Palaeoecological evidence for changes over the past 200 years in chironomid communities of a shallow lake exposed to cyanobacterial toxins

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Abstract In spite of the existence of a number of papers applying the biomarker approach using pigments or akinetes to discuss the impact of cyanobacteria on the functioning of lakes in the palaeoenvironmental context, their sedimentary imprint in the form of microcystins (MCs) has never been taken into consideration. Our objective was to reconstruct 200 years of the development of chironomid assemblages in a shallow Polish lake with notoriously blooming toxic cyanobacteria. A 50-cm long sediment core sampled from the central part of the lake was sliced every 1 cm, dated, and analysed for the subfossil remains of Chironomidae and concentrations of MCs. The fauna underwent four distinct stages in its recent history: three periods when the assemblages were typical of hypertrophic conditions (the first one comprising the entire nineteenth century, and the second and third one including approximately the last 30 years of the twentieth century), separated by a "eutrophic" assemblage during the first 70 years of the twentieth century. The typical features of the "eutrophic" midge assemblage were high total densities and high values of taxonomic richness and diversity. This period coincided with relatively low concentrations of MCs in the sediments. Sediments from the hypertrophic periods were distinguished by taxonomic impoverishment of chironomids, low Shannon diversity index, a strong decrease in chironomid numbers, and high concentrations of MCs. Significant negative correlations with MCs along the sediment profile were observed for eurytopic chironomids. MC concentrations were also negatively but not significantly correlated with total density of chironomids, their benthic and epiphytic assemblages, species richness, and diversity index.

Keywords Subfossil chironomids · Zoobenthos · Sediments · Cyanobacteria · Microcystins · Eutrophication

Introduction

During the last century, eutrophication in lakes accelerated significantly as a result of human impact, often leading to hypertrophy (Harper 1992). A noticeable symptom of excessive lake fertility is cyanobacterial blooms (e.g. Reynolds and Walsby 1975; Jensen et al. 1994; Anderson et al. 2002). The blooms result in far-reaching transformations in lakes' ecosystems, including deterioration of oxygen conditions, changes in the structure of bottom sediments,

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disappearance of submerged plants, and as a consequence profound alterations in the structure of other communities, including impoverishment of their species diversity (Scheffer 1998).

There are many papers that use a biomarker approach such as pigments or akinetes to trace cyanobacteria in sediment cores and discuss them in a palaeoenvironmental context (e.g. Hall et al. 1999; Francis 2001; Eilers et al. 2004; Waters et al. 2005; Routh et al. 2009).

The effect of cyanobacteria can be strengthened by microcystins (MCs), toxic metabolites that they secrete (Carmichael 1992; Anderson et al. 2002). Their effect on aquatic life, and particularly insects, is still little studied (Kotak et al. 1996; Chen and Xie 2008). MCs can cause acute effects (e.g. reduction in survivorship, feeding inhibition, paralysis), chronic effects (e.g. reduction in growth and fecundity), biochemical alterations, and changes in life cycle in aquatic invertebrates such as mussels and crustaceans (Ferrão-Filho and Kozlowsky-Suzuki 2011).

Traces of MCs are detectable not only in the superficial sediment layers, but also in the deeper sediments deposited in the past (Pawlik-Skowrońska et al. 2010). This permits analysis of the impact of cyanobacterials on aquatic communities in long-term history. The present paper is a first such attempt. The objective of the study is to reconstruct 200 years of development of chironomid assemblages in Lake Syczyńskie, a small and shallow lake with notoriously blooming toxic cyanobacteria, described by Pawlik-Skowrońska et al. (2010).

Study area

Lake Syczyńskie is located in the Łęczna-Włodawa Lakeland, within the Cretaceous elevation of the Chełm Hills (518170N and 238140E) (Fig. 1). It is small (surface area 5.71 ha) and shallow (maximum depth 2.9 m) with two small inflows and one outflow. The lake's southern shore is adjacent to a carbonate peatland. The other part of the lake borders on the small village Syczyn. The lake's drainage basin is relatively large (458.2 ha) in relation to the lake's surface area. This results in the high value of the Ohle's index (80.2). This index (quotient of total drainage area and lake area) measures a potential magnitude of the pressure exerted by a drainage basin on a lake; the higher the value of a lake index, the

Fig. 1 Location of Łęczna-Włodawa Lakeland and Lake Syczyńskie

stronger the basin's effect on the lake (Bajkiewicz-Grabowska 2001).

