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

Jie C. Chang (jie.chang@uqconnect.edu.au) 1, 2

James Shulmeister (james.shulmeister@uq.edu.au) 1

Darren R. Gröcke (d.r.grocke@durham.ac.uk) 4

Craig A. Woodward (c.woodward1@uq.edu.au) 1, 3

Affiliation:
1. School of Earth and Environmental Sciences, University of Queensland Level 4, Chamberlain Building (35), St Lucia, Brisbane, 4072 Queensland, Australia
2. Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences 73 Beijing E Rd, Xuanwu, Nanjing, Jiangsu, China, 210008
3. Institute for Environmental Research, Australian Nuclear Science and Technology Organisation, New Illawarra Rd, Lucas Heights, 2234 New South Wales, Australia
4. Stable Isotope Biogeochemistry Laboratory (SIBL), Durham University, Durham DH1 3LE, United Kingdom

Key Words:
Chironomid head capsules, Tanypodinae, δ18O, 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 (δ¹⁸O) 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 δ¹⁸O of lake water. Inferences can then be made about other paleoenvironmental variables based on reconstructions of past lake water δ¹⁸O. Previous studies have demonstrated that δ¹⁸O values from a variety of sources can be used as paleoenvironmental proxies and a relationship between lake water δ¹⁸O and δ¹⁸O 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 δ¹⁸O 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 chironomid-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 δ18O 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 δ18O of lake water is strongly influenced by climatic variables including regional air temperature, precipitation and evaporation. In non-arid environments, the δ18O of lake water is closely correlated with the δ18O 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 δ18O of chitin reflects the δ18O of lake water and hence, can be applied as a proxy to infer precipitation δ18O values and changes in temperature. The application of this method to down-core records is based on the assumption that the same relationship between δ18O 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 δ18O of ocean water linked to changing zonal wind fields on glacial to interglacial time scales may also affect precipitation δ18O.

Previous analyses from sixteen lakes of south-eastern Australia suggested δ18O 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 δ¹⁸O 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 δ¹⁸O from a different group of chironomids (subfamily: Tanypodinae) to determine if we could improve on the performance of *Chironomus* spp. HC δ¹⁸O. 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 δ¹⁸O analysis from the last deglaciation and the Holocene (Rees and Cwynar 2010). In addition, it would also allow direct comparison between transfer function and δ¹⁸O based temperature reconstructions.

In the study presented in this paper we analysed δ¹⁸O on Tanypodinae from nine south-east Australian lakes. We investigated the relationship between the δ¹⁸O of Tanypodinae HCs and the δ¹⁸O of lake water, δ¹⁸O 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 δ¹⁸O from *Chironomus* spp. HCs in Australian lakes (Chang et al. 2016). This allowed us to compare the relationships of δ¹⁸O *Chironomus* spp. and Tanypodinae HCs with δ¹⁸O of lake water, δ¹⁸O precipitation and temperature respectively. We explore the potential of applying the δ¹⁸O of Tanypodinae HCs as a temperature proxy and investigate the implication of the variations of δ¹⁸O 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 $\pm 0.04 \%$. 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 $\delta^{18}$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 $\delta^{18}$O values of the HCs vs. $\delta^{18}$O lake water, $\delta^{18}$O precipitation as well as temperatures was examined.

Lake water samples for $\delta^{18}$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 $\delta^{18}$O values were calibrated using three Purdue internal standards relative to Vienna-Standard Mean Ocean Water (VSMOW) (Coplen 1995). Average uncertainties for lake water $\delta^{18}$O were $\pm$ 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 $\delta^{18}$O of Tanypodinae HCs, precipitation, lake water and MFT, MAT data are within an acceptable range ($\pm$ 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 $\delta^{18}$O values of Tanypodinae HCs and $\delta^{18}$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 $\delta^{18}$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 δ¹⁸O 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 δ¹⁸O values on HCs from two samples (Lake Samuel and Plimsoll Lake) that were directly comparable (measured in this study) showed that δ¹⁸O of Chironomus spp. HC was more enriched than Tanypodinae (Table 1). The results showed that the lake water δ¹⁸O values varied from −7.0 ‰ (Lake Albina) to 13.0 ‰ (Lake Cartcarrong), precipitation δ¹⁸O values ranged from −7.2 ‰ (Lake Albina) to −4.7 ‰ (Lake Cartcarrong) (Table 1).

