CrossMark

ORIGINAL PAPER

Can stable oxygen and hydrogen isotopes from Australian subfossil *Chironomus* head capsules be used as proxies for past temperature change?

Jie Christine Chang · James Shulmeister · Craig Woodward · Greg Michalski

Abstract This paper presents the first systematic investigation of stable isotopes (δ^{18}O and δ^2H) from the subfossil chironomid head capsules (HCs) of a single taxon (*Chironomus*). The study focuses on sixteen south-eastern Australian lakes and investigates the potential of *Chironomus* spp. HC stable isotopes to reconstruct past temperature changes from these lakes. The relationship between δ^{18}O values of *Chironomus* spp. HCs from Australian oligotrophic to mesotrophic lakes in humid areas and air temperature appears robust (r = 0.88) and in line with results from European lakes. Similar results were obtained for δ^2H values and temperature (r = 0.94). For lakes that are located in semi-arid and sub-humid areas that have high evaporation compared to precipitation, and often long water residence time, the temperature relationship is not robust. This is due to the evaporative enrichment of δ^{18}O values in lake water and the effect of salinity and higher nutrient status. Vital effects may also play a role. The overall findings of this study are that both *Chironomus* spp. HC δ^{18}O and δ^2H are potentially valuable tools for reconstructing temperature in cooler, low nutrient and low salinity lakes of Australia. In warmer, sub-humid to semi-arid regions, δ^{18}O and δ^2H may provide valuable information about past changes in hydrological conditions.

Keywords Chironomid head capsules · δ^{18}O · δ^2H · South-east Australia · Vital effects · Nutrients ·

J. C. Chang (✉) · J. Shulmeister · C. Woodward
School of Geography, Planning and Environmental Management, University of Queensland, Level 4, Chamberlain Building (35), St Lucia, Brisbane, QLD 4072, Australia
e-mail: jie.chang@uqconnect.edu.au

J. Shulmeister
e-mail: james.shulmeister@uq.edu.au

C. Woodward
e-mail: c.woodward1@uq.edu.au

J. C. Chang
Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, 73 Beijing E Rd, Xuanwu, Nanjing 210008, Jiangsu, China

C. Woodward
Institute for Environmental Research, Australian Nuclear Science and Technology Organisation, New Illawarra Rd, Lucas Heights, NSW 2234, Australia

G. Michalski
Department of Earth and Atmospheric Sciences, Purdue University, 550 Stadium Mall Dr., West Lafayette, IN 47907, USA
e-mail: gmichals@purdue.edu

Springer

Salinity · Aridity · Evaporation · Temperature reconstructions

Introduction

Proxies that can provide long-term and reliable paleoclimate records are key to reconstructing and understanding past climate systems. Ideally these proxies should be widely distributed and common in the environment. There are very limited proxies for reconstructing temperature and rainfall in Australia. All of these proxies have limited ranges, precision and/or other constraints. Chironomids (Diptera: Chironomidae) are non-biting midges whose larvae occur in virtually all permanent and semi-permanent terrestrial water bodies (Cranston 1995). The subfossil head capsules (HCs) of chironomid larvae are preserved in lake sediment and the structure of these chitinous exoskeleton elements is usually well preserved (Walker 1987). The growth, species distribution and life cycles of chironomid larvae are strongly controlled by water temperature (Walker 1987; Eggermont and Heiri 2012). Based on these characteristics, transfer functions based on chironomid species assemblages have been developed from various parts of the world for paleoclimatic and environmental inferences (Lotter et al. 1999; Larocque et al. 2001; Woodward and Shulmeister 2006; Walker and Cwynar 2006; Rees et al. 2008; Massaferro et al. 2014; Chang et al. 2015a). These functions have been successfully applied to past species assemblage data and have provided several seasonal (usually summer) temperature reconstructions (Woodward and Shulmeister 2007; Rees and Cwynar 2010; Samartin et al. 2012; Chang et al. 2015b). However, it is also important to be able to validate these reconstructions using other techniques.

In addition to chironomid-based transfer functions, other studies have shown that the stable oxygen isotope ratio (δ^{18}O) of the chitinous structures of organisms including chironomids (Wooller et al. 2004, 2008; Wang et al. 2009), terrestrial and aquatic beetles (Gröcke et al. 2006; van Hardenbroek et al. 2012) and cladocerans (Verbruggen et al. 2011; Schilder et al. 2015) is largely a reflection of lake water δ^{18}O which is a function of the δ^{18}O of local precipitation and local modifying effects of lake morphology and hydrology. More recently, δ^{18}O

variations in subfossil chironomid HCs were applied to quantitatively characterize the late glacial lake water δ^{18}O changes and, indirectly, past air temperature changes near Rotsee, Switzerland (Verbruggen et al. 2010b). Stable isotopes provide an opportunity to directly cross-validate temperature from chironomid based transfer functions. Compared to δ^{18}O, fewer studies (Belle et al. 2015) are available for the measurements of stable hydrogen isotope ratio (δ^2H) on chironomid HCs. Results from Belle et al. (2015) suggests that δ^2H used in conjunction with stable carbon isotope ratio (δ^{13}C), may be useful to distinguish the long-term evolution and the relative contribution of the two pathways of methanogenesis when applied to chironomid HCs from lake sediment cores.

There are two key assumptions that need to be considered before δ^{18}O and δ^2H measurements from chironomid HCs in lake sediment can be used as proxies for temperature. Firstly, it is often assumed that the modern and past isotopic composition of lake water reflects mean annual precipitation (MAP) (Rozanski et al. 1993). While this is generally true in humid regions, evaporation affects the stable isotopic composition of water via evaporative enrichment of the heavy isotope (Leng et al. 2006). More recently Jones et al. (2016) shows that mass balance models can explain up to 74 % of the measured lake water isotope variability. Therefore although linkages between environmental change and isotopic variability may be more complex, shallow lakes in non-humid areas still have the potential to be used in calibration sets in conjunction with modelling and monitoring. Thus testing the application of δ^{18}O and δ^2H of chitinous organisms is worthwhile and it remains to be done in these non-humid settings. Lake morphology and stratification can also affect lake water δ^{18}O and δ^2H values. The lake needs to be volumetrically large enough but un-stratified for its isotopic composition to reflect the average isotope values in MAP (Leng et al. 2006). However, stratification may protect a large portion of the water from evaporative enrichment (Verbruggen et al. 2011). Lake water δ^{18}O values may approximate those of precipitation in stratified lakes if there are regular mixing events (e.g. winter overturn) when considered at annual-decadal timescales.

Secondly, it is sometimes assumed that the isotopic composition of the HCs is in equilibrium with the lake water and that there are no vital effects. Vital effects include but are not limited to trophic fractionation,

offset between larvae and HCs, relative contribution of diet and water to the isotopic composition of organisms, and feeding behaviour, all of which might modify HC isotopic values. These vital effects could be induced by organism metabolic processes (Heiri et al. 2012; Frossard et al. 2013; Soto et al. 2013) and diet. Such effects have been examined extensively in carbonate organisms include foraminifera (Erez 1978), ostracods (von Grafenstein et al. 1999), and coccoliths (Ziveri et al. 2003). There has been very little work on the vital effects of organic chitin material, such as the HCs of chironomids but only for $\delta^{13}C$ (van Hardenbroek et al. 2014) and fossil beetles (Gröcke et al. 2006; van Hardenbroek et al. 2012). It remains to be determined whether similar taxon dependent processes affect $\delta^{18}O$ and δ^2H fractionation in chironomid HCs.

In an attempt to address some of these issues in chironomids, Wang et al. (2009), Soto et al. (2013) and Belle et al. (2015) quantified how the $\delta^{18}O$ and δ^2H of water and diet influenced the $\delta^{18}O$ and δ^2H of chironomid larvae and HCs. Results of these studies revealed that both water and diet affect the $\delta^{18}O$ and δ^2H isotopic composition of chironomid larvae. Wang et al. (2009) suggests ~ 70 % of the oxygen in the total organic composition is derived from the water of the larval habitat and diet dominates the hydrogen isotope ratios of chironomid larvae, only 30 % of hydrogen in the chironomid larvae is derived from the ambient water. Soto et al. (2013) shows that the percentage of water contribution to chironomids hydrogen tissue was 47 % (53 % came from diet). Belle et al. (2015) suggested 85 % of the total hydrogen in the chironomid biomass is derived from the diet. However, these laboratory experiments are all based on feeding the chironomid larvae powdered algae (e.g. Spirulina) as a controlled diet. In natural aquatic environment, the total proportion of hydrogen in the chironomid larvae that is derived from ambient water could be higher if the consumption of δ^2H in the natural diet is considered. For instance, in the case of chitinous bryozoans van Hardenbroek et al. (2016) found that δ^2H of the chitinous remains were significantly correlated with lake water δ^2H and suggested this was partly caused by δ^2H of bryozoan diet reflecting lake water δ^2H. Differences in metabolic pathways perhaps also influenced $\delta^{18}O$ and δ^2H fractionation in different taxa. This is the first field based investigation of the relationship between δ^2H of lake water and chironomid HCs from the Southern Hemisphere.

Here we present the study of stable isotope ($\delta^{18}O$ and δ^2H) analyses on chironomid HCs from south-east Australian lakes. Based on previous research (Grey et al. 2004a, b; van Hardenbroek et al. 2014), it is possible that HCs of different chironomid taxa exhibit differences in stable isotopic composition (perhaps via vital effects) and will thus display different relationships to climatic or environmental variables. We therefore performed the $\delta^{18}O$ and δ^2H analyses on HCs from a single genus (Chironomus spp.) because it is the most abundant and widespread genus in south-eastern Australian lakes (Chang et al. 2015a). In addition this taxon is relatively large and easy to pick from sediment samples. We investigate the relationship between the $\delta^{18}O$ and δ^2H values of Chironomus spp. HCs and how this relates to the isotopic composition of lake water and local precipitation. The main goal of this study was to examine the feasibility of the application of $\delta^{18}O$ and δ^2H of Chironomus spp. HCs as a proxy to reconstruct past changes in temperature in south-eastern Australia.

