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ORIGINAL RESEARCH ARTICLE

Bioaccumulation of microcystins in invasive bivalves: A case study from the boreal lagoon ecosystem☆

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Summary In the current study we present the first report on the bioaccumulation of microcystins (MC) in zebra mussel *Dreissena polymorpha* from the eutrophic brackish water Curonian Lagoon. The bioaccumulation capacity was related to age structure of mussels and ambient environmental conditions. We also discuss the relevant implications of these findings for biomonitoring of toxic cyanobacteria blooms in the Curonian Lagoon and potential consequences for *D. polymorpha* cultivation activities considered for the futures as remediation measure. Samples for the analysis were collected twice per year, in June and September, in 2006, 2007 and 2008, from two sites within the littoral zone of the lagoon. The highest microcystin concentrations were measured in mussels larger than 30 mm length and sampled in 2006 (when a severe toxic cyanobacteria bloom occurred). In the following years, a consistent reduction in bioaccumulated MC concentration was noticed. However, certain amount of microcystin was recorded in mussel tissues in 2007 and 2008, when no cyanotoxins were reported in the phytoplankton. Considering high depuration rates and presence of cyanotoxins in the bottom sediments well after the recorded toxic blooms, we assume mechanism of secondary contamination when microcystin residuals could be uptaken by mussels with resuspended sediment particles.

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1. Introduction

Toxic algal blooms are of a particular concern in eutrophic aquatic ecosystems, where natural or anthropogenically induced nutrient enrichment leads to enhanced algae and cyanobacteria biomass (Sutcliffe and Jones, 1992). About 300 microalgae species were reported as forming so-called algal blooms. Nearly one fourth of these species have a potential to produce toxic compounds (Hallegraeff et al., 2003). Some of algal toxins may bioaccumulate in aquatic organisms and be transferred through a food chain, reaching critically high concentrations at higher trophic levels (Cazenave et al., 2005; Ferrão-Filho and Kozlowski-Suzuki, 2011; Landsberg, 2002; Rhodes et al., 2001). Due to the wide toxicological effects of these compounds, including neurotoxicity, hepatoxicity, cytotoxicity and dermatoxicity, there is a risk of health hazard for humans, domestic animals and wildlife related to the toxic algal blooms in aquatic ecosystems (Carmichael, 2001; Kujbida et al., 2006; Van Dolah, 2000). Among the toxins produced by cyanobacteria microcystins (hepatotoxins) are probably the most hazardous ones in terms of impact on human health (Carmichael, 1994; Chorus and Bartram, 1999; Funari and Testai, 2008). Microcystins (MC) are very stable (Jones and Orr, 1994; Tsuji et al., 1994), not destroyed by the common water treatment methods (Keijola et al., 1988; Rositano and Nicholson, 1994) and therefore considered having high potential for bioaccumulation in aquatic organisms (Figueiredo et al., 2004; Funari and Testai, 2008; Jonasson et al., 2010; Vasconcelos, 1995).

In this study we address the bioaccumulation of microcystins by the invasive zebra mussel *Dreissena polymorpha* (Pallas 1771), widely distributed and being acknowledged as powerful biofilter (Karatayev and Burlakova, 1994; Karatayev et al., 2002; Nicholls, 2001; Vanderploeg et al., 2002; Zaiko and Daunys, 2012). *D. polymorpha* has an intrinsically high clearance rate that is approximately 10 times that of other freshwater filter-feeding bivalves (Vanderploeg et al., 2002). On the other hand, zebra mussel filtration capacity is highly dependent on the environmental conditions and population structure, and may vary in a wide range (Zaiko and Daunys, 2012).

These bivalves can efficiently accumulate micropollutants, are easy to collect in large numbers and are sedentary, reflecting site specific pollution (Bervoets et al., 2005; Hendriks et al., 1998; Voets et al., 2006). Being themselves resistant to a broad range of environmental conditions (Claudi and Mackie, 1993) and to various types of pollution (Bervoets et al., 2005), they are considered as a proper object for biomonitoring studies (Bervoets et al., 2005; Smolders et al., 2003). Their bioaccumulation abilities may imply important ecological consequences. Zebra mussels are important food source for some fish and water birds thus might be an agent for toxic substances transfer through the food web (Tucker et al., 1996; Zimmermann et al., 1997).

