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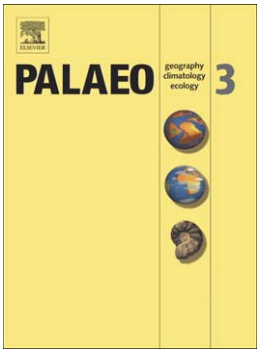

A chironomid based transfer function for reconstructing summer temperatures in south eastern Australia

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A chironomid based transfer function for reconstructing summer temperatures in south eastern Australia

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Abstract

We present a new chironomid based temperature transfer function which was developed from a training set of 33 natural and artificial lakes from southeast Australia from subtropical Queensland to cool temperate Tasmania. Multivariate statistical analyses (CCA, pCCA) were used to study the distribution of chironomids in relation to the environmental and climatic variables. Seven out of eighteen available variables were significantly ($P < 0.05$) related to chironomid species variation and these were mean February temperature (9.5%), pH (9.5%), specific conductance (8.2%), total phosphorous (8%), potential evapotranspiration (8%), chlorophyll *a* (6.9%) and water depth (6.2%). Further pCCA analyses show that mean February temperature (MFT) is the most robust and independent variable explaining chironomid species variation. The best MFT transfer function was a partial least squares (PLS) model with a coefficient of determination ($r^2_{Jackknifed}$) of 0.69, a root mean squared error of prediction (RMSEP) of 2.32˚C, and maximum bias of 2.15°C. Chironomid assemblages from actively managed reservoirs appear to match assemblages from equivalent natural lakes in similar climates and therefore can be included in the development of the chironomid transfer function. Although we cannot completely rule out some degree of endemism in the Tasmanian chironomid fauna, our analyses show that the degree of endemism is greatly reduced. Therefore, integrating the existing chironomid transfer function for Tasmania (Rees et al. 2008) with this new model is a real possibility.

1. Introduction

In temperate Australia, high-resolution continuous palaeoenvironmental records are scarce due to the relative paucity of permanent water bodies (Chang et al. 2014). As a result, palaeoenvironmental research is focussed on a few regions where these records exist (Petherick et al. 2011). Continuous records that extend back to the last glaciation maximum (LGM: c. 21,000 yrs ago) are rare and geographical coverage is poor (Petherick et al. 2013). Pollen is the most widely used proxy for these palaeoenvironmental reconstructions, and most of the reconstructions of climate in Australia are qualitative. This has limited the climate inferences that can be made from these records, which is unfortunate, as there are few estimates of the absolute scale of temperature change between glacial and interglacial times. Bioclimatic modelling has been used with some success (D'Costa and Kershaw 1997) but the technique does not allow biotic effects to be easily separated from climate change (Jeschke and Strayer 2008). Statistical approaches using pollen transfer functions have been developed for mainland Australia (Cook and Van der Kaars 2006) and for Tasmania (Fletcher and Thomas 2010). The former has not been widely applied and the latter is appropriate only to Tasmania. Transfer functions have also been developed using other organisms, such as diatoms (Tibby 2004; Tibby and Haberle 2007), but diatoms are used primarily for salinity and other limnological variables rather than temperature estimates. Molluscs (Edney et al. 1990; D'Costa et al. 1993) and beetles (Porch et al. 2009; Sniderman et al. 2009) have also been applied but a critical gap remains in the tools that are available to determine past changes in temperature in Australia.

Chironomids (Diptera: Chironomidae) have been widely used as a proxy in palaeoclimate and palaeoenvironmental studies (Walker and Paterson 1985; Hofmann 1986; Walker 1987). Since temperature is a dominant factor in every aspect of the chironomid life cycle (e.g. egg hatching, larval and pupal development, adult emergence), many studies have

focused on the influence of temperature on the distribution and abundance of chironomids, in the context of past climate change (Walker 2002; Porinchu and MacDonald 2003; Walker and Cwynar 2006).

Most chironomid-based reconstructions have been carried out in temperate and sub-polar regions of the Northern Hemisphere, including areas in central and northern Europe (Olander et al. 1999; Larocque et al. 2001; Brodersen and Anderson 2002; Luoto 2009; Heiri et al. 2011) and northern North America (Porinchu et al. 2002; Barley et al. 2006; Porinchu et al. 2009; Brodersen et al. 2008; Medeiros and Quinlan 2011). There are only few applications in the Southern Hemisphere and these are from New Zealand (Woodward and Shulmeister 2006; Dieffenbacher-Krall et al. 2007), Tasmania (Rees et al. 2008), east Africa (Eggermont et al. 2010) and South America (Massaferro and Larocque-Tobler 2013).

Early palaeoecological and palaeoclimate investigations of chironomids from the Southern Hemisphere were restricted to semi-quantitative interpretations because the numerical techniques for creating transfer functions were still in an early stage of development. For example, Schakau (1993) investigated the modern chironomid distribution from New Zealand lakes using cluster analysis and ordinations. Classification techniques were applied down-core to interpret the fluctuations in fossil chironomid abundances and species from a 6,000 year record from Lake Grasmere in New Zealand (Schakau 1991) and a glacial transition to Holocene record from Blue Lake in alpine Mount Kosciusko (Fig. 1), Australia.

Dimitriadis and Cranston (2001) performed the first quantitative reconstruction based on chironomids from eastern Australian lakes. Instead of using chironomid head capsules in the training set, they used the presence and abundance of chironomid exuviae from 68 water bodies in eastern Australia. Dimitriadis and Cranston (2001) then used the mutual-climate-ranges (MCRs) of the pupal exuviae in the training set to create a Holocene climate reconstruction from Lake Barrine (Atherton Tableland, northeast Queensland, Fig. 1) based on

the down-core chironomid head-capsule record. They inferred up to 6˚C temperature change in the Holocene.

The first Southern Hemisphere transfer function based on chironomid head capsules was developed by Woodward and Shulmeister (2006) for New Zealand. Both summer temperature and chlorophyll *a* (Chl *a*) transfer functions were presented. The summer temperature transfer function was successfully applied to a record spanning the marine oxygen isotope stage 3/2 transition (~26,600 - 24,500 cal yr BP) from lake deposits in Lyndon Stream, New Zealand (Woodward and Shulmeister 2007). A second independent chironomid model for New Zealand was produced by Dieffenbacher-Krall et al. (2007), which also yields satisfactory reconstructions.

In Australia, a head capsule based chironomid transfer function was developed by Rees et al. (2008) for summer temperature from Tasmanian lakes. This transfer function was applied to produce late-glacial (~16,000 cal yr BP) to late Holocene summer temperature reconstructions from Eagle Tarn and Platypus Tarn in Mount Field National Park, Tasmania (Fig. 1) (Rees and Cwynar 2010a). Although the Tasmanian chironomid transfer function appears to be robust, biogeographical controls on the Tasmanian chironomid taxa may prevent the application of the Tasmanian transfer function to mainland sites.

Concerns over the influence of biogeography on Tasmanian and mainland Australian chironomid taxa stem from a chironomid exuviae survey of eastern Australian lakes by Wright and Burgin (2007). Wright and Burgin argue for the presence of 23 endemic taxa from Tasmania, including 5 genera, 4 species and 14 undescribed morpho-species. Despite this assertion, Wright and Burgin's study does not completely rule out the possibility of applying the Tasmanian transfer function to the mainland or producing a combined mainland and Tasmanian transfer function. Wright and Burgin (2007) did not include mainland alpine lakes in their study and the degree of Tasmanian endemism may be over-estimated. The level of

taxonomic resolution provided by exuviae is typically higher than for chironomid head capsules. Even if there are endemic chironomid species in Tasmania, their presence might not dramatically affect the composition of sub-fossil chironomid head capsule assemblages.

Here we present a temperature transfer function based on sub-fossil assemblages of Chironomidae (non-biting midges), from 33 southeast Australian lakes. We included 7 lakes from Tasmania in this total as an initial test of the feasibility of producing a chironomid training set combining both mainland and Tasmanian lakes. We assessed Wright and Burgin's argument for Tasmanian chironomid endemism using our new training set and published information on the distribution of Wright and Burgin's endemic taxa. A combined training set would be desirable because it is difficult to find non-impacted, permanent freshwater lakes spread continuously along a long temperature gradient on mainland Australia. Due to the rarity of freshwater bodies on the mainland, we also investigated the possibility of including artificial water-bodies in the training set.

2. Materials and methods

2.1 Study sites

This data set comprises 25 natural lakes and 8 artificial water bodies located in the south-east Australia (Table 1, Fig. 1). The transect covers a distance of 2500 km along the east coast of Australia from Kureelpa, Queensland to Mount Field National Park, Tasmania (25.96˚S to 42.67˚S, 140.18˚E to 153.26˚E) (Table 1, Fig. 1). The climate ranges from sub-tropical in the north, to cool temperate in the south, and hence there are large temperature and precipitation gradients in the data set. Elevation of the sites ranges from sea level to ~ 2000 m above mean sea level (a.s.l) (Table 1a), corresponding to estimated mean February air

temperatures (MFT) of 10.7–24.7˚C (Table 1a). A detailed description of the climate, vegetation and geology of the study area is provided in Chang et al (2014).