Approximately 94 % of the catchment is used for agriculture, concentrated mostly on fertile black earth soils and rendzinas (Smal et al. 2005). Annual external loading of P and N to the lake is very high. The loads of nitrogen and phosphorous are 28 and 10 times higher, respectively, than the dangerous level, according to Vollenweider's (1976) criteria (Smal et al. 2005). Chlorophyll-a concentrations, water transparency, total phosphorus (TP), and electric conductivity (EC) are typical of hypertrophic, phytoplankton-dominated lakes (Table 1). The near-bottom waters are well oxygenated (Table 1), probably due to lake shallowness. This allows mixing of the water column by wind in the summer, and enables photosynthetic activity by benthic/sessile algae in year-round, including the winter. Consequently, even under ice cover, the oxygen saturation does not drop below 48 % (Kornijów, unpubl.).

The lake is surrounded by a 5–30-m wide belt of emergent vegetation, mainly *Phragmites australis* (Cav.) Trin. ex Steud. Submerged plants barely exist, except for some stray shoots of *Ceratophyllum demersum* L. (Kornijów et al. 2002). The phytoplankton is totally dominated by cyanobacteria *Plantothrix agardhii* (Gom.) Anagn. and Kom.—the main producer of toxic MCs (Pawlik-Skowrońska et al. 2008).

Materials and methods

A 50-cm long sediment core was sampled in April 2005 from the central part of the lake by means of a

Table 1 Physical–chemical characteristics of water of Lake Syczyńskie

pH[a]	8.52
O_2 (mg dm^{-3})[b]	6.0–14.8
Secchi Disc, SD (m)[a]	0.3
EC (μs cm^{-1})[a]	514
TP (mg dm^{-3})[a]	0.239
N_{tot} (mg dm^{-3})[a]	1.7
Chl-a (μg dm^{-3})[c]	162–358
Concentrations of intracellular microcystins (μg equivalent MC-LR dcm^{-3})[d] in water	50–90
TSI	81.2

EC, electric conductivity; TP, total phosphorus; TSI, Carlson's Trophic State Index determined on the basis of Chl-a; SD and TP, following Kornijów et al. (2002)

[a] Mean values for the period May–October 2001, following Smal et al. (2005)

[b] Values from the monthly monitoring programme in 2005–2009 (Kornijów, unpublished)

[c] Following Kornijów et al. (2002)

[d] Following Pawlik-Skowrońska et al. (2010)

Table 2 Results of the Pearson correlation between concentrations of microcystins in bottom sediments and total numbers of chironomids, numbers of their ecological assemblages, and most abundant taxa

Variable	r	p	n
Total Chironomidae	−0.3227	NS	22
Tanypodinae	0.1560	NS	22
Tanytarsini	−0.2934	NS	22
Benthic Chironomidae	−0.3423	NS	22
Epiphytic Chironomidae	−0.1858	NS	22
Eurytopic Chironomidae	−0.4915	*	21
Chironomus sp.	−0.4436	*	21
Glyptotendipes sp.	0.1329	NS	21
Endochironomus sp.	−0.2915	NS	22
Dicrotendipes sp.	−0.4337	NS	22
Cricotopus sp.	−0.1617	NS	22
Total richness	−0.3516	NS	22
Shannon index	−0.2285	NS	22

