

**Toxic *Nodularia spumigena*
blooms in the coastal
waters of the Gulf of
Gdańsk: a ten-year
survey***

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Abstract

In the Baltic Sea, summer blooms of the filamentous, nitrogen-fixing cyanobacterium *Nodularia spumigena* are favoured by high P concentrations at low N:P ratios and a salinity range of 5–13 PSU. The blooms are initiated by calm and sunny weather, an elevated surface water temperature and thermal stratification. The mass occurrence of *N. spumigena* in coastal waters is a matter of special concern, as the cyanobacterium produces nodularin, a potent pentapeptide hepatotoxin. In the Gulf of Gdańsk, the large-scale occurrence of *N. spumigena* was recorded for the first time in 1994. Blooms of a similar intensity occurred in 2001, 2003 and 2004. Nodularin concentrations in freeze-dried bloom samples varied from 0.01 to 4.01 mg g⁻¹ d.w. In the coastal waters of the Gulf of Gdańsk, cell-bound nodularin concentrations in 2004 and 2005 attained maxima of 25 852 ± 107 μg dm⁻³ and

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$3964 \pm 125 \mu\text{g dm}^{-3}$, respectively. Microscopic analysis revealed the presence of diverse *Nodularia* forms, with the dominance of curved filaments in bloom samples. The results of *in situ* studies and remote sensing measurements indicate a high frequency and intensity of cyanobacterial blooms in the Gulf of Gdańsk in the last ten years.

1. Introduction

The mass occurrence of toxic phytoplankton organisms has a negative impact on water quality and the sustainable development of an aquatic ecosystem. The blooms pose a potential health threat, especially when they occur in drinking and recreational waters. Numerous incidents where humans and animals have allegedly been poisoned by toxic cyanobacteria have been reported by Francis (1878), Edler et al. (1985), Nehring (1993) and Kupier-Goodman et al. (1999). In fresh waters, such blooms are formed mostly by *Microcystis*, *Anabaena*, *Planktothrix*, *Aphanizomenon* and *Nostoc* species. In marine and brackish environments, toxic cyanobacteria are less common. Nevertheless, in some seas nuisance blooms of *Nodularia spumigena*, *Trichodesmium* or *Lyngbya* regularly occur.

Palaeolimnological studies based on analyses of zeaxanthin as a pigment biomarker have revealed that a high number of nitrogen-fixing cyanobacteria occurred in the Baltic Sea about 7000 years B.P. This was soon after the inflows of saline, nutrient-rich waters from the North Sea changed the freshwater Ancylus Lake into the brackish Litorina Sea (Bianchi et al. 2000). When the halocline developed, limited vertical mixing of the water masses resulted in anoxic conditions in the sediments and the release of phosphate bound to ferric iron. This in turn created favourable conditions for the development of cyanobacteria. Since cyanobacteria blooms occurred in the Baltic long before human-induced eutrophication, they could be regarded as a natural phenomenon of this ecosystem.

The presence of *N. spumigena* as a dominant species in the open waters of the Baltic Sea was recorded for the first time in late-July and early-August 1939 (Finni et al. 2001). In the 1960s mass occurrences of this cyanobacterium in the Baltic Proper and southern Baltic were regular. In the late 1960s and 1970s, several intensive nutrient-rich, highly saline inflows from the North Sea and a rise in the surface water temperature caused an increase in the abundance of this phytoplankton species. The remote sensing methods introduced in the mid-1970s improved our ability to determine the spatial and temporal dynamics of the blooms (Kahru 1997). Blooms of positively buoyant, gas-vesicle-forming cyanobacteria can be monitored by satellite systems as they accumulate at the sea surface. Satellite images in

tandem with *in situ* measurements indicate that in the last twenty years cyanobacterial blooms have intensified (Lavender & Groom 2001).

At present, the summer phytoplankton community in the Baltic is dominated by two cyanobacterial species: *Aphanizomenon flos-aquae* (L.) Ralfs ex Bornet & Flahault and *Nodularia spumigena* Mertens ex Bornet & Flahault. The latter is a matter of particular concern as it produces nodularin, a potent cyclic pentapeptide hepatotoxin with $LD_{50} = 50 \mu\text{g kg}^{-1}$ b.w. (body weight). A low N:P ratio is considered to be one of the most important factors responsible for the increase in the intensity and frequency of cyanobacterial blooms. Apart from the two essential macronutrients, P and N, heterocystous and nitrogen-fixing cyanobacteria exhibit a high demand for Fe and Mo (Stal et al. 2003). These elements are components of nitrogenase, the enzyme catalysing N_2 -fixation in cyanobacteria; iron is also a component of ferredoxin, which plays the role of electron donor to nitrogenase. The growth of *N. spumigena* is stimulated by high irradiance, calm weather, rising surface water temperature and distinct thermal stratification. The optimum salinity range for *N. spumigena* bloom development is 5–13 PSU (Sivonen et al. 1989, Lehtimäki et al. 1994).

