Towards more accurate temperature reconstructions based on oxygen isotopes of subfossil chironomid head-capsules in Australia

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Key Words:

Chironomid head capsules, Tanypodinae, δ^{18}O, south-east Australia, vital effects, seasonality, temperature reconstructions

Abstract

This study investigates the potential of applying stable oxygen isotopes ($\delta^{18}O$) from head capsules (HCs) of subfossil chironomids (subfamily Tanypodinae) to reconstruct past temperature changes from south-eastern Australia. The study reports $\delta^{18}O$ results from Tanypodinae HCs in nine lakes. The relationship between $\delta^{18}O$ values of Tanypodinae HCs in lakes and summer (February) air temperature is robust ($r = 0.84$) supporting its potential to be applied as a temperature proxy in the Australian region. The comparison of these results with the $\delta^{18}O$ values measured on *Chironomus* spp. HCs from the same lakes reveals differences between the two groups. $\delta^{18}O$ values of Tanypodinae HCs have a stronger correlation with the $\delta^{18}O$ of lake water, $\delta^{18}O$ of precipitation and air temperature as compared with *Chironomus* $\delta^{18}O$ values. This suggests that Tanypodinae HCs are superior targets to *Chironomus* spp. for temperature reconstructions. Our data indicate that the $\delta^{18}O$ of Tanypodinae HCs could perform as well as the assemblage based chironomid transfer function from the Australian mainland. We recommend analysis of HCs from additional lakes to develop a more robust calibration curve relating Tanypodinae HC $\delta^{18}O$ to temperature.

Introduction

The stable oxygen isotopic composition ($\delta^{18}O$) of tissue from aquatic organisms can be used to infer the isotopic composition of the host lake water (Sauer et al. 2001; Schimmelmann and DeNiro 1986; Wooller et al. 2004). This is because oxygen for biosynthesis is acquired primarily from lake water; either directly or indirectly via diet (Schimmelmann and DeNiro 1986; Soto et al. 2013). The calcareous and chitinous parts of aquatic organisms are often fossilised and preserved in the lake sediment which provide opportunities to reconstruct past changes in $\delta^{18}O$ of lake water. Inferences can then be made about other paleoenvironmental variables based on reconstructions of past lake water $\delta^{18}O$. Previous studies have demonstrated that $\delta^{18}O$ values from a variety of sources can be used as paleoenvironmental proxies and a relationship between lake water $\delta^{18}O$ and $\delta^{18}O$ from these sources has been established. This includes calcareous materials (Andrews et al. 1997; Stuiver 1970), aquatic cellulose (Wolfe et al. 2007), biogenic silica (Leng and Marshall 2004) and the chitinous remains of aquatic invertebrates (chironomids, cladocera and aquatic beetles) (Gröcke et al. 2006; Gröcke et al. 2011; van Hardenbroek et al. 2012; Verbruggen et al. 2011; Wooller et al. 2004).

The use of $\delta^{18}O$ signatures from the chitinous remains of invertebrates as a paleoenvironmental proxy has received increased attention over the last few decades. This is because they are commonly present in lake sediments, usually well preserved and the morphotypes can be identified and manually selected before analyses. Isolation of sample targets by manual picking is advantageous as it reduces the risk of contamination and provides the opportunity to compare isotopic signatures from different taxonomic groups. Among these chitinous remains, subfossil chironomid head capsules (HCs) have been targeted in a few studies (Chang et al. 2016; Frossard et al. 2013; Verbruggen et al. 2011; Verbruggen et al. 2010; Wooller et al. 2004). One of the advantages of targeting subfossil chironomids is that an independent temperature proxy can be derived from the application of a transfer function based on species assemblages (Chang et al. 2015a; Chang et al. 2015b; Heiri et al. 2011; Larocque et al. 2001; Massaferro et al. 2014; Rees and Cwynar 2010; Rees et al. 2008) and thus the results of the stable isotope analysis can be cross-verified using an independent technique from the same samples. In addition, because of the widespread use of chiromomid-based transfer functions, the environmental tolerances of these organisms are relatively well known (Brooks and Birks 2001; Walker 1987; Walker et al. 1991).

Previous studies have demonstrated that the $\delta^{18}O$ from chitinous subfossil remains can provide insights into past changes of climate and environment (Wooller et al., 2004; Wooller et al., 2008; Verbruggen et al., 2010). This is because the $\delta^{18}O$ of lake water is strongly influenced by climatic variables including regional air temperature, precipitation and evaporation. In non-arid environments, the $\delta^{18}O$ of lake water is closely correlated with the $\delta^{18}O$ of precipitation, which in many instances, is strongly influenced by the air temperature (Gibson et al. 2016; Gibson et al. 2002; Jones et al. 2016). Therefore, the $\delta^{18}O$ of chitin reflects the $\delta^{18}O$ of lake water and hence, can be applied as a proxy to infer precipitation $\delta^{18}O$ values and changes in temperature. The application of this method to down-core records is based on the assumption that the same relationship between $\delta^{18}O$ of precipitation and lake water and temperature prevailed in the past as is observed at the present. We acknowledge however, that this likely to vary where precipitation source has changed in the region due to changes in atmospheric circulation pattern. Changes in $\delta^{18}O$ of ocean water linked to changing zonal wind fields on glacial to interglacial time scales may also affect precipitation $\delta^{18}O$.

Previous analyses from sixteen lakes of south-eastern Australia suggested $\delta^{18}O$ of *Chironomus* HCs is potentially a valuable tool for reconstructing temperature in cooler, low nutrient and low salinity lakes but that ‘vital effects’ may also play a role (Chang et al. 2016). The ‘vital effects' may stem from, but are not limited to the insect physiology and foraging behaviour of the chironomid larvae. *Chironomus* spp. HC $\delta^{18}O$ is a promising temperature proxy, but did not perform as well as the assemblage-based chironomid transfer function for mainland Australia (Chang et al. 2015a). Here, we examine HC $\delta^{18}O$ from a different group of chironomids (subfamily: Tanypodinae) to determine if we could improve on the performance of *Chironomus* spp. HC $\delta^{18}O$. We chose to analyse the HCs from the subfamily Tanypodinae because it is one of the most abundant and wide-spread subfamilies in south-east Australian lakes (Chang et al. 2015a), second only to the genus *Chironomus*. It has a maximum abundance ~34% in modern lake surface samples from the south-eastern Australian training set (Chang et al. 2015a; Rees et al. 2008). Tanypodinae are also common (e.g. up to 40% from Eagle Tarn in Tasmania) in down-core samples that have been used for previous Australian paleo-temperature reconstructions derived from chironomid transfer functions (Rees et al. 2008), suggesting that enough HCs would be available for $\delta^{18}O$ analysis from the last deglaciation and the Holocene (Rees and Cwynar 2010). In addition, it would also allow direct comparison between transfer function and $\delta^{18}O$ based temperature reconstructions.

In the study presented in this paper we analysed $\delta^{18}O$ on Tanypodinae from nine south-east Australian lakes. We investigated the relationship between the $\delta^{18}O$ of Tanypodinae HCs and the $\delta^{18}O$ of lake water, $\delta^{18}O$ of precipitation and seasonal temperatures (summer and mean annual). Five of the samples used in this study were part of a previous study which examined $\delta^{18}O$ from *Chironomus* spp. HCs in Australian lakes (Chang et al. 2016). This allowed us to compare the relationships of $\delta^{18}O$ *Chironomus* spp. and *Tanypodinae* HCs with $\delta^{18}O$ of lake water, $\delta^{18}O$ precipitation and temperature respectively. We explore the potential of applying the $\delta^{18}O$ of Tanypodinae HCs as a temperature proxy and investigate the implication of the variations of $\delta^{18}O$ values between the HCs of the two chironomid taxa. Finally, we compare the potential of applying this method with the chironomid assemblage-based transfer function from the same region.

Regional setting

Nine lakes located in south-east Australia (Fig. 1) were examined in this study. This included four lakes from Victoria, four from Tasmania and one from the Australian Alps in New South Wales. The elevation of the sites ranged from sea level to c. ~2000 m above mean sea level (a.m.s.l) (Fig. 1). The lakes from Victoria are located in humid areas that have a winter dominated rainfall regimes. The four lakes from Tasmania all come from humid locations with westerly derived winter and spring rainfall. Lake Albina is located on the Snowy Mountains in the Australian Alps (Fig. 1), which is the coolest and highest area of Australia. The precipitation (including significant snowfall) is winter and spring dominant. Among the Victorian lakes, Lake Catani on Mount Buffalo is an artificial reservoir established in 1910 AD. It was created by construction of a dam across Eurobin Creek, which originates from Haunted Gorge (1450 m a.m.s.l.) (National-Parks-Service 1996). Highlands Waters and Lake Samuel in the central highlands of Tasmania are also artificial though in these cases the basins were flooded to create lakes for trout fishing.

