Toxic cyanobacteria blooms in the Lithuanian part of the Curonian Lagoon

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Abstract

The phenomenon of cyanobacteria (blue-green algae) blooms in the Baltic and the surrounding freshwater bodies has been known for several decades. The presence of cyanobacterial toxic metabolites in the Curonian Lagoon has been investigated and demonstrated for the first time in this work (2006–07). *Microcystis aeruginosa* was the most common and widely distributed species in the 2006 blooms. *Nodularia spumigena* was present in the northern part of the Curonian Lagoon, following the intrusion of brackish water from the Baltic Sea; this is the first time that this nodularin-(NOD)-producing cyanobacterium has been recorded in the lagoon. With the aid of high-performance liquid chromatography (HPLC), four microcystins (MC-LR, MC-RR, MC-LY, MC-YR) and nodularin were detected in 2006. The presence of these cyanobacterial hepatotoxic cyclic peptides was

additionally confirmed by enzyme-linked immunosorbent assay (ELISA) and protein phosphatase inhibition assay (PP1). Microcystin-LR, the most frequent of them, was present in every sample at quite high concentrations (from < 0.1 to 134.2 μg dm^{-3}). In 2007, no cyanobacterial bloom was recorded and cyanotoxins were detected in only 4% of the investigated samples. A comparably high concentration of nodularin was detected in the northern part of the Curonian Lagoon. In one sample dimethylated MC-RR was also detected (concentration 7.5 μg dm^{-3}).

1. Introduction

In terms of morphology, physiology and metabolism, cyanobacteria (blue-green algae) are one of the most diverse groups of gram-negative photosynthetic prokaryotes (Codd 1995). Due to their capacity for oxygenic photosynthesis, cyanobacteria can rapidly become dominant in aquatic and terrestrial habitats by forming intensive blooms. These can have a strong negative effect on water quality, as certain species of cyanobacteria are capable of producing toxins.

The development of cyanobacterial blooms has become a serious problem in recent decades, because many bloom-forming species are reported to be able to produce secondary metabolites toxic to many organisms, including humans. The WHO expert group developed a guideline value of 20 000 cells cm^{-3} for safe practice in managing recreational waters, as well as a safe level of 1.0 μg microcystin-LR equivalent per litre for drinking water quality (WHO 1998). Over 100 species of cyanobacteria from 40 genera are reported to be toxigenic. However, *Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Microcystis, Nostoc, Nodularia* and *Planktothrix* (*Oscillatoria*) are recognised as the most important genera with toxigenic species (Carmichael 2001). In the Baltic Sea, summer blooms of cyanobacteria have been observed for several decades (Horstmann 1975); they usually form during July-August and are dominated by *Aphanizomenon flos-aquae* and the toxic *Nodularia spumigena*.

Toxins produced by cyanobacteria are very diverse in their chemical structure and biological activity. Hepatotoxins, including microcystins and nodularin, are the most frequent group of cyanotoxins. Microcystins are produced mainly by freshwater species, but they have also been reported from the Baltic (Karlsson et al. 2005). Nodularin is produced only by *N. spumigena*, which occurs most commonly in the brackish waters of the Baltic Sea (Sivonen et al. 1989, Pliński & Jóźwiak 1999, Stal et al. 2003) and in the estuaries, coastal lagoons and saline lakes of Australia (Francis 1878, Jones et al. 1994, Blackburn et al. 1996).

There are many reports from all over the world about animal deaths, as well as human diseases and occasional fatalities related to cyanobacterial

blooms (Kupier-Goodman et al. 1999). At least 50% of blooms are toxic in bioassays. The potentially toxic cyanobacteria commonly found in the freshwater Curonian Lagoon include the unicellular, colony-forming species *Microcystis aeruginosa* and the filamentous genera *Anabaena, Oscillatoria, Aphanizomenon*. In this study *Nodularia spumigena* was detected in northern part of the Curonian Lagoon for the first time; it is the most common toxic species in the Baltic Sea (Pliński & Jóźwiak 1999).

The aim of this study was to determine the frequency and intensity of cyanobacterial blooms in the Curonian Lagoon and to evaluate their toxicity.

