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Dynamics of toxic HABs in the Curonian Lagoon, Baltic Sea during 2010-2013

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Summary
Identification of cyanobacterial hepatotoxins using HPLC-MS-MS demostrated the presence of several Microcystin-congeners in Curonian lagoon, 2010-2013. MC–LR, -RR, -YR and its demethylated forms were most abundant and frequent. The positive correlation between total content of dissolved and intracellular microcystins was found with biomass of 3 *Microcystis* species. *M. viridis* was most responsible for microcystin producing in the study area. *Aphanisomemon flos-aqua* dominance correlates with low MCs content.

Introduction
Curonian Lagoon, is a hyper-trophic, mostly freshwater water body, with well-developed commercial fishery and recreational activity. Since late 1990s, a continual increase of frequency, scale and duration of harmful algal blooms (HABs) occurs. Frequent cases of mass mortality of hydrobionts during HABs, including cool period, let to suppose toxic cyanobacterial metabolites as one of the reasons. In 2010-2013 we tried to monitor dynamics of cyanobacterial toxins in the Lagoon coastal zone in National Park. Composition and abundance of phytoplankton was studied seasonally and during presence of visual signs of bloom; the qualitative screening of microcystins in water by a rapid immunochromatographic test was performed. Positive samples were checked by high pressure liquid chromatography (HPLC)-tandem mass-spectrometry.

Materials and methods
Material collected at 6 littoral and 2 pelagic sampling sites during May – November, 2011–2013. In the first year of observation (2010) only samples for toxin detection were collected during bloom events 2010 (July, August and October). Phytoplankton sampling and further analysis were performed according to HELCOM guidelines. Abraxis Microcystin Strip Test for recreational waters was used for a microcystins/nodularins field screening. The method of HPLC-tandem mass-spectrometry with high-resolution has been used for reliable confirmation of cyanotoxins presence, identification of their structures. Water samples were filtered and divided into filtrate (contains dissolved toxins) and phytoplankton biomass (contains intracellular toxins). Later these parts of sample were analyzed separately. Liquid chromatograph-mass-spectrometer-LTQ OrbiTrap with linear and orbital ion traps and electrospray-ionization in positive mode (ESI+) was used. For analysis of experimental tandem spectra the Mass Frontier 5.0 QuickStart program was used.

Results and Discussion
The lagoon is classified as hypertrophic from 2000s (Alexandrov 2010). During 2011-2013, study area remains hypertrophic, average summer biomass exceeds 10 gm^{-3}. Bloom-forming cyanobacteria belong to Aphanizomenon, Anabaena, Microcystis, Planktothrix, and Woronichinia genera, a number of potentially toxic species were marked between them. During 2010-2013 the most frequent dominants were *Anabaena flos-aquae, Aphanisomemon flos-aquae, Microcystis viridis, Planktothrix agardhii.*

Field screening have shown the presence of various microcystins in water from all sampling sites during summer-autumn period (23.07.10, 18.11.10, 22.07.11, 30.08.11, 19.09.11, 02.11.11, 24.11.2011, 23.05.2012, 27.06.2012, 06.07.2012, 09.08.2012, 08.10.2012, 23.11.2012, 27.07.2013, 16.09.2013, 15.10.2013). It was confirmed and quantified by HPLC – MS – MS, several peaks of microcystins content during the year were identified: first usually in July, then in August and in some autumn months.

<u>2010</u> ΣMCs in water was low and varies in 0.04 – 0.34 μg L^{-1}, MC–LR prevailed. MC -RR, - YR and its demethylated congeners were also detected. Intracellular content of microcystins was 0.17 – 0.81 μg L^{-1} (Table 1). MC–LR and -RR were prevailing with maximal content in July during polydominant cyanobacterial bloom (*Microcystis aeruginosa, M. viridis, M. wesenbergii, Aph. flos-aquae,* others)

<u>2011</u> During 2011 most abundant MC congeners were MC–LR, -RR, -YR and its demethylated variants. ΣMCs in water reaches 194.8 μg L^{-1}, maximal content of MC–LR (93.7 μg g^{-1}) was detected in the end of July, along with a high content of MC-RR (64.4 μg L^{-1}). Intracellular content of ΣMCs reach 3660 μg g^{-1} (Table 1). *Aph.flos-aquae* was dominant in sampling sites (biomass up to 407.4 g·m^{-3}), but strong positive correlation of ΣMCs, as well as dissolved MC–LR, has been found with biomass of *Microcystis* species. During autumn hyper-bloom of *Aph. flos-aquae* (biomass to 3912.7 g·m^{-3}), ΣMCs content was low –1.3 μg L^{-1} .

<u>2012</u> In the end of August absolute maximum of dissolved ΣMCs (152.6 – 290.5 μg L^{-1}) was detected, but in pelagic zone of lagoon. In the littoral maximal value of ΣMCs 141.2 was μg L^{-1} . Content of MC–LR was high, up to 65.5 μg L^{-1}, but the main MC-congeners were MC-RR (45.1 – 167.4 μg L^{-1}) and demethyl-MC–RR (26.8 – 68.4 μg L^{-1}). Intracellular content of ΣMCs varied 1.3-658.2 μg g^{-1} (Table 1), maxima were measured in July, August and October. Dominant species *Aph. flos-aquae* and *Microcystis spp.* could change each other in time and space or co-dominate.

<u>2013</u> In 2913 the content of dissolved MCs was relatively low, maximum (ΣMCs = 14.9 μg L^{-1}) was measured in October (Table 1), main congeners were demethyl-MC-RR and MC-RR. Intrecellular content of ΣMCs varied in wide range, maximum was recorded in October for demethyl-MC–RR ($[M+H]^{+}$= 1024) (up to 3618 μg g^{-1}). Dominants *Aph. flos-aquae* and *Microcystis spp.* changed each other. The positive correlation between ΣMCs and MC-LR content in water and biomass of 3 *Microcystis* species has been found. *M. viridis*, dominanting among Microcystis genus, was most responsible for microcystin producing in water bodies. *Microcystis spp.* blooming in terminal phase is followed very fast by *Aph.flos-aqua* dominance, but its toxic metabolytes stayed in water/decaying biomass long time. Pure domination of *Aph.flos-aqua* coincides with low ΣMCs content, but can correlates with sharp neurotoxic effect.

Table 1 The ranges of summary microcystins and MC – LR content in Curonian Lagoon in 2010-2013.

Years	Water sample, μg L^{-1}		Biomass sample, μg g^{-1}(d.w.)		Domination species
	ΣMCs	MC - LR	ΣMCs	MC - LR	
2010	0.04-0,34	0.01 – 0.15	0.2 – 0.8 (w.w.)	0.01-0.17 (w.w.)	*M. aeruginosa, M. viridis, M. wesenbergii, Aph. flos-aquae*
2011	0.24 – 194.76	<LOD- 93.68	304.3 – 3660.7	12 - 1714	*Aph. flos-aquae*
2012	0.04 – 18.8 152.6 – 290.5 *	0.01 - 334 25 – 65 *	1.27 – 658.2	0,05 - 151,5	*Aph. flos-aquae, Microcystis spp.*
2013	2.2 – 14.9	0.07 – 0.46	25 - 4719	0,3 - 77	*Aph. flos-aquae, Microcystis spp.*

* pelagic sampling sites

References

Alexandrov, S.V. 2010. The effect of climate change on the level of euthrophication of Curonian Lagoon. Vestnik RGU, Natural sciences, Kaliningrad, 1: 49-57.