Causes of spatial distribution of subfossil diatom and chironomid assemblages in surface sediments of a remote deep island lake

Pedro Miguel Raposeiro[1], Alberto Saez[2], Santiago Giralt[3], Ana Cristina Costa[1] and Vítor Gonçalves[1]

[1]*CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Pólo dos Açores, Azores, Portugal and Departamento de Biologia, Faculdade de Ciências e Tecnologias, Universidade dos Açores, 9501-801 Ponta Delgada, Açores, Portugal*

[2] *Departament of Earth and Ocean Dynamics. Faculty of Earth Sciences. Universitat de Barcelona. Martí i Franques s/n, E-08028 Barcelona, Spain*

[3] *Institute of Earth Sciences Jaume Almera (ICTJA-CSIC), Lluís Solé i Sabaris s/n, E-08028 Barcelona, Spain*

**email:* pedro.mv.raposeiro@uac.pt *; phone;* +351296650480

10 Abstract

11 Until recently, the distribution of diatom and chironomid species assemblages and their attributes (e.g. species
12 richness and diversity) in relation to water depth and sedimentary environments have been identified but not quantified.
13 The influence of environmental variables on assemblage distribution and taxa richness in a deep, monomitic lake in São
14 Miguel Island is assessed. Attention is given to community variation along a depth gradient. Surface-sediment diatom and
15 chironomid assemblages were collected along three transects from shoreline to the centre deep basin of the lake at a
16 resolution of 1 m water depth. Linear and unimodal regressions were used to test taxon richness, taxon diversity and
17 taxon evenness versus water depth of each transept. A hump-shaped relationship between species richness and water
18 depth was noted, with a peak occurring at mid depth levels, meaning that samples located at that depth better represented
19 the total subfossil assemblage of lake Azul. Also, diatom and chironomid assemblages in Lake Azul, and taphomomic
20 effects, were influenced by sedimentary environmental processes depending on the lake morphology. Based on the
21 present results, in order to determine ideal coring locations for lake studies combining diatom and chironomid analyses,
22 an understanding of the spatial distribution of these biota is essential.

23 Keywords

24 Diatom, chironomid, spatial distribution; deep lakes;; species diversity;

25

Introduction

Lacustrine sediments are excellent archives of past climatic and environmental changes because they can record past air or water temperatures (Heiri et al. 2011; Verbruggen et al. 2011), precipitation (Nichols et al. 2009) and nutrient levels (Verbruggen et al. 2011) among other environmental variables. A large set of proxies, like the remains of organisms preserved in the sedimentary record (e.g. diatoms and chironomids) are commonly used to reconstruct these environmental and climatic changes through time. However, it is of paramount importance to hold an exhaustive and precise knowledge of the lake processes to infer these changes. This is especially significant for the biota where a comprehensive ecological understanding about their spatial distribution in lakes is essential. Conceptually, the distribution of subfossil assemblages in sediments of deep lakes, depend on: 1) their spatial distribution when they were alive (Kattel et al. 2006; van Hardenbroek et al. 2010) and 2) and their potential post-depositional redistribution (Eggermont et al. 2007; Frey 1988).

In fact, subfossils of diatoms and chironomids are not evenly distributed in lakes, especially in those with significant habitat and sedimentary environment variability (Eggermont et al. 2007; Heiri 2004).. While the epilimnion is characterized by presence of light, diversity in habitats, well-oxygenized, warm waters whereas the hypolimnion often absence of light, relative homogeneity of soft-sediment habitat, oxygen depletion and cold waters. For instance, light, nutrients, disturbance and substrate type are among the most frequently reported environmental controls of phytobenthos (Cantonati et al. 2009), while in chironomid assemblages water temperature, food availability, hypolimnetic oxygen, and distribution of aquatic macrophytes play a crucial role on their distribution (Cao et al. 2014; Heiri 2004; Laird et al. 2010; Wang et al. 2012).

Depth has a crucial role in determining environmental factors in lakes, such as light intensity, nutrient availability, and disturbance regime (e.g. wave action; mixing water depth), and consequently shaping the biological assemblages' distribution. Some authors (Laird et al. 2010; Peters and Lodge 2010) reported that species richness, diversity and evenness tend to be higher in shallow zones than in offshore deep zones due to higher habitat complexity in the former (e.g. presence of macrophytes), and this pattern seems universal (Kovalenko et al. 2011). So, the relationship between lake depth and its species diversity could provide important information of the characteristic on the environment that can be used to help understand the biodiversity drivers. Even though the shape of the relationship could be highly variable (VER REFS), hump-shaped relationship are the most common in aquatic systems (Dodson et al. 2000; Mittelbach et al. 2001).

Sedimentation and taphonomic processes can also have significant impact on the spatial distribution and composition of subfossil assemblages (Anderson 1990; Skinner et al. 2014), but certain taxa are more affected by these

processes than others (Cao et al. 2014; Eggermont et al. 2007). According to Alin and Cohen (2004), two transitions are inherent to the formation of the fossil record: transition from life to death assemblage and from death to fossil assemblage. Several factors, such as microtopography, geology, hydrology, and differential watershed land use can control the flux of fossil remains to the lake (Heggen et al. 2012), affecting the spatial distribution of the subfossil assemblages. For example, Hakanson (1977) demonstrated that slope is crucial for sedimentation process, concluding that sediment will not accumulate on slopes higher them 14%, below 4%, slope has no effect. Despite of the knowledge that fossil assemblages in lake sediments are commonly irregularly distributed, paleolimology studies are usually based on one sediment core, which in most cases, is recovered in offshore deep-water sedimentary conditions (Brodersen et al. 2001; Heiri et al. 2011; Raposeiro et al. 2017; Skov et al. 2010).

By ignoring natural within-lake heterogeneity of fossil assemblages, and therefore the complex interactions of environmental and sedimentation/taphonomic drivers this simple approach can lead to imprecise paleolimnological reconstructions, such as climatic and/or environmental reconstructions (Zhang et al. 2012). Consequently, an in-lake characterization of fossil distribution should be made before any paleolimnological reconstruction.

Freshwater diatom assemblages are highly diverse and ubiquitous, and constitute an important proxy to estimate primary production in lakes (Hall and Smol 1999). Similarly, chironomids are an important proxy to assess secondary production in lakes (Anderson et al. 2012). These two groups colonize many habitats both within the littoral zone and deep lake environments (Cao et al. 2012; Laird et al. 2010). Therefore, diatoms and chironomids are ideal proxies for assessing if they follow the IDH in deep lakes.

The distribution of diatoms and chironomids in surface sediments of Azorean lakes has already been assessed (Pereira et al. 2014), but while the surface sediments were collected for 41 lakes, only three different locations in each lake were sampled. So, detailed in-lake studies of multiple surface samples are lacking from the Azores archipelago.

Here, we assessed the spatial distribution of subfossil diatom and chironomid assemblages in surface sediments from a deep lake relating them to the main environmental, geochemical and sediment variables in the Azores archipelago (North Atlantic) using a multi-core approach. From the serial surface sampling of several transects, we predict that: a) environmental variables (e.g. light, nutrients, dissolved oxygen) are important indicators of where species occur (Matthews-Bird et al. 2016; Soininen and Weckström 2009; Yang et al. 2009); b) diversity-depth relationship in the lake be 'hump-shaped', where higher species diversity occurs at an intermediate depth (Chase and Leibold 2002; Dodson et al. 2000; Flöder and Sommer 1999; Mittelbach et al. 2001); c) sedimentation and taphonomic processes affects the spatial distribution of the subfossil assemblages (Alin and Cohen 2004; Wang et al. 2012).

Methodology

Study area

The Azores archipelago is a group of nine oceanic volcanic islands located in the mid North Atlantic, roughly 1500 km from Europe and 1900 km from America. At the western end of São Miguel Island lies the Sete Cidades volcanic caldera (Fig. 1A), which includes in its interior eight craters, four of them partially occupied by lakes dominated by clastic sedimentation. The largest lakes inside the caldera are Lakes Azul and Verde, which are hydrologically connected but separated as sedimentary basins by a shallow and narrow bedrock threshold. Lake Azul has a surface of 3.59 km^2, 28 m of maximum depth and it is located at 259 meters above sea level.

Lake Azul morphology and sedimentary environments

The morphology of the coast line of Lake Azul (Fig. 1A) is conditioned by: (a) the internal steep vent slope of the main Sete Cidades Caldera forming the convex NE littoral zone of the lake, (b) the emerged volcanic vents of Caldeira do Alferes to the NW and Lake Santiago vents to the SE with concave morphology, (c) the occurrence of the Cerrado das Freiras deltaic system in NE forming a delta plain and S part of the lake littoral (Sete Cidades village); and (d) the existence of two alluvial distributary systems, one in Sete Cidades village coast and the other in the NE coast of the lake.

Bottom lake basin shows several morphological areas mainly configured by the orientation NW-SE of Mosteiros graben faults system (Moore 1991). From the S to the N, 5 main geomorphological units can be distinguished (Fig.1B): (1) a shallow platform-ramp (from 2 to 12 m of water depth, 0.8-2.1º slope angle) occupying the southern half part of the lake which corresponds to a raised block of the Mosteiros graben; (2) a platform slope zone dipping to the NE (from 12 to 25 m of water depth, 3.2º slope angle) which occupies a bit less than one third of the lake basin and conforms the slope formed by a normal fault plane; (3) a deep plain, from 25 to 28 m depth (< 0.6º angle of slope), which represents the upper surface of a fall block of the graben occupying less than one third of the lake basin, (4) a very dip slope unit located between 27 and 0 m of water depth determined by the internal slope of the main caldera vent (>15º angle of slope) and the delta front (10º slope angle) which occupies the northern half of lake margin, and (5) alluvial littoral areas found at 0-2 m of water depth located in littoral fringe of the NW and SW lake margins. Unit 1 (platform-ramp) is mainly covered by fine sediments and some patches of aquatic plant remains whereas alluvial littoral zones are sandy and accumulate terrestrial plant remains (see Fig. 1B). The delta front slope (unit 4) is composed of sand to gravelly coarse deposits while the northern dip caldera margin is occupied by big blocks accumulated from subaerial rock avalanches.

115

116 Field sampling and laboratory methods

117 Seventy-five surface sediment samples (1cm thick) from Lake Azul were taken in 2013 with an UWITEC-90mm
118 gravity corer along three transects representative of the lake topography. According to Raposeiro et al. (2017), the first
119 centimetre of surface sediments of the deep plain of Lake Azul, corresponds to approximately 3 years of sedimentation.
120 Samples were taken at each 1 m of water depth from the deep plain to the shoreline in three different transects (Fig. 1B).
121 The location of the transects was chosen to cover of the main geomorphological units. GPS coordinates and water depth
122 were determined for each sampling point (see Table S1).

123 Transect 1 extends along the lake in a NE/SW orientation, from the deep plain to the alluvial village coast,
124 crossing the platform slope and platform-ramp. Transect 2 extends along an E/W orientation, from the deep plain to the
125 alluvial western lake coast, crossing the vent slope. Transect 3 extends along the lake in a NW/SE orientation, from the
126 deep plain to the delta front of Cerrado das Freiras (Fig. 1B).

127 Core samples of approximately 1 cm^3 were weighted, dried for 24 h at 60°C and weighted again to estimate dry
128 density ($g.cm^{-3}$). The sand and plant fractions were estimated in smear slides using visual estimation charts (Terry and
129 Chilingar 1955) in four defined areas per sample at 200x magnification under binocular. Samples were then grouped by
130 percentage ranks of sand and plant fractions. Organic matter (LOI_{550}) and residual mineral matter (LOI_{925}) were measured
131 by loss on ignition (LOI) at 550 °C for 4 h and at 925 °C for 2 h, respectively (Heiri et al. 2001). Water temperature (T),
132 pH, dissolved oxygen (DO) and electric conductivity (EC) in the water column were compiled from the data collected as
133 part of the European Water Framework Directive - Regional Monitoring Programme (monthly measured *in situ* with
134 multiparametric field probes) between the 2009 - 2012 period. This approach gives a more realistic perspective of the last
135 3 years of past environmental conditions than single spot measurements, which are often used in paleolimnology
136 modelling. Light intensity was taken every 1 m of water depth from the surface to the maximum depth using a data-
137 logging sensor (HOBO Pendant Temperature/Light Data Logger 64k, Onset) (see Table 1 and Table S2).

138 Sediment samples of 0.5 g dry mass were prepared for diatom analysis following the procedure of Renberg
139 (1990). Diatom slides were mounted with Naphrax, and at least 500 valves were counted per sample at 1000x
140 magnification using a ZEISS AXIOIMAGE A1 microscope. Diatoms were identified by reference to standard floras
141 (Krammer and Lange-Bertalot 1986; Krammer and Lange-Bertalot 1988; Krammer and Lange-Bertalot 1991a; Krammer
142 and Lange-Bertalot 1991b; Krammer and Lange-Bertalot 2000). The nomenclature used has been updated according to
143 the most recent publications, as indicated in the OMNIDIA v5.3 database (Lecointe et al. 1993). Diatom species
144 abundances are expressed as percentages, calculated using the total number of valves recorded for each sample.