2. Material and methods

2.1. Study area and sampling

The Curonian Lagoon (Figure 1) – the largest European lagoon – is a shallow water body (total area 1584 km^2, mean depth 3.8 m; maximum depth 5 m). Situated in the southern part of the Baltic Sea, the lagoon receives water from the River Nemunas, the third-largest contributor (after the Vistula and Oder) of total nitrogen and phosphorus to the Baltic Sea (Stalnåcke et al. 1999). The salinity of the water in the northern part of the lagoon fluctuates between 0.1 and 7 PSU; marine, brackish and freshwater species inhabit the lagoon.

Bloom samples of cyanobacteria were collected from seven different stations in the Curonian Lagoon from July to October (2006) and from May to October (2007). The phytoplankton was preserved with Lugol's solution. Sub-samples were examined to determine the contribution of different cyanobacteria species to the phytoplankton population. Water samples (100 cm^3) were passed through Whatman GF/C filters, which were then stored deep-frozen at $-20°C$ until toxin analysis.

Water temperature, salinity, pH, and light intensity were measured with a MultiLineF/Set-3 instrument (WTW, USA) at the same time as the phytoplankton samples were taken. The statistical relationship between the physical environmental factors and the presence of cyanobacterial toxins was also examined.

2.2. Analysis of toxins

2.2.1. Liquid chromatographic method

The filters with cyanobacterial material were suspended in 90% methanol (1 cm^3) and sonicated for 5 min with a HD 2070 Sonopuls ultrasonic disrupter equipped with a MS 72 probe (Bandelin, Berlin, Germany; 20 kHz, 25% duty cycle). Then, another portion of 90% methanol (1 cm^3)

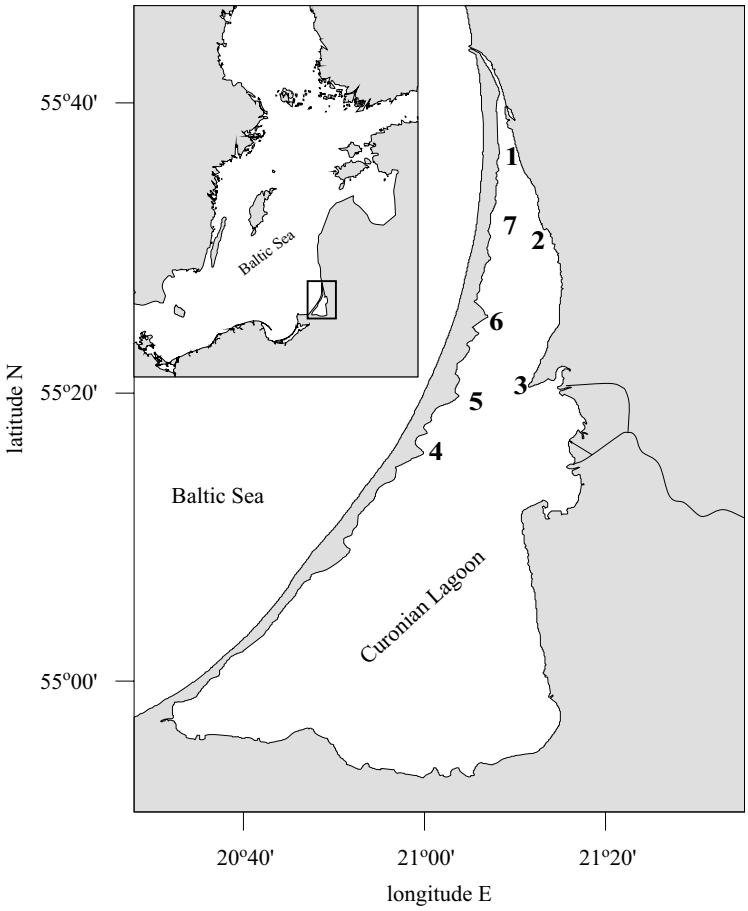

Figure 1. Sampling site locations in the Curonian Lagoon, south-eastern Baltic Sea