2.2 Chironomid collection and analysis

The lakes were sampled during the Southern Hemisphere summer (January or February) of 2012 and 2013 (Fig. 1 and Table 1). Lakes were selected along an altitudinal and latitudinal range to ensure a long temperature gradient. Where possible we sampled shallow to medium depth (between 1-10 m) lakes to ensure a close relationship between bottom water temperature and air temperature. The sampling of very deep (> 30 m), stratified lakes was avoided to eliminate the effect of hypolimnetic anoxia on the chironomid species assemblages (Little and Smol 2001). A minimum of three sediment cores were collected using a Glew Mini Corer (Glew 1991) at the deepest point or lake centre where bathymetry was not available. The top 2 cm of each core were extruded on site and packaged at 0.5 cm intervals in Whirlpak® sample bags. Sediment samples were refrigerated prior to analysis.

Sediment samples were processed for chironomid analysis following the method outlined in Hofmann (1986) with the following modification. Samples were deflocculated in warm 10% KOH for 20 minutes and washed on a 90 µm mesh with distilled water. Samples were transferred to a Bogorov counting tray and examined under a dissection microscope at 50 × magnification. Chironomid head capsules were hand-picked using fine forceps onto a glass slide, until a minimum of 100 head capsules were obtained (when possible). Chironomid head-capsules were mounted on glass slides in a drop of Euparal® and covered with a glass coverslip. Head-capsules were mounted ventral side up to assist identification. Chironomid species were identified using a compound light microscope at 400 × magnification, following

the published identification guides by Cranston (2000a, 2010), Brooks et al. (2007) and Dieffenbacher-Krall et al. (2008).

Several studies have examined the minimum number of chironomid head capsules that should be extracted to provide representative samples. Larocque (2001) found that 50 head capsules is the minimum required to provide an accurate temperature estimate, but counts 90 head capsules or above will give much better representation of taxa in the assemblage. Quinlan and Smol (2001) concurred with this finding, whereas Heiri and Lotter (2001) argued that the minimum count size is model and location dependent. We therefore used rarefaction analysis in R (version 2.11.1, R Development Core Team 2010) and the Vegan package (version 2.0-10, Oksanen et al. 2013) to test how representative different sample sizes were in our training set. A plot of observed species richness vs predicted species richness was derived using the 'estimateR' function which uses Chao's method (Chao 1987) to estimate actual richness. We also created multiple rarefaction curves for each site based on multiple random sub-samples from the full species pool. This allowed us to visualise the effect of simulated increased sampling intensity on the observed species richness.

2.3 Lake water chemistry and environmental variables

Water samples for chemical analyses were collected from 30 cm below the water surface at the location where the core samples were taken. Untreated water samples were collected for the analysis of major ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, HCO_3^-, Cl^-, SO_4^{2-}) (Table 1b) and total nitrogen/total phosphorous (TN/TP) analysis. These samples were kept frozen until analysis. A 1000 ml water sample was filtered for Chl *a* through a 4.7 cm diameter GF/F filter (0.45 µm pore size). The Chl *a* filter was wrapped in foil and kept frozen for subsequent analysis. The 125 ml water sample was filtered using a syringe and a Whatman® syringe filter

(Supor® Membrane 0.45 µm pore size) and the filtered water was frozen in the bottle for later analysis for dissolved reactive phosphorus (DRP) and reactive nitrogen (NO_X). Total dissolved solids (TDS), pH, specific conductance (COND) and turbidity (TURB) were also obtained from water chemistry analyses which was carried out by the Forensic and Scientific Services, in Brisbane, Queensland. In the field, water temperature, oxidation reduction potential (ORP), pH, dissolved oxygen (DO), specific conductance (COND), total dissolved solids (TDS), salinity (SAL), turbidity (TURB) were recorded from 30 cm below the water surface using an Aquaread® multi-parameter meter. Lake depth at the sampling point was measured using a Speedtech® portable depth sounder.

Climate variables were obtained using the combination of the WorldClim program (available from http://www.worldclim.org/bioclim, accessed 20 August 2013) and ArcGIS 10.1. Worldclim data for Australia is based on climate surfaces 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, mean February temperature (MFT), mean annual temperature (MAT) and mean annual precipitation (Precip) were considered. The potential evapotranspiration (PET) values were obtained from the Global Potential Evapo-Transpiration (Global-PET) and Global Aridity Index (Global-Aridity) data set (CGIAR-CSI, available from http://www.cgiar-csi.org/data/global-aridity-and-pet-database, accessed 20 August 2013).

2.4 Statistical analyses

2.4.1 Test for Tasmanian endemism and difference between natural and artificial lakes

A principal components analysis (PCA) of the chironomid taxa data were run for the 33 lakes. We tested to determine if the chironomid assemblages were significantly different in mainland lakes compared to Tasmanian lakes to look for potential biogeographical effects. We also tested for significant differences between natural and artificial lakes. The distribution of both sets PCA axis scores were assessed for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965) respectively. The data were normally distributed around the mean so that a student t-test was appropriate to test significance of the results.

We first performed a t-test ($\alpha = 0.05$, two sample assuming unequal variances) on sample axis scores from a principal components analysis (PCA) of the chironomid taxa based on head-capsule assemblages for Tasmania vs. mainland lakes. This is not an exhaustive test for endemism as it is based on the taxonomic resolution possible with head capsules. In order to further test for endemism in the Tasmanian chironomid taxa we performed a literature search for records of Wright and Burgin (2007)'s endemic Tasmanian taxa, and searched the Australian National Insect Collection records (Atlas of Living Australia database: http://bie.ala.org.au/species/CHIRONOMIDAE, accessed 20 July 2014). The same t-test was performed to natural vs. artificial lakes.

2.4.2 Selection of environmental variables and model development

Constrained ordinations were performed using CANOCO version 4.5 (ter Braak and Šmilauer 2002) to determine which variable(s) explained a significant proportion of the variation in the chironomid species data. Prior to ordination, climate and lake water chemistry data were assessed for normality in Minitab 16® using the Shapiro–Wilk test (Shapiro and Wilk 1965), and through measurements of skewness and kurtosis (Zar 1999). All variables apart

from MAT, MFT and pH were normalized using a $\log_{10}$ transformation. Chironomid species were used in the form of square root transformed percentage data.

A detrended correspondence analysis (DCA) (with rare taxa down-weighted) of the chironomid data was used to determine whether linear or unimodal methods were appropriate for selecting the best candidates for model construction. The gradient length for DCA axis 1 was 2.067 standard deviation units, which means that a unimodal technique (Canonical correspondence analysis, CCA) is appropriate (Birks 1998). CCAs were run to determine which environmental variables explained the highest and most significant amount of the variation in the chironomid species data. The environmental variable with highest variance inflation factor (VIF) was removed after each CCA and the CCA was repeated until all VIFs were less than 20 (ter Braak and Šmilauer 2002). The ability of the remaining environmental variables to explain a statistically significant amount of the variation in the chironomid species data was determined using a series of CCAs with manual forward selection and a Monte Carlo permutation test (999 unrestricted permutations) (ter Braak and Šmilauer 2002). Variables that were significant ($p \leq 0.05$) were retained for further analyses. To test the strength of the explanatory power of each of the significant variables for the chironomid distribution, a series of partial canonical correspondence analyses (pCCAs) were performed with the remaining significant variables included as co-variables. This step was used to distinguish between indirect and direct relationships between the environmental variables and the chironomid species data. Only environmental variables that retained their significance after this step were considered for transfer function development. CCA bi-plots of sample and species scores were generated using CanoDraw (ter Braak and Šmilauer 2002). Chironomid taxon response curves for significant variables ($p \leq 0.05$) were generated in CanoDraw using Generalized Linear Models (GLMs) with a Poisson distribution.

Transfer functions for the significant environmental variable(s) selected in the CCAs and pCCAs were developed in the computer program C2 (Juggins 2005). A detrended canonical correspondence analysis (DCCA) was used to determine whether chironomids were responding in a linear or unimodal fashion along the gradient of the environmental variable selected for model development (Birks 1995). Leave-one-out, cross-validation was used as this technique is more robust for data sets with fewer than 80 sites (Kim and Han 1997).

Transfer functions were selected based on the performance of the jack-knifed coefficient of determination ($r^2_{jackknifed}$), average bias of jack-knifed predictions ($AveBias_{jack}$), maximum bias of jack-knifed predictions ($MaxBias_{jack}$), and root mean square error of prediction (RMSEP) (Birks 1998). Additional components were only included in the model if the addition of an additional component reduced the RMSEP by at least 5% (Birks 1998).

3. Results

3.1 Chironomid Taxa

The average sample size in the training set is 128 head capsules and only two samples (Chaffey Dam (n = 0) and Lake Cootapatamba (n = 59) produced fewer than 100 head capsules. Counts of this level are generally regarded as reliable (Heiri and Lotter 2001). Rarefaction analysis (Fig. 2) indicated that the sample size for most sites was adequate for including all of the common chironomid species. There is a significant correlation between observed and predicted species richness (Fig. 2a) and only one site (Lake Cootapatamba) produced a low head-capsule count (59) which possibly under-represents the full chironomid species pool. All head capsules from the top 2 cm of the Cootapatamba sediment core were used. The plot of multiple rarefaction curves (Fig. 2b) indicates that the minimum number of

counted head-capsules that is sufficient to capture the actual species richness varies from site to site.