145 Abundance estimates were combined with knowledge of habitat preference to generate percentage weighted habitat
146 scores for planktonic, tychoplanktonic and benthic diatoms.
147 For chironomid analysis, sediment samples of 5.0 (±0.02) g of wet mass were prepared by deflocculating them
148 in 10% KOH at approximately 75°C for at least 15 min (Brooks et al. 2007). The sediments were passed through two
149 sieves of 200 and 90 µm mesh size. Head capsules were hand sorted from a Bogorov counting chamber under a stereo-
150 microscope (40x magnification – Zeiss Stemi), mounted in Euparal after dehydration and identified using a microscope
151 (ZEISS AXIOIMAGE A1) at 100x–400x magnification. Identification was largely based on mentum characteristics, as
152 described in Brooks et al. (2007), and was performed to the highest possible taxonomic resolution, commonly species
153 morphotypes. Taxonomical nomenclature was updated following Brooks et al. (2007). Chironomid head capsules
154 concentrations were calculated as head-capsule abundance per gram of wet sediment ($HC.g^{-1}$).
155
156 Data analysis
157 Species (diatoms) and taxon (chironomids) richness were estimated by counting the numbers of species and
158 taxon identified in each sample. Shannon diversity index (H) (Shannon and Weaver 1963) and Hills N2 (Hill 1973) were
159 used as an indicator of species diversity and species evenness (calculated as the total number of taxon), respectively. In
160 diatom assemblages, a minimum of 400 valves has been accepted as representative (VER REFS), while in chironomid, a
161 minimum of 50 head capsules is required to be representative (Heiri and Lotter 2001; Larocque 2001). However, the
162 concentration of the head capsules varies a lot between different depths in a lake (VER REFS) sometimes resulting in
163 counter lower than 50. Considering of the dependence of observed number of taxa on sampling effort, caution should be
164 taken on the interpretation of these results. Therefore, we used individual based rarefaction analyses (on chironomid
165 assemblages) to compare taxon richness between samples (i.e. depths), with unequal sample counts, which enables to
166 assess the adequacy of sampling counts in terms of taxa detection (Gotelli and Colwell, 2001). All the analyses were
167 calculated using PRIMER 6.0 (Clarke and Gorley 2006).
168 For diatoms, only taxa that reached a relative abundance of 5% on at least one sample were included in
169 multivariate statistical analyses. Multivariate analyses were performed after fourth root transformation of relative
170 (diatoms) and absolute (chironomids) abundance prior to numerical analyses to reduce differences in scale (Clarke and
171 Gorley 2006).
172 Linear and unimodal regressions were used to test taxon richness (S) and rarefied number of taxa richness (ES),
173 taxon diversity (H') and taxon evenness (N2) versus water depth of each transept. The adjusted R-squared was used to
174 select the model that explained more variation, and therefore the best model for that data set. In the first model, we fitted

175 a linear model between taxon richness (S), taxon diversity (H') and taxon evenness (N2) versus water depth, using the
176 function y = ax+b; where y is the metric variable and x is the water depth at sampling site. In the second model, we
177 adjusted to the data a quadratic function: y = ax2 + bx + c, where y is the metric variable, x is the water depth variable
178 and a, b and c are constants; a < 0 indicates a hump-shaped curve. The curve vertex (i.e. the maximum value in a hump-
179 shaped curve) is given by x = – (b/2a) and y = a(–(b/2a))2 + b(–(b/2a)) + c. The analyses were performed using R version
180 3.1.2 (RCoreTeam 2015) and vegan package (Oksanen et al. 2015).

181 Cluster analysis was used to identify homogeneous groups of diatom and chironomid assemblages and a
182 SIMPROF test was applied to detect significant zonations. According to Clarke and Gorley (2006), SIMPROF tests data
183 against the null hypothesis of "absence of structure" without requiring *a priori* groupings. A SIMPER analysis was then
184 applied to the whole data matrix to identify the principal taxa associated with the clusters of taxonomic groups and link
185 them with the corresponding zonation. Links between the water physico-chemistry and the subfossil diatom and
186 chironomid assemblages were assessed using distance-based multivariate analysis for a linear model – DistLM (Anderson
187 et al. 2008). DistLM analyses and models the relationship between the multivariate data cloud for one or more predictor
188 variables (Anderson et al. 2008). DistLM allows predictor variables to be fitted individually or together in user specified
189 sets. The DistLM routine was based on the AIC model selection criterion (Burnham and Anderson 2004) using a step-
190 wise selection procedure. The AIC was used to select the model that explained more variation, i.e. the 'best' model for
191 that particular data set. To minimize redundancy between environmental variables, whenever two variables were highly
192 correlated ($r > 0.7$ or $r < -0.7$), the variable with higher overall mean correlation was excluded from further analysis
193 (LOI_{925}; T, EC, DO and pH – see Table S3). LOI_{550} was as surrogates of LOI_{925}, while depth was chosen as surrogates of
194 water variables (temperature, electric conductivity, pH and dissolved oxygen). The environmental data were prior
195 normalized to reduce differences in scale (Clarke and Gorley 2006).

196 For visual interpretation of the resulting model in multi-dimensional space we used distance based redundancy
197 analysis (dbRDA) to investigate the relationship between physicochemical variables (Anderson et al. 2008). Each vector
198 begins at the centre of the circle (the origin) and ends at a given xy coordinates. The length and direction of each vector
199 indicates the strength and sign of the relationship between the given variable and the dbRDA axes. The overlaying vector
200 illustrates the relationship between the five variables identified by the DistLM procedure.

201 The permutation procedure ANOSIM was applied to the Bray-Curtis rank similarity matrices for biological
202 variables to test for differences and the level of significance between transects. R-values obtained in ANOSIM tests range
203 between -1 and 1, where 1 indicates high levels of within group similarity and -1 points out that there are higher similarity
204 levels between samples across groups than within groups (Clarke and Gorley 2006). A SIMPER analysis was then applied

205 to identify the principal taxa associated with the corresponding transect. Primer 6.0 and PERMANOVA + for PRIMER
206 software were used (Anderson et al. 2008; Clarke and Gorley 2006).
207

208 Results

209 Water and sediment variables

210 A statistical summary for selected physical and chemical variables in sediment and in water column is provided
211 in Table S2 and in Figures 2 and 3. The sand fraction and plant debris of the lake sediment varies between transects,
212 slopes degrees and sedimentary environments. Transect 1 shows a low sand influx (some isolated terrigenous silicate
213 grains) in platform-ramp and platform slope peaking at 7 and 12 m of water depth (0.6º) of the outer platform and at 14-
214 18 m depth of the rise slope platform (1.8º). Plant debris accumulates in shallow littoral waters (0-5 m depth; 5-10% plant
215 remains). Transect 2 shows a medium content of sand (1-5% silicate grains) that is arranged in an upward sequence of
216 increasing coarseness that occupies the complete vent slope talus (5.2º). This sequence reflects clastic inputs in the lake
217 from the stream flowing to the lake from the NW. Sand maximum is present in alluvial sediments of NW platform found
218 between 1 and 4 m of water depth (>10% silicate grains). Transect 3 shows the maximum slope (9.9º), and displays sand
219 and plant debris contents carried from Cerrado das Freiras delta system. The sand fraction conforms to an increasingly
220 coarse upward prograding delta sequence from the base (some isolated silicate grains) to the top of the slope at the
221 shoreline (>10% silicate grains).

222 The presence of aquatic and terrestrial plant remains in the sediments ranged between their complete absence
223 (e.g. deepest plain samples) to a maximum of 50-80% plant remains found at 13-14 m of water depth in Transect 3.
224 Transect 1 presented maxima plant remains in a narrow fringe of the village littoral at 0-5 m of water depth and occupied
225 by distal alluvial deposits (1-5% plant remains). In Transect 2, a progressive increasing in plant remains, from vent slope
226 (no plant remains) to a maximum in the narrow littoral NW platform was observed (1-5% plant remains). Transect 3 was
227 characterized by three maximum intervals of plant remains at 14-15, 17 and 19 m of water depth and by their absence in
228 the deep plain, at 20-25 m of water depth. A gradual decrease from the alluvial to 8 m depth of the delta front was
229 observed.

230 The organic content of the lake sediments ranged from 3.1% found at 2 m of water depth of the delta front in
231 Transect 3 to 18.9% located at 2 m depth of the platform ramp in Transect 1. In general, LOI_{550} tended to be lower in
232 shallow-water samples. In Transect 1, LOI_{550} followed a U shape curve, with a minimum value at 14 m depth. In Transect
233 2, a gradual decrease from the deep plain sediments (17.0%) to the NW platform (7.9%) was observed. Four minimum
234 intervals of LOI_{550} were observed along Transect 3 at 6, 10, 12 and 16 m of water depth, respectively. A similar pattern

235 was observed with the residual mineral matter that varied from 0.3 (Transect 3 – 3 m depth) to 4.6% (Transect 2 – 22 m
236 depth).

237 Higher values of conductivity (mean±SE; 111.7 ± 3.4 µS.cm^{-1}) were present near the bottom in the deepest areas
238 of the lake whereas lower values were found between 8 and 11 m of water depth. In the opposite direction temperature
239 (mean±SE; 15.6 ± 0.3ºC), dissolved oxygen (mean±SE; 7.9 ± 0.5 mg. L^{-1}) and pH (mean±SE; 7.7 ± 0.1) presented higher
240 values between 1-5 m of water depth while lower values were detected in the deepest areas. The mean lower depth limit
241 of the light zone was around 10 m.

242

243 Diatom assemblages

244 A total of 154 diatom species from 47 genera were identified from the surface sediments of the 72 sampled
245 locations. The most abundant genera were *Aulacoseira* (31.5%) and *Fragilaria* s. l. (10.6%). Most species were
246 extremely rare, with only 30.5% of the species reaching a maximum relative abundance above 1% (Fig. 4). From the 154
247 diatom taxa identified, only *Aulacoseira ambigua* occurred in all 72 studied locations, and only 27 taxa occurred in more
248 than 50% of the samples. These included species such as *Achnanthidium minutissimum, Aulacoseira granulata,*
249 *Cocconeis euglypta, Eolimna subrotundata, Fragilaria crotonensis, Fragilaria tenera, Navicula rhyncocephala,*
250 *Pseudostaurosira brevistriata, Staurosira elliptica* and *Tabellaria flocculosa*, which are all ubiquitous diatoms.
251 Taxa richness ranged from 60 taxa recorded at the middle depth in Transect 3 (14 m of water depth - T3_14) to 26 taxa in
252 the deep platform of Transect 3 (23 m deep - T3_23), with a mean richness of 32 taxa per sampling point. Species such as
253 *Fragilaria crotonensis, Fragilaria tenera, Eolimna subrotundata, Aulacoseira ambigua, Psammothidium* sp. and
254 *Staurosira elliptica* were among the taxa with the highest mean abundances (Fig. 4).
255 The overall average number of taxa (S: 38.9±0.7) and diversity (H': 2.6±0.1) was higher below 20 m depth (Diat-1 zone),
256 when compared to the deeper zone (Diat-2 zone; S: 27.7±1.1; H': 1.1±0.1; respectively) (Fig. 5). These trends in taxon
257 richness and diversity were consistent across the three transects and were highly correlated (S: r = 0.56–0.66, p <0.001;
258 H': r = 0.79–0.89, p <0.001). The diversity of habitats present in the littoral zone (e.g. macrophytes, stones, sand),
259 associated with the availability of light, is reflected in a much higher percentage of benthic and tychoplanktonic diatoms
260 such as *Eolimna* spp., *Psammothidium* sp. and *Pseudostaurosira brevistriata* in samples from shallow sites of all
261 transects. Planktonic taxa like *Aulacoseira ambigua*, *Fragilaria crotonensis* and *Fragilaria tenera* were most abundant in
262 diatom assemblages in deeper zones. Species evenness (Hill's N2) was greatest between 5 and 15 meters depth (Fig. 4)
263 declining to around 20 meters depth and reaching lowest values in the deeper zone (> 20 meters depth). This pattern
264 shows that more taxa occur at higher abundances in the shallow zone when compared to the deeper one. In the shallow

zone, the lowest evenness occurs between 2 and 5 meters depth. These trends were consistent across the studied transects (r = 0.55–0.81, p <0.001).

The three transects display a significant negative relationship between diatom species diversity (R^2_{adj}=0.34–0.78; p<0.001), species evenness (R^2_{adj}=0.36–0.45; p<0.01) and water depth (Table 2). However, the quadratic model fitted the data better for both species diversity (R^2_{adj}=0.78–0.81; p<0.001) and species evenness (R^2_{adj}=0.56–0.74; p<0.001) than the linear model (Table 2) suggesting that the unimodal model was superior and had better support to explain the diatom species diversity and evenness along the depth gradient. The curve vertex for the diatom diversity and evenness was highest at intermediate water depth (hump = 8.6–10.5 m deep).

Cluster analysis shows that at 77% similarity samples are subdivided into two main clusters (Fig. 6). Also, the SIMPROF test identifies these two significant diatom assemblages. One cluster comprises most of the samples present from 0 to 20 m of water depth (Diat-1) and the remaining samples from the deepest zones are grouped in the second cluster (>20m depth; Diat-2).

