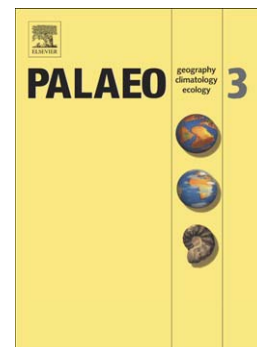


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Impact of the historical introduction of exotic fishes on the chironomid community of Lake Azul (Azores Islands)

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Running head: Impact of top predators introduction in a natural lake

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Abstract (300 words)

Little is known about the effect of top predator introduction in historically fishless communities, especially on remote islands. This issue is important because it might strongly affect climate reconstructions derived from biota assemblages such as chironomids. Head capsule larval remains of chironomids have been studied in a 660 years lacustrine sedimentary sequence from Lake Azul (Sao Miguel Island, Azores archipelago) to assess the extent and timescale of the effect of the predator introduction occurring in this historically fishless lake. Analysis of similarity showed that the chironomid assemblage was statistically different before and after predator introduction ($R=0.78$; $p<0.001$). Abundance of chironomids was about 40% greater in the fishless lake period compared to the period in the presence of predator. Results show major change in chironomid assemblage coinciding with the first time of goldfish introduction (around 1790 AD), followed by carp (1890 AD) and pike (1979 AD) introductions. The composition of feeding group guilds changed following a pattern characterized by a decrease in abundance of detritivorous and predaceous taxa and an increase in abundance of grazing chironomid taxa. This study suggests that predator introduction was the most important factor affecting the chironomid assemblages in this natural, Azorean fishless lake, but predators did not affect all chironomid species. Other external forcings like major climate oscillations, anthropogenic activities in the catchment basin, and volcanic eruptions seem to play an additional role. The latest stage of the warm and arid Medieval Climate Anomaly (1000 - 1300 AD) favoured the occurrence of some warm-adapted chironomid taxa, which were absent through the Little Ice Age (ca. 1450 - 1850 AD) cool period.

1. Introduction

Today it is accepted that the introduction of non-native species is one of the main threats to autochthonous biodiversity globally and one of the major reasons for the worldwide biodiversity decline (e.g. Mooney and Cleland, 2001; Vitousek et al., 1996). Among these introductions, there is the particular case of freshwater fishes' introductions into highly restricted, susceptible and vulnerable environments, such as lakes (Fritts and Rodda, 1998; Gurevitch and Padilla, 2004; Sax et al., 2002). The disastrous ecological consequences of these introductions are direct and indirect impacts on food web structure and ecosystem function (Findlay et al., 2005). In lakes, a strong effect of top predators is common, with direct impacts on the diversity and abundance of prey populations (e.g., native fishes or invertebrates) and cascading effects down to lower trophic levels (Bystrom et al., 2007; Findlay et al., 2005). For example, introducing planktivore fishes in a fishless lake can decrease zooplankton densities, lowering grazing pressure on phytoplankton and resulting in increased chlorophyll *a* concentration and consequently reduced water clarity (Buchaca et al., 2011; Findlay et al., 2005; Skov et al., 2010). Also, increased nutrient availability through sediment disturbance and excretion by fish has direct effects on primary producers (quantity and diversity) and subsequent bottom-up consequences on food webs (Du et al., 2015). On the other hand, climate reconstructions developed from transfer functions that use biological assemblages, such as chironomids and diatoms (Fritz et al., 1991; Heiri et al., 2014), assume that the main driver that triggers the observed oscillations in their assemblages is climate fluctuations. Therefore, the introduction of top predators in lacustrine ecosystems might have significant effects on these climate reconstructions. To our best knowledge, these possible effects have not been properly assessed.

Oceanic islands are considered fragile ecosystems and highly vulnerable to biological invasion such as introduction of non-native species (Sax and Gaines, 2008). The Azores is a remote oceanic archipelago located in the middle of the Atlantic Ocean where fishless lakes were common landscape features before human settlement.

In the Azores archipelago, biodiversity impairment is a well-recognized consequence of biological invasions resulting from man-mediated introductions, especially of terrestrial flora (Silva et al., 2008). The impact of several fish introductions made between the late 19th and early 20th centuries (Flor de Lima, 1993; Vicente, 1956) in the naturally fishless Azorean lakes is poorly studied (Skov et al., 2010) although ecological problems in Azorean freshwater lakes are known to be a consequence of the recent introduction of exotic species such as the weed *Egeria densa*, crayfish *Procambarus clarkii* (Costa et al., 1996), and the freshwater limpet *Ferrissia fragilis* (Raposeiro et al., 2011a). Azorean lakes have simple food webs and low diversity typical of remote island systems (Matias et al., 2016; Raposeiro et al., 2012, 2016). Chironomids, one of the most abundant and diverse families of freshwater macroinvertebrates in the Azores archipelago (Raposeiro et al., 2011b; Raposeiro et al., 2009), are an important element in aquatic food webs, constituting approximately 50% of secondary production in lakes (Armitage et al., 1995). Consequently, chironomids play a crucial role in benthic food webs and in the trophic structure of ecosystems (Armitage et al., 1995). Chironomids are important prey for fish, but also play a key role as macroinvertebrate predators (Armitage et al., 1995). For example, the predaceous *Procladius culiciformis* (Linnaeus) exhibited a positive feeding preference for ostracods, cladocerans and chironomids, and a negative choice for rotifers (Vodopich and Cowell, 1984). Chironomids are also sensitive to a variety of disturbances of anthropogenic and natural origin including changes in land use (Belle et al., 2016; Raposeiro et al., 2011b), climate (Heiri et al., 2011; Verbruggen et al., 2011), nutrient input (Brooks et al., 2001; Langdon et al., 2010), and predation pressure (Gilinsky, 1984; Goyke and Hershey, 1992; Skov et al., 2010).

The chitinised head capsules of chironomid larvae are well preserved in lake sediments (Skov et al., 2010), which make chironomids useful indicators of past environmental conditions (Walker et al., 1995; Cao et al., 2014) allowing us to uncover the main paleoecological and paleolimnological oscillations that have taken place in lake ecosystems. They can be useful proxies for past introductions and changes in fish population as the subfamily Tanypodinae

includes both free-swimming and crawling predators (Vallenduuk and Moller Pillot, 2007) whose abundances are largely influenced by fish predation (Skov et al., 2010). Despite the potential of fossil chironomids as proxies for past fish introductions, there has been scarce information to reconstruct ecological conditions for this indicator group based on information from sedimentary archives (Skov et al., 2010).

In spite of morphological similarities of chironomid larvae, they display more differences in feeding strategies than any other aquatic insect group, and they can be grouped in several general feeding categories (Berg et al., 1995). Feeding groups (e.g. predators, grazers, detritivores) have been assigned for each taxon as in Wilson and Ruse (2005) and more recently in the work of Moller Pillot for the Netherlands and Adjacent Lowlands (Moller Pillot, 2009, 2014; Vallenduuk and Moller Pillot, 2007). The classification of chironomid larvae into functional feeding groups is a useful tool that enhances our understanding of lake nutrient cycling and trophic interactions that impact lake integrity and function (Blocksom et al., 2002). Moreover, changes in the trophic state of a lake are often followed by shifts of chironomid assemblages (Frossard et al., 2014; Frossard et al., 2013).