Study sites

Fifteen natural lakes and one reservoir located in south-east Australia (ESM 1, Fig. 1) were included in this dataset. The dataset covers the latitudes between 30°S and 41.6°S and elevation of the sites ranged from sea level to c. ~ 2000 m above mean sea level (a.s.l) (ESM 1). The sixteen waterbodies included in this dataset had a large spatial distribution across eastern Australia. One lake (LLL) was located on top of New England Tablelands (Fig. 1), which lies in sub-tropical latitudes but maintains a cool temperate climate due to high elevation (ESM 1). Summer is the dominant season for the source of precipitation for this site. Three lakes were from Mt Kosciusko in the Australian Alps (Fig. 1), which is the coolest and highest (ESM 1) area of Australia and the precipitation (with significant snowfall) is winter and spring dominant. Ten lakes were from western Victoria, where a true Mediterranean climate exists with warm dry summers and cool wet winters. The region consists of semi-arid to sub-humid areas and the lakes are highly susceptible to both natural and agricultural related eutrophication and salinization (Chang et al. 2014). Two lakes were from north-western Tasmania (Fig. 1) and this is the region that is humid and precipitation is winter and spring dominant (similar to Australian Alps).
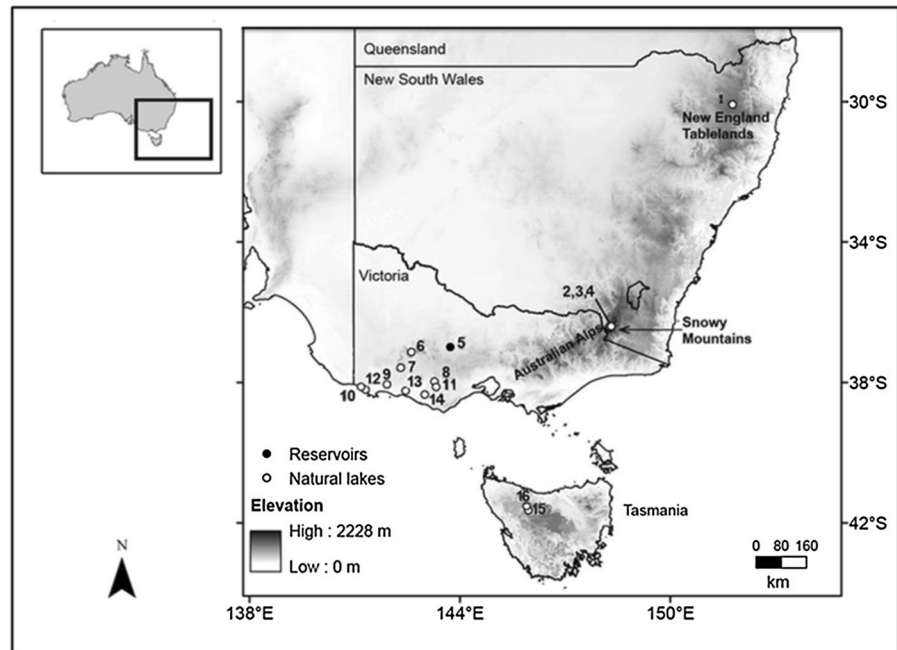
Detailed descriptions of the climate, vegetation and geology of the study area were presented in Chang et al. (2014).

Materials and methods

Sample collection

All lakes were sampled during the summer (January and February) of 2012 and 2013. A minimum of three sediment cores were taken using a Glew Mini Corer (Glew 1991) from the deepest point or the geographical centre of each lake when bathymetric information is not available. The top 2 cm of each core were extruded on site and packaged at 0.5-cm intervals into Whirlpaks®. Lake water for stable isotope analyses was sampled from the location where the core samples were taken, at approximately 30 cm below the water surface. They were collected into pre-washed and labelled polyethylene bottles. All bottles were sealed to prevent evaporation. Sediment and water samples were refrigerated until analysis.

Lake water samples were collected at the same time for the analysis of the concentrations of major ions, total nitrogen/total phosphorus (TN/TP), Chlorophyll *a* (Chl *a*), pH and specific conductance (COND). In the field, water temperature, oxidation reduction potential

(ORP), dissolved oxygen (DO), total dissolved solids, salinity and turbidity were also recorded using an Aquaread multi-parameter meter (AQUAREAD, Kent, UK). Detailed methodology for water chemistry analyses was outlined in Chang et al. (2014) and analytical results are presented in ESM 1.

Climatic and stable isotopes in precipitation data

Climate variables were interpolated from Australian climate stations using the combination of the World-Clim program (available from http://www.worldclim.org/bioclim, accessed 20 January, 2014) and ArcGIS 10.1. WorldClim data for Australia are derived from around 600 nation-wide weather stations that have climate records spanning the years 1950–2000 (http://www.bom.gov.au/climate/data/stations/, accessed 20 January, 2014). For this study, only mean annual air temperature (MAT) and precipitation (MAP) were considered (ESM 1), because the spatial variation in $\delta^{18}O$ in precipitation is usually strongly related to MAT along latitudinal gradients (Rozanski et al. 1993). The *Chironomus* spp. HCs we picked are 4th instars. We are aware of the fact that 4th instars are formed by *Chironomus* larvae in both autumn and spring, at least in northern European conditions (Goddeeris et al. 2001). A consequence of these moulting times is that the stable isotope composition

of the 4th instar mentum may relate to either late autumn or spring conditions. As a result, we chose to reconstruct MAT from the chitin of *Chironomus* spp. HCs rather than mean summer temperature as would be constructed from the transfer functions (Chang et al. [2015a](); Rees et al. [2008]()). This reflects both the normal approach to stable isotope data (Wooller et al. [2004](); Verbruggen et al. [2010a]()) and our uncertainty about when the chitin is formed. Potential evapotranspiration (PET) and Aridity Index (AI) values were obtained from the Global Potential Evapo-Transpiration (Global-PET) and Global Aridity Index (Global-Aridity) dataset (CGIAR-CSI, available from http://www.cgiar-csi.org/data/globalaridity-and-pet-database, accessed 20 March 2015) (ESM 1). Stable isotopes in precipitation data were obtained from the Global Network of Isotopes in Precipitation (GNIP) data set (IAEA/WMO, [2015]()) and interpolated using ArcGIS 10.1 for each site (Bowen and Revenaugh [2003]()) (ESM 1).

Stable isotope sample preparation

Sediment treatment and stable isotope sample preparation were performed in the laboratories at the School of Geography, Planning and Environmental Management, The University of Queensland. Both sample preparation protocols developed and used in Wang et al. ([2008]()) and Verbruggen et al. ([2011]()) were considered and employed with some modifications. Sediment samples were deflocculated in a cold 10 % solution of potassium hydroxide (KOH) for two hours at room temperature and subsequently sieved with a 180 mm mesh-size sieve (van Hardenbroek et al. [2010]()). The top 2 cm was processed for chironomid HCs from three surface core samples. Sieved residues were stored in vials with distilled water. HCs of genus *Chironomus* (*Chironomus* spp.) were picked from the sieved residue under a dissection microscope at $50\times$ magnification using fine forceps and placed into pre-labelled vials. Verbruggen et al. ([2010a]()) suggested that chemical pre-treatment of chironomid HCs could affect the resulting $\delta^{18}O$ values. Therefore, a physical treatment was used instead where after a minimum of 150 HCs was picked into the vials, they were placed in an ultrasonic bath for 20–30 s to separate contaminants. The HCs were then examined for contaminants under the microscope and later transferred to a pre-weighed silver cup of 3.5×5 mm (Costech Analytical Technologies, INC., code: 041066). This was based on a modified two-step transfer protocol (Wang

et al. [2008]()), which had an advantage of allowing any contaminants to be detached and manually separated during the process. Silver cups containing the HCs were allowed to dry for several days in a covered Petri dish at room temperature. The silver cups were again weighed and, if a minimum of 100 µg was present, folded and shape-trimmed (Verbruggen et al. [2011]()). Samples were subsequently measured for stable oxygen ($\delta^{18}O$) and hydrogen (δ^2H) isotope ratios in Purdue Stable Isotope laboratory (PSI) facility, West Lafayette, USA.

Stable isotope analyses

$\delta^{18}O$ and δ^2H analysis of the lake water samples were prepared by vacuum pump filtration and samples were placed in 10-ml Gas Chromatography (GC) vials. 0.3-µl aliquots were auto-injected on the PSI lab high Temperature Conversion Elemental Analyzer (TC/EA, Thermo Fisher Scientific) by a GC-PAL autosampler. The injected water sample was pyrolyzed under reducing conditions at 1400 °C to produce H_2 and CO gases. These were separated chromatographically in a helium carrier gas stream and introduced sequentially into the ion source of an isotope ratio mass spectrometer (IRMS, Thermo Fisher Scientific) (Delta V Plus, ThermoFinnigan) for isotope ratio determination (Gehre et al. [2004]()). Six sequential injections were made for each sample and the reported values represent the average of final three injections (Nielson and Bowen [2010]()). All lake water stable isotope data were calibrated using repeated analysis of three internal standards (PT, UT and PZ) relative to Vienna-Standard Mean Ocean Water and Standard Light Antarctic Precipitation (VSMOW/SLAP) (Coplen [1995]()). All data were reported as per mil (‰) relative to the VSMOW standard. Average uncertainties for lake water were ± 0.44 ‰ for $\delta^{18}O$ and ± 3.6 ‰ for δ^2H.