Materials and Methods

All lakes were sampled during the summer (January and February) of 2012 and 2013. The sampling method follows Chang et al. (2016). Three short sediment cores and lake water samples for stable isotope analyses were collected from the centre of the lakes. A Glew Mini Corer (Glew 1991) was used to collect sediment cores and the top two centimetres of each core were extruded on site and packaged at 0.5-cm intervals into Whirlpac® bags. Lake water samples were collected into polyethylene bottles and sealed carefully to prevent evaporation and isotopic fractionation. All samples were refrigerated until analysed.

Precipitation $\delta^{18}O$ and temperature data

Stable oxygen isotopes ($\delta^{18}O$) values in precipitation data were obtained from the Global Network of Isotopes in Precipitation (GNIP) data set (IAEA/WMO 2015). The GNIP $\delta^{18}O$ in precipitation surface (raster file) was imported into ArcMap (ArcGIS 10.1) and the annual average $\delta^{18}O$ values were extracted for each study site (Bowen and Revenaugh 2003). WorldClim climate surfaces (WorldClim Program, available from http://www.worldclim.org/bioclim, accessed 24 July, 2015) were also imported into ArcMap so the relevant climate variables could be extracted for each of our study sites (Chang et al. 2015a; Chang et al. 2014). The WorldClim climate surface is based on Australian instrumental records (Hijmans et al. 2005) derived from around 600 nation-wide weather stations spanning the years 1950–2000 (http://www.bom.gov.au/climate/data/stations/, accessed 24 July, 2015). We chose to test both mean annual air temperature (MAT) and mean February temperature (MFT) against the $\delta^{18}O$ values of chironomid HCs in this study because MAT is usually derived from chironomid-based stable isotope data (Chang et al. 2016; Mayr et al. 2015; Verbruggen et al. 2011; Wooller et al. 2004), whereas it is known that the $\delta^{18}O$ values reflect the growth season for the chironomids, which is summer time. It is reasonable to expect that the chitin in the instars is also formed at this time. Hence, we have also compared our results to MFT as a summer proxy.

Sample preparation and analysis

Preparation for the analyses of $\delta^{18}O$ of chironomid HC samples was performed in the laboratories at the School of Earth and Environmental Sciences, University of Queensland, following the same method as in Chang et al. (2016). The protocol is a modified version of the method developed and used in Wang et al. (2008) and Verbruggen et al. (2011). Surface sediment samples were deflocculated using a cold 10% solution of potassium hydroxide for 20–30 minutes and washed through a 90 micron sieve using distilled water. HCs were hand-picked from the residue under a dissection microscope at 50x magnification and placed into pre-labelled vials. The samples were then placed in an ultrasonic bath for 20–30 seconds and contaminants (e.g. sediment) were removed manually under the dissection microscope. The treated clean HCs were transferred to a pre-weighed silver cup (Costech Analytical Technologies, INC., code: 041066) and allowed to dry at room temperature. When a minimum of 100 µg of head capsules was reached the silver cups were weighed, folded and shape-trimmed. The samples were shipped by air to the Stable Isotope Biogeochemistry Laboratory at Durham University for oxygen isotope analysis.

The HCs were stored in the Stable Isotope Biogeochemistry Laboratory in Durham University at room temperature for seven days prior to analysis for the samples to equilibrate with the laboratory environment. Analyses were performed using a TC/EA connected to a Thermo Scientific Delta V Advantage isotope-ratio mass-spectrometer. The oxygen isotope results were internally calibrated against several keratin standards. The samples and internal standards are stringently calibrated against the international standards IAEA 602 and IAEA 601. Repeated measurement of the internal and international standards gave standard deviations (1 SD) of ± 0.04 ‰. Oxygen isotope values are reported in standard delta notation ($\delta^{18}O$) in per mil (‰) against Vienna Standard Mean Ocean Water (VSMOW) (Coplen 1995). A total of eleven HC samples were successfully measured and these include Tanypodinae HC samples from all lakes and two *Chironomus* spp. HC samples from Lake Samuel and Plimsoll Lake. We do not attempt to directly compare absolute δ^{18}O values of *Chironomus* spp. HC from this study with the values derived in Chang et al. (2016), since samples were analysed from different laboratory and a slightly different protocol was used. Instead, the strength of the correlation in the relationships between the δ^{18}O values of the HCs vs. δ^{18}O lake water, δ^{18}O precipitation as well as temperatures was examined.

Lake water samples for δ^{18}O analyses were transported, prepared and analysed at Purdue Stable Isotope laboratory facility, West Lafayette, USA and these values were reported in Chang et al. (2016). These samples were analysed using a High Temperature Conversion Elemental Analyzer (TC/EA, Thermo Fisher Scientific) injected using a GC-PAL auto-sampler. The TC/EA was connected to an isotope ratio mass spectrometer (IRMS, Thermo Fisher Scientific) (Delta V Plus, ThermoFinnigan) for isotope ratio determination (Gehre et al. 2004). The lake water δ^{18}O values were calibrated using three Purdue internal standards relative to Vienna-Standard Mean Ocean Water (VSMOW) (Coplen 1995). Average uncertainties for lake water δ^{18}O were ± 0.44‰.

We assessed each of the data sets using kurtosis and skewness and results show that the kurtosis and skewness values for each sets of the δ^{18}O of Tanypodinae HCs, precipitation, lake water and MFT, MAT data are within an acceptable range (± 2) (Table 1). This suggests that no evidence exists for a non-normal distribution (Field 2000; Trochim and Donnelly 2006). Pearson's correlation analysis was then applied in Grapher 8.0 to examine the relationships between the δ^{18}O values of Tanypodinae HCs and δ^{18}O values of lake water, local precipitation and temperature (mean summer and annual) in the nine lakes. The correlation coefficient (r) was used to assess the strength of these relationships. The same statistical methods were then applied to the five lakes with δ^{18}O values from both Tanypodinae (this study) and *Chironomus* spp. (Chang et al. 2016), to compare the strength of these relationships in the two different taxa. We then applied a ‘One step’ Bonferroni-type correction for the critical p-values in this set of significance tests to detect the ‘false positives’(García 2004).

Results

The $\delta^{18}O$ values of Tanypodinae HCs from the nine lakes range from 16.4 ‰ to 30.7 ‰ (Table 1). The lowest value was obtained from Lake Catani (Fig. 1, Table 1) and the highest value came from Lake Cartcarrong (Fig. 1, Table 1). Lake Catani and Lake Cartcarrong are located in the high country (Mt Buffalo ~1300 m. a.m.s.l) and lowland volcanic plains of Victoria, respectively. The $\delta^{18}O$ values on HCs from two samples (Lake Samuel and Plimsoll Lake) that were directly comparable (measured in this study) showed that $\delta^{18}O$ of *Chironomus* spp. HC was more enriched than Tanypodinae (Table 1). The results showed that the lake water $\delta^{18}O$ values varied from –7.0 ‰ (Lake Albina) to 13.0 ‰ (Lake Cartcarrong), precipitation $\delta^{18}O$ values ranged from –7.2 ‰ (Lake Albina) to –4.7 ‰ (Lake Cartcarrong) (Table 1).

A strong correlation between the $\delta^{18}O$ of precipitation and lake water (r = 0.74) was observed (Fig. 2, Table 2) and $\delta^{18}O$ of precipitation was also strongly correlated with the local temperature (r = 0.83 for MAT and r = 0.89 for MFT) (Fig. 2, Table 2). The correlation between $\delta^{18}O$ of lake water and temperature was also strong (r = 0.77 for MAT and r = 0.72 for MFT) (Table 2).

Pearson’s correlation analysis on $\delta^{18}O$ of Tanypodinae HCs against $\delta^{18}O$ of lake water, $\delta^{18}O$ of precipitation, mean annual temperature (MAT) and mean February temperature (MFT) was performed with all nine lakes included and with Lake Catani left out, respectively (Table 2, Figs. 3–5). We explored the effect of removing Lake Catani because the $\delta^{18}O$ of lake water was particularly low with respect to the $\delta^{18}O$ of precipitation (Table 1, Fig. 2). The strength of the correlations between $\delta^{18}O$ of Tanypodinae HCs and all of the four tested variables were enhanced when Lake Catani was removed. Removal of Lake Catani improved the correlation between $\delta^{18}O$ of Tanypodinae HCs and all variables. R values increased for all correlations including $\delta^{18}O$ of lake water (r increased from 0.69 to 0.76, Fig. 3); $\delta^{18}O$ of precipitation (r increased from 0.40 to 0.76, Fig. 4); MAT (r increased from 0.72 to 0.80), and MFT (r increased from 0.63 to 0.84) (Fig. 5).