* $p < 0.05$

60-mm diameter UWITEC sampler pushed to the sediments using a 6-m long rod. The core was sliced to 1 cm layers using a sediment slicer (Kornijów 2009), placed in bags, and stored at 4 °C until analyses could be carried out. Up to a depth of 20 cm, the sediment was analysed at a resolution of 1 cm, and deeper every 5 cm. In total, 26 sediment layers were studied. The general laboratory procedure for chironomid analysis followed that described by Hofman (1986), with minor modifications. Subsamples of wet sediment were deflocculated with 8 % KOH at a temperature of 30–32 °C for 3 days. Then, the material was sieved through 180 μm mesh, and dehydrated with three successive treatments of ethanol (75, 90, and 99 %). Individual head capsules were picked with forceps from a Bogorov counting chamber under 40× magnification using a stereomicroscope. Individuals were identified to the lowest possible taxonomic level, according to Wiederholm (1983), Walker (2000) and Brooks et al. (2007). Two half head capsules of the same taxon were counted as one complete head capsule. A minimum of 50 head capsules (HC) was extracted from each sediment sample (Heiri and Lotter 2001; Quinlan and Smol 2001) except for the following sediment sections: 5 cm (HC = 30), 7 cm (HC = 38), 9 cm (HC = 33), 10 cm (HC = 0), 11 cm (HC = 10), 12 cm (HC = 33), 40 cm (HC = 32), and 45 cm (HC = 48). The midge larvae encountered were divided into three ecological assemblages: epiphytic, benthic, and eurytopic, depending on their substrate preferences (Tarkowska-Kukuryk and Kornijów 2008; Tarkowska-Kukuryk 2011). Tanytarsini was distinguished as a separate group due to the difficulties in the identification of their remains to the genera level.

Data were compiled and a chironomid percentage diagram was produced by means of Tilia 2.0 version (Grimm 1993). Cluster analysis (Grimm 1987) using Orloci's chord distance was applied to identify groups of samples with similar chironomid assemblages, and to divide them into local chironomid assemblage zones (LCAZ). The chord distance was chosen as distance coefficient given many zero entries in the chironomid subfossil matrix (Legendre and Legendre 1998).

We applied the Shannon–Wiener index H') to describe chironomid diversity:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

where S—number of species per sample; p_i—relative abundance of ith taxon; $=n_i/N$, N—total number of all

individuals; n_i—number of individuals of ith taxon; ln—log to base 2.

Pearson's correlation coefficients were calculated to identify significant associations between densities of midge taxa and concentrations of MCs. Before calculations, outliers in both data sets were identified and rejected by means of the Dixon's Q test (Dean and Dixon 1951).

The age of the sediment layers, calculated based on a constant rate of supply of unsupported ^{210}Pb model, and mean sedimentation rate during the last 150 years (3.4 mm year^{-1}) are discussed by Gąsiorowski (2008). Samples for concentrations of microcystins were collected in the same time, place, and manner as those for chironomids. The total MC concentration was analyzed and determined by gas chromatography/mass spectrometry (Pawlik-Skowrońska et al. 2010). The method is based on oxidation of Adda (specific aminoacid present in all iso forms of MCs together with degraded ones) to 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB) and determination of MMPB as methyl ester. The MC concentrations were expressed as µg equivalent MC-LR g^{-1} dry weight (DW) of sediment, where L and R are the symbols of amino acids that are incorporated in MCs.

Results

Changes in chironomid assemblages in a sediment profile

A total of 807 chironomid head capsules belonging to 17 taxa were found in 26 sediment samples retrieved from the core (Fig. 2). The material analysed was dominated by epiphytic forms constituting 58.5 % of total numbers. Benthic forms were less abundant, amounting to 16 % of the total. Eurytopic taxa constituted 10 %. Tanytarsini and undetermined Chironomidae constituted 12 and 3.5 %, respectively.

Based on the analysis of similarities in percentage contributions of fossil chironomids contained in the entire sediment core, four local chironomid assemblage zones (LCAZ) were distinguished (Fig. 2). The oldest sediments analyzed (LCAZ-I, 50–30 cm) contained very changeable abundance of HC, achieving maxima at 50 cm and 35 cm (Fig. 2). The fauna was constituted mostly of littoral epiphytic taxa, with *Cricotopus* sp. and *Glyptotendipes* sp. as dominants.

The benthic assemblage made insignificant contributions and was dominated by pelophilous benthic *Chironomus* sp., most numerous at 40 cm depth. Eurytopic larvae and Tanytarsini were rare, being most abundant in the bottom part of the zone. The period was distinguished by relatively low species richness and diversity. The MC concentrations were quite high, especially at 40 and 35 cm depth (Fig. 2).