One site (Chaffey Dam) had no head capsules and was removed from the dataset for further analyses. 43 chironomid taxa were identified and counted from the training set. Five rare species were removed from further analyses as they have a maximum abundance of less than 2% and/or occurred in fewer than two lakes (Brooks and Birks 2001).

3.2 Test for Tasmanian endemism and difference between natural and artificial lakes

Chironomid species assemblages for the seven Tasmanian lakes show no significant difference to mainland lakes based on t-test results (Fig. 3a, Supplementary Table 1a). 14 of Wright and Burgin's 23 endemic taxa are undescribed morpho-species (Supplementary Table 2), so it is not possible to assess these taxa for endemism. The remaining 9 endemic taxa comprise 5 genera and 4 species. Head capsules from one of Wright and Burgin's (2007) endemic Tasmanian taxa (*Thienemanniella* sp.) were found on the Australian mainland in Blue Lake and Lake Albina (Mount Kosciuszko), from our training set. Head capsules from Wright and Burgin's (2007) other 4 "endemic" Tasmanian genera (*Apsectrotanypus* sp., Pentaneurini genus E, *Nanocladius* sp., Orthocladiinae "MO5" (Now = *Echinocladius* sp. Cranston)) were not found in our training set but have been previously recorded from mainland sites by Marchant et al (1999), Cranston (2000a), Cranston (2000b), and Krosch (2011) respectively (Supplementary Table 2). Wright and Burgin's (2007) four endemic Tasmanian species (*Botryocladius australoalpinus, B. grapeth, Riethia plumosa, Tanytarsus liepae*) were not identified in the training set, but have been previously recorded from the Australian mainland by Cranston and Edward (1999), Cranston and Edward (1999), Cranston (Australian National

Insect Collection, available from http://bie.ala.org.au/species/CHIRONOMIDAE, accessed 20 July 2014), and Cranston (2000a) respectively (Supplementary Table 2).

The t-test of PCA axis scores shows that there is no significant difference in the chironomid assemblages from natural and artificial water bodies on PCA axis 1, but there is on PCA axis 2 (Fig. 3b and Supplementary Table 1b). PCA axis 2 mainly separates warm stenotherms (low PCA axis 2 scores, e.g. *Dicrotendipes, Kiefferulus, Cladopelma*) from cold stenotherms (high PCA axis 2 scores, e.g. *Parakiefferiella*) (Supplementary Fig. 1a). Warm stenotherms are more common in three high altitude, shallow artificial lakes (not reservoirs) (Highland Waters (LD), Lake Samuel (LS) and Lake Cantani (LCN)) than other high altitude, natural lakes (Supplementary Fig. 1b).

3.3 Selection of environmental variables and model development

Individual ions (Table 1b), precipitation (Precip), and mean annual temperature (MAT) were excluded prior to ordination. Cation and anion gradients are correlated in PCA space and are better represented by specific conductance (see Chang et al. 2014: Supplementary Fig. 1). Rainfall is usually a secondary effect on chironomids species distribution where its effect on chironomids is through influencing or altering the lake water chemistry by dilution and in-lake macrophyte composition and structure through changes in water depth. However, potential evapotranspiration (PET) was included in this dataset since evaporative balance drives salinity and nutrient gradients in sub-humid and semi-arid areas, especially for shallow lakes with endorheic basins (Chang et al. 2014). Chang et al (2014) concluded that PET is a much stronger climate driver for lake water chemistry and nutrient status changes of the east coast Australian waterbodies than rainfall alone. The choice of summer temperatures as a control on chironomids is routine for the alpine lakes and those in more temperate settings. Only two of

our sites (UQ lakes, Lake Poona) are not located in temperate settings as defined by the Köppen-Geiger climate classification (Kottek et al. 2006), so it is reasonable to assume that summer is the prime breeding period for chironomids in this study also.

Total nitrogen (TN) produced the highest VIF in a CCA with all selected environmental variables and chironomid species and it was therefore not considered for further analyses. The remaining seven variables individually accounted for a significant portion ($p \leq 0.05$) of the variance (Fig. 4). In order of explanatory power, these were mean February temperature (9.5%), pH (9.5%), specific conductance (8.2%), total phosphorous (8%), potential evapotranspiration (8%), Chl *a* (6.9%) and water depth (6.2%).

South eastern Australian lakes in this training set cover large productivity and temperature gradients. The first four axes of the CCA constrained by the seven significant environmental variables (Fig. 4) account for 28% of the variance in the chironomid species data (Table 2). Depth, MFT and pH are significantly correlated to the first axis and Depth, TP, pH and specific conductance are correlated with the second axis. Mean February temperature (MFT) shows the strongest correlation with the first axis (Table 2). Total phosphorous (TP) shows the strongest correlation with the second axis (Table 2).

Partial CCA's were then undertaken to determine the direct and indirect effects of each of the seven significant variables. The pCCA results show that lake water depth (DEPTH), nutrient variables (TP, Chl *a*) and specific conductance (COND) are confounded, while potential evapotranspiration (PET) is correlated with specific conductance. pH retained 7.7% of variance explained and remained significant ($p \leq 0.05$) after the pCCAs with all other significant variables partialled out (Table 3). Although MFT and PET appear to be confounded, PET is the dependent variable because evaporation is partially a function of temperature and not vice-versa. In summary, pH and MFT are the two primary independent parameters that

should be considered for model construction. They both explained the largest amount of variance (both 9.5%) (Table 3).

Although pH is also a good candidate for a transfer function, the focus here is on developing a palaeoclimate proxy and MFT is a more useful parameter. The taxon response curves and a plot of chironomid species turnover with respect to temperature (Fig. 5 and Fig. 6) show that the major taxa ($p \leq 0.05$ and $N2 \geq 5$) which are dominant at warm sites are *Polypedilum* spp., *Parachironomus* spp., *Coelopynia pruinosa*., *Paratanytarsus* spp., *Tanytarsus lactescens*. and *Procladius* spp. Taxa that are typical of cool sites include *Paralimnophyes* morphotype 1and *Botryocladius*. However, many taxa may respond to other environmental variables as well (e.g. *Cladopelma, Dicrotendipes* and *Kiefferulus,* see Fig 6 and Table 4). A few taxa such as *Chironomus, Procladius, Pentaneurini* and undifferentiated *Tanytarsini* contain many species each of which is likely to have different environmental responses but cannot be separated from similar morphotypes.

3.4 The transfer function

The DCCA results (gradient length = 1.07 standard deviation units) suggest a linear response of chironomid taxa along the mean February temperature gradient (Birks 1998), therefore, a partial least squares (PLS) model was appropriate for the transfer function construction (ter Braak and Juggins 1993) for MFT (Table 5) in C2 (Juggins 2005). The third component of the PLS model (with 3 components, jack-knifing, including 33 lakes and 38 non-rare species, Table 5) was selected based on the criteria of Birks (1998). It produced a coefficient of determination ($r^2_{\text{Jackknifed}}$) of 0.69, $\text{RMSEP}_{\text{jack}}$ of 2.32˚C, maximum $\text{bias}_{\text{jack}}$ of 2.15˚C and an $\text{AveBias}_{\text{jack}}$ of 0.07 ˚C (Table 5, Fig. 7).

4. Discussion

4.1 Can we include artificial lakes in temperature training sets?

A recent study (Chang et al. 2014) showed that reservoirs and other artificial water bodies respond to stressors in their catchments in a similar fashion to natural lakes. Despite the general preference for natural lakes for temperature training sets, this observation is not unexpected as human impacts that could change water quality in reservoir catchments are generally limited. We might expect chironomid species composition in reservoirs and natural lakes in environmentally equivalent settings to be similar.

However, we also note that for three high elevation artificial lakes (not reservoirs) (LD, LS and LCN), chironomid assemblages resemble lowland eutrophic lakes, with high values of *Cladopelma, Dircrotendipes* and *Kiefferiulus*, (see Supplementary Fig. 1, Fig 6 and Table 4). These three artificial water bodies comprise two nutrient rich trout stocked lakes in Tasmania and a shallow, productive recreation lake on Mt Buffalo (Table 1a). If the two Tasmanian lakes are excluded, the distinction between warm and cold lakes is markedly diminished (Fig 6). On Mt Buffalo, the shallowness of Lake Cantani increases its mean temperature in summer, making it resemble a lower elevation lake due to the increased abundance of *Cladopelma*.

In summary, chironomid assemblages from true reservoirs appear to match assemblages from equivalent natural lakes in similar climates. We should be cautious about including other types of artificial water bodies, especially those where the food web or trophic status might be significantly altered.

4.2 Endemism and other considerations

In our dataset there was no significant difference between the chironomid assemblages from Tasmania and the Australian mainland (Fig. 3a, Supplementary Table 1a) for the taxonomic resolution that is available for sub-fossil head capsules. This test can be considered a first order test of the possibility for combining Tasmanian and mainland training sets. As we will mention below, we did detect a genus in our dataset (*Thienemanniella* sp.) that was previously considered endemic to Tasmania (Wright and Burgin 2007), but this does not fully rule out some degree of Tasmanian endemism. To do this we need to have chironomid distribution records with the same taxonomic resolution that was provided in Wright and Burgin's (2007) study on Australian chironomid biogeography.