was added. The samples were sonicated in a bath for 10 min (Sonorex, Bandelin, Berlin, Germany), vortexed for 1 min and left to stand for 2 hours at room temperature. After centrifugation at 12 000 g for 15 min the supernatant was separated and analysed (Waters HPLC system: 626 pump, 600S controller, 917plus auto-sampler, 996 photodiodarray detector (PDA); Milford, MA, USA). Isocratic elution was performed on a Waters Symmetry RP-18 column (5 μm; 150 mm $\times$ 3.9 mm I.D.) using a mobile phase of acetonitrile:water (32:68), both constituents containing 0.05% TFA, at a flow rate of 1 cm^3 min^{-1}. The auto-injection volume was 2×10^{-5} dm^3. Microcystins (RR, LR, LA, LF, and LW) and nodularin standards were purchased from Alexis Biochemicals (San Diego, CA, USA). Concentrated solutions of toxins (10 μg cm^{-3}) were prepared in methanol. All reagents

used for the analyses were of HPLC grade. Methanol and acetonitrile were purchased from J. T. Baker (Deventer, The Netherlands). Deionised-distilled water was obtained using the Milli-Q ultra-pure water system (Millipore, Bedford, MA, USA). The cyanotoxins were identified by their retention times and characteristic absorption spectra with maxima at 238 nm. All HPLC data were collected and processed using Waters Millennium 32 Software. The hepatotoxins were analysed quantitatively using a calibration curve based on peak area measurements for standard solutions.

2.2.2. Immunological assay

In the study cyanobacterial extracts were diluted in Milli-Q water (5–10 000 times) and analysed by enzyme-linked immunosorbent assay (ELISA). The ELISA test kit was purchased from EnviroGuard (Strategic Diagnostics, Newark, DE, USA) and used according to the manufacturers' instructions.

2.2.3. Protein phosphatase 1 inhibition assay (PPIA)

The cyanobacterial extracts prepared for ELISA were also analysed by colorimetric protein phosphatase inhibition assay (PPIA). The PPIA was carried out on a 96-well microplate according to the method described by Rapala et al. (2002). Bovine serum albumin (BSA) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Dithiothreitol (DTT), $MgCl_2 \times 6H_2O$, $MnCl_2 \times 4H_2O$, Na_2SO_4, *p*-nitrophenyl phosphate (*p*-NNP), tris-(hydroxymethyl)-aminomethane (Tris) were of analytical grade. The substrate and enzyme buffers were prepared immediately before the test. Catalytic subunits (2.5) of commercially available enzyme (PP1; New England Biolabs, USA) were diluted in 1.5×10^{-3} dm^3 of the enzyme buffer. Subsequently 1×10^{-5} dm^3 of standard solutions or sample were added to the well and mixed with 1×10^{-5} dm^3 of PP1 in buffer. After 5 min incubation, 20×10^{-5} dm^3 of *p*-NPP in buffer solution was added to each well. The content of the wells was mixed by swirling the plate sideways. After 2-hour incubation at 37°C the absorbance of the solutions was measured. The plates with ELISA and PPIA tests were read on a VERSAmax microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 450 nm (ELISA) or 405 nm (PPIA), respectively.

2.2.4. Statistical analysis

To compare the three different methods of toxin detection, the data were analysed using multiple regression analysis (Statistics Soft V. 6). The cyanobacterial toxins detected were related to the environmental variables

using redundancy analysis (RDA) as one of the multivariate statistical methods. 'Redundancy' expresses how much of the variance in one set of variables can be explained by the other. In this case, RDA was considered to be more appropriate than CCA (canonical correspondence analysis), since there were many zero values and in general the relationship between different toxins and explanatory variables was quite linear. The Brodgar software (Highland Statistics Ltd.) statistical package was used for the analysis. A confidence level of 0.05 was accepted for the statistical tests to differentiate between statistically significant and insignificant relationships.