The LCAZ-II (25–12 cm) showed the highest concentrations of HC. They dropped at about 11 cm, corresponding to the year ∼1975. The relative abundance of benthic and eurytopic taxa increased, whilst that of epiphytic forms decreased (Fig. 2). Epiphytic chironomids were still dominated by *Cricotopus* sp. and *Glyptotendipes* sp., benthic chironomids were dominated by *Chironomus* sp., and *Dicrotendipes* sp. was most abundant amongst eurytopic chironomids. Tanytarsini reached their highest relative numbers in this zone. Several other genera also occur for the first time here: *Ablabesmyia*, *Procladius*, *Polypedilum*, *Microtendipes*, *Limnophyes*, *Cladopelma*, and *Propsilocerus*. Consequently, the total species richness and species diversity were the highest in the whole sediment profile, but tended to decrease in the top section of this zone. The MC concentrations in the sediment record were lowest in zone LCAZ-II, and, except for one peak at 17 cm, they did not fluctuate significantly (Fig. 2).

The beginning of LCAZ-III (11–7 cm) involved a rapid decrease in the concentrations of subfossil chironomids until their total disappearance at the level of 10 cm. The highest numbers were reached by pelophilous *Chironomus* sp. Another benthic chironomid, *Propsilocerus lacustris* Kieff., increased slightly, as did *Procladius* sp. Amongst epiphytic chironomids, the highest contribution was that of *Cricotopus* sp., followed by *Endochironomus* sp. The remaining taxa in this zone occurred sporadically, and *Psectrocladius* sp. disappeared. In the uppermost layer of the zone, Tanytarsini appeared again (Fig. 2). The total richness and diversity index were close to zero in the bottom part but increased starting from 9 cm. MCs tripled their concentrations, being highest between 11 and 9 cm.

The zone represented by the youngest sediments, LCAZ-IV (6–0 cm), was distinguished by the growing numbers of head capsules, and relatively constant values of both species richness and diversity, with minima in the intermediate sequence (Fig. 2). Benthic

Fig. 2 Relative abundance
of chironomid taxa, their
assemblages, total richness,
total numbers of head
capsules, diversity index
(H'), and concentrations of
microcystins
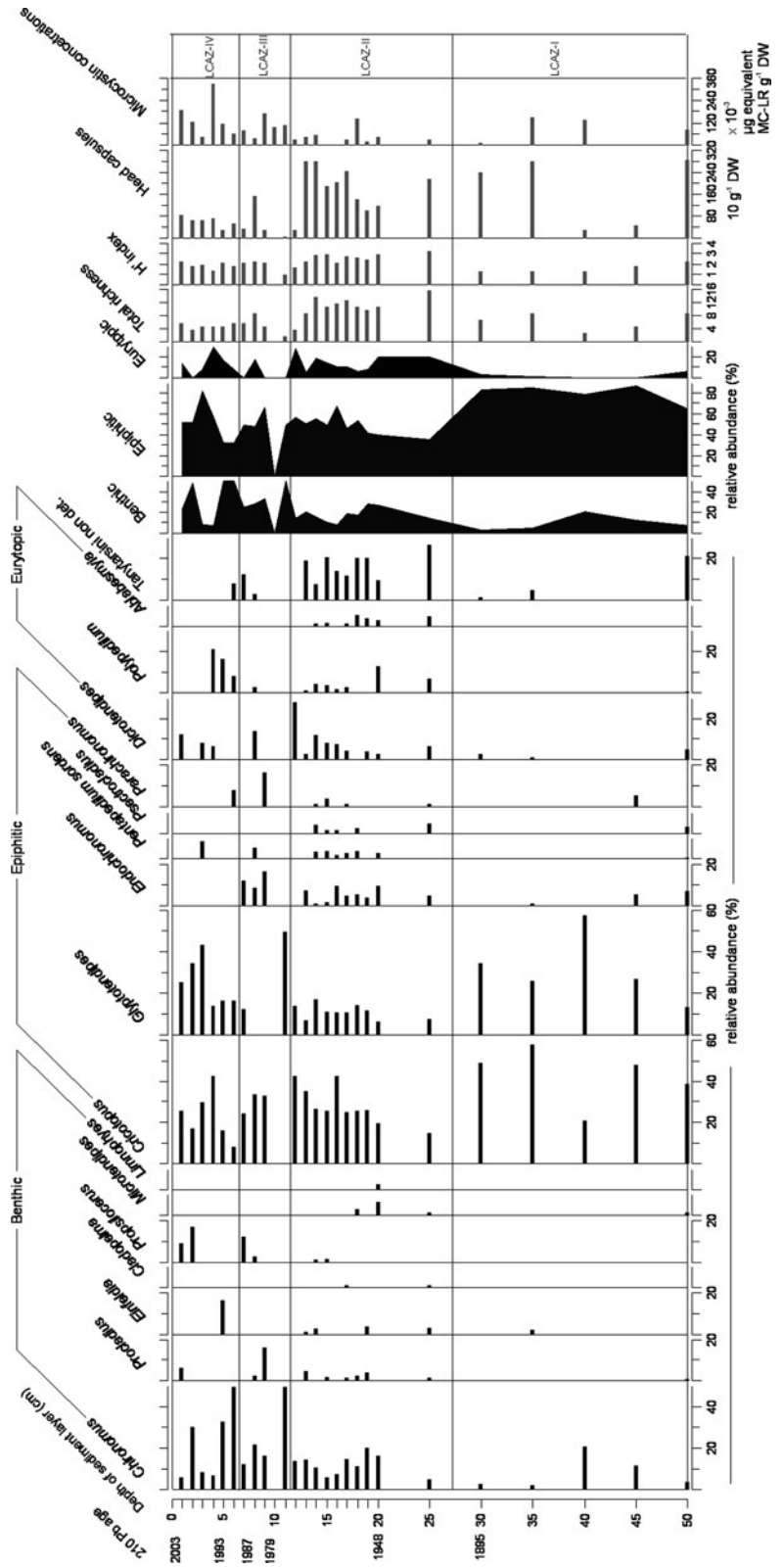
larvae were dominated by *Chironomus* sp., but some other benthic taxa appeared again-*Einfeldia* sp. at 5 cm depth and *P. lacustris* at 2 and 3 cm. In the epiphytic assemblage, *Cricotopus* sp. and *Glyptotendipes* sp. prevailed. *Endochironomus* sp., abundant in lower zones, disappeared completely. Tanytarsini occurred only at the bottom of the zone. The concentrations of MCs increased markedly, and reached the highest recorded value exceeding 0.300 µg equivalent to MC-LR g^{-1} DW (Fig. 2).