In this study, we address the effects of the introduction of top predators (fish) in Lake Azul (Azores archipelago) using a paleoecological approach to quantify chironomid remains in lake sediments before and after the historical introduction of predators. We hypothesized that the introduction of predators would lead to (i) a decrease in head capsule abundance of chironomids in sediments, due to direct predation on chironomid assemblages (Skov et al., 2010), (ii) an alteration in the composition of chironomid assemblages (Johnson et al., 1990), and (iii) an alteration in the functional diversity of feeding guilds of chironomid assemblages (Armitage et al., 1995).

1.1 Lake Azul geographic and limnological settings

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 (Fig. 1a, b). At the western

end of São Miguel Island lies the Sete Cidades volcanic caldera (Fig. 1c) which is occupied by eight craters, four of them partially filled by lakes. The greatest lakes inside the caldera are Lake Azul and Lake Verde, which are hydrologically connected but separated as sedimentary basins by a shallow and narrow substratum threshold. Lake Azul has a surface area of 4.35 km², 28 m of maximum depth and is located at 259 meters above sea level. The lake basin has two main morphological areas (Fig. 1d): (1) a shallow platform (from 0 to 12 m depth) occupying the southwestern part of the lake, and (2) a deep plateau, 25 - 28 m deep, in the northeastern part of the lake.

Limnological data for Lake Azul covers the last 30 years, with a more complete and detailed dataset for the last 10 years (Table 1). Based on these observations and data from Medeiros et al. (1983) and Gonçalves (2008), Lake Azul is a mesotrophic, monomictic lake, with a well-developed thermocline between April and September at 11-20 m of water depth. The macrophyte community is dominated by the exotic *Egeria densa*, followed by the native *Myriophyllum alterniflorum* in the shallow zones of the lake. The recent phytoplankton communities are dominated by green algae (Chlorophyceae and Zygnematophyceae) and diatoms (Bacillariophyceae). The most dominant planktonic diatom is *Aulacoseira ambigua*. A diverse benthic diatom community is present in epilithic and epiphytic littoral habitats. *Achnantheidium minutissimum*, *Encyonopsis cesatii*, *Navicula notha* are the most abundant benthic diatoms (Gonçalves, 2008). Chironomids dominate the benthic invertebrates, followed by Oligochaeta and by invasive gastropods (Raposeiro et al., 2011a; Raposeiro et al., 2016).

1.2. Historical data on fish introductions in the lake

Since the establishment of the first settlers on São Miguel Island, lakes have been affected by anthropogenic disturbances, including predator introductions (see Table 2). According to Kottelat and Freyhof (2007), the goldfish *Carassius auratus* (Linnaeus, 1758) may have arrived from the Portugal mainland in 1611 AD and the first reference in Azorean literature was in 1792 AD for Furnas Lake (Valois e Silva, 1886). These authors say “a few years ago, someone introduced the fish that existed in tanks into the surrounding lakes, which have been

reproducing considerably”. Later, Godman (1870) stated: “At some time these lakes (Sete Cidades lakes and Furnas Lake) have been stocked with Goldfish (*Cyprinus auratus*), which have since increased in number that they literally swarm so that I can hand-catch them easily”. The fish stocking in Azorean lakes started in 1879 AD and Lake Azul was not an exception (Vicente, 1956). In this lake, *Achondrostoma oligolepis* (Robalo, Doadrio, Almada & Kottelat, 2005) was introduced, followed by *Salmo trutta fario* Linnaeus, 1758 and *Salmo stomachicus* Günther, 1866, in 1880 AD and 1882 AD, respectively. Later, in 1889 AD, *Salmo trutta lacustris* Linnaeus, 1758 was introduced. According to Moreira da Silva and Cabral (1983), these latter three species did not become established due to the lack of spawning zones. Two varieties of carp (common and mirror carp - *Cyprinus carpio carpio* Linnaeus, 1758) were introduced in Lake Azul in 1890 AD. The last introductions in the nineteen-century were the largemouth black bass, *Micropterus salmoides* (Lacepède, 1802) and the European perch, *Perca fluviatilis* Linnaeus, 1758, in 1898 AD. Later, regional forest services (Junta Geral Distrital de Ponta Delgada), introduced in 1941 AD the rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), but this species did not become established (Moreira da Silva and Cabral, 1983). The same institution twice introduced the Northern pike, *Esox lucius* Linnaeus, 1758, in 1979 AD and 1991 AD for recreational purposes. The Pike-perch - *Sander lucioperca* (Linnaeus, 1758), was also introduced by these services in 1981 AD, without success (Flor de Lima, 1993). The last successful introduction was conducted in 1982 AD with the transfer of Roach, *Rutilus rutilus* (Linnaeus, 1758), from Lake Furnas (where it was introduced in 1885 AD) to Lake Azul (Moreira da Silva and Cabral, 1983). To our knowledge, no data are available on the abundance in terms of biomass, on fish communities of Lake Azul, however, *C. carpio* is the most abundance species, in similar Azorean lakes (Bio et al., 2008; Skov et al., 2010).

2. Material and methods

2.1. Coring, sampling and organic chemistry

A coring campaign in Lake Azul was conducted in September 2011 and a total of 15 cores, up to 1.70 m long, were retrieved using a 60 mm diameter UWITEC gravity corer from UWITEC floating platform. All cores were sealed, transported to the laboratory and kept in a cold room at +4 °C until their opening.

All cores were split longitudinally in two halves and lithologically described to establish the main sedimentological features, and lithologically correlated in order to select the best core to conduct the paleoenvironmental reconstructions (Rubio-Inglés et al., submitted). Core AZ11_02 (1.70 m long) located in the deep basin of the lake (Fig. 1d), was chosen to perform lithological, geochemical, chronological and biological analyses. Core AZ11_02 was photographed using the high-resolution CCD camera of the XRF AVAATECH core scanner of the Universitat de Barcelona (UB, Spain) and subsampled to characterize the Lake Azul sediments using a multiproxy approach.

Samples for bulk organic geochemistry were obtained every 1 cm, manually ground using an agatha mill and analyzed for total carbon (TC) and total nitrogen (TN) (RSD, 5% of the measurements) using a Finnigan delta Plus EA-CF-IRMS spectrometer at the Centres Científics i Tecnològics of the Universitat de Barcelona (SCT-UB). Additionally, mineralogical analyses were carried out every centimeter by X-ray diffraction (XRD) using an automatic X-ray diffractometer SIEMENS-D500, Cu-K α , 40 kV, 30 mA and graphite monochromator at the Institute of Earth Sciences Jaume Almera (ICTJA-CSIC, Spain) in order to check the carbonate content of the sediments. The carbonate content was below the detection limit of the XRD, and consequently, TC was considered to be equal to Total Organic Carbon (TOC).

