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Accumulation and effects of cyanobacterial microcystins and anatoxin-a on benthic larvae of *Chironomus* spp. (Diptera: Chironomidae)

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Abstract. Larvae of Chironomidae are distributed world-wide and are very abundant in eutrophic water bodies affected by cyanobacterial blooms. However, there is little information on the effect of cyanobacteria and their metabolites on these aquatic organisms. Our studies revealed that benthic species of *Chironomus* inhabiting a hypertrophic lake where blooms of microcystin (MC) and/or anatoxin-a (ANTX)-producing filamentous *Planktothrix agardhii*, *Dolichospermum* spp. and *Cuspidothrix issatschenkoi* occur, fed on these cyanobacteria and accumulated cyanotoxins. Up to 3.2 μg MCs g^{-1} F.W. and up to 185 μg ANTX g^{-1} F.W. were detected. Of the four MC variants detected in the cyanobacterial biomass [Asp^3, Dhb^7]MC-RR and MC-LR prevailed, whereas in the larvae it was [Asp^3, Dhb^7]MC-RR and MC-LA. The effect of pure MC-LR and ANTX as well as crude extracts of MC-producing *P. agardhii* and ANTX-producing *D. lemmermannii* on lake and riverine larvae of *Chironomus* spp. was also compared. The assays revealed that pure cyanotoxins (concentrations: 0.83–3.32 mg L^{-1}) were generally less toxic to riverine larvae than cyanobacterial extracts containing approximately 10-times less toxins. The survival of both the lake and riverine *Chironomus* larvae did not decrease when exposed to environmentally relevant concentrations of cyanotoxins (< 0.20 mg L^{-1}). The larvae were also highly resistant to higher amounts (up to 0.35 mg ANTX L^{-1} and 0.42 mg MCs L^{-1}) of extracellular toxins. In the natural environment, *Chironomus* larvae exposed to toxins contained in cyanobacterial prey, dissolved in water and/or bound to bottom sediments may be very important vectors of cyanotoxins to higher levels in aquatic food chains. To the best of our knowledge, this is the first report on the accumulation of ANTX and effects of cyanotoxins on *Chironomus* larvae.

INTRODUCITON

Water blooms formed by cyanobacteria that produce various toxins, such as hepatotoxic microcystins (MCs), neurotoxic anatoxin-a (ANTX) etc. (Carmichael, 1992; Welker & Döhren, 2006), which may affect aquatic organisms at various levels in the food chain (Ferrão-Filho & Kozlowsky-Suzuki, 2011). The sensitivity of hydrobionts to exposure to cyanotoxins differs greatly. There is little information on the effect of cyanobacterial toxins on benthic insect larvae, such as *Chironomus* spp., which are widespread and abundant in eutrophic water bodies (Armiatge et al., 1995; Frouz et al., 2003; Chen & Xie, 2008). Larvae of *Chironomidae* play an important role in aquatic ecosystems, e.g. as a prey of fish, and are good indicators of water quality (Armiatge et al., 1995).

Toxin-producing cyanobacteria develop abundantly in the pelagic zone (Sivonen et al., 1990; Pawlik-Skowrońska et al., 2008), but may also form mats (e.g. *Oscillatoria limosa* At. ex Gom. and *Oscillatoria tenuis* C. Agardh) on the bottom of various water bodies (Mez et al., 1997). Planktonic species, like the microcystin-producing *Planktothrix agardhii* (Gom.) Anagn. et Kom., *Microcystis* spp. and *Dolichospermum* spp. (syn. *Anabaena*), which may produce both MCs and ANTX, overwinter on bottom sediments (Hašler et al., 2004). As a consequence, MCs, ANTX and other cyanotoxins bind to lake sediments (Pawlik-Skowrońska et al., 2010; Klitzke et al., 2011), particularly to the upper layers, where they may affect the benthic fauna. Currently little is known about the accumulation and influence of cyanotoxins on benthic macro-invertebrates (Kotak et al., 1996; Chen & Xie, 2008; Ibelings & Havens, 2008), in particular, the consequences of exposing the aquatic fauna to ANTX. There are several reports that exposure of fish to ANTX in the laboratory (Oberemm et al., 1999; Osswald et al. 2007) and under natural (Pawlik-Skowrońska et al., 2012) conditions results in it accumulating and damaging the tissue of edible fish. There is no information on ANTX accumulation and effect of cyanotoxins on benthic larvae of *Chironomus* spp. As chironomids are non-selective feeders (Ali, 1990) cyanobacteria (including potential toxin-producers, like *Anabaena*, *Oscillatoria*, *Lyngbya* and *Microcystis*) are an essential source of food for the larvae. For example, cyanobacteria are predominant (52–84% of food) in guts of larvae of *Chironomus crassicaudatus* Malloch living in a lake in Florida (Ali, 1990). Therefore, in eutrophic waters where cyanobacterial blooms occur, there is the possibility that cyanotoxins will be in the trophic chain and are potential hazard for fish and their consumers.

