ORIGINAL ARTICLE

Is there a common threshold to subfossil chironomid assemblages at 16 m water depth? Evidence from the Tibetan Plateau

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ABSTRACT

Fluctuating lake levels are an important driver of ecosystem change, and changes in the precipitation/evaporation balance of a region can lead to undesirable changes in ecosystem functioning. Large-scale changes in hydrology will become increasingly more likely as a result of ongoing climate change in the coming century. This is especially true for the Tibetan Plateau, which plays a crucial role as the "Asian water tower" for the surrounding densely populated regions. Chironomids (Diptera: Chironomidae) have proven to be one of the most valuable bioindicators for monitoring and reconstructing the development of aquatic ecosystems. Besides temperature, water depth and salinity are two of the most important environmental factors affecting chironomids. To study the relationship between chironomids and water depth, we analyzed surface sediment samples of two large Tibetan lakes, Selin Co and Taro Co. These lakes have similar environmental conditions (*e.g.* elevation, temperature and oxygenation) but show strong differences in salinity (7-10 and 0.5 ppt, respectively). Our results show that the chironomid assemblages in both lakes have similar water depths at which the fauna abruptly changes in composition, despite different faunal assemblages. The most important boundaries were identified at 0.8 and 16 m water depth. While the uppermost meter, the "splash zone", is characterized by distinctly different conditions, resulting from waves and changing water levels, the cause of the lower zone boundary remains enigmatic. Even though none of the measured water depth-related factors, such as water temperature, oxygen content, sediment properties, light intensity or macrophyte vegetation, show a distinct change at 16 m water depth, comparison to other records show that a similar change in the chironomid fauna occurs at 16 m water depth in large, deep lakes around the world. We propose that this boundary might be connected to water pressure influencing the living conditions of the larvae or the absolute distance to the surface that has to be covered for the chironomid larvae to hatch. We conclude that water depth either directly or indirectly exerts a strong control on the chironomid assemblages even under different salinities, resulting in distribution patterns that can be used to reconstruct past fluctuations in water depths.

INTRODUCTION

Understanding the development of large lakes is of key importance to understand and predict consequences of ongoing climate change. This is especially true for lakes situated on the Tibetan Plateau, which not only serve as water storage, but also as water suppliers due to evaporation and atmospheric water transport (Lu *et al.,* 2005). While glaciers account for the main amount of stored water (Immerzeel *et al.,* 2010), the lakes with their combined surface area of about 50,000 km^2 (Zhang *et al.,* 2019) are crucial for the water budget of the downwind situated, densely populated regions (Lu *et al.,* 2005). Ongoing climate change has a strong impact on the Tibetan Plateau. First, warming is twice as strong on the plateau relative to the global average (Qiu, 2014). Second, strengthening of the summer monsoon is leading to increased precipitation (Zhu *et al.,* 2019). Because many of the large Tibetan lakes are closed systems, changes in the water supply lead to changes in lake surface area and therefore to the amount of evaporated and subsequently transported water (Jiang *et al.,* 2017). To be able to predict future changes to the hydrological balance of these unique closed-basin lakes, knowledge of past changes in their water level is of crucial importance. Such information can be obtained by lake level reconstructions using a range of different approaches.

One of the most effective bioindicators for reconstructing lake level oscillations are subfossil

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chironomid (Diptera: Chironomidae) remains in lake sediment records (Brooks, 2006). Whereas the flying adults ensure dispersal, the aquatic larvae are adapted to a particular habitat (Armitage *et al.,* 2013). Because the larvae of most chironomid species live connected to water plants, in sediment, or on the lake sediment surface, water depth has a strong impact on the within-lake distribution patterns of individual taxa, and as such, on the spatial heterogeneity of the chironomid fauna of a lake (Engels and Cwynar, 2011). After molting or death, larval head capsules, due to their high chitin content, are preserved in lake sediments (Brooks *et al.,* 2007). Subfossil larval head capsule assemblages have been shown to resemble the living larval community of a lake (van Hardenbroek *et al.*, 2011; Tarrats *et al.,* 2018).

In Tibetan lakes, water depth is the second most important factor for chironomid distribution following electrical conductivity (Plank, 2010). Salinity, often measured through electrical conductivity (Schwarz *et al.,* 2017), is often related to water depth in closed lake systems, where the amount of ions is relatively constant, but salinity changes as water volume changes (Kasper *et al*., 2013, 2015). Therefore, water depth reconstructions for this region are often based on salinity-reconstructions as the signaling variable (Doberschütz *et al*., 2014; Haberzettl *et al*., 2005, Laug *et al*., 2020). Water depth reconstructions using salinity as an indirect indicator are only qualitative, though (Ohlendorf *et al*. 2013, 2014), whereas reconstructions based on chironomid-water depth transfer functions can result in quantitative results (Kurek and Cwynar, 2009a; Cwynar *et al.,* 2012). Chironomid-water depth transfer functions for shallow freshwater lakes (Engels *et al.,* 2012) as well as chironomid-salinity transfer functions (Eggermont *et al.,* 2006, Dickson *et al.,* 2014) exist, but little attention has been paid to changes in the relationship between chironomids and water depth in deep lakes and under different salinities. To enable reliable chironomid-based water depth reconstructions for saline closed-basin lakes such as those on the Tibetan Plateau, we need more information on changes in chironomid assemblages along the water depth gradient. We specifically need to focus on studying lakes with different salinity to differentiate between the effects of salinity and water depth.

In this study, surface sediment samples from two Central Tibetan lakes, Selin Co and Taro Co, were analyzed for their chironomid head capsule assemblages. Both lakes are large, closed lakes at similar elevation (Guo *et al.*, 2016; Yu *et al.*, 2019), affected by similar air temperatures (Wang and Dou, 1998; Zhang *et al.*, 2011) and well oxygenated to the bottom of the lake (Turner *et al.*, 2016; Yu *et al.*, 2019). They differ in salinity, Selin Co is a saline lake (Yu *et al.*, 2019) and Taro Co is a freshwater lake (Haberzettl *et al.*, 2015).

The goals of this study are to (1) analyze the subfossil chironomid distribution along the water depth gradient of each lake, (2) identify major zones of transition along the depth gradient and (3) discuss similarities or differences in the observed distribution patterns with regard to the difference in salinity between the two lakes.

Study sites

Selin Co (87°39–92°26′ E, 30°03′–33°40′ N), situated at 4552 m a. s. l. on the Central Tibetan Plateau (Yu *et al.,* 2019; Fig. 1), is the largest lake of Tibet with a surface area of 2389 km^2 (Zhu *et al.,* 2019). The area of the lake varies: mainly due to a monsoon driven precipitation increase, the lake level rose rapidly over the course of the recent decades (Jiang *et al.,* 2017) and the lake's catchment shows a range of lake terraces representing past highstands up to 110 m above the modern lake level (Shi *et al.,* 2017a,2017 b). Considering its large surface area, the lake is relatively shallow (max. water depth ca. 59 m; Gyawali *et al.,* 2019). Selin Co is mostly saline, showing total ion concentrations of 11.07 g L^{-1} and a pH of 8-10, measured 2017 in the center of the lake (Yu *et al.,* 2019). The occurrence of aquatic macrophytes is limited to less saline areas of the lake, in particular to the western side of the lake around the inflows of the Ali and Zhagen river, the north around the inflow of the Zhajia river and a freshwater sub-basin in the south of lake Selin Co (Yu *et al.,* 2019). The lake is well oxygenated down to the bottom throughout the year, dimictic and features a thermocline located between 22 and 30 m water depth (Yu *et al.,* 2019). The Selin Co area is characterized by a cold and dry high-alpine, continental climate, featuring a mean annual temperature of 0.7°C (average January air temperature is -12.5°C, average July air temperature is 10°C; Zhang *et al.,* 2011).