The chronology model was based on both a ^{210}Pb profile and five radiocarbon AMS dates (Fig. 2). The ^{210}Pb in excess activity was determined by alpha spectrometry at the Department of Physics, Institute of Science and Environmental Technology (Autonomous University of Barcelona) for the first 21 cm of the AZ11_02 core in order to obtain the sedimentation rate changes for the last 100 years. The age model of the rest of the core was built using five AMS ^{14}C dates analyzed at Beta Analytic Inc. (Miami, FL, USA). The radiocarbon dates were

calibrated using the CALIB 7.0 and the IntCal13 curve (Reimer et al., 2013) and selecting the median of the 95.4 % distribution (2σ probability interval). Owing to the lack of plant macrofossil remains, concentrated bulk palynological material was selected for radiocarbon dating.

2.2. Lake sedimentary record and dating

The entire lacustrine sedimentary sequence of Lake Azul was 1.7 m long in the deep offshore part of the lake, overlying a hard layer of volcanic rocks unit (ash and lapilli). Three very thin muddy lacustrine layers, between 133 cm and 108 cm of core depth, were found interbedding the uppermost part of this volcanoclastic unit (Rubio-Inglés et al., submitted) (Fig. 3). Over this, volcanic deposits and, from the base to the top of the sedimentary record, three lithological lacustrine units were recognized. Unit 2 (from 103 to 85 cm) is composed of light grey laminated mud, rich in volcanic particles. This unit is interpreted as fine clastic deposits reworked from previously deposited volcanic ash sediments in the catchment. Unit 3 (from 85 to 61 cm) is formed from brown-greenish laminated fine to coarse silts and brown mud with frequent dark layers rich in terrestrial vascular plant debris and barren of chironomids and diatoms. Unit 3 has been interpreted as relatively shallow lacustrine deposits. Unit 4 (from 61 to top of the sequence) is made up of strongly to poorly laminated light silty clays. This last unit has been interpreted as relatively deep fine lacustrine deposits interbedding lobe deposits generated by gravity currents, as flood events favoured in the steep lake margins by the strong precipitation events and by human activities in the catchment.

The chronological model was built using a new age-depth modelling approach called Dynamic Age Model (DAM), based on both ^{210}Pb profile and radiocarbon dates (Rubio-Inglés et al., submitted). The DAM calculates the age of the samples of a given historical sequence distributing the time along the profile according to the amount of terrigenous material present in the samples. The radiocarbon date of the base of Unit 2 indicates that the lacustrine sedimentary sequence accumulated over the last ca 660 cal. years BP (Fig. 2). Furthermore, this radiocarbon

date also confirms that uppermost volcanic unit corresponds to the last recorded eruption in Sao Miguel island dated at 660 +/- 60 years BP (Queiroz et al., 2008).

2.3. Chironomid analyses

Analysis of the chironomid larval head capsules was conducted on subsamples of 2 cm³, following the procedure described by Brooks et al. (2007). The samples were deflocculated in 10 % KOH, heated to 70 °C for 5 min, sieved and separated into two size fractions (90 and 212 µm). Head capsules (HC) were sorted under a stereo-microscope (40x magnification – Zeiss Stemi). Chironomid larval head capsules were mounted with Euparal mounting medium and identified using a microscope at 100x–400x magnification (ZEISS AXIOIMAGE A1).

Identification of HC was mainly based on mentum characteristics, as described in Brooks et al. (2007), and was done to the lowest taxonomic resolution, usually species morphotypes, using Brooks et al. (2007) taxonomical nomenclature. The relative abundance of each taxon was presented as a percentage of the total abundance in each centimeter.

2.4. Statistical analyses

The multivariate analyses were performed in PRIMER v6.0 (Clarke and Gorley, 2006). Absolute abundance data was fourth root transformed prior to analyses to reduce the influence of very abundant taxa (Clarke and Gorley, 2006), and a resemblance matrix was subsequently formed using a Bray–Curtis measure. Cluster analysis was used to identify groups of chironomid assemblages and a SIMPROF test (test for significant sign of assembly among samples that have no pre-defined grouping) was applied to detect significant zonations. The null hypothesis of no internal group assembly in the full set of samples was rejected when the significance level (p-value) was < 0.01. ANOSIM was used to test for significant differences between, before and after fish introduction groups and to assess the differences between the distinct groups of introductions (see Table 2). The groups were defined according to the timing of introduction of fish species or fish groups (in close temporal proximity - e.g. Carp and Pike group), based on historical literature. These introductions were grouped in four general

categories (Table 2). Fishless group (115-36 cm), defined by the absence of fish communities (115-36 cm); Goldfish group (35-20 cm), defined by the introduction of the *Carassius auratus* around 1792 AD; Carp group (20-8 cm), defined by the introduction of *Achondrostoma oligolepis*, *Salmo stomachicus*, *Salmo trutta lacustris*, *Cyprinus carpio*, *Micropterus salmoides* and *Perca fluviatilis*; and Pike group (7-0 cm) defined by the introduction of *Esox lucius*, *Sander lucioperca* and *Rutilus rutilus*. ANOSIM is a non-parametric analysis which tests for whether differences among samples within pre-defined groups. According to Clarke and Gorley (2006), R values range between -1 and 1; a value of 1 indicates high levels of within group similarity, while -1 indicates that similarity levels between samples across groups are higher than within groups.

PERMDISP compares the variation in measures of assemblage similarity between groups (Anderson and Braak, 2003; Anderson et al., 2008). Low dispersal reveals more similar assemblages across sampling intervals within that group while groups with more variable assemblage similarity measures indicate more heterogeneous chironomid assemblages. SIMPER analysis was applied to the whole data matrix to identify the species contributing the most to the formation of the clusters of taxonomic groups (Clarke and Gorley, 2006). Principle Coordinates Ordination (PCO) using Bray-Curtis similarity was used to visualize the structure of chironomid assemblage and feeding groups patterns in the sediment record. The feeding groups were reported for each taxon based on the recent studies on chironomid autecology (Moller Pillot, 2009, 2014; Vallenduuk and Moller Pillot, 2007; Wilson and Ruse, 2005).

For data analyses, some samples of Unit 1 (between 104 and 115 cm of core depth) and samples from the bottom of Unit 2 - reworked volcaniclastic material (from 93 to 103 cm of core depth), as well as the samples from flood event deposits (between 40 and 44 cm and at 53, 58, 60 and 63 cm of core depth - see Fig. 3), were removed because all of these deposits are considered to be instantaneously deposited due to flash flood processes (Rubio-Inglés et al., submitted).

3. Results

3.1. Chironomid assemblages in Lake Azul sediments

A total of 19 Chironomidae taxa distributed among 15 genera and 3 subfamilies were identified from the head capsules present in the sediments of core AZ11-02 (Fig. 3). The Orthocladiinae and Chironominae showed the greatest richness (9 and 8 taxa, respectively) followed by Tanypodinae (2 taxa). *Micropsectra contracta*-type (occurred in 84% of samples), *Psectrocladius sordidellus* type (67%) and *Micropsectra* type A (64%) were the dominant taxa in the studied core. Subdominant taxa varied substantially but generally included *Polypedilum nubifer*-type, *Macropelopia*-type and *Chiromonus plumosus*-type.