The *Chironomus* spp. HC samples were stored in the PSI facility at room temperature for seven days prior to analysis for the samples to equilibrate with the analytical environment. The TC/EA coupled to IRMS were used to determine ratios of $\delta^{18}O$ and δ^2H for *Chironomus* spp. HCs (sample weight >100-µg). Samples were transferred to a Zero Blank Auto-sampler (Costech Analytical) interfaced with the TC/EA. They were pyrolyzed at 1400 °C in an oxygen-free environment to produce H_2 and CO gases, which were chromatographically separated and introduced sequentially to the source of the IRMS (Gehre et al. [2004]()). Two blanks were measured at

the start of every run. Three commercial keratin laboratory standards (powdered KHS, FH and UH) were used to normalize the results. $\delta^{18}O$ and δ^2H values were calibrated against primary reference materials (Nielson and Bowen 2010) and all data were reported as per mil (‰) relative to the VSMOW standard (Coplen 1995). Average precisions of ± 0.8 and ± 4.0 ‰ for $\delta^{18}O$ and δ^2H of *Chironomus* spp. HCs were achieved respectively.

Statistical analyses

Regression analyses and redundancy analyses (RDAs) were applied to calculate variance partitioning on a single response variable (Borcard et al. 1992) in CANOCO version 4.5 (ter Braak and Šmilauer 2002). The correlation significance, correlation coefficient and the independence of the variable correlations between the $\delta^{18}O$ and δ^2H data of *Chironomus* spp. HCs against climatic and environmental variables were tested with a Monte Carlo permutation test (999 unrestricted permutations) (ter Braak and Šmilauer 2002). A Bonferroni-type correction (One-step) for 'false positives' was applied in this set of repeated significance tests to correct the critical p values (García 2004). The purpose of these analyses was also to determine if fractionation or variable offsets were constant or varied with changes in the environment.

Changes between isotopic compositions of two substances are given as apparent fractionation factor (α). The α values for *Chironomus* spp. and lake water of $\delta^{18}O$ and δ^2H is defined as:

$$\alpha_{\delta^{18}O(\text{Chironomus spp.}-\text{water})} = \frac{R_{\text{Chironomus spp.}}}{R_{\text{water}}}$$
$$= \frac{\delta^{18}O_{\text{Chironomus spp.}} + 1000}{\delta^{18}O_{\text{water}} + 1000}$$
(1)

$$\alpha_{\delta^2H(\text{Chironomus spp.}-\text{water})} = \frac{R_{\text{Chironomus spp.}}}{R_{\text{water}}}$$
$$= \frac{\delta^2H_{\text{Chironomus spp.}} + 1000}{\delta^2H_{\text{water}} + 1000}$$
(2)

(Kendall and Caldwell 1998) respectively. We are aware of that the α values do not only reflect fractionation between oxygen/hydrogen in water and oxygen/hydrogen incorporated into *Chironomus* spp. HCs. The key limitations of these calculations are that these values (α) may be influenced by differences in diet between the study lakes and seasonal variations in lake water $\delta^{18}O$ and δ^2H. However, the accurate differences in α values could only be calculated in laboratory settings. Since our analyses of HCs are based on the same genus (*Chironomus* spp.) sampled during the same season, we assume that the diet differences and the effects of seasonal variation on the α values are relatively constant among the study lakes.

For testing correlations between α values and environmental and climatic variables, the natural logarithm of α ($\ln\alpha$) was used by convention (Clark and Fritz 1997) and was multiplied by 10^3 to adapt to the ‰ notation for δ values. Both regression and RDA (CANOCO version 4.5) (Borcard et al. 1992) were used to test the correlation significance, correlation coefficient and the independence of the variable correlations between $\alpha_{[\delta18O(\text{Chironomus spp.-water})]}$ and $\alpha_{[\delta2H(\text{Chironomus spp.-water})]}$ against climatic and environmental variables, respectively. A 'One-step' Bonferroni-type correction was also applied for the critical p values in this set of significance tests (García 2004).

Results

The $\delta^{18}O$ and δ^2H analytical results on HCs of *Chironomus* spp. and the respective host lake water were obtained from sixteen south-eastern Australian waterbodies (Table 1). Apparent fractionation factors (α) between *Chironomus* spp. HCs and host lake water were calculated applying Eqs. 1 and 2 for $\delta^{18}O$ and δ^2H, respectively and results are presented in Table 1.

Stable oxygen isotope ($\delta^{18}O$) analytical results

The annual averaged of $\delta^{18}O$ of precipitation varied between -7.20 and -4.65 ‰ VSMOW and was positively correlated (r = 0.84) with the lake water $\delta^{18}O$ (Fig. 2a), which varied between -6.17 and 12.97 ‰ VSMOW. Lake water $\delta^{18}O$ in a number of sites from sub-humid areas was significantly enriched relative to precipitation (Table 1; Fig. 2a) and the enrichment ranged between -0.56 and 17.71 ‰ (Table 1). The measured $\delta^{18}O$ values from the chitinous HCs of *Chironomus* spp. from the surface

Table 1 Stable isotope analyses (δ18O and δ2H) results of south-eastern Australian modelled precipitation, lake water, *Chironomus* spp. head capsules (HCs) and the fractionation factor between *Chironomus* spp. HCs and lake water (α)

No.	Lake Name	Lake	δ18O of modelled precipitation VSMOW	δ18O of lake water VSMOW	δ18O of Chiro. spp. HCs VSMOW	δ18O of enrichment in lake water VSMOW	α [δ18O] (Chironomus spp.—water)	δ2H of modelled precipitation VSMOW	δ2H of lake water VSMOW	δ2H of Chiro. spp. HCs VSMOW	δ2H enrichment in lake water VSMOW	α [δ2H] (Chironomus spp.—water)
1	Little Llangothlin Lagoon	LLL	−6.7	−1.25	18.7	5.45	19.78	−39.13	−34.04	−91.7	5.09	−61.55
2	Blue Lake	BL	−7.2	−7.76	12.27	−0.56	19.99	−44.27	−50.69	−84.82	−6.42	−36.61
3	Lake Albina	LA	−7.2	−7.02	13.27	0.18	20.23	−44.27	−54.33	−101.17	−10.06	−50.8
4	Lake Cootaptamba	CTL	−7.2	−6.53	13.1	0.67	19.56	−44.27	−44.49	−94.87	−0.22	−54.17
5	Nuggety Gully Resrvoir	NGR	−4.8	11.27	15.77	16.07	4.44	−26.61	57.81	−63.29	84.42	−121.58
6	Lake Fyans	LFY	−4.91	3.57	15.28	8.48	11.61	−27.47	28.15	−48.18	55.62	−77.13
7	Freshwater Lake	FWL	−5.01	5.57	15.94	10.58	10.26	−28.3	40.8	−54.7	69.1	−96.25
8	Lake Tooliorook	LTK	−4.91	1.05	16.88	5.96	15.69	−27.61	27.87	−73.17	55.48	−103.47
9	Lake Surprise	LSP	−4.91	1.18	16.8	6.09	15.47	−27.7	27.48	−75.4	55.18	−105.5
10	Lake Mombeong	LMB	−4.65	3.34	16.28	7.99	12.82	−26.71	28.63	−72.82	55.34	−103.83
11	Lake Terangpom	LTP	−4.81	10.54	13.91	15.35	3.33	−26.94	56.67	−55.32	83.61	−112.04
12	Swan Lake	SWL	−4.72	4.02	13.63	8.74	9.52	−26.46	43.26	−69.18	69.72	−114.04
13	Lake Cartcarrong	LCT	−4.74	12.97	15.09	17.71	2.09	−26.52	61.63	−70	88.15	−132.38
14	Lake Elingamite	LEM	−4.85	5.52	16.09	10.37	10.45	−27.27	51.33	−50.93	78.6	102.33
15	Lake Lila	WP	−6.21	−6.17	12.43	0.04	18.54	−38.17	−34.55	−79.53	3.62	−47.71
16	Lake Lea Pond	LEA	−5.73	2.43	15.22	8.16	12.67	−34.6	17.26	−75.81	51.86	−95.95

