Combined effects of nutrients and trace metals on chironomid composition and morphology in a heavily polluted lake in central China since the early 20th century

Yanmin Cao[1], Enlou Zhang[2*], Hongqu Tang[3*], Peter Langdon[4], Dongliang Ning[2], Wenxiu Zheng[2]

1) Engineering Research Center for Protection and Utilization of Bioresource in Ethnic Area of southern China, South-Central University for Nationalities, Wuhan 430074, P. R. China

2) State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography & Limnology, Chinese Academy of Sciences, Nanjing 210008, P. R. China

3) Research Centre of Hydrobiology, Jinan University, Guangzhou 510632, P. R. China

4) Geography and Environment, University of Southampton, Southampton SO17 1BJ, UK

Corresponding author: Enlou Zhang, Email: elzhang@niglas.ac.cn;

Hongqu Tang, Email: townningt@gmail.com

Abstract

Eutrophication and trace metal pollution seriously threaten the health of lake ecosystems; however, little is known about the response of zoobenthos to their combined impacts. In order to detect their effects on the biotic community of a lake, subfossil chironomids were analyzed in a sediment core from Sanliqi Lake, a hypereutrophic and severely metal-polluted lake located in the middle reaches of the Yangtze River in central China. The sediment core provides a record of environmental changes since the 1930s. Increases in pollutant concentrations began before the 1990s, and increases in total P and Pb began from the 1950s. Significant increases in nutrient and metal concentrations in the 1990s document the acceleration of eutrophication and metals pollution. As a consequence, macrophyte-related chironomid taxa (e.g. *Cricotopus sylvestris*-type and *Dicrotendipes* sp.) which dominated the subfossil assemblages prior to the 1990s were replaced by pollution-tolerant species (i.e., *Tanypus chinensis*-type and *Procladius choreus*-type) thereafter. Chironomid diversity gradually decreased from the 1950s with an abrupt change occurring in 1995. Multivariate statistical analyses reveal that subfossil chironomid assemblages are significantly correlated with total N, Pb and Cd, highlighting the combined impact of nutrients and trace metals on the chironomid communities. In addition, the relative abundance of *Procladius choreus*-type with mouthpart deformities increased over time and is significantly positively correlated with trace metals and nutrients. Nevertheless, further laboratory studies to assess the linkage between sediment contamination and mouthpart deformities are needed in order to enhance the utility of the latter as an indicator of environmental health.

Keywords: morphological deformity, chironomid assemblages, eutrophication, trace metal, Yangtze floodplain

1. Introduction

Eutrophication in freshwater ecosystems is considered to be one of the most serious environmental problems and needs to be addressed urgently (Smith, 2003) before lakes are degraded irreversibly (Carpenter et al., 1999). Heavy metals threaten the water quality of aquatic ecosystems because toxic metals (e.g., Ag, Au, Cd, Hg and Pb) may inhibit the metabolism of organisms. Although some metals (e.g., As, Cu, Cr, Fe, Mn and Zn) are indispensable for the physiological functioning of organisms, they become toxic when they exceed critical thresholds (Mason & Jenkins, 1995). Large quantities of heavy metals from mining operations and industrial sewage are discharged into rivers and lakes and cause various adverse effects such as compositional changes and reductions in the richness of species assemblages (Rippey et al., 2008). In addition, metals contamination can result in the miniaturization of community structures because of the elimination of intolerant larger-bodied invertebrates (Fleeger et al., 2003; von der Ohe & Liess, 2004; Campbell et al., 2008). Moreover, metal toxicity can physiologically disrupt the ion balance within zoobenthos bodies and reduce their respiratory efficiency, causing morphological deformities in their mouthparts (Dickman & Rygiel, 1996; Vinogradov, 2000; Vermeulen et al., 2000; Ilyashuk et al., 2003). Chironomidae (Insecta, Diptera) are one of the most abundant zoobenthic families in freshwater systems. As an important functional group in the soft substrata of lakes and rivers, chironomids have been widely used in water quality assessment (Saether, 1979; Wiederholm, 1980; Meriläinen et al., 2000; Raunio et al., 2007) and in paleolimnological studies.