Abundance of chironomid HC showed large variations along the core, ranging from 0 to 127 head capsules per cubic centimetre (HC.cm^{-3}) of sediment (mean of 5.4 HC.cm^{-3}). Also, chironomid taxon richness exhibited large variation along the core, with a maximum number of 9 taxa (65 and 83 cm core depth) and a minimum of 0, during both the flood and volcanic instantaneous events. The cluster analysis indicated a split into two significantly different zones (Fig. 4, SIMPROF Global test $\pi = 5.29$, $p < 0.01$ – core depth 37 cm). SIMPER analysis revealed a dissimilarity of 63.4% between these two zones (Table 3). *Micropsectra contracta*-type (22.7% - Zone 1), *Micropsectra* type A (15.6% - Zone 1), *Paratanytarsus penicillatus*-type (13.7% - Zone 2), *Psectrocladius sordidellus*-type (13.4% - Zone 2) and *Chiromonus plumosus*-type (8.2% - Zone 2) were the species that contributed most to the dissimilarity.

The differences in assemblage composition between the two zones were further supported by the PCO analyses. The first two PCO axes explained 82.6% of total variation (Fig. 5a). The first axis of the ordination (71.3% of total variation) is the only significant axis and it separates the two different zones revealed by the SIMPROF. Therefore, the two chironomid zones are:

Zone 1: ca. 1290 – ca. 1780 AD (115-37 cm)

Zone 1 comprises the lower part of the core which corresponds to lithological units 1, 2 and 3 with 69.5% average similarity (Table 3). The zone is characterized by the dominance of oligotrophic-mesotrophic, oxyphilous and detritivore species, such as *Micropsectra contracta*-

type (48.9%) and *Micropsectra* type A (24.6%), as revealed by the negative PCO1 scores.

Assemblages also included the predator *Macropelopia* type (10.2%) and the detritivore *Polypedilum nubifer*-type (4.7%). Head capsules abundances are generally high in this zone, ranging from 9 to 127 HC.cm⁻³ and an average of 34 HC.cm⁻³ (Fig. 3).

On the other hand, Shannon Diversity (H') and Pielou's evenness (J') indexes are generally low in this zone with an average of 1.3 and 0.8, respectively. These results indicate dominance by relatively few taxa.

The percentage of TOC of this zone is low, ranging from 0.5 to 1.6 % wt and an average of 0.9 % wt (Fig. 6). In the bottom sediments TOC content increases gradually toward the top of this zone. Both the percentage and trend of nitrogen (TN) are similar to that of TOC (Fig. 6). With an average of 0.1% the percentage of nitrogen content ranges from 0.1 to 0.2 %. According to historical sources and the chronological model of the core, all samples from this zone were deposited during the period that fish communities were absent.

Zone 2: ca. 1780 – ca. 2011 AD (37-0 cm)

Zone 2 corresponds to the uppermost part of the studied core, mostly formed by sediments of the lithological unit 4 and with an average similarity of 66.6%. Several important and abrupt changes occur in Zone 2. A progressive decrease in the concentration of HC, reflected by the decrease of detritivorous taxa, such as *Micropsectra contracta*-type and *Micropsectra* type A (from 48.9% to 22.8%; from 24.6% to 3.9%), was observed. These taxa were gradually replaced by the detritivore *Psectrocladius sordidellus*-type (22.7%) and the grazer *Paratanytarsus penicillatus*-type (19.3%). In addition, an increase of the detritivore/grazers, hypoxia-tolerant, eutrophic species like *Chiromonus plumosus*-type (11.82%) and *Glyptotendipes pallens*-type (10.5%) was observed and associated with positive PCO1 scores. This biostratigraphic zone is also characterized by the appearance of other eutrophic taxa: *Parachironomus varus*-type (2.7%) and *Cricotopus sylvestris*-type (0.7%), which is reflected in the increase of the Shannon Diversity index (H'), to an average of 1.5. An increase in the Pielou's evenness (average=0.9)

was also observed, reflecting taxonomic homogenization. The TOC percentages of this zone are higher than that of Zone 1, ranging from 1.5 to 2.4 % wt, with an average of 2.4 % wt. A similar pattern was observed for TN. According to both the chronological model of the core and the historical sources, sediments of all samples contained in this zone were deposited within the period where the fish communities were present.

3.2. Community relationships between chironomids and predator introduction

3.2.1. Taxonomic composition

ANOSIM analysis showed that the chironomid assemblages were statistically different before and after predator introduction ($R=0.88$; $p<0.001$). Pairwise comparison revealed that the first introduction (*Caurassius auratus*) around 1790 resulted in highly significant differences in chironomid assemblages ($R=0.82$; $p<0.001$; Table 4) and high dissimilarities between the Fishless vs Goldfish group (53.2%). The comparison between the Fishless and the Goldfish groups identified five good discriminating taxa: *Micropsectra* type A, *Paratanytarsus penicillatus*-type, *Macropelopia* type and *Glyptotendipes pallens*-type. Average abundance of *Paratanytarsus penicillatus*-type and *Psectrocladius sordidellus*-type increased (0.1 HC.cm^{-3} vs 1.3 HC.cm^{-3} and 0.8 HC.cm^{-3} vs 1.2 HC.cm^{-3} , respectively) after the introduction of goldfish, while the *Glyptotendipes pallens*-type was absent in the fishless group (0.0 HC.cm^{-3} vs 0.8 HC.cm^{-3}); *Micropsectra* type A and *Macropelopia* type decreased their abundance (1.6 HC.cm^{-3} vs 0.4 HC.cm^{-3} and 1.2 HC.cm^{-3} vs 0.1 HC.cm^{-3} , respectively). After the introduction of several fish species during the stocking phase (Carp introduction), the main changes in the chironomid community were a reduction in abundance of *Micropsectra*-type A and *Micropsectra contracta*-type, *Micropsectra insignilobus*-type and an increase in the abundance of the *Chironomus plumosus*-type, *Parachironomus varus*-type and *Glyptotendipes pallens*-type. *Macropelopia* type, *Procladius* sp., *Paralimnophyes* sp., *Limnophyes* sp. and *Micropsectra insignilobus*-type species were absent in this phase. Finally, with the introduction of the Pike group there was an increase in the abundance of *Polypedilum nubifer*-type (0.1 HC.cm^{-3} vs 1.0 HC.cm^{-3}), *Micropsectra contracta*-type (0.6 HC.cm^{-3} vs 0.7 HC.cm^{-3}) and *Psectrocladius sordidellus*-type

(1.3 HC.cm⁻³ vs 1.4 HC.cm⁻³) and a decrease in the abundance of *Paratanytarsus penicillatus*-type (1.3 HC.cm⁻³ vs 0.9 HC.cm⁻³), *Chironomus plumosus*-type (1.3 HC.cm⁻³ vs 1.1 HC.cm⁻³) and *Glyptotendipes pallens*-type (1.2 HC.cm⁻³ vs 1.1 HC.cm⁻³).

The differences in assemblage composition associated with the introduction of different predators were further supported by the PCO analyses (Fig. 5a). There was a significant difference of dispersions (PERMDISP; F=5.33; p<0.005) after the predator introduction (e.g. Goldfish group – 11.2 ±2.0 HC.cm⁻³), whereas fishless lake samples formed a distinct cluster with comparatively little variation (21.05±1.3 HC.cm⁻³).