Selin Co is an endorheic lake featuring four permanent inflows, Zhajia in the north, Boqu in the east, and Ali as well as Zhagen in the west. It is fed by the biggest inland lake catchment of the Tibetan Plateau, which extends over 59,383 km^2, and includes the Geladandong glacier, the Tanggula mountain range in the north and Mt. Gyagang in the south (Meng *et al.,* 2012). Besides glacial meltwater delivered by all four permanent rivers, precipitation and evaporation control the lake level (Lei *et al.,* 2013). Miocene lacustrine limestone marl, sandstone and conglomerate, Early Cretaceous limestone, mudstone and sandstone, and Quaternary mud, sands and gravel make up the catchment geology (Rowley 2006, Wu *et al.,* 2017). The vegetation around the lake is dominated by alpine meadow plants and is shaped by animal husbandry composed primarily of sheep, yak and goats (Zhu *et al.,* 2019).

Taro Co (31°03’–31°13’ N, 83°55’–84°20’ E) is a deep (max. water depth 132 m) lake situated at 4566 m asl in the western part of the Central Tibetan Plateau (Guo *et al.,* 2016; Fig. 1). Nowadays it is an endorheic lake extending over an area of 488 km^2 (Wang and Dou 1998). Over the course of the last decades, the lake only slightly increased in size (Wu *et al.,* 2017), but lake terraces in the catchment provide evidence of past lake levels that were up to 53 m higher than the present day lake level (Alivernini *et al.,* 2018). Taro Co is a freshwater lake (mean conductivity 1.0 mS cm^{-1}, alkalinity 6.9 mmol L^{-1}, pH 9.4; Alivernini *et al.,* 2018). It is well oxygenated down to the bottom throughout the year, featuring a thermocline located around 20 m water depth (Turner *et al.,* 2016). Aquatic macrophytes, in particular *Stuckenia pectinata* and *Batrachium bungei* (Guo *et al.,* 2016), densely grow all over the lake basin at depths down to 40 m (Turner *et al.,* 2016). The climate of the Taro Co region is high-alpine, semi-arid, continental, with a mean annual temperature of 0-2°C (Wang and Dou, 1998).

Taro Co is fed by several inflows, most notably the Buduo River originating in the glaciers of the Gangdise Mountains in the South of Taro Co (Zheng *et al.,* 1989). Glacial meltwater is the most important water source out of the almost 7000 km^2 large lake catchment (Ma *et al.,* 2004), which mainly consists of mudstone, siltstone, conglomerates, marine clastic rocks, siliceous mudstone and marlstone (Bureau of Geology and Mineral Resources of Xizang Autonomous Region, 1993). The catchment vegetation is mainly composed of alpine steppe and alpine meadows (Ma *et al.,* 2014).

METHODS

Fieldwork and laboratory analysis

A total of 132 surface sediment samples were taken in July of 2016 (12 samples), 2017 (52 samples) and 2018 (68 samples) at Selin Co using an Ekman-Birge grab sampler (Fig. 2). The uppermost two centimeter surface sediment was collected for each sampling location and used for chironomid analysis. Sample depths ranged from 0.15 to 51.6 m. We collected 75 water quality measurements including water depth, total dissolved solids (TDS), pH, conductivity, turbidity, temperature and optical dissolved oxygen (ODO) using an YSI Exo 2 sensor.

A total of 57 surface sediment samples were taken using an Ekman-Birge grab sampler in September 2011 (10 samples), 2012 (24 samples) and 2014 (23 samples) at Taro Co (Fig. 3). The uppermost centimeter of sediment was used for chironomid analysis. Sample depth ranged from 0.3 to 125 m. A total of 36 water quality measurements including water depth, pH, conductivity, temperature, dissolved oxygen and alkalinity were obtained using multi-probe sensors (Hydrolab DS5 and YSI multiple sensors), producing a vertical water quality profile for the deepest part of each lake (Fig. 4).

Sample preparation for chironomid analysis followed Walker *et al.* (1991). After treatment of 1-2 g sediment with 10% potassium hydroxide, heating up to 85°C and rinsing through a 100 µm mesh sieve, larval head capsules were handpicked from the residue and mounted in

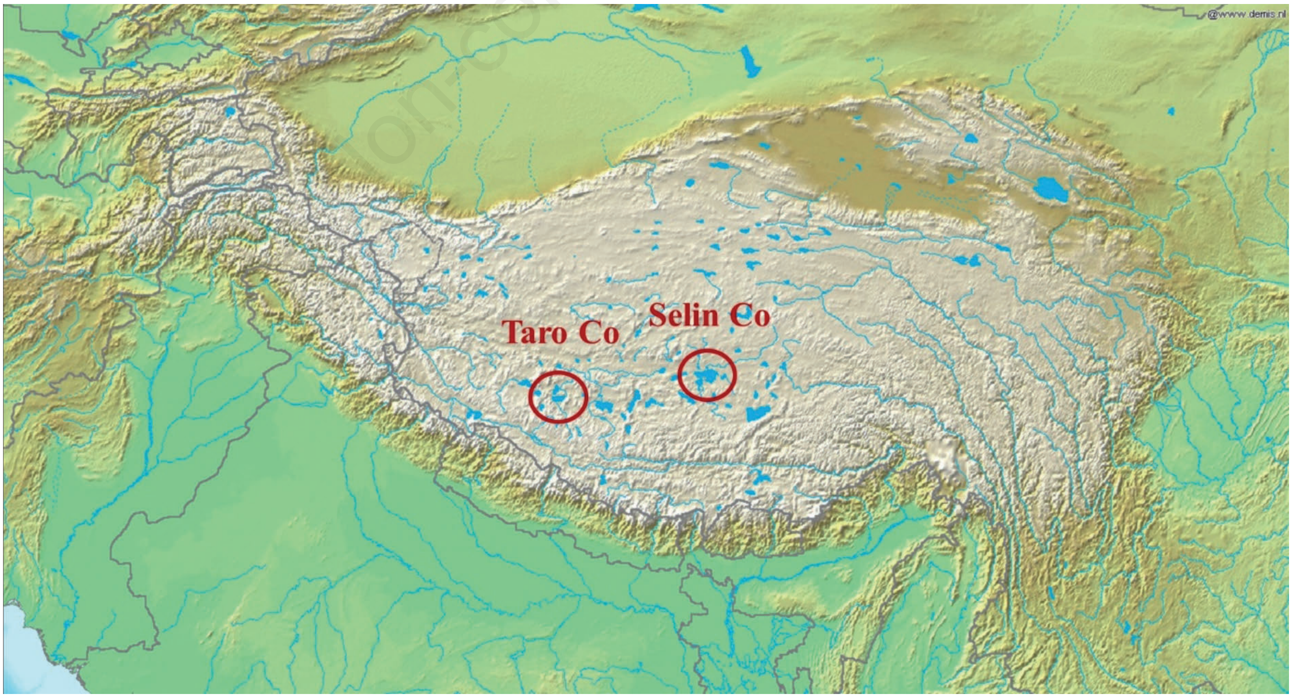

Fig. 1. Location map of the Central Tibetan lakes Selin Co and Taro Co (map source: http://www.demis.nl).