We hypothesized that *Chironomus* larvae inhabiting water bodies affected by perennial blooms of toxigenic

cyanobacteria accumulate and are tolerant of cyanotoxins. The effects of MCs and ANTX on lake and riverine populations of *Chironomus* were experimentally compared.

MATERIAL AND METHODS

Study area, sampling and analysis of cyanobacteria

This study was carried out in the shallow flow-through Lake Syczyńskie (E. Poland) in which perennial blooms of toxigenic cyanobacteria occur (Pawlik-Skowrońska et al., 2008; Toporowska et al., 2010). Water samples for chemical analyses and for qualitative and quantitative analyses of cyanobacteria and cyanotoxins were collected from the uppermost (0–0.5 m) and near-bottom (2.4–2.8 m) water layers once a month (April–November) in 2008. The biomass of cyanobacteria was based on counts and measurement of algae obtained using an inverted microscope (Utermöhl, 1958). For cyanobacteria with straight filaments, a length of 100 µm was counted as one individual. One coil of coiled *Dolichospermum* spp. (syn. *Anabaena*) and one colony of coccoid cyanobacteria were recognised as individuals. The taxonomic identification was carried out mostly following Komárek (1996), Komárek & Anagnostidis (2005) and Wacklin et al. (2009).

Organisms

Chironomus larvae were collected by means of a hand net (diameter of 40 cm and mesh size of 250 µm) from Lake Syczyńskie 5 times (from April to October) in 2008. Larvae were separated in a laboratory and stored in organic sediments (temp. 3–4°C) until used in the toxicological experiments. In toxicological experiments, *Chironomus* larvae (commercially available) collected from River Przemsza (S. Poland) were also used. The nomenclature of Chironomidae larvae followed Wiederholm (1983). Both the lake and riverine organisms were over 20 mm long.

Lake larvae for cyanotoxin analyses weighed from 0.08–5.28 g F.W. and were frozen (–20°C) until the day of toxin extraction. Photographic documentation of the gut contents of the lake *Chironomus* sp. was done using a camera mounted on a light microscope.

Physical-chemical analyses

The physical-chemical parameters of the water in the uppermost and bottom layers of the lake were measured once a month. Biogenic nutrients were determined according to Golterman (1971) and chlorophyll-a according to PN-ISO 10260 (2000). The Carlson Trophic State Index (TSI), based on water transparency measurements (Secchi disc), was calculated according to Carlson (1977).

Cyanotoxin extraction

For the determination of intra- and extracellular MCs and ANTX, 0.5–1.0 L of lake water was filtered through Whatman GF/C filters, and extracts of the filtered off phytoplankton were prepared using ultrasonication (3 times for 5 min, 50 W, ultrasonic homogeniser Sonoplus, Bandelin) in 75% (v/v) methanol (Merck, pure p.a.) containing 0.002 M HCl. After centrifugation (14,000 rpm for 10 min, 17°C), supernatants were collected and frozen (–20°C) until required for cyanotoxin analysis. Crude extracts of the scum of *Planktothrix agardhii* and *Dolichospermum lemmermannii* Richt. in Lemm. (collected in the lake) were prepared using ultrasonication (3 times for 5 min). After centrifugation, supernatants were frozen (–20°C). *Chironomus* larvae were first homogenised in acidified 75% (v/v) methanol (Merck, pure p.a.) and then ultrasonicated (2 times for 5 min). After centrifugation (14,000 rpm for 10 min, 17°C), supernatants were collected and purified with n-hexane (1:1, v/v) and then frozen.

HPLC-DAD analysis of microcystins

The HPLC-photodiode array detection system (Shimadzu) was used for microcystin detection and identification. The detection range was 200–300 nm. MC-LR, -RR, -YR, -LA, -LY, - LW, -LF, -WR (Alexis) were used as standards. Extracts were separated using acetonitrile (Merck) acidified with 0.05% trifluoroacetic acid (gradient 30–100%) at a flow rate of 0.7 ml/min in a RP-18 Purosphere column (125 × 3 mm, Merck).

HPLC-FLD analysis of anatoxin-a

ANTX in extracts of cyanobacteria and *Chironomus* was determined using liquid chromatography (HPLC, Beckman) with fluorescence detection (Shimadzu) according to James et al. (1998) and Furey et al. (2005). For ANTX derivatization, 10% NBD-F (4-fluoro-7-nitrobenzofuran; Fluka) was used. The detector parameters were as follows: excitation wavelength 470 nm and emission wavelength 530 nm. Extract separation was obtained using a RP-18 Purosphere column (125 × 3 mm, Merck) and TFA (0.05%) acidified acetonitrile at a flow rate of 0.6 ml/min. For identification and quantitative determinations, standard ANTX (Tocris, Bioscence) was used.