Five genera (including *Thienemanniella* sp.) and four species that Wright and Burgin (2007) identified as Tasmanian endemics have actually been reported from the Australian mainland (Supplementary Table 2). This means that the basis for Tasmanian endemism now relies on the presence of 14 undescribed morpho-species in Wright and Burgin's (2007) dataset. It was not possible for us to further test for endemism in records of adult and larval chironomid distributions for morpho-species that are based on exuviae alone. Further collection of exuviae from mainland lakes and rearing of chironomid larvae to possibly associate morpho-species with other life stages of described species is required to rule out endemism. In the absence of this data, alternative methods for testing for endemism would include combining our dataset with Rees et al. (2008) and splitting the pooled dataset into Tasmanian and Mainland sites. Mainland sites can then be used to reconstruct temperatures from Tasmanian sites and vice-versa. This technique has been used to compare trans-Atlantic chironomid datasets (Lotter et al., 1999). At this stage we can only conclude that claims for endemism are greatly diminished, but we do not expect future efforts to combine datasets to be thwarted by endemism.

There is no reason for a high degree of Tasmanian endemism when changes in sea-level over the Quaternary are considered. Tasmania is currently isolated from the mainland by the c. 250 km wide expanse of water known as the Bass Strait. However, during glacial periods, such as the last glacial maximum (LGM, c. 21,000 years ago), global sea level was c. 120 m lower than present so that Tasmania was connected to the mainland. In fact, since Bass Strait is c.75 m deep at its deepest point, it is connected with the mainland during most stadials. Given that the connection between Tasmania and the mainland occurs during cold phases, this would facilitate the dispersal of cold stenotherms from Tasmania north onto the mainland. Wright and Burgin (2007) did not sample the Kosciuszko lakes and these represent the one likely high altitude refugium for cold stenotherms that could have migrated north during past glaciations. From these lines of evidence, we conclude that a transfer function based on summer temperature is feasible for south eastern Australia.

4.3 Reconciliation and integration with Tasmanian transfer function

We recognize that the temperature error in this transfer function is relatively high in comparison to other transfer functions. This reflects the long scalar length of the temperature gradient which extends from sub-tropical to sub-alpine locations, some 14°C. The RMSEP is 2.3°C which represents 16% of the scalar length. This is comparable with the recently developed western Irish chironomid-based calibration set (Potito et al., in press). The Potito et al. (In press) dataset has a RMSEP of 0.57°C, but the temperature range this dataset covers is only 3.8°C; so the error represents 15% of the scalar length. Furthermore, it has been observed that data sets with large temperature gradients naturally have larger errors (Walker and Cwynar 2006) but this in no way diminishes the value of the reconstruction.

The number of water bodies used in the study is small (33), but this is a function of the rarity of lakes on continental SE Australia. There are additional natural lakes to sample but they are exclusively in areas and elevations that we have already sampled. When we established this study we attempted to focus on natural water bodies. It is clear that in order to extend the training set, more reservoirs will need to be included. Even allowing for this, there is a gap between summer temperatures of c. 13.3-14.8˚C for which there are no ideal candidate lakes or reservoirs. There are some reservoirs at these temperatures (e.g. Lake Jindabyne) but they are exceptionally large and deep lakes, and require both alternative sampling strategies and some analyses and consideration before including in the data set. The other alternative is to integrate this model and data set with the Tasmania model and data set of Rees et al (2008). We have deliberately replicated some of the sites (e.g. Eagle Tarn) from Rees et al (2008) so that the models can be compared and harmonised in due course and this is an obvious next step for this research.

4.4 Value of the transfer function

This transfer function has relatively large errors for detecting change during periods of relative climate stability (i.e. in the Holocene). However, on longer time scales, the expected change from Last Glacial Maximum (LGM) to the Holocene in south eastern Australia is between 8 to 10˚C (Galloway 1965; Miller et al. 1997). The precision of the current transfer function is ample to constrain climate change of this magnitude. Transfer functions with relatively large errors may still be able to provide a reliable indication of variation in temperature through time and this should be tested using the method developed by Telford and Birks (2011).

5. Conclusions

We constructed a February mean temperature transfer function based on the modern distribution of Chironomids (Diptera: Chironomidae) species in southeast Australia. The training set comprises 33 natural and artificial lakes in locations that span the subtropics to the alpine zone. The February mean temperature model is statistically robust with an $r^2_{Jackknifed}$ of 0.69, a RMSEP of 2.32˚C and a maximum bias of 2.15 °C. The transfer function is suitable for the reconstruction of summer temperatures during the LGM and the late glacial to Holocene transition in south eastern Australia. In a context where there are few reliable and no continuous estimates of palaeo-temperature available from mainland Australia, the transfer function represents a significant advance for palaeoclimatological studies.

We also conclude that chironomids assemblages in actively managed reservoirs (non-impacted) show no significant difference to natural lakes in the same climate and vegetation zones, and therefore can be included for transfer function development. Although we cannot completely rule out some degree of endemism in the Tasmanian chironomid fauna, our analyses show that the degree of endemism indicated by earlier studies is greatly reduced. This raises the real possibility of integrating the existing chironomid transfer function for Tasmania with this new model for the SE Australian mainland.

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Fig. 1 Map of eastern Australia with the 34 study sites identified, the numbers correspond to the lake names and numbers in Table 1.

Fig. 2 (a) Plot of observed species richness vs predicted species richness (b) Rarefaction curves for individual sites indicating estimated species richness with respect to increasing sub-sample size. Rarefaction curves begin to flatten once true species richness is achieved. Together, these plots indicate that sample sizes are adequate for most samples to capture all but the most rare species. Only one site (CTL) with a low head capsule count may possibly underestimate the true species richness. Minimum sample size varied from site to site and counts as low as 50 may be sufficient to capture the actual species richness (e.g. LPO).

Fig. 3 PCA plots for exploring the difference between (a) mainland and Tasmanian lakes and (b) for natural and artificial lakes. PCA axis 1 and axis 2 explains 16.9% and 10.8% of the variance in chironomid species data respectively. A t-test was performed on the sample score means for each (Supplementary Table 1a and 1b). The sample size for the Tasmania and artificial lakes is small (< 10). There are no significant differences apparent between axis 1 scores for both tests. There are no differences in axis 2 scores either for Tasmania vs mainland lakes, but there is for natural vs artificial lakes. Warm taxa are over-represented in artificial lakes that are not reservoirs (Supplementary Figure 1).

Fig. 4 CCA Biplots of (a) sample and (b) species scores constrained to seven environmental variables that individually explain a significant ($p < 0.05$) proportion of the chironomid species data. Sites codes correspond to site names in Table 1. Taxon numbers correspond to taxa in Table 6. Sites and taxa in warmer environments tend to plot in the upper left quadrant. Taxa typical of warmer sitesinclude *Harnischia* spp., *Cryptochironomus* spp., *Polypedilum* spp., *Coelopynia pruinosa*, *Cladopelma* spp., *Paratanytarsus* spp., *Procladius* spp., *Riethia* spp., while sites and taxa in colder environments tend to plot in the lower right hand quadrant. Taxa typical of cold sites include *Paralimnophyes* morphotype 3, *Parakiefferiella* morphotype 1, *Orthoclad* type 1, *Orthoclad* type 4, *Pseudosmittia 2*, and *Botrycladius*. Eutrophic sites and taxa typical of these environments tend to plot in the lower left hand quadrant, while oligotrophic sites and taxa typical of these environments tend to plot in the upper right hand quadrant.

Fig. 5 Taxon response curves for taxa that show a significant response to temperature ($p < 0.05$) using a generalised linear model with Poisson distribution (ter Braak and Smilauer 2002). (a) Taxa which are more common at lower temperatures, such as *Paralimnophyes* morphotype 1 and *Parakiefferiella* morphotype 2 respond strongly to cooling in temperature (b) Taxa which are more common at higher temperatures demonstrate weaker but still significant responses. Examples include *Procladius*, *Polypedilum* spp. and *Tanytarsus lactescens*.

Fig. 6 Stratigraphy diagram of the 38 non-rare taxa included in the final model, where observed mean February temperature is on the y-axis and taxon abundance is in percentage. Taxa such as *Cladopelma*, *Dicrotendipes* and *Kiefferulus* (*) show high abundance in lowland warm lakes but are also present in highland artificial lakes (Grey bars).

Fig. 7 Performance of the three component PLS model where (a) shows the predicted versus observed mean February temperature and (b) displays residuals of the predicted versus observed mean February temperature. Note that the model has a potential to over predict temperatures from some very shallow high altitude lakes by up to ~6˚C. These lakes have increased mean water temperature in summer and chironomid assemblages may resemble lower elevation sites.