The results from Pearson's correlation analyses from the five lakes (Table 1) that have $\delta^{18}O$ values from both Tanypodinae and *Chironomus* spp. HCs showed that $\delta^{18}O$ values of Tanypodinae HCs are more strongly correlated to the four tested variables (Table 3). These are r = 0.80 ($\delta^{18}O$ of Tanypodinae HCs) against r = 0.33 ($\delta^{18}O$ of *Chironomus* spp. HCs) for $\delta^{18}O$ of lake water; r = 0.71 ($\delta^{18}O$ of Tanypodinae HCs) against r = 0.50 ($\delta^{18}O$ of *Chironomus* spp. HCs) for $\delta^{18}O$ of precipitation; r = 0.80 ($\delta^{18}O$ of Tanypodinae HCs) against r = 0.44 ($\delta^{18}O$ of *Chironomus* spp. HCs) for MAT and r = 0.86 ($\delta^{18}O$ of Tanypodinae HCs) against r = 0.41 ($\delta^{18}O$ of *Chironomus* spp. HCs) for MFT.

Discussion

The strong correlation between temperature and $\delta^{18}O$ of precipitation in the nine lakes (r = 0.83 for MAT and r = 0.89 for MFT) (Fig. 2A) suggests that in the temperate humid south-eastern region of Australia, temperature is the dominant factor influencing $\delta^{18}O$ in precipitation (Jones et al. 2016). The relatively strong correlation between $\delta^{18}O$ of lake water and precipitation (r = 0.74, Fig. 2B) suggests the water in the lakes mainly originates from the regional rainfall in spring and winter; other factors probably also played important roles in determining the relationship between the $\delta^{18}O$ of lake water and precipitation, for instance, aridity and residence time (Chang et al. 2016; Chang et al. 2014). Two lakes, Lake Cartcarrong and Lake

Catani deviate from the relationship apparent in the remaining lakes (Fig. 2B). The high δ^{18}O value of Lake Cartcarrong lake water was possibly due to being located in an area that is more arid than all the other lakes. The lake is also the warmest and shallowest (1.1 m deep) and may thus be more susceptible to evaporative enrichment than the other sites.

In contrast, Lake Catani was characterized by low δ^{18}O value of lake water with respect to precipitation, inferring a much lower average lake water temperature value than the modelled local air temperature value based on WorldClim. The site receives a stream inflow from Eurobin Creek, south-west of the basin, which originates from a higher elevation area all year round. More critical than the modest difference in elevation of the source area for the stream is that Mt Buffalo is a ski resort and snow banks persist late into the summer in sheltered locations (Parks-Victoria 2016). Snowbank feeding of the inflow stream would ensure that water temperatures would be lower during the summer than predicted from the climate surface models (i.e. WorldClim). In contrast, the highest lake in the data set (Lake Albina, Fig. 1) has no inflow stream and meltwater from its catchment is likely to be warmed during the slower seepage to the lake. Alternatively, as the δ^{18}O for precipitation is modelled for an annual average in WorldClim therefore the modelled output does not necessarily represent the synoptic climate patterns that resulted in the precipitation during sampling.

Lake Catani was also characterized by an unexpectedly low δ^{18}O value of Tanypodinae HCs (Table 1) (Fig. 3A). The δ^{18}O of Tanypodinae HCs is a very poor reflection of the δ^{18}O of precipitation in Lake Catani (Fig. 4A). The same applies to the temperature (both MAT and MFT) correlations from this site (Fig. 5A and 5C). We propose the following hypothesis for these observations: as discussed above, Lake Catani may receive inflow of snowbank meltwater and this has affected the correlation between the δ^{18}O of lake water and precipitation and because of that, the lake water $\delta^{18}O$ is a poor inference of the local air temperature. We therefore re-analysed all the data after removing Lake Catani.

Relationship between $\delta^{18}O$ of Tanypodinae HCs and air temperatures

There is a strong correlation (r = 0.76) between the $\delta^{18}O$ of Tanypodinae HCs and lake water of the remaining eight south-eastern Australia lakes (Fig. 3B). An identical strength of correlation (r = 0.76) was obtained between the $\delta^{18}O$ of Tanypodinae HCs and $\delta^{18}O$ of precipitation indicating that it potentially could also be used to infer the $\delta^{18}O$ of precipitation (Fig. 4B). This is further confirmed as a stronger correlation (r = 0.80, Fig. 5B) between the $\delta^{18}O$ of Tanypodinae HCs and mean annual temperature (MAT) was obtained. $\delta^{18}O$ of precipitation is strongly related to air temperature in this region, but chironomid based inferences of $\delta^{18}O$ of precipitation may also be useful in other areas where there are strong source effects based on changes in circulation.

We note that the $\delta^{18}O$ value of Tanypodinae HCs from Lake Albina deviates in the correlation relationship compared to the interpolated MAT from the site (Fig. 5A, B). Lake Albina is the only true Alpine lake (c.~2000 a.m.s.l) that is located above the tree-line among all the sites tested and is ice-covered during winter and spring (June – November) (Green 2012). It is likely that the $\delta^{18}O$ value of Tanypodinae HCs from Lake Albina in particular, represents only the summer season temperature as the growth of chironomid larvae and the incorporation of chitin into the chironomid HCs may only occur during the ice-free period in this lake. The $\delta^{18}O$ value of Tanypodinae HCs and MFT showed a stronger correlation (r = 0.84) than MAT. This is due to the fact that Lake Albina results agrees better with the relationship between $\delta^{18}O$ of Tanypodinae and MFT than the one between $\delta^{18}O$ of Tanypodinae and MAT. In summary, the results from this study demonstrated that the $\delta^{18}O$ of Tanypodinae HCs has the potential to be applied as a proxy to infer past temperature change in south-eastern Australia and when high elevation lakes (i.e. lakes that freeze during winter-spring) are examined, it may be a better proxy for summer temperatures than mean annual temperatures.

$\delta^{18}O$ values of Tanypodinae vs. Chironomus spp. HCs

This study suggests $\delta^{18}O$ data from Tanypodinae HCs may be a better indicator of the $\delta^{18}O$ of lake water and precipitation than the $\delta^{18}O$ of *Chironomus* spp. HCs from the five lakes (Table 3) for which we have overlapping data. For these lakes, $\delta^{18}O$ values of Tanypodinae HCs have stronger positive correlation with temperatures and in particular with summer (MFT) temperature (r = 0.86, Table 3). A clear difference in $\delta^{18}O$ values between Tanypodinae and *Chironomus* spp. HCs were observed when samples were prepared and analysed from the same sub-samples simultaneously from Lake Plimsoll and Lake Samuel, respectively (Table 1). *Chironomus* spp. HC $\delta^{18}O$ was enriched in comparison with Tanypodinae HC $\delta^{18}O$ values from both sites (Table 1).

These results are unsurprising because the larvae of Tanypodinae and *Chironomus* spp. differ with respect to habitat, foraging behaviour (Hershey 1987) and physiology. *Chironomus* spp. larvae are tube-dwelling, in contrast, most species of Tanypodinae larvae are free-living (Charbonneau et al. 1997; Oliver 1971) and this facilitates gas exchange, including oxygen between the larvae and the host lake water. Though most of the oxygen (but not all) in chironomid biomass originates from lake water, changing the amount of dissolved O_2 could still play a role in determining $\delta^{18}O$. Therefore, reduced gas-exchange and replenishing of O_2 for *Chironomus* spp. could affect the $\delta^{18}O$ values in the chironomid HCs. It is not unexpected that $\delta^{18}O$ values of Tanypodinae HCs have a much stronger correlation (r = 0.80 vs. r = 0.33) with the $\delta^{18}O$ of lake water and the local climates, for instance, *Chironomus* spp. larvae could take up large proportions of biomass originating from bacteria, including methane oxidizers (Grey et al. 2004a; Grey et al. 2004b). We therefore acknowledge that there are other reasons why $\delta^{18}O$ of *Chironomus* spp. may deviate from $\delta^{18}O$ of Tanypodinae HCs. Microbial processes can lead to strong fractionation in the chironomid tubes, which would affect $\delta^{18}O$ values of *Chironomus* spp. more than $\delta^{18}O$ values of Tanypodinae. Diet is perhaps another important factor that could have contributed to the difference in the relationships between $\delta^{18}O$ values of the two types of HCs vs. the $\delta^{18}O$ lake water (Wang et al. 2009). *Chironomus* spp. larvae are collectors-gatherers with a few filterers, and mainly shredders-herbivores while some are miners (Armitage et al. 1995; Johnson 1987). Tanypodinae are generally predators (engulfers and piercers) and their food includes Oligochaeta and many other prey (Armitage et al. 1995; Baker and McLachlan 1979). How this may have enhanced the relationship between the $\delta^{18}O$ Tanypodinae HCs and lake water $\delta^{18}O$ needs further investigation. We propose a testable hypothesis here that Tanypodinae which have a wide range of prey may provide a more integrated $\delta^{18}O$ signal that is more similar to the overall lake water $\delta^{18}O$, whereas *Chironomus* HCs may represent a more habitat and food-specific $\delta^{18}O$ signal.