Deformed mouthparts and compositional shifts when exposed to pollutants make chironomids a reliable indicator for detecting heavy metal pollution (Dermott, 1991; Ilyashuk et al., 2003; Odume et al., 2012). However, many lowland lakes are exposed to both eutrophication and heavy metal pollution

(Cattaneo et al., 2008), resulting in the dominance of nutrient- and metals-tolerant species in biotic assemblages (Reavie et al., 2005). Because of the complex interaction of multiple stressors, it is a challenging task to determine whether the changes are induced by metals, nutrient enrichment, or by other toxic substances (Davies et al., 2004; Reavie et al., 2005). To circumvent this problem, several researchers have focused on lakes impacted by a single perturbation (e.g. heavy metals) (Cattaneo et al., 2008), and then determined the response of the aquatic biota to the stressor (Davies et al., 2004). However, lake ecosystems are usually exposed to more than one type of pollutant (Chen et al., 2014). The inducement of changes on the individual level (e.g., survival and mouthpart deformities) caused by exposure to multiple stressors is not fully understood, since little is known about the effects of complex interactions between different types and abundances of pollutants (Meregalli & Ollevier, 2001; Arambourou et al., 2013; Di Veroli et al., 2012; 2014).

The Yangtze floodplain possesses one of the largest groups of freshwater lakes in China; however, many lakes in the region are significantly affected by both nutrient and metals pollution caused by intensive agricultural and industrial activities (Yang et al., 2010). Daye City, located in the middle reaches of the Yangtze floodplain, has a long history as an important national centre for mining and metallurgy, with copper mining in Daye City dating back several thousand years. A large volume of untreated sewage containing high concentrations of metals (e.g., Cu, Mn and Zn) from local mining enterprises is directly discharged into the surrounding surface waters. Daye Lake and its 15 sub-lakes suffer from both eutrophication and metals pollution (Li & Zhang, 2010) and thus provide ideal sites for studying the combined effects of nutrients and heavy metals on the lake biota. However, currently available reports from these lakes are focused solely on metals, including their sources, distribution and changes in concentration during the last decade (Lu et al., 2012; Mao et al., 2013), and few studies have addressed the impacts of pollutants on organisms (Chen et al., 2013, 2014). In the case of paleolimnological studies of Daye Lake and its sub-lakes, only diatoms have been studied in order to detect the relationships between pollutants and biota, and this work provides a reference for further studies on the environmental reconstruction of these lakes (Chen et al., 2014).

In the present study we aim (1) to detect compositional changes and mentum deformities in chironomid assemblages in a sediment record from a severely polluted lake; and (2) to illustrate the effects of anthropogenic nutrients and metals on the chironomid community since the early 20th century. We hypothesize that excessive nutrient and metal loadings would induce sharp changes in the chironomid community composition and cause distinct abnormalities in their mouthparts. To verify this hypothesis, a sediment core was sampled from the most severely polluted sub-lake (Sanliqi Lake). Chironomids and geochemical variables were measured to obtain a history of environmental changes since the 1930s. It is expected that our results will provide a basis for future biomonitoring and paleolimnological studies in the region.

2. Materials and methods

2.1 The study site

Sanliqi Lake, one of the sub-lakes of Daye Lake, has an area of 2 km^2 and an average water depth of 2 m (Fig. 1). The catchment is located in the subtropical climate zone, with a mean annual temperature of 17-18 ℃ and a mean annual precipitation of ~1500 mm. The lake is fed by surface runoff from the western part of the catchment, and the pH of the lake water is 7.2-8.5. An enormous amount of sewage and industrial waste (mainly from the local mining and smelting industries) is discharged into the lake via two main inflows located in the northwest and southeast parts of the basin lake (Fig. 1). The average concentrations of total nitrogen and total phosphorus during 2000-2009 were 4066 μg·L^{-1} and 295 μg·L^{-1}, respectively; and Cd reached to 30 μg·L^{-1} in 2002 (Li & Zhang, 2010).

2.2 Sample collection and laboratory analyses

A 32-cm-long sediment core was collected from the deepest part of Sanliqi Lake in May 2012 using a modified Kajak gravity corer. The core was extruded at a 1-cm interval in the field, and the samples were stored at 4 ℃ for multi-proxy analyses, including chironomids and the concentrations of total nitrogen (TN), total phosphorus (TP), total organic carbon (TOC) and trace metals.