3.2.2 Feeding group composition

When ordered by taxonomic composition similarities, sediment layers were grouped according to different times/types of predator introduction (Fig. 5a). However, these differences dissipated when plotting samples by chironomids' feeding group similarities (Fig. 5b). The first axis of the ordination is positively correlated with the presence of fish in the lake (PCO1 explained 89.2% of total variation). ANOSIM analysis revealed significant differences in feeding group composition between each introduction phase (R=0.51; p<0.001; see Table 5). Pairwise comparison also revealed that the first introduction (*Caurassius auratus*) resulted in higher significant difference in feeding groups composition (R=0.64; p>0.001) than in subsequent phases. SIMPER results revealed changes in the absolute concentration of HC of detritivores (3.7 HC.cm⁻³ vs 1.6 HC.cm⁻³), predators (2.0 HC.cm⁻³ vs 0.1 HC.cm⁻³) and grazers (0.1 HC.cm⁻³ vs 0.5 HC.cm⁻³), with an average dissimilarity of 32.6% between fishless and goldfish groups.

With the introduction of the Carp, the chironomid feeding group composition also changed, mainly as result of the absence of predators, an increase of grazers (0.5 HC.cm⁻³ vs 0.7 HC.cm⁻³), and a decrease in the abundance in detritivorous taxa (1.7 HC.cm⁻³ vs 1.0 HC.cm⁻³). Finally, with the introduction of the Pike group, there the abundance of grazers decreased further (0.7 HC.cm⁻³ vs 0.3 HC.cm⁻³). PCO analyses also indicated changes in the feeding structure of assemblages between the different times/types of introductions (Fig. 5b). Predators and grazers

were the two main feeding groups contributing to the PCO1 scores. Predators were associated with positive PCO1 scores, and grazers with negative PCO1 scores.

4. Discussion

The presence or absence of top predators was found to have a strong effect on the chironomid assemblage structure in Lake Azul, as revealed by PCO, ANOSIM and SIMPROF analyses (Tables 4-5 and Fig. 4-5). This is consistent with other findings comparing the community structure of fishless and fish containing lakes (e.g., Schilling et al., 2009). In fact, the presence of fish plays a strong role in shaping the structure of most aquatic insects communities in lakes, sometimes greater than the role of lake origin or physiography (Bendell and McNicol, 1987; Binckley and Resetarits, 2005; Schilling et al., 2009). Fish preferentially prey on large insects (Pope and Hannelly, 2013), such as Ephemeroptera, Trichoptera and Odonata, which are poorly represented in the Azores archipelago (Raposeiro et al., 2012, 2016). Chironomids, however, being the most abundant and diverse family of freshwater macroinvertebrates in the Azores archipelago (Raposeiro et al., 2009, 2012), are the available prey for introduced predators. Studies elsewhere have shown predation by fish to be an important driver in shaping chironomid communities (Goyke and Hershey, 1992; Milardi et al., 2016; Mousavi et al., 2002; Rieradevall et al., 1995; Sayer et al., 2016).

Understanding how fish introductions might be driving changes in chironomid community is not straightforward due to scarce information available about the ecology of most Azorean midge taxa. However, the ecological requirements of chironomid taxa have universal validity because even though the species might not be the same from one region to another, genera display comparable ecological requirements (Brundin, 1956; Mousavi et al., 2002; Oliver, 1971; Saether, 1975). Many of the chironomid genera present in Lake Azul have a Holarctic distribution (Raposeiro et al., 2009) and their ecology is well studied, and so we can infer tentative conclusions about their response to predator introductions in Azorean lakes.

4.1 Fishless conditions (until ca. 1790 AD)

Prior to introduction of predators in Lake Azul the concentration of head capsules is higher indicating that chironomid assemblages were more abundant in the past. The exception occurred during the volcanic eruptive phase (Unit 1) when falling ash increased the sedimentation rate and hence diminished the concentration of chironomids in the sediment. Tephra inputs into the lake during volcanic eruptions led to the complete absence of chironomid head capsules, most probably either by the dilution effect of the almost instantaneous deposition of large amount of particulate sediments, or because of unsuitable extreme volcanic conditions for most species to survive. A later recovery of the chironomid assemblages occurred once the tephra deposition had terminated (± 2 years). A similar pattern was observed in Lake Mascardi, in Argentina, where Massafiero and Corley (1998) found a complete absence of chironomid head capsules, followed by sharp changes in chironomid diversity after an eruptive episode.

The dominant taxa, *Micropsectra*-type A, *Micropsectra contracta*-type and *Macropelopia* are considered indicators of good water quality including oxygen-rich conditions (Mousavi et al., 2002; Oliver, 1971) and low TOC content (Brodersen and Quinlan, 2006; Brooks et al., 2007). Some *Micropsectra* species dominate deep ultraoligotrophic alpine lakes (Frossard et al., 2014; Frossard et al., 2013), while *Macropelopia* is characteristic of acidic lakes (Brooks et al., 2007; Brundin, 1956). Tanypodinae distribution and abundance is strongly associated with the absence of fish (Ganshorn, 2002). Moreover, species associated with fishless habitats, such as *Macropelopia* and *Procladius*, tend to be large, active free-swimmers, compared with the small, slow-moving, tube-dwelling taxa that cohabit with fishes (Mousavi et al., 2002; Oliver, 1971). Our results corroborate the descriptions of the Azorean chronicler, Gaspar Frutuoso (1522–1591), who highlighted the clean waters of Lake Azul. Probably, at his time, in XVIth century, Lake Azul had effective trophic functioning, explaining the small organic matter accumulation in the sediment.

In addition to fishes, climate oscillations, volcanic eruptions and anthropogenic activities in the catchment could play a role in shaping the chironomid assemblages. The occurrence of some thermophilic taxa, such as *Chironomus plumosus*-type seem to be controlled by climate factors

(Brooks, 2000; Heiri et al., 2011). In lake Azul, they were present during the period of 1280-1440 AD, the latest stages of the warm and arid Medieval Climate Anomaly, and absent during the Little Ice Age (1450 - 1700 AD). On Pico, another island in the Azores archipelago, Björck et al. (2006), defined a transition from dry to wet conditions (c. 1450-1550 AD). According to Rull et al. (submitted), Azul lake level progressively rose from AD 1289-1771, suggesting a general trend from drier to wetter climate.

4.2. Goldfish introduction (ca. 1787 AD)

At about 1787 AD (near 36 cm of core depth) distinct changes occurred in chironomid concentration and diversity, as well as in the organic matter composition (Figs. 3, 5 and 6), which correspond to the first introduction of a top predator (*Carassius auratus*) in the lake (Table 2). The main changes in the chironomid assemblage were associated with a substantial reduction in the numbers of chironomid head capsules, changes in the composition of the chironomid assemblage, and changes in the structure of the feeding groups of chironomids. These results suggest that predators had a strong effect on chironomid assemblages, exploiting the initial high densities of these invertebrates. The presence of large, free-swimming larvae, more vulnerable to fish predation, such as *Macropelopia*, were more abundant at times of the fishless lake, consistent with the pattern found in the literature (Becker et al., 2010). Several authors reported a rapid increase in abundance of Goldfish in Lake Azul. For example, (Walker, 1886) stated: “In these waters are to be found innumerable golden carp (*Carassius auratus*)[...]. The size that these fish have here attained, in spite of the apparent scarcity of food and grasses, is astonishing, and is certainly an inducement to other wealthy proprietors to stock the numerous lakes in the island with this valuable food supply.”