The $\delta^{18}O$ values of *Chironomus* spp. HCs are higher than the $\delta^{18}O$ values (by 2.6 – 6.6 ‰) of Tanypodinae HCs from the same samples where the absolute values are directly comparable. This difference may relate to the effect of haemoglobin effects on $^{18}O/^{16}O$ fractionation in *Chironomus* spp. larvae (Chang et al. 2016). This effect is not evident for Tanypodinae as this subfamily usually does not use haemoglobin for oxy-regulation (Osmulski and Leyko 1986). Some studies have argued that haemoglobin is present in some metabolic processes within Tanypodinae larvae (e.g. nerves) (Osmulski and Leyko 1986) but there is general agreement that *Chironomus* spp. is the taxon that uses haemoglobin most efficiently in the metabolic process (Ramakrishnan 2002; Walshe 1950). The further enrichment of $\delta^{18}O$ in *Chironomus* spp. HCs may be due to the scavenging of oxygen by haemoglobin (Warwick 1992). This active biological process preferentially uses ^{16}O over ^{18}O leaving a relatively larger proportion of ^{18}O for incorporation into the insect cuticle. Haemoglobin is abundant in *Chironomus* spp. larvae, but not in Tanypodinae larvae and therefore δ^{18}O values of Tanypodinae larvae may be more strongly correlated with lake water. This hypothesis requires further testing to confirm.

In summary, the δ^{18}O values of the subfamily Tanypodinae HCs are better indicators of δ^{18}O of lake water and show more promising results as a proxy to infer air temperatures (both mean annual and summer) as compared to *Chironomus* spp. HCs. We argue that the most likely mechanisms to explain this difference relate to habitat, diet and insect physiology (the presence vs. absence of haemoglobin) between the two taxa. Based on our results, we suggest that Tanypodinae are a better target than *Chironmus* spp. HCs as a proxy to reconstruct temperature changes from south-eastern Australian lakes. We believe that these observations may hold worldwide and recommend Tanypodinae for the use in paleoclimate reconstructions.

We applied a simple linear regression analysis (at 95% confidence interval) to quantify the relationship between the δ^{18}O of Tanypodnae HCs and temperature in Microsoft Excel 2013. We used the linear regression equations presented in Figure 5B and 5D to compute Tanypodinae-inferred MAT and MFT values (Table 4). These values were compared with the temperature values for the lakes derived from the WorldClim surface model based on interpolated climate surfaces from weather stations (Table 1). We observed that the δ^{18}O of Tanypodinae HCs under-predicted MFT by a maximum of 2.20 °C (Swan Lake) and over-predicted by a maximum of 1.79 °C (Lake Albina), which is a range of 3.99 °C in the residuals. We also calculated the root-mean-square-error (RMSE) for this correlation using the following equation:

Eq. 1.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (X_{obs,i} - X_{model,i})^2}{n}}$$

where X_{obs} represents the observed values based on WorldClim data and X_{model} is the output values by applying the $\delta^{18}O$ Tanypodinae HCs calibration curve at time/place *i*. The results show that the RMSE of the $\delta^{18}O$ Tanypodinae predicted MFT is 1.50 °C. We found that much larger uncertainties resulted from deriving MAT using the same method (a range of 6.57 °C) with a RMSE of 1.98 °C (Table 4).

We then compared the results with the predicted MFT values using a chironomid assemblage based transfer function (Chang et al. 2015a) (Table 1). This transfer function model was developed based on 33 lakes from south-eastern Australia and includes all the eight lakes tested for $\delta^{18}O$ in this study. The transfer function model under-predicted MFT by 0.09 °C in Plimsoll Lake and similar to the $\delta^{18}O$ of Tanypodinae HCs, over-predicted MFT up to 1.78 °C in Lake Samuel (range 1.90 °C). It has a RMSE of 1.07 °C, calculated using the same method by applying Eq. 1. These observations suggest that the $\delta^{18}O$ of Tanypodinae HCs has the potential capability to quantify MFTs to a similar precision as the transfer function method when more lakes are incorporated and the calibration refined. We also recommend a comparison between $\delta^{18}O$ reconstructions from some ^{210}Pb dated records with historical temperature records. This would confirm if the space-for-time approach that is typically used for calibration datasets is applicable down-core for the $\delta^{18}O$ dataset. This would test if changes in synoptic weather patterns and moisture sources are more important than temperature at the local scale.

Conclusions

This study reports $\delta^{18}O$ results from Tanypodinae HCs in nine south-east Australia lakes. The relationship between $\delta^{18}O$ values of Tanypodinae HCs and summer (February) and mean annual air temperature both appear robust (r = 0.84 for mean February, r = 0.80 for mean annual). We also compared these results with the $\delta^{18}O$ values measured on *Chironomus* spp.

HCs (Chang et al. 2016) from five of the same lakes. Results show $\delta^{18}O$ values of Tanypodinae HCs have a stronger correlation with both the $\delta^{18}O$ of lake water and air temperatures than that of *Chironomus* spp. HCs. Differences in $\delta^{18}O$ values between the two types of HCs were observed in the same lakes samples that were directly comparable. Overall, this study suggests $\delta^{18}O$ of Tanypodinae HCs has better potential to be used as a temperature proxy than *Chironomus* spp. We have no reason to expect that this effect will be confined to Australia as it is likely related to the physiology and diet of *Chironomus* spp. and it may therefore be useful to generally exclude *Chironomus* spp. HCs from samples when reconstructing past temperatures from the $\delta^{18}O$ of chironomid head capsules.

In contrast to the usual approach of deriving mean annual temperatures from isotope analyses of chironomids in northern Europe (Verbruggen et al. 2011) and North America (Wooller et al. 2004), mean summer (February) temperatures show a stronger correlation with Tanypodinae HCs when high altitude lake that freeze during winter are included. This suggests that in high elevation lakes, $\delta^{18}O$ records based on Tanypodinae reflect $\delta^{18}O$ of lakes water and precipitation during the summer and when these values are used to estimate past air temperature change, it reflects variations during the summer months. It is worth investigating whether this effect applies in other regions.

The MFT values derived from the $\delta^{18}O$ calibration curve of Tanypodinae HCs demonstrated comparable results with the MFTs calculated using a transfer function based on chironomid assemblage from south-eastern Australia (Chang et al. 2015a) albeit with larger errors (RMSE = 1.50 °C for the $\delta^{18}O$ Tanypodinae HCs and 1.07 °C for the transfer function). We recommend the construction of a more robust calibration based on a wider range of lakes in the south-east mainland and Tasmania. As core records show that Tanypodinae are a major subfamily present during the glacial times (e.g. from Eagle Tarn, Tasmania) (Rees and Cwynar 2010), application of the δ^{18}O of the HCs to infer glacial to inter-glacial climate change, as an independent tool in south-eastern Australia is promising.

Acknowledgements

We thank the Australian Research Council for providing Discovery Project Grant DP110103081 to financially support the field work and sample analyses of this work. We thank Lydia Mackenzie and Abdollah Jarihani for field assistance; the Department of Primary Industries, Water and Environment (DPIWE), the Department of Sustainability and Environment (DSE) and the Department of Environment and Heritage Protection (DEHP) for the permission of sample collection. Oxygen isotope analyses of the chironomids were funded kindly by DRG at the Stable Isotope Biogeochemistry Laboratory at Durham University. We thank two anonymous reviewers and the editor for their insights and helpful comments that have greatly improved the manuscript.

References

Andrews, J. E., R. Riding, and P. F. Dennis. 1997. The stable isotope record of environmental and climatic signals in modern terrestrial microbial carbonates from Europe. Palaeogeography, Palaeoclimatology, Palaeoecology **129:** 171-189.

Armitage, P. D., L. C. Pinder, and P. S. Cranston. 1995. The habitats of chironomid larvae, p. 107-133. *In* P. D. Armitage, L. C. Pinder and P. S. Cranston [eds.], The Chironomidae: Biology and ecology of non-biting midges. Springer.