Another implication of cyanotoxins bioaccumulation by zebra mussel is related to its potential use for water quality remediation, recently addressed in several studies (Elliott et al., 2008; Goedkoop et al., 2011; Orlova et al., 2004; Reeders and Bij de Vaate, 1990; Stybel et al., 2009). These issues are particularly relevant for the large transitional ecosystems, such as the Baltic Sea brackish lagoons, with a well pronounced anthropogenically induced eutrophication (Chuseve et al., 2012). Such an option is considered for the Curonian Lagoon as well, and possible *pros* and *cons* being analyzed within the Baltic Sea Region Programme project SUBMARINER ("Sustainable Uses of Baltic Marine Resources"). Since the harvested mussel biomass is not suitable for human consumption, it is often advised for utilization in husbandry as chicken feed, fertilizer or aquafeed for fishfarms (Lindahl et al., 2005; Schernewski et al., 2012; Stybel et al., 2009). Therefore it is important to identify and assess the potential risks of transfer of bioaccumulated toxic substances.

In this study, we present the potential of zebra mussel to be used as indicator of toxic cyanobacteria occurrence in a eutrophic brakish water coastal lagoon and relation of its bioaccumulative capcity to the age structure and ambient environmental conditions. We also discuss the possible consequences for other aquatic organisms and relevant implications of these findings for *D. polymorpha* cultivation activities (e.g., utilization of the zebra mussel biomass in husbandry).

2. Material and methods

2.1. Study area and sampling

The Curonian Lagoon is a large (1,584 km^2), shallow (average depth ~3.8 m) and mainly freshwater coastal body connected to the south-eastern Baltic Sea by a narrow (0.4–1.1 km) Klaipeda strait (Fig. 1). The Nemunas River brings 98% of the total freshwater runoff and enters the lagoon in its central area, dividing the water body into two different parts (Gasiūnaitė et al., 2008). The northern part is a transitory riverine-like system transporting fresh water into the sea, where salinity may episodically increase up to 5–6 psu during wind driven short-term inflow events. Seawater inflows of 1–6 days duration are most common and the seawater intrusions are usually restricted to the northern part of the lagoon in rare cases propagating ≥40 km into the lagoon. The lacustrine southern part is characterized by a relatively closed water circulation and lower current velocities. Therefore, it serves as a main depositional area of the lagoon (Daunys et al., 2006; Galkus and Jokšas, 1997; Gasiūnaitė, 2000; Pustelnikov, 1983).

Most likely, the zebra mussel *D. polymorpha* was introduced into the Curonian Lagoon in the early 1800s. The molluscs would have been attached to timber rafts transported via the Central European invasion corridor (Olenin et al., 1999). However, it may have spread much earlier. According to palaeontological data, *Dreissena* could have existed in the Baltic Sea area during the last interglacial, later becoming extinct, before being re-introduced in the early 1800s (Buynevich et al., 2011; Starobogatov and Andreyeva, 1994).

Zebra mussels are now very abundant in the Curonian Lagoon. They occupy the hard substrates (boulders, embankments, hydrotechnical structures) and soft bottoms (sand, silt or mud) down to 3–4 m depth (Zaiko et al., 2010). The largest area occupied by a zebra mussel community is located in the central part of the lagoon (Gasiūnaitė et al., 2008; Olenina, 1997; Zaiko et al., 2009).

Zebra mussels (*D. polymorpha*) were collected twice per year, in June and September, in 2006, 2007 and 2008, from two sites within the area of the natural zebra mussel