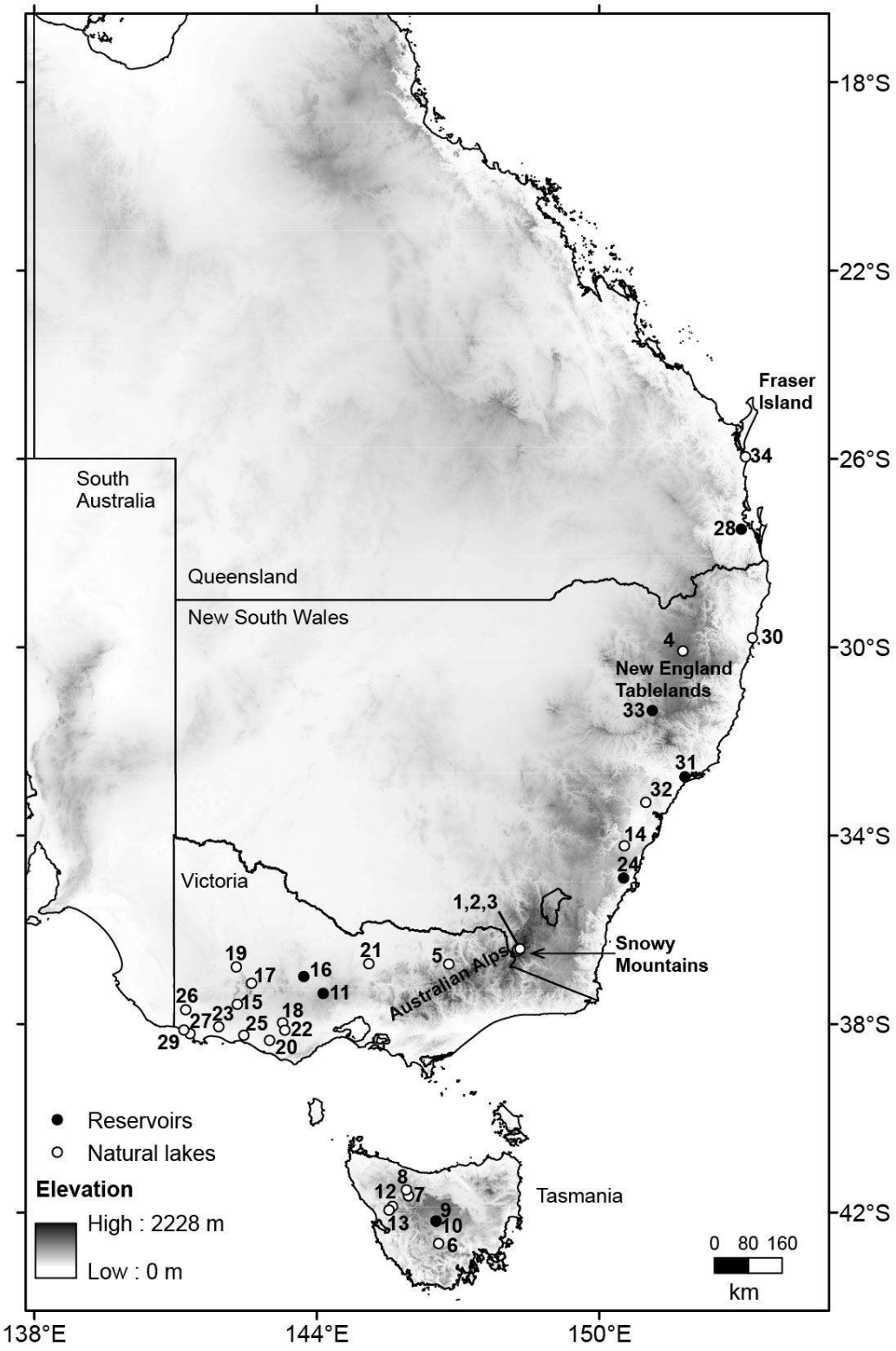

Figure 1

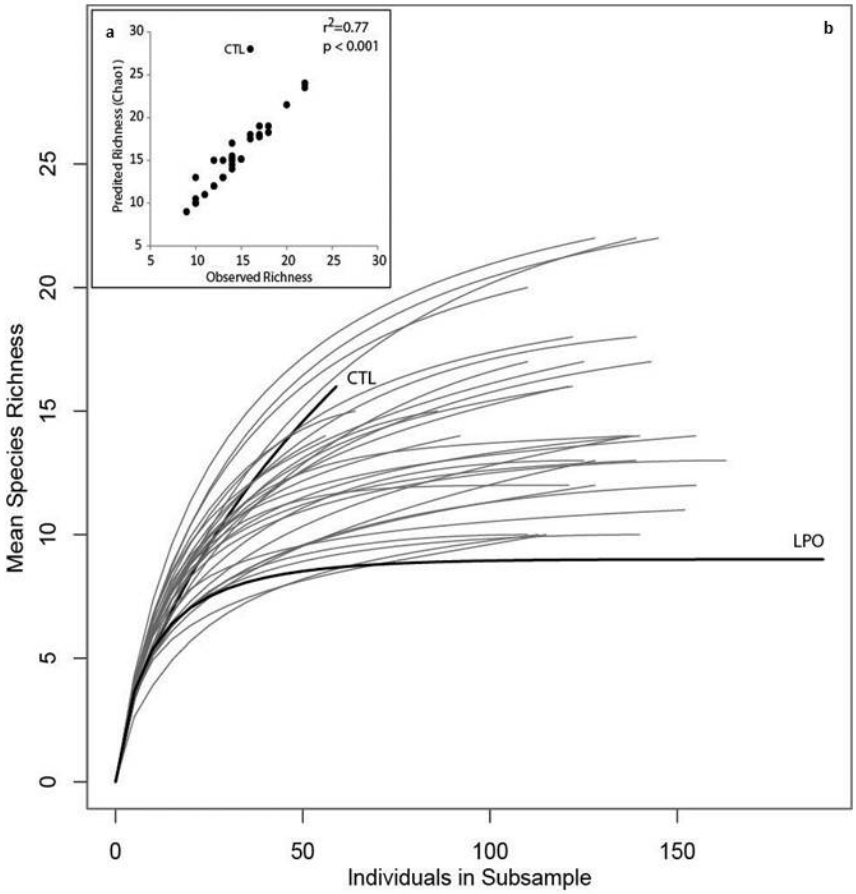

Figure 2

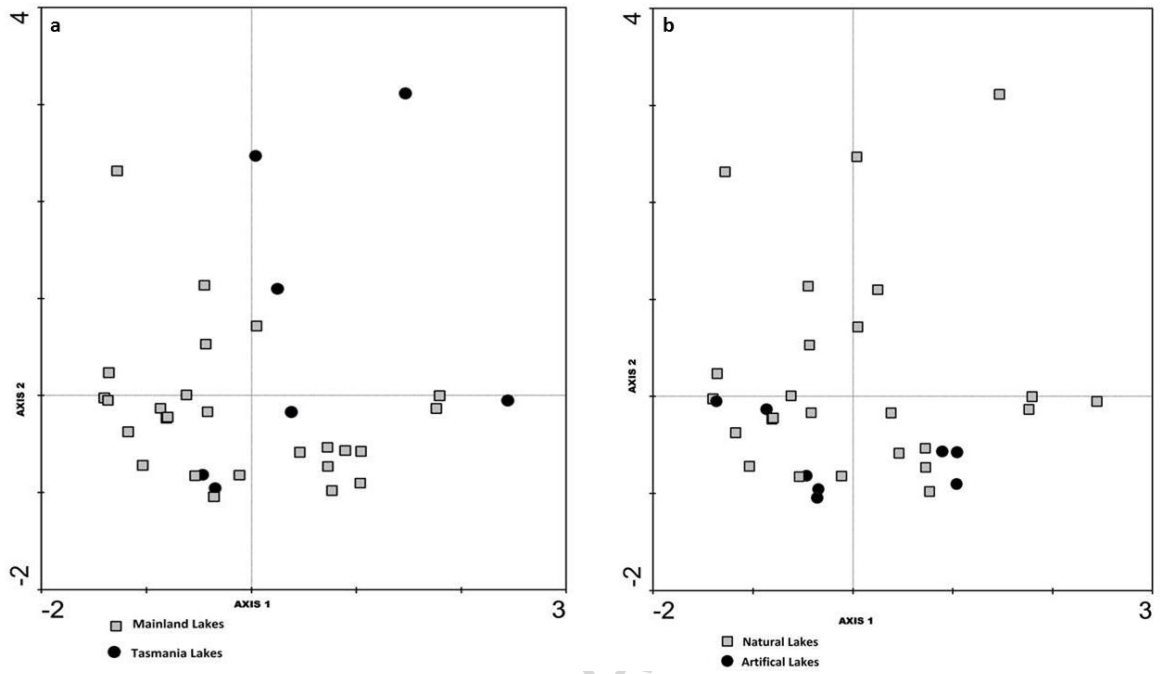

Figure 3

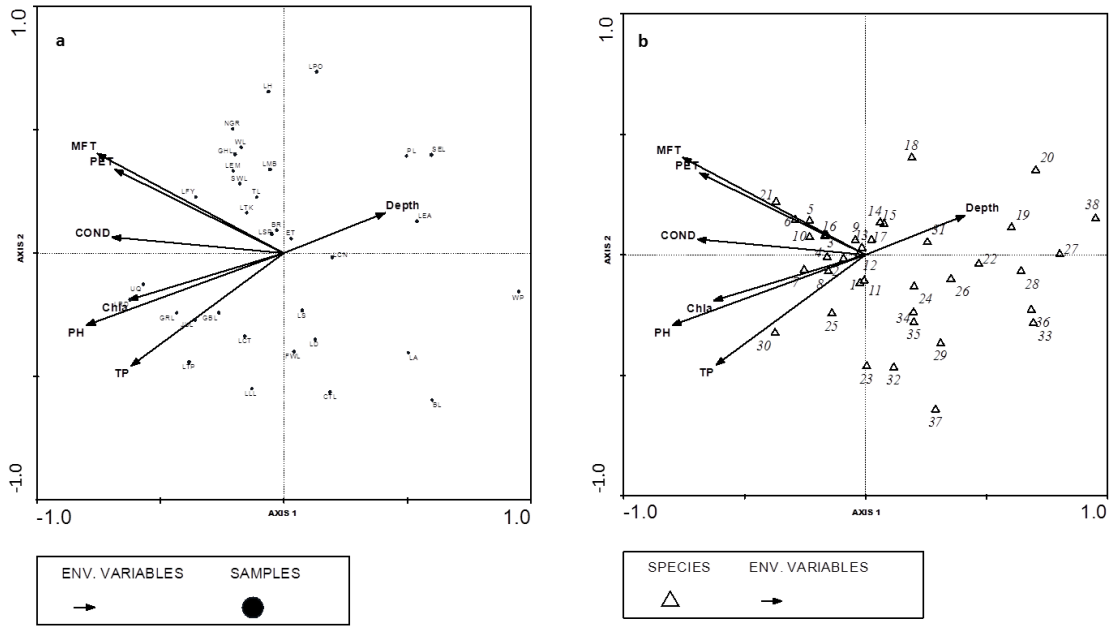

Figure 4

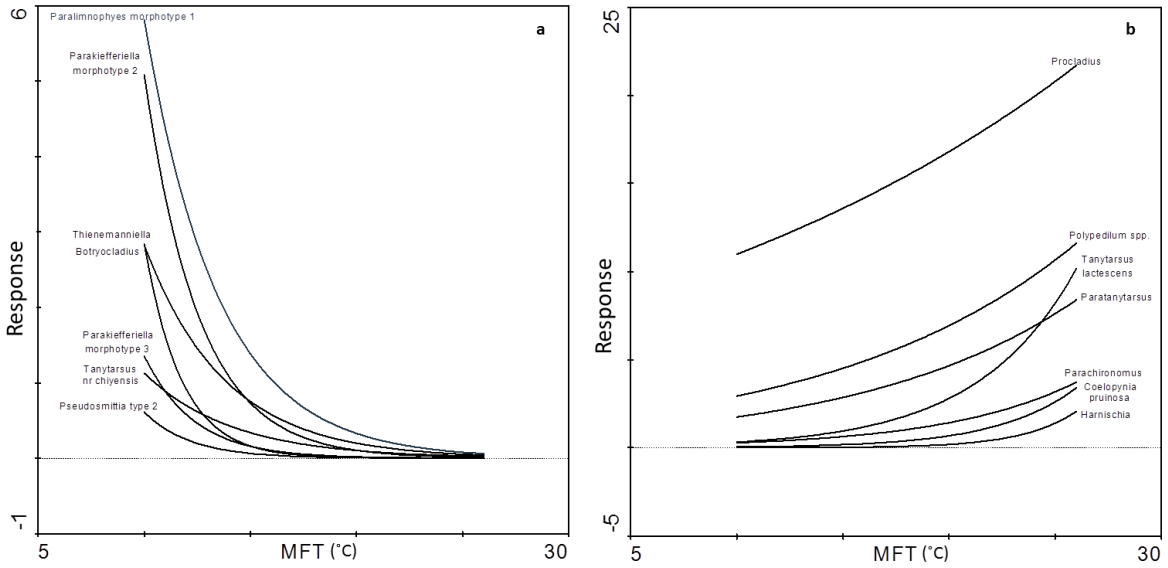

Figure 5

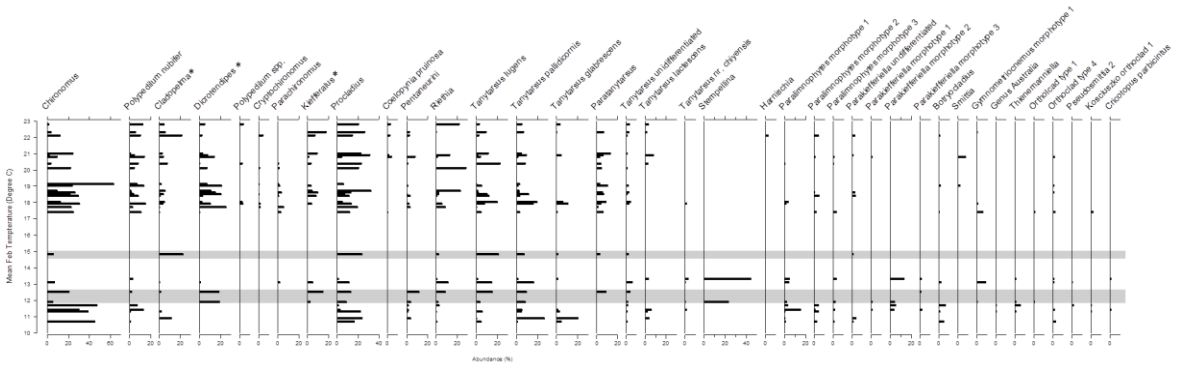

Figure 6

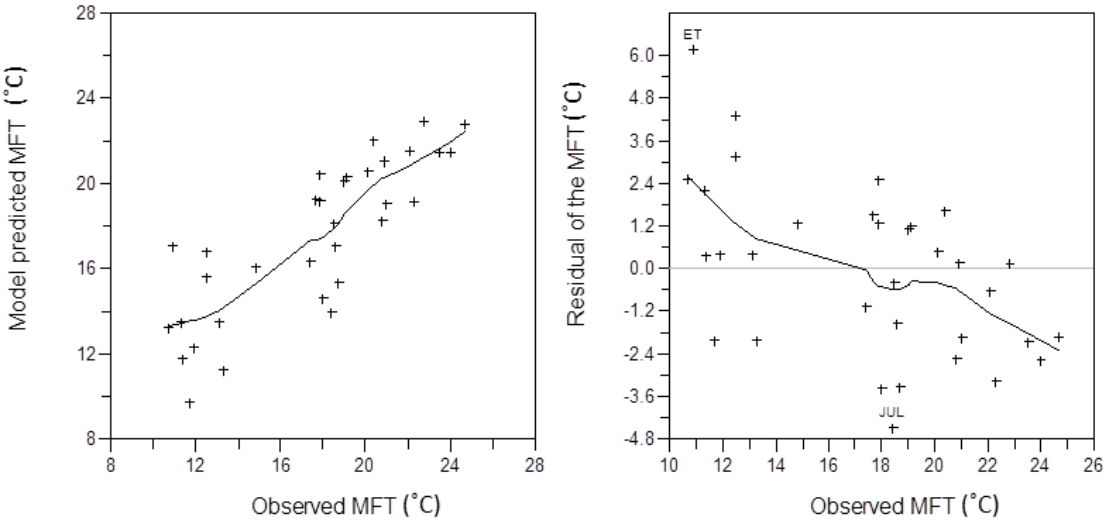

Figure 7

Table 1 (a) Selected climatic and environmental variables for the thirty-four water bodies sampled from Southeast Australia (b) Major ions measurement for the thirty-four water bodies sampled from Southeast Australia

Table 2 CCA summary of the seven significant variables including canonical co-efficients and t-values of the environmental variables with the ordination axes including 33 lakes and 38 non-rare species

Table 3 Partial CCAs of the seven significant ($p \leq 0.05$) environmental variables alone and with the effects of other significant variables partialled out for 33 lakes with 38 non-rare species included.

Table 4 List of Chironomid taxa enumerated in this study along with data on distribution and environmental significance

Table 5 Performance of partial least squares (PLS) model for reconstructing mean February temperature of southeast Australia using 33 lakes and 38 non-rare chironomid species. The bold indicates the model of choice.