A strong correlation between the δ¹⁸O of precipitation and lake water (r = 0.74) was observed (Fig. 2, Table 2) and δ¹⁸O 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 δ¹⁸O 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 δ¹⁸O of Tanypodinae HCs against δ¹⁸O of lake water, δ¹⁸O 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 δ¹⁸O of lake water was particularly low with respect to the δ¹⁸O 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 $\delta^{18}O$
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 $\delta^{18}O$ 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 $\delta^{18}O$ 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 $\delta^{18}O$ value of Tanypodinae HCs
(Table 1) (Fig. 3A). The $\delta^{18}O$ of Tanypodinae HCs is a very poor reflection of the $\delta^{18}O$ 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 $\delta^{18}O$ 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.$\sim$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 \( (r = 0.86, \text{Table 3}) \). 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 \( (r = 0.80 \text{ vs. } r = 0.33) \)
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 $^{16}\text{O}$ over $^{18}\text{O}$ leaving a relatively larger proportion of $^{18}\text{O}$ for incorporation into the insect cuticle. Haemoglobin is abundant in *Chironomus* spp. larvae, but not in Tanypodinae larvae and therefore δ$^{18}\text{O}$ values of Tanypodinae larvae may be more strongly correlated with lake water. This hypothesis requires further testing to confirm.

In summary, the δ$^{18}\text{O}$ values of the subfamily Tanypodinae HCs are better indicators of δ$^{18}\text{O}$ 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 *Chironomus* 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$^{18}\text{O}$ of Tanypodinae 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$^{18}\text{O}$ 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:

\[ RMSE = \sqrt{\frac{\sum_{i=1}^{n} (X_{\text{obs}} - X_{\text{model}})^2}{n}} \]

Eq. 1.
where $X_{\text{obs}}$ represents the observed values based on WorldClim data and $X_{\text{model}}$ 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 δ¹⁸O values of Tanypodinae
HCs have a stronger correlation with both the δ¹⁸O of lake water and air temperatures than that
of Chironomus spp. HCs. Differences in δ¹⁸O values between the two types of HCs were
observed in the same lakes samples that were directly comparable. Overall, this study suggests
δ¹⁸O 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 δ¹⁸O 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, δ¹⁸O records based on Tanypodinae reflect δ¹⁸O 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 δ¹⁸O 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 δ¹⁸O 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 δ¹⁸O 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


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 δ18O of precipitation (lakes labelled in circles) and a plot of mean February temperature (MFT) against δ18O of precipitation (lakes labelled in squares). δ18O 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 δ18O of precipitation (B) Plot of δ18O of lake water against δ18O 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. 18O in Lake Cartcarrong (closed triangle) is enriched with respect to precipitation, while 18O in Lake Catani (closed circle) is depleted with respect precipitation. Other lakes plot close to the correlation relationship.

Fig. 3. (A) Plot of δ18O of Tanypodinae HCs against δ18O 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 δ18O of
Tanypodinae HCs against δ¹⁸O 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 δ¹⁸O of Tanypodinae HCs against δ¹⁸O of precipitation 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 δ¹⁸O of Tanypodinae of HCs against δ¹⁸O of precipitation improves (r = 0.76, p > adjusted critical p-value, at the P = 0.05 level) dramatically when Lake Catani is removed.

**Fig. 5.** (A) Plot of δ¹⁸O of Tanypodinae HCs against 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 δ¹⁸O of Tanypodinae HCs against mean February 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 critical p-value, at the P = 0.05 level). The correlation between δ¹⁸O 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 (δ¹⁸O) of chironomid head capsules (HCs), precipitation and lake water from nine lakes in south-eastern Australia. For δ¹⁸O 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 δ¹⁸O 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 (δ¹⁸O) 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 (δ¹⁸O) 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 δ¹⁸O 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 δ¹⁸O of Tanypodinae HCs (δ¹⁸O(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 3

A.) $r = 0.69$

B.) $r = 0.76$

Figure 4

A.) $r = 0.4$

B.) $r = 0.76$
Figure 5

A) $r = 0.72$

Mean Annual Temperature (MAT °C) vs. δ18O of Tanypodinae HCs (% VSMOW)

B) $r = 0.80$

$\tau^2 = 0.64$

$\text{MAT} = 0.78 \times \delta^{18} \text{O HCs - 9.20}$

C) $r = 0.63$

Mean February Temperature (MFT °C) vs. δ18O of Tanypodinae HCs (% VSMOW)