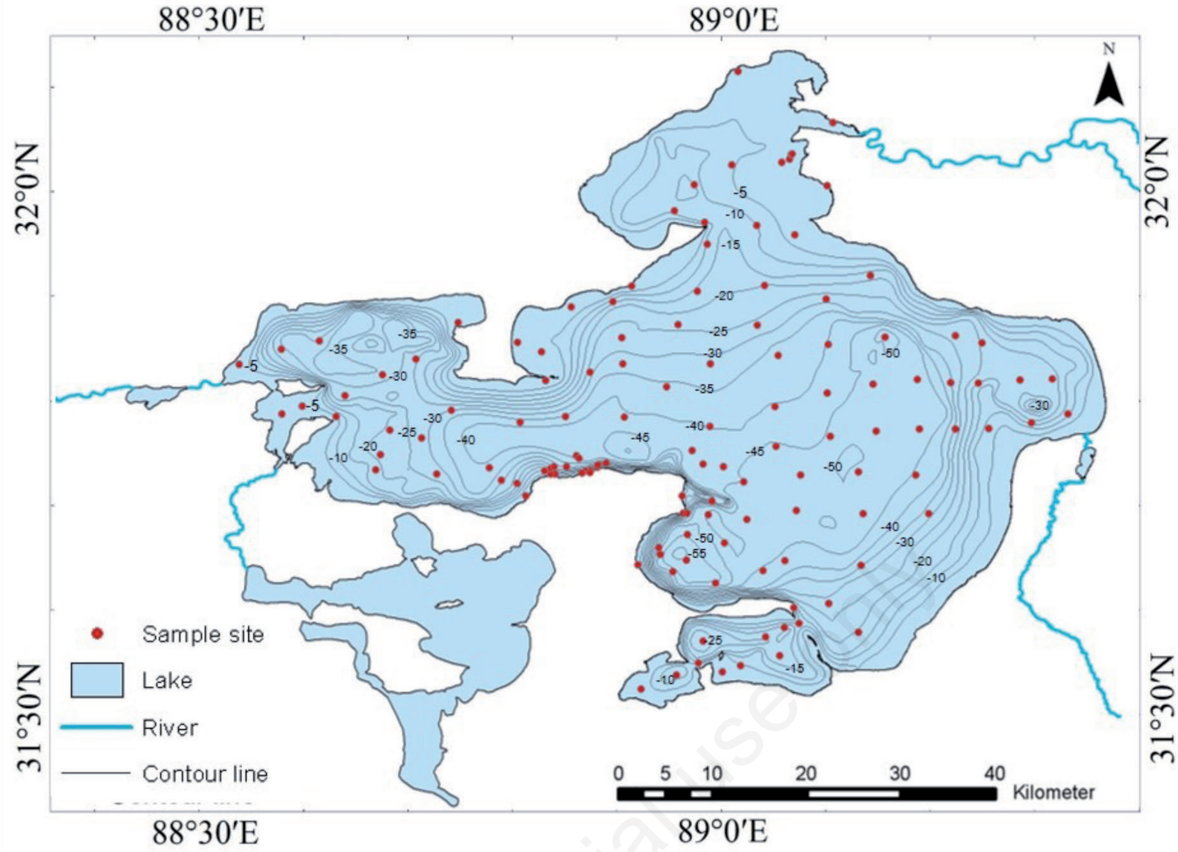

Fig. 2. Bathymetric map of Selin Co and inflows. Red dots indicate the surface sediment sample locations (modified from Yu *et al*., 2019. J. Great Lakes Res. 45:1069-1079; with permission).

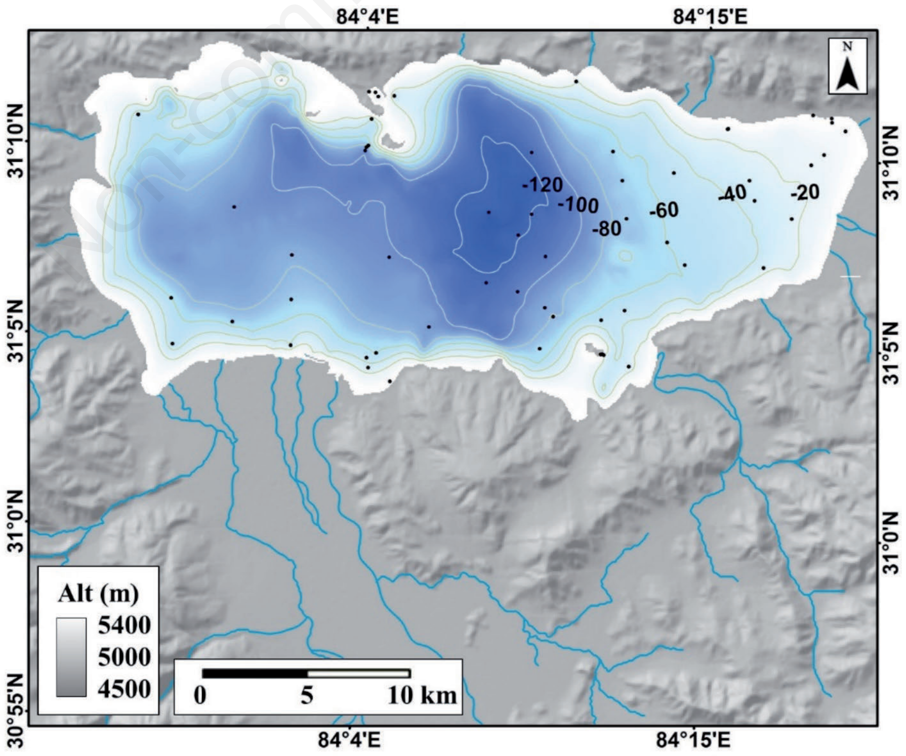

Fig. 3. Bathymetric map of Taro Co, inflows and catchment. Black dots indicate the surface sediment sample locations.

Euparal©. If sufficient material was available, the process was repeated until at least 50 head capsules were found.

Identification of the subfossil larval remains followed Brooks *et al.* (2007), Rieradevall and Brooks (2001) and Bitušík and Hamerlík (2014). Additionally, we used Zelentsov (1989), Plank (2010) and Laug (2019) to identify chironomid taxa not found in these European guides. Pictures of chironomid morphotypes found in our samples that are not included in Brooks *et al*. (2007) are included in the supplementary material (Supplementary Figs. S1-3). Head capsules that resemble those of the newly described *Acricotopus* indet. morphotype *incurvatus* (Laug *et al.,* 2019) are listed in this study as *Acricotopus* type I. In order to calculate the water content of the samples, a subset of the samples was freeze-dried before further preparation. Overall head capsule concentrations were calculated using count numbers from dry and wet samples in relation to the measured or calculated dry weight.

Numerical analysis

A minimum of 50 head capsules is often recommended as a sufficient count sum for numerical analysis (Larocque, 2001; Quinlan *et al.,* 2001). Due to the low concentrations of chironomid remains in our study material, and despite our large sample sizes of up to 120 g per sample, it was not possible to reach this count sum for all our samples. Because of the extremely low chironomid diversity in Selin Co, and in order to include as much ecological information as possible, samples with count sums between 10 and 50 were nevertheless included in our analyses.

It was not always possible to identify head capsules belonging to the genus *Chironomus* to morphotype level due to poor preservation. In samples including specimens that could not be identified below genus level, these identifications were later split into morphotypes, *Chironomus anthracinus*-type and *Chironomus plumosus*-type according to the ratio of head capsules identified to morphotype level. In most cases, *Paratanytarsus* specimens were not identified to morphotype, because the mandibles, necessary for further identification, were missing. Therefore, all *Paratanytarsus* were grouped into a single taxon. Rare taxa, occurring in fewer than 2 samples with abundances over 2%, were excluded from further statistical analysis. Head capsules of the closely related Diptera family Ceratopogonidae were included in the chironomid sum. The raw count datasets were uploaded to the data repository Pangaea (PDI-23027 for Selin Co and PDI-23028 for Taro Co, respectively).