3. Results

3.1. Environmental factors

Table 1 shows the physical parameter data gathered during the cyanobacterial bloom (July–October 2006 and May–October 2007) at 7 different stations in the Curonian Lagoon. In 2006, the temperature varied from 17.4 to 28.7°C, in 2007 from 14.0 to 26.4°C. The highest temperature was recorded in the middle of August, the lowest temperature at the end of October (2006 and 2007); pH varied from 8.2 to 9.8 (2006) and from 8.1 to 9.6 (2007). The salinity ranged from 0.00 to 6.00 (2006) and from 0.00 to 4.4 (2007), depending on the station. The highest salinities were recorded in the northern part of the Curonian Lagoon (stations 1, 7, 6) – 6.0 PSU (2006) and 4.4 PSU (2007). Light intensity was measured at the same time at all stations: it ranged from 111 to 365 μmol m^{-2} s^{-1} (2006) and from 62 to 182 μmol m^{-2} s^{-1} (2007).

3.2. Distribution of cyanobacterial toxins in water at selected stations

In 2006–07, phytoplankton samples were collected from seven stations located in an area of the lagoon used for recreation and fishing. Microscopic analysis of scum collected from the surface waters in 2006 showed the presence of many potentially toxic cyanobacterial species with the dominance of the genera *Microcystis, Planktothrix* and *Anabaena*: they respectively made up 55, 30 and 10% of all the samples. *Woronichinia* was rarely recorded – it was found only in 5% of the samples. In 2007, cyanobacteria from the genera *Planktothrix*, *Limnothrix* and *Woronichinia* were detected, but they did not form a bloom in the Curonian Lagoon. Hepatotoxin production by cyanobacteria was determined by HPLC-PDA. Four different microcystin analogues – MC-LR, MC-RR, MC-LY and MC-YR – were detected in the lagoon in 2006. Microcystins were detected in 60 out of 75 samples collected at all the stations (Table 2), microcystin-LR being the most

Table 1. Physical characteristics of the Curonian Lagoon (2006–2007)

Parameters	Stations																				
	1			2			3			4			5			6			7		
	n	min–max	Me	n	min–max	Me	n	min–max	Me	n	min–max	Me	n	min–max	Me	n	min–max	Me	n	min–max	Me
	2006																				
T [°C]	10	19.6–27.0	23	13	19.2–24.0	22	10	18.0–26.3	22	10	18.4–25.0	22.2	12	17.6–28.7	22.4	11	17.4–24.0	22.1	9	19.0–24.0	22
LI [μmol m^{-2} s^{-1}]	10	112–359	180	13	113–358	179	10	111–357	178	7	112–357	81	6	111–359	180	6	112–359	181	6	111–356	176
pH	10	9.1–9.8	9.1	13	8.2–9.1	8.8	10	8.1–9.2	9.0	7	8.2–8.7	8.5	6	8.6–8.9	8.8	6	8.6–8.7	8.6	6	8.4–9.1	8.6
salinity [PSU]	10	0.04–1.4	0.5	13	0.0–0.4	0	10	0.0	0.0	10	0.00–0.04	0.0	12	0.0–0.08	0.01	11	0.0–0.09	0.01	9	0.0–6.0	0.4
	2007																				
T [°C]	12	16.0–25.4	19	12	15.0–24.2	22	12	16.0–25.5	20	10	19.0–26.4	21	10	15.0–24.6	21	10	16.0–25.1	20	10	14.0–24.6	19.6
LI [μmol m^{-2} s^{-1}]	12	60–180	110	12	56–174	105	12	70–174	107	10	80–186	118	10	78–182	112	10	79–180	110	10	62–181	108
pH	12	8.2–9.4	8.8	12	8.4–9.2	8.1–9.1	12	8.8–9.6	8.9	10	8.9–9.6	9.2	10	8.2–8.8	8.6	10	8.1–9.2	8.6	10	8.2–8.9	8.6
salinity [PSU]	12	0.00–4.4	0.8	12	0.00–1.2	0.2	12	0.00	0.0	10	0.00	0.00	10	0.0–0.7	0.0	10	0.0–1.4	0.2	10	0.0–4.4	0.4

T [°C] – water temperature; LI [μmol m^{-2} s^{-1}] – light intensity; Me – median; n – number of investigated samples.