Response of chironomids to MC concentrations

The correlations between the concentrations of MCs and the abundance of chironomid ecological assemblages were negative, but significant ($r = -0.4915$) only in the case of eurytopic assemblage (Table 2). The total head capsule concentrations, chironomid species richness and diversity correlated negatively with MCs as well ($r = -0.3516$, $r = -0.3227$ and $r = -0.2285$, respectively), but none of these correlations were significant.

The most abundant taxa correlated negatively with MCs, but significant relationships were found only for *Chironomus* sp. larvae ($r = -0.4436$). There were only two taxa, *Glyptotendipes* sp. and Tanypodinae, that showed positive, but very weak and statistically insignificant, correlations with MCs (Table 2).

Discussion

This study reveals that the lowest productivity and MC concentrations in the lake's recent history occurred in the first 70 years of the twentieth century (LCAZ-II). This coincides with the highest concentrations of head capsules in the material, and higher taxonomic richness and diversity compared with other zones. The lake's productivity suggests a eutrophic character in this period. Cyanobacterial blooms were not intensive, as evidenced by the consistently low values of MC concentrations in LCAZ-II. In the late 1960s, numerous patches of *C. demersum* L. and *Potamogeton natans* L. were still found in the lake (Kowalczyk 1979), suggesting relatively good light conditions that allowed the development of elodeids. This is reflected in the fossil chironomid material by high species richness of epiphytic forms and large contributions of Tanytarsini.

The sediments from the earlier period, including the nineteenth century (zone LCAZ-I), and the later two periods, comprising about 25 last years of the twentieth century (LCAZ-III and LCAZ-IV), are distinguished by taxonomic impoverishment of chironomids, low values of the species richness and diversity index, and a strong decrease in the total chironomid numbers. These coincided with MC concentrations up to five times higher than in LCAZ-II, suggesting intensive cyanobacterial blooms and hypertrophic conditions. The poor ecological state of the lake is confirmed by the low numbers of Tanytarsini. They are recognized as inhabitants of weakly eutrophicated waters (Saether 1979), which is related to their small individual size and reduced efficacy of haemoglobin (Bryce and Hobart 1972).