Coinciding with the introduction of *Carassius auratus* an increase in the percentages of carbon and of nitrogen in the sediment was observed. This is probably related to the detritivorous bottom feeding strategy of goldfish. Goldfish consume benthic invertebrates and macrophytes, and additionally release nutrients into the water from the sediment (Richardson et al., 1995) and by excretion (Lamarra, 1975). The nutrients originally present in the sediment would be a new

source of N and P to the primary producers when sediments are resuspended by disturbance due to fishes' foraging behaviour. This nutrient load was amplified by the increase in anthropogenic activities in the catchment, especially cutting of native vegetation for timber, and land clearing for agriculture and livestock production (Moreira, 1987; Rull et al., submitted). The introduction of fish affected the type and ratio of nutrients available in the system. The change in nutrient availability lead to a change primary production rates and a shift in the predator:grazer ratio was observed. The introduction of Goldfish coincided with an increase in chironomid grazer abundance, with the appearance of the littoral *Polypedilum nubifer*-type, and the increase of *Paratanytarsus penicillatus*-type. Both these taxa are associated with macrophyte *Myriophyllum* which was recorded at this time (Rull et al., submitted.). Macrophytes provide a refuge from top predators and a habitat for macrophyte-associated species (Langdon et al., 2010). The reduction in the concentration of head capsules of the free-swimming larvae of predaceous tanypods after the fish introduction suggests increased predation.

Other paleoecological studies in Azorean lakes have focused on changes in chironomid abundance and Cladocera associated with fish introductions (Buchaca et al., 2011; Skov et al., 2010). However, these studies only examined changes from ca. 1860 AD, in Fogo Lake, and from ca. 1960 AD in Furnas Lake, missing the first fish introductions.

4.3. Fish stocking introductions (1879 AD)

Fish stocking in Azorean lakes started in 1879 AD when several introductions took place (see Table 2) and stocking continues today. A change at core depth 20 cm (ca. 1875 AD) is indicated by ANOSIM analysis mostly due to the decrease in abundance of cold and oligotrophic taxa, such as *Micropsectra* -type A and *Micropsectra contracta*-type (Brooks et al., 2007; Heiri et al., 2011), and an increase in the abundance of warmer and meso/eutrophic taxa *Chiromonus plumosus*-type and *Glyptotendipes pallens*-type (Brooks et al., 2007; Osborne et al., 2000). This suggests that the introduced fish species had a large effect on chironomid assemblages. Moreira da Silva and Cabral (1983) conducted a survey of the fish fauna of Lake Azul, and reported the presence of chironomid in the diet of *Carassius auratus*, *Cyprinus carpio* and *Perca fluviatilis*.

These findings are similar to others studies that highlighted benthic invertebrates as a significant part of the diet of *Achondrostoma oligolepis*, *Cyprinus carpio*, *Micropterus salmoides* and *Perca fluviatilis* whose abundance led to a reduction of benthic invertebrate abundance, diversity, evenness, and richness (Lammens and Hoogenboezem, 1991; Miller and Crowl, 2006; Rieradevall et al., 1995). Also, Weber and Brown (2009) reported a decreased of macroinvertebrates abundance as a direct response to *C. carpio* introduction in lakes.

The observed increase in the percentages of both carbon and nitrogen in the sediments (Fig. 6) is probably related to the Cyprinidae feeding strategy (*C. carpio* and *A. oligolepis*). Cyprinids connect pelagic and benthic food webs through benthic scavenging activities and via excretion (Lamarra, 1975). For example, the carp bottom-feeding mode, sucking food along with sediment, and discarding inedible material (Lammens and Hoogenboezem, 1991), may result in consumption or uprooting of macrophytes, and resuspension of algal cells and particules from the sediment and therefore increasing nutrient and turbidity levels (Miller and Crowl, 2006; Richardson et al., 1995). Moreira da Silva and Cabral (1983) reported that the gut contents of the Cyprinidae fishes present in Lake Azul were mainly diatoms (*Aulacoseira* sp., *Epithemia* sp., *Navicula* sp. and *Fragilaria* sp.) commonly found in the sediments of Azorean lakes (Pereira et al., 2014). Carp also excrete huge quantities of nutrients into the water column that become available to algal communities accelerating their growth and production (Matsuzaki et al., 2007).

Bottom-up theory predicts that this increase in nutrients should result in increased phytoplankton, enhancing eutrophication (Drenner and Hambright, 1999). These changes lead to an increase of grazer taxa in chironomid-feeding groups (Fig. 5). Similar results were reported from Fogo lake, where carp introduction was followed by a major decrease in the abundance of chironomids and an increase in nitrogen and carbon loads resulting in a turnover from a benthic to a pelagic ecosystem (Skov et al., 2010). The disturbance of the sediment caused by the carp may have increased turbidity (Miller and Crowl, 2006; Richardson et al., 1995) therefore reducing the light availability and consequently algal production, leading to a

change in the oxygen conditions in the hypolimnion. In the stratified zone of lakes like Lake Azul, the reduction in oxygen supply, food quantity and quality are the most important factors shaping the composition of the profundal chironomid fauna (Frossard et al., 2014; Frossard et al., 2013; Heinis and Davids, 1993). Behavioral and physiological adaptations of profundal taxa make them less sensitive to variations in the oxygen availability (Frossard et al., 2014; Frossard et al., 2013; Heinis and Crommentuijn, 1992). *Micropsectra*, which dominated prior to fish introduction, prefers oxygen-rich environments, especially above the stratified zone (Frossard et al., 2014). Favourable oxygen conditions prevailed in the hypolimnion of Lake Azul from at least 1280 AD to the time of the fish stocking (Carp introduction). After that, there was a drastic change in profundal chironomids assemblages suggesting severe hypolimnetic oxygen conditions. From AD 1879, the rapid decrease in the majority of oxyphilous profundal taxa infer hypoxia, reinforced by the appearance of *Chironomus* and *Procladius*, two taxa that are well adapted to live in low oxygen environments (Little et al., 2000). At present, Lake Azul is monomictic, developing anoxia in the hypolimnion during summer stratification (Raposeiro et al., 2016).