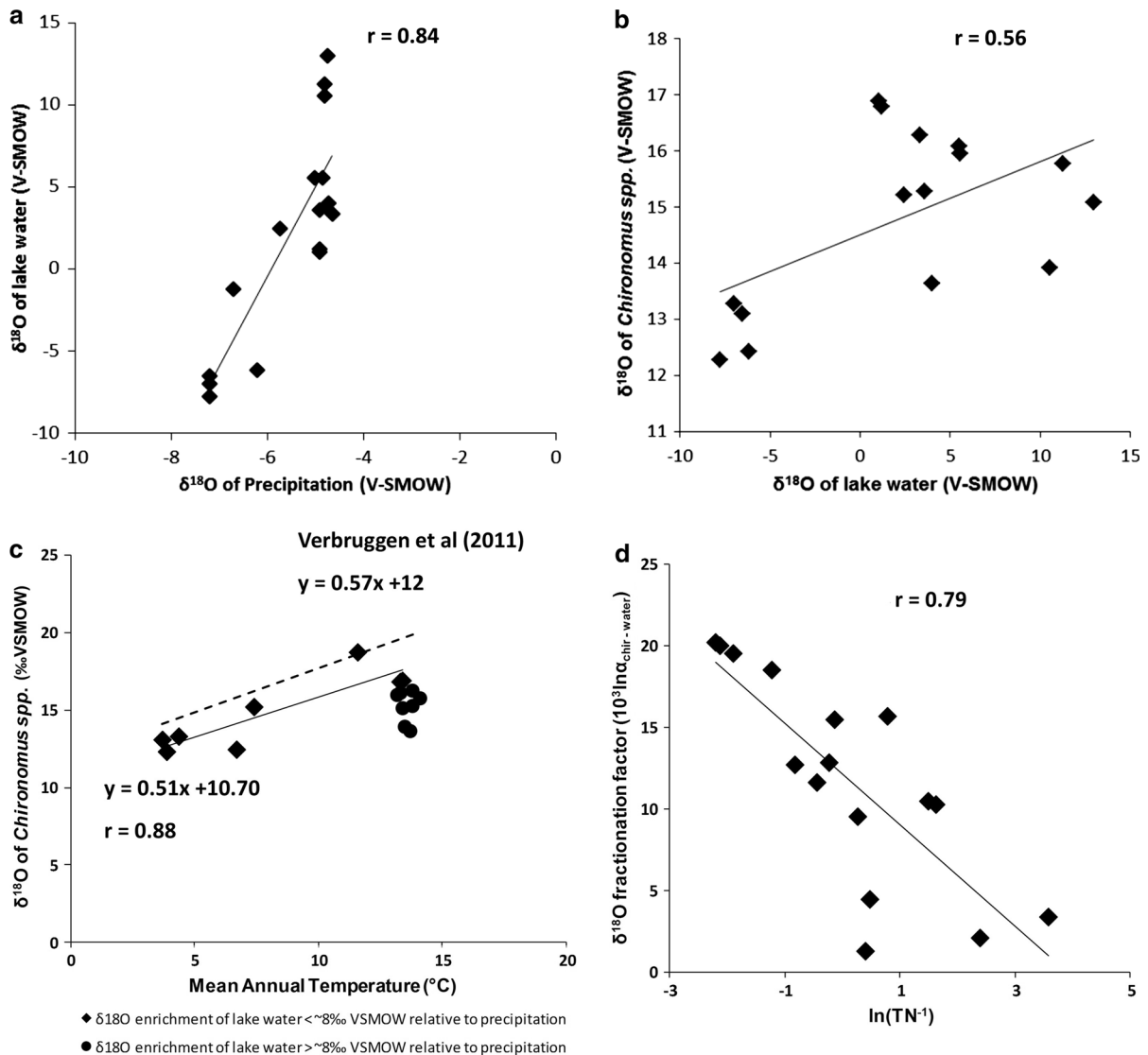**Fig. 2 a** Plot of $\delta^{18}O$ of precipitation against $\delta^{18}O$ of lake water. The $\delta^{18}O$ of precipitation and lake water is correlated but significant enrichment of lake water is observed where the $\delta^{18}O$ of precipitation is around -5 ‰ VSMOW. **b** The plot of $\delta^{18}O$ of lake water against *Chironomus* spp. HCs showed no significant correlation ($p = 0.13$) between the two data sets, suggesting that the oxygen stable isotopic composition of *Chironomus* spp. HCs do not always reflect changes in ambient water. **c** Plot of mean annual air temperature (MAT) against $\delta^{18}O$ of *Chironomus* spp. HCs with all sixteen lakes and plot of MAT against

$\delta^{18}O$ of *Chironomus* spp. HCs with only the eight lakes that have $\delta^{18}O$ enrichment of lake water less than ~ 8 ‰ relative to precipitation $\delta^{18}O$, respectively. There is a strong correlation ($r = 0.88$) between MAT and $\delta^{18}O$ of *Chironomus* spp. HCs for the later subset (*solid line*). The slope and intercept values were very similar to those observed by Verbruggen et al. (2011) in Europe (*dashed line*). **d** Plot of total nitrogen (TN) against the apparent fractionation factor ($\alpha_{[\delta 18O(chiro-water)]}$), suggesting lake eutrophication is an important consideration for the interpretation of oxygen isotope fractionation in *Chironomus* spp. HCs

sediments of all sixteen south-eastern Australian lakes showed no significant correlation ($p > 0.05$) with the surface lake water isotopic composition (Fig. 2b; Table 2). The measured $\delta^{18}O$ values from the HCs of *Chironomus* spp. showed a close linear relationship

($r = 0.88$) with MAT for sites (eight lakes) that have $\delta^{18}O$ enrichment of lake water less than ~ 8 ‰ VSMOW relative to precipitation $\delta^{18}O$ values (Fig. 2c; Table 2). The remaining eight lakes have $\delta^{18}O$ enrichment of lake water larger than ~ 8 ‰

Table 2 Summary of results from statistical analyses of stable oxygen isotope (δ^{18}O) data, details of each step of RDAs are included in ESM 2 and 3

Tested relationship	Correlation coefficient (r)	p value (at 95 % confidence level)	Correlation
δ^{18}O precipitation versus δ^{18}O lake water	r = 0.84	4.00×10^{-5} Significant	Positive strong and significant correlation with unevenly distributed data
δ^{18}O *Chironomus* spp. HCs versus δ^{18}O lake water	r = 0.56	0.31 Not significant	No strong or significant correlation
δ^{18}O *Chironomus* spp. HCs versus MAT (ALL sites)	r = 0.74	0.006 Significant	Positive and significant correlation, unevenly distributed data
δ^{18}O *Chironomus* spp. HCs versus MAT (sites with the δ^{18}O enrichment of lake water less than ~ 8 ‰ relative to precipitation δ^{18}O)	r = 0.88	0.0039 Significant	Positive strong and significant correlation with evenly distributed data

Tested variables	Significant variables ($p < 0.05$) and the respective correlation coefficient (r) values	Variable that explained the largest % variance	Variables that interact
Regression and redundancy analyses against environmental variables			
δ^{18}O *Chironomus* spp. HCs values	MAT (0.65), AI (0.73), PET (0.59), pH (0.50), COND (0.52)	AI (53.7 %)	MAT
Apparent fractionation factor values ($\alpha[\delta^{18}$O (*Chironomus* spp.—water)])	MAT (0.69), TN (0.80), TP (0.69), Chl$a$ (0.53), depth (0.57), COND (0.74), AI (0.66)	TN (64.4 %)	COND

VSMOW relative to precipitation δ^{18}O values and do not follow the same relationship (Fig. 2c; Table 2).

Regression analyses of δ^{18}O of *Chironomus* spp. HCs values against climatic and environmental variables showed that five variables were significantly correlated ($p < 0.05$) to variation in δ^{18}O of the HCs of *Chironomus* spp.. These were MAT, AI, PET, pH and COND (Table 2). RDAs (Borcard et al. 1992) were then performed using the δ^{18}O of *Chironomus* spp. HCs values against these five variables (ESM 2). Results showed that AI was the variable that explained the largest percent (53.7 %) of the total variance in the δ^{18}O *Chironomus* spp. HCs data (Table 2), although it did not retain its significance when MAT was partialled out (ESM 2). The RDA on MAT showed that when AI was partialled out, it only explained 0.9 % of the total variance (ESM 2). AI and MAT are correlated and this is due to the effect of temperature on evaporation of the lake water.

Regression analyses of the δ^{18}O apparent fractionation factor between the chironomid HCs and lake water ($\alpha_{[\delta18O(Chironomus\ spp.-water)]}$) values with respect to climatic and environmental variables showed that seven variables: MAT, TN, TP, Chl a, depth, COND and AI were significantly correlated ($p < 0.05$) (Table 2). RDAs (Borcard et al. 1992) were performed using the $\alpha_{[\delta18O(Chironomus\ spp.-water)]}$ values against all seven variables (ESM 3). TN was the variable that explained the largest percent (64.4 %) of the total variance in the $\alpha_{[\delta18O(Chironomus\ spp.-water)]}$ data (Table 2). There is a strong interaction between COND and TN but TN is more independent and explains a larger proportion of variance (Table 2, ESM 3). The regression plot of the values of $\alpha_{Chironomus\ spp.-water}$ for δ^{18}O against TN showed a close negative correlation (with correlation coefficient r = 0.79) (Fig. 2d).