Because the gradient lengths of the first axes of detrended correspondence analyses of the square root transformed chironomid abundance datasets were 2.28 (Selin Co) and 1.47 (Taro Co), linear methods were selected as most appropriate for subsequent analyses (Birks, 1998). Correlations between the environmental factors were calculated using Pearson R correlation coefficient tests (Pearson, 1895). If environmental factors

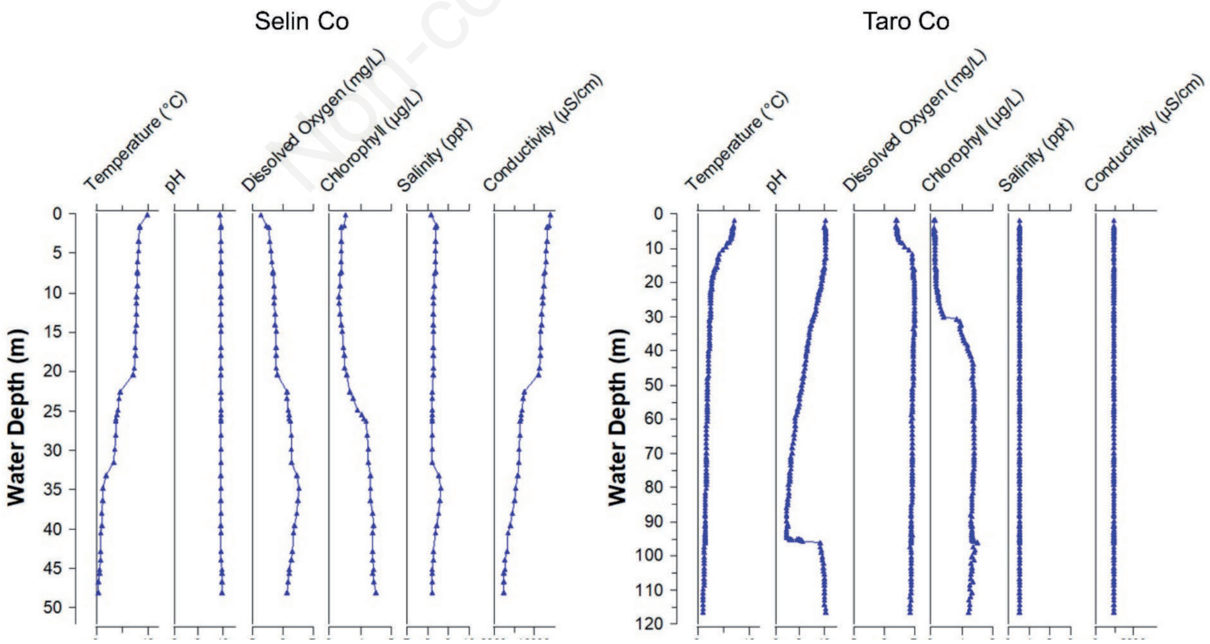

Fig. 4. Vertical distribution of the basic environmental variables (temperature, pH, dissolved oxygen, chlorophyll, salinity and conductivity) of Selin Co (left) and Taro Co (right).

were correlated significantly, one was chosen for subsequent ordinations. We used water depth instead of water temperature (Pearson's r of -0.92 in Selin Co and -0.77 in Taro Co) and salinity instead of conductivity (Pearson's r of 0.99 in Selin Co and 0.3 in Taro Co). Redundancy analysis (RDA) was used to evaluate the environmental factors driving spatial trends in the chironomid distribution of each lake (Legendre and Legendre, 1998). For this analysis, only samples with measurements of environmental conditions and a sufficient chironomid head capsule count (10 HC in Selin Co, 50 HC in Taro Co) were taken into account. For Selin Co, our dataset for numerical analysis contains 39 samples that span a water depth gradient of 0.68-46.2 m. The dataset for Taro Co includes 36 samples and spans a water depth gradient from 4.2 to 125 m.

Zonation along the water depth gradient was achieved using cluster analysis based on square root transformed percentages of the chironomid data (Everitt, 2011). As our dataset contained a low number of samples with identical water depth values, sample depths for identical samples were changed by 10 cm to allow the method to run. We repeated our analyses using different permutations to ensure that these artificial changes to our data did not influence the final zonation results. As the chironomid head capsule concentration in the Selin Co samples is low, many samples did not reach the minimum count sum of 50 typically required for numerical analyses such as zonation (Quinlan and Smol, 2001). To establish the influence of low count sums on the zonation results, we ran multiple cluster analyses based on different minimum head capsule counts (10, 25 and 50). For each run with higher head capsule cut-levels, more samples had to be removed prior to analysis. The number of statistically significant zones was determined using a broken-stick model (Bennett, 1996). All statistical analyses were performed with R (R Core Team, 2016) using the package 'vegan' (Oksanen *et al.*, 2016). Chironomid abundances along the depth gradient were plotted using C2 (Juggins, 2014).

RESULTS

Selin Co

A total of 132 surface sediment samples were analyzed for Selin Co. The samples contained an average of 25.8 head capsules (HC) per sample. A count sum of at least 50 HC was achieved in 32 samples and a count sum of at least ten HC in an additional 26 samples. The head capsule concentration was generally low (3.0 HC g^{-1} dry sediment), showing highest values between 10 and 20 m water depth (max. 23.9 HC g^{-1} dry sediment). In water depths of more than 32.9 m, low concentrations led to counts of zero for most samples, and below 35 m water depth only three samples contained at least ten HC. These three samples were all located relatively close to the shoreline at 155, 404 and 1910 m distance. We identified a total of 34 taxa in the samples from Selin Co. Nine of these were only identified to subfamily level as characteristics necessary for further identification were missing. Sixteen taxa were identified as being rare, occurring in few samples and in low abundances, and were thus excluded from statistical analyses.

The Orthocladiinae taxa *Acricotopus* type I, *Cricotopus intersectus*-type and *Psectrocladius sordidellus*-type occur in high abundances along the entire depth gradient. Semiterrestrial taxa such as *Pseudosmittia* type B and *Ceratopogonidae* and lotic taxa occur in the shallowest samples, *Chironomus anthracinus*-type occurs between 6.1 and 16.8 m water depth and *Procladius* is abundant in samples deeper than 16.8 m water depth (Fig. 5).

Redundancy analysis showed that water depth was the most important factor driving chironomid distribution, explaining 7.5% of the variance in the chironomid data (Fig. 6). Indipendent of the minimum head capsule count used in the cluster analysis, one statistically significant zonal boundary was identified along the water depth gradient. The position was at 16.25, 14.05 or 19.5 m water depth, for CONISS runs using a minimum head capsule count of 10, 25 or 50 HC, respectively (Supplementary Fig. 4-9). Additionally, a boundary between 2.5 and 6.1 m water depth was visually established in order to seperate the assemblages in which mainly semiterrestrial and lotic taxa were found from those dominated by lentic taxa. Between 2.5 and 6.1 m water depth, no samples with at least 10 HC were present, resulting in a relatively large uncertainty with regard to the exact position of the upper boundary.

Zone I (≤2.5 m)

The shallowest 2.5 m (6/15 samples ≥10 HC) include two types of samples. The first type, only occuring up to half a meter water depth, is dominated by *Pseudosmittia*-type B and Ceratopogonidae. The other sample type lacks both of these taxa and is characterized by *Cricotopus intersectus*-type including high concentratons of a morphotype with a very dark mentum and a striped HC. Concentrations in Zone I vary strongly. While the first sample type features one of the highest concentrations of Selin Co (28.5 HC g^{-1} dry sediment), concentrations of the samples dominated by *C. intersectus*-type are relatively low (ca. 0.87 HC g^{-1} dry sediment) and almost every second sample above 2.5 m water depth did not contain any chironomid head capsules.

Zone II (6.1-16.8 m)

Our second zone extends from 6.1-16.8 m water depth (15/19 samples ≥10 HC) and is dominated by *Chironomus* *anthracinus*-type in combination with *Cricotopus intersectus*-type percentages lower than in Zone I, but higher than those of deeper water depths. *Acricotopus* type I is present at levels of up to 46%. *Psectrocladius sordidellus*-type reaches percentages of up to 20% in Zone II. Concentrations in Zone II (on average. 7.8 HC g^{-1} dry sediment) are higher than in Zone I. Samples empty of chironomid HC are limited to water depths ≤9.3 m water depth and highest concentrations were found at 10-14 m water depth.