Initially, studies of the diversity of Baltic *Nodularia* were based solely on microscopic observations and measurements of different types of cells (vegetative cells, heterocytes and akinetes). These measurements showed that individual strains of *Nodularia* differ with respect to cell size, length and diameter of trichomes, and the degree of coiling. Some strains were characterised by the ability to synthesise gas vesicles and to produce toxin. All *Nodularia* strains were classified by Komárek et al. (1993) into seven species: four planktonic, with gas vesicles (*N. spumigena*, *N. baltica*, *N. litorea* and *N. crassa*) and three benthic, without gas vesicles (*N. harveyana*, *N. spherocarpa* and *N. willei*). It was observed, however, that after longer incubation under culture conditions, some of the morphological features changed and therefore could not be further used as a criterion for identification (Laamanen et al. 2001). Quite often, the gas vesicles disappeared and the trichomes became mostly straight or slightly curved. Verification of *Nodularia* taxonomy became possible following the application of genetic methods. On the basis of DNA sequence data (PC-IGS, *gvpA*-IGS and rRNA-ITS) Barker et al. (1999) concluded that there was no correlation between genotype and such morphological features of the cyanobacterium as trichome width, degree of coiling or gas vesicle size. The considerable diversity of these morphological features under different environmental conditions suggested that they are not genetically determined. According to Lehtimäki et al. (2000), analyses of the nucleotide sequence in 16S rRNA indicated great similarity in the genotypes of

cyanobacteria from the genus *Nodularia*. This gene is conserved, and cannot be used as a tool in the taxonomic identification of *Nodularia* species; nevertheless, there was a noticeable difference in gene sequence between toxic and non-toxic *Nodularia* species. Finally, it was concluded that in the Baltic there are only three species of *Nodularia*: one planktonic and toxin-producing, with gas vesicles, which fits the description of *N. spumigena*, and two benthic, non-nodularin-producing species, without gas vesicles, namely, *N. sphaerocarpa* and *N. harveyana* (Laamanen et al. 2001, Janson & Granéli 2002, Lyra et al. 2005). The latter two are rather rare and are observed mostly in the coastal waters of the Baltic.

The latest studies have shown that nodularin is not the only toxin produced by *Nodularia* from the Baltic Sea. In some isolates of *N. spumigena*, as well as in many other cyanobacteria, a neurotoxic amino acid, β -N-methylamino-L-alanine (BMAA), was detected (Cox et al. 2005). Benthic strains of *N. harveyana*, but not of *N. sphaerocarpa*, turned out to be highly cytotoxic to mammalian cells (Surakka et al. 2005). Additionally, most cyanobacteria, including *N. spumigena*, produce lipopolysaccharide (LPS) endotoxins: these are contact irritants and highly inflammatory agents known to be cell-wall components of Gram-negative bacteria (Martin et al. 1989).

In this work, the dynamics of the *N. spumigena* bloom in 2004 and 2005 have been studied. Additionally, the general trend in the *N. spumigena* bloom and toxin production in the Gulf of Gdańsk in the last 10 years is analysed. The application of different methods to study cyanobacterial bloom distribution and dynamics is also discussed. Satellite remote-sensing methods are among the most promising ones used in studies of algal bloom dynamics, as they can provide additional qualitative and quantitative data on the blooms. From the physical point of view, algal blooms form aggregations of suspended organic matter. Their optical properties can vary because, for example, of the composition of optically active pigments specific to different groups of species. Analysis of the water-leaving radiance spectrum enables them to be identified even on the basis of remote sensing (Lin et al. 1999). If the aggregations occur at or close to the sea surface then, owing to strong light scattering, they can be seen from the satellite level as well. Because of the large-scale accumulations of biomass at the surface produced by *N. spumigena* in the Baltic Sea, the blooms of this cyanobacterium are easily detectable with almost any visible imagery.

Massive blooms of toxic *N. spumigena* have a negative impact on the ecosystem. Because of the high probability of adverse health effects, they also restrict the use of bathing beaches and reduce the recreational values of popular tourist destinations on the Gulf. We therefore consider the

studies of *N. spumigena* bloom dynamics and measurements of nodularin concentration in coastal areas of the Gulf of Gdańsk to be of a high priority.

2. Material and methods

2.1. Sampling

The r/v 'Oceanograf 2' of the Institute of Oceanography, University of Gdańsk, was used for sampling. Water and phytoplankton samples were collected during cruises off the coast near Gdynia (54°58'N, 18°34'E), Sopot (54°27'N, 18°36'E), Gdańsk (54°25'N, 18°38'E) and Świbno (54°21'N, 18°38'E) in 2004 (7, 14, 21, 28, 31 July and 4, 11, 18 August) and 2005 (28 June, 1, 8, 12, 14 and 20 July). In 2004 and 2005, from the beginning of June until the end of September samples were collected once a week from one onshore station situated at the end of the pier in Gdynia. Occasionally, samples were also taken from other sites on the coast of the gulf. When a *N. spumigena* bloom occurred, sampling was performed every day, or even twice a day. The sampling locations are shown in Fig. 1. Water samples with suspended organisms for nodularin and chlorophyll *a* (Chl*a*) analysis were collected in 1-dm³ plastic bottles and kept in a cool and dark place until filtration in the laboratory. Within less than 7 hours, each sample was