Table 2. Hepatotoxin concentrations at all the investigated stations in the Curonian Lagoon in 2006–2007

Toxins	Stations																				
Concentration	1			2			3			4			5			6			7		
[μg dm^{-3}]	I_{min}–I_{max}	$\overline{X}$	S^2	I_{min}–I_{max}	$\overline{X}$	S^2	I_{min}–I_{max}	$\overline{X}$	S^2	I_{min}–I_{max}	$\overline{X}$	S^2	I_{min}–I_{max}	$\overline{X}$	S^2	I_{min}–I_{max}	$\overline{X}$	S^2	I_{min}–I_{max}	$\overline{X}$	S^2
	2006																				
MC-?	n.d.–4.29	1.74	2.36	n.d.–4.16	2.28	1.89	n.d.–4.62	1.47	3.21	n.d.–17.35	2.53	2.3	n.d.–3.44	1.15	1.91	n.d.–2.12	0.94	0.66	n.d.–1.37	0.27	0.22
MC-LR	n.d.–1.14	0.18	0.16	n.d.–1.96	0.51	0.29	n.d.–0.71	0.08	0.06	n.d.–134.25	13.71	1794.22	n.d.–11.21	1.42	10.17	n.d.–7.89	1.89	9.07	n.d.–20.96	6.22	64.733
MC-YR	n.d.–1.99	0.22	0.44	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.–20.67	2.14	41.11	n.d.–1.35	0.29	0.23	n.d.–0.85	0.08	0.07	n.d.–1.91	0.21	0.41
MC-RR	n.d.–19.32	2.14	4.48	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.–13.52	7.83	25.79	n.d.–18.90	3.89	43.43	n.d.–9.18	2.09	13.65	n.d.–30.69	3.77	103.01
MC-LY	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.–0.61	0.05	0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
NOD	n.d.–5.18	1.07	3.36	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.85	n.d.	n.d.	n.d.–1.14	0.09	0.11	n.d.–2.95	0.77	1.23	n.d.–5.81	1.63	4.45
E [%]	100			76.92			50			70			75			100			100		
	2007																				
dmMC-RR	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.–7.5	0.75	2.31	n.d.	n.d.	n.d.
NOD	n.d.–284.6	30.63	6730.29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.–7.89	0.42	1.81
E [%]	25			0			0			0			0			8.33			5.26		

n.d. – not detected; I_{min}–I_{max} – minimum and maximum toxin concentrations at a station; S^2 – dispersion; $\overline{X}$ – abundance index, average toxins in all investigated samples; E [%] – number of samples in which toxins were detected.

abundant toxin in the samples collected there (concentration from < 0.1 to 134.2 μg dm^{-3}). The maximum concentration (134.2 μg dm^{-3}) was detected in water collected at station 4 in mid-August. Microcystin-RR and microcystin-YR were detected mostly in the western part of the lagoon (stations 4, 5, 6, 7). The concentrations of these toxins varied from < 0.1 to 30.7 μg dm^{-3} MC-RR (highest concentration measured at the end of July) and from < 0.1 to 20.67 μg dm^{-3} MC-YR (station 4 in mid-August). A very low concentration of microcystin-LY (0.61 μg dm^{-3}) was detected in only one sample from station 5 in August. In all the samples there was one microcystin analogue that could not be identified (MC-?).

In 2006, the brackish water cyanobacterial species *N. spumigena* was detected for the first time in the northern part of the Curonian Lagoon (stations 1, 7). Nodularin was detected there (concentration < 0.1–5.8 μg dm^{-3}) in 24% of all samples analyses that year; the toxin occurred in water samples where *N. spumigena* was present. In 2007, nodularin was detected in only 4% of samples (concentration range < 0.1–284.6 μg dm^{-3}) at stations 1 and 7 at the end of August; the dominant species in these samples was *Nodularia spumigena*. That year, dmMC-RR (7.5 μg dm^{-3}) was also detected in one sample collected at station 6 in mid-July; the dominant species in this sample was *Planktothrix agardhii*.