Toxicological experiments

Toxicity of MC-LR and ANTX standards and extracts of cyanobacterial scum containing MCs or ANTX, for larvae of *Chironomus* spp. indigenous to Lake Syczyńskie and those collected from a river was evaluated using 48- and 96-h bioassays. The survival of larvae exposed to cyanotoxins was estimated. The death of organisms was verified by touching them with tweezers. Series of three dilutions of cyanotoxin standards (0.83–3.32 mg L^{-1}), three dilutions of crude extract of *P. agardhii* (0.11–0.91 mg MCs L^{-1}) and four dilutions of the crude extract of *D. lemmermannii* (0.06–0.35 mg ANTX L^{-1}) were prepared in standard freshwater. The assays were performed twice, each time in three replicates in darkness, at room temperature (20 ± 1°C). Due to the low number of larvae from Lake Syczyńskie, the assays were only done using extracts of cyanobacteria. The influence of 1% methanol (the maximum concentration of the solvent for MC-LR) on the survival of lake and riverine larvae was also determined and no effect was observed.

RESULTS

Bloom-forming cyanobacteria and cyanotoxin production

In the shallow hypertrophic (TSI_{SD} = 65–82) lake, the physical-chemical conditions supporting mass development of cyanobacteria were similar at the surface and the near-bottom of the lake (Table 1). The vertical distribution of cyanobacteria and oxygen concentration in the lake water were also homogenous. The seasonal average values of the total biomass of cyanobacteria ranged from 1.2 mg L^{-1} in spring to 92.9 mg L^{-1} in autumn (the maximum exceeded 100 mg L^{-1}). In spring, ANTX-producing Nostocales with filamentous *Dolichospermum* cf. *heterosporum* (Nyg.), *D. flos-aquae* (Lyng.) Breb. ex Born. et Flath., *D. vigueri* Denis et Frémy and *Aphanizomenon gracile* (Lemm.) Lemm. predominated (making up 59%, 5%, 5% and 26%, respectively, of the total biomass of Nostocales). *Cuspidothrix* (syn. *Aphanizomenon*) *issatschenkoi* (Usachev) Rajaniemi was present in the lake in spring and summer, but in low quantities (up to

0.22 mg L^{-1}). In other seasons, the Nostocales biomass was smaller than that of Oscillatoriales, which consisted mainly of *Planktothrix agardhii*, the main bloom-forming and microcystin-producing species. The total biomass of coccoid Chrooccocales [predominately the non-toxic *Aphanocapsa incerta* (Lemm.) Cronb. et Kom., *A. holsatica* (Lemm.) Cronb. et Kom. and potentially toxic *Microcystis aeruginosa* (Kütz.)] was the lowest among the cyanobacteria (Table 1).

The concentrations of cyanotoxins (MCs and ANTX) in the lake were similar in the surface and bottom water layers (Fig. 1). The average seasonal concentrations of intracellular MCs increased from 1.22 µg L^{-1} in spring to 17.23 in summer and 56.78 µg L^{-1} in autumn (Fig. 1A). Extracellular forms were present in much lower concentrations (0.16–2.18 µg L^{-1}). The desmethyl derivative of MC-RR – [Asp^3, Dhb^7]MC-RR predominated (91.9––96.2%) in the total concentration of cell-bound MCs. However, MC-LR > MC-LA > MC-YR were also detected (Table 2). Average seasonal concentrations of intracellular ANTX in the lake water changed over a narrower range: 1.51–2.71 µg L^{-1} (Fig. 1B). The cyanotoxin was only detected in spring and summer during mass development of Nostocales. Extracellular ANTX concentration reached up to 1.73 µg L^{-1} at the same time. In April, when *Dolichospermum* spp. formed a water bloom,

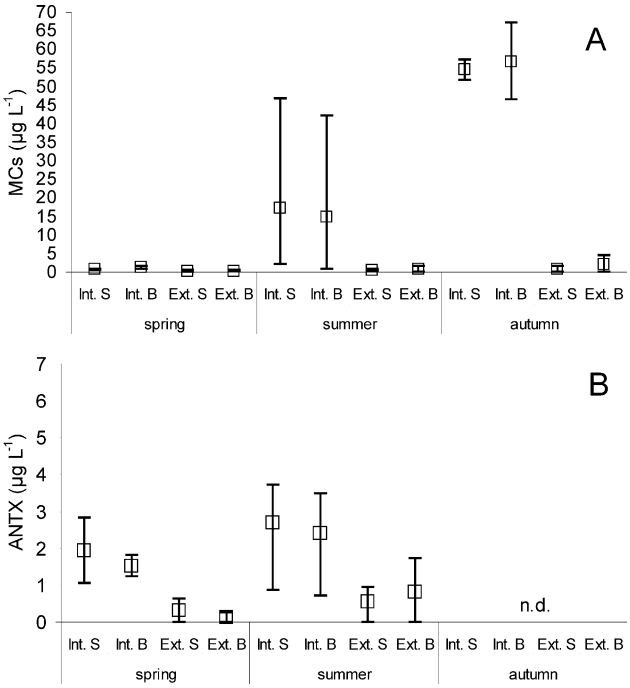

Fig. 1. Distribution of intra- (Int.) and extracellular (Ext.) microcystins (A) and anatoxin-a (B) in the surface (S) and bottom (B) layers of water in Lake Syczyńskie (seasonal mean values; n = 2–3, and range of values). n.d. – not detected.