This cluster analysis, together with the SIMPER analysis of similarity (Table S4), enabled identification of the characteristic species within each group that defines each biozone. SIMPER analysis revealed a dissimilarity of 24.4% between the two biozones. *Fragilaria crotonensis* (10.0%), *Staurosira pseudoconstruens* (9.6%), *Eolimna subrotundata* (9.0%), *Eolimna uthermoehlii* (8.4%) and *Psammothidium* sp. (8.4%) were the species that contributed most to the dissimilarity. Diat-1 zone, with 86.3% average similarity, is characterized by the dominance of benthic diatoms belonging to the genera *Eolimna, Achnanthidium, Navicula* and *Nitzschia*, and tychoplanktonic diatoms such as the *Staurosira pseudoconstruens* and *Pseudostaurosira brevistriata* (Fig. 4A and Table S4). The samples grouped in Diat-2 zone were characterized by the dominance of the planktonic taxa *Aulacoseira ambigua, A. granulata, Asterionella formosa, Fragilaria crotonensis* and *F. tenera* (Fig. 4A and Table S4).

This grouping pattern is also evident in the dbRDA scores applied to the diatom assemblages where planktonic species display low dbRDA axis 1 score, whereas the benthic and tycoplanktonic species presents higher scores on the first dbRDA axis (Fig. 6B). According to DistLM procedure, all physical and chemical variables (AIC=333.1.; R^2=0.52; number of variables=5) were important to explain the diatom distribution along the lake depth gradient (p<0.006; Table 3). The water column variables were the most important variables to explain diatom assemblages' distribution in Lake Azul, where depth (surrogate of temperature, pH, dissolved oxygen and electrical conductivity) and light explained 34 and 7% of fitted variance, respectively.

The dbRDA illustrates how these zones were clearly separated by their location along the water depth gradient and these physical and chemical variables (Fig. 6B). The first two dbRDA axes explained 89.5% of the relationship

between the diatom assemblages and the measured physical and chemical variables, and 46.5% of the total variability in the assemblage data. The first dbRDA axis was strongly related to depth, while the second axis was related to habitat availability (sand fraction and plant remains in sediment).

ANOSIM results (Table 4) showed that diatom assemblages differed significantly among transects (R=0.101; Significance P<0.1%). SIMPER results (Table S4) revealed dissimilarity levels among transects that ranged from 39 to 43%, which were reflected by the percentage abundance in *Eolimna subrotundata* (10.5% in Transect 1; 18.4% in Transect 2; 7.0% in Transect 3), *Aulacoseira ambigua* (26.7% in Transect 1; 20.0% in Transect 2; 23.8% in Transect 3) *Psammothidium* sp. (3.5% in Transect 1; 3.8% in Transect 2; 9.3% in Transect 3) and *Pseudostaurosira brevistriata* (6.1% in Transect 1; 3.1% in Transect 2; 6.8% in Transect 3). These taxa were identified as the main species contributing to the observed dissimilarity (> 40%) between transects.

Chironomid assemblages

A total of 1,340 chironomid head capsules, which belong to 3 subfamilies, 12 genera and 17 infra-genera taxa, were recovered from the 72 surface sediment samples. Chironominae (9 taxa) showed the highest taxa richness, followed by Orthocladiinae (6 taxa) and Tanypodinae (2 taxa). The most abundant taxa with abundances exceeding 5% were *Psectrocladius sordidellus* (48.3%), *Micropsectra* contracta (23.3%), *Chironomus anthracinus* (7.2%) and *Glyptotendipes barbipes* (5.4%). Several taxa, such as *Psectrocladius sordidellu*s and *Micropsectra contracta*, occurred in most samples all over the lake, albeit with low abundances in the deep plain of Lake Azul (Fig. 4B).

The samples showed low densities of head capsules of chironomid larvae, with a mean of 18.3±1.8 HC. g^{-1} reaching their maximum at 10 m water deep (Fig. 4B). Taxon richness in each sample ranged from 1 (below 20 m of water depth) to 9 (7 m water deep). The overall average number of taxa (S: 1.8±0.2) and diversity (H': 0.4±0.0) was lower in the deeper zone (>20m), when compared to the benthic zone (S: 5.1±0.3; H': 1.2±0.1, respectively). These trends in taxon richness and diversity were consistent across the three transects and were correlated (S: r = 0.56–0.69, p <0.01; H': r = 0.50–0.47, p <0.05; Fig. 5).

In the shallow zone, the lowest evenness (Hill's N2) occurred between 2 to 5 meters depth in all transects. Taxon evenness was generally greatest between 5 and 15 meters depth (Fig. 5), declining to around 20 meters depth and reaching the lowest values in the deepest zone (> 20 meters depth). These trends were consistent in transects T1 and T3 (r = 0.47, p <0.05), but not along transept T2 (r = 0.40-0.45, p >0.05). As in the diatom models, the three transects for chironomid assemblages display a significant negative relationship between chironomid taxon richness (R^2_{adj}=0.31–0.51; p<0.01), taxon diversity (R^2_{adj}=0.37–0.50; p<0.001), taxon evenness (R^2_{adj}=0.24–0.34; p<0.01) and water depth (Table 2).

However, the quadratic model fitted the data better for taxon richness (R^2_{adj}=0.43–0.65; p<0.01), taxon diversity (R^2_{adj}=0.57–0.67; p<0.001) and taxon evenness (R^2_{adj}=0.39–0.54; p<0.001) than the linear one (Table 2) suggesting that the unimodal model was superior and had better support to explain the chironomid assemblages along the depth gradient. The curve vertex for the chironomid richness, diversity and evenness was highest at intermediate water depth (hump = 7.7–10.6 m deep).

Cluster analysis at similarity values of 50%, and supported by SIMPROF routine, identifies three chironomid assemblages (Fig. 7A): (i) the Chiro-1 zone, which corresponds to the near-shore and mid-depth benthic taxa, and two deeper ones (ii) Chiro-2 and (iii) Chiro-3.

Chiro-1 zone is characterized by the dominance of littoral and detritivore species such as *Psectrocladius sordidellus* (40.0%) and *Micropsectra contracta* (31.5%). Assemblages in this biozone also included sub-littoral species like *Chironomus athracinus* (12.5%) and *Glyptotendipes barbipes* (4.1%). Head capsule abundances are generally higher in this zone than the other two, ranging from 0 to 64 HC.g^{-1}, with an average of 23 HC.g^{-1} (SE±1.9). Chiro-1 assemblages occurred in shallow littoral areas characterized by the presence of aquatic macrophytes remains, higher water temperature, dissolved oxygen and pH, and lower conductivity and organic matter (LOI_{550}).

Chiro-2 and Chiro-3 assemblages were found in the profound zone of Lake Azul characterized by colder waters, low dissolved oxygen and pH, higher conductivity and organic matter concentration and the absence of light and plant remains. A rapid decrease in the abundance of head capsules, reflected by the decrease of *Psectrocladius sordidellus* and *Micropsectra contracta*, was observed. Head capsule abundances are particularly low in these Chiro-2 and Chiro-3 zones ranging from 1 to 7 HC.g^{-1} and 1 to 2 HC.g^{-1}, with an average of 3 HC.g^{-1} (SE±0.6) and 2 HC.g^{-1} (SE±0.3), respectively.

SIMPER analysis indicated that the average similarity index was low in Chiro-1 zone (61.0%), increasing in Chiro-2 (65.6%) and Chiro-3 (73.6%), which suggests greater homogeneity on deeper zones (Table S5). Three taxa contributed extensively to the differences between zones, *Psectrocladius sordidellus*, *Micropsectra contracta* and *Chironomus plumosus* accounted for 46.4% of the overall dissimilarity between Chiro-1 and Chiro-2 zones, 50.2% between Chiro-1 and Chiro-3 zones, and 83.7% between Chiro-2 and Chiro-3 zones.

The first two statistically significant axes in the initial dbRDA between all chironomids and the five exploratory variables explained 29.0% of the total variation in the chironomid assemblages. Among the five determinants (Table 3), two water column variables and two sediment variables formed the best set of variables after Best procedure (AIC=479.8; R^2=0.32; number of variables=4), which explained a statistically significant amount of the total variation in the chironomid data.

354 Using only these four significant variables, the first two dbRDA axes explained 89.9% of the relationship
355 between the chironomid assemblages and the measured environmental variables, and 28.6% of the total variability in the
356 community data (Fig. 7B). The first dbRDA axis accounted for 77.6% of the explained variation in the chironomid
357 assemblages and was strongly related to depth, explaining 24.7% of the total variance in the chironomid assemblages.
358 The second axis was related to sediment variables, such as the plant remains and LOI_{550}, and explained 12.3% of the total
359 variation and 3.9% of the total variance in the chironomid assemblages.
360 The ANOSIM test showed that chironomid assemblages significantly differ among transects (R=0.101;
361 Significance p<0.1%; Table 4). Dissimilarity levels, revelled by SIMPER analysis (Table S5), were lower between
362 Transect 1 and Transect 2 (48%), and Transect 2 and Transect 3 (52%), than between Transect 1 and Transect 3 (55%).
363 *Psectrocladius sordidellus*, *Micropsectra contracta*, *Chironomus athracinus* and *Glyptotendipes barbipes* (with more than
364 10% of the total difference each individually in the composition patterns) were the principal taxa contributing to the
365 observed dissimilarity between transects. On average, higher concentration of head capsules occurred in Transect 1 (21
366 $HC.g^{-1}$), and Transect 2 (19 $HC.g^{-1}$) in contrast with Transect 3 (15 $HC.g^{-1}$). Regarding species richness, deltaic conditions
367 in Transect 3 lead to an increase of species richness (15 taxa), than platform and vent slope conditions of Transect 1 (12
368 taxa) and Transect 2 (11 taxa), respectively. In fact, *Orthocladius* sp. and *Pseudosmittia* sp., two taxa associated to lotic
369 systems, were exclusive to Transect 3, probably transported by floods occurring at the mouth of the river delta (Fig 1B).
370

371 Discussion

372 Resource availability cause spatial heterogeneity of biota in lake Azul

373 Described data for Azul Lake shows a significant spatial heterogeneity in the distribution of the main diatom and
374 chironomid assemblages. Living diatom and chironomid communities are well known to show shifts in species
375 composition with water depth (especially in deep, seasonally stratified lakes) through their response to conductivity,
376 temperature, oxygen availability and substrate type (Gonçalves et al. 2015; Raposeiro et al. 2011). The results of the
377 DistLM ordination revealed that depth was found to have the greatest explanatory power for both assemblages (34% and
378 18% for diatoms and chironomids, respectively). The good correlation with depth gradient is probably a result of the
379 original habitat of the subfossil assemblages, as the flora and fauna changes with water depth (Eggermont et al. 2007;
380 Heggen et al. 2012). In this study, depth is negatively correlated with temperature, dissolved oxygen and pH.
381 Conductivity in Azorean lakes is mainly related to CO_2 production from organic matter decomposition (Cruz et al. 2006)
382 that prevails in deeper, low oxygen waters. According to Talling (2009) values of electric conductivity in lake
383 environments, especially in stratified lakes, are closely linked to food availability and lake productivity. Therefore,

384 electric conductivity is an important regulator (associated predominantly with redox potential - oxygen availability) in
385 spatial distribution of diatom and chironomid assemblages. The correlation between electric conductivity, nutrients
386 availability and oxygen concentration in Lake Azul are closely related to water-depth gradient. This pattern constrains
387 some species to littoral shallow zone, or mid–depth (mid-slope and platform) zone distributions, whereas others restrict
388 their distribution in the deeper offshore zone. This pattern was also found in other lakes where diatom and chironomid
389 assemblages could change in a sequential manner from the near-shore shallow zone to the deep along a gradient of water
390 depth (Verbruggen et al. 2011; Wang et al. 2012). For example, in Lake Azul *Pseudostaurosira brevistriata*, a benthic
391 high profile taxon, reached abundances higher than 20% only in samples from less than 5 m of water depth. Also,
392 *Psectrocladius sordinellus*, a sensitive taxon to hypoxia (Luoto 2009), was more abundant in the littoral zone. In the
393 opposite direction, .Taxon richness of diatom and chironomid assemblages follows a unimodal or 'hump-shaped' pattern
394 along all the lake depth transects, with maximum richness occurring at mid depths (~10 m depth) (Fig. 5; Table 2). This
395 pattern has also been found for different freshwater taxonomic groups (macrophytes, macroalgal, macroinvertebrates) in
396 several other lakes (e.g. Chase and Leibold 2002; Townsend et al. 1997). So, this unimodal response curve is probably
397 caused by limitation of the biological assemblages by the high and drastic e□ects of disturbance on the littoral zone of
398 Azul lake, moderate disturbance on the middle depth zone and a more constant environmental on the bottom. The littoral
399 zone of Lake Azul presents high levels of disturbance such as increased natural stress from turbulence (e.g. wind, waves),
400 and water level fluctuations, among other factors, may contribute to explain the lower diversity on littoral zones of lakes
401 (Gabel et al. 2012; King et al. 2006). For example, waves suspend fine sediment from littoral areas and deposit into
402 offshore areas (ver refs) and water fluctuations accelerate this sediment focusing processes. So, through the interplay of
403 direct (e.g., physiological stress) and indirect (e.g., habitat alteration) mechanisms, water level fluctuations structure
404 spatio-temporal heterogeneity that shape the littoral zone assemblage composition and richness (ver refs). This idea
405 support our results, were in the littoral zone (especially between 2 and 5 meters depth) were observed lower levels of
406 diversity and evenness for diatoms and chironomid assemblages. This trend is more marked in the chironomid
407 assemblages because taxa distribution and abundance are largely determined by the spatio-temporal hierarchy of habitat
408 and resource heterogeneity of the littoral zone (Heino 2008; Tolonen and Hämäläinen 2010). The less marked trend
409 observed in diatoms response may be explained by rapid algal turnover rates (VER REFS), although a small reduction of
410 epilithic and epipelic; increase of motile, epipsammic and epilithic taxa was observed, respectively. It seems that
411 environmental heterogeneity, more than the habitat diversity, is crucial in controlling the assemblage composition in Azul
412 Lake. This pattern,also described in the literature for diatom and invertebrates' assemblages (Ptacnik et al. 2008; Shurin et
413 al. 2007), could be explained by Connell's intermediate disturbance hypothesis (Connell 1978). An alternative hypothesis