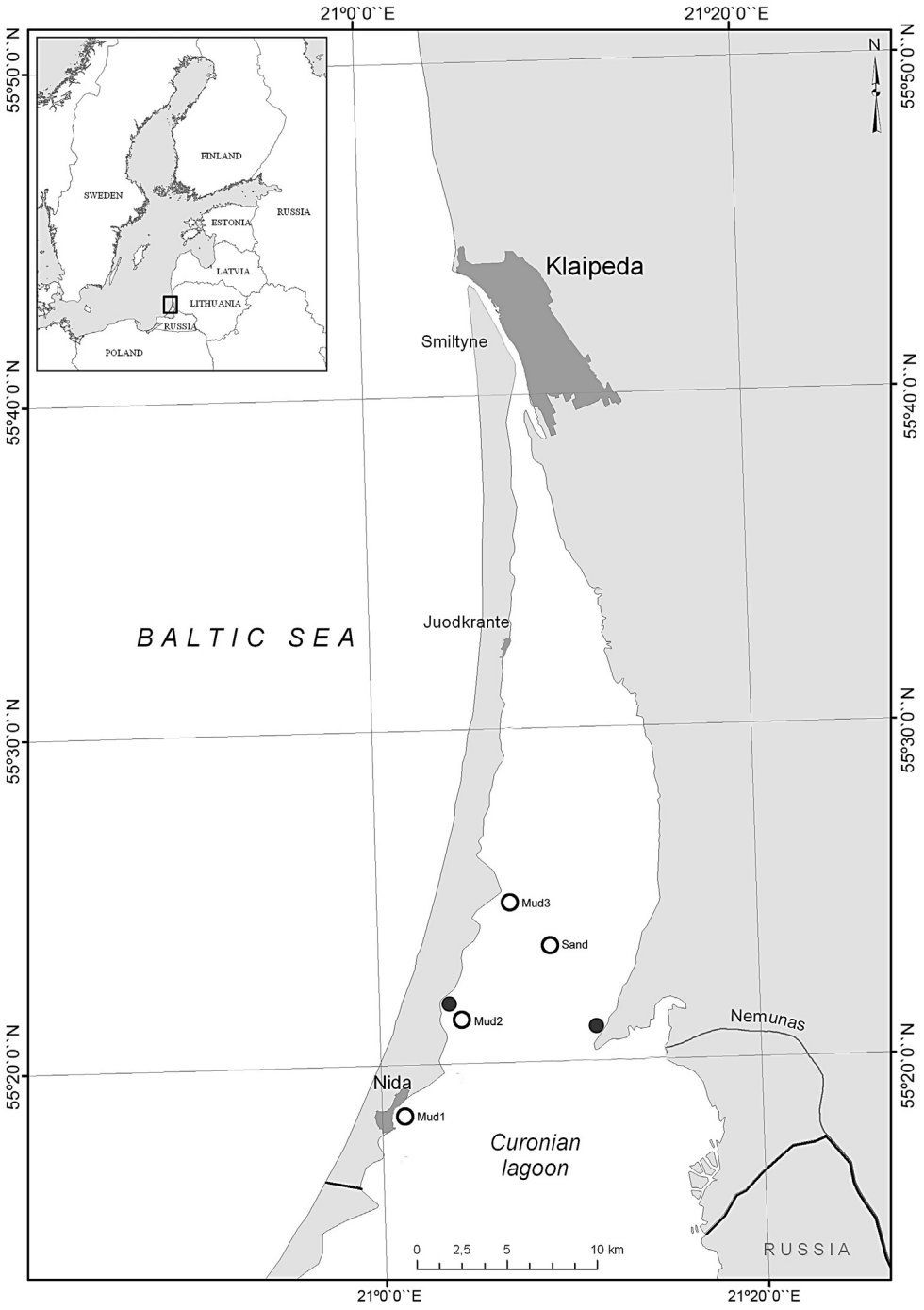

Figure 1 Location of the sampling sites in the central part of the Curonian Lagoon. Filled circles indicate zebra mussel sampling sites, open circles — sediment sampling sites (from muddy and sandy substrates accordingly).

distribution (Fig. 1). Clumps of mussels were collected manually by wading in the littoral, at 1—1.5 m depth. After collection, mussels were immediately transported to the laboratory, where individual mussels were separated from clumps and frozen at −20°C until analysis of toxins was performed. Three size classes of the collected mussels were distinguished: <10 mm length, 10—30 mm length and >30 mm length. In total, 108 mussels were collected and analyzed.

Sediment core samples were collected from a boat in 2008, July and October. In total, four sites were sampled: sandy sediment in the open lagoon (Sand), muddy sediment in the littoral zone (Mud1), muddy sediment in the open lagoon (Mud2, Mud3) (Fig. 1). 1 cm^3 of the upper 0—5 cm section of each core was removed and stored frozen into 15 ml centrifuges tubes until the later analysis.