^{137}Cs and ^{210}Pb dating was used to establish a chronology for the core. The measurements were made using EG&G Ortec well-type coaxial low background germanium detectors (HPGe GWL-120-15). Geochemical measurements were made using standard techniques and detailed information is provided in Chen et al. (2014).

Chironomid head capsules (hcs) in the sediment were extracted according to the standard procedure described by Brooks et al. (2007). Wet sediment samples were deflocculated in 10% KOH in a water bath at 75 °C for 15 min, and then sieved through 212 and 90 μm meshes. The residue was transferred to a grooved Perspex sorting tray and examined manually under a stereo-zoom microscope at 25× magnification with fine forceps. The hcs were permanently mounted on slides using Hydromatrix®, ventral side uppermost, and subsequently identified at ×100-400 magnification using the taxonomy of Brooks et al. (2007), with reference to Wiederholm (1983), Rieradevall & Brooks (2001) and Yan & Wang (2006). A minimum of 50 (typically more than 75) identifiable whole head capsules from each sample is considered to be representative of the extant fauna (Heiri & Lotter, 2001; Larocque, 2001; Quinlan & Smol, 2001).

Morphological abnormalities of head capsules were also assessed in each sample. The mentum and ligula of the mouthparts were selected as the targeted structures, because of the wide response range of mentum to types of contaminations (i.e. organic and inorganic matters) (Odume et al., 2012) and the high abundance of *Procladius choreus*-type in the Sanliqi Lake sediment samples. Deformed menta and ligula were distinguished according to Warwick (1991), Dickman et al. (1992), de Bisthoven et al. (1998), Martinez et al. (2002, 2006), Odume et al. (2012) and Pramesthy et al. (2014). Broken and worn head capsules were considered as normal individuals, while obvious abnormalities (e.g., extra, missing, fused and bizarrely-shaped teeth) were identified as deformed (Salmelin et al., 2015). According to Warwick (1991) and Brooks et al. (2007), the normal ligula of *Procladius choreus*-type IV instars has five teeth. Accordingly the specimens in our samples with less than or more than this number of teeth in the ligula were counted as deformed individuals. A morphological deformity incidence (DI) was used to measure the frequency of abnormal individuals (Warwick & Tisdale, 1988; Warwick, 1989). Deformities among *Tanytarsus* sp. mentum and *Procladius choreus*-type ligula were counted separately and calculated as mentum DI (%) and ligula DI (%), respectively. The total DI (%) is the sum of ligula DI of *Procladius choreus*-type and mentum DI of *Tanytarsus* sp.

2.3 Statistical analyses

The relative abundance of each chironomid taxon was calculated for further analyses. Stratigraphic diagrams of the major taxa were plotted using TILIAGRAPH 2.0.b.5, and major assemblage zones were identified using squared-chord distance within a constrained cluster analysis using the program TILIA 2.0.b.4 (Grimm, 1993). The Shannon-Wiener index (H'; Shannon & Weaver 1963) was calculated to estimate biodiversity changes using the program PRIMER 5.0 (Clarke, 1993).

Taxon percentages were square-root transformed prior to ordination analyses, and only taxa occurring in at least two samples with ≥2% abundance were included in subsequent analyses.

Detrended correspondence analysis (DCA) revealed that the gradient length of the chironomid data was 2.1 standard deviations, indicating that linear analysis was appropriate for ordination analyses (Birks, 1995). Principal component analysis (PCA) and redundancy analysis (RDA) were employed to identify the distribution of chironomid taxa and to illuminate the relationships between midges and explanatory variables (heavy metals and nutrients). The sedimentary data were $\log_{10}(x+1)$ transformed to standardize the variance prior to the analysis. Ordination analyses were performed using CANOCO v. 4.5 (ter Braak & Šmilauer, 2002).