414 to explain the species richness differences across ecosystems is the environmental heterogeneity, by which more
415 heterogeneous environments support a higher number of species (e.g. Maestre and Reynolds 2006; Oliver et al. 2010).
416 However, the effects of the environmental heterogeneity and moderate disturbance on species richness are difficult to
417 distinguish because both drivers frequently co-occur (Graham and Duda 2011). This might not be the case in lakes since
418 spatial heterogeneity is normally higher in the littoral zone than in the offshore one (Lampert and Sommer 2007; Peters
419 and Lodge 2010).
420 In general, biological assemblages of the upper littoral are less diverse and are constituted of generalist and cosmopolitan
421 taxa, while in the lower littoral they are more diverse and contain more specialist and relict taxa. For example, Dodson et
422 al. (2000) reported that diatom species richness depended on the available energy in North American lakes and found that
423 once the primary productivity increased the community responded positively in both abundance and species richness.
424 Similarly, the peak in the chironomid taxon richness corresponded with peaks in food resources (Brodersen et al. 2001).
425 Based in our findings, it seems that the peaks in chironomid species richness and abundance at 8.3–9.9 m depth indicate a
426 positive association between the maximum species richness of diatoms found at 8.6–9.7 m depth and the maximum food
427 resource diversity occurring at this lake zone. Light quality, quantity and availability are crucial, especially for diatoms
428 allowing the co-existence of more species in the littoral zone (Moos et al. 2005). Compared to the deep offshore plain,
429 higher species richness in mid-depth zone (Diat-1 and Chiro-1 zones) was observed in Azul Lake. This is like the results
430 of Wang et al. (2012) for the diatoms subfossil assemblages, supporting the idea that maximum diversity of diatoms on
431 lakes occur with low light and high nutrient availability. The same abundance pattern for the chironomid subfossil
432 assemblages was observed before (Kurek and Cwynar 2009; Zhang et al. 2013).
433 The formation of the descendent part of the humped shape back curve is cause by increasing dominance of diatoms
434 species group, and by the drastic drop of chironomid head capsules. This decrease chironomid signal is well displayed in
435 Fig. (VER), especially below the 20m depth (Chiro-2; Chiro-3 zones), with the dominance of planktonic taxa (diatoms;
436 Diat-2;) and profundal taxa (chironomids) associated, with increasing depth. First, the species evenness (Hill's N2), then
437 diversity and species richness started to decrease with increasing depth. The dominance of planktonic taxa in higher
438 depths is probably due to a higher sinking rate of heavier/larger species, lower buoyancy or reduced mobility. This effect
439 is amplified by increased stratification and reduced mixing (Hampton et al. 2014; Reynolds 1984). For example, the
440 larger planktonic forms of diatom such as *Aulacoseira ambigua, Fragilaria crotonensis* and *Aulacoseira granulata*,
441 reached abundances higher than 60% only in samples below 20 m of water depth supporting this idea. The oxygen
442 tolerant chironomid taxa like the *Chironomus* group and the *Glyptotendipes* groups, presented a higher relative abundance
443 below 18 m of water depth, where low hypolimnetic oxygen levels are present and can reach anoxia in late summer

(Gonçalves 2008). So, lake Azul data confirm dissolved oxygen has one of the most important factors affecting chironomid distribution which limiting the presence in deeper sediments to low oxygen tolerant taxa, similar to what was observed by several authors for other lakes (Frossard et al. 2013; Luoto 2009; Luoto and Salonen 2010).

Some studies highlighted that both diatoms and chironomid remains are first deposited near to where they live, that could be later transported to other areas of the lake by sediment erosion and re-deposition (Heiri 2004; Yang et al. 2009). Although they can be transported to the deepest parts of the lake by currents set up by the runoff in the margins of the lake or gravity flows generated into the lake causing sediment erosion and re-deposition, the extent to which subfossil remains are moved offshore clearly depends upon lake morphometrics (Frey 1988). These sedimentation processes have affected differently the three analysed transects in Lake Azul (see below).

Sedimentation and taphonomic processes affects the spatial distribution

Percentage of sand fraction is also one of the best explicative variables to understand the spatial distribution of diatom and chironomid assemblages in Lake Azul. This sediment variable has been previously reported as influencing subfossil assemblage composition (Cao et al. 2012; Eggermont et al. 2007). A higher percentage of sand fraction reveals intensive erosion from the lake shore and can provide information about the hydrodynamic intensity in lake Azul. For example, the presence of coarser grains may indicate enhanced water currents that can transport fine particles away; in contrast, the dominance of finer grain particles may be indicative of weak hydrodynamism. This is particular visible in lake Azul, where higher clastic influxes were correlated with steeper slopes, especially in delta front slopes (Figure 1). The enhanced hydrodynamic water currents, especially in Transect 3, suggested by the coarser sedimentary particles presence, would favour the occurrence of higher flow conditions. These hydrodynamic conditions were confirmed by the presence of several taxa associated to lotic systems and well represented in Azorean streams (Gonçalves et al. 2015; Raposeiro et al. 2011; Raposeiro et al. 2009; Raposeiro et al. 2013), such as *Cocconeis euglypta*, *Eunotia exigua, Fragilaria capucina* and *Eolimna uthermoehlii*, for the diatom assemblages, and *Orthocladius* sp. and *Pseudosmitthia* sp. in the chironomid assemblages. The change of substrate from silt to coarse sand also affects the distribution of both biological assemblages. This was evident in diatom assemblages where epilithic and epipsammic taxa, such as *Geissleria decussis*, *Psammothidium* sp. or *Planothidium daui*, were only found in sandy sites. With respect to the chironomids, Tanytarsini taxa showed the highest abundance on silt areas whereas Chironomini taxa were more abundant in sandy habitats. These patterns are well described in Pinder's revision of the habitats of chironomid larvae (Pinder 1986), and could be explained by the distribution of the burrowing and tube building larvae and larvae living on the sediment surface. To avoid possible biases in the past environmental reconstructions, especially in deep stratified lakes, it is very

474 important to consider the origin of subfossil remains collected from the offshore (Millet et al. 2010). As discussed above,
475 littoral sediment erosion and transport to offshore areas is a frequent mechanism occurring in clastic lakes (Brodersen and
476 Lindegaard 1999; Heiri 2004), but the range to which organism remains are susceptible to be transported to offshore
477 zones depends mostly on the particular processes of erosion/transport/deposition of sediments occurring in each lake. For
478 example, Wang et al. (2012) examined the taxonomic distribution of subfossil assemblages of a deep-water lake in China
479 and found that most organisms in deep assemblages had been transported out from the littoral zone or from the slope to
480 offshore areas. Brodin (1986) concluded that the offshore transport of littoral remains was minimal by showing that a
481 high proportion of remains eroded from littoral habitats seemed to deposit preferentially in the sediment accumulated
482 close to the thermocline depth.

483 In our study, transport of littoral subfossil diatoms and chironomids to the offshore plain of lake Azul seems to
484 be variable between the different transects. In Transect 1 and Transect 2 sediment transport processes did not notably
485 affect biological assemblages. Transect 1 was characterized by low clastic influx in both the platform and the platform
486 slope, with two medium fringes of clastic accumulations in the outer platform (8–12 m deep). Terrestrial plant remains
487 were concentrated in a narrow fringe of the littoral (3–5 m of water depth) occupied by distal alluvial deposits in the
488 southern lake littoral area. Transect 2 presented steeper slopes, with increasing plant remains upwards towards the vent
489 slope. Also, the most abundant terrestrial plant remains and medium clastic influx (>10% terrigenous silicate grains) was
490 observed in the alluvial narrow NW-platform. In both transects a decreasing trend of both the ratio benthic vs planktonic
491 diatoms and the chironomid head capsule abundance with respect to an increasing water depth was evident (Figure 2),
492 with a maximum species diversity observed between 6 and 12 m of water depth. Diatoms characteristic of littoral
493 habitats, such as *Eolimna* spp., *Staurosira* spp. and *Pseudostaurosira* spp., were present in relative high abundances in
494 sediments above 12 m water depth (in Transect 1 platform-ramp and in Transect 2 alluvial plain and vent slop units) but
495 rapidly decreased in deeper areas. In these two transects, biological assemblages were mostly controlled by the
496 environmental variables. In contrast, Transect 3 shows strong indications of sediment transport processes that
497 significantly influenced biological assemblages. The influence of sediment transport processes was more evident in the
498 four intervals of maxima species richness of diatoms and chironomids that are related to the sand fraction particles and
499 LOI_{550} between 2-16 m depth. In this transect, littoral species appear with high abundance in deeper zones (e.g. 26.4% of
500 *Pseudostaurosira brevistriata* at 10 m, 13.3% of *Eolimna subrotundata* at 15 m or 5.6% of *Staurosira elliptica* at 16 m)
501 associated with increased sand fraction, plant remains in the sediments and LOI_{550}. The enhancement of transportation of
502 littoral subfossils from littoral to deeper zones in Transect 3 could be related to its location in the Cerrado das Freiras
503 delta, where sediment transportation and re-deposition is expected to be higher. The fact that Transect 3 has the steepest

slope of the three studied transects associated with enhanced runoff and torrential and highly erosive flow regimes that occur in the delta slope almost immediately after episodes of rainfall in Azorean lotic systems (Raposeiro et al. 2013) increase sediment transport in the NE area of the lake (along Transect 3).

However, deep plain sedimentary environment has quite distinct profound assemblages in lake Azul as identified by the SIMPROF routine (Zones: Diat-2; Chiro-2; Chiro-3) compared with those in shallower areas. According to Gonçalves (2008), Lake Azul displays severe anoxia stratification during the summer period below 20 m of water depth. This corresponds with the observed separation of diatom and chironomid assemblages in the profound lake zone. In fact, the drastic change observed in the benthic vs planktonic ratio and the decrease of abundance of chironomid head capsules suggest that a high proportion of the subfossil remains are deposited directly from the water column, and arrival of particulate sediments transported from the littoral to offshore deep plain seems minimal. However, in lake Azul during the fall overturn small amounts of the benthic diatoms and chironomid head capsules are probably deposited in deep plain of the lake. The mechanism of this kind of transport is called "sediment focusing" that is a more or less continuous process of erosion of sediments from the shallow margins of the lake and re-deposition on deeper lake zones. (Davis et al. 1984). These authors found that this process of transport, which is strongest during the period of isothermal conditions, is responsible for about 40% of the total annual sediment influx to the lake bottom at a depth of 10 meters.

Implications to paleolimnological studies

The observed fossil assemblages' gradient from near-shore to offshore can reflect the real living diatoms and chironomid compositions close to their living habitat, especially in areas that present a gentle slope. Although in areas with a steeper slope, the taphomomic (transport) effects have a strong effect on the spatial distribution of subfossil assemblages. The ability to determine environmental changes in lakes is a powerful tool for use in reconstructing past environmental and predicting future environmental change (such as climate change). Although, as we could see in lake Azul, complex interactions affect local assemblages' signals in deep lakes, and therefore is crucial to increase efforts with multiple-core (spatially distributed in deep lakes) to support the interpretation of the subfossil assemblages, as well the interactions of local assemblages' environmental variables (e.g. light, temperature, dissolved oxygen, among others) in relation to water depth.