Zone III (≥16.8 m)

Procladius is the dominant genus below 16.8 m water depth (36/93 samples ≥10 HC). It has the most distinct distribution of all taxa. It occurs in high abundances limited to 16.8–32.9 m water depth. While *Chironomus anthracinus*-type and *Cricotopus intersectus*-type show low abundances, *Acricotopus* type I and *Psectrocladius sordidellus*-type show percentages similar to those in Zone II. Concentrations in Zone III are relatively high between 16.9 and 32.9 m (on avg. 3.8 HC g^{-1} dry sediment) and show the highest values around 20 m water depth. Below 35 m water depth the concentration is mostly very low (on avg. 0.5 HC g^{-1} dry sediment).

Taro Co

We analyzed 57 surface sediment samples containing on average 143.4 HC per sample and reached a count sum of at least 50 HC in each sample. The head capsule concentration was relatively high (in average 35.3 HC g^{-1} dry weight), with highest values obtained between 40 and 60 m water depth (max. 284 HC g^{-1} dry sediment) and with minimum values of just over 2 HC g^{-1} dry sediment for the deepest part of the lake (Fig. 7). In total, 57 taxa were identified in Taro Co, including nine taxa that were not identified to morphotype level and 37 rare taxa.

The most abundant chironomid taxa in the Taro Co samples were the Tanytarsini taxa *Micropsectra radialis*-type, dominant in all samples ≥16.25 m water depth, and *Paratanytarsus*. The only exception from this pattern are the uppermost 65 cm in which *Acricotopus lucens*-type, *Pseudosmittia* type B and *Tanytarsus* 'no spur', a group composed of the morphotypes *T. gracilentus*-type, *T. lugens*-type and the rare *T. mendax*-type in the Taro Co samples, dominate the assemblages (Fig. 7).

Redundancy analysis showed that water depth was the most important factor driving chironomid distribution (Fig. 8), explaining 29.2% of the variance in the chironomid dataset. Along the water depth gradient, two zonal

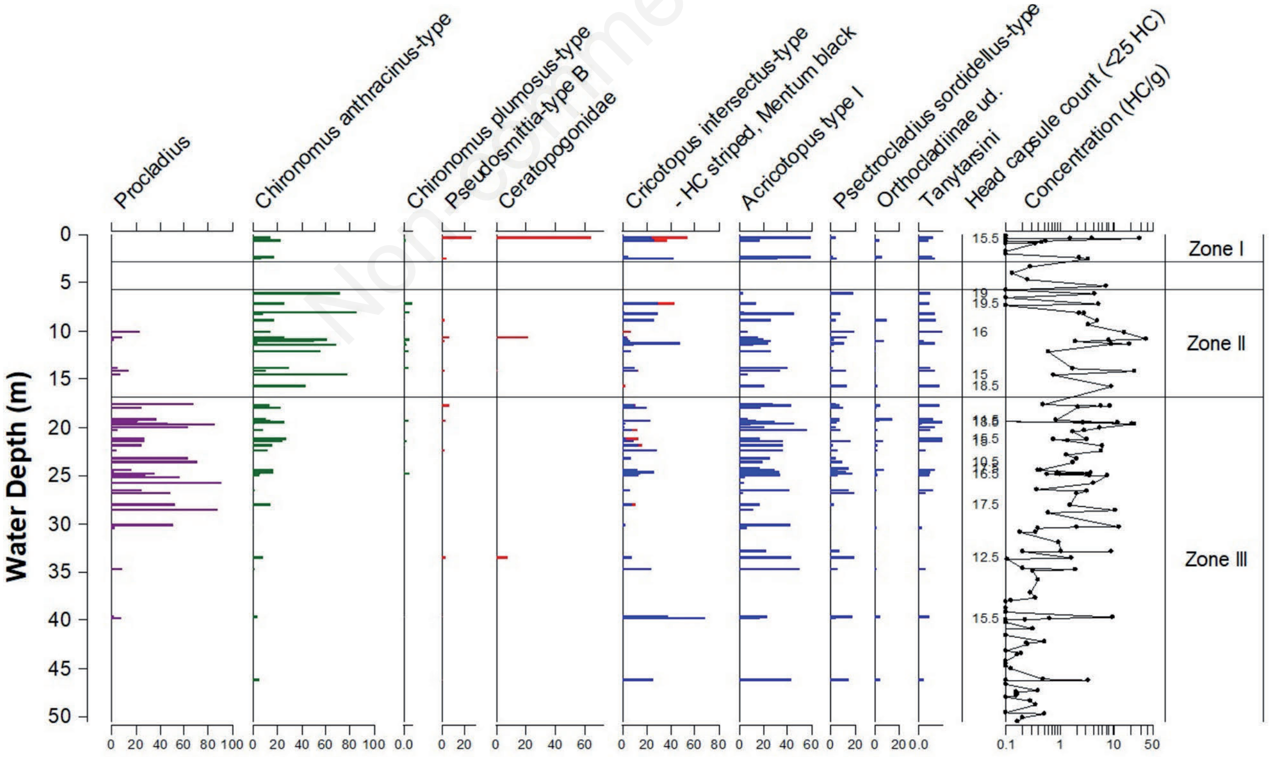

Fig. 5. Chironomid assemblages of Selin Co in percent of the total chironomid sum (including Ceratopogonidae). Head capsule count is given for samples with <25 head capsules. Concentrations are given in head capsules per gram dry sediment. Taxa with highest abundances in Zone III violet, Zone II green and Zone I red. Taxa without clear association to any zone blue.

boundaries were identified using a cluster analysis (Supplementary Fig. 10), the first at 0.65 m, the second at 16.25 m water depth. Both boundaries are statistically significant following a broken-stick model (Supplementary Fig. 11).

Zone I (≤0.65 m)

The shallowest 65 cm (2 samples) are dominated by *Pseudosmittia* type B, *Acricotopus lucens*-type and *Tanytarsus* 'no spur', which account for more than 80% of all HC in Zone I. Chironomid concentrations are relatively high at 27 HC g^{-1} dry sediment.

Zone II (0.65-16.25 m)

All three taxa that characterize Zone I are almost completely absent from Zone II (10 samples). Instead, *Paratanytarsus* dominates. Between 0.65 and 5.7 m water depth, *Chironomus anthracinus*-type occurs in high abundances and several Orthocladiinae (e.g. *Cricotopus shilovae*-type) morphotypes are present as well. With the exception of one sample, chironomid concentrations are lower than in Zone I (on average 15 HC g^{-1} dry sediment).

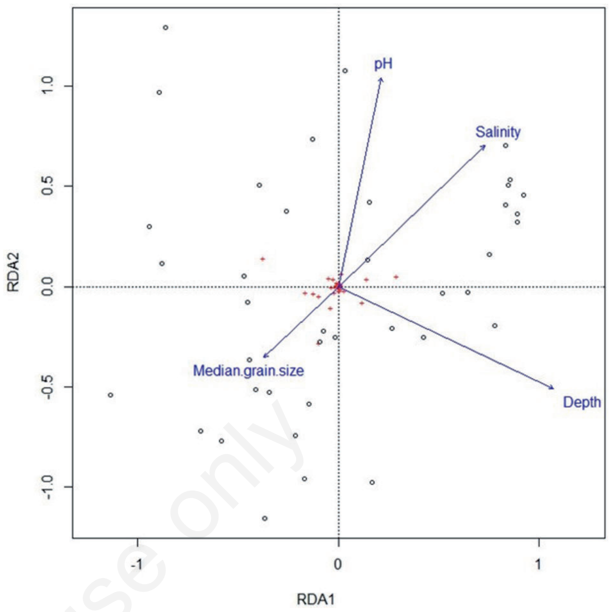

Fig. 6. Results of the redundancy analysis (RDA) of Selin Co.