4.4. Pike introduction (1979 AD)

Pike was first stocked in Lake Azul in 1979 AD and again in 1991 AD (Flor de Lima, 1993; Moreira da Silva and Cabral, 1983). The chironomid assemblage exhibited a progressive compositional change following the time of pike introduction. *Polypedilum nubifer*-type and *Micropsectra contracta*-type became more abundant, and *Paratanytarsus penicillatus*-type decreased. These changes lead to a change in the chironomid-feeding group from a co-dominance during the carp phase to a clear dominance of detritivorous taxa. The most likely explanation is the cascading effects of pike predation on the existing fish species. As pike is an opportunistic predator that had been associated to the decline and eradication of several fish species (Sepulveda et al., 2013). The decrease in the abundance of the fishes that include chironomid as significant part of the diet, leads to a significant effect on chironomid assemblages. There was a significant increase in numbers of *Esox lucius* captured from 0.2% to

5%, while there was a sharp decline in captures of *Perca fluviatilis* from 89.9% to 58% (Borges 2001; Moreira da Silva and Cabral 1983)

In fact, pike stockings are used worldwide in lake restoration projects (Jeppesen and Sammelkorpi, 2002), with the objective of increasing predation pressure on young zooplanktivorous fish, such as roach *Rutilus rutilus*, carp *Cyprinus carpio*, and perch *P. fluviatilis*, that predate on and limit zooplankton and invertebrate densities (Sondergaard et al., 2007). Piscivores such as pike diminish abundance of their fish prey, and consequently reduce predation on large zooplankton as well as fish-induced resuspension of sediments (Weber and Brown, 2009). This results in reduced release of nutrients from sediments into the water column (Weber and Brown, 2009). Following the introduction of pike into Lake Azul there was a decrease in carbon and nitrogen in the sediment (Fig. 6).

5. Conclusions and Final remarks

The introduction of top predators markedly affected the chironomid assemblages of the lacustrine ecosystems of Lake Azul. The results also highlight that not all taxa are affected equally. Volcanic eruptions, climate oscillations and anthropogenic activities play an additional role in shaping and structuring the lake biotic communities. The most significant change in lake Azul occurred with the first introduction of fish (Goldfish), when there were significant changes in the abundance, composition and structure of the chironomid feeding groups. The subsequent carp introduction further changed the composition of the chironomid assemblage and modified the structure of the feeding groups of chironomids, probably via direct predation and cascading impact. The carp introduction, changed the profundal chironomids assemblage and hypolimnetic oxygen conditions, probably increasing eutrophication processes in the lake.

For lakes in the Azores archipelago, chironomid species such as free-swimming larvae of predaceous tanypods (e.g. *Macropelopia*), *Micropsectra* and *Polypedilum nubifer*-group, appears sensitive to the introduction of top predators, while during the fishless lake period

Chironomus plumosus-type, owing to their ecological temperature requirements, seem to be more triggered by climate fluctuations.

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Tables

Table 1 - Physical and chemical characteristics of Lake Azul (mean values from 2003-2013; data compiled from Regional Monitoring Programme)

Variable	Value
<u>Environmental</u>	
Altitude (m)	260
Surface area (Km ²)	3.6
Drainage area (ha)	506
Non native floret area (%)	20
Scrub area (%)	71
<u>Water chemistry</u>	
Temperature (°C)	16.4
pH	7.5
Conductivity (µS/cm)	95.0
Oxygen (% saturation)	81
Transparency (m)	3.5
Chlorophyll <i>a</i> epilimnion(µg l ⁻¹)	44.7
Alkalinity (mg CaCO ₃ l ⁻¹)	25.2
Total nitrogen (mg N l ⁻¹)	0.39
Total phosphorus (µg P l ⁻¹)	0.018

Table 2 – Predator introductions ordered by chronology of introduction in Lake Azul. ANOSIM groups were established according to the time of introduction.

Year	Fish introductions	Common name	ANOSIM Group	Reference
Before 1791	Fishless		Fishless	Fructuoso (1977)
1792	First reference of presence of <i>C. auratus</i>	Goldfish	Goldfish	Valois and Silva (1886)
1879	<i>A. oligolepis</i>	Ruivaco		
1880	<i>S. trutta fario</i> *	Brown trout		
1882	<i>S. stomachicus</i> *	Gillaroo		
1889	<i>S. trutta lacustris</i> *	Brown trout	Carp	Vicente (1956)
1890	<i>C. carpio carpio</i>	Carp		
1898	<i>M. salmoides</i>	Largemouth bass		
	<i>P. fluviatilis</i>	European perch		
1941	<i>O. mykiss</i> *	Rainbow trout	-	Vicente (1956)
1979				
1991	<i>E. lucius</i>	Pike		
1981	<i>S. lucioperca</i> *	Pike-perch	Pike	Flor de Lima (1993)
1983	<i>R. rutilus</i>	Roach		

* Failed introductions - not established in the wild

Table 3 - Summary of SIMPER analysis results on the occurrence and relative abundance of chironomid taxa

	Average	Average		
	Abundance	Abundance	% Contribution	Cumulative %
Taxa	Zone 1	Zone 2	to dissimilarity	contribution
Average dissimilarity: 63.43				
<i>Micropsectra contracta</i> -type	48.9	22.8	22.7	22.7
<i>Micropsectra</i> type A	24.6	3.9	15.6	38.2
<i>Paratanytarsus penicillatus</i> -type	0.4	19.4	13.7	51.9
<i>Psectrocladius sordidellus</i> -type	4.6	22.7	13.4	65.4
<i>Chiromonus plumosus</i> -type	0.6	11.8	8.2	73.6
<i>Glypotendipes pallens</i> -type.	0.0	10.5	7.6	81.2

Table 4 - Results of one-way ANOSIM tests (global and pairwise tests) and SIMPER dissimilarity results based on chironomid assemblages in the studied sediment core, in relation to the predator introduction. ↑↓ indicate the direction of percentage change among different predator introduction.

ANOSIM	R Statistic	%Significance Level	% Dissi	SIMPER Results Taxa most responsible for dissimilarity (%)
Global test	0.78	0.1		
<u>Pairwise comparison</u>				
				↑ <i>Polypedilum nubifer</i> -type (25.5)
				↑ <i>Micropsectra contracta</i> -type (17.6)
Pike vs Carp	0.54	0.1	26.9	↓ <i>Parachironomus varus</i> -type (14.6)
				↓ <i>Paratanytarsus penicillatus</i> -type (12.5)
				↓ <i>Micropsectra contracta</i> -type (22.5)
Carp vs Goldfish	0.50	0.1	37.9	↑ <i>Glyptotendipes pallens</i> -type (14.9)
				↓ <i>Micropsectra</i> -type A (14.1)
				↑ <i>Paratanytarsus penicillatus</i> -type (15.5)
Goldfish vs Fishless	0.82	0.1	53.2	↓ <i>Macropelopia</i> type (13.2)
				↓ <i>Polypedilum nubifer</i> -type (11.5)

Table 5 - Results of one-way ANOSIM tests (global and pairwise tests) and SIMPER dissimilarity results based on chironomid feeding groups in the studied sediment core, in relation to the predator introduction. ↑↓ indicate the direction of percentage change among different predator introduction.