In this study, species richness, diversity and evenness follows a "hump-shaped", with maximum richness occurring at mid depths (8-10 m depth), meaning that samples located at that depth better represented the total subfossil assemblage of lake Azul. So, based on these findings, coring in the offshore deeper zone of lake Azul will consistently highlight planktonic production, less diverse subfossil assemblages, with low concentration of chironomid head capsules

534 which limits the assessment of littoral and shallow habitat diversity. Also, most of the littoral samples were species-poor,
535 with a large degree of variability among samples. These patterns have a paramount importance in paleoecological studies,
536 since a single core taken in offshore would be unrepresentative of the local community for the whole lake basin. Several
537 examples could be found on the literature, with opposite directions. Frey (1988) showed that littoral assemblages often
538 show a different response to long-term changes than offshore communities, while Belle et al. (2015) showed that
539 chironomid assemblages exhibited a uniform spatial distribution at deep lake in France. Other authors, reported the
540 greater variation of the fossil assemblages on the offshore zone of the lake contributes to the overall prediction error
541 associated with based inference models (Heiri et al. 2003). Nevertheless, Heiri (2004) studied subfossil assemblages of
542 surface sediments several Norwegian lakes and concluded that, even though individual transect should great variability
543 among each other, but the dominant taxa were present in most of the samples.
544 Consequently, using the multi-proxy combining with multi-site approach can then be combined into regional
545 climate and environmental reconstructions to offer additional insights. In fact, Kurek and Cwynar (2009) proposed that
546 multiple, within-lake sampling of gradients is a powerful approach to improve the performance statistics of transfer
547 functions and consequently to paleo reconstruction.
548
549 Final remarks
550 In deep lakes, such as lake Azul, with a variable gradient (smooth and steep), a depth greater than 10 m, and with
551 littoral zones affected locally by intra-basin transport currents, subfossil assemblages such as diatoms and chironomids
552 show great spatial heterogeneity along the depth gradient. This spatial heterogeneity is a product of the superposition of
553 ecological and geological factors. To conduct paleolimnological studies based on these organisms, coring location
554 strategy of the target lake (number and eater depth) must be established according to the objective to be achieved. For
555 studies in which one wants to obtain a knowledge of the lake variations or to recognize the species diversity, the
556 appropriate is to extract sedimentary sequences located in the mid depth zones, with intermediate disturbance, free of
557 transport (soft slopes). If the objective is to know study the major ecological changes that the lake has undergone
558 throughout its history, as it is appropriate to core the deep offshore areas. Based on the present results, the previous
559 studies of surficial biota distribution in large, deep lakes are essential in order to make overall suggestions about ‘ideal’
560 coring sites for multi-proxy paleolimnological studies involving both aquatic and terrestrial organisms.
561

562 Acknowledgments

563 Part of this study was financed by Fundação para a Ciência e Tecnologia (SFRH/BPD/99461/2014) and the PaleoNAO,
564 RapidNAO and PaleoModes projects of the Spanish Ministry of Education (CGL2010-15767, CGL2013-40608-R, and
565 CGL2016-75281-C2-1-R respectively). Thanks, are given to the Freshwater Ecology Group from the University of the
566 Azores for all the help in field and laboratory work. The surveys performed comply with the current laws of Portugal.

567

568 **References**

569

570 Alin, S. R. & A. S. Cohen, 2004. The live, the dead, and the very dead: taphonomic calibration of the recent
571 record of paleoecological change in Lake Tanganyika, East Africa. Paleobiology 30(1):44-81
572 doi:10.1666/0094-8373(2004)030<0044:tltdat>2.0.co;2.

573 Anderson, M. J., R. N. Gorley & K. R. Clarke, 2008. PERMANOVA + for PRIMER: Guide to Software and
574 Statistical Methods. PRIMER-E, Plymouth, UK.

575 Anderson, N. J., 1990. Spatial pattern of recent sediment and diatom accumulation in a small, monomictic,
576 eutrophic lake. Journal of Paleolimnology 3(2):143-160 doi:10.1007/BF00414068.

577 Anderson, T. J., R. S. Stelzer, H. G. Drecktrah & S. L. Eggert, 2012. Secondary production of Chironomidae in
578 a large eutrophic lake: implications for lake sturgeon production. Freshwater Science 31(2):365-378
579 doi:10.1899/11-042.1.

580 Belle, S., L. Millet, F. Gillet, V. Verneaux & M. Magny, 2015. Assemblages and paleo-diet variability of
581 subfossil Chironomidae (Diptera) from a deep lake (Lake Grand Maclu, France), vol 755.

582 Brodersen, K. P. & C. Lindegaard, 1999. Classification, assessment and trophic reconstruction of Danish lakes
583 using chironomids. Freshwater Biology 42(1):143-157 doi:10.1046/j.1365-2427.1999.00457.x.

584 Brodersen, K. P., B. V. Odgaard, O. Vestergaard & N. J. Anderson, 2001. Chironomid stratigraphy in the
585 shallow and eutrophic Lake Søbygaard, Denmark: chironomid–macrophyte co-occurrence. Freshwater
586 Biology 46(2):253-267 doi:10.1046/j.1365-2427.2001.00652.x.

587 Brodin, Y. W., 1986. The Postglacial History of Lake Flarken, Southern Sweden, Interpreted from Subfossil
588 Insect Remains. Internationale Revue der gesamten Hydrobiologie und Hydrographie 71(3):371-432
589 doi:10.1002/iroh.19860710313.

590 Brooks, S. J., P. G. Langdon & O. Heiri, 2007. The identificiation and Use of Palaeartic Chironomidae Larvae
591 in Palaeoecology. Quarternary Research Association, London.

592 Burnham, K. P. & D. R. Anderson, 2004. Multimodel Inference: Understanding AIC and BIC in Model
593 Selection. Sociological Methods & Research 33(2):261-304 doi:10.1177/0049124104268644.

Cantonati, M., S. Scola, N. Angeli, G. Guella & R. Frassanito, 2009. Environmental controls of epilithic diatom depth-distribution in an oligotrophic lake characterized by marked water-level fluctuations. European Journal of Phycology 44(1):15-29 doi:10.1080/09670260802079335.

Cao, Y., E. Zhang, X. Chen, N. John Anderson & J. Shen, 2012. Spatial distribution of subfossil Chironomidae in surface sediments of a large, shallow and hypertrophic lake (Taihu, SE China). Hydrobiologia 691(1):59-70 doi:10.1007/s10750-012-1030-3.

Cao, Y., E. Zhang, P. G. Langdon, E. Liu & J. Shen, 2014. Chironomid-inferred environmental change over the past 1400 years in the shallow, eutrophic Taibai Lake (south-east China): Separating impacts of climate and human activity. The Holocene doi:10.1177/0959683614522308.

Chase, J. M. & M. A. Leibold, 2002. Spatial scale dictates the productivity-biodiversity relationship. Nature 416(6879):427-430 doi:10.1038/416427a.

Clarke, K. R. & R. N. Gorley, 2006. PRIMER v6: User manual/tutorial. PRIMER-E , Plymouth, UK:115 pp.

Connell, J. H., 1978. Diversity in Tropical Rain Forests and Coral Reefs. Science 199(4335):1302-1310 doi:10.1126/science.199.4335.1302.

Cruz, J. V., P. Antunes, C. Amaral, Z. França & J. C. Nunes, 2006. Volcanic lakes of the Azores archipelago (Portugal): Geological setting and geochemical characterization. Journal of Volcanology and Geothermal Research 156(1-2):135-157 doi:10.1016/j.jvolgeores.2006.03.008.

Dodson, S. I., S. E. Arnott & K. L. Cottingham, 2000. The relationship in lake communities between primary productivity and species richness. Ecology 81(10):2662-2679 doi:10.1890/0012-9658(2000)081[2662:TRILCB]2.0.CO;2.

Eggermont, H., P. De Deyne & D. Verschuren, 2007. Spatial variability of chironomid death assemblages in the surface sediments of a fluctuating tropical lake (Lake Naivasha, Kenya). Journal of Paleolimnology 38(3):309-328 doi:10.1007/s10933-006-9075-9.

Flöder, S. & U. Sommer, 1999. Diversity in planktonic communities: An experimental test of the intermediate disturbance hypothesis. Limnology and Oceanography 44(4):1114-1119 doi:10.4319/lo.1999.44.4.1114.

Frey, D. G., 1988. Littoral and offshore communities of diatoms, cladocerans and dipterous larvae, and their interpretation in paleolimnology. Journal of Paleolimnology 1(3):179-191 doi:10.1007/bf00177764.

Frossard, V., L. Millet, V. Verneaux, J.-P. Jenny, F. Arnaud, M. Magny, J. Poulenard & M.-E. Perga, 2013. Chironomid assemblages in cores from multiple water depths reflect oxygen-driven changes in a deep French lake over the last 150 years. Journal of Paleolimnology 50(3):257-273 doi:10.1007/s10933-013-9722-x.

Gabel, F., X. F. Garcia, I. Schnauder & M. T. Pusch, 2012. Effects of ship-induced waves on littoral benthic invertebrates. Freshwater Biology 57(12):2425-2435 doi:10.1111/fwb.12011.

Gonçalves, V., 2008. Contribuição do estudo das microalgas para a avaliação da qualidade ecológica das lagoas dos Açores: fitoplâncton e diatomáceas bentónicas. Universidade dos Açores.

630 Gonçalves, V., H. Marques & P. Raposeiro, 2015. Diatom assemblages and their associated environmental
631 drivers in isolated oceanic island streams (Azores archipelago as case study). Hydrobiologia
632 751(1):89-103 doi:10.1007/s10750-015-2174-8.

633 Graham, J. H. & J. J. Duda, 2011. The Humpbacked Species Richness-Curve: A Contingent Rule for
634 Community Ecology. International Journal of Ecology 2011:1-15 doi:10.1155/2011/868426.

635 Hall, R. I. & J. P. Smol, 1999. Diatoms as indicators of lake eutrophication. In Stoermer, E. F. & J. P. Smol
636 (eds) The Diatoms. Cambridge University Press, Cambridge, 128-168.

637 Hampton, S. E., D. K. Gray, L. R. Izmest'eva, M. V. Moore & T. Ozersky, 2014. The Rise and Fall of Plankton:
638 Long-Term Changes in the Vertical Distribution of Algae and Grazers in Lake Baikal, Siberia. PLoS
639 One 9(2):e88920 doi:10.1371/journal.pone.0088920.

640 Heggen, M. P., H. H. Birks, O. Heiri, J.-A. Grytnes & H. J. B. Birks, 2012. Are fossil assemblages in a single
641 sediment core from a small lake representative of total deposition of mite, chironomid, and plant
642 macrofossil remains? Journal of Paleolimnology 48(4):669-691 doi:10.1007/s10933-012-9637-y.

643 Heiri, O., 2004. Within-lake variability of subfossil chironomid assemblages in shallow Norwegian lakes.
644 Journal of Paleolimnology 32(1):67-84 doi:10.1023/B:JOPL.0000025289.30038.e9.

645 Heiri, O., S. J. Brooks, H. J. B. Birks & A. F. Lotter, 2011. A 274-lake calibration data-set and inference model
646 for chironomid-based summer air temperature reconstruction in Europe. Quaternary Science Reviews
647 30(23-24):3445-3456 doi:10.1016/j.quascirev.2011.09.006.

648 Heiri, O., A. Lotter & G. Lemcke, 2001. Loss on ignition as a method for estimating organic and carbonate
649 content in sediments: reproducibility and comparability of results. Journal of Paleolimnology
650 25(1):101-110 doi:10.1023/A:1008119611481.

651 Heiri, O. & A. F. Lotter, 2001. Effect of low count sums on quantitative environmental reconstructions: an
652 example using subfossil chironomids. Journal of Paleolimnology 26(3):343-350
653 doi:10.1023/a:1017568913302.

654 Heiri, O., A. F. Lotter, S. Hausmann & F. Kienast, 2003. A chironomid-based Holocene summer air
655 temperature reconstruction from the Swiss Alps. The Holocene 13(4):477-484
656 doi:10.1191/0959683603hl640ft.

657 Hill, M. O., 1973. Diversity and Evenness: A Unifying Notation and Its Consequences. Ecology 54(2):427-432
658 doi:10.2307/1934352.

659 Kattel, G. R., R. W. Battarbee, A. Mackay & H. J. B. Birks, 2006. Are cladoceran fossils in lake sediment
660 samples a biased reflection of the communities from which they are derived? Journal of
661 Paleolimnology 38(2):157-181 doi:10.1007/s10933-006-9073-y.

662 King, L., G. Clarke, H. Bennion, M. Kelly & M. Yallop, 2006. Recommendations for sampling littoral diatoms
663 in lakes for ecological status assessments. Journal of Applied Phycology 18(1):15-25
664 doi:10.1007/s10811-005-9009-3.

665 Kovalenko, K. E., S. M. Thomaz & D. M. Warfe, 2011. Habitat complexity: approaches and future directions.
666 Hydrobiologia 685(1):1-17 doi:10.1007/s10750-011-0974-z.

667 Krammer, K. & H. Lange-Bertalot, 1986. Bacillariophyceae 1/2 Naviculaceae. In Ettl, H. (ed) Süsswasserflora
668 von Mitteleuropa. G. Fisher Verlag, Jena, 876.

669 Krammer, K. & H. Lange-Bertalot, 1988. Bacillariophyceae 2/2 Bacillariaceae, Epithemiaceae, Surirellaceae.
670 In Ettl, H. (ed) Süsswasserflora von Mitteleuropa. G. Fisher Verlag, Jena, 576.

671 Krammer, K. & H. Lange-Bertalot, 1991a. Bacillariophyceae. 2/3: Centrales, Fragilariaceae, Eunotiaceae. In
672 Ettl, H. (ed) Süsswasserflora von Mitteleuropa. G. Fisher Verlag, Jena, 576.