3. Results

3.1 Chronology

Unsupported ^{210}Pb ($^{210}Pb_{ex}$) and ^{137}Cs activities in the sediment profile are shown in Fig. 2. The $^{210}Pb_{ex}$ distribution exhibits an exponential decay (r = -0.82, $p < 0.001$), with several fluctuations in the uppermost 8 cm of the core which can be attributed to the physical or biological mixing common in shallow lakes. However, the total ^{210}Pb activity does not reach radioactive equilibrium with the supporting ^{226}Ra. The ^{137}Cs profile exhibits a well-resolved peak at 28 cm depth which probably records the 1963 atmospheric maximum as a result of nuclear weapons testing. In order to improve the accuracy of the chronology, the ^{137}Cs 1963 peak was used as an independent dated reference level. The total $^{210}Pb_{ex}$ inventory was determined by the $^{210}Pb_{ex}$ inventory above the reference level, the age of the reference level and the ^{210}Pb radioactive decay constant (Appleby, 2001). The final age-depth model was calculated using the constant rate of supply (CRS) model, together with the ^{137}Cs 1963 peak as a reference level. Chen et al. (2014) also calculated the dates for the same core using two time markers, the ^{137}Cs peak and a peak in spheroidal carbonaceous particles (SCP). Consequently, the age model in the present paper differs slightly from that of Chen et al. (2014). Based on the composite CRS model, the age of the base of the core is 1913 and that of the top of the core is 2011 AD. The average sedimentation rate for the entire core is 0.59 cm yr^{-1}; however, the sedimentation rate was substantially lower (<0.3 cm yr^{-1}) prior to 1970 and it gradually increased to 1.79 cm yr^{-1} in the uppermost sediments (Fig 2).

3.2 Historical changes in heavy metals and nutrients

Both metals and nutrients are remarkably enriched in the Sanliqi Lake sediment core (Fig. 3). In the sediments below 19-cm-depth (ca. 1985 AD), Cr and TN maintain roughly constant values of 100 and 1800 mg/kg, respectively; while Cd, Zn and TP exhibit slight increases although their concentrations are low (Cd <100 mg/kg, Zn <1500 mg/kg and TP <1400 mg/kg). Subsequently, the concentrations of pollutants increase greatly, as indicated by rapid increases in Cd, TN and TP. This was followed by a short interval of uniform values from 12 to 8 cm (between 1996 and 2004 AD). Subsequently, however, the metals and nutrients commence another period of rapid increase, with Zn and TN content reaching 9.7×10^3 mg/kg and 6.2×10^3 mg/kg, respectively. The Pb profile differs from the other metals in that it increases rapidly below 13 cm (1995 AD) and then decreases above 7 cm (2005 AD) following an interval of steady values from 11 cm to 8 cm. In summary, several pollutants (especially TP and Pb) began to rise since 1950s, and then increased considerably around 1990s.

3.3 Chironomid stratigraphy

A total of 3777 hcs, consisting of 46 taxa and 36 genera belonging to 3 subfamilies (Chironominae includes two tribes of Chironomini and Tanytarsini) (Fig. 4), were observed in the 32 sediment samples. Two samples with the lowest counts (from 2 cm and 3 cm depth) were combined in order to meet the minimum requirement of hcs, and an average of 118 hcs was counted for each sample

(Fig. 5). *Procladius choreus*-type is the dominant taxon throughout the core, with a mean representation of 48%. Two main stratigraphic zones were identified by CONISS with the boundary between them at 13.5 cm (ca. 1995); in addition, zone I (below 13 cm; from 1932 to 1995) was further subdivided into subzone Ia (32-31 cm; before 1950) and subzone Ib (30-13 cm; ca. 1950-1995) (Fig. 4). Subzone Ia is characterized by high abundances of plant-related taxa, such as *Paratanytarsus* sp. (18.5%), *Cricotopus sylvestris*-type (14.9%), *Dicrotendipes nervosus*-type (12.8%), *Polypedilum* sp. (8.9%) and *Ablabesmyia* sp. (5.2%), while *Microchironomus tener*-type larvae (8.2%) are also well represented. In subzone Ib, the representations of the aforementioned five epiphytic taxa are reduced to 4.7%, 3.8%, 5.9%, 2.8% and 1.8%, respectively. The mean percentage of *Microchironomus tabarui*-type (15.0%) and *Procladius choreus*-type (39.7%) larvae is an order of magnitude higher than in subzone Ia. *Tanypus chinensis*-type also increases in this subzone despite its low abundance. In addition, species diversity decreases slightly (Fig. 5). In Zone II (above 13 cm; after 1995), both *Procladius choreus*-type and *Tanypus chinensis*-type are the dominant species, with mean abundances of 65% and 25%, respectively. In contrast, aquatic macrophyte-related taxa (e.g. *Ablabesmyia* sp., *C. sylvestris*-type, *D. nervosus*-type and *Polypedilum* sp.) (Fig. 4 and 5), and some other common taxa (e.g. *Microchironomus* sp. and *Tanytarsus* sp.), either decrease markedly or disappear completely. Shannon-Wiener diversity decreases significantly in this zone ($p < 0.05$) but increases slightly in the uppermost samples. There is a significant change in the scores for PCA axis 1 after 1995 ($p < 0.001$), which confirms the two clusters (Fig. 5).