3.3. Comparison of different methods of cyanotoxin detection

HPLC equipped with a diode array detector, ELISA and PPIA all revealed the presence of microcystins in 80% of bloom samples collected in 2006. Multiple regression analysis was applied to compare the concentrations of microcystins determined by HPLC, ELISA and PPIA in environmental samples of cyanobacteria. Microcystin concentrations measured by HPLC were compared with the results obtained by PPIA and ELISA. There was a strong positive correlation between the microcystin concentrations determined by HPLC and ELISA ($y = 0.3704 + 0.0547 \times x$; $r = 0.78$; $p = 0.0015$). The positive relation between the microcystin concentrations determined by HPLC and PPIA was weak and statistically insignificant ($y = 0.9866 + 0.0469 \times x$; $r = 0.43$; $p = 0.123$).

3.4. The role of physical factors

Redundancy analysis (RDA) was used to evaluate the relationship between environmental factors and cyanotoxin abundance. This showed that cyanotoxins (microcystin-LR, microcystin-RR, microcystin-YR, microcystin-LY and the unknown microcystin) were positively related to the water temperature, light intensity, but that nodularin was positively related to salinity [PSU] and pH.

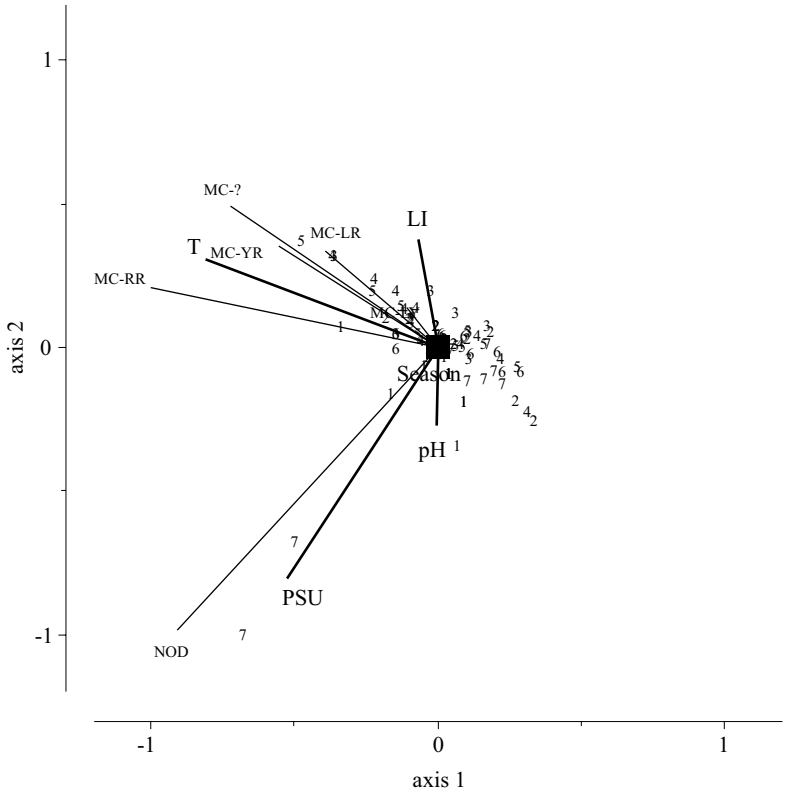

Figure 2. Biplot obtained by Redundancy Analysis. Light grey lines show response variables: MC-LR, MC-RR, MC-?, MC-YR, MC-LR, MC-LY, nodularin; bold lines indicate the explanatory variables: pH, salinity [PSU], temperature (T), light intensity (LI), season (summer, autumn); the numbers denote the stations

Table 3. Relationship between environmental factors and variation in cyanotoxin abundance. The total sum of all eigenvalues is 0.22 and the total inertia is 1. The second column shows the increase in explained variation following the addition of an extra explanatory variable. The third column shows the eigenvalue as a % using only one explanatory variable

Variable	Cond. effects	Eigenvalue as %	F-statistic	p-value
temperature (T)	0.1	43.16	7.644	0.010
salinity [PSU]	0.08	38.62	7.051	0.040
season	0.02	4.85	1.531	0.180
light intensity (LI)	0.01	8.36	1.154	0.390
pH	0.01	4.82	1.202	0.320

Five ambient physical variables (water temperature, light intensity, salinity, pH, season) explained 22% of the variation in six different toxin concentrations, as can be seen from the 2-D plot (14% on axis 1 and 8% on axis 2) (Figure 2). The results of forward selection and permutation tests, presented in Table 3, indicate that cyanotoxins were significantly associated with water temperature (MC; $p = 0.01$), and salinity (NOD; $p = 0.04$).