673 Krammer, K. & H. Lange-Bertalot, 1991b. Bacillariophyceae. 2/4: Achnanthaceae. In Ettl, H. (ed)
674 Süsswasserflora von Mitteleuropa. G. Fisher Verlag, Jena, 576.

675 Krammer, K. & H. Lange-Bertalot, 2000. Bacillariophyceae. 2/5: English and French translation of the keys.
676 In Ettl, H. (ed) Süsswasserflora von Mitteleuropa. G. Fisher Verlag, Jena, 576.

677 Kurek, J. & L. C. Cwynar, 2009. The potential of site-specific and local chironomid-based inference models
678 for reconstructing past lake levels. Journal of Paleolimnology 42(1):37-50 doi:10.1007/s10933-008-
679 9246-y.

680 Laird, K. R., M. V. Kingsbury & B. F. Cumming, 2010. Diatom habitats, species diversity and water-depth
681 inference models across surface-sediment transects in Worth Lake, northwest Ontario, Canada. Journal
682 of Paleolimnology 44(4):1009-1024 doi:10.1007/s10933-010-9470-0.

683 Lampert, W. & U. Sommer, 2007. Limnoecology - The Ecology of Lakes and Streams. Oxford University
684 Press, New York.

685 Larocque, I., 2001. How many chironomid head capsules are enough? A statistical approach to determine
686 sample size for palaeoclimatic reconstructions. Palaeogeography, Palaeoclimatology, Palaeoecology
687 172(1):133-142 doi:https://doi.org/10.1016/S0031-0182(01)00278-4.

688 Lecointe, C., M. Coste & J. Prygiel, 1993. Omnidia: software for taxonomy, calculation of diatom indices and
689 inventories management. Hydrobiologia 269-270(1):509-513.

690 Luoto, T. P., 2009. A Finnish chironomid- and chaoborid-based inference model for reconstructing past lake
691 levels. Quaternary Science Reviews 28(15-16):1481-1489 doi:10.1016/j.quascirev.2009.01.015.

692 Luoto, T. P. & V. P. Salonen, 2010. Fossil midge larvae (Diptera: Chironomidae) as quantitative indicators of
693 late-winter hypolimnetic oxygen in southern Finland: a calibration model, case studies and
694 potentialities. Boreal Environment Research 15(1):1-18.

695 Maestre, F. T. & J. F. Reynolds, 2006. Spatial heterogeneity in soil nutrient supply modulates nutrient and
696 biomass responses to multiple global change drivers in model grassland communities. Global Change
697 Biology 12(12):2431-2441 doi:10.1111/j.1365-2486.2006.01262.x.

698 Matthews-Bird, F., W. D. Gosling, A. L. Coe, M. Bush, F. E. Mayle, Y. Axford & S. J. Brooks, 2016.
699 Environmental controls on the distribution and diversity of lentic Chironomidae (Insecta: Diptera)

700 across an altitudinal gradient in tropical South America. Ecology and Evolution 6(1):91-112
701 doi:10.1002/ece3.1833.

702 Millet, L., C. Giguet-Covex, V. Verneaux, J.-C. Druart, T. Adatte & F. Arnaud, 2010. Reconstruction of the
703 recent history of a large deep prealpine lake (Lake Bourget, France) using subfossil chironomids,
704 diatoms, and organic matter analysis: towards the definition of a lake-specific reference state. Journal
705 of Paleolimnology 44(4):963-978 doi:10.1007/s10933-010-9467-8.

706 Mittelbach, G. G., C. F. Steiner, S. M. Scheiner, K. L. Gross, H. L. Reynolds, R. B. Waide, M. R. Willig, S. I.
707 Dodson & L. Gough, 2001. What is the observed relationship between species richness and
708 productivity? Ecology 82(9):2381-2396 doi:10.1890/0012-9658(2001)082[2381:WITORB]2.0.CO;2.

709 Moos, M. T., K. R. Laird & B. F. Cumming, 2005. Diatom assemblages and water depth in Lake 239
710 (Experimental Lakes Area, Ontario): implications for paleoclimatic studies. Journal of Paleolimnology
711 34(2):217-227 doi:10.1007/s10933-005-2382-8.

712 Nichols, J. E., M. Walcott, R. Bradley, J. Pilcher & Y. Huang, 2009. Quantitative assessment of precipitation
713 seasonality and summer surface wetness using ombrotrophic sediments from an Arctic Norwegian
714 peatland. Quaternary Research 72:443-451 doi:dx.doi.org/10.1016/j.yqres.2009.07.007.

715 Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, Peter Solymos,
716 M. H. H. Stevens & H. Wagner, 2015. Vegan: Community Ecology Package. R package version
717 2.2.Available at http://CRAN.R-project.org/package=vegan.

718 Oliver, T., D. B. Roy, J. K. Hill, T. Brereton & C. D. Thomas, 2010. Heterogeneous landscapes promote
719 population stability. Ecology Letters 13(4):473-484 doi:10.1111/j.1461-0248.2010.01441.x.

720 Peters, J. A. & D. M. Lodge, 2010. Lake Ecosystem Ecology: A Global Perspective. In Likens, G. E. (ed) Lake
721 Ecosystem Ecology: A Global Perspective. Adademic Press, San Diego, 18-26.

722 Pinder, L. C. V., 1986. Biology of Freshwater Chironomidae. Annual Review of Entomology 31(1):1-23
723 doi:doi:10.1146/annurev.en.31.010186.000245.

724 Ptacnik, R., A. G. Solimini, T. Andersen, T. Tamminen, P. Brettum, L. Lepistö, E. Willén & S. Rekolainen,
725 2008. Diversity predicts stability and resource use efficiency in natural phytoplankton communities.
726 Proceedings of the National Academy of Sciences 105(13):5134-5138 doi:10.1073/pnas.0708328105.

727 Raposeiro, P. M., A. C. Costa & S. H. Hughes, 2011. Environmental factors - spatial and temporal variation of
728 chironomid communities in oceanic island streams (Azores archipelago). Annales de Limnologie -
729 International Journal of Limnology 47(0):325-338 doi:doi:10.1051/limn/2011048.

730 Raposeiro, P. M., S. J. Hughes & A. C. Costa, 2009. Chironomidae (Diptera: Insecta) in oceanic islands: New
731 records for the Azores and biogeographic notes. Annales de Limnologie - International Journal of
732 Limnology 45(2):59-67 doi:10.1051/limn/2009012.

733 Raposeiro, P. M., S. J. Hughes & A. C. Costa, 2013. Environmental drivers – spatial and temporal variation of
734 macroinvertebrate communities in island streams: the case of the Azores Archipelago. Fundamental
735 and Applied Limnology / Archiv fur Hydrobiologie 182(4):337-350 doi:10.1127/1863-
736 9135/2013/0384.

737 Raposeiro, P. M., M. J. Rubio, A. González, A. Hernández, G. Sánchez-López, D. Vázquez-Loureiro, V. Rull,
738 R. Bao, A. C. Costa, V. Gonçalves, A. Sáez & S. Giralt, 2017. Impact of the historical introduction of
739 exotic fishes on the chironomid community of Lake Azul (Azores Islands). Palaeogeography,
740 Palaeoclimatology, Palaeoecology 466:77-88 doi:http://dx.doi.org/10.1016/j.palaeo.2016.11.015.

741 Renberg, I., 1990. A procedure for preparing large sets of diatom slides from sediment cores. Journal of
742 Paleolimnology 4(1):87-90 doi:10.1007/BF00208301.

743 Reynolds, C. S., 1984. The ecology of freshwater phytoplankton. Cambridge University Press, Cambridge,
744 UK.

745 Shannon, C. E. & W. Weaver, 1963. A Mathematical Theory of Communication. University of Illinois Press.

746 Shurin, J. B., S. E. Arnott, H. Hillebrand, A. Longmuir, B. Pinel-Alloul, M. Winder & N. D. Yan, 2007.
747 Diversity-stability relationship varies with latitude in zooplankton. Ecology Letters 10(2):127-34
748 doi:10.1111/j.1461-0248.2006.01009.x.

749 Skinner, D., R. Oliver, K. Aldridge & J. Brookes, 2014. Extreme water level decline effects sediment
750 distribution and composition in Lake Alexandrina, South Australia. Limnology 15(2):117-126
751 doi:10.1007/s10201-013-0422-z.

752 Skov, T., T. Buchaca, S. Amsinck, F. Landkildehus, B. Odgaard, J. Azevedo, V. Gonçalves, P. Raposeiro, T.
753 Andersen & E. Jeppesen, 2010. Using invertebrate remains and pigments in the sediment to infer
754 changes in trophic structure after fish introduction in Lake Fogo: a crater lake in the Azores.
755 Hydrobiologia 654(1):13-25 doi:10.1007/s10750-010-0325-5.

756 Soininen, J. & J. Weckström, 2009. Diatom community structure along environmental and spatial gradients in
757 lakes and streams. Fundamental and Applied Limnology / Archiv fur Hydrobiologie 174(3):205-213.

758 Talling, J. F., 2009. Electrical Conductance — A Versatile Guide in Freshwater Science. Freshwater Reviews
759 2(1):65-78 doi:10.1608/frj-2.1.4.

760 Terry, R. D. & G. V. Chilingar, 1955. Summary of "Concerning some additional aids in studying sedimentary
761 formations," by M. S. Shvetsov. Journal of Sedimentary Research 25(3):229-234
762 doi:10.1306/74D70466-2B21-11D7-8648000102C1865D.

763 Townsend, C. R., M. R. Scarsbrook & S. Dolédec, 1997. The intermediate disturbance hypothesis, refugia, and
764 biodiversity in streams. Limnology and Oceanography 42(5):938-949 doi:10.4319/lo.1997.42.5.0938.

765 van Hardenbroek, M., O. Heiri, M. F. Wilhelm & A. F. Lotter, 2010. How representative are subfossil
766 assemblages of Chironomidae and common benthic invertebrates for the living fauna of Lake De
767 Waay, the Netherlands? Aquatic Sciences 73(2):247-259 doi:10.1007/s00027-010-0173-4.

768 Verbruggen, F., O. Heiri, J. J. MerilÄInen & A. F. Lotter, 2011. Subfossil chironomid assemblages in deep,
769 stratified European lakes: relationships with temperature, trophic state and oxygen. Freshwater
770 Biology 56(3):407-423 doi:10.1111/j.1365-2427.2010.02508.x.

771 Wang, Q., X. Yang, P. Hamilton & E. Zhang, 2012. Linking spatial distributions of sediment diatom
772 assemblages with hydrological depth profiles in a plateau deep-water lake system of subtropical
773 China. Fottea 12(1):59-73 doi:10.5507/fot.2012.005.

774 Yang, H., R. J. Flower & R. W. Battarbee, 2009. Influence of environmental and spatial variables on the
775 distribution of surface sediment diatoms in an upland loch, Scotland. Acta Botanica Croatica
776 68(2):367-380.

777 Zhang, E., Y. Cao, P. Langdon, Q. Wang, J. Shen & X. Yang, 2013. Within-lake variability of subfossil
778 chironomid assemblage in a large, deep subtropical lake (Lugu lake, southwest China). Journal of
779 Paleolimnology 72(1) doi:10.4081/jlimol.2013.e10.