Stable hydrogen isotope (δ^2H) analytical results

For δ^2H, the annual average δ^2H values of precipitation varied between -44.27 and -26.46 ‰ VSMOW and was closely correlated with the lake water δ^2H values which varied between -6.17 and 12.97 ‰ VSMOW (r = 0.96) (Fig. 3a; Table 3). Lake water
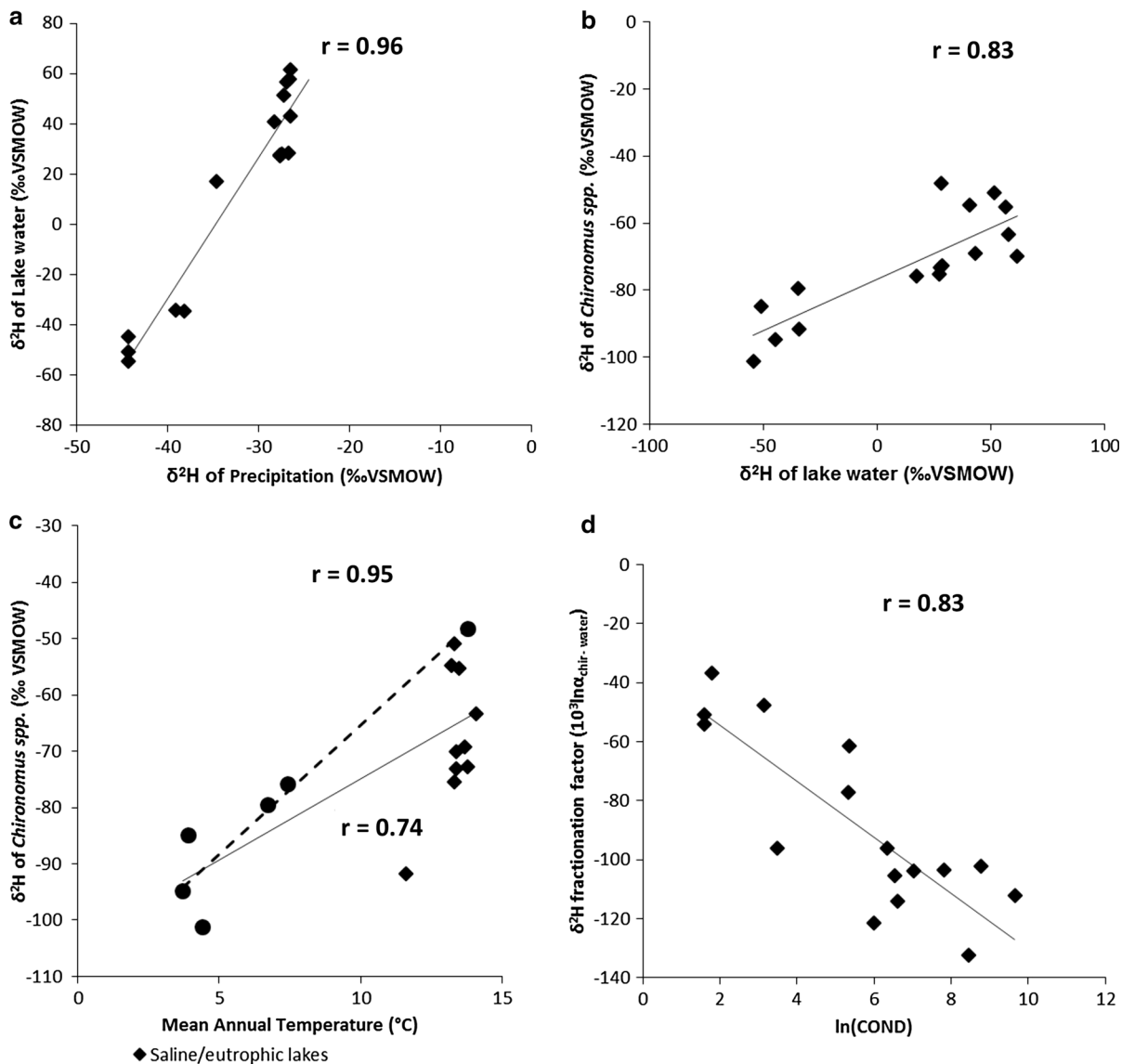
Fig. 3 a Plot of δ^2H of precipitation against δ^2H of lake water show a strong relationship. **b** Plot of δ^2H of lake water aginst δ^2H of *Chironomus* spp. HCs, which also displays a close correlation. **c** Plot of mean annual air temperature (MAT) against δ^2H of *Chironomus* spp. HCs for all lakes (*solid line*) and also for the eight low nutrient lakes that had a ln(COND) less than ∼6 (*dashed line*) (ESM 1). The observed correlation is stronger for low conductivity non-eutrophic sites. **d** Plot of ln(COND) against $10^3 ln$ ($\alpha_{\delta2H(chiro-water)}$), showing increasing fractionation between δ^2H of *Chironomus* spp. HCs and δ^2H of lake water as conductivity increases

δ^2H from non-humid area was significantly enriched in relative to precipitation and the enrichment ranges between −10.07 and 88.15 ‰ (Table 1). The measured δ^2H values from the chitinous HCs of *Chironomus* spp. from the surface sediments of the sixteen south-eastern Australian lakes showed a significant positive correlation (r = 0.83, $p < 0.05$) with host water isotopic composition (Fig. 3b; Table 3).

Regression analyses of δ^2H of *Chironomus* spp. HC values against the chosen variables showed that seven variables (MAT, TN, TP, Chl *a*, Depth, COND and AI) were significantly correlated ($p < 0.05$) (Table 3). RDAs (Borcard et al. 1992) were then performed using the δ^2H of *Chironomus* spp. values against these seven variables (ESM 4). Results showed that MAT was the variable that explained the largest percent (55.2 %) of

Table 3 Summary of results from statistical analyses (RDAs) for stable hydrogen isotope (δ^2H), details of each step of RDAs are presented in ESM 4 and 5

Tested relationship	Correlation coefficient (r)	*p* value (at 95 % confidence interval)	Correlation	
δ^2H precipitation versus δ^2H lake water	r = 0.96	3.80×10^{-9} Significant	Positive strong and significant correlation with unevenly distributed data	
δ^2H *Chironomus* spp. HCs versus δ^2H lake water	r = 0.83	6.66×10^{-5} Significant	Positive strong and significant correlation with evenly distributed data	
δ^2H *Chironomus* spp. HCs versus MAT (ALL sites)	r = 0.74	9.80×10^{-4} Significant	Positive and significant correlation with unevenly distributed data	
δ^2H *Chironomus* spp. HCs versus MAT (with only non-eutrophic and freshwater sites)	r = 0.94	0.0042 Significant	Positive strong and significant correlation	
Tested variables	Significant variables ($p < 0.05$) and the respective correlation coefficient (r) values		Variable that explained the largest % variance	Variables that interact
Regression and redundancy analyses against environmental variables				
δ^2H *Chironomus* spp. HCs values	MAT (0.74), TN (0.68), TP (0.51), Chl*a* (0.52), Depth (0.61), COND (0.71), AI (0.67)		MAT (55.2 %)	COND, TN, AI
Apparent fractionation factor values ($\alpha[\delta^2H$ (*Chironomus* spp.—water)])	TN (0.76), TP (0.61), Chl*a* (0.6), COND (0.83), Depth (0.6), MAT (0.82) and AI (0.80)		COND (68.4 %)	None

the total variance in the δ^2H *Chironomus* spp. HC data (Table 3). The explanatory power of MAT was reduced by partialling out COND, AI and TN (ESM 4). The reduction in explanatory power was greatest after partialling out COND and AI (ESM 4). However, MAT appears to be the most independent variable since the explanatory power of AI and COND is almost eliminated by partialling out MAT (Table 3, ESM 4). COND, TN, AI and MAT are correlated and this is because of the effect of temperature on evaporation and the concentration of ions and nutrients in the lake waters. The regression plot of δ^2H values of *Chironomus* spp. HCs against MAT showed a positive and significant ($p < 0.05$) correlation with a correlation coefficient of r = 0.74 with all sites included (Fig. 3c; Table 3). When saline (COND > 500 µs cm^{-1}) and eutrophic lakes (TN > 1 mg L^{-1}) were excluded (ESM 1), the remaining six lakes showed a much higher linear correlation coefficient with r = 0.94 (Fig. 3c; Table 3).

Regression analyses of the fractionation ($\alpha_{Chironomus \ spp.-water}$) values for δ^2H against climatic and environmental variables showed that TN, TP, Chl *a*, COND, Depth, MAT and AI were significantly correlated ($p < 0.05$) (Table 3). The partial RDAs

(Borcard et al. 1992) showed that COND was the sole variable that retained significance ($p < 0.05$) when all other variables were partialled out and explained 68.4 % of the total variance in the $\alpha_{Chironomus \ spp.-water}$ values for δ^2H (Table 3, ESM 5). The regression plot of δ^2H fractionation values against COND has a correlation coefficient of r = 0.84 (Fig. 3d).

Discussion

Laboratory analytical considerations

The application of stable isotope analyses on subfossil chironomids is still at an early stage of development and a standard chitin sample purification protocol for chironomid HCs is not yet established. There have been only three studies that reported the measurement of δ^2H on chironomid larvae (Deines et al. 2009; Wang et al. 2009; Soto et al. 2013) and HCs (Belle et al. 2015) respectively. Studies on keratin material (Wassenaar and Hobson 2003) suggested that the use of hydrogen isotope ratios needs to be considered carefully because of the rapid, partial exchange of

loosely bound hydroxyl and/or amine hydrogen atoms with the environment. Here, hydrogen isotope ratios were not corrected for the contribution of exchangeable hydrogen. This was because at the time of analysis, a set of exchange-calibrated chitin standards was not yet established at PSI (Nielson and Bowen 2010). The amount of exchangeable hydrogen is usually expressed as percentage of the total amount of hydrogen and is 15.3 ± 2.9 % in chitin (Schimmelmann et al. 1993). Hydrogen atoms in exchangeable hydroxyl and amine groups should have the same isotopic composition across all chitin samples analysed at the same time under the same conditions. This concern was discussed in detail in Nielson and Bowen (2010) for the analysis of shrimp chitin from PSI. As all samples reported in this study were processed, pretreated and analysed using internally consistent methods, the relative offsets should be constant. Consequently while the absolute δ^2H values of *Chironomus* spp. HCs obtained from this study will not be suitable to compare directly with other studies, the trends should still yield useful information and are worthy of discussion.

Relationship between $\delta^{18}O$ of lake water and regional climate

Due to evaporative enrichment in non-humid areas and the wide range spatial distribution of the sites, there is a disparity in between the $\delta^{18}O$ of source water (i.e. seasonal variations in $\delta^{18}O$ of precipitation) and the $\delta^{18}O$ of lake water. Therefore, it is not surprising that although the $\delta^{18}O$ of precipitation was correlated with lake water (Fig. 2a), the sites are unevenly distributed around the regression line.