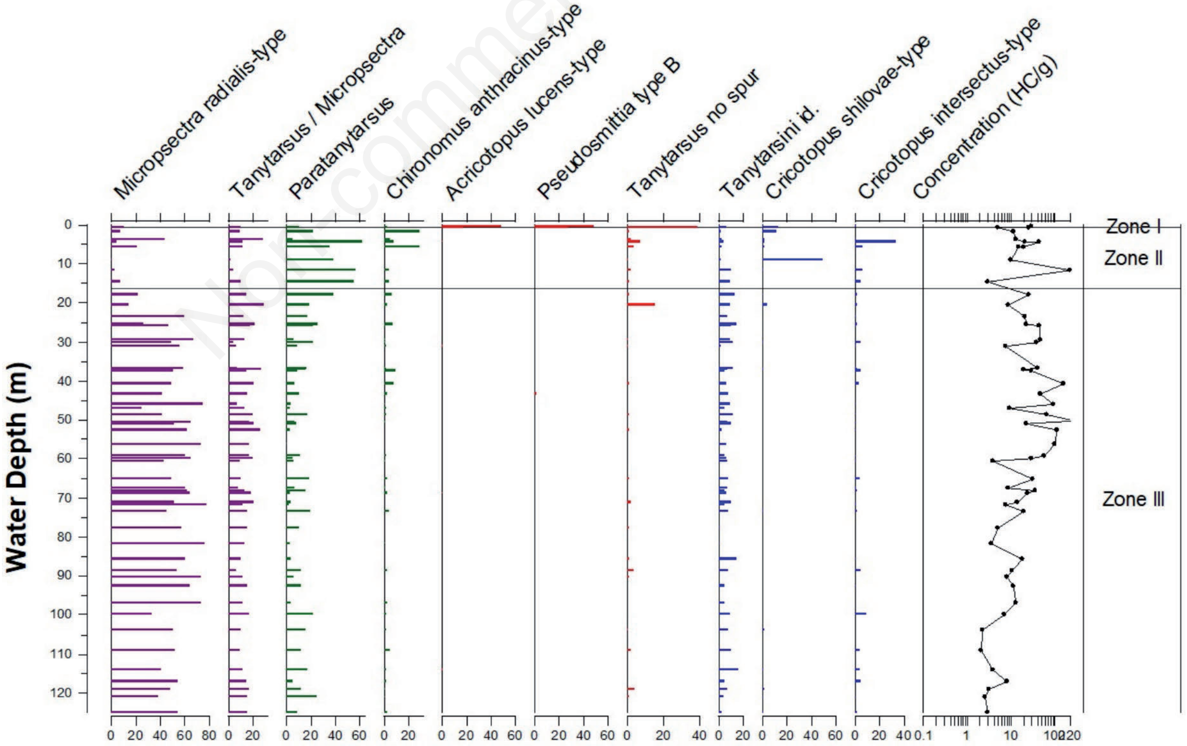

Fig. 7. Chironomid assemblages of Taro Co in percent of the total chironomid sum. Concentrations are given in head capsules per gram dry sediment. Taxa with highest abundances in Zone III violet, Zone II green and Zone I red. Taxa without clear association to any zone blue. For graphical representation, the taxa *Tanytarsus gracilentus*-type, *T. lugens*-type and *T. mendax*-type were added to the more broadly defined taxon *Tanytarsus* 'no spur'.

Zone III (≥16.25 m)

Micropsectra radialis-type is the dominant taxon in Zone III (45 samples), where *Paratanytarsus* shows lower percentage-abundances. Chironomid concentrations are similar to those of the other zones between 16 and 40 m water depth (on average 27 HC g^{-1} dry sediment), are very high between 40 and 60 m water depth (on average 90 HC g^{-1} dry sediment) and decrease in water depths exceeding 60 m (on average 11 HC g^{-1} dry sediment).

DISCUSSION

Major trends

Selin Co

The combination of Selin Co's high elevation, high level of salinity and absence of macrophyte vegetation means that the habitat is not suitable for many chironomid species. Consequently, chironomid diversity and head capsule concentration are low compared to other sites (Engels *et al.*, 2020; Motta and Massaferro, 2019). The prominence of Orthocladiinae is well known to be associated with mountain lakes (Hamerlík *et al.,* 2017) and some of the common Orthocladiinae morphotypes are well adapted to the conditions in Selin Co: *Cricotopus intersectus*-type and *Psectrocladius sordidellus*-type are adapted to high salinity (Plank, 2010; Zhang *et al.,* 2007) and *Acricotopus* indet. morphotype *incurvatus* was found in sandy sediments with sparse aquatic vegetation (Laug *et al.,* 2019). The only two taxa of high abundances that aren't part of the subfamily Orthocladiinae are *Chironomus anthracinus*-type and *Procladius*. Both taxa are interpreted as generalists (Moller Pillot, 2013a; Vallenduuk and Moller Pillot, 2013), and have been shown to occur in high abundance at high salinities (Mocq and Hare, 2018; Zhang *et al.,* 2007). The most important environmental condition explaining the within-lake patterns of chironomid distribution is water depth (Fig. 6). This finding matches results from other datasets studying chironomid distribution within lakes (Kurek and Cwynar, 2009b; Chen *et al.,* 2014; Raposeiro *et al.,* 2018).

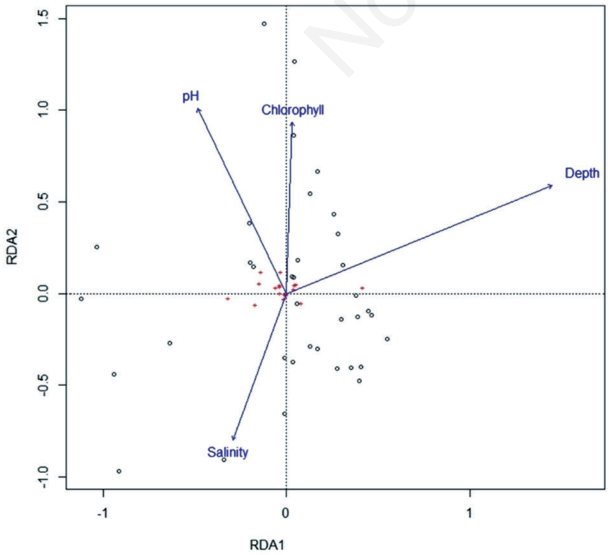

Fig. 8. Results of the redundancy analysis (RDA) of Taro Co.

Two types of chironomid assemblages occur in the shallowest zone (0-2.5 m) of Selin Co. The first assemblage type is made up by *Pseudosmittia* type B and Ceratopogonidae. Both taxa are known to inhabit a range of habitat types but are most commonly interpreted as indicating (semi-) terrestrial habitats (Brooks *et al.*, 2007). For instance, many species in the genus *Pseudosmittia* inhabit terrestrial or semi-terrestrial habitats (Strenzke, 1950) but some species also occur in shallow zones of lakes. Similarly, whilst the family of Ceratopogonidae is a large and species-rich family, the larvae of many species of biting midges can be found in terrestrial habitats (Foote and Pratt, 1954) or in shallow freshwater habitats. For instance, Engels et al. (2012) observed that subfossil remains of Ceratopogonidae most commonly occurred in surface sediment samples from shallow areas of a set of eight ponds, with highest abundances in samples retrieved from water depths of 2 m or less. Finally, in Tibet, Ceratopogonidae larvae have been observed to live in semi-terrestrial habitats (Turner, *personal observation*). Therefore, we interpret the assemblage type dominated by these two taxa as indicative for a shallow near-shoreline habitat, a particular habitat shaped by regular water cover and -level changes (Lorenz and Herdendorf, 1982). The second assemblage type is characterized by *Cricotopus intersectus*-type head capsules, which are striped and feature a very dark mentum, and are thus slightly different from specimens of this morphotype as typically encountered in e.g. European lake sediment records (Brooks *et al.,* 2007). This morphotype is known to contain a number of different species, including those specialized in lotic habitats (Moller Pillot, 2013b). This assumption is further supported by the fact that this type is not limited to the "splash zone", because it is not directly influenced by (changes in) water level, but rather seems to be influenced by the distance from an inflow. As slopes vary in height and shape around the lake, the zonal boundary is influenced by multiple parameters, potentially explaining why it is not statistically significant. Strikingly, the concentrations between samples inside this zone vary between very high where the "splash zone" is inhabited, low in the inflow-based samples and void of chironomid head capsules in about half of the zone's samples, specifically those samples characterized by coarse sand and gravel.