780 Zhang, E., B. Zheng, Y. Cao, G. Gao & J. Shen, 2012. Influence of environmental parameters on the
781 distribution of subfossil chironomids in surface sediments of Bosten lake (Xinjiang, China). Journal of
782 Limnology 71(2):31 doi:10.4081/jlimnol.2012.e31.
783

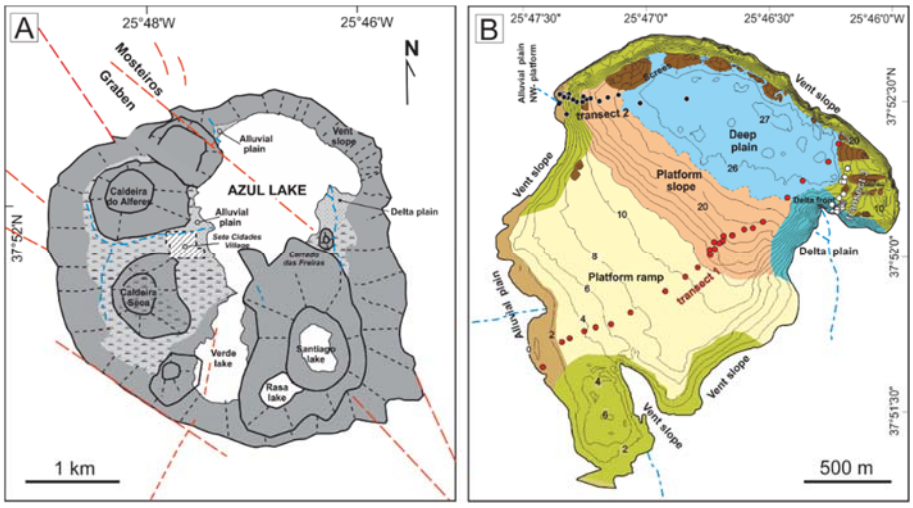

Figure 1 – A) Geographical location of the study lake, Lake Azul, São Miguel, Azores, Portugal. B) Location of the: i) three transects (T) in Azul Lake (dots are sampling sites, black lines with numbers are depths): T1 in red, T2 in black and T3 in grey; ii) main geomorphological units: 1) shallow platform-ramp (sand yellow); 2) platform slope (melon orange); 3) deep plain (sky blue); 4) dip slope (pea green); 5) alluvial littoral (light brown)

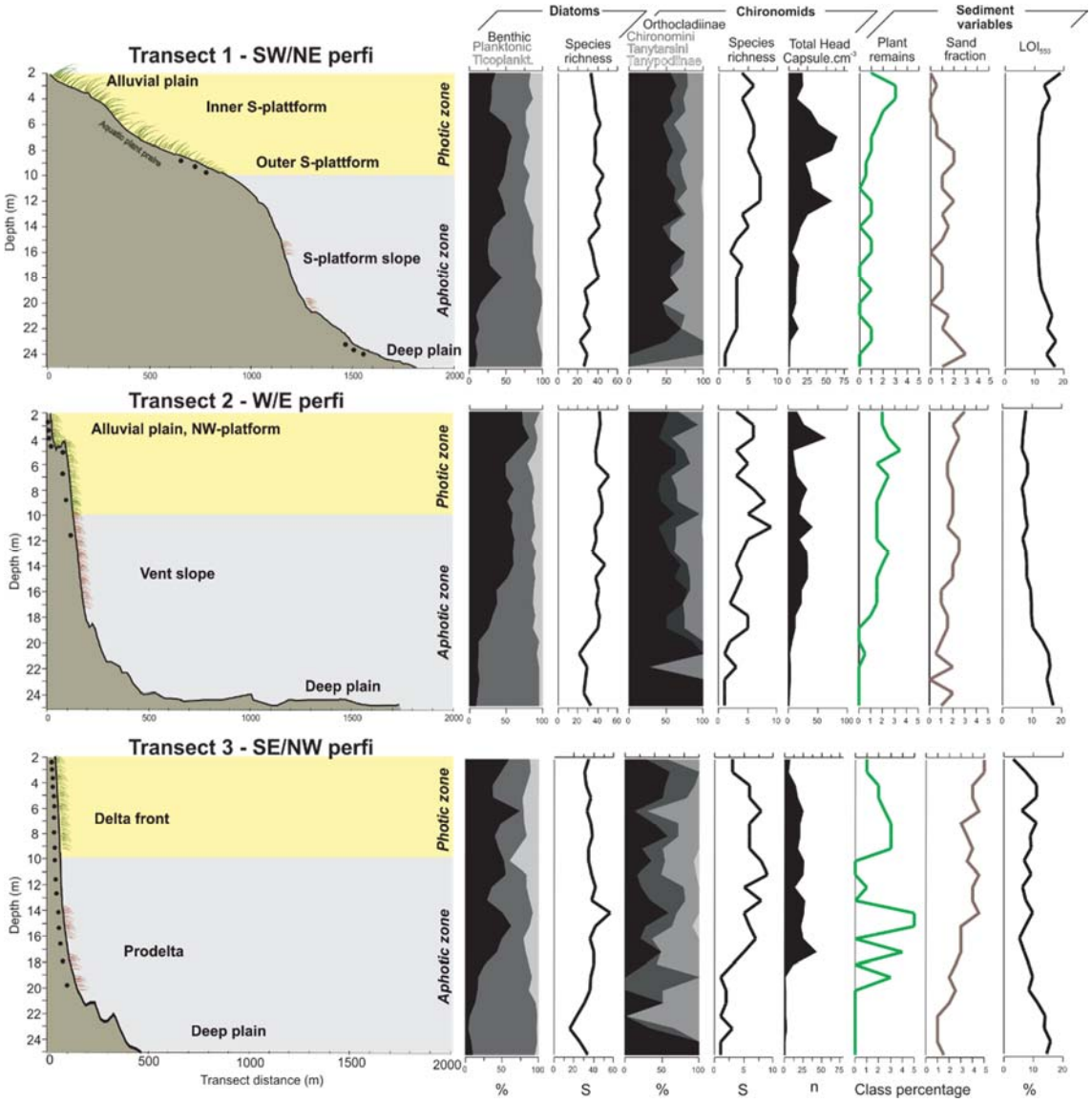

Figure **2** - Schematic profiles of the three transects performed in Lake Azul and subfossil distribution biological assemblages and sediment variables along depth gradient.

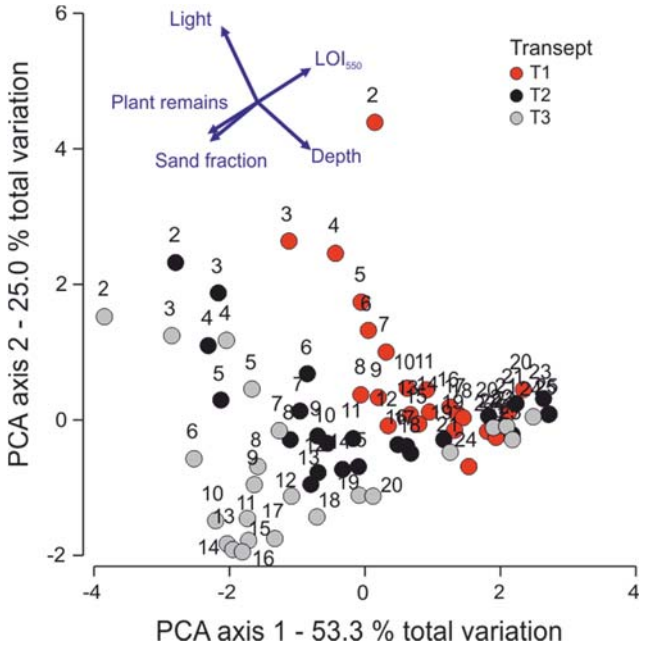

Figure **3** - PCA of all sampling sites in transect 1 (T1), transect 2 (T2) and transect 3 (T3) based on environmental variables showing axis 1 (PCA axis 1) and 2 (PCA axis 2). Solid vectors represent environmental variables, and the length of the vector is the measure of the importance of the variable.

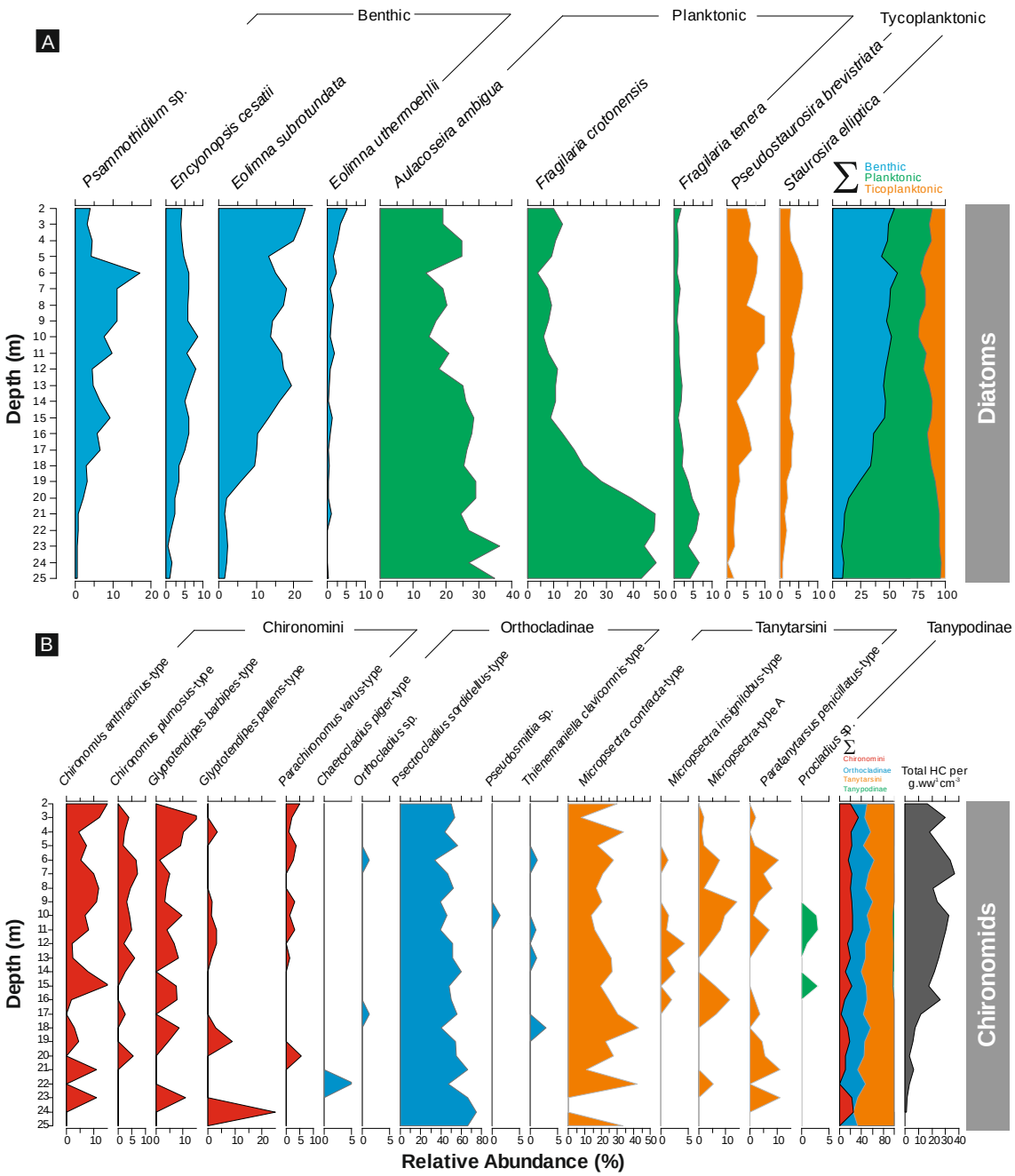

Figure **4** – Mean distribution (average across transects) of the most abundant (**A)** diatoms (>5% of relative abundance; only the 9 most abundant taxa are individually represented and (**B)** chironomids (>5% of relative abundance; the Head Capsule (HC) concentration is also given).

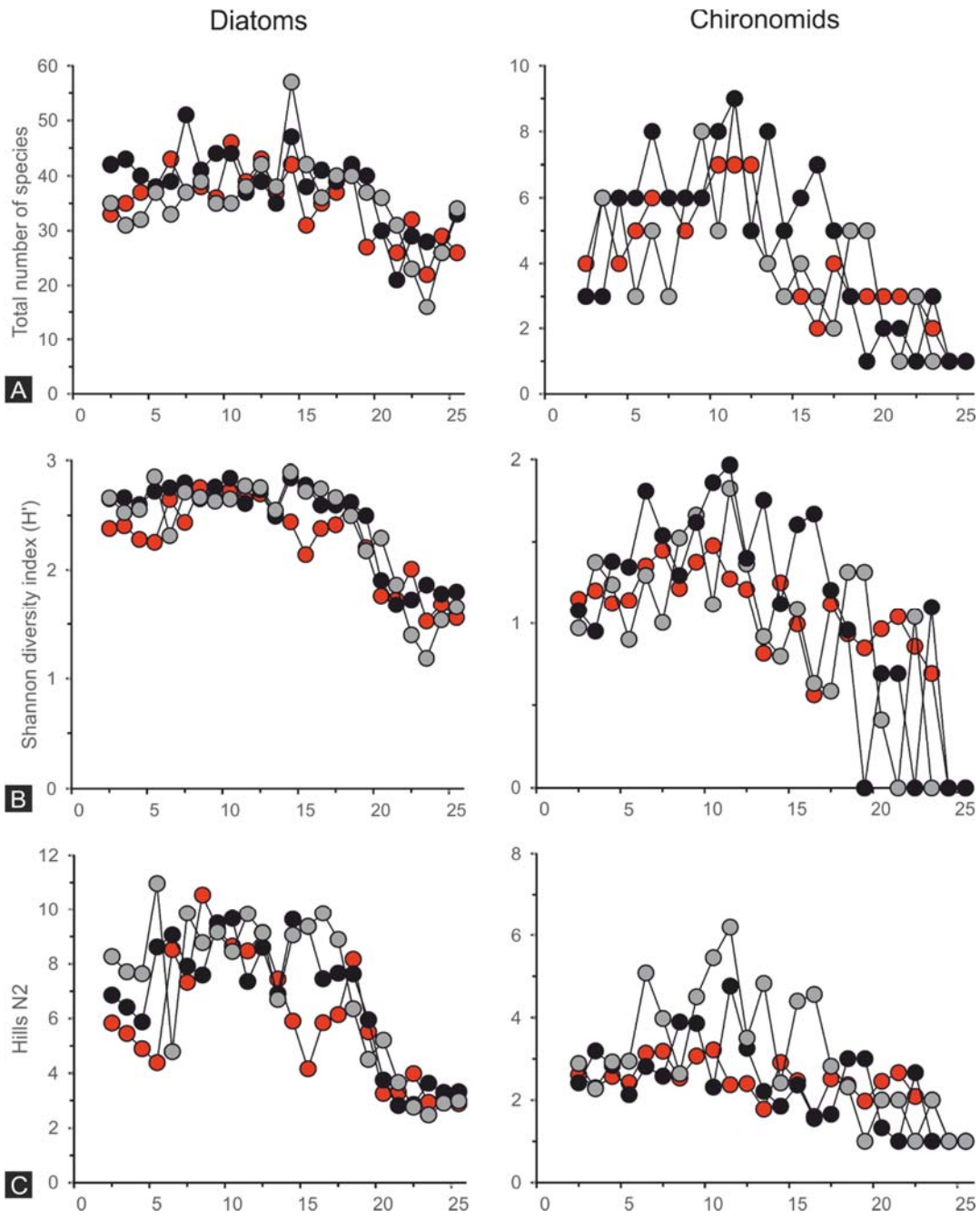

Figure **5** - Summary of species diversity (diatoms and chironomids) for the three transepts: Transect T1, red circle; Transect T2, black circle, Transect T3, grey circle. **A)** Taxon richness as represented by the total number of taxon in each sampling point; **B)** Taxon diversity as represented by Shannon diversity index based on taxa in each sample; **C)** Taxon evenness as represented by Hills N2 based on taxa in each sample