ANOSIM	R Statistic	%Significance Level	% Dissi	SIMPER Results Taxa most responsible for dissimilarity (%)
Global test	0.51	0.1		
<u>Pairwise comparison</u>				
				↓ <i>Grazer</i> (50.2)
Pike vs Carp	0.52	0.1	25.9	↑ <i>Detritivorous</i> (46.8)
				↓ <i>Detritivorous</i> (72.1)
Goldfish vs Carp	0.16	2.0	30.0	↑ <i>Grazer</i> (31.3)
				↓ <i>Predator</i> (6.6)
				↓ <i>Detritivorous</i> (48.5)
Goldfish vs Fishless	0.64	0.1	53.4	↓ <i>Predator</i> (39.5)
				↑ <i>Grazer</i> (12.0)

Figures

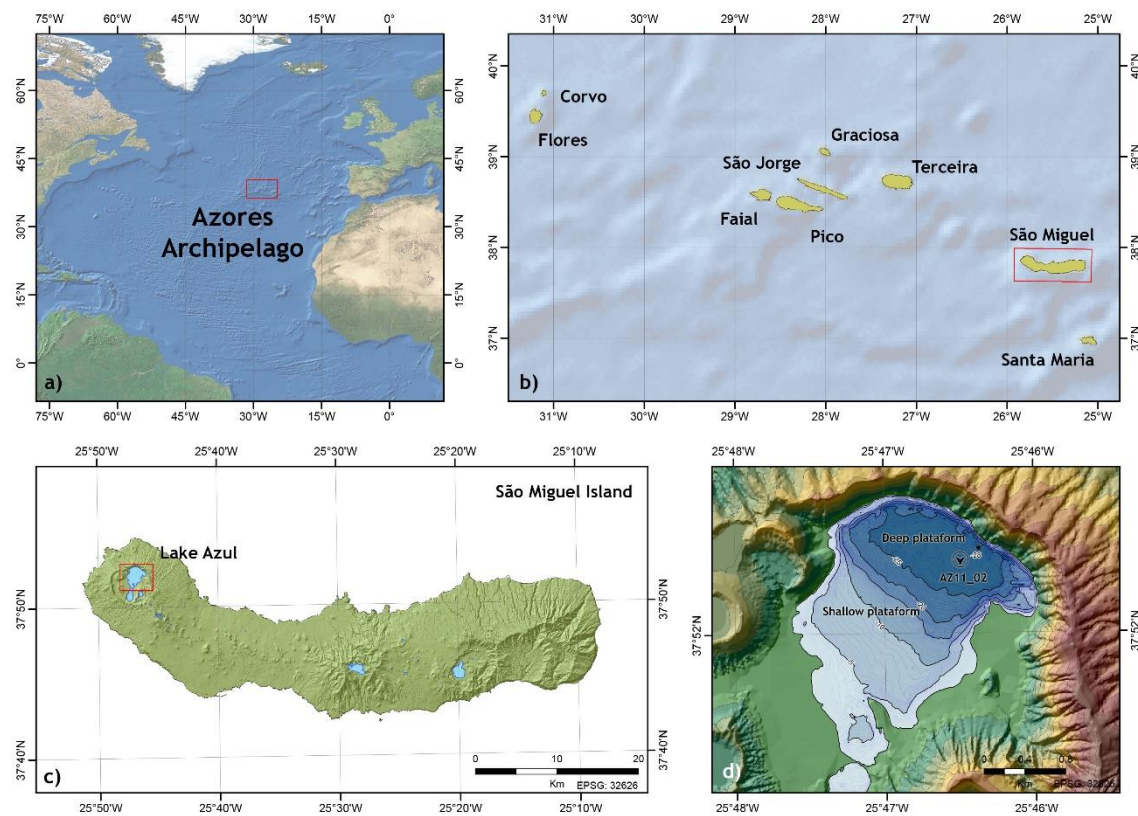


Figure 1 – Geographical location of the study lake, Lake Azul, São Miguel, Azores, Portugal. A) - Azores Archipelago in the Atlantic Ocean highlighted by a square; B) Location of São Miguel island; C) Location of Lake Azul; D) - Location of AZ11_02 in Azul lake

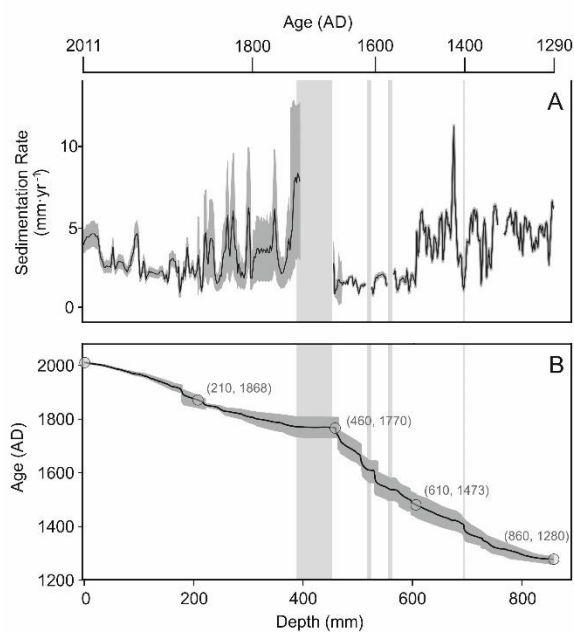


Figure 2 – A) Sedimentation rate profile from the core AZ11_02; B) Age model profile obtained from the application of the Novel Dynamic Age Model (adapted from Rubio-Inglés et al., submitted). Vertical grey bands correspond to rapid (instantaneous-like) flood events.

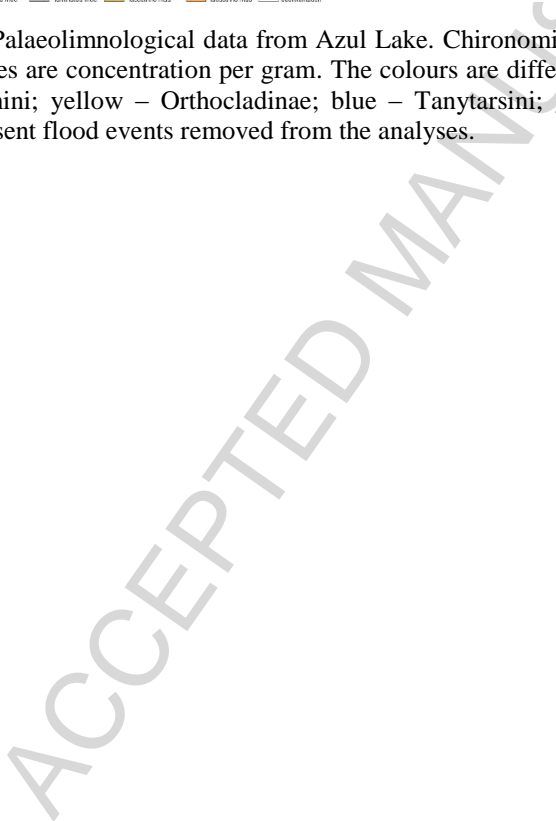


Figure 3 – Palaeolimnological data from Azul Lake. Chironomid remains are indicated as percentages of head capsules are concentration per gram. The colours are different chironomid families/subfamilies (red – Chironomini; yellow – Orthocladinae; blue – Tanytarsini; green – Tanypodinae). Horizontal white bands represent flood events removed from the analyses.