TABLE. 1. Physical-chemical and biological characteristics of the surface (S) and bottom (B) layers of water in Lake Syczyńskie (seasonal mean values) in 2008.

Parameters	Water layer	Spring	Summer	Autumn
Water temperature (°C)	S	18.4	21.6	9.9
	B	17.5	21.0	10.0
pH	S	8.1	8.1	7.8
	B	8.0	8.1	7.8
Conductivity (µS cm^{-1})	S	560	331	385
	B	564	344	390
N-NH_4 (mg L^{-1})	S	0.182	0.316	0.509
	B	0.221	0.324	0.540
N-NO_3 (mg L^{-1})	S	0.030	0.064	0.196
	B	0.029	0.068	0.269
P-PO_4 (mg L^{-1})	S	0.021	0.022	0.093
	B	0.025	0.022	0.096
Oxygen concentration (mg L^{-1})	S	12.5	10.4	14.2
	B	12.3	9.4	14.2
Transparency – SD (m)		0.70	0.40	0.22
Chlorophyll-a (µg L^{-1})	S	51.3	202.3	237.8
	B	48.9	197.2	224.6
Chroococcales biomass (mg L^{-1})	S	0.15	0.36	2.46
	B	0.19	0.27	2.92
Nostocales biomass (mg L^{-1})	S	0.95	4.17	0.04
	B	0.88	4.38	0.01
Oscillatoriales biomass (mg L^{-1})	S	0.06	27.13	90.40
	B	0.65	27.70	84.47
TSI_{SD}		65	73	82
[a] Content of MCs in lake sediments (1–7 cm) in 2005 (µg eq. MC-LR g^{-1} D.W.)		0.91 (1 cm) – 0.13 (7 cm)		

[a] – following Pawlik-Skowrońska et al., 2010. Contribution of different taxa of cyanobacteria to the total biomass ≥ 5%; Chroococcales: *Aphanocapsa* spp., *Microcystis aeruginosa*; Nostocales: *Dolichospermum* spp., *Aphanizomenon gracile*; Oscillatoriales: *Planktothrix agardhii*, *Planktolyngbya limnetica*.

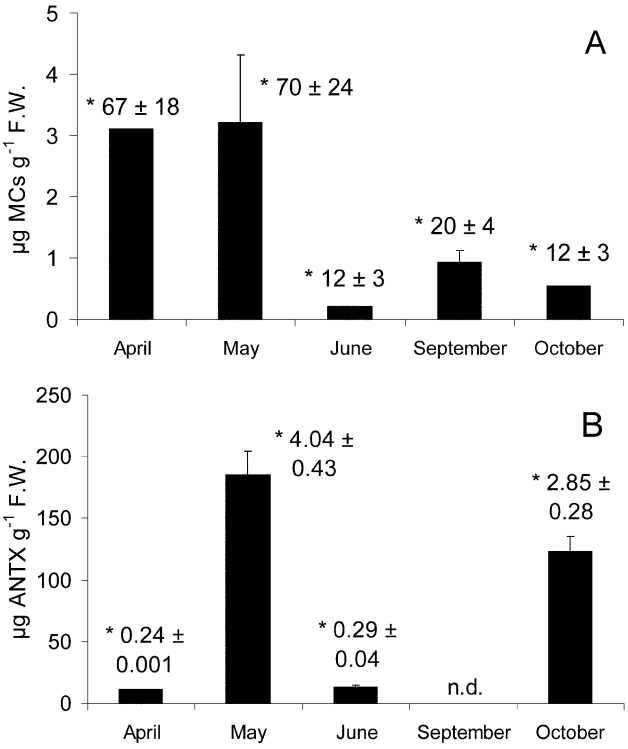

Fig. 2. Contents of microcystins (A) and anatoxin-a (B) in *Chironomus* sp. larvae collected in Lake Syczyńskie (mean values ± SD, n = 2–3). * – content of MCs (ng) and ANTX (µg) per individual. n.d. – not determined.