The second zone (6.5-16.25 m) is characterized by high abundances of *Chironomus anthracinus*-type accompanied by several Orthocladiinae taxa. *Chironomus anthracinus*-type can tolerate a range of different environmental conditions, and is known to occur in deeper, profundal habitats in for example northeast USA (Engels and Cwynar, 2011). On first sight, this might seem contradictory to the findings of this study, because in Selin Co it occurs in the second shallowest zone. In most chironomid studies, the line between shallow, littoral and deep, profundal habitats is drawn at 5-6 m water depth though. In Selin Co, the gap of samples containing sufficient HC between 2.5 and 6.1 m masks the whole littoral. Therefore, this second zone might represent the profundal habitats preferred by *Chironomus anthracinus*-type. The exact depth of the boundary between Zone II and III depends on the minimum head capsule counts chosen as a cut-level in the analysis (16.25, 14.05 or 19.05 m water depth for 10, 25 or 50 HC cut-levels, respectively). This is partly due to the low and variable number of samples included in the analyses with higher minimum count sums, especially in the 10-20 m water depth interval (8, 5 or 3 samples for 10, 25 or 50 HC, respectively). The identification of the exact position of the water depth threshold for Selin Co is further complicated by the fact that the lake has a series of steep areas, probably formed by past lake level fluctuations, between 5 and 20 m water depth. As taxon richness is relatively low in our samples, we prefer the zonation run that includes a higher number of samples that are more evenly distributed along the depth gradient over the zonation run with a higher head capsule count. Therefore, we used the results of a CONISS run with a minimum cut-level of 10 HC in the discussion below.

In the deepest zone at Selin Co, *Procladius* replaces *Chironomus anthracinus*-type as the most abundant taxon. Several Orthocladiinae taxa continue to be present in Zone III. Although *Procladius* has been found widely spread along the water depth gradient (Plank, 2010; Chen *et al.,* 2014), it was shown to play an important role in the deepest part of lakes, e.g. in Southwest China (Zhang *et al.,* 2013), comparable to Zone III in Selin Co.

The most important Orthocladiinae taxa are independent of water depth. While ecological information on the recently described Tibetan morphotype *Acricotopus* indet. morphotype *incurvatus* is still limited

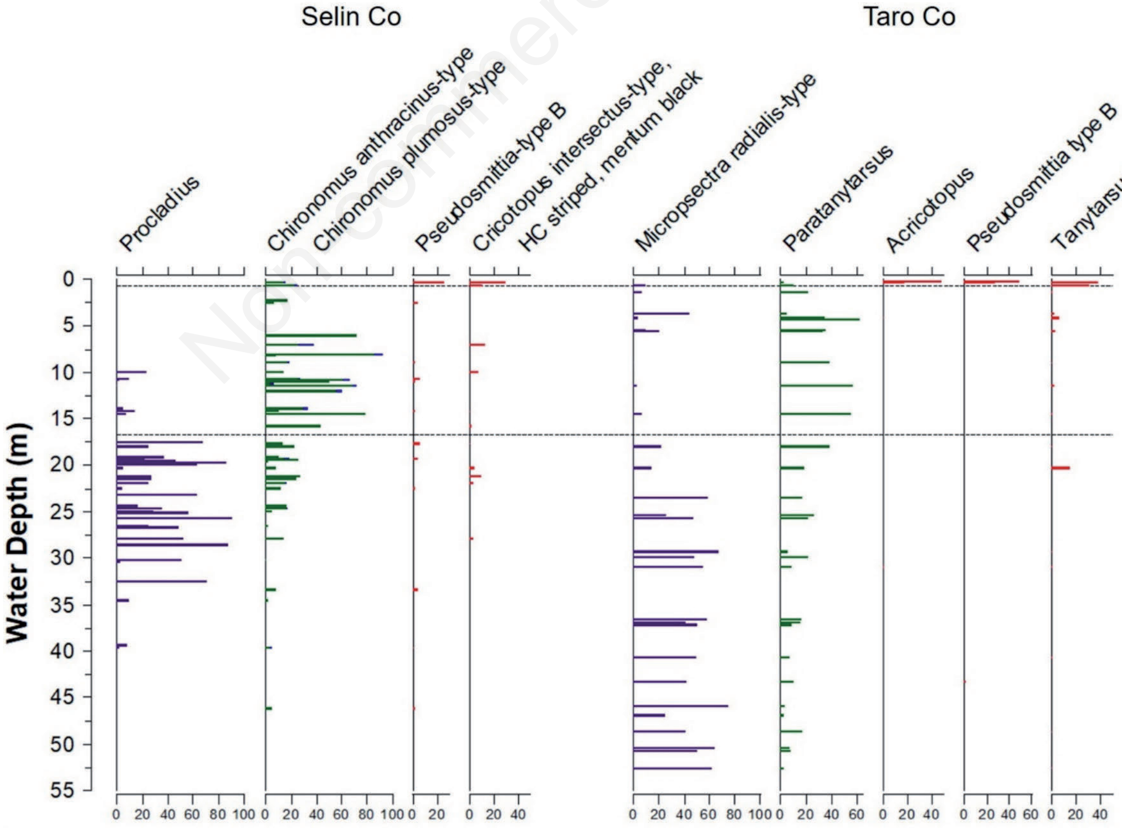

Fig. 9. Comparison of the zone characterizing chironomid taxa in percent of the total chironomid sum for the upper 55 m water depth of Selin Co and Taro Co. Taxa with highest abundances in Zone III violet, Zone II green and Zone I red.

(Laug *et al.,* 2019), both *Cricotopus intersectus*-type and *Psectrocladius sordidellus*-type are associated with aquatic macrophytes (Moller Pillot, 2013b) and are therefore most commonly found in littoral areas (Engels and Cwynar, 2011). Both taxa contain multiple species featuring different habitat requirements (Zhang *et al.,* 2007) and both this study and e.g. Raposeiro *et al.* (2018) show the even distribution along a water depth gradient of *Psectrocladius sordidellus*-type. As aquatic macrophytes play no important role in Selin Co, their distribution cannot be connected to the presence or absence of *Psectrocladius sordidellus*-type, possibly explaining the observed water depth independence.

Taro Co

Unlike in Selin Co, salinity levels are low in Taro Co, making the lake a less hostile environment for many chironomid species. As a result, chironomid diversity, head capsule concentration and maximum depth of chironomid distribution are all higher in Taro Co. The dominant two taxa in Taro Co, *Micropsectra radialis*-type and *Paratanytarsus*, are widespread across the Tibetan plateau, and tend to occur in lakes with a low salinity (Zhang *et al*., 2007; Plank, 2010). Similar to Selin Co, the most important environmental parameter explaining the variation in chironomid distribution is water depth (Fig. 6).

The characteristic taxa for the shallow zone in Taro Co are *Acricotopus lucens*-type and *Pseudosmittia* type B. *A. lucens*-type is known to occur in small water bodies with macrophytes and in the littoral area of lakes (Hirvenoja 1973; Andersen *et al*., 2013), as well as in bog pools and small streams (Brooks *et al*., 2007). Similar to the shallow-water assemblage type for Selin Co, the occurrence of both *A. lucens*-type and *Pseudosmittia* type B in Taro Co is interpreted to indicate a shallow-water habitat, potentially reflecting the "splash zone" of the lake. *Pseudosmittia* type B occurs in this habitat in both lakes, regardless of the large difference in salinity. In contrast to Selin Co, no inflow-based chironomid assemblages are found in Taro Co. This is most likely explained by a higher chironomid production in Taro Co, overriding any lotic input. As a result, the shallow zone at Taro Co does not extend below the uppermost 65 cm and its distinction is statistically significant.