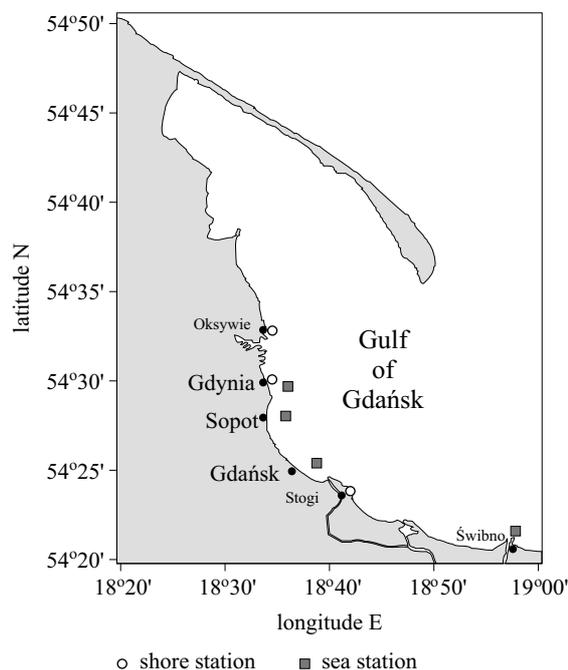


Fig. 1. Sampling locations in the coastal waters of the Gulf of Gdańsk

passed through a Watman GF/F glass microfibre filter disc (2.5 cm i.d.). The volume of the sample ranged from 5 to 250 cm³, depending on the intensity of the bloom, as determined by visual inspection. The filters were immediately frozen in a freezer and kept there until analysis. Concentrated phytoplankton samples were collected with a 100 μm mesh plankton net towed horizontally through the surface layer. Plankton was freeze-dried and stored at -20°C prior to toxin extraction and analysis. Samples for microscopic determination of phytoplankton were fixed in Lugol's solution.

2.2. Sample extraction and analyses

Thawed filters or 10 mg of lyophilised cyanobacterial cells were placed in 2 cm³ microcentrifuge tubes, to which 1 cm³ of 90% methanol was added. The extracts were prepared by a 15-min bath sonication (Sonorex, Bandeline, Berlin, Germany) followed by a 1-min probe sonication with an ultrasonic disrupter (HD 2070 Sonopuls-Bandeline, Berlin, Germany). After centrifugation at 8000 × g for 15 min, nodularin and Chla concentrations were determined in the supernatants. Chla was determined spectrophotometrically (Shimadzu UV-1202 UV-Vis, Australia) by absorbance measurements at 665 (A₆₆₅) and 750 nm (A₇₅₀) using the equation: C_{chla} = 13.42 (A₆₆₅ - A₇₅₀) [μg cm⁻³] (Mackinney 1941). To determine the dissolved nodularin concentration, a 1 dm³ filtered water sample was concentrated by solid phase extraction (SPE) on Sep-Pak Vac C18 cartridges (1 g, Waters, Massachusetts, USA), which had been activated with 15 cm³ of 100% methanol, followed by 15 cm³ of MilliQ water. The filtered water sample was introduced into the cartridge at a rate of about 7 cm³ min⁻¹. The cartridge was then rinsed with 5 cm³ of MilliQ water. The fraction containing nodularin was eluted with 15 cm³ of 100% methanol; the solvent was removed by rotary evaporation at 35°C. The residue was redissolved in 1 cm³ of 30% methanol and analysed with HPLC. All samples were prepared in triplicate.

2.3. HPLC analyses

Quantitative determination of cell-bound and extracellular nodularin concentration was carried out with the Waters HPLC system equipped with a photodiode-array detector (Waters, Milford, MA, USA) set at 238 nm. Isocratic elution of a mobile phase consisting of acetonitrile : water (32:68) both containing 0.05% trifluoroacetic acid (TFA) was used. The flow rate was set to 1 cm³ min⁻¹ and the separation took place on a Waters Symmetry RP-18 column (5 μm; 150 mm × 3.9 mm i.d.). Injections of 10 μl were made using a Waters 917plus autosampler.

The nodularin standard was purchased from Calbiochem (LaJolla, USA), gradient grade acetonitrile and water were obtained from Baker (Deventer, The Netherlands), and TFA of protein sequencing grade was obtained from Fluka (Buchs, Switzerland). The water was purified to 18.2 M Ω on an Ultra Pure Water System from Millipore (Milford, USA).

2.4. Microscopic analyses

Cyanobacteria abundance and biomass were determined with an inverted microscope (Nikon TMS, Tokyo, Japan) according to the Utermöhl method, as described by Edler (1979). 5 and 10 cm³ counting chambers were used. The biomass was calculated assuming a cylindrical shape of the trichomes and a carbon content of 0.11 pgC μm^{-3} . The length and width of *N. spumigena* cells were measured with an eyepiece micrometer. 50 independent measurements were made for each cell type.

2.5. Satellite detection of cyanobacterial bloom

In our work we used the reflectances measured by the AVHRR¹ (*Advanced Very High Resolution Radiometer*) visible (1) and near-IR channels (2) continuously recorded by the satellite receiving station of the Institute of Oceanography, University of Gdańsk (IO UG). The spatial resolution of the maps derived on the basis of these data was 1 km. To minimise the effect of atmospheric diversity, AVHRR spectral channels 1 and 2 were subtracted according to the method described by Svejksky & Shandley (2001). Further in this work, this parameter is called the turbidity index *TI*. Turbidity is not specific to algal blooms, but it can be used as a quantitative estimate of their intensity, once the existence of the bloom has been detected by *in situ* measurements or true-colour images.

Apart from optical and near-IR, the AVHRR thermal infrared channels were used to determine the sea surface temperature (SST). This was done in the standard way, to an accuracy of the order of 0.5°C.

3. Results

Microscopic analyses of samples collected in the Gulf of Gdańsk in July and August 2004 and 2005 confirmed the dominance of cyanobacteria in the summer phytoplankton community. Blooms were formed mainly by nitrogen-fixing species – *A. flos-aquae*, *N. spumigena* and, to a lesser extent, several species of *Anabaena* (Fig. 2). Near the Vistula River mouth, freshwater cyanobacteria of the genera *Microcystis* and *Planktothrix* were

¹AVHRR is the sensor routinely used to obtain an overview of the algae situation in the Baltic Sea (Rud & Kahru 1995).