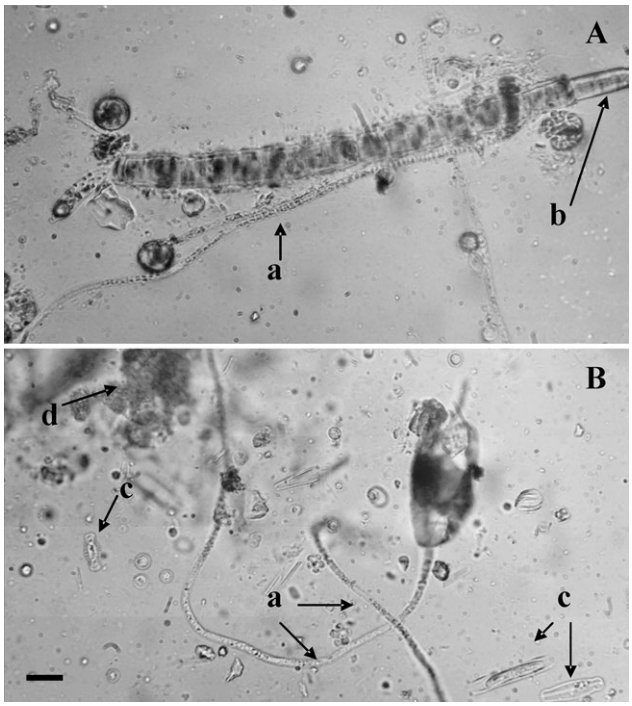

Fig. 3. The contents (A–B) of guts of *Chironomus* sp. inhabiting Lake Syczyńskie: cyanobacteria a – *P. agardhii*, b – *O. limosa*; c – diatoms; d – detritus. Scale = 10 µm.

the surface scum mostly consisting (98%) of *D. flos-aquae* contained 439 µg ANTX L^{-1}. The benthic cyanobacteria *Oscillatoria limosa*, common in the lake, also produced MCs and there was 189 µg MCs L^{-1} in the benthic mat.

Cyanotoxins in the *Chironomus* larvae from the lake

As a consequence of long-term cyanobacterial blooms, their relatively homogenous distribution in the water column and simultaneous occurrence of MCs and ANTX in the surface and near-bottom water layers, both cyanotoxins occurred in the *Chironomus* larvae inhabiting the lake (Fig. 2). The filaments of *P. agardhii* and *O. limosa* were found in guts of lake larvae (Fig. 3). Microcystins' and anatoxins' accumulation were found in larvae collected throughout the study period and a higher content of ANTX than MCs was detected. The highest content of MCs in *Chironomus* sp. (Fig. 2A) was found in spring (3.2 µg g^{-1} F.W. of larvae; 70 ng per organism) during a multispecies bloom of cyanobacteria (mainly Nostocales). In summer and autumn (during a bloom mainly formed by MC-producing *P. agardhii*), it decreased to 0.21–0.93 µg g^{-1} F.W. of *Chironomus* sp. The desmethyl derivative of MC-RR (66–100%) and MC-LA (34%) were detected in the larvae. The highest content of ANTX (Fig. 2B; 185.4 µg g^{-1} F.W.; 4.04 µg per organism) was found in larvae collected in May in the littoral zone, where two weeks earlier a surface scum of ANTX-producing *D. flos-aquae* occurred.

TABLE 2. The percentage of the different variants of microcystins identified in the cyanobacterial biomass collected from August to November.

Month	MC variants			
	[Asp^3, Dhb^7] MC-RR	MC-LR	MC-LA	MC-YR
VIII	90.6	4.0	3.8	1.6
IX	94.4	4.1	0	1.5
X	96.2	3.8	0	0
XI	91.9	8.1	0	0

Effect of cyanotoxins on *Chironomus* larvae

To estimate the direct influence of cyanotoxins on *Chironomus* larvae, pure standards of MC-LR and ANTX and the extracellular toxins present in crude extracts of cyanobacteria were tested. The experiments carried out on the lake and riverine larvae of *Chironomus* sp. revealed that pure MC-LR was slightly more toxic than ANTX to the riverine larvae (Figs 4A, 5A). MC-LR at the highest concentration used (3.32 mg L^{-1}) after 96 h of exposure caused a decrease in the survival of riverine larvae to approximately 61%, whereas ANTX under the same conditions caused a decrease to approximately 83% in comparison with the controls. The cyanobacterial extracts containing approximately 10-times less MCs or ANTX (Figs 4B, C, 5B, C) were more toxic than standard cyanotoxins for the lake and riverine *Chironomus* spp. The crude extract of *D. lemmermannii* (Figs 5B, C), containing ANTX, seemed to be slightly more toxic for the lake and riverine larvae than that of MCs-containing *P. agardhii* (Figs 4B, C), at very similar cyanotoxin concentrations (0.11–0.35 mg L^{-1}). However, in the extract of