3.4 Occurrence of deformed subfossils in the sediment samples

All identifiable subfossil chironomid hcs were screened for deformities in the mentum and ligula. Total DI ranges from 0.7% to 10.7%, with the exception of samples from the following depths (in cm):

1, 4, 10, 14, 19, 20, 24, 29 and 30 which are zero. Below 13.5 cm the frequencies of deformities in *Tanytarsus* sp. is higher than in *Procladius choreus*-type (Ligula DI ranges from 0-1.6%). Consistent with the dominance of *Procladius choreus*-type, ligula DI (ranging from 0-7.1%) increases dramatically above 13.5 cm. Calculation of a Spearman rank correlation coefficient indicates that the ligula DI increases significantly ($r = 0.47$, $p < 0.01$) over time.

3.5 Correlations between environmental variables and changes in chironomid communities

The RDA results reveal that TN, Pb and Cd are significantly correlated with chironomid communities (Fig. 6), explaining 62.5% of the variance in the chironomid data. Furthermore, partial RDAs demonstrate that the pure effects of the three significant factors capture 21.3%, 8.9% and 5.5% of the total variance, respectively. In RDA ordination biplots, samples from Zones I and II are clearly distinguished along RDA axis 1, with Zone I samples in the left part and Zone II samples in the right part. Samples are distributed along axis 2. The older samples are scattered in the upper part and younger samples in the lower part of the plot for zone I, and the inverse is the case for zone II samples. Among the primary taxa, two taxa (*Procladius choreus*-type and *Tanypus chinensis*-type) are positively correlated with three significant variables, while the other taxa are negatively correlated with these variables. In addition, most of the macrophyte-related chironomid taxa (e. g. *Ablabesmyia* sp., *C. sylvestris*-type, and *Polypedilum* sp.) are located in the positive sector of axis 2 and nutrient-tolerant taxa (i.e. *Microchironomus* sp. and *Chironomus plumosus*-type - not shown) are located in the negative sector. In addition, calculation of Spearman rank correlation coefficients reveals significant positive relationships between ligula DI and metals and nutrients ($r = 0.43$-0.44, $p < 0.05$ for Cd, Cr and TN; $r = 0.46$, $p < 0.01$ for both Zn and TP).

4 Discussion

4.1 Status of the lake prior to the 1990s

Before 1995, the chironomid assemblages were diverse and characterized by high abundances of *Paratanytarsus* sp., *Ablabesmyia* sp., *Dicrotendipes* sp., *C. sylvestris*-type and *Polypedilum* sp., and these taxa were notably dominant until ca. 1950. In the lakes of the UK and Denmark, the abundances of specific chironomid taxa (e.g. *Dicrotendipes* sp.) are significantly associated with the richness of the aquatic macrophyte flora (Langdon et al., 2010). Stands of *Potamogeton* are often preferred by *C. sylvestris*-type and *Polypedilum* sp. owing to the large quantity of epiphyton attached to *Potamogeton*, while *Chara* may favor the growth of *Glyptotendipes* sp. larvae (Berg, 1950; Brodersen et al., 2001). *Dicrotendipes* sp. also has a preference for a plant substratum, as well as for eutrophic and productive habitats (Brooks et al., 2007; Zhang et al., 2012). The differentiation of epiphytic and non-epiphytic taxa along RDA axis 2 indicates that RDA axis 2 may be related to the status of aquatic macrophytes which provide a vital habitat for the colonization of zoobenthos (Berg, 1949; Brodersen et al., 2001; Papas, 2007). High abundances of macrophyte-dwelling chironomid taxa in Zone I are in accordance with the abundance of macrophyte-associated diatoms (e.g. *Gyrosigma acuminatum* and *Gomphonema parvulum*) before the 1990s (Chen et al., 2014), which in turn is consistent with documentary evidence which reported abundant *Trapa bispinosa* in Sanliqi Lake before the 1990s. Although chironomid indices suggest a decreasing tendency during 1950s to 1990s (decrease in plant-related taxa in Fig. 5), several lines of evidence indicate that Sanliqi Lake was in a macrophyte-dominated state before the 1990s.