D) $r = 0.84$

$\tau^2 = 0.71$

$\text{MFT} = 0.68 \times \delta^{18} \text{O HCs - 2.13}$
<table>
<thead>
<tr>
<th>Lake name</th>
<th>Code</th>
<th>Altitude</th>
<th>δ¹⁸O chironomid HCs</th>
<th>δ¹⁸O of precipitation</th>
<th>δ¹⁸O of lake water</th>
<th>MAT</th>
<th>MFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Albina*</td>
<td>LA</td>
<td>1919</td>
<td>-7.20</td>
<td>-7.02</td>
<td>4.4</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td>Swan Lake*</td>
<td>SWL</td>
<td>23</td>
<td>-4.75</td>
<td>4.02</td>
<td>13.7</td>
<td>17.7</td>
<td></td>
</tr>
<tr>
<td>Lake Cartcarrong*</td>
<td>LCT</td>
<td>90</td>
<td>-4.74</td>
<td>12.97</td>
<td>13.4</td>
<td>17.9</td>
<td></td>
</tr>
<tr>
<td>Lake Surprise*</td>
<td>LSP</td>
<td>107</td>
<td>-4.91</td>
<td>1.18</td>
<td>13.3</td>
<td>17.9</td>
<td></td>
</tr>
<tr>
<td>Highland Waters</td>
<td>LD</td>
<td>756</td>
<td>-6.75</td>
<td>-1.65</td>
<td>7.6</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>Plimsoll Lake</td>
<td>PL</td>
<td>523</td>
<td>-6.37</td>
<td>-2.49</td>
<td>9.0</td>
<td>13.1</td>
<td></td>
</tr>
<tr>
<td>Lake Lea Pond*</td>
<td>LEA</td>
<td>837</td>
<td>-5.73</td>
<td>2.43</td>
<td>7.4</td>
<td>11.9</td>
<td></td>
</tr>
<tr>
<td>Lake Cantani</td>
<td>LCN</td>
<td>1300</td>
<td>-5.02</td>
<td>-1.72</td>
<td>7.9</td>
<td>14.8</td>
<td></td>
</tr>
<tr>
<td>Lake Samuel</td>
<td>LS</td>
<td>766</td>
<td>-6.75</td>
<td>-2.65</td>
<td>7.5</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>Kurtosis</td>
<td></td>
<td></td>
<td>0.18</td>
<td>-1.96</td>
<td>1.97</td>
<td>-1.22</td>
<td></td>
</tr>
<tr>
<td>Skewness</td>
<td></td>
<td></td>
<td>0.15</td>
<td>-0.18</td>
<td>1.09</td>
<td>0.29</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Correlation Coefficient (r)</th>
<th>δ¹⁸O of lake water</th>
<th>δ¹⁸O of precipitation</th>
<th>Mean annual temperature</th>
<th>Mean February temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ¹⁸O of lake water</td>
<td>-</td>
<td>0.74</td>
<td>0.77</td>
<td>0.72</td>
</tr>
<tr>
<td>δ¹⁸O of precipitation</td>
<td>-</td>
<td>-</td>
<td>0.83</td>
<td>0.89</td>
</tr>
<tr>
<td>δ¹⁸O Tanypodinae HCs (with 9 lakes)</td>
<td>0.69</td>
<td>0.40</td>
<td>0.72</td>
<td>0.63</td>
</tr>
<tr>
<td>δ¹⁸O Tanypodinae HCs (with Lake Catani removed)</td>
<td>0.76</td>
<td>0.76</td>
<td>0.80</td>
<td>0.84</td>
</tr>
</tbody>
</table>
### Table 3

<table>
<thead>
<tr>
<th>Correlation Coefficient (r)</th>
<th>$\delta^{18}$O of lake water</th>
<th>$\delta^{18}$O of precipitation</th>
<th>Mean annual temperature (MAT)</th>
<th>Mean February temperature (MFT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta^{18}$O <em>Chironomus</em> spp. HCs</td>
<td>0.33</td>
<td>0.50</td>
<td>0.44</td>
<td>0.41</td>
</tr>
<tr>
<td>$\delta^{18}$O Tanypodinae HCs</td>
<td>0.80</td>
<td>0.71</td>
<td>0.80</td>
<td>0.86</td>
</tr>
</tbody>
</table>

### Table 4

#### A.

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

#### B.

<table>
<thead>
<tr>
<th>Descriptive statistics</th>
<th>$\Delta$MFT based on $\delta^{18}$O(t)</th>
<th>$\Delta$MFT based on the TF</th>
<th>$\Delta$MAT based on $\delta^{18}$O(t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Deviation</td>
<td>1.61 °C</td>
<td>0.71 °C</td>
<td>2.11 °C</td>
</tr>
<tr>
<td>Sample Variance</td>
<td>2.59 °C</td>
<td>0.51 °C</td>
<td>4.46 °C</td>
</tr>
<tr>
<td>Range</td>
<td>3.99 °C</td>
<td>1.87 °C</td>
<td>6.57 °C</td>
</tr>
<tr>
<td>RMSE</td>
<td>1.50 °C</td>
<td>1.07 °C</td>
<td>1.98 °C</td>
</tr>
</tbody>
</table>