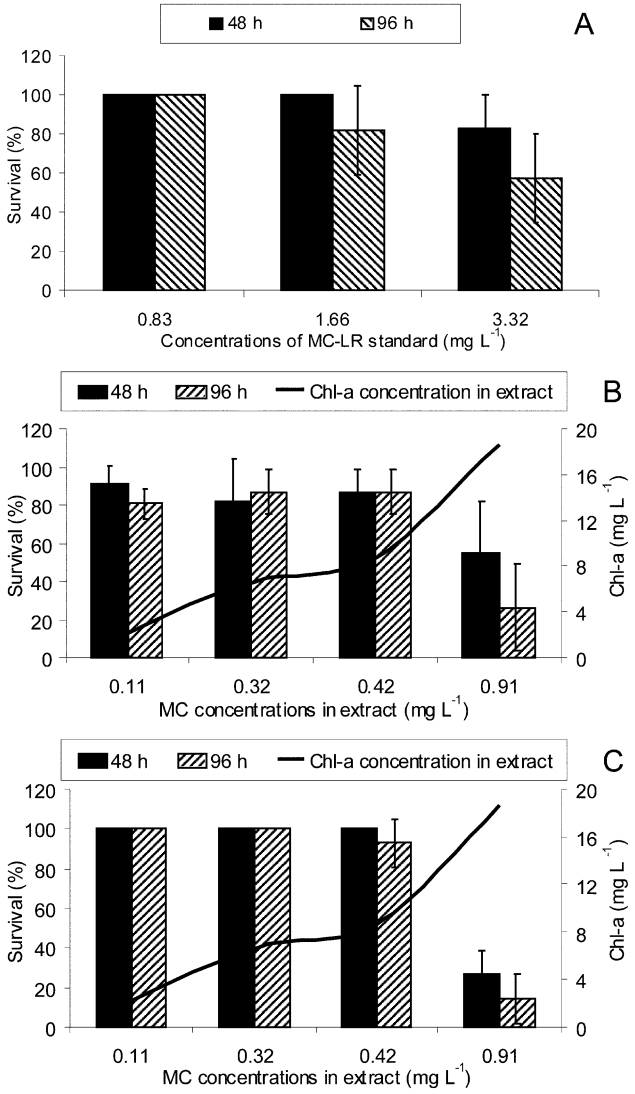

Fig. 4. Influence of pure MC-LR (A) and *P. agardhii* extract containing different concentrations of MCs (B and C) on survival of *Chironomus* spp. larvae after 48 and 96 h of exposure (mean values ± SD; n = 6). Survival of organisms in controls was set as 100%. A, B – riverine larvae, C – lake larvae.

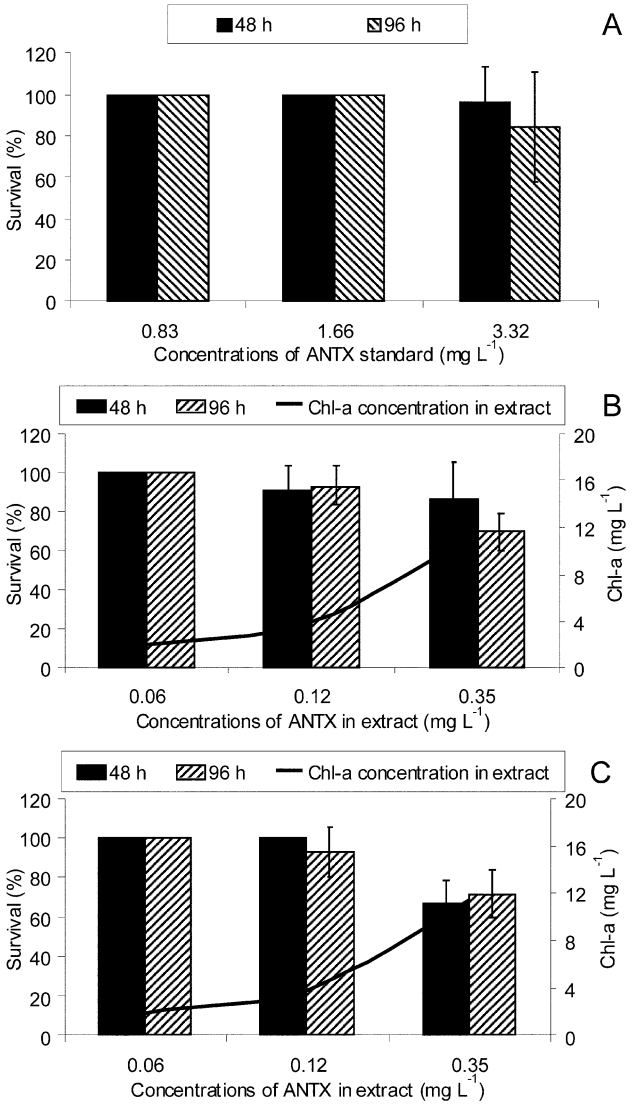

Fig. 5. Influence of pure ANTX (A) and *D. lemmermannii* extract containing different concentrations of ANTX (B, C) on survival of *Chironomus* spp. larvae after 48 and 96 h of exposure (mean values ± SD; n = 6). Survival of organisms in controls was set as 100%. A, B – riverine larvae, C – lake larvae.