Baker, A. S., and A. J. McLachlan. 1979. Food preferences of tanypodinae larvae (Diptera: Chironomidae). Hydrobiologia **62:** 283-288.

Bowen, G. J., and J. Revenaugh. 2003. Interpolating the isotopic composition of modern meteoric precipitation. Water Resources Research **39:** 1299-1312.

Brooks, S. J., and H. J. B. Birks. 2001. Chironomid-inferred air temperatures from Lateglacial and Holocene sites in north-west Europe: progress and problems. Quaternary Science Reviews **20:** 1723-1741.

Chang, J. C., J. Shulmeister, and C. Woodward. 2015a. A chironomid based transfer function for reconstructing summer temperatures in southeastern Australia. Palaeogeography, Palaeoclimatology, Palaeoecology **423:** 109-121.

Chang, J. C., J. Shulmeister, C. Woodward, and G. Michalski. 2016. Can stable oxygen and hydrogen isotopes from Australian subfossil Chironomus head capsules be used as proxies for past temperature change? Journal of Paleolimnology **56:** 331-348.

Chang, J. C., J. Shulmeister, C. Woodward, L. Steinberger, J. Tibby, and C. Barr. 2015b. A chironomid-inferred summer temperature reconstruction from subtropical Australia during the last glacial maximum (LGM) and the last deglaciation. Quaternary Science Reviews **122:** 282-292.

Chang, J. C., C. Woodward, and J. Shulmeister. 2014. A snapshot of the limnology of eastern Australian water bodies spanning the tropics to Tasmania: the land-use, climate, limnology nexus. Marine and Freshwater Research **65:** 872-883.

Charbonneau, P., L. Hare, and R. Carignan. 1997. Use of X-ray images and a contrasting agent to study the behavior of animals in soft sediments. Limnology and Oceanography **42:** 1823-1828.

Coplen, T. B. 1995. Reporting of stable hydrogen, carbon, and oxygen isotopic abundances. Geothermics **24:** 707-712.

Field, A. P. 2000. Discovering Statistics Using SPSS for Windows: Advanced Techniques for the Beginner. SAGE.

García, L. V. 2004. Escaping the Bonferroni iron claw in ecological studies. Oikos **105:** 657-663.

Gehre, M., H. Geilmann, J. Richter, R. A. Werner, and W. A. Brand. 2004. Continuous flow 2H/1H and 18O/16O analysis of water samples with dual inlet precision. Rapid Communications in Mass Spectrometry **18:** 2650-2660.

Gibson, J. J., S. J. Birks, and Y. Yi. 2016. Stable isotope mass balance of lakes: a contemporary perspective. Quaternary Science Reviews **131, Part B:** 316-328.

Gibson, J. J., E. E. Prepas, and P. McEachern. 2002. Quantitative comparison of lake throughflow, residency, and catchment runoff using stable isotopes: modelling and results from a regional survey of Boreal lakes. Journal of Hydrology **262:** 128-144.

Glew, J. R. 1991. Miniature gravity corer for recovering short sediment cores. Journal of Paleolimnology **5:** 285-287.

Green, K. 2012. Intra- and inter-annual changes in chemistry of Australian glacial lakes. Marine and Freshwater Research **63:** 513-527.

Grey, J., A. Kelly, and R. I. Jones. 2004a. High intraspecific variability in carbon and nitrogen stable isotope ratios of lake chironomid larvae. Limnology and Oceanography **49:** 239-244.

Grey, J., A. Kelly, S. Ward, N. Sommerwerk, and R. I. Jones. 2004b. Seasonal changes in the stable isotope values of lake-dwelling chironomid larvae in relation to feeding and life cycle variability. Freshwater Biology **49:** 681-689.

Gröcke, D. R., A. Schimmelmann, S. Elias, and R. F. Miller. 2006. Stable hydrogen-isotope ratios in beetle chitin: preliminary European data and re-interpretation of North American data. Quaternary Science Reviews **25:** 1850-1864.

Gröcke, D. R., M. van Hardenbroek, P. E. Sauer, and S. A. Elias. 2011. Hydrogen Isotopes in Beetle Chitin, p. 105-116. *In* N. S. Gupta [ed.], Chitin: Formation and Diagenesis. Springer Netherlands.

Heiri, O., S. J. Brooks, H. J. B. Birks, and A. F. Lotter. 2011. A 274-lake calibration data-set and inference model for chironomid-based summer air temperature reconstruction in Europe. Quaternary Science Reviews **30:** 3445-3456.

Hershey, A. E. 1987. Tubes and Foraging Behavior in Larval Chironomidae: Implications for Predator Avoidance. Oecologia **73:** 236-241.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology **25:** 1965-1978.

IAEA/WMO. 2015. Global network of isotopes in precipitation. http://www.iaea.org/water. The International Atomic Energy Agency and the World Meteorological Organization, accessed Oct 2015.

Johnson, R. K. 1987. Seasonal variation in diet of Chironomus plumosus (L.) and C. anthracinus Zett. (Diptera: Chironomidae) in mesotrophic Lake Erken. Freshwater Biology **17:** 525-532.

Jones, M. D. and others 2016. Comparisons of observed and modelled lake δ18O variability. Quaternary Science Reviews **131, Part B:** 329-340.

Larocque, I., R. I. Hall, and E. Grahn. 2001. Chironomids as indicators of climate change: a 100 ‐ lake training set from a subarctic region of northern Sweden (Lapland). Journal of Paleolimnology **26:** 307-322.

Leng, M. J., and J. D. Marshall. 2004. Palaeoclimate interpretation of stable isotope data from lake sediment archives. Quaternary Science Reviews **23:** 811-831.

Massaferro, J., I. Larocque-Tobler, S. J. Brooks, M. Vandergoes, A. Dieffenbacher-Krall, and P. Moreno. 2014. Quantifying climate change in Huelmo mire (Chile, Northwestern Patagonia) during the Last Glacial Termination using a newly developed chironomid-based temperature model. Palaeogeography, Palaeoclimatology, Palaeoecology **399:** 214-224.

Mayr, C. and others 2015. Oxygen isotope ratios of chironomids, aquatic macrophytes and ostracods for lake-water isotopic reconstructions – Results of a calibration study in Patagonia. Journal of Hydrology **529, Part 2:** 600-607.

National-Parks-Service. 1996. Mount Buffalo National Park Management Plan. *In* D. o. N. R. a. E. Victoria [ed.].

Oliver, D. R. 1971. Life History of the Chironomidae. Annual Review of Entomology **16:** 211-230.

Osmulski, P., and W. Leyko. 1986. Structure, function and physiological role of chironomus haemoglobin. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry **85:** 701-722.

Parks-Victoria. 2016. Mount Buffalo National Park, http://parkweb.vic.gov.au/explore/parks/mount-buffalo-national-park, accessed 15/07/2016.

Ramakrishnan, S. 2002. The Role of Recombination in the Evolution of Globin Genes in Chironomus Riparius. University of Wisconsin-Milwaukee.

Rees, A. B. H., and L. C. Cwynar. 2010. Evidence for early postglacial warming in Mount Field National Park, Tasmania. Quaternary Science Reviews **29:** 443-454.

Rees, A. B. H., L. C. Cwynar, and P. S. Cranston. 2008. Midges (Chironomidae, Ceratopogonidae, Chaoboridae) as a temperature proxy: a training set from Tasmania, Australia. Journal of Paleolimnology **40:** 1159-1178.

Sauer, P. E., G. H. Miller, and J. T. Overpeck. 2001. Oxygen isotope ratios of organic matter in arctic lakes as a paleoclimate proxy: field and laboratory investigations. Journal of Paleolimnology **25:** 43-64.

Schimmelmann, A., and M. J. DeNiro. 1986. Stable isotopic studies on chitin. III. The D/H and 18O/16O ratios in arthropod chitin. Geochimica et Cosmochimica Acta **50:** 1485-1496.

Soto, D. X., L. I. Wassenaar, and K. A. Hobson. 2013. Stable hydrogen and oxygen isotopes in aquatic food webs are tracers of diet and provenance. Functional Ecology **27:** 535-543.

Stuiver, M. 1970. Oxygen and carbon isotope ratios of fresh-water carbonates as climatic indicators. Journal of Geophysical Research **75:** 5247-5257.

Trochim, W., and J. P. Donnelly. 2006. The Research Methods Knowledge Base. Cengage Learning.

van Hardenbroek, M., D. R. Gröcke, P. E. Sauer, and S. A. Elias. 2012. North American transect of stable hydrogen and oxygen isotopes in water beetles from a museum collection. Journal of Paleolimnology **48:** 461-470.