We observe that $\delta^{18}O$ of lake water is closely correlated with $\delta^{18}O$ of precipitation for lakes in humid climates. There is a wide scatter of the $\delta^{18}O$ of lake water values in lakes with similar precipitation $\delta^{18}O$ values, specifically when $\delta^{18}O$ precipitation is >5 ‰ VSMOW (Fig. 2a). Sites with precipitation $\delta^{18}O$ values >5 ‰ VSMOW are located in sub-humid areas (Fig. 2a; Table 1). Evaporative enrichment of $\delta^{18}O$ in lake water is obviously important at these sites and variations in enrichment probably reflect differences in local evaporation rates and residence time (Fig. 4a) as the lighter isotope ($\delta^{16}O$) is preferentially
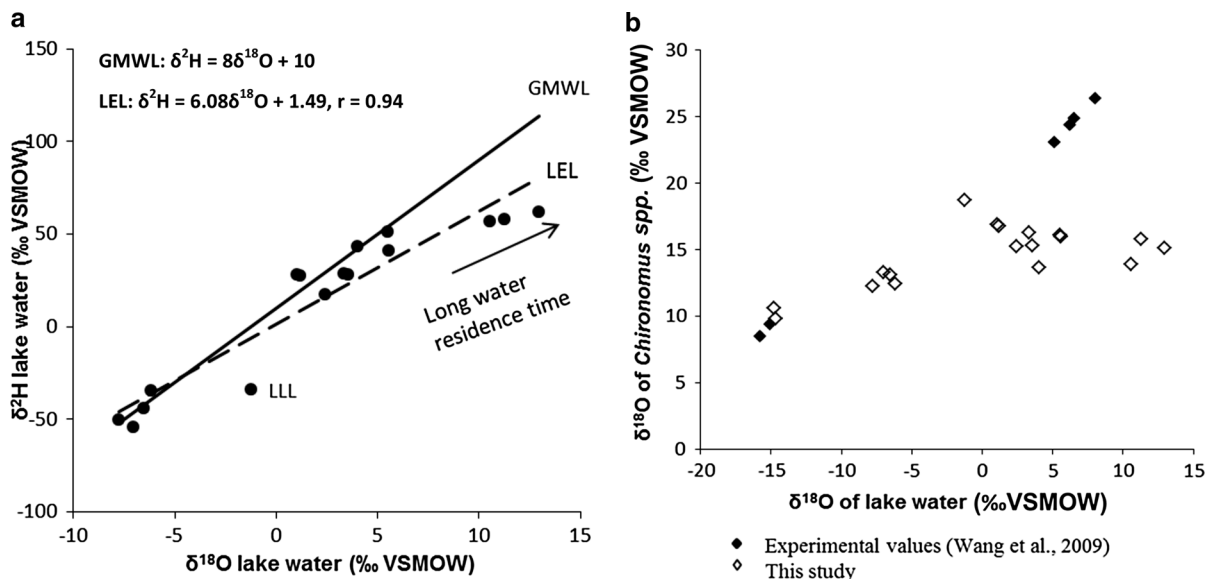

Fig. 4 a A local evaporation line (LEL) for lakes was derived from plotting $\delta^{18}O$ against δ^2H values measured from lake water (*dashed line*). Except for Little Llangothlin Lagoon (LLL) from northern New South Wales, the remaining fifteen lakes follow either the global meteoric water line (GMWL) (*solid line*) or LEL, and imply that these lakes are able to track regional climate variations. **b** Plot of the experimental chironomid $\delta^{18}O$ values against $\delta^{18}O$ of host water in Wang et al. (2009) compared to values derived from this study. Eight of the lakes from this study (CTL, BL, LA, WP, LEA, LLL, LSP, LTK), fall along the regression line of Wang et al. (2009). These are oligotrophic and mesotrophic lakes come from the Australian Alps near Mt Kosciuszko and from Tasmania, and also from two fresher western Victorian lakes

lost due to evaporation (Leng and Marshall 2004; Henderson and Shuman 2009; Jones et al. 2016). Lakes with enriched $\delta^{18}O$ with respect to source water are closed maar lakes and all are located in western Victoria. In fact, in two of the western Victorian lakes (LTP and LCT) and one reservoir (NGR), the $\delta^{18}O$ values exceed 10 ‰ VSMOW (Fig. 4a) which is considered large and this case, long residence times (Fig. 4a) are likely the primary cause (Henderson and Shuman 2009; Barton et al. 2007; Chivas et al. 1993). However, lake water δ^2H and $\delta^{18}O$ at these sites closely tracks the isotopic composition of precipitation (r = 0.84 for δ^2H and r = 0.96 for $\delta^{18}O$; Figs. 2a, 3a). In summary, Australia has a high inter-annual variability in rainfall and in seasonally arid regions, such as western Victoria, closed basin shallow lakes do not track rainfall isotope variability. Using the modelling method suggested in Jones et al. (2016), these lakes may still be suitable to be included in a calibration set for paleoclimate reconstructions.

Lake trophic status affects $\delta^{18}O$ fractionation

A few previous studies (Wang et al. 2009; Verbruggen et al. 2011; Mayr et al. 2015) suggested that $\delta^{18}O$ of chironomid HCs can serve as a useful tool for lake water reconstructions for $\delta^{18}O$. However, these results were only replicated for lakes in non-arid environments in this study (Fig. 2c). One of the possible explanations is that insect metabolism and/or respiration in *Chironomus* spp. plays a part in the fractionation between $\delta^{18}O$ of lake water and HCs of *Chironomus* spp.. Other possible causes include (a) different food sources in different lake ecosystems (e.g. saline vs. freshwater, coastal vs. montane lakes), and (b) salinity, which influences through osmosis the structure (Galat et al. 1988) and isotopic fractionation of *Chironomus* spp. exoskeletons. Figure 4b shows the experimental chironomid $\delta^{18}O$ values against $\delta^{18}O$ of host water in Wang et al. (2009) compared to this study. Sites where there is a strong relationship between $\delta^{18}O$ of lake water and $\delta^{18}O$ *Chironomus* spp. HCs are mostly oligotrophic to mesotrophic lakes in humid areas. In most of the eutrophic to hypereutrophic lakes (ESM 1) in non-humid areas, the relationship does not apply.

The moderately strong correlation (r = 0.79) between TN and $\alpha_{[\delta18O(Chironomus\ spp.-water)]}$ (Fig. 2d), shows that lake trophic status is an important

consideration for the interpretation of $\delta^{18}O$ data for *Chironomus* spp. HCs. Ten out of sixteen of these lakes are eutrophic to hypereutrophic (ESM 1), eight out of these, which are coincident with the sites where we observed vital effects in $\alpha_{[\delta18O(Chironomus\ spp.-water)]}$, also have very high algal production (large Chl *a* values). We highlight that as productivity pathways move from macrophytes to algae and subsequently as algae are consumed by bacteria in anoxic sediments (after death) (Granéli and Solander 1988; France 1995; LaZerte and Sxulados 1982), there is a strong depletion of carbon and oxygen isotopes in lake water, as reflected by the enrichment of both carbon and oxygen isotopes (Teranes et al. 1999) and also deuterium (Whiticar 1999) in eutrophic lake sediments. This is because $\delta^{12}C$ and $\delta^{16}O$ are preferentially selected over $\delta^{13}C$ and $\delta^{18}O$. As a result, the lake waters are comparatively enriched in $\delta^{13}C$ and $\delta^{18}O$ and this is reflected in enrichment of *Chironomus* spp. chitin (Table 1). Shallow lakes located in sub-humid regions, with high residence times are highly susceptible to this effect (Chang et al. 2014). These lakes provide challenging conditions for biota but *Chironomus* spp. is dominant in most lakes of this type. It reached a maximum abundance of 63.7 % [i.e. Lake Toolirook (LTK), Victoria (Table 1)] in lowland eutrophic to hypereutrophic lakes and is present in 94 % of the lakes sampled in Chang et al. (2015a) in south-eastern Australia. This is similar to New Zealand (Woodward and Shulmeister 2006), where in some warm lowland eutrophic lakes, *Chironomus* spp. reached a maximum abundance of 80 %. One of the key features that allows *Chironomus* spp. to adapt to low oxygen levels and eutrophic conditions is that they have the ability to metabolise and respire through the use of haemoglobin (Osmulski and Leyko 1986; Walker 1987; Brodersen et al. 2004). A consequence of this may be that scavenging of oxygen by haemoglobin, which is an active process, is likely to take all of the available oxygen from oxygen depleted water, thereby not selecting against heavier isotopes. It is possible the process is in analogous to the carbon isotope discrimination in plants suggested in previous studies (Farquhar et al. 1982, 1989). However, we do not rule out other mechanisms affecting $\delta^{18}O$ values of *Chironomus* spp. HCs. Examples of such mechanisms include feeding on different food sources between lakes, seasonal variations, and differences in the length of the larval stage (seasonal or mean annual signal).

In summary, $\delta^{18}O$ values of *Chironomus* spp. HCs from eight out of sixteen sites within the dataset appear to reflect lake water $\delta^{18}O$ and those HCs come from lakes in humid and/or cooler regions. High lake nutrient status is associated with more arid regions and the dominance of *Chironomus* spp. in the species composition. We reaffirm the findings of Mayr et al. (2015) that for non-humid regions where $\delta^{18}O$ of precipitation is not a priori correlated with MAT, it is challenging to use $\delta^{18}O$ values in *Chironomus* spp. HCs for reconstruction of $\delta^{18}O$ in precipitation and consequently paleo-temperature.

Relationship between $\delta^{18}O$ of *Chironomus* spp. HCs and regional air temperature

The previous discussion highlighted the large variable offsets on HCs of *Chironomus* spp. $\delta^{18}O$ in western Victorian lakes, and other lakes subject to eutrophication and evaporative enrichment. A close correlation between HCs of *Chironomus* spp. $\delta^{18}O$ and MAT was still found in lakes that have a $\delta^{18}O$ enrichment less than ~ 8 ‰ of lake water compared to precipitation values. The slope (0.51) and intercept (10.7 $\pm$ 0.8 ‰) values of the regression obtained in these eight south-eastern Australian lakes were very similar to Verbruggen et al.'s (2011) European dataset, which had a slope of 0.57 and an intercept value of 12 $\pm$ 0.32 ‰ (Fig. 2c). It is of interest to note that, as the $\delta^{18}O$ analyses of chironomid HCs from Verbruggen et al. (2011) were based on a mixed taxon assemblage, including *Heterotrissocladius subpilosus*-type, *Cladotanytarsus*, and *Cricotopus* (Verbruggen et al. 2011). Although results from this study inferred that metabolic effects could have an effect on the $\delta^{18}O$ of *Chironomus* spp. HCs, this only appears to be true in some environments (i.e. nutrient-rich lakes).