D. lemmermannii containing 0.35 mg ANTX L^{-1}, there was a higher concentration of chlorophyll-a (12 mg L^{-1}) than in the *P. agardhii* extract (7 mg L^{-1}). This was a consequence of extracting a greater biomass of *D. lemmermannii* in order to obtain a similar cyanotoxin concentration. This possibly accounts for the different contents of other, unidentified cyanobacterial metabolites. There was no clear difference between the response of lake and riverine *Chironomus* larvae to the same cyanobacterial extracts, despite the greater accumulations of cyanotoxins in the larvae from the lake.

DISCUSSION

Despite the extensive studies on cyanobacterial metabolites (Carmichael, 1992; Welker & Döhren, 2006) and their influence on living organisms (Ibelings & Havens, 2008; Ferrão-Filho & Kozlowsky-Suzuki, 2011), there is little information on their accumulation and effects on some zoohydrobionts, particularly the benthic larvae of insects that are a very important component of aquatic food webs. Our study showed that in shallow eutrophicated reservoirs with a homogenous distribution of cyanobacteria and their toxins in the water, the *Chironomus* larvae may be affected by cyanotoxins contained in the cyanobacterial biomass, dissolved in the water and/or bound to bottom sediments. As reported by Pawlik-Skowrońska et al. (2008, 2010), microcystins were present in the lake in cell-bound, dissolved and sediment-bound forms. The surface layers of sediments (1–7 cm) in the *Planktothrix*-dominated Lake Syczyńskie were rich in microcystins (0.91–0.13 µg MC-LR eq. g^{-1} D.W). ANTX produced by several species of *Dolichospermum* and *Aphanizomenon* can also bind to bottom sediments (e.g. 47–656 µg ANTX kg^{-1}; Klitzke et al., 2011). This study revealed simultaneous accumulation of MCs and ANTX in benthic *Chironomus* sp. during

multispecies water blooms formed by MC and/or ANTX-producing filamentous cyanobacteria. The lake larvae examined accumulated similar amounts of MCs (up to 20.1 μg g^{-1} D.W., mostly dm MC-RR), like the *Chironomus* sp. (up to 11.54 μg MC-RR and MC-LR g^{-1} D.W.) in the eutrophic Lake Chaohu (China) affected by blooms of *Microcystis* sp. and *Dolichospermum* sp. (Chen & Xie, 2008). Currently there is no information on ANTX accumulation in insect larvae in their natural environments. Contents of ANTX in the larvae from Lake Syczyńskie were much higher than those of MCs despite the 1.5–20-times higher concentrations of intracellular MCs in the lake water. Beside the planktonic, benthic cyanobacteria also can be an important source of ANTX. As reported by Aráoz et al. (2005), *Oscillatoria formosa* Bory and *Oscillatoria* sp. produce ANTX. Filaments of *Oscillatoria limosa* were found in the guts of *Chironomus* larvae from Lake Syczyńskie. The contents of cyanotoxins found in the benthic larvae seem to be a consequence of their high contents in the biomass of cyanobacteria, their main food (Ali, 1990; Frouz et al., 2004), and in the lake water. The highest amounts of MCs and ANTX were recorded in the spring population of *Chironomus* sp. inhabiting Lake Syczyńskie, which followed a period when *P. agardhii* overwintered on sediments, the development of a benthic mat of *O. limosa* and a mass development of *D. flos-aque* in the lake. In early spring, extracellular concentrations of cyanotoxins in water may also be high (11 μg eq. MC-LR L^{-1}; Pawlik-Skowrońska et al., 2008) and may affect aquatic organisms. Beside the abundant cyanobacteria and species structure the life cycle of chironomids also seems to be a very important factor determining the accumulation of cyanotoxins in these organisms. The life cycle of *Chironomus* spp. (from egg to adult) can last from 12–14 days to more than a year, depending on the species and environmental conditions (Armiatge et al., 1995; Frouz et al., 2003; Kajak & Prus, 2003). Hence, the observed differences in the cyanotoxin content in *Chironomus* larvae may depend on the duration of their development in the lake. Larvae of *Chironomus* spp. may be an important vector of both MCs and ANTX to organisms higher in the food chain. They are an essential prey of fish (Kajak & Prus, 2003; Kornijów & Pęczuła, 2005), in which the accumulation of cyanotoxins (Pawlik-Skowrońska et al., 2012) is also recorded.