Verbruggen, F., O. Heiri, G. J. Reichart, C. Blaga, and A. F. Lotter. 2011. Stable oxygen isotopes in chironomid and cladoceran remains as indicators for lake-water δ18O. Limnology and Oceanography **56:** 2071-2079.

Walker, I. R. 1987. Chironomidae (Diptera) in paleoecology. Quaternary Science Reviews **6:** 29-40.

Walker, I. R., J. P. Smol, D. R. Engstrom, and H. J. B. Birks. 1991. An Assessment of Chironomidae as Quantitative Indicators of Past Climatic Change. Canadian Journal of Fisheries and Aquatic Sciences **48:** 975-987.

Walshe, B. M. 1950. The function of haemoglobin in Chironomus Plumosus under natural conditions. Journal of Experimental Biology **27:** 73-95.

Wang, Y. V., D. M. O'Brien, J. Jenson, D. Francis, and M. J. Wooller. 2009. The influence of diet and water on the stable oxygen and hydrogen isotope composition of Chironomidae (Diptera) with paleoecological implications. Oecologia **160:** 225-233.

Warwick, W. F. 1992. The effect of trophic/contaminant interactions on chironomid community structure and succession (Diptera: Chironomidae). Netherland Journal of Aquatic Ecology **26:** 563-575.

Wolfe, B. B. and others 2007. Progress in isotope paleohydrology using lake sediment cellulose. Journal of Paleolimnology **37:** 221-231.

Wooller, M. J., D. Francis, M. L. Fogel, G. H. Miller, I. R. Walker, and A. P. Wolfe. 2004. Quantitative paleotemperature estimates from δ18O of chironomid head capsules preserved in arctic lake sediments. Journal of Paleolimnology **31:** 267-274.

Figure captions and legends

Fig. 1. The location of the nine studied lakes from south-eastern Australia, with mean annual precipitation displayed as background information. All lakes are distributed in the range of c. > 800 - 2500 mm of mean annual precipitation.

Fig. 2. (A) Plot of mean annual temperature (MAT) against δ^{18}O of precipitation (lakes labelled in circles) and a plot of mean February temperature (MFT) against δ^{18}O of precipitation (lakes labelled in squares). δ^{18}O of precipitation shows a slightly stronger relationship with MFT (solid line) than with MAT (dashed line) ($r = 0.89$, p < adjusted critical p-value against $r = 0.83$, p < adjusted critical p-value, at the $P = 0.05$ level). Both MFT (closed square) and MAT (closed circle) for Lake Catani are poorly correlated to the δ^{18}O of precipitation (B) Plot of δ^{18}O of lake water against δ^{18}O of precipitation. This shows a strong correlation ($r = 0.74$, p > adjusted critical p-value, at the $P = 0.05$ level) from nine south-eastern Australian lakes. ^{18}O in Lake Cartcarrong (closed triangle) is enriched with respect to precipitation, while ^{18}O in Lake Catani (closed circle) is depleted with respect precipitation. Other lakes plot close to the correlation relationship.

Fig. 3. (A) Plot of δ^{18}O of Tanypodinae HCs agaist δ^{18}O of lake water showing that there is a moderate correlation ($r = 0.69$, p > adjusted critical p-value, at the $P = 0.05$ level) between these measurements while Lake Catani (closed circle) is an outlier. (B) The re-plotted δ^{18}O of

Tanypodinae HCs against δ^{18}O of lake water with the removal of Lake Catani displays an improved correlation (r = 0.76, p > adjusted critical p-value, at the P = 0.05 level).

Fig. 4. (A) Plot of δ^{18}O of Tanypodinae HCs against δ^{18}O of preciptation displays a moderate strong correlation (r = 0.40, p > adjusted critical p-value, at the P = 0.05 level) where Lake Catani (closed circle) appears as an outlier. (B) Plot of δ^{18}O of Tanypodinae of HCs against δ^{18}O of precipitation improves (r = 0.76, p > adjusted critcial p-value, at the P = 0.05 level) dramatically when Lake Catani is removed.

Fig. 5. (A) Plot of δ^{18}O of Tanypodinae HCs aginst mean annual temperature (MAT) with all nine lakes included (where Lake Catani is labelled with closed circle and Lake Albina is with closed diamond) and (B) without Lake Catani. Again the strength of the correlation improves (from r = 0.72, p > adjusted critical p-value to r = 0.80, p > adjusted critical p-value, at the P = 0.05 level). Lake Albina (closed diamond) also shows a large deviation from the correlation relationship in this MAT plot. (C) Plot of δ^{18}O of Tanypodinae HCs against mean Feburary temperature (MFT) with all lakes and (D) with Lake Catani excluded. Here also the strength and significance level of the correlation improves notably (from r = 0.63, p > adjusted critical p-value to r = 0.84, p < adjusted critifal p-value, at the P = 0.05 level). The correlation between δ^{18}O of Tanypodinae HCs and MFT is stronger than for MAT (r = 0.84 vs. r = 0.80) due to the better fit of Lake Albina (closed diamond) with the relationship observed for the other lakes.

Table captions

Table 1 Stable oxygen isotopic composition (δ^{18}O) of chironomid head capsules (HCs), precipitation and lake water from nine lakes in south-eastern Australia. For δ^{18}O of chironomid HCs, (T) indicates the values measured on Tanypodinae HCs whereas (C) indicates the measurement for *Chironomus* spp. HCs. Air temperature values for mean annual temperature

(MAT) and mean February temperature (MFT) are both presented. The (*) indicates sites with $\delta^{18}O$ of *Chironomus* spp. HCs measurements reported in Chang et al. (2016). The data show no evidence for distributions being significantly different from normal because the values of kurtosis and skewness are less than +/- 2 in each data sets respectively.

Table 2 Results from Pearson's correlation analyses in stable oxygen isotope ($\delta^{18}O$) data of lake water, precipitation and Tanypodinae head capsules (HCs) in south-eastern Australian lakes. This shows a summary of the correlation coefficient (r) values from Figures 2-5.

Table 3 Summary of results from Pearson's correlation analyses for stable oxygen isotope ($\delta^{18}O$) data on Tanypodinae and *Chironomus* spp. HCs against the tested variables. These are from five (lakes indicated with (*) in Table 1) out of the nine lakes which have HCs of both taxa analysed for $\delta^{18}O$ from the same sediment samples. Tanypodinae display much stronger relationships than *Chironomus* spp. do. Due to the limited number of data points available for this comparison, we acknowledge that these statistical values should be used with caution.

Table 4 (A) Results showing mean February temperature (MFT) and mean annual temperature (MAT) calculated using the equations presented in Figures 5d and 5b respectively based on the calibration curve of $\delta^{18}O$ of Tanypodinae HCs ($\delta^{18}O(t)$) from eight lakes. The MFT values were compared to MFTs predicted from the chironomid species assemblages based transfer function (TF) model using 33 south-eastern Australian lakes (Chang et al. 2015a). The observed MFT and MAT values of each site are climate surface interpolations obtained from WordClim data presented in Table 1. (B) The descriptive statistics including the standard deviation, sample variance, range and root-mean-squared-error (RMSE) analyses performed on the residuals when the model-based results were compared.