The intermediate zone at Taro Co is characterized by *Paratanytarsus*, a genus associated to macrophytes (Beckett *et al.,* 1992, Tarkowska-Kukuryk, 2014). In a study on several lakes of the Plymouth Aquifer, Engels and Cwynar, (2011) found it connected to shallow water up to 10 m water depth. Even though in Taro Co the lower boundary occurs at greater depth, the habitats described in Engels and Cwynar (2011) and the one described for this study match. Whilst speculative, two potential reasons for the extended prevalence of *Paratanytarsus* up to 16.8 m water depth are the reduced competition of the Tibetan chironomid fauna relative to comparable sites elsewhere, or the extend of macrophyte growth into the deep areas of Taro Co. The chironomid diversity in Selin Co and Taro Co is much lower than the average diversity encountered in lakes with a comparable July air temperature in mid- to high-latitudinal areas across the Northern Hemisphere, which typically feature 10-20 common taxa even in samples with low count sums (Engels et al., 2020). As such, it can be hypothesized that competition for food sources, habitats *etc.* is lower in the Tibetan lakes and that the taxa that are present can therefore occupy a larger range of habitats.

Zone III is dominated by *Micropsectra radialis*-type, a species group known for its prevalence in the deepest part of lakes (Kurek and Cwynar, 2009b). Even though the transition between Zone II and III is gradual and its exact position therefore remains uncertain, it is the most important division of the chironomid distribution in Taro Co.

Comparison of distribution patterns

As expected for two lakes with such differing salinity, the species composition of the chironomid faunas of Selin Co and Taro Co is distinctly different. Chironomid concentrations are generally higher in freshwater lake Taro Co. The changes in concentration with increasing water depth follow a similar pattern in both lakes – highest in intermediate depth –but differ in the deep areas. In Selin Co, signs of chironomid life were found up to 35 m water depth, while the head capsule concentration remained relatively high up to the deepest part of Taro Co. The lakes differ in their faunal composition, but show a similar zonation. Distinct chironomid assemblages occur in the shallowest part of each of the lakes, the splash zone. This is followed by an intermediate zone between ca 4 to 16m, and, below 16-17 m, a zone characterized by chironomid taxa specialized on the deepest parts of lakes (Fig. 9). While the boundary between "splash zone" and main water body is well known (Lorenz and Herdendorf, 1982) and is recognized in many studies on within-lake distribution patterns of chironomids (Kurek and Cwynar, 2009b), the deeper boundary was not discussed previously and shall be focused on in more detail.

Zone boundary 16-17 m water depth

In both lakes, the zone boundary at 16-17 m water depth is statistically significant, but does not match with changes in any of the measured environmental conditions. The most plausible environmental condition forming a distinct distribution boundary would have been water temperature, because it changes rapidly around a thermocline in stratified lakes (Wetzel, 2001). In both studied lakes though, the thermocline is positioned well below the chironomid distribution boundary, between 22 and 30 m in Selin Co (Yu *et al.,* 2019) and around 20 m in Taro Co (Turner *et al.,* 2016).

Several other hydrological parameters – pH, salinity and, most importantly, oxygen – often vary across the thermocline, and all these parameters can have an important impact on chironomid distribution (Mocq and Hare, 2018, Dickson *et al.,* 2014, Quinlan and Smol, 2001). Neither in Selin Co nor in Taro Co do these parameters change drastically at 16-17 m and they are therefore unlikely to be the drivers of the large-scale reorganizations of the subfossil chironomid fauna that we observe at this depth. Sediment properties, especially grain size, can be used as an indicator for different flow conditions (Blais and Kalff, 1995) which influence chironomid assemblages (Schäffer *et al.,* 2019). Grain size was homogenous for the deeper areas of Selin Co (Yu *et al*., 2019) and is therefore unlikely to be a driver of the change observed in chironomid assemblages around 16 m water depth.

In many lakes, the distribution of chironomid taxa correlates with macrophyte distribution (Brodersen *et al.,* 2001). The difference in the aquatic macroflora is one of the main differentiating factors between Selin Co, where macrophytes are limited to freshwater areas, and Taro Co, where macrophytes are abundant down to 40 m water depth. In both cases, there is no change in the vegetation at 16-17 m water depth.

Taking into account that the environmental factors discussed above are unlikely to be responsible for the distribution boundary observed at 16-17 m water depth, we have to speculate what might explain the transition in the chironomid fauna. First, there could be additional important environmental parameters that we did not measure. However, our dataset does include some of the most well-known drivers of chironomid distribution. Additional unexplored parameters could include the distance to the surface or the related water pressure. One possible impact of water pressure on chironomid survival is through oxygen supply. Some chironomid larvae build tubes and keep the water in it well oxygenated through undulating motions (Armitage *et al.,* 2013). These movements might have various effects depending on water pressure from the overlying water body, calling for corresponding adaptations. Another life stage affected by water pressure and distance to the surface is the process of eclosion when the pupae have to reach the surface (Armitage *et al.,* 2013). By trapping air bubbles, they are able to slowly float upwards and thus live in great depths, in lake Baikal even down to 1360 m (Linevich, 1971). In both cases, special adaptations for life in deep lakes are necessary, possibly leading to the observed distribution boundary.

Such an organism-specific distribution boundary would affect other aquatic organisms only indirectly through predator-prey interactions. Appropriately, Yu *et al.* (2019) showed for Selin Co that the diatom distribution indeed changes at a different depth, at 22 m, the position of the thermocline. Similarly, ostracod studies at Taro Co showed no change at 16-17 m, but rather at 40 m water depth (Guo *et al.*, 2016). Both studies can be seen as support of the thesis that the distribution boundary is connected to chironomid physiology rather than as a general ecological threshold.

Global comparison

In order to estimate the significance of the zonal boundary observed at 16-17 m water depth, comparisons to other chironomid datasets were made. The most similar study to ours dealt with eight lakes in East North America (Engels and Cwynar, 2011). Long Pond, the only lake studied by Engels and Cwynar (2011) that was deeper than 16 m, showed a chironomid distribution boundary at a similar water depth (14.8 or 16.0 m, depending on the statistical approach). Apart from this single-lake dataset, a similar distribution boundary has been found by Barley *et al.* (2006) who assembled a northwest North American training set for chironomids, showing a distinct change at 15 m water depth.

A distributional boundary is not visible in all available datasets, though. In Lugu Lake, Yunnan, southwest China, a shift in the chironomid assemblages at 17 m water depth was found, but it was not statistically significant, probably overlaid by the effects of the thermocline and the extent of macrophyte vegetation situated at the same water depth (Zhang *et al.,* 2013). When studying maar lakes in northwest North America, Kurek and Cwynar (2009b) found a comparable distribution boundary (15.3 m water depth) in only one out of three deep lakes. A possible reason for the lack of a similar transition in the other two lakes might be the steep shorelines of the maar lakes resulting in increased transportation diluting boundaries.

A distribution boundary was completely absent in the Spanish lake Enol, that showed a relatively homogeneous chironomid composition along the depth gradient. We propose that this is most likely due to the small extend of lake Enol (0.1 km^2) and the connected small distances that can easily be bridged through head capsule transportation (Tarrats *et al.,* 2018). Raposeiro *et al.* (2018) found the most important change for chironomid distribution at 20 m water depth in Azul Lake, Azores. All three transects analyzed in that study featured a steep slope that also covered 16-17 m water depth.

In conclusion, we find compelling evidence for a transition in chironomid distribution patterns along the water depth gradient from a range of studies across different continents and climate zones.

CONCLUSIONS

The study of the two Tibetan lakes Selin Co and Taro Co revealed patterns of the chironomid distribution along the water depth gradient that are independent of salinity and their corresponding chironomid community. The pattern shows two important thresholds at around 0.8 and 16 m water depth and can be found in comparable lakes worldwide. While the shallowest, semi-terrestrial, habitats are distinctly different, the threshold at 16 m water depth is apparently specific for chironomids. Further studies are necessary to discover the driving mechanisms as well as associated factors responsible for the exact position of the boundary.

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