4. Discussion

Most of the frequently bloom-forming cyanobacteria in the Curonian Lagoon belong to the genera *Microcystis, Planktothrix, Anabaena, Aphanizomenon* and *Woronichinia* (Olenina 1997, Pilkaitytė 2003). In 2006 the cyanobacterial bloom assemblages were dominated by different *Microcystis* species. In 2007, however, no cyanobacteria blooms were recorded, presumably because of the unfavourable weather conditions. *Nodularia spumigena* was present in only a few samples. During our investigations we detected the toxic *N. spumigena* for the first time in the Curonian Lagoon. Like many other countries (Chorus & Bartram (eds.) 1999), Lithuania has not escaped the problems caused by toxic cyanobacteria blooms.

The different methods of toxin analysis have different sensitivities and selectivities. Some of them (PPIA) provide information on bloom toxicity, some are important for the quantitative analysis of individual toxins. Other methods are important for the analysis of MC and NOD when their concentrations are below the detection limit of HPLC. With the use of HPLC, PPIA and ELISA to analyse samples from the Curonian Lagoon five microcystin variants (MC-LR, MC-LY, MC-RR, MC-YR, dmMC-RR) and nodularin were detected. Microcystin was detected in 75% of the analysed bloom samples collected in 2006. This percentage lies within the range of results from other European surveys, e.g. 66% in Denmark (Henriksen 1996), 66% in Germany (Fastner et al. 2001), 60% in Portugal (Vasconcelos 2001), 53% in Belgium and Luxembourg (Willame et al. 2005). In some regions a higher ratio may occur, e.g. 90% in Czech Republic (Maršalek et al. 2001). 72% of all the samples (co-)dominated by *Microcystis* spp. analysed in the current study were found to contain at least one microcystin variant. The other genera (*Planktothrix*, *Anabaena* and *Woronichinia*) were found in 7 to 12% of the samples. As documented by Sivonen & Jones (1999) microcystin-LR was the most frequently encountered MC variant in other studies. In phytoplankton samples from the Curonian Lagoon MC-LR was the most abundant MC variant. Of the other microcystins, MC-LY, a rare but highly toxic variant harmful to hydrobionts and humans (Sivonen & Jones 1999), was detected only in one sample (station 5, 2006). In 2007 only one variant of microcystin (dmMC-RR) was detected in just

one of all the samples analysed. The presence of the toxic *N. spumigena* was recorded for the first time in the Curonian Lagoon. There, the nodularin concentration tended to follow a north-south pattern, being the highest in the northern part and the lowest in the south-central part (station 4 in 2006); this could be explained by the corresponding salinity gradient. Further, longer-term studies will be required to reveal the trends in toxic cyanobacteria blooms in the lagoon and the principal regulatory factors.

According to published data, nutrients, physical and hydrological factors can influence the abundance and biomass of different cyanobacteria species (van der Westhuizen & Eloff 1985), Sivonen et al. 1989, Shapiro 1990, Romo 1994, Lehtimaki et al. 1994, Yunes et al. 1994, 1998, Olenina 1997, Walsh et al. 1997, Rapala & Sivonen 1998, Song et al. 1998, Chorus & Bartram (eds.) 1999, Pliński & Jóźwiak 1999, Pliński & Dziopa 2001, Pilkaitytė 2003, Stal et al. 2003, Tonk et al. 2005, Pilkaitytė & Razinkovas 2007) However, only a few papers (Rapala & Sivonen 1998, Salomon et al. 2003, Tonk et al. 2005) have described how environmental factors can affect cyanobacterial growth and toxin production. According to Pilkaitytė & Razinkovas (2007), the summer phytoplankton community in the Curonian Lagoon is limited by nitrogen, with phosphorus being the secondary limiting factor. In general, inorganic nutrient concentrations in the hypereutrophic Curonian Lagoon are too high to limit the total plankton biomass, which is governed mostly by ambient physical factors.