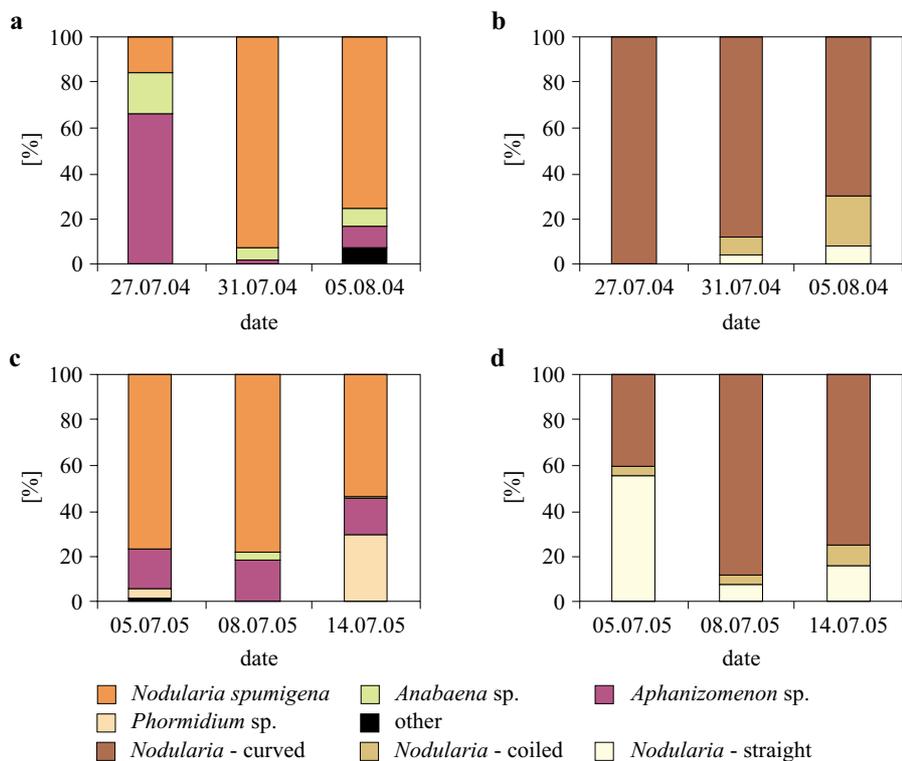


Fig. 2. Cyanobacterial genera and *Nodularia spumigena* forms in bloom samples collected in 2004 (a, b) and 2005 (c, d)

observed. In the last two years, elevated numbers of *Phormidium* sp. were also found. In the coastal waters of the Gulf of Gdańsk, at least three forms of *N. spumigena* filaments were present: straight, curved and

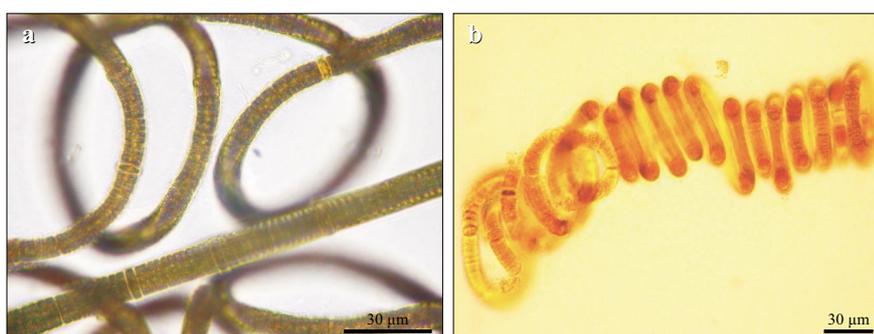


Fig. 3. Different forms of *Nodularia spumigena* occurring in the Gulf of Gdańsk: curved and straight (a), coiled (b). Scale bar 30 μ m

coiled (Fig. 3a, b). All strains had discoid vegetative cells, with widths from $9.2 \pm 1.6 \mu\text{m}$ (coiled form) to $14.0 \pm 1.3 \mu\text{m}$ (straight form), and lengths from $2.5 \pm 0.1 \mu\text{m}$ (all forms) to $3.8 \pm 0.2 \mu\text{m}$ (coiled form). On average, more heterocytes were present in coiled *N. spumigena* filaments (every 12.1 ± 2.3 vegetative cells) rather than in straight or curved filaments (every 14.4 ± 2.1 vegetative cells). Fig. 4 shows the abundance of the three *N. spumigena* forms during bloom development in 2004 and 2005. On 27 July 2004, just before the peak of the bloom, only the curved form was observed. On 31 July, the contribution of the three forms was as follows:

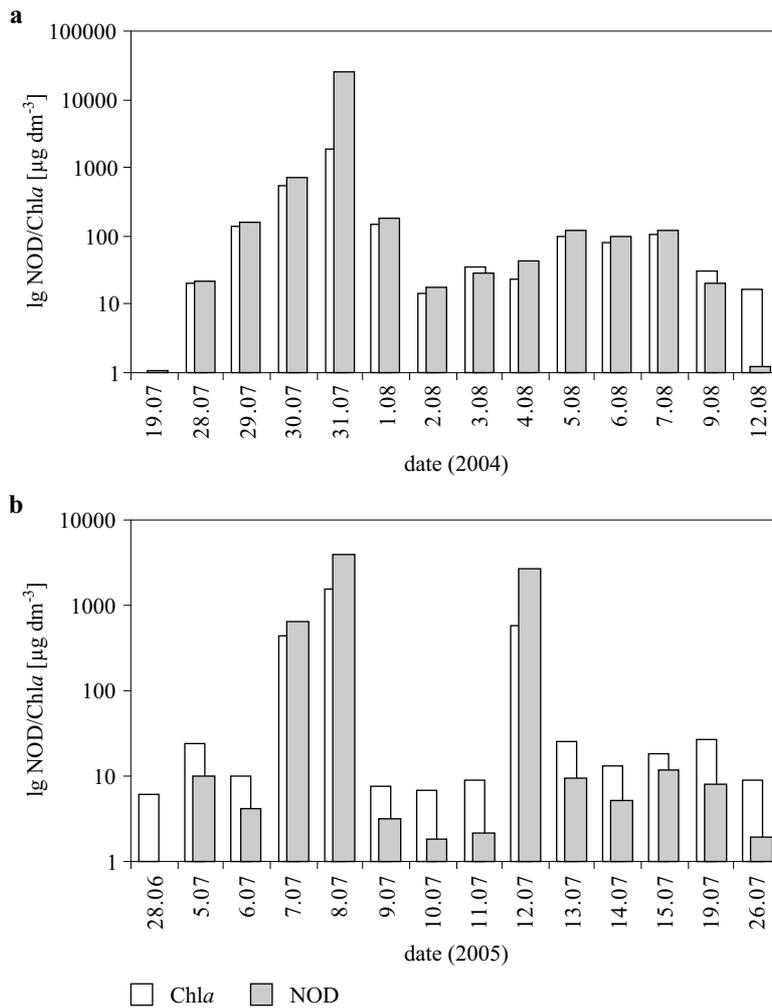


Fig. 4. Nodularin and chlorophyll *a* concentrations in the Gulf of Gdańsk during *Nodularia spumigena* blooms in 2004 (a) and 2005 (b). Concentrations are given on a log scale

straight $871\,640 \pm 8304$ cells dm^{-3} , curved $1\,826\,790 \pm 38\,752$ cells dm^{-3} , and coiled $20\,872\,380 \pm 293\,583$ cells dm^{-3} . During the bloom, the *N. spumigena* biomass ranged from 0.1 to 1647.7 mgC dm^{-3} . At the beginning of summer 2005 the straight *N. spumigena* form dominated. As in the previous year, all three forms co-occurred when the population reached its highest cell number. On 8 July, there were $227\,624 \pm 24\,113$ cells dm^{-3} of the straight form, $2\,650\,875 \pm 17\,906$ cells dm^{-3} of the coiled form and $120\,626 \pm 23\,936$ cells dm^{-3} of the curved form. During the 2005 bloom, the *N. spumigena* biomass ranged from 0.9 to 40.3 mgC dm^{-3} .

In summer 2004 and 2005, single *N. spumigena* filaments appeared in the Gulf of Gdańsk as early as the end of May and the beginning of June. In 2005, they were found in coastal waters before the temperature exceeded 13°C (24 May). When the weather became warmer, and the water temperature reached over 16°C , the *N. spumigena* population increased in biomass. In 2004, nodularin was detected for the first time in samples collected on 6 July (0.3 ± 0.1 $\mu\text{g dm}^{-3}$). During the next three weeks, the surface water temperature rose from 15.5 to 17.7°C . Toxin concentrations were rather low and ranged from < 0.1 to 2.9 $\mu\text{g dm}^{-3}$, and Chl *a* concentrations reached 14.4 $\mu\text{g dm}^{-3}$ (Fig. 4). At the end of July the increase in surface water temperature to $> 20^\circ\text{C}$ stimulated the mass development of cyanobacteria (Fig. 5). *N. spumigena* became the prevailing phytoplankton component and on 31 July, in the coastal waters off Gdynia, it attained its maximum cell number. On the same day, the highest cell-bound nodularin concentrations in water ($25\,852 \pm 107$ $\mu\text{g dm}^{-3}$) and freeze-dried phytoplankton (4.01 ± 0.07 mg g^{-1} d.w.) were recorded; the Chl *a* concentration was $18\,528 \pm 121$ $\mu\text{g dm}^{-3}$. However, there were some other stations (e.g. Stogi, situated to the east of Gdańsk), where *N. spumigena* blooms occurred only sporadically and where the nodularin concentration did not exceed 34.5 $\mu\text{g dm}^{-3}$ during the whole summer. By 11 August, the *N. spumigena* bloom was practically over, both in the Gulf of Gdańsk and in the open sea, but nodularin was detected in phytoplankton samples till the beginning of September (0.27 ± 0.05 mg g^{-1}). The dynamic changes in bloom development and the extent of the bloom in summer 2004 were also observed in turbidity index maps derived from the AVHRR data recorded by the receiving station of the Institute of Oceanography, University of Gdańsk (Fig. 5). According to the satellite images, the turbidity index on 28 July was still rather low ($TI < 0.02$) and the water temperature did not exceed 20°C . In the image taken three days later, on 31 July, strong signals with a turbidity index locally reaching about 0.025 were visible in the whole Gulf of Gdańsk. On that day, the data from the AVHRR on the NOAA series of satellites also showed an elevated surface water temperature. According to