Table 1a

No	Lake Name	Code	Coordinates	ALT	Depth	PET	Precip	MFT	MAT	TP	TN	Chl	PH	COND
			Metric	m	m	mm/Year	mm/Year	°C	°C	mg/L	mg/L	µg/L	-	µs/cm
1	Lake Cootaptamba	CTL	S36.46 E148.26	2048	3	673	1691	10.7	3.7	0.011	0.15	1	6.47	5
2	Lake Albina	LA	S36.42 E148.27	1919	9	728	1693	11.3	4.4	0.007	0.12	1	6.6	6
3	Blue Lake	BL	S36.41 E148.32	1901	28	724	1708	11.7	3.9	0.007	0.11	2	6.44	5
4	Little Llangothlin Lagoon	LLL	S30.09 E151.78	1361	3	1142	944	17.4	11.6	0.16	1.5	31	8.11	212
5	Lake Catani	LCN	S36.73 E146.81	1301	1.6	940	1441	14.8	7.9	0.015	0.28	2	6.53	12
6	Eagle Tarn	ET	S42.67 E146.59	1056	0.2	710	1410	10.9	6.3	0.021	0.41	1	7.39	47
7	Lake Lila	WP	S41.65 E145.96	957	13	720	2207	11.4	6.7	0.019	0.29	1	4.91	23
8	Lake Lea Pond	LEA	S41.53 E145.91	837	0.75	1484	2095	11.9	7.4	0.028	0.44	5	4.83	33
9	Lakes Samuel	LS	S42.18 E146.53	766	1.2	755	1010	12.5	7.5	0.024	0.47	8	7.38	68
10	Highland Waters	LD	S42.19 E146.54	756	1.2	1455	1019	12.5	7.6	0.043	0.57	9	7.22	67
11	Jubilee Lake	JUL	S37.35 E144.14	558	1.3	805	877	18.4	11.9	0.11	0.78	5	8.95	194
12	Lake Selina	SEL	S41.88 E145.61	528	7.3	811	2733	13.3	8.9	0.003	0.16	1	5.5	29
13	Lake Plimsoll Pond	PL	S41.96 E145.54	523	1.3	1457	2968	13.1	9	0.01	0.38	6	4.8	44
14	Thirlmere Lakes	TL	S34.22 E150.54	311	1.8	1068	1027	20.4	15	0.051	3.2	65	6.17	249
15	Freshwater Lake	FWL	S37.59 E142.32	227	2.1	802	660	18.6	13.2	0.56	5.1	60	6.74	564
16	Nuggety Gully Resrvoir	NGR	S37.00 E143.73	221	1.2	801	537	21.0	14.1	0.041	1.6	11	7.02	400
17	Lake Fyans	LFY	S37.14 E142.62	219	2.7	1367	595	20.1	13.8	0.019	0.64	3	8.79	206
18	Lake Tooliorook	LTK	S37.98 E143.28	167	3	1539	591	19.1	13.4	0.12	2.2	1	9.17	2470
19	Green Lake	GRL	S36.79 E142.30	149	2.1	1465	492	20.8	14.3	0.18	1.5	16	8.97	553
20	Lake Elingamite	LEM	S38.35 E143.00	147	1.2	1241	891	18.5	13.3	0.11	4.4	22	7.98	6420
21	Reedy Lake	LRD	S36.72 E145.11	143	0.5	1139	577	22.1	14.8	0.52	5.3	80	8.23	959
22	Lake Terangpom	LTP	S38.14 E143.33	133	0.5	1239	649	19.0	13.5	3.6	36	90	8.68	15700
23	Lake Surprise	LSP	S38.06 E141.92	107	6	1208	763	17.9	13.3	0.031	0.88	9	8.08	698
24	Bamerang Reservoir	BR	S34.90 E150.52	103	25	1114	1232	20.9	16.2	0.011	0.3	4	7.7	91
25	Lake Cartcarrong	LCT	S38.24 E142.45	90	1.1	1241	835	17.9	13.4	1.1	11	24	8.33	4700
26	Grubbed Lake	GBL	S37.70 E141.22	78	1.7	1272	721	18.7	13.7	0.12	3.2	36	7.89	345
27	Swan Lake	SWL	S38.21 E141.31	23	1.1	1058	797	17.7	13.7	0.044	1.3	10	7.61	744
28	UQ lake	UQ	S27.50 E153.02	23	1.3	1275	1150	24.7	20.3	0.27	1.1	69	7.5	210
29	Lake Mombeong	LMB	S38.13 E140.18	21	4.8	1096	793	18.0	13.8	0.021	0.79	5	8.03	1140
30	Lake Hiawatha	LH	S29.81 E153.26	20	16.8	1054	1465	23.5	19.1	0.006	0.2	3	6.84	117
31	Grahamstown Lake	GHL	S32.76 E151.82	16	11.4	1144	1124	22.8	17.8	0.013	0.4	4	7.74	197
32	Wellums Lake	WL	S33.30 E150.99	13	3	1021	953	22.3	17.5	0.025	0.56	11	6.75	134
33	Chaffey Dam	LPO	S31.35 E151.12	517	9.7	1501	779	21.8	20.3	0.009	0.44	12	4.7	45
34	Poona Lake	CHD	S25.96 E153.11	158	28	1128	1555	24.0	15.6	0.031	0.46	4	8.3	185

Table 1b

No	Lake Name	Lake	Coordinates	Na+	K+	Ca2+	Mg2+	HCO3-	Cl-	SO42-
				mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
1	Lake Cootaptamba	CTL	S36.46 E148.26	1	0.1	0.1	0.1	7	0.2	1
2	Lake Albina	LA	S36.42 E148.27	1	0.1	0.4	0.1	6	0.1	1
3	Blue Lake	BL	S36.41 E148.32	1	0.1	0.2	0.1	6	0.1	1
4	Little Llangothlin Lagoon	LLL	S30.09 E151.78	9	5	15	11	109	9.1	1
5	Lake Catani	LCN	S36.73 E146.81	2	0.3	0.4	0.2	6	2	1
6	Eagle Tarn	ET	S42.67 E146.59	3	0.1	3.4	1.7	25	4.4	1
7	Lake Lila	WP	S41.65 E145.96	2	0.2	0.3	0.4	2	3.7	1
8	Lake Lea Pond	LEA	S41.53 E145.91	4	0.3	0.2	0.5	2	6.5	1
9	Lakes Samuel	LS	S42.18 E146.53	6	0.1	3.8	2.2	27	8.9	1
10	Highland Waters	LD	S42.19 E146.54	6	0	3.4	2.1	26	9.6	1
11	Jubilee Lake	JUL	S37.35 E144.14	21	1.1	6.2	8.5	81	19	1.2
12	Lake Selina	SEL	S41.88 E145.61	3	0.1	0.6	0.5	3	5.9	1
13	Lake Plimsoll Pond	PL	S41.96 E145.54	5	0.2	0.4	0.7	2	8.9	1
14	Thirlmere Lakes	TL	S34.22 E150.54	32	3.9	1.6	2.9	6	48	28
15	Freshwater Lake	FWL	S37.59 E142.32	77	13	9	12	41	130	22
16	Nuggety Gully Resrvoir	NGR	S37.00 E143.73	44	14	8	9.2	26	100	1
17	Lake Fyans	LFY	S37.14 E142.62	24	2.6	6.4	5.2	49	36	6.3
18	Lake Tooliorook	LTK	S37.98 E143.28	353	11	16	85	124	690	62
19	Green Lake	GRL	S36.79 E142.30	75	9.6	14	12	112	100	13.2
20	Lake Elingamite	LEM	S38.35 E143.00	976	41	65	197	259	2000	223
21	Reedy Lake	LRD	S36.72 E145.11	147	21	22	17	246	170	8.5
22	Lake Terangpom	LTP	S38.14 E143.33	3000	164	10	458	1810	4800	418
23	Lake Surprise	LSP	S38.06 E141.92	85	9.6	18	26	235	100	2.3
24	Bamerang Reservoir	BR	S34.90 E150.52	8	1.1	3.8	2.5	24	12	2.9
25	Lake Cartcarrong	LCT	S38.24 E142.45	705	21	96	185	565	1100	378
26	Grubbed Lake	GBL	S37.70 E141.22	36	13	15	9.3	107	50	1.5
27	Swan Lake	SWL	S38.21 E141.31	85	4.8	40	13	91	150	45
28	UQ lake	UQ	S27.50 E153.02	17	3.2	14	4.7	55	19	13
29	Lake Mombeong	LMB	S38.13 E140.18	143	3.6	46	20	91	280	52
30	Lake Hiawatha	LH	S29.81 E153.26	17	0.8	0.6	1.9	6	28	2.6
31	Grahamstown Lake	GHL	S32.76 E151.82	23	2.5	4.5	4.7	27	32	15
32	Wellums Lake	WL	S33.30 E150.99	18	2.7	1.2	2.6	9	30	3
33	Chaffey Dam	LPO	S31.35 E151.12	8	0.9	0.5	1	2	13	2
34	Poona Lake	CHD	S25.96 E153.11	14	1.6	6.7	11	89	6.4	8.5

Table 2

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalue	0.199	0.109	0.078	0.042
Cum % var. spp.	13.0	20.2	25.3	28.0
Cum % var. spp. - env. relation	38.5	59.7	74.8	83.0
Regression/canonical co-efficient				
Variable				
Depth	0.384	-0.463	0.704	-0.813
MFT	**-0.745**	0.523	0.869	-0.193
PET	0.262	0.230	-0.645	0.336
TP	0.277	**-1.009**	0.002	-1.034
Chla	-0.302	-0.402	0.097	0.432
pH	-0.650	-0.654	0.506	0.804
COND	0.115	0.912	-0.545	-0.991
t-values for regression co-efficients				
FR explained	0.385	0.212	0.151	0.082
Variable				
Depth	2.136	-2.432	3.453	-3.316
MFT	-2.336	1.547	2.401	-0.442
PET	0.824	0.684	-1.787	0.775
TP	0.835	-2.871	0.005	-2.285
Chla	-1.032	-1.297	0.291	1.083
pH	-3.207	-3.045	2.200	2.904
COND	0.443	3.316	-1.850	-2.797