Our results confirm that lakes that are not affected by significant $\delta^{18}O$ enrichment are suitable for paleo-temperature reconstructions using $\delta^{18}O$ of *Chironomus* spp. HCs as a proxy. Consequently temperature reconstructions from $\delta^{18}O$ *Chironomus* spp. HCs in Australia are applicable only to areas such as Tasmania and the higher altitude areas of the south-eastern mainland as long as these lakes remain in a humid climate. The correlation between $\delta^{18}O$ *Chironomus* spp. HCs and temperature will be affected if climate zones have shifted in the past (e.g. from the LGM to the Holocene). This is an important aspect to consider when applying this proxy for paleoclimate reconstructions.

The δ^2H of precipitation, lake water, *Chironomus* spp. HCs and air temperature

Our analyses of lake water δ^2H revealed a close correlation with δ^2H of precipitation (Fig. 3a) and the enrichment of δ^2H in lake water was not as strongly affected by local evaporation and residence time as the $\delta^{18}O$ results (Fig. 3a). The δ^2H values of *Chironomus* spp. HCs and lake water showed a moderately strong correlation (r = 0.83) (Fig. 3b), which suggests a large proportion of *Chironomus* spp. HC δ^2H was derived from or exchanged with lake water. At first glance, this observation does not appear to agree with the laboratory culturing experiments of Wang et al. (2009) and Belle et al. (2015). However, *Chironomus* mainly feeds on algal detritus, where the algae contain δ^2H values derived from lake water (Zhang and Sachs 2007). A similar correlation between lake water δ^2H and δ^2H of Bryozoa was also observed by van Hardenbroek et al. (2016). In addition, the inter-specific dietary variability has been reduced in our study as the chironomids samples were from a single genus.

The $\alpha_{[\delta2H(Chironomus\ spp.-water)]}$ values of δ^2H of *Chironomus* spp. HCs and lake water follow a conductivity (COND) gradient (Fig. 3d) suggesting that conductivity is a key factor in understanding the relationship between δ^2H of *Chironomus* spp. HCs and δ^2H of lake water. However, without further laboratory experiments and more testing, we cannot provide an insight of how conductivity affects the fractionation process. In freshwater lakes (COND < ~ 500 μs cm^{-1}, i.e. ln(COND) < 6, Behar 1997) that support diverse aquatic life, MAT and COND co-varied (ESM 6) and there are potentially three reasons for this observation. First, lake order (i.e. the landscape position of lakes) effect may be driving the salinity gradient (Riera et al. 2000) where high altitude (cooler temperature) low order lakes are often dilute (e.g. Mt Kosciusko lakes, Fig. 1) and lowland (warmer temperature) high order lakes have higher salinity. Secondly, when COND is >500 μs cm^{-1} (e.g. most of the Victorian lakes, ESM 1), the changes in COND are driven by catchment hydrology, i.e. residence time, which affects the evaporative enrichment of salts. Finally, the overall pattern is likely due to the fact that regional evaporation is a function of temperature.

In summary, high conductivity in lakes obscures any relationship between δ^2H of *Chironomus* spp. HCs

and MAT but for freshwater lakes there is a strong correlation obtained between δ^2H of *Chironomus* spp. HCs and MAT (Fig. 3c). Since conductivity is closely related to lake eutrophication and salinity (Chang et al. 2014) within the south-eastern Australian dataset, this means that temperature values can only be derived from oligotrophic and mesotrophic lakes. These lakes are often found in the cold temperate and high altitude areas in south-eastern Australia and are the same lakes that are suitable for δ^{18}O temperature estimates.

Conclusions

Stable isotopes (^{18}O and δ^2H) analysed on HCs of the species from chironomid genus *Chironomus* in sixteen south-eastern Australian lakes can be used as proxies for past temperature change. For δ^{18}O, regional aridity and its consequential issues are critical. Eight sub-humid and semi-arid lakes in our dataset have strong evaporative effects, which causes high concentrations of nutrients and salts that create ecological stress for aquatic plants and animals. In these lakes, *Chironomus* spp. HCs and lake water are both δ^{18}O-enriched but their δ^{18}O values are not related. We propose that the possession of haemoglobin by *Chironomus* spp., which is an adaptation to low oxygen conditions, may be an important factor that affects the δ^{18}O values and oxygen isotope fractionation in *Chironomus* spp. HCs in these environments. Consequently, *Chironomus* spp. HC δ^{18}O do not show close correlation with regional temperatures. In contrast, for lakes in humid settings and where nutrient concentrations are low, the relationship between δ^{18}O of *Chironomus* spp. HCs and MAT appears robust.

Although, the δ^2H values obtained on *Chironomus* spp. HCs in this study were not calibrated for the contribution of exchangeable hydrogen and the absolute values are not reliable, the trends in the data are robust and yield a similar story to δ^{18}O. For freshwater lakes, the δ^2H values of *Chironomus* spp. HCs showed that it is a promising proxy for MAT reconstructions. δ^2H values of *Chironomus* spp. HCs have the apparent advantage that they appear to be less influenced by vital effects (such as changes in oxygen availability in lakes). The δ^2H values of *Chironomus* spp. HCs are also less strongly affected by evaporative enrichment. Since salinity and nutrient status are closely related in this dataset (Chang et al. 2014), the overall findings of

this paper are that both δ^{18}O and δ^2H are potentially valuable tools for reconstructing temperature in low nutrient lakes in humid parts of Australia (e.g. Tasmania and the south-eastern highlands). Challenges remain for understanding how each of the different in-lake variables and their confounding effects influence the δ^{18}O and δ^2H fractionation from *Chironomus* HCs. Modern calibration studies and culturing experiments would be valuable to disentangle these effects.

References

Barton AB, Herczeg AL, Dalhaus PG, Cox JW (2007) A geo-chemical approach to determining the hydrological regime of wetlands in a volcanic plain, south-eastern Australia. In: Groundwater and ecosystems. IAH Congress, Lisbon, p 8

Behar S (1997) Definition of water quality parameters. Testing the waters: chemical and physical vital signs of a river. River Watch Network, Montpelier

Belle S, Verneaux V, Millet L, Parent C, Magny M (2015) A case study of the past CH4 cycle in lakes by the combined use of dual isotopes (carbon and hydrogen) and ancient DNA of methane-oxidizing bacteria: rearing experiment and application to Lake Remoray (eastern France). Aquat Ecol 49:279–291

Borcard D, Legendre P, Drapeau P (1992) Partialling out the spatial component of ecological variation. Ecology 73:1045–1055

Bowen GJ, Revenaugh J (2003) Interpolating the isotopic composition of modern meteoric precipitation. Water Resour Res 39:1299–1312

Brodersen KP, Pedersen O, Lindegaard C, Hamburger K (2004) Chironomids (Diptera) and oxy-regulatory capacity: an experimental approach to paleolimnological interpretation. Limnol Oceanogr 49:1549–1559

Chang JC, Woodward C, Shulmeister J (2014) A snapshot of the limnology of eastern Australian water bodies spanning the tropics to Tasmania: the land-use, climate, limnology nexus. Mar Freshw Res 65:872–883

Chang J, Shulmeister J, Woodward C (2015a) A chironomid based transfer function for reconstructing summer temperatures in south eastern Australia. Palaeogeogr Palaeoclimatol Palaeoecol 423:109–121

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

Chivas AR, De Deckker P, Cali JA, Chapman A, Kiss EG, Shelley JM (1993) Coupled stable isotope and trace element measurements of lacustrine carbonates as paleoclimatic indicators. In: Swart PK, Lohmann KC, Mckenzie J, Savin S (eds) Climate change in continental isotopic records. American Geophysical Union, Washington, DC, pp 113–121

Clark ID, Fritz P (1997) Environmental isotopes in hydrogeology. CRC Press, Boca Raton

Coplen TB (1995) Reporting of stable hydrogen, carbon, and oxygen isotopic abundances. Geothermics 24:707–712

Cranston PS (1995) Introduction. In: Armitage PD, Cranston PS, Pinder LCV (eds) The chironomidae. The biology and ecology of non-biting midges. Chapman and Hall, London, pp 1–7

Deines P, Wooller MJ, Grey J (2009) Unravelling complexities in benthic food webs using a dual stable isotope (hydrogen and carbon) approach. Freshw Biol 54:2243–2251

Eggermont H, Heiri O (2012) The chironomid-temperature relationship: expression in nature and palaeoenvironmental implications. Biol Rev 87:430–456

Erez J (1978) Vital effect on stable-isotope composition seen in foraminifera and coral skeletons. Nature 273:199–202

Farquhar G, O'Leary M, Berry J (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Funct Plant Biol 9:121–137

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537

France RL (1995) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Mar Ecol Prog Ser 124:307–312

Frossard V, Belle S, Verneaux V, Millet L, Magny M (2013) A study of the $\delta^{13}C$ offset between chironomid larvae and their exuvial head capsules: implications for palaeoecology. J Paleolimnol 50:379–386

Galat DL, Coleman M, Robinson R (1988) Experimental effects of elevated salinity on three benthic invertebrates in Pyramid Lake, Nevada. Hydrobiologia 158:133–144

García LV (2004) Escaping the Bonferroni iron claw in ecological studies. Oikos 105:657–663

Gehre M, Geilmann H, Richter J, Werner RA, Brand WA (2004) Continuous flow 2H/1H and 18O/16O analysis of water samples with dual inlet precision. Rapid Commun Mass Spectrom 18:2650–2660

Glew J (1991) Miniature gravity corer for recovering short sediment cores. J Paleolimnol 5:285–287

Goddeeris BR, Vermeulen AC, De Geest E, Jacobs H, Baert B, Ollevier F (2001) Diapause induction in the third and

fourth instar of *Chironomus riparius* (Diptera) from Belgian lowland brooks. Arch Hydrobiol 150:307–327

