of bacteria from Baltic sediments, a rapid loss of nodularin was recorded (Kankaanpää et al. 2009, Mazur-Marzec et al. 2009). The enzymatic activity of Baltic surface water was lower; the apparent decrease in NOD concentration was only observed when water collected during the *N. spumigena* bloom was used. In laboratory experiments with individual bacterial isolates from the natural environment, the cyanotoxin-degrading ability was rarely detected. Either mainly the non-cyanotoxin-degrading bacterial strains were isolated and used in the experiments, or microbial degradation is mediated by a number of enzymes produced by more than one micro-organism.

5. Conclusions

The mass occurrence of cyanobacteria, especially the toxin-producing species, is undoubtedly one of the most important problems of the Baltic Sea. It is a natural consequence of burning environmental issues, such as the high discharge of nutrients into the sea, the spread of anoxia in near-bottom layers, and global climate changes. Studies of the ecological consequences of the blooms have indicated that the most harmful effects are related to the occurrence of a high cyanobacterial biomass, mainly of heterocystous species, which supply an additional pool of nitrogen to the Baltic. This is followed by a reduction in biodiversity, oxygen depletion and an increase in hydrogen sulphide in the near-bottom layers of the sea. Accumulation of cyanobacterial hepatotoxins in zooplankton, mussels, fish and birds has been documented, but their harmful effects on the biota are not clear-cut. While the toxins turned out to have a negative impact on vertebrates, including fish, most aquatic organisms at lower trophic levels appear to have evolved resistance to these compounds.

There are also important socio-economic consequences of the blooms: they may reduce the recreational value and attractiveness of Baltic seaside resorts; the adverse effects on human health following contact with toxic cyanobacteria have been proven; and the quantity and quality of fish caught in areas of cyanobacterial blooms may be reduced, which impacts on fishermen's income.

An improvement in the ecological status of the Baltic Sea with respect to cyanobacterial blooms may be expected if further reduction in external nutrient loads results in a significant limitation of internal nutrient sources, phosphorus in particular.

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