Cyanobacteria have generally higher temperature optima for growth than other phytoplankton, and temperature has been considered the most important factor contributing to cyanobacterial dominance (van der Westhuizen & Eloff 1985, Rapala 1998). This can explain why in temperate and boreal water bodies most cyanobacteria bloom in summer (Pliński & Dziopa 2001). The results of this study also showed that temperature was a very important factor stimulating growth and toxicity of cyanobacteria, including *Microcystis aeruginosa* from the Curonian Lagoon. According to Rapala & Sivonen (1998), temperature affects the production of MC-RR. All five microcystin variants detected in our samples were strongly correlated with temperature, whereas the nodularin correlation with temperature was comparatively low. The impact of salinity on *N. spumigena* was probably crucial for the occurrence of the species (Mazur-Marzec et al. 2005).

Salinity is another important factor controlling the growth of cyanobacteria (Stal et al. 2003). Studies in the Patos Lagoon (Brazil) showed that *M. aeruginosa* blooms are formed in freshwater, and under discharge conditions reach the estuarine area. The intracellular MC concentrations fell as salinity increased (Yunes et al. 1994, 1998). In the Curonian Lagoon the

correlation between microcystin concentrations and salinity was relatively low. To explain this fact, two factors should be considered: salinity may regulate the structure of the cyanobacterial community in the lagoon, e.g. the increasing salinity may reduce the population of microcystin producers. Alternatively, salinity may influence toxin production per cell. In the case of *N. spumigena*, a toxic brackish-water species, salinity is the main factor determining its distribution, growth, biomass and toxin production (Lehtimaki et al. 1994, Mazur-Marzec et al. 2005). Field observations and laboratory studies indicate that salinity is an effective barrier to the mass development of *Nodularia*. Despite the fact that intensive blooms of cyanobacteria are frequent in the Baltic Sea, they do not occur in adjacent waters of salinity beyond the range regarded as optimal for their growth, i.e. from 5 to 13 PSU (Sivonen et al. 1989, Lehtimaki et al. 1994). This could be one of the reasons why *N. spumigena* was detected mainly in the northern part of the Curonian Lagoon (stations 1, 7), where the salinity is occasionally as high as 7 PSU. Nodularin concentrations in the south-western Curonian Lagoon (station 4, 5, 6) were very low, which could have been due to the wind-driven transport of water from the north-west. The present results show a strongly positive, significant relation between salinity and nodularin concentration.

The results of this study do not show any significant impact of light intensity on microcystin and nodularin concentrations (in environmental samples) – the effect of irradiance on the growth and toxicity of cyanobacteria is unclear and may also be genus- and species-specific (Pliński & Dziopa 2001) – and neither do the results of Redundancy Analysis display any statistical confidence for the relationship between pH and the occurrence of cyanotoxin.

The aim of numerous culture experiments and a large part of the field work has been to determine the factors influencing toxic cyanobacterial growth and the production of different toxins, but there is still no consensus regarding the key factors.

5. Conclusions

A preliminary study concerning the toxin concentration and toxicity of cyanobacterial blooms was carried out in the nearly freshwater Curonian Lagoon in 2006–07. Mass toxic blooms of *Microcystis aeruginosa* were recorded in 2006, and the brackish, toxic cyanobacterium *Nodularia spumigena* was found there for the first time. This latter event was presumably due to an intrusion of Baltic Sea water into the lagoon. The quite high microcystin and nodularin concentrations recorded in the lagoon, widely used for recreation

and fishing could cause a threat to human health. Existing knowledge on toxin-producing cyanobacteria in the Curonian Lagoon is very sparse, so the inclusion of cyanotoxin measurements in the monitoring programme would be beneficial both for water management and ecological studies in this water body.

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