Granéli W, Solander D (1988) Influence of aquatic macrophytes on phosphorus cycling in lakes. In: Persson G, Jansson M (eds) Phosphorus in freshwater ecosystems. Springer, Berlin, pp 245–266

Grey J, Kelly A, Jones RI (2004a) High intraspecific variability in carbon and nitrogen stable isotope ratios of lake chironomid larvae. Limnol Oceanogr 49:239–240

Grey J, Kelly A, Ward S, Sommerwerk N, Jones RI (2004b) Seasonal changes in the stable isotope values of lake-dwelling chironomid larvae in relation to feeding and life cycle variability. Freshw Biol 49:681–689

Gröcke DR, Schimmelmann A, Elias S, Miller RF (2006) Stable hydrogen-isotope ratios in beetle chitin: preliminary European data and re-interpretation of North American data. Quat Sci Rev 25:1850–1864

Heiri O, Schilder J, van Hardenbroek M (2012) Stable isotopic analysis of fossil chironomids as an approach to environmental reconstruction: state of development and future challenges. Fauna Nor S.l 31:7

Henderson AK, Shuman BN (2009) Hydrogen and oxygen isotopic compositions of lake water in the western United States. Geol Soc Am Bull 121:1179–1189

IAEA/WMO (2015) Global network of isotopes in precipitation. The GNIP database. http://www.iaea.org/water. Accessed Oct 2015

Jones MD, Cuthbert MO, Leng MJ, McGowan S, Mariethoz G, Arrowsmith C, Sloane HJ, Humphrey KK, Cross I (2016) Comparisons of observed and modelled lake δ18O variability. Quat Sci Rev 31(Part B):329–340

Kendall C, Caldwell EA (1998) Fundamentals of isotope geochemistry. In: Kendall C, McDonnell JJ (eds) Isotope tracers in catchment hydrology. Elsevier, Amsterdam, pp 51–86

Larocque I, Hall RI, Grahn E (2001) Chironomids as indicators of climate change: a 100-lake training set from a subarctic region of northern Sweden (Lapland). J Paleolimnol 26:307–322

LaZerte BD, Sxulados JE (1982) Stable carbon isotope ratio of submerged freshwater macrophyte. Limnol Oceanogr 27:413–418

Leng MJ, Marshall JD (2004) Palaeoclimate interpretation of stable isotope data from lake sediment archives. Quat Sci Rev 23:811–831

Leng MJ, Lamb AL, Heaton TH, Marshall JD, Wolfe BB, Jones MD, Holmes JA, Arrowsmith C (2006) In: Leng MJ (ed) Isotopes in lake sediments. Isotopes in palaeoenvironmental research. Springer, Berlin, pp 147–184

Lotter AF, Walker IR, Brooks SJ, Hofmann W (1999) An intercontinental comparison of chironomid palaeotemperature inference models: Europe vs North America. Quat Sci Rev 18:717–735

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

Mayr C, Laprida C, Lücke A, Martín RS, Massaferro J, Ramón-Mercau J, Wissel H (2015) Oxygen isotope ratios of chironomids, aquatic macrophytes and ostracods for lake-water isotopic reconstructions—results of a calibration study in Patagonia. J Hydrol 529(Part 2):600–607

Nielson KE, Bowen GJ (2010) Hydrogen and oxygen in brine shrimp chitin reflect environmental water and dietary isotopic composition. Geochim Cosmochim Acta 74:1812–1822

Osmulski P, Leyko W (1986) Structure, function and physiological role of chironomus haemoglobin. Comp Biochem Physiol Part B: Biochem Mol Biol 85:701–722

Rees ABH, Cwynar LC (2010) Evidence for early postglacial warming in Mount Field National Park, Tasmania. Quat Sci Rev 29:443–454

Rees AH, Cwynar L, Cranston P (2008) Midges (Chironomidae, Ceratopogonidae, Chaoboridae) as a temperature proxy: a training set from Tasmania, Australia. J Paleolimnol 40:1159–1178

Riera JL, Magnuson JJ, Kratz TK, Webster KE (2000) A geomorphic template for the analysis of lake districts applied to the Northern Highland Lake District, Wisconsin, U.S.A. Freshw Biol 43:301–318

Rozanski K, Araguás-Araguás L, Gonfiantini R (1993) Isotopic patterns in modern global precipitation. Geophys Monogr Ser 78:1–36

Samartin S, Heiri O, Vescovi E, Brooks SJ, Tinner W (2012) Lateglacial and early Holocene summer temperatures in the southern Swiss Alps reconstructed using fossil chironomids. J Quat Sci 27:279–289

Schilder J, Tellenbach C, Möst M, Spaak P, van Hardenbroek M, Wooller MJ, Heiri O (2015) The stable isotopic composition of Daphnia ephippia reflects changes in $\delta^{13}C$ and $\delta^{18}O$ values of food and water. Biogeosciences 12:3819–3830

Schimmelmann A, Miller RF, Leavitt SW (1993) Hydrogen isotopic exchange and stable isotope ratios in cellulose, wood, chitin, and amino compounds. In: Swart PK, Lohmann KC, Mckenzie J, Savin S (eds) Climate change in continental isotopic records. American Geophysical Union, Washington, DC, pp 367–379

Soto DX, Wassenaar LI, Hobson KA (2013) Stable hydrogen and oxygen isotopes in aquatic food webs are tracers of diet and provenance. Funct Ecol 27:535–543

ter Braak C, Šmilauer P (2002) CANOCO reference manual and CanoDraw for windows user's guide: software for canonical community ordination (version 4.5), Microcomputer Power, Itaca. www.canoco.com

Teranes JL, McKenzie JA, Lotter AF, Sturm M (1999) Stable isotope response to lake eutrophication: calibration of a high-resolution lacustrine sequence from Baldeggersee, Switzerland. Limnol Oceanogr 44:320–333

van Hardenbroek M, Heiri O, Grey J, Bodelier PLE, Verbruggen F, Lotter AF (2010) Fossil chironomid $\delta^{13}C$ as a proxy for past methanogenic contribution to benthic food webs in lakes? J Paleolimnol 43:235–245

van Hardenbroek M, Gröcke D, Sauer P, Elias S (2012) North American transect of stable hydrogen and oxygen isotopes in water beetles from a museum collection. J Paleolimnol 48:461–470

van Hardenbroek M, Lotter AF, Bastviken D, Andersen TJ, Heiri O (2014) Taxon-specific $\delta^{13}C$ analysis of chitinous invertebrate remains in sediments from Strandsjön, Sweden. J Paleolimnol 52:95–105

van Hardenbroek M, Leuenberger M, Hartikainen H, Okamura B, Heiri O (2016) Bryozoan stable carbon and hydrogen isotopes: relationships between the isotopic composition of zooids, statoblasts and lake water. Hydrobiologia 765:209–223

Verbruggen F, Heiri O, Reichart GJ, De Leeuw JW, Nierop KGJ, Lotter AF (2010a) Effects of chemical pretreatments on $\delta^{18}O$ measurements, chemical composition, and morphology of chironomid head capsules. J Paleolimnol 43:857–872

Verbruggen F, Heiri O, Reichart GJ, Lotter AF (2010b) Chironomid $\delta^{18}O$ as a proxy for past lake water $\delta^{18}O$: a Lateglacial record from Rotsee (Switzerland). Quat Sci Rev 29:2271–2279

Verbruggen F, Heiri O, Reichart GJ, Blaga C, Lotter AF (2011) Stable oxygen isotopes in chironomid and cladoceran remains as indicators for lake-water $\delta^{18}O$. Limnol Oceanogr 56:2071–2079

von Grafenstein U, Erlernkeuser H, Trimborn P (1999) Oxygen and carbon isotopes in modern fresh-water ostracod valves: assessing vital offsets and autecological effects of interest for palaeoclimate studies. Palaeogeogr Palaeoclimatol Palaeoecol 148:133–152

Walker IR (1987) Chironomidae (Diptera) in paleoecology. Quat Sci Rev 6:29–40

Walker IR, Cwynar LC (2006) Midges and palaeotemperature reconstruction—the North American experience. Quat Sci Rev 25:1911–1925

Wang Y, Francis DR, O'Brien DM, Wooller MJ (2008) A protocol for preparing subfossil chironomid head capsules (Diptera: Chironomidae) for stable isotope analysis in paleoclimate reconstruction and considerations of contamination sources. J Paleolimnol 40:771–781

Wang YV, O'Brien DM, Jenson J, Francis D, Wooller MJ (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

Wassenaar LI, Hobson KA (2003) Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. Isot Environ Health Stud 39:211–217

Whiticar MJ (1999) Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. Chem Geol 161:291–314

Woodward CA, Shulmeister J (2006) New Zealand chironomids as proxies for human-induced and natural environmental change: transfer functions for temperature and lake production (chlorophyll a). J Paleolimnol 36:407–429

Woodward CA, Shulmeister J (2007) Chironomid-based reconstructions of summer air temperature from lake deposits in Lyndon Stream, New Zealand spanning the MIS 3/2 transition. Quat Sci Rev 27:142–154

Wooller MJ, Francis D, Fogel ML, Miller U, Walker IR, Wolfe AP (2004) Quantitative paleotemperature estimates from $\delta^{18}O$ of chironomid head capsules preserved in arctic lake sediments. J Paleolimnol 31:267–274

Wooller M, Wang YM, Axford Y (2008) A multiple stable isotope record of Late Quaternary limnological changes and

chironomid paleoecology from northeastern Iceland. J Paleolimnol 40:63–77

Zhang Z, Sachs JP (2007) Hydrogen isotope fractionation in freshwater algae: I. Variations among lipids and species. Org Geochem 38:582–608

Ziveri P, Stoll H, Probert I, Klaas C, Geisen M, Ganssen G, Young J (2003) Stable isotope 'vital effects' in coccolith calcite. Earth Planet Sci Lett 210:137–149