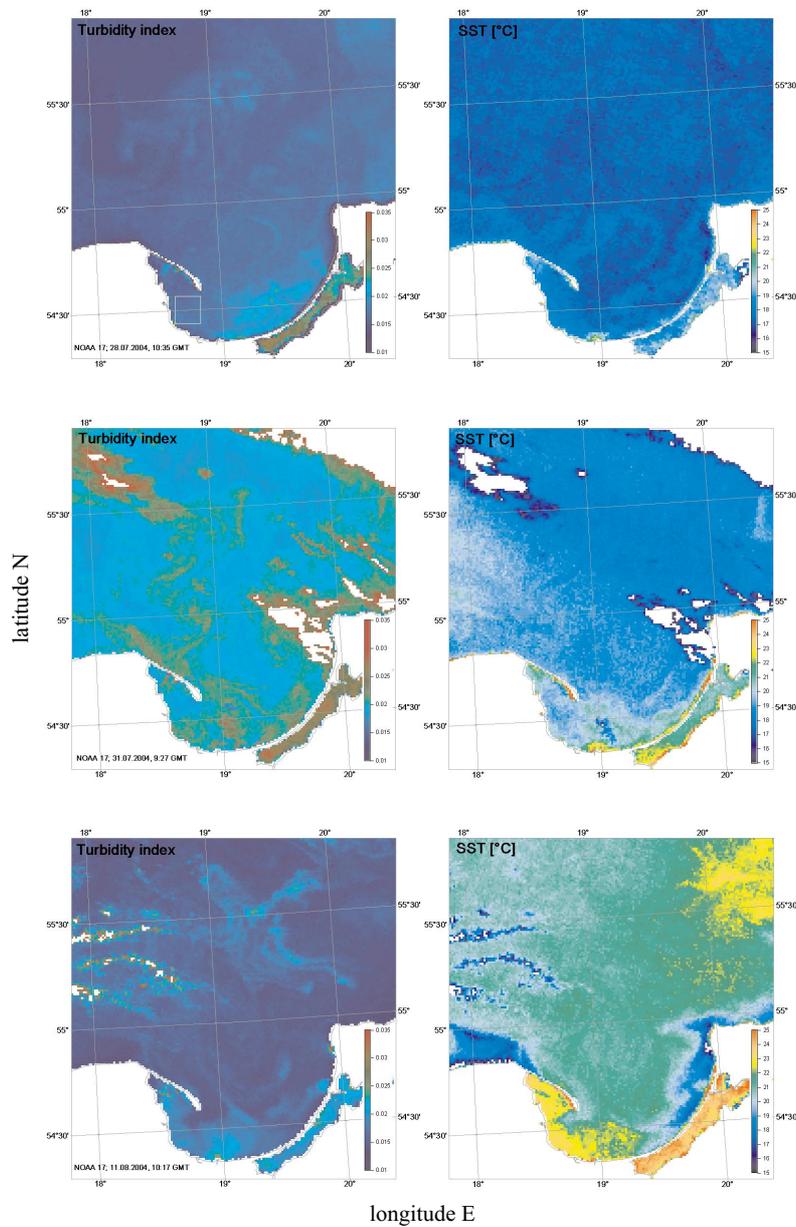


Fig. 5. NOAA/AVHRR images of surface cyanobacterial blooms in the Gulf of Gdańsk in summer 2004 taken on 28 July, 31 July and 11 August

the data received on 11 August, there was a decrease in the turbidity index ($TI < 0.019$), which confirmed the retreat of the cyanobacterial blooms in the Gulf. During the period of high biomass of the toxic cyanobacterium

in late July – early August 2004, fish kills were observed. The toxin was detected in blue mussels, fish and sediments collected in the Gulf of Gdańsk (personal communication). On occasion, the accumulations of scum along the shore led to beach closures. In 2004, there were quite large numbers of people complaining of skin irritation and allergic reactions after contact with the cyanobacteria.

In the first week of July 2005, the changes in Chl a (from 6.0 to 34.5 $\mu\text{g dm}^{-3}$) and nodularin concentrations (from below the detection limit to 642.1 $\mu\text{g dm}^{-3}$) both indicated the sudden burgeoning of a *N. spumigena* bloom (Fig. 4). In July, the weather was rather warm, with a surface water temperature of $>20^{\circ}\text{C}$. *N. spumigena* was dominant in the phytoplankton community of the coastal waters of the Gulf of Gdańsk from 5 to 14 July. On 6 July, just before the peak of the bloom, when the cell-bound nodularin concentration was $4.2 \pm 0.2 \mu\text{g dm}^{-3}$, the extracellular toxin concentration in the water amounted to $0.9 \pm 0.2 \mu\text{g dm}^{-3}$. The highest average concentrations of cell-bound nodularin and Chl a were recorded in samples collected on 8 July in coastal waters off Gdynia ($3964 \pm 125 \mu\text{g dm}^{-3}$ and $1542 \pm 212 \mu\text{g dm}^{-3}$) and on 12 July in waters off Gdańsk ($2688 \pm 312 \mu\text{g dm}^{-3}$ and $577 \pm 52 \mu\text{g dm}^{-3}$). In freeze-dried phytoplankton samples there was $2.36 \pm 0.10 \mu\text{g g}^{-1}$ d.w. of nodularin (Fig. 6). During the peak of the cyanobacteria bloom, the concentration of extracellular toxin in the water off bathing beaches ranged from 68 to