2.2. Extraction of toxins

ELISA and protein phosphatase 1 inhibition assay (PPIA 1) are the most sensitive methods widely used for determination of microcystin (Adamovsky et al., 2007; Amorim and Vasconcelos, 1999; Babica et al., 2006; Kankaanpaa et al., 2007;

Msagati et al., 2006; Nicholson et al., 2007; Sipia et al., 2006; Yu et al., 2002). ELISA is often advised for the analyses of cyanobacterial toxins when their concentrations are lower than high-performance liquid chromatography (HPLC) detection limit (Mazur-Marzec et al., 2006). However, occasionally ELISA can give false positive results, therefore to confirm the occurrence of microcystin in samples PPIA was additionally employed.

Mussels and sediment samples were lyophilized (TEGA, Germany) and then extracted using 30 ml of pure methanol per 1 g mussel and sediment dry weight. Extracts were disrupted by sonication (5 min) and then centrifuged for 15 min at 10,000 rpm 20°C. The solvents were removed by rotary evaporation and the residue was re-dissolved in 1 ml of MiliQ water. After that samples were vortexed for 1 min and then centrifuged for 15 min at 12,000 rpm 20°C. Later on, the samples were subjected to solid phase extraction on Sep-Pak Vac C18 cartridges (200 mg, Waters, Massachusetts, USA).

Chlorophyll *a* was extracted by adding 80% ethanol to sediment samples (Jespersen and Christoffersen, 1987). After 24 h samples were centrifuged and obtained supernatant analyzed spectrophotometrically according to Lorenzen (1967).

2.2.1. ELISA and protein phosphatase 1 inhibition assay (PPIA)

Extracts of mussels and sediments were diluted in MilliQ water (10—5,000 times) and analyzed by enzyme-linked immunosorbent assay (ELISA). The ELISA test was performed using the EnviroGuard kit (Strategic Diagnostics, Newark, DE, USA), according to the manufacturers' instructions. The same extracts were also analyzed by colorimetric protein phosphatase 1 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), $MgCl_2{\cdot}6H_2O$, $MnCl_2{\cdot}4H_2O$, Na_2SO_4, *p*-nitrophenyl phosphate (*p*-NNP — the substrate), tris-(hydroxymethyl)-aminomethane (Tris) were of analytical grade. The substrate and enzyme buffers were prepared immediately before the test. Catalytic subunits (2.5 U) of commercially available enzyme (PP1; New England Biolabs, USA) were diluted in 1.5 ml of the enzyme buffer. Subsequently 10 μl of standard solutions or sample were added to the well and mixed with 10 μl of PP1 in buffer. After 5 min incubation, 200 μl 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-h incubation at 37°C, the absorbance of the solutions was measured. The plates with ELISA and PPIA tests were read on VERSAmax microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 450 nm (ELISA) or 405 nm (PPIA), respectively.

2.2.2. Statistical analysis

An exploratory data analysis showed that the values of microcystin concentrations in *D. polymorpha* tissues (including zero values) had considerably right-skewed distributions with several very apparent outliers. Therefore it was decided to apply log-transformation for the data, in order to minimize the effect of outliers. Since general assumptions of the parametric analysis methods (Shapiro—Wilk normality test, $p < 0.014$; Fligner—Killeen test of homogeneity of variances, $p < 0.05$) were not met neither before nor after transformation applied, the further data analysis was performed using non-parametric methods.

To compare the results of microcystin concentrations gained by two different analysis methods (ELISA and PPIA), the non-parametric Wilcoxon signed rank test was applied. Non-parametric Kruskal—Wallis test was applied to compare microcystin concentrations found in muddy and sandy sediments in 2008. The multivariate effects of the studied factors — time of sampling (combining year and month), sampling site and mussel size — on the concentration of microcystins in the mussel tissues were analyzed by statistical program PRIMER 6 & PERMANOVA (Anderson, 2001, 2005). The test-statistic is a multivariate analogue to Fisher's *F*-ratio and is calculated directly from any symmetric distance or dissimilarity matrix. *p*-Values are then obtained using permutations. In the current study the Euclidean distance similarity measure was used to construct the similarity matrices. The statistical differences between the factor levels were assessed by four-way PERMANOVA with "time of sampling" (6 levels), "size" (3 levels) and "location" (2 levels) as factors. The permutation of raw data was used as this method is recommended in the case of relatively small sample size (Anderson and Robinson, 2001). When a factor and/or interaction was identified as significant ($p < 0.05$), post hoc PERMANOVA pair-wise tests were conducted to detect which levels were responsible for significant differences. Multiple regression was applied to the log-transformed microcystin concentrations data from the zebra mussel tissues with mussels size and sampling time as explanatory variables.