4.2 Increased pollution since the 1990s

The abundance of epiphytic chironomids decreased markedly in Sanliqi Lake after 1990, lagging behind nutrient and metal enrichment due to the large influx of waste water. Generally, aquatic macrophytes can enhance the clarity and water quality of lakes by promoting the deposition of nutrients and metals within the water column (cf., Scheffer et al., 1993). However, TP and Pb increased throughout the sequence and may have caused a gradual decline in aquatic macrophytes, thereby reducing the amount of favorable substrate for the development of a diverse midge fauna and thus leading to a decline in species diversity. After reaching a threshold level, the macrophytes suddenly collapsed and finally disappeared, and induced sharp shifts in chironomids around 1995. It was reported that the Secchi depth of Daye Lake had decreased by 1 m in the mid-1990s compared with the 1950s, and the number of fish species as well as the total catch were reduced by 52% and 70%, respectively, over the same interval (Deng & Xie, 1995). In addition, the commercial harvesting of aquatic plants (e.g., lotus, water chestnut and reed) decreased significantly. The process of degradation was reflected by decreases in epiphytic chironomids and an increase in pollutant-tolerant *Procladius choreus*-type.

The larva of *Tanypus chinensis*-type prefers mesotrophic to hypertrophic waters with soft substrata, and it can tolerate eutrophic conditions (Wang, 1994). However, little is known about its tolerance to metals pollution (Shang et al., 2010). In the present study, positive correlations between *Tanypus chinensis*-type abundance and sedimentary concentrations of nutrients and metals indicate that *Tanypus chinensis*-type larvae are tolerant of both eutrophic and metals-polluted habitats. *Procladius choreus*-type is another pollution-tolerant taxon that can adapt to both a broad range of trophic conditions (Wiederholm, 1981) as well as anoxia in European lakes (Heiri & Lotter, 2003). After 1995, both *Tanypus chinensis*-type and *Procladius choreus*-type increased at the expense of phytophilous species, indicating the significant degradation of the aquatic environment of Sanliqi Lake. This interpretation is supported by the fossil diatom record of the site which reveals a decrease in the mesotrophic *Aulacoseira granulate*, but an increase in nutrient and metals-tolerant *Nitzschia palea* (Chen et al., 2014). Effects of nutrients on chironomids are mainly indirect, often mediated by oxygen availability and substrate changes such as macrophytes' decline. *M. tabarui*-type, a common eutrophic chironomid species in the Yangtze floodplain lakes (Zhang et al., 2012), is negatively correlated with nutrient concentrations in the present study, probably because the hypereutrophic status of Sanliqi Lake (mean TP concentration of 295 μg L^{-1} between 2000-2009) greatly exceeded the optimum level for the taxon in this region (~135 TP μg L^{-1}) (Zhang et al., 2006). Thus severe eutrophication inhibited its ability to survive after 2000 and it became locally extinct. The larvae of some *Microchironomus* species can survive in high-salinity sewage canals in China (Tang, 2006); however, as observed in the present study, the percentages of *M. tabarui*-type, larvae decrease with increasing metals concentration (Fig 3), indicating that their growth is inhibited in conditions of severe metals pollution. It is unexpected that *M. tener*-type, a mesotrophic species in this region, increases when the metal concentrations are increasing. It probably need more data to understand its response to metals and the difference from that of *M. tabarui*-type.