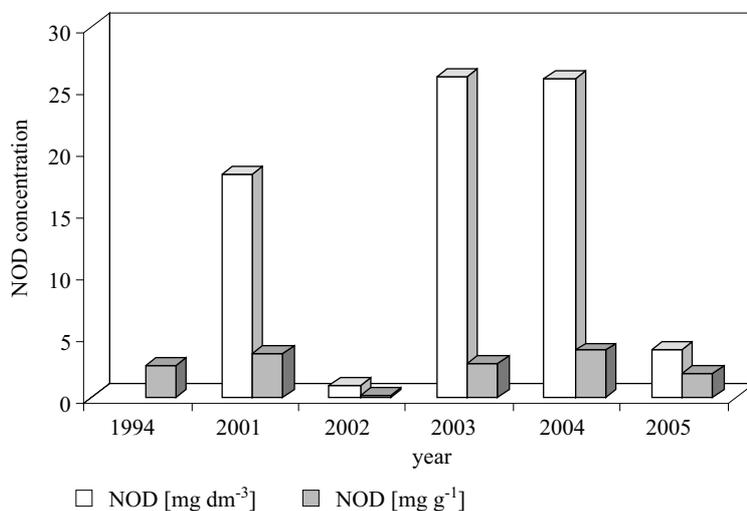


Fig. 6. Maximum concentrations of cell-bound nodularin (NOD) in water and freeze-dried phytoplankton samples measured during the most intensive blooms of *Nodularia spumigena* in 1994–2005

95 $\mu\text{g dm}^{-3}$. Thereafter, the *N. spumigena* abundance decreased abruptly. Three days later, the cell-bound nodularin concentration had dropped to $< 2 \mu\text{g dm}^{-3}$, and extracellular nodularin was not detected. Single filaments of the cyanobacterium were present in phytoplankton samples even in September, but concentrations of the toxin were below the HPLC detection limit (0.5 ng per 10 μl injection). The amount of nodularin produced in cultures by an isolated strain of *N. spumigena* (NSGG-1) was found to range from 2.14 to 5.90 $\mu\text{g g}^{-1}$ d.w., depending on the culture conditions.

4. Discussion

The Gulf of Gdańsk is a highly eutrophic water basin characterised by a low N:P ratio, which favours the mass growth of nitrogen-fixing cyanobacteria. Apart from the high intensity and frequency of the blooms, a decrease in the diversity of cyanobacteria was observed (Wiktor & Pliński 1992). In this water body, the mass occurrence of toxic *N. spumigena* was recorded for the first time in 1994 (Pliński & Józwiak 1999). Both in July and August 1994, *N. spumigena* was the most abundant species, with cell numbers reaching 16 million and 10 million cells dm^{-3} respectively (Witek & Pliński 1998). Until then, *A. flos-aquae* had dominated in the phytoplankton community of the Gulf. This seasonal shift in dominance, also observed in other parts of the Baltic Sea, can be attributed to the warming of surface waters and the slightly different temperature preferences of the two species. Published results (Kononen 1992, Wasmund 1997, Konoshina et al. 2003) and our observations (Pliński & Józwiak 1999, Mazur & Pliński, 2003) show that growth of the hepatotoxin-producing *N. spumigena* is strongly temperature-dependent and is optimal at 25–28°C. For *A. flos-aquae* the optimum temperature for growth is several degrees lower (16–22°C). *N. spumigena* is also more tolerant towards higher irradiance and accumulates mainly in the surface layer. This position in the water column gives better access to atmospheric nitrogen and light, both of which are required for photosynthesis and other biochemical processes. However, high temperature and irradiance are not the only prerequisites for a *N. spumigena* bloom to occur. The summer of 2002 was exceptionally warm and sunny, with water temperatures $> 20^\circ\text{C}$, but the highest nodularin concentration recorded in the Gulf of Gdańsk that year was only 12.6 $\mu\text{g dm}^{-3}$ (Mazur & Pliński 2003). The impact of environmental factors on cyanobacterial bloom formation in the Baltic was studied by Wasmund (1997), Kiirikki et al. (2001), Stal et al. (2003) and Moisander et al. (2003), among others. These authors demonstrated that excess phosphorus at a low N:P ratio, an elevated water temperature and light irradiance, stratified water and moderate salinity all stimulate the

growth of nitrogen-fixing *N. spumigena*. They also pondered the role of iron and sulphate as bloom controlling factors. Janssen et al. (2004) emphasised the impact of hydrographic factors, including the North Atlantic Oscillation, on cyanobacterial blooms. In the Gulf of Gdańsk, conditions favour the mass development of toxic *N. spumigena*; nevertheless, the factors governing the year-to-year fluctuation in the dynamics of the blooms are not yet fully understood.

In the last ten years, the most intensive *N. spumigena* blooms in the Gulf of Gdańsk were recorded in 1994, 2001, 2003 and 2004 (Fig. 6). In these years the maximum nodularin concentrations in lyophilised phytoplankton samples were 2.6, 3.5, 2.8 and 3.8 mg g⁻¹ d.w. respectively. In 2005, the bloom occurred quite early, i.e. at the beginning of July, but with a rather moderate intensity, and a maximum nodularin concentration of 1.9 mg g⁻¹ d.w. Analyses of filtered water samples collected during the bloom and three days after the peak of the bloom showed a dramatic fall in nodularin concentration. This toxin is known to be very stable: dissolved in MilliQ water, it can persist for several days or even weeks (Twist & Codd 1997, Mazur & Pliński 2001). The observed decrease in nodularin concentration might be a result of water mixing and/or toxin degradation by the natural microbial community. Heresztyn & Nicholson (1997) reported that the half-life of nodularin in water collected during a *N. spumigena* bloom was 24 h.