Table 3

Variable	covariable	λ_1	λ_1/λ_2	% Variance explained	P-value
Depth	none	0.095	0.302	6.2	0.016
	MFT	0.100	0.412	7.2	0.002
	PET	0.092	0.347	6.5	0.014
	TP	0.062	0.207	4.4	0.163
	Chla	0.064	0.221	4.5	0.133
	pH	0.077	0.304	5.6	0.031
	COND	0.067	0.240	4.7	0.089
	MFT, PET,TP, Chla, pH, COND	0.071	0.353	6.5	0.034
MFT	None	0.145	0.562	9.5	0.001
	Depth	0.151	0.621	10.5	0.001
	PET	0.062	0.240	4.4	0.150
	TP	0.125	0.508	8.9	0.001
	Chla	0.112	0.446	7.9	0.001
	pH	0.100	0.450	7.2	0.004
	COND	0.095	0.380	6.8	0.007
	Depth, TP, Chla, pH, COND	0.069	0.332	6.2	0.029
	Depth, PET,TP, Chla, pH, COND	0.057	0.284	5.3	0.139
PET	None	0.123	0.449	8.0	0.010
	MFT	0.040	0.155	2.9	0.468
	Depth	0.120	0.453	8.3	0.009
	TP	0.100	0.377	7.1	0.012
	Chla	0.082	0.306	5.8	0.035
	pH	0.081	0.343	5.8	0.044
	COND	0.069	0.261	4.9	0.075
	MFT, COND	0.036	0.145	2.7	0.523
	Depth, MFT, TP, Chla, pH, COND	0.034	0.169	3.2	0.529
TP	None	0.122	0.408	8.0	0.003
	MFT	0.102	0.439	7.4	0.004
	PET	0.099	0.374	7.0	0.009
	Depth	0.089	0.297	6.2	0.025
	Chla	0.058	0.201	4.1	0.173
	pH	0.066	0.261	4.8	0.083
	COND	0.086	0.308	6.1	0.012
	Chla, pH	0.063	0.278	4.8	0.107
	Depth, MFT, PET, Chla, pH, COND	0.046	0.229	4.4	0.282
Chla	none	0.106	0.367	6.9	0.004
	MFT	0.073	0.291	5.3	0.032
	PET	0.066	0.246	4.7	0.080
	Depth	0.075	0.260	5.2	0.050
	TP	0.042	0.145	3.0	0.517
	pH	0.065	0.261	4.7	0.068
	COND	0.046	0.167	3.2	0.456
	Depth, MFT, PET, TP, pH, COND	0.029	0.144	2.8	0.765
pH	None	0.146	0.574	9.5	0.001
	MFT	0.101	0.462	7.3	0.003
	PET	0.105	0.445	7.4	0.004
	Depth	0.129	0.514	9.0	0.002
	TP	0.091	0.360	6.4	0.005
	Chla	0.106	0.426	7.4	0.002
	COND	0.091	0.361	6.4	0.011
	Depth, MFT, PET, TP, Chla, COND	0.085	0.423	7.7	0.013
COND	None	0.125	0.448	8.2	0.002
	MFT	0.075	0.300	5.4	0.044
	PET	0.071	0.269	5.1	0.055
	Depth	0.097	0.348	6.8	0.004
	TP	0.089	0.319	6.3	0.015
	Chla	0.065	0.236	4.5	0.127
	pH	0.069	0.274	5.0	0.053
	PET, Chla, pH	0.059	0.251	4.7	0.114
	Depth, MFT, PET, TP, Chla, pH	0.058	0.289	5.4	0.125

Table 4

No.	Taxa name	N	Hill's N2	Maximum	Mean	PLS β-coefficent (Jack-knifed)	Environmental variables that have a significant relationship to the taxon ($p \leq 0.05$) based on the GLM results
1	*Chironomus* Meigen	31	19.8	63.7	19.3	-0.2	-
2	*Polypedilum nubifer* Skuse	27	19.6	15.8	6.0	0.9	MFT
3	*Cladopelma* Kieffer	24	12.0	23.2	4.2	0.3	Depth
4	*Dicrotendipes* Kieffer	24	14.3	25.8	6.6	-0.1	Chla, Depth, TP, COND
5	*Polypedilum* spp. Kieffer	6	5.1	4.4	0.5	0.9	MFT
6	*Cryptochironomus* Kieffer	8	5.7	4.7	0.4	0.2	COND
7	*Parachironomus* Lenz	11	6.9	10.9	1.2	0.4	Chla, MFT, TP
8	*Kiefferalus martini* Freeman	16	11.7	18.5	3.5	0.3	Chla, Depth
9	*Procladius* Skuse	33	24.9	32.8	15.3	0.1	-
10	*Coelopynia pruinosa* Freeman	8	5.9	4.7	0.6	1.2	MFT
11	*Pentaneurini* undifferentiated	17	10.0	12.3	1.8	-0.7	COND
12	*Riethia* Kieffer	21	9.8	28.9	4.8	-0.3	COND
13	*Tanytarsus lugens* type	29	16.8	23.1	6.6	-0.3	-
14	*Tanytarsus pallidicornis* type	28	17.7	27.0	7.2	-0.3	TP
15	*Tanytarsus glabrescens* type	18	8.2	20.9	2.6	-0.4	Chla, TP, pH
16	*Paratanytarsus* Skuse	22	17.2	14.3	4.0	0.6	Chla, MFT, COND
17	*Tanytarsus* undifferentiated	22	17.3	6.4	1.9	0.1	MFT
18	*Tanytarsus lactescens* type	10	5.2	27.0	2.4	0.5	Depth, MFT, TP, pH
19	*Tanytarsus nr chinensis*	5	3.8	3.6	0.3	-0.3	MFT, pH
20	*Stempellina* Thienemann & Bause	2	1.8	44.6	2.1	0	Chla, MFT, TP, pH
21	*Harnischia* Kieffer	4	2.9	3.1	0.2	1.2	MFT
22	*Paralimnophytes* type 1Unofficial morphotype	13	5.5	15.8	1.3	-0.5	Chla, MFT, COND, pH
23	*Paralimnophytes* type 2Unofficial morphotype	13	8.6	5.4	1.0	-0.3	Chla
24	*Paralimnophytes* type 3 Unofficial morphotype	9	6.9	3.6	0.5	-0.3	-
25	*Parakiefferiella* undifferentiated	7	6.0	4.3	0.6	-0.8	Depth
26	*Parakiefferiella* typel Unofficial morphotype	3	3.0	1.6	0.1	-0.3	-
27	*Parakiefferiella* type 2 Unofficial morphotype	4	3.1	13.7	0.9	0	Chla, MFT, TP, COND, pH
28	*Parakiefferiella* type 3 Unofficial morphotype	4	3.2	2.9	0.2	-1.7	Chla, MFT, COND, pH
29	*Botrycladius* Cranston & Edward	9	5.4	7.0	0.7	-0.6	MFT
30	*Smittia* Holmgren	3	1.9	7.8	0.3	0.3	-
31	*Gymnometriocnemus* type 1 Unofficial morphotype	7	3.2	8.8	0.6	0.1	-
32	Genus Australia	2	2.0	1.2	0.1	-1.1	-
33	*Thienemanniella* Kieffer	4	2.7	5.8	0.3	-0.3	Chla, Depth, MFT, TP, COND
34	Ortholcad type 1 Unoffical morphotype	3	2.5	1.8	0.1	-0.8	Chla
35	Orthoclad type 4 Unoffical morphotype	8	7.3	3.4	0.5	-0.3	-
36	*Pseudosmittia* type 2 Unofficial morphotype	2	1.6	2.3	0.1	0.2	MFT, TP, COND
37	Kosciuszko Orthoclad type 1 Unoffical morphotype	3	2.4	2.7	0.1	-0.4	TP
38	*Cricotopus* 'Parbicintus'	2	2.0	1.4	0.1	-0.1	Chla, Depth, MFT, TP, pH

Table 5

Method		r^2 (apparent)	r^2_{Jack}	$RMSEP_{Jack}$	$MaxBias_{Jack}$	$Av\ Bias_{Jack}$	% reduced
PLS	Component 1	0.67	0.45	3.15	4.39	0.13	-
	Component 2	0.87	0.57	2.80	3.28	0.18	10.85
	Component 3	**0.93**	**0.69**	**2.33**	**2.15**	**0.07**	**16.35**
	Component 4	0.94	0.67	2.45	2.07	-0.01	-4.30
	Component 5	0.95	0.66	2.56	1.83	-0.05	-4.56

Highlights

- First chironomid-based transfer function from mainland Australia
- Reconstructs mean February temperatures and will give new tool for quantitative palaeoclimate estimates from Australia
- Reservoirs were included in the development of the training set.
- Integrating the existing chironomid transfer function from Tasmania with this new model is a real possibility.