4.3 Relative effects of nutrients and metals on midge assemblages

The ordination analysis results indicate that at Sanliqi Lake nutrient concentrations are likely more important than metals in explaining the changes throughout chironomid assemblages, probably as the result of phytoplankton blooms and degradation of the macrophyte community. Compositional changes in chironomids began in the 1950s, well before the concentrations of most of the pollutants (with the exception of Pb and TP) began to increase. The increase in Pb concentrations prior to the 1990s was even more significant than for TP, as indicated by the gradients of the curves in Fig. 3. The effects of TP on Sanliqi Lake should not be underestimated because of its relative insignificance in the results of the RDA, and at the same time the importance of metals (mainly Pb) in altering the chironomid composition before the 1990s should be emphasized. Therefore the major shift in chironomid assemblages which occurred around 1995 may be attributed to the combined effects of nutrients and metals, presumably mediated through high turbidity and the sudden elimination of submerged macrophytes. However, despite the high concentrations of metals, their relative effects on the biota were likely less than those of nutrients. Both nutrient enrichment and alkaline conditions within lakes would cause metal precipitation and reduce their biological availability and thereby buffering their impacts on the biota (Wang & Dei, 2001; Brooks et al., 2005). Comparison with the results of a diatom analysis of the same sediment core (Chen et al., 2014) reveals that both diatom and chironomid assemblages changed abruptly at 14 cm depth. In the study of Chen et al. (2014), nutrients captured more variance in the diatom assemblages before 1999, and planktonic diatom species replaced epiphytic taxa. Subsequently, the dominance of metal-tolerant diatoms was reinforced by the continuous high level of metal pollution. In our study, although nutrients were more important than metals in explaining variations in the overall chironomid stratigraphy throughout the core, nutrients were insignificant in the individual ordination analysis for both zones I and II (data were not shown here). These results indicate that it is difficult to disentangle the complex effects of nutrient and metals pollution on ecosystems.

The continuous interactions of slow and fast drivers over time can sometimes result in abrupt ecological changes, which can be termed regime shifts or tipping points (Randsalu-Wendrup et al., 2016). Freshwater ecosystems are complex and typically resilient to external stressors via complicated feedback mechanisms. Increases in single and/or interactions of stressors can, over time, reduce the resilience of ecosystem so it reaches a threshold (the so-called tipping point), and at this stage the lake may tip from a clear water state to a turbid water state (Vanacker et al., 2015). In this study, external inputs of metals and nutrients persisted from the 1950s. Chironomids showed slight changes in composition and diversity, which were likely responding to macrophyte decreases through intensified pollutions prior to 1995. It is inferred that a critical threshold was reached and a regime shift occurred in Sanliqi Lake around 1995, as shown by the sudden shift in chironomid community. Similar phenomena have been found in several other (now) eutrophic lakes in this region (Wang et al., 2012). To test the occurrence of a tipping point at this site, and associated underlying ecological mechanisms, would require higher resolution chironomid data, in addition to data from other ecological groups.

4.4 Chironomid deformity frequency as a potential pollution indicator

Exposure to heavily-contaminated environments increases the incidence of deformities in chironomid larvae (Cushman, 1984; Dickman et al., 1989). In a biomonitoring experiment, the incidence of mouthpart abnormalities in Chironomidae has been used as a biomarker of water quality degradation (Odume et al., 2012; Planello et al., 2015). In Sanliqi Lake, the percentage of ligula deformities increased significantly over time, and statistical analyses indicates a significant relationship with pollutant concentrations. However, the occurrences are relatively low in such a severely-polluted lake compared with other studies on extant chironomids. For example, the DI values of larval chironomids were 5-25% in severely polluted lakes and the values were 0.7-0.8% even in unpolluted lakes in Sweden (Wiederholm, 1984). 26% of deformed chironomids were detected in sediments near point-source locations in the Niagara River watershed, which were severely polluted by wastewater contaminated by both heavy metals and oil wasters (Cd up to 20 g $\cdot kg^{-1}$) (Dickman & Rygiel, 1996). More than 8% of mentum deformities occurred in the Swartkops River in South Africa, where the concentrations of Cd and Sn were 11.9 mg kg^{-1} and 1586 mg kg^{-1}, respectively (Binning & Baird, 2001; Odume et al., 2012). However, these results are difficult to compare since different researchers use different definitions of deformity (Salmelin et al., 2015). Generally, dose-response relationships between specific contaminant and larvae deformities can reliably be determined in laboratory bioassays. However, linkages between deformities and pollutant concentrations in field conditions are ambiguous owing to the complex interactions among multiple pollutants (Martinez et al., 2006; Di Veroli et al., 2014). In the present study, statistically significant relationships between the incidence of *Procladius choreus*-type deformities and abiotic factors confirm the sensitivity of deformity to the level of contaminants. Complex antagonistic interactions and the combined stresses from multiple pollutants may reduce the extent of deformation, thus decreasing biotic deformity (Di Veroli et al., 2010); and this may explain the relatively low deformity frequency in this study. Alternatively, there may be an ‘amount effect’ of the specific metals present in Sanliqi Lake, and/or the combinations and abundances of specific metals may be important. In addition, metal precipitation caused by nutrients and alkaline conditions probably protects the biota from heavy metal toxicity in both eutrophic and metals-polluted lakes (Wang & Dei, 2001; Brooks et al., 2005; Chen et al., 2014). This mechanism between nutrients and metals may have afforded some protection for specific chironomid taxa from the toxic effects of other contaminants. Clearly, further studies of the relationship between deformities and various environmental stressors in such heavily-polluted aquatic ecosystems are needed.