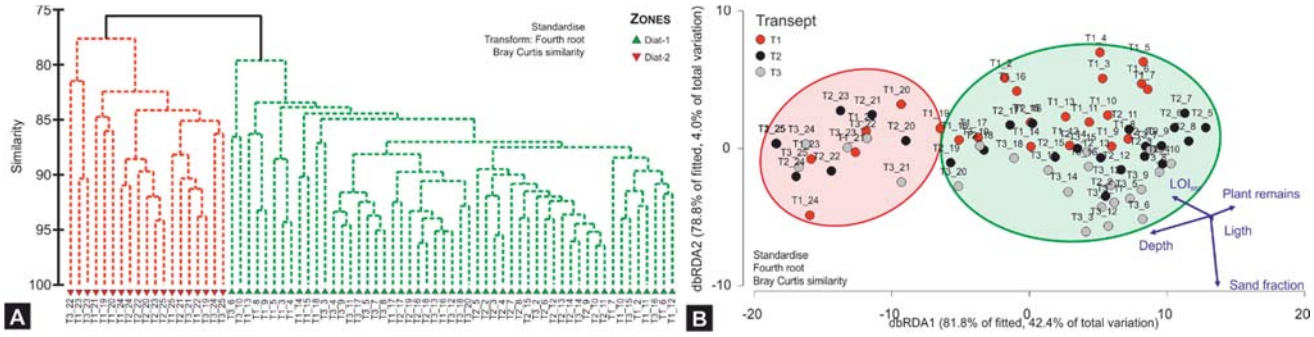

Figure 6 – A) Group-average cluster analysis on subfossil diatom assemblages in Lake Azul surface sediments. The similarity profile (SIMPROF) test ($p < 0.01$) defines non-random zones in the analyses (solid lines); B) Distance-based RDA ordination of first and second fitted axes relating the physical and chemical variables to subfossil diatom assemblages. Vectors projections are given for the physical and chemical variables selected by the DistLM routine. Length and direction of the vectors represent the strength and direction of the relationship.

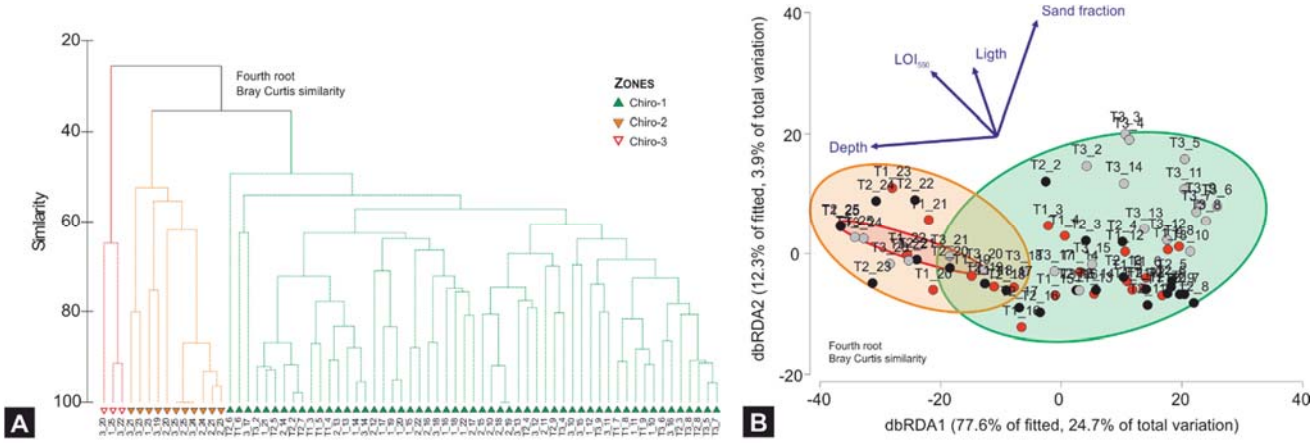

Figure 7 – A) Group-average cluster analysis on subfossil chironomid assemblages in Lake Azul surface sediments. The similarity profile (SIMPROF) test ($p < 0.01$) defines non-random zones in the analyses (solid lines); B) Distance-based RDA ordination of first and second fitted axes relating the physical and chemical to subfossil chiromid assemblages. Vectors projections are given for the physical and chemical variables selected by the DistLM routine. Length and direction of the vectors represent the strength and direction of the relationship.

Table **1** - Water temperature (T), pH, dissolved oxygen (DO), electric conductivity (EC) and light in the water column (data collected as part of the European Water Framework directive - Regional Monitoring Programme - monthly measured *in situ* with multiparametric field probes, during 2009 to 2012)

Depth (m)	**T (ºC)**	**DO (mg.l-1)**	**pH**	**EC (µS.cm-1)**	**Light (Lux)**
1	17.4	10.1	8.1	108.6	764.3
2	17.3	10.2	8.1	105.8	387.5
3	17.2	10.2	8.0	103.4	261.9
4	17.2	10.3	8.0	101.3	233.2
5	17.1	10.2	8.0	99.7	136.3
6	17.0	10.2	8.0	98.5	96.9
7	16.9	10.1	7.9	97.6	53.8
8	16.7	10.0	7.9	97.2	32.3
9	16.6	9.8	7.9	97.2	21.5
10	16.5	9.6	7.8	97.6	3.6
11	16.3	9.4	7.8	98.3	0.0
12	16.1	9.1	7.8	99.5	0.0
13	16.0	8.9	7.7	101.1	0.0
14	15.8	8.5	7.7	103.1	0.0
15	15.6	8.2	7.7	105.5	0.0
16	15.4	7.8	7.6	108.3	0.0
17	15.2	7.4	7.6	111.5	0.0
18	14.9	7.0	7.5	115.1	0.0
19	14.7	6.5	7.5	119.1	0.0
20	14.4	6.0	7.4	123.5	0.0
21	14.2	5.4	7.4	128.3	0.0
22	13.9	4.9	7.3	133.5	0.0
23	13.6	4.3	7.3	139.1	0.0
24	13.3	3.6	7.2	145.2	0.0
25	13.0	3.0	7.2	151.6	0.0
Average	15.7	8.0	7.7	111.6	79.7
Max	24.8	16.7	9.6	149.0	764.3
Min	11.7	0.2	6.5	81.0	0.0

Table 2 - Fits to the linear and quadratic models for diatom and chironomid subfossils along the depth gradient

Fossil assemblage	Linear			Quadratic			
	Model	R^2	R^2_{adj}	Model	R^2	R^2_{ajd}	Hump location (m)
Diatom							
Species richness							
Transept 1	$S=42.10-0.53d^{**}$	0.37	0.34	$S=32.59+1.38d-0.07d^{2***}$	0.61	0.58	9.8^{***}
Transept 2	$S=46.73-0.66d^{***}$	0.45	0.43	$S=39.78+0.73d-0.05d^{2***}$	0.56	0.51	8.1^{**}
Transept 3	$S=37.21-0.28d^{NS}$	0.07	0.02	$S=25.25+2.52d-0.10d^{2**}$	0.43	0.37	12.1^{***}
Shannon diversity							
Transept 1	$H'=2.79-0.04d^{***}$	0.48	0.46	$H'=2.13+0.14d-0.01d^{2***}$	0.80	0.78	9.7^{***}
Transept 2	$H'=3.05-0.04d^{***}$	0.59	0.57	$H'=2.45+0.08d-0.01d^{2***}$	0.83	0.81	8.6^{***}
Transept 3	$H'=3.05-0.05d^{***}$	0.80	0.78	$H'=2.22+0.12d-0.01d^{2***}$	0.80	0.78	9.5^{***}
Evenness Hill's N2							
Transept 1	$N2=8.42-0.18d^{**}$	0.29	0.26	$N2=4.46+0.62d-0.03d^{2***}$	0.60	0.56	10.5^{***}
Transept 2	$N2=9.54-0.21d^{**}$	0.41	0.38	$N2=5.32+0.64d-0.03d^{2***}$	0.76	0.74	10.2^{***}
Transept 3	$N2=10.59-0.26d^{**}$	0.47	0.45	$N2=6.47+0.56d-0.03d^{2***}$	0.72	0.69	9.2^{***}
Chironomid							
Taxon richness							
Transept 1	$S=6.72-0.19d^{***}$	0.53	0.51	$S=4.54+0.31d-0.02d^{2***}$	0.68	0.65	7.7^{***}
Transept 2	$S=6.33-0.18d^{**}$	0.34	0.31	$S=3.85+0.32d-0.02d^{2**}$	0.48	0.43	8.7^{**}
Transept 3	$S=7.47-0.21d^{**}$	0.35	0.32	$S=3.03+0.68d-0.03d^{2***}$	0.67	0.64	10.3^{***}
Rarefied taxon richness ($R_{S=5}$)							
Transept 1	$S=3.06-0.04d^{***}$	0.37	0.35	$S=2.54+0.06d-0.00d^{2***}$	0.51	0.46	8.3^{**}
Transept 2	$S=3.34-0.07d^{**}$	0.38	0.35	$S=2.43+0.11d-0.01d^{2**}$	0.52	0.48	8.4^{**}
Transept 3	$S=4.14-0.10d^{**}$	0.53	0.51	$S=3.13+0.10d-0.01d^{2***}$	0.64	0.61	6.8^{***}
Shannon diversity							
Transept 1	$H'=1.53-0.04d^{***}$	0.51	0.50	$H'=1.02+0.06d-0.01d^{2***}$	0.70	0.67	8.3^{***}
Transept 2	$H'=1.61-0.05d^{***}$	0.44	0.41	$H'=0.94+0.09d-0.01d^{2***}$	0.60	0.57	8.5^{***}
Transept 3	$H'=1.87-0.05d^{***}$	0.40	0.37	$H'=0.85+0.15d-0.01d^{2***}$	0.69	0.66	9.9^{***}
Evenness Hill's N2							
Transept 1	$N2=3.05-0.05d^{**}$	0.37	0.34	$N2=2.39+0.08d-0.01d^{2***}$	0.50	0.45	8.3^{**}
Transept 2	$N2=3.51-0.08d^{**}$	0.33	0.31	$N2=2.51+0.12d-0.01d^{2**}$	0.44	0.39	8.0^{**}
Transept 3	$N2=4.57-0.11d^{**}$	0.27	0.24	$N2=2.00+0.41d-0.02d^{2***}$	0.58	0.54	10.6^{***}

Table 3 - Summary of DistLM procedure for physical and chemical variables for subfossil diatoms and chironomids.

	Diatoms				Chironomids			
Variable	SS (trace)	Pseudo-F	P	Prop.	SS(trace)	Pseudo-F	P	Prop.
Plant Remains [d]	2307.9	7.0	0.001	0.17	10407	8.6	0.001	0.11
Sand fraction [c,d]	1244.9	14.1	0.002	0.09	7580	6.0	0.001	0.08
LOI 550 [d]	3434.0	23.3	0.001	0.25	12698	10.7	0.001	0.14
Depth [c,d]	4616.5	35.5	0.001	0.34	16438	14.6	0.001	0.18
Light [c,d]	880.1	4.8	0.006	0.07	2729	2.0	0.06	0.03

Variables selected by Step-wise procedure for: [d] diatoms and [c] chironomids

Table **4** – ANOSIM analysis for subfossil diatom and chironomid assemblages in Azul lake results when testing for differences between transects (Significance $^{*}p<5\%$ and $^{**}p<1\%$*)*

	Diatom assemblages		**Chironomid assemblages**	
Tests	R-value	Significant level %	R-value	Significant level %
Global	0.101	0.1^{**}	0.131	0.1^{**}
Transect 1 vs Transect 2	0.167	0.3^{**}	0.167	0.2^{**}
Transect 1 vs Transect 3	0.062	0.3^{**}	0.062	2.4^{*}
Transect 2 vs Transect 3	0.17	1.5^{*}	0.17	0.1^{**}