Blooms of *N. spumigena* are regular occurrences in the whole Baltic Sea. Thanks to remote sensing measurements, it has been possible to determine that the surface accumulation of cyanobacteria can cover over 100 000 km² of the Baltic Proper (Hansson 2006). The highest nodularin concentration – 18.1 mg g⁻¹ d.w. – was recorded in a sample from the Bothnian Sea (Kononen et al. 1993), but the average nodularin concentration in bloom samples ranged from <0.1 mg g⁻¹ d.w. to 6.0 mg g⁻¹ d.w. (Sivonen et al. 1989, Kankaanpää et al. 2001, Laamanen et al. 2001). There is a lack of systematic data on nodularin concentrations in the Baltic Sea. The quantity of the toxin has usually been referred to the dry weight of phytoplankton sample, which does not reflect the actual concentration in the environment. The contribution of toxic cyanobacteria to the phytoplankton community varies. Moreover, different strains of *N. spumigena* are characterised by different rates of nodularin production, which are additionally modified by environmental conditions. The *N. spumigena* strain BY1, isolated from the Arkona Sea, was found to produce 4–16 mg of nodularin per g d.w. (Lehtimäki et al. 1997), whereas the NSGG-1 strain isolated from the Gulf of Gdańsk produced 2.14–5.90 mg of nodularin per g d.w.

For over 10 years, toxic blooms of cyanobacteria have affected the Gulf of Gdańsk. The beaches along the coast of the Gulf are an important tourist attraction in the region. Measures should be taken to control the toxic blooms, as detected concentrations of nodularin far exceed the provisional guideline level for recreational waters ($2\text{--}4\ \mu\text{g dm}^{-3}$; first alert level) (Falconer et al. 1999). There are different methods that could be used to monitor toxic *N. spumigena* blooms. Most frequently, the biomass of potentially toxic species has been determined by spectrophotometric measurements of chlorophyll *a* concentration and by microscopic counts of phytoplankton. However, the correlation between Chl*a* and nodularin concentration is rather poor, from $R^2 = 0.466$ (Henriksen 2005) to $R^2 = 0.89$ (Schlüter et al. 2004). In our studies, carried out in the coastal waters of the Gulf of Gdańsk, the correlation was $R^2 = 0.64$. To identify an organism and determine its abundance, analyses of phytoplankton pigments have often been used (Jeffrey et al. 1997). A 4-keto-myxoxanthophyll-like pigment was found to be a good indicator of the presence of *N. spumigena* and nodularin (Schlüter et al. 2004). In the western Baltic Sea, nodularin was positively correlated with the concentration of this 4-keto-myxoxanthophyll-like pigment (0.97). Based on studies carried out in the Sound and Køge Bay, Henriksen (2005) showed that *N. spumigena* trichome counts could also provide an estimate of nodularin concentration. He found a good correlation between cell-bound nodularin and *N. spumigena* abundance ($R^2 = 0.944$), biomass ($R^2 = 0.939$), and the concentrations of the three major *N. spumigena* carotenoids: echinenon ($R^2 = 0.823$), cantaxanthin ($R^2 = 0.916$) and cis-cantaxanthin ($R^2 = 0.938$). Analyses of nodularin concentrations in water and in phytoplankton samples by HPLC are the standard, recommended method for the control of toxic *N. spumigena* blooms. However, collecting data representative of a whole water body solely on the basis of chemical and microscopic analyses of samples collected during cruises is rather difficult, and probably impossible. Cyanobacterial blooms are heterogenic in character: ‘patches’ of surface cyanobacterial aggregations change their position with the wind and currents so that at two separate sampling sites at any one time, the concentrations of cells and cell-bound nodularin can differ significantly. During mass blooms of *N. spumigena* in the Gulf of Gdańsk, point measurements of nodularin concentrations in the surface water and the mean value of the remotely determined turbidity index show unexpectedly good agreement (Fig. 7). This could be explained by the fact that the floating accumulations are formed mainly by the nodularin-producing *N. spumigena*. The results have demonstrated that the satellite system for detecting and determining toxic blooms, even at its present level of development,

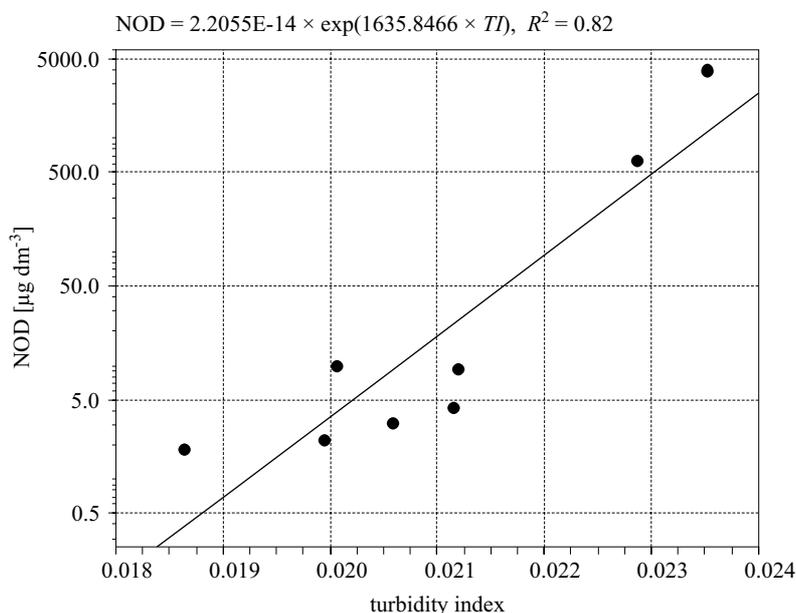


Fig. 7. Relationship between turbidity index and concentration of cell-bound nodularin in the surface waters of the Gulf of Gdańsk in 2004

can adequately complement time-consuming *in situ* sampling followed by laboratory analyses. Further experiments are planned to investigate the applicability of this comparatively easy satellite method for estimating the total quantities of nodularin over as broad an area as possible.

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