5 Conclusions

Sanliqi Lake has suffered from multiple sources of environmental stress over the past 100 years. Nutrient and metal inputs increased since the 1950s causing a gradual decline of the macrophyte-related chironomid taxa. After the 1990s, enhanced eutrophication and metals accumulation may have led to the final disappearance of macrophytes and thus to a significant compositional turnover in chironomid assemblages, characterized by the replacement of plant-preferred taxa by pollution-tolerant taxa, together with a sharp decrease in species richness. The deformity index of *Procladius choreus*-type is a potential biotic indicator of pollution in Sanliqi Lake and is closely correlated with the concentrations of nutrients and metals; however, further modern ecological or experimental studies are necessary to fully assess its application to biomonitoring and paleolimnology.

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Figure captions

Fig. 1 Map of the study area and location of the sediment core obtained from Sanliqi Lake.

Fig. 2 Profiles of $^{210}Pb_{ex}$ and ^{137}Cs activity, age-depth plot and sedimentation rate for the sediment core from Sanliqi Lake.

Fig. 3 Contents of metals and nutrients in the sediment core from Sanliqi Lake. The grey horizontal line is the boundary between two chironomid assemblage zones.

Fig. 4 Stratigraphic diagrams of chironomids in the Sanliqi Lake sediment core. Only taxa present in at least two samples with ≥2% abundance were included.

Fig. 5 Profiles of chironomid indices for the sediment core from Sanliqi Lake. Plant-related taxa (%) is the total abundance of plant-related chironomid taxa, such as *Ablabesmyia* sp., *Cricotopus sylvestris*-type, *Dicrotendipes* sp., *Glyptotendipes* sp., *Paratanytarsus* sp. and *Polypedilum* sp.. Ligula DI (%) is the morphological deformity incidence of *Procladius choreus*-type, and DI (%) is the sum of ligula DI and mentum DI of *Tanytarsus* sp..

Fig. 6 a) Redundancy analysis (RDA) biplots of sample – environment, which shows a plot of the chironomid assemblage samples that can be tracked through time, against the main pollutants (TN, Cd and Pb). The directional trend is for the chironomids to track towards changes in pollutants/metals in zone 2, but not in zone 1; b) RDA of major chironomid taxa (average percentage exceeding 1%) -

environment. Only significant environmental variables are shown. Blue, black and grey arrows in b) respectively represent macrophyte-related taxa, pollutant-tolerant taxa and other taxa with abundances >1%. Abbreviations: *Ab* = *Ablabesmyia* sp.; *Ce* = *Corynoneura edwardsi*-type; *Cs* = *Cricotopus sylvestris*-type; *Dn* = *Dicrotendipes nervosus*-type; *Me* = *Microchironomus tener*-type; *Ma* = *Microchironomus tabarui*-type; *Pa* = *Paratanytarsus* undiff.; *Pp* = *Paratanytarsus penicillatus*-type; *Pn* = *Polypedilum nubeculosum*-type; *Pr* = *Procladius choreus*-type; *Tp* = *Tanypus chinensis*-type; *Tt* = *Tanytarsus* undiff.; *Tm* = *Tanytarsus mendax*-type; *Ta* = *Tanytarsus pallidicornis*-type.