Figure 1

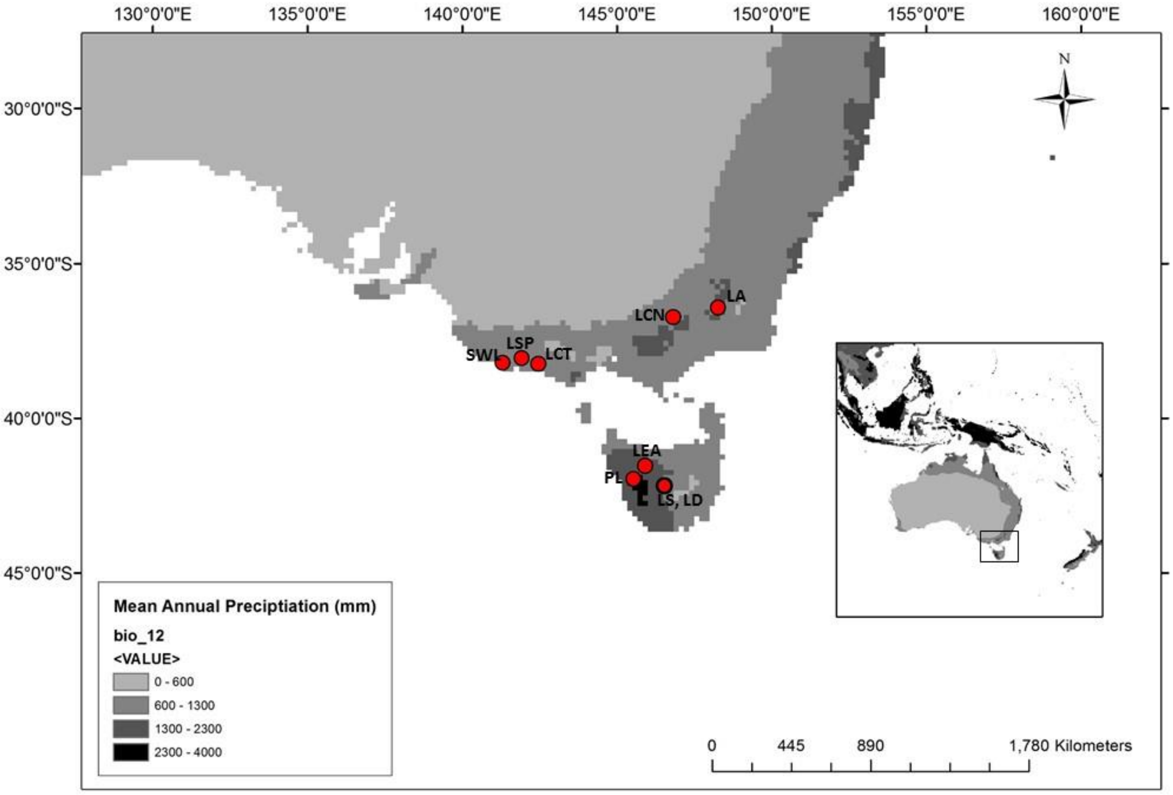

Figure 2

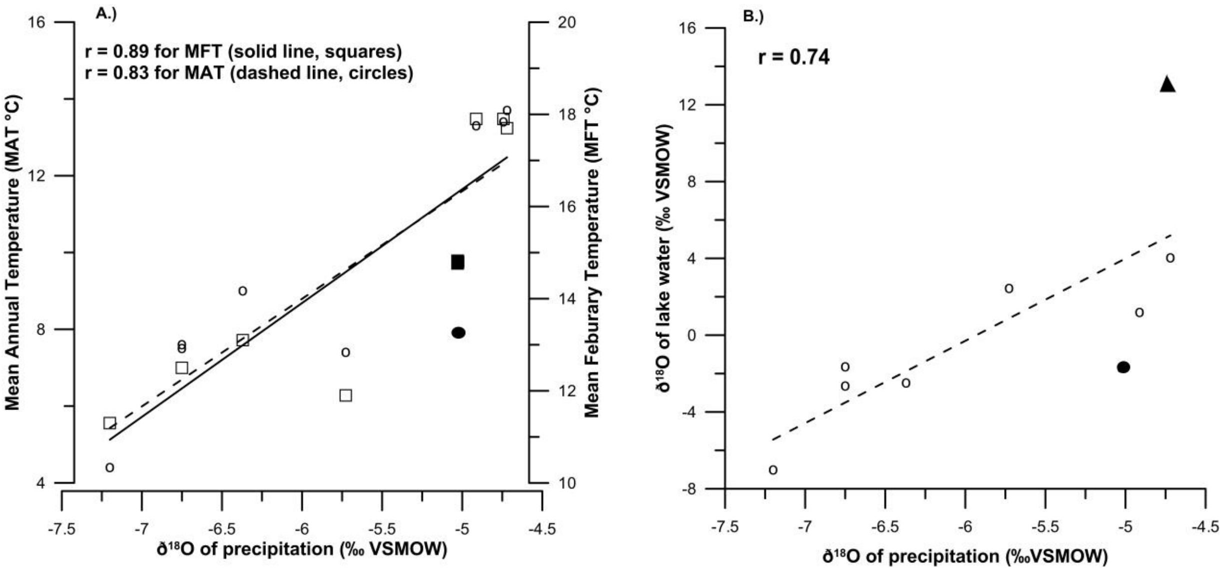

Figure 3

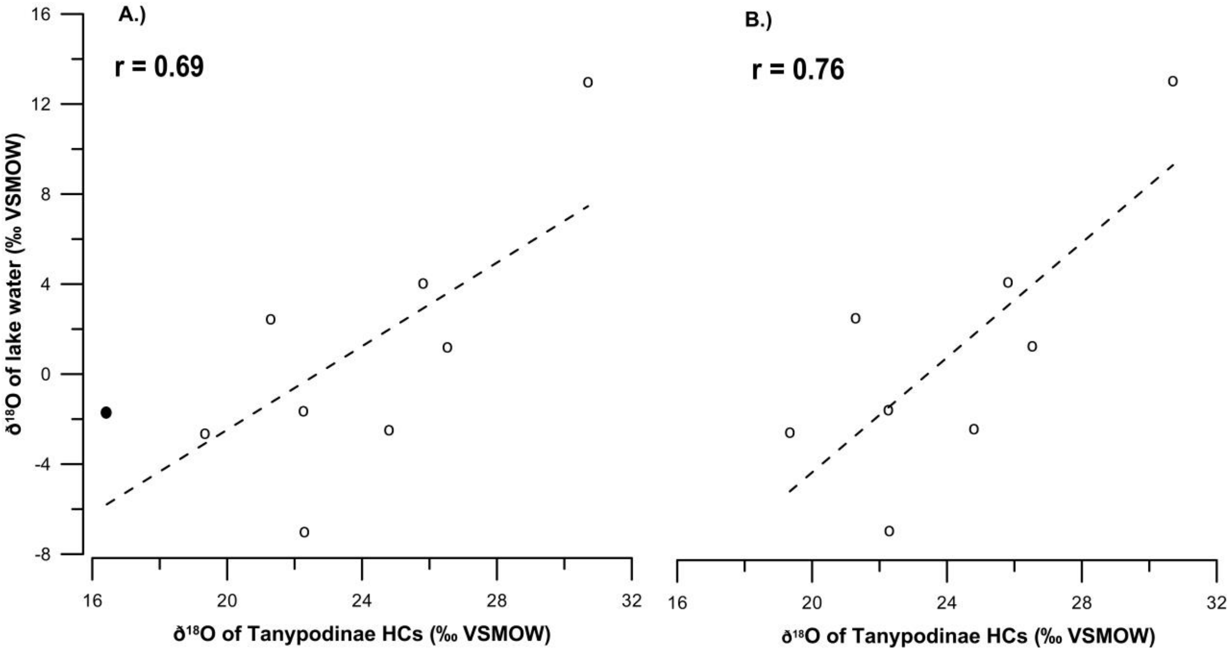

Figure 4

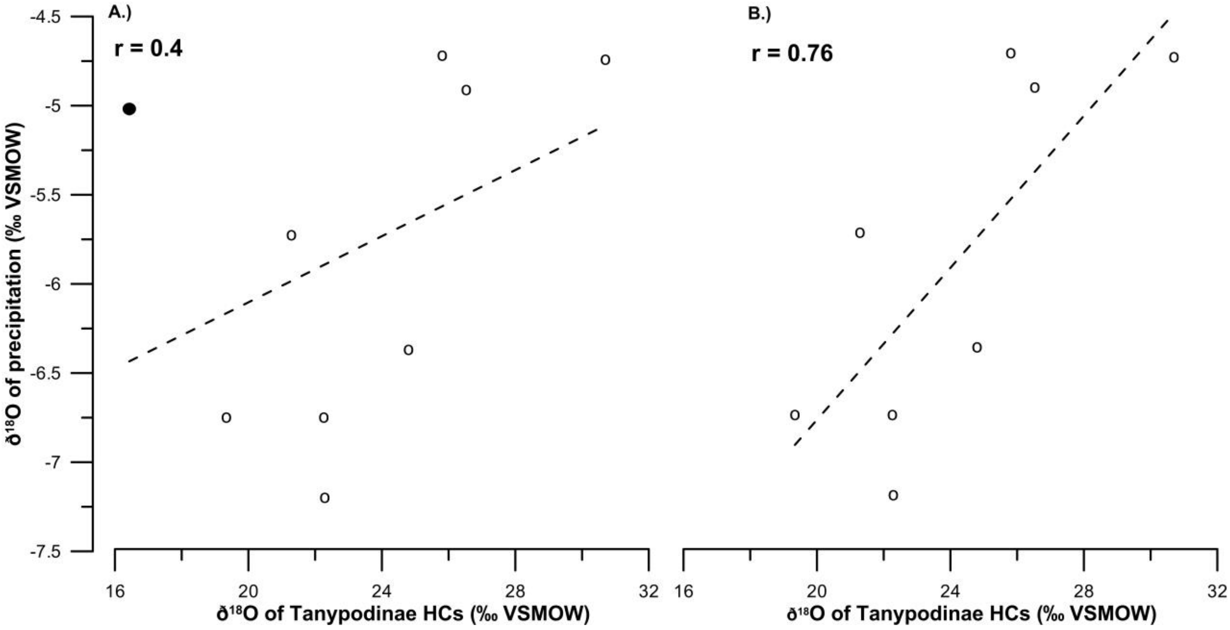

Figure 5

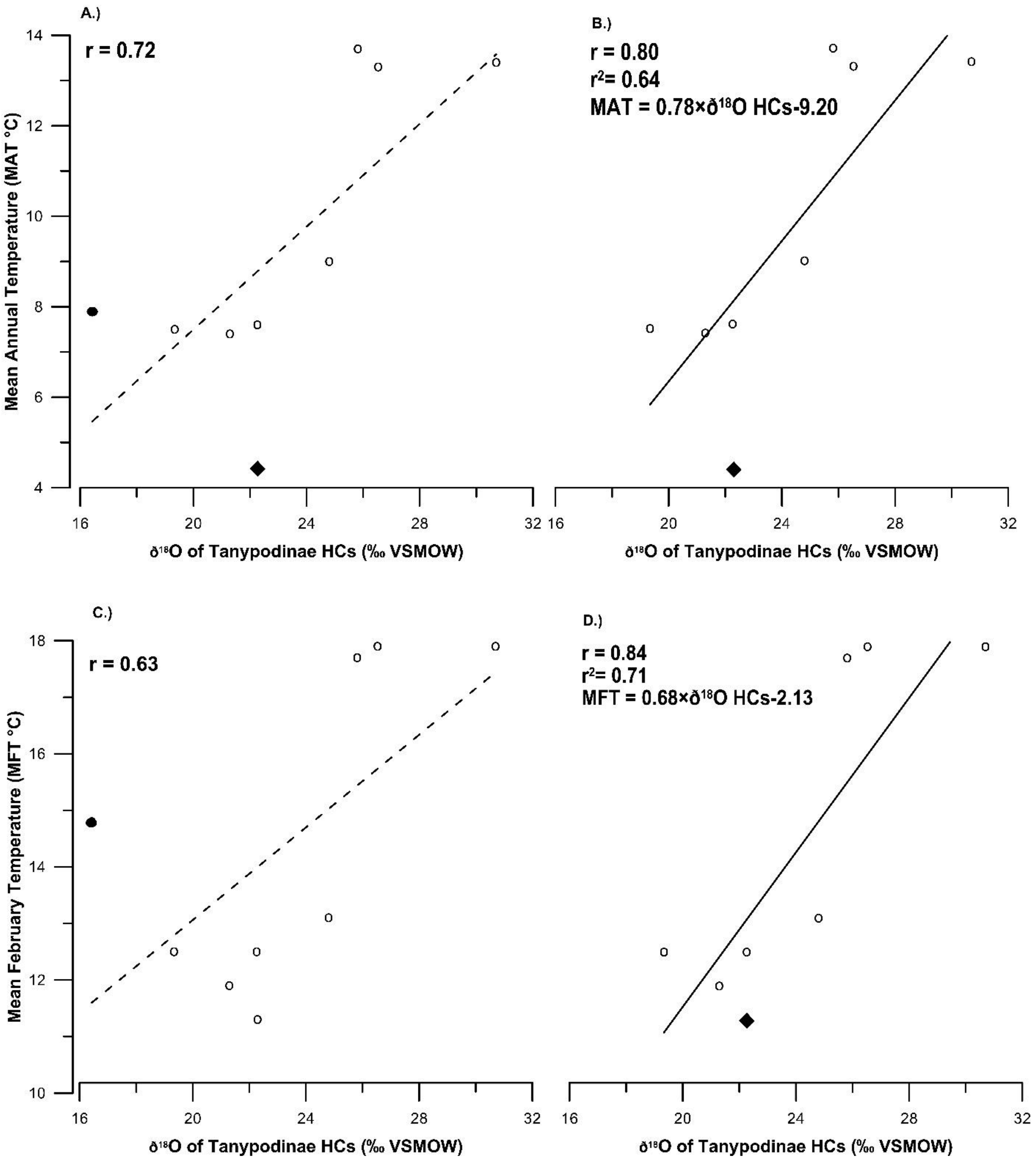
A.)
r = 0.72
Mean Annual Temperature (MAT °C)
14
12
10
8
6
4
16
20
24
28
32
ð¹⁸O of Tanypodinae HCs (‰ VSMOW)
B.)
r = 0.80
r² = 0.64
MAT = 0.78×ð¹⁸O HCs-9.20
16
20
24
28
32
ð¹⁸O of Tanypodinae HCs (‰ VSMOW)
C.)
r = 0.63
Mean February Temperature (MFT °C)
18
16
14
12
10
16
20
24
28
32
ð¹⁸O of Tanypodinae HCs (‰ VSMOW)
D.)
r = 0.84
r² = 0.71
MFT = 0.68×ð¹⁸O HCs-2.13
16
20
24
28
32
ð¹⁸O of Tanypodinae HCs (‰ VSMOW)

Table 1

Lake name	Code	Altitude	δ^{18}O chironomid HCs	δ^{18}O of precipitation	δ^{18}O of lake water	MAT	MFT
		M	‰ VSMOW	‰ VSMOW	‰ VSMOW	°C	°C
Lake Albina*	LA	1919	22.29 (T)	-7.20	-7.02	4.4	11.3
Swan Lake*	SWL	23	25.81 (T)	-4.75	4.02	13.7	17.7
Lake Cartcarrong*	LCT	90	30.70 (T)	-4.74	12.97	13.4	17.9
Lake Surprise*	LSP	107	26.53 (T)	-4.91	1.18	13.3	17.9
Highland Waters	LD	756	22.26 (T)	-6.75	-1.65	7.6	12.5
Plimsoll Lake	PL	523	24.80 (T)/27.44 (C)	-6.37	-2.49	9.0	13.1
Lake Lea Pond*	LEA	837	21.29 (T)	-5.73	2.43	7.4	11.9
Lake Cantani	LCN	1300	16.43 (T)	-5.02	-1.72	7.9	14.8
Lake Samuel	LS	766	19.34 (T)/25.98 (C)	-6.75	-2.65	7.5	12.5
		Kurtosis	0.18	-1.96	1.97	-1.22	-1.84
		Skewness	0.15	-0.18	1.09	0.29	0.47

Table 2