Our experimental study also revealed that different populations of larvae (inhabiting the hypertrophic Lake Syczyńskie or a river free of cyanobacterial blooms) were resistant to high concentrations of extracellular MCs and ANTX contained in crude cyanobacterial extracts. The larvae used in this study could belong to different species. For example, in lakes and fish-ponds *C. plumosus* (L.) is the most abundant (Matěna, 1995, Panis et al., 1996) while in polluted rivers *C. riparius* Meigen is abundant (Groenendijk et al., 1998). Larval survival decreased when the cyanotoxin concentrations were much higher than those recorded in the lake. Altogether, this indicates that *Chironomus* larvae may possess some specific defence against cyanotoxins. Forcella et al. (2007) recorded an increase in specific enzymes, including glutathione-S-transferase, glutathione peroxidase and glutathione synthase, in *Chironomus* larvae under oxidative stress. Both MCs and ANTX cause oxidative stress in aquatic organisms (Blaha et al., 2004), and glutathione (GSH), a strong antioxidant, plays an essential role in coping with this stress. Glutathione-S-transferase conjugates microcystins with GSH to metabolise them into less harmful compounds (Pflugmacher et al., 1998), which can be physiologically degraded and excreted from cells. Detoxification of MCs under natural conditions takes several weeks and is dependent on many environmental and biological factors (Harada et al., 1996; Ozawa et al., 2003), animal species (Zhang et al., 2009) or phase of development (Oberemm et al., 1999). ANTX is decomposed to non-toxic dihydroanatoxin-a and epoxyanatoxin-a (Harada et al., 1993), and its half-life in water reservoirs is shorter than that of MCs (Hardy, 2008). In sediments, which are reached by very little light, the persistence of ANTX can be higher than in the lake water, because it undergoes rapid photochemical degradation in sunlight (Chorus & Bartram, 1999). In spite of the lower stability of ANTX, it may be accumulated in benthic larvae. That is because, as reported previously in an experiment with fish (Kolmakov & Gladyshew, 2003), MCs and/or ANTX-containing Nostocales (e.g. *Dolichospermum flos-aquae*, *Aphanizomenon flos-aquae*) were completely digested and assimilated. As reported by Ali (1990), filamentous cyanobacteria are an essential source of food for benthic insect larvae, and the abundance of *C. crassicaudatus* larvae increased with increase in abundance of cyanobacteria (Ali et al., 2002). In a laboratory feeding experiment larvae of the chironomid *Glyptotendipes paripes* Edwards were able to complete their development when fed on *Lyngbya* cf. *aeruginosa* Kütz. ex Gom. and *Anabaena flos-aquae* whereas those fed on *Microcystis* were smaller and many failed to complete their development (Frouz et al., 2004). The *Chironomus* larvae from Lake Syczyńskie also contained cyanobacteria in their guts, including among others *O. limosa* and *P. agardhii*. The high contents of ANTX and MCs recorded in the *Chironomus* sp. indicate a high probability of cyanotoxins being transfered to benthos-feeding and omnivorous fish. This creates potential human health risk.

The comparative bioassays of acute toxicity, carried out on the lake and riverine *Chironomus* larvae, revealed no clear differences in the resistance of the two populations to extracts of cyanobacteria containing extracellular MCs or ANTX. The cyanotoxins present in the larvae collected from the lake did not decrease their resistance to toxins. This may account for the existence of *Chironomus* larvae in water bodies affected by blooms of toxigenic cyanobacteria. Generally, both the larvae from the river and the lake were more resistant to pure MC-LR and ANTX than to extracts of cyanobacteria containing approximately 10-times less MCs or ANTX. Some planktonic *Dolichospermum* spp. and benthic *Anabaena* species, in addition to ANTX may also produce other neurotoxins, like

anatoxin-a(S) and cytotoxic oligopeptides (e.g. anabaenolysins; Jokela et al., 2012). *Planktothrix* spp., in addition to MCs may also produce other bioactive oligopeptides (e.g. aeruginosins 205a, -B 538, microviridins D–F), which have a negative effect on aquatic invertebrates (Shin et al., 1996; Blom et al., 2003). Moreover, the constituents of cyanobacterial extracts may have a synergistic effect or increase the uptake rate of anatoxin-a. The synergy between anatoxin-a and other cyanotoxins, such as microcystin-LR, is described for mice (Fitzgeorge et al., 1994). This may account for the stronger negative effect of crude cyanobacterial extracts. The resistance of *Chironomus* spp. living in the presence of cyanobacterial blooms and in a bloom-free ecosystem to high concentrations of cyanotoxins, however, may be due to their very high content of the antioxidant GSH and GSH/GSSG ratio (a biomarker of antioxidant potential). As stated by Forcella et al. (2007), *Chironomus riparius* Meigen contained 200–300 m GSH g^{-1} F.W. and the GSH/GSSG ratio was also high (23.7–28.8). The recently reported ability of daphnids to biodegrade MC by producing MC-GSH conjugates (Wojtal-Frankiewicz et al., 2013) indicates that it is likely that chironomid larvae, which also have a high GSH content, can quickly adapt to environmental threats such as cyanotoxins.

CONCLUSIONS

Larvae of *Chironomus* sp., inhabiting eutrophicated water bodies with multispecies blooms of toxigenic cyanobacteria, accumulate simultaneously both microcystins and anatoxin-a. The larvae, independent of their habitat, appeared to be very resistant to high concentrations of extracellular cyanotoxins and may be an important vector of both MCs and ANTX to organisms higher in the food chain.

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