We do not have enough information to specify the exact causes for the hypertrophication of the lake in the nineteenth century. It can be assumed that it was related to the proximity of the village and agriculture, as determined repeatedly in various studies (e.g. Bechmann et al. 2005; Smal et al. 2005). The high ratio of catchment area to lake area could have reinforced the high nutrient loading and hastened the lake's degradation (Harper 1992).

Another deterioration of the environmental conditions (LCAZ-III), indicated by changes in chironomid fauna and elevated concentrations of MC commenced at the beginning of the 1980s. The reason for the degradation of the ecosystem was probably long-lasting (1972–1997) dumping of manure into the lake from the nearby cattle and hog farm, a fact corroborated by the testimony of the local residents. The high contribution of typical epiphytic midges, such as *Glyptotendipes* sp. and *Cricotopus* sp. in this period, suggests luxuriant development of vegetation, at least emergent vegetation in the form of expanding *Phragmites* belt. Nowadays, both *Glyptotendipes* and *Cricotopus* are important components of the epiphytic fauna inhabiting reed stems in Lake Syczyńskie (Halkiewicz 2005; Tarkowska-Kukuryk 2011). This is in agreement with the opinion, considering these chironomid genera as typical indicators of highly eutrophicated lakes (Brodersen et al. 2001; Brodersen and Quinlan 2006). According to some authors (Ali 1990; Frouz et al. 2004), *Glyptotendipes* larvae can utilize cyanobacteria as a source of food. This might explain the weak correlation between concentrations of the midge and MCs (Table 2).

High accumulation of organic matter derived from manure in the 1980s could enhance oxygen demand in the bottom sediments, which are fine detritus gyttja. However, the sediments were pinkish in the top ca. 0.5 cm depth, and beige brown below this depth. This seems to exclude anoxic conditions, but not hypoxia, at least at the water sediment interface. Although many invertebrates, including chironomids, have physiological and behavioural adaptations to cope with oxygen shortages (Kornijów and Moss 2002; Brodersen and Quinlan 2006, and the references therein), the hypoxic conditions must have eliminated some chironomids living in the deeper parts of the lake, except for those resistant to oxygen deficits (*Chironomus* and *Procladius*). Another chironomid, *P. lacustris*, tolerant of high nutrient levels (Kornijów and Halkiewicz 2007) even increased its density (Fig. 2).

Manure has not been dumped in the lake since 1997, but the nutrient load accumulated in the sediment can be a significant source of the so-called internal eutrophication (Scheffer 1998; Kowalczewska-Madura et al. 2010). In addition, agricultural activity in the catchment and poor sewage management (Smal et al. 2005; Dawidek et al. 2009) are currently important factors determining the quality and productivity of the lake. This results in year-round cyanobacterial blooms and high production of MCs (Pawlik-Skowrońska et al. 2008). However, the chironomid fauna shows some signs of revival, manifested by the increase in HC concentrations, and the stable species richness and diversity values (Fig. 2, zone LCAZ-IV).

So far, MC toxicity, specifically to insects, including chironomids, has received limited attention (Ferrão-Filho and Kozlowsky-Suzuki 2011). No studies regarding the issue have been carried out in the scope of paleolimnology, although there are several papers that use pigments to trace cyanobacteria in sediment cores to reconstruct lake history (e.g. Waters et al. 2005; Routh et al. 2009).

In Lake Syczyńskie, particularly in winter, decaying cells of *Planktothrix agardii* cover the sediments and release high amounts of MCs throughout the year. The toxins are extremely stable due to their chemical structure (Pawlik-Skowrońska et al. 2008). It is very likely that their long persistence in the water may hamper the development of populations of benthic midge larvae. The negative correlation between MCs

and several chironomid taxa (Table 2) supports this supposition. The majority of the correlations obtained were not significant, but one should consider that information derived from each sediment layer is of cumulative character.

The structuring role of cyanobacterial blooms towards chironomid populations may result not only from the harmful effect of the toxins, but also from alterations in the habitat conditions caused by cells densely packed in the scum. Scum was often reported to dramatically worsen water oxygenation (discussed above), light climate, and pH values (e.g. Zohary 1985; Scheffer 1998).

The approach we present here broadens the possibility of tracking the changes in palaeoenvironment, suggesting the functional linkages between the symptoms of lake hypertrophication in the form of MCs production by cyanophytes and the development of other biotic components, such as chironomids, as an example.

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