3. Results

Microcystin concentration in mussel tissues varied from values below the detection limit to 139 ng/gDW when measured with ELISA test and from values below the detection limit to 284 ng/gDW when measured with PPIA. Although the pair-wise comparison of the two applied sample analysis methods, ELISA and PPIA, has shown no significant differences in the obtained results ($W = 1.13$, $p = 0.26$), in concentrations higher than 10 ng/g DW PPIA tended to give greater values. In order not to lose any data and minimize the undesirable bias, the results of the both tests were considered in the multivariate analysis as response variables. The site location was the only environmental factor recorded along with the sampling date and the size of analyzed mussels; however it appeared to be not statistically significant in relation to the measured microcystin concentrations. The statistical significance of the factors and their interactions obtained with PERMANOVA analysis are presented in Table 1.

The post hoc PERMANOVA pair-wise test indicated significant differentiation of the 2006 data ($p < 0.05$), as well as data got from the smallest (<10 mm) mussel size group ($p < 0.05$) (Fig. 2). The highest microcystin concentrations were measured or determined in mussels longer than 30 mm collected in 2006. Then, in the following years, a consistent reduction in the MC concentration was noticed (Fig. 3).

Microcystin concentration measured in sediments in 2008 with ELISA test varied between 0.80 and 28.20 ng/g DW, and between 0.02 and 38.07 ng/g DW when measured

Table 1 PERMANOVA based on Euclidean distances of the obtained microcystins concentrations in zebra mussels in response to the time of sampling, location, size group and their interactions.

Source	*df*	MS	Pseudo-*F*	*P* (perm)
Time	5	43.26	393.01	0.001
Location	1	0.26	2.19	0.132
Size	2	9.98	83.74	0.001
Time × location	5	0.58	4.89	0.003
Time × size	10	9.38	78.69	0.001
Location × size	2	0.44	3.72	0.03
Time × location × size	10	0.52	4.41	0.001
Residual	72	0.12		
Total	107			

with PPIA. Yet, the pairwise comparison of the results obtained by the two applied analysis methods, has not shown any significant difference ($W = 1.22$; $p = 0.63$). Significantly higher concentrations were observed in muddy bottom habitats, comparing to the sandy ones (KW-H = 13.29; $p = 0.004$). Chlorophyll *a* concentration at the surface sediment layer corresponded well with the microcystin concentrations in sediments (Fig. 4) and varied between 22.11 mg/m^3 (in sandy bottom) and 39.94 mg/m^3 (in muddy bottom) in July 2008, and between 26.19—77.91 mg/m^3 in October 2008 (in sandy and muddy bottom respectively).

4. Discussion

Not all cyanotoxins provided by ecosystem are assimilated effectively by filter-feeding organisms since part of them may be rejected as faeces or pseudo-faeces. The other part may be irreversibly bound to protein phosphatases or metabolized (Vasconcelos, 1995). Variation in microcystin accumulation rates reported earlier was predominantly related to species intrinsic features, mainly due to uptake routes and detoxification abilities (Zurawell et al., 2005). Accumulation

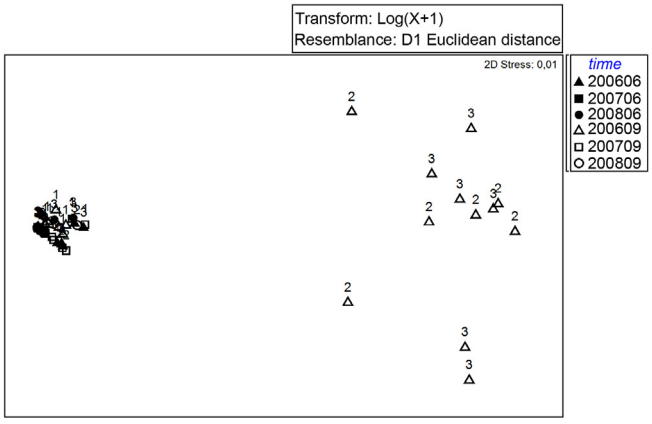

Figure 2 A non-metric MDS plot visualizing the multivariate distance among microcystin concentrations in mussels of different sizes (1 — <10 mm length; 2 — 10—30 mm; 3 — >30 mm), sampled during 3 sampling sessions (June 2006; September 2006; June 2007; September 2007; June 2008; September 2008).

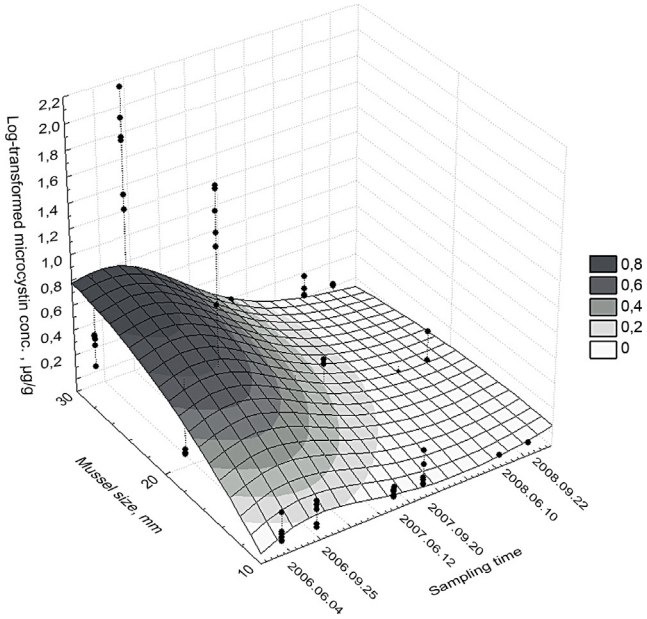

Figure 3 Log-transformed microcystin concentrations obtained from zebra mussel tissues as a function of mussel size and sampling time. Circle spots indicate actual measurements and the surface represents the regression model, color scale indicates the respective microcystin concentration levels.

abilities might differ among mollusks due to their feeding habits (grazing, filtering), respiration mode (aerial, aquatic), specific ecological, physiological traits and life history strategy (Dillon, 2000; Gérard et al., 2008).

However, there are evidences that bioaccumulation and depuration rates of filter-feeding bivalves are also highly influenced by environmental factors, mainly by temperature (Bayne et al., 1977; Yokoyama and Park, 2003), salinity (Amorim and Vasconcelos, 1999) and food (seston) quality and availability (Hawkins et al., 2001). The higher risk of contamination with cyanotoxins is related to the direct exposure of mollusks to the heavy cyanobacteria blooms (Amorim and Vasconcelos, 1999).

In the current study the highest concentrations of microcystin were detected in large mussels (≥30 mm length) collected in 2006. These findings are consistent with the results of toxicological plankton study conducted in 2006—2008 (Paldavičienė et al., 2009). In 2006, heavy cyanobacteria bloom with a distinct prevalence of *Microcystis aeruginosa* was reported. That year, intracellular microcystin (predominantly microcyctin-LR) was detected in 75% of the samples collected during the bloom, with concentrations ranging from <0.1 to 134.2 μg/l. In 2007, cyanobacteria from the genera *Planktothrix*, *Limnothrix*, *Woronichinia* were detected, but they did not form a bloom in the Curonian Lagoon. Cyanotoxins were detected only in 4% of all investigated samples in 2007. In the next year (2008), *Aphanizomenon flos-aquae* dominated the cyanobacterial community, however, no cyanotoxins were reported in the samples (unpublished study results). Therefore our results showed that bioaccumulated MC concentration coincided well with the production of toxins by cyanobacteria, and was reducing gradually due to depuration and natural shift of mussels in the population.

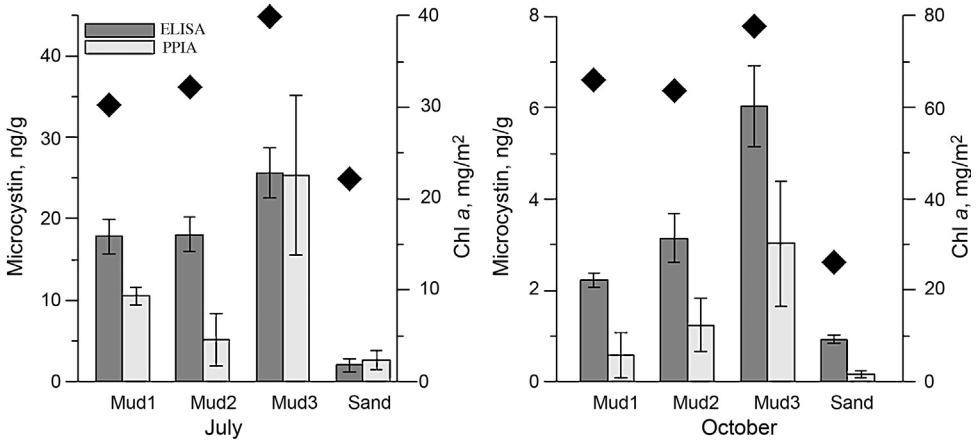

Figure 4 Microcystin concentration in the upper sediment layer collected in 2008.

The size of bioaccumulating organisms may also play an important role since this parameter is related to the filtration and depuration rates (Amorim and Vasconcelos, 1999). Thus there could be at least several explanations of the current results indicating higher microcystin concentrations in larger mussels comparing to the small ones.

Adult zebra mussels can exploit cyanobacteria as food in the water column, irrespective of the size, shape, form and toxicity of these phytoplankton species. It is also known that zebra mussels could alter phytoplankton communities and promote Microcystis (Fahnenstiel et al., 1995; Vanderploeg et al., 2002; Woller-Skar, 2009). Large mussels even seem to prefer cyanobacteria over other phytoplankton groups and detritus. Mussels larvae, on the contrary, can effectively filter and utilize small-sized cyanobacteria only if the latter do not contain (much) microcystin (Naddafi, 2007). The larvae show higher mortality, decrease in growth and fecundity rates when fed upon MC containing strains of cyanobacteria than if MC is lacking (Gérard and Poullain, 2005; Gérard et al., 2009; Lance et al., 2007). In contrast, the adult mussels easily survive on a diet of toxic cyanobacteria (Dionisio Pires et al., 2004). The toxic bloom in 2006 was reported in mid-August (Paldavičienė et al., 2009), after the first settlement peak of zebra mussels spat in June (unpublished study results), and well before the late settlement (in August—September) occur. It means that in September (when the highest microcystin concentrations were detected in zebra mussel tissues) there was a higher probability to find among newly settled mussels (<10 mm length) those that have not been (or have been marginally) exposed to the toxic bloom during their larval and post-veliger stages.

The morphological characteristics of cyanobacteria, like cell or colony size may also affect the bioaccumulation capacities of zebra mussels. According to earlier findings, toxins are mainly produced by cyanobacteria which form larger colonies (>500 μm) (Chorus and Bartram, 1999; Kurmayer et al., 2002). These forms are less available for consumption by young mussels or larvae.

Large bivalves (like *Unio*, *Anodonta* and *Dreissena*) may accumulate a notable quantity of cyanotoxins in the field, but seem to be rather insensitive to them (Bij de Vaate et al., 2010; Ibelings et al., 2005; Watanabe et al., 1996). The bigger and older mussels could have experienced contact with toxic cyanobacterial blooms more than once or for a longer period during their life. Thus, there is a probability to find some residual concentrations of cyanotoxins in mollusk tissues a certain time after exposure due to incomplete depuration (Mazur-Marzec et al., 2006). Our results, as well as results of other studies (Amorim and Vasconcelos, 1999; Yokoyama and Park, 2003), confirm the long-lasting persistence of microcystin in environment and filter-feeding organisms. Even devoid of cyanotoxins in water, a certain amount of toxins have been detected both in sediment samples and zebra mussel tissues two years after exposure to the toxic bloom (Figs. 2 and 3). The increased stability of the toxin might be a result from slower biodegradation at low water temperatures or/and from the binding of hepatoxins to sediment particles (Mazur-Marzec et al., 2006). Also, it is known that in temperate waters, vegetative filaments of potentially toxic cyanobacteria may form benthic overwintering populations (Gérard et al., 2009). As we did find considerable concentrations of microcystins in the bottom sediments at all sites sampled in 2008 (Fig. 4), when no toxic bloom was detected, it is possible to hypothesize that microcystins absorbed to the sediment particles could have persisted from previous years. That is consistent with a number of studies (Chen et al., 2005; Lahti et al., 1997; Latour et al., 2007; Zakaria et al., 2007) stating that microcystins and their degradation products could persist in bottom sediments for more than a decade (Pawlik-Skowrońska et al., 2010). Therefore, considering the resuspension as one of the most common phenomena in the shallow Curonian lagoon (Pilkaitytė and Razinkovas, 2006), residuals of toxic compounds could be uptake by mussels with resuspended sediment particles not only in 2006 but also in 2007 and 2008 when no toxic blooms were detected. Resuspension also could explain the presence of comparatively high microcystin concentrations in mollusks well after the toxic blooms the same year (Fig. 5) as zebra mussels is known for quite high depuration rates (Dionisio Pires et al., 2004). On the other hand, toxic cyanobacteria could have be also present but not detected in the water column in 2007 and 2008, due to low density or great spatio-temporal variability, despite the obvious mechanism of secondary contamination.

Due to their feeding behaviour, generally wide distribution and abundance, close association with benthic sediments and

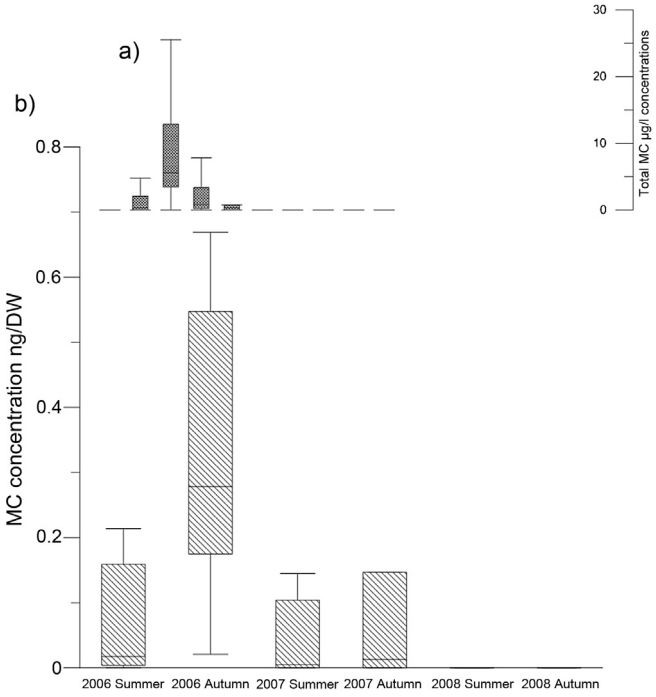

Figure 5 Microcystin concentration in phytoplankton (upper figure a) and in *Dresissena polymorpha* during the in situ investitation (b) in 2006—2008.

relatively sedentary nature, zebra mussels are considered as a proper indicator of water contamination (Lefcort et al., 2002; Salanki, 2000). Based on the current results and taking into account that mussels accumulate microcystins both by grazing upon toxic phytoplankton and uptaking the dissolved (extracellular) toxins (Prepas et al., 1997), we suggest zebra mussels as a good biomonitor of cyanotoxins in the ecosystem. Toxic compounds bound in mussel tissues may have important implications for the good environmental status of ecosystem, socio-economic aspects and even human health.

From the Curonian Lagoon it is known that zebra mussels are consumed by vimba (*Vimba vimba*), white bream (*Blicca bjorkna*), roach (*Rutilus rutilus*), invasive round gobies (*Neogobius melanostomus*) and some other benthophagous fish and waterfowl (Kublickas, 1959). Although, the smaller individuals are usually preferred (Nagelkerke et al., 1995; Ray and Corkum, 1997). However, the analysis of microcystins distribution in the foodweb showed no evidence of biomagnification occurring through the benthic food chain based on Dreissena (Ibelings et al., 2005).

Another implication is related to the potential use of zebra mussels in water quality remediation and subsequent utilization of the cultured biomass. Our data suggest that utilization of *D. polymorpha* cultured under toxic bloom conditions may pose some risk for husbandry or add to intoxication of economically important aquatic species. Due to higher bioaccumulation capacity and incomplete depuration long time after exposure, larger mussels are of a higher concern comparing to the young ones. Therefore for remediation of coastal lagoons, we suggest considering seasonal (May—October) zebra mussel cultivation approach. This would ensure sufficiently effective extraction of nutrients by newly settled mussels avoiding the risk of severe intoxication with cyanotoxins. Anyway, proper monitoring of cyanotoxin concentration in the water during the cultivation season should be undertaken.

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