Correlation Coefficient (r)	δ^{18}O of lake water	δ^{18}O of precipitation	Mean annual temperature	Mean February temperature
δ^{18}O of lake water	-	0.74	0.77	0.72
δ^{18}O of precipitation	-	-	0.83	0.89
δ^{18}O Tanypodinae HCs (with 9 lakes)	0.69	0.40	0.72	0.63
δ^{18}O Tanypodinae HCs (with Lake Catani removed)	0.76	0.76	0.80	0.84

Table 3

Correlation Coefficient (r)	$\delta^{18}O$ of lake water	$\delta^{18}O$ of precipitation	Mean annual temperature (MAT)	Mean February temperature (MFT)
$\delta^{18}O$ *Chironomus* spp. HCs	0.33	0.50	0.44	0.41
$\delta^{18}O$ Tanypodinae HCs	0.80	0.71	0.80	0.86

Table 4

A.

Lakes Name	MFT calculated based on $\delta^{18}O(t)$	MFT modelled based on the TF method	ΔMFT for $\delta^{18}O(t)$ modelled and observed	ΔMFT for the TF modelled and observed	MAT modelled based on $\delta^{18}O(t)$	ΔMAT for $\delta^{18}O(t)$ modelled and observed
	°C	°C	°C	°C	°C	°C
Lake Albina	13.09	12.36	1.79	1.06	8.11	3.71
Swan Lake	15.50	18.64	-2.20	0.94	10.84	-2.86
Lake Cartcarrong	18.84	18.16	0.94	0.26	14.64	1.24
Lake Surprise	15.99	19.57	-1.91	1.67	11.40	-1.90
Highland Waters	13.07	13.51	0.57	1.01	8.09	0.49
Plimsoll Lake	14.81	13.01	1.71	-0.09	10.06	1.06
Lake Lea	12.41	11.90	0.51	0.00	7.33	-0.07
Lake Samuel	11.08	14.28	-1.42	1.78	5.82	-1.68

B.

Descriptive statistics	ΔMFT based on $\delta^{18}O(t)$	ΔMFT based on the TF	ΔMAT based on $\delta^{18}O(t)$
Standard Deviation	1.61 °C	0.71 °C	2.11 °C
Sample Variance	2.59 °C	0.51 °C	4.46 °C
Range	3.99 °C	1.87 °C	6.57 °C
RMSE	1.50 °C	1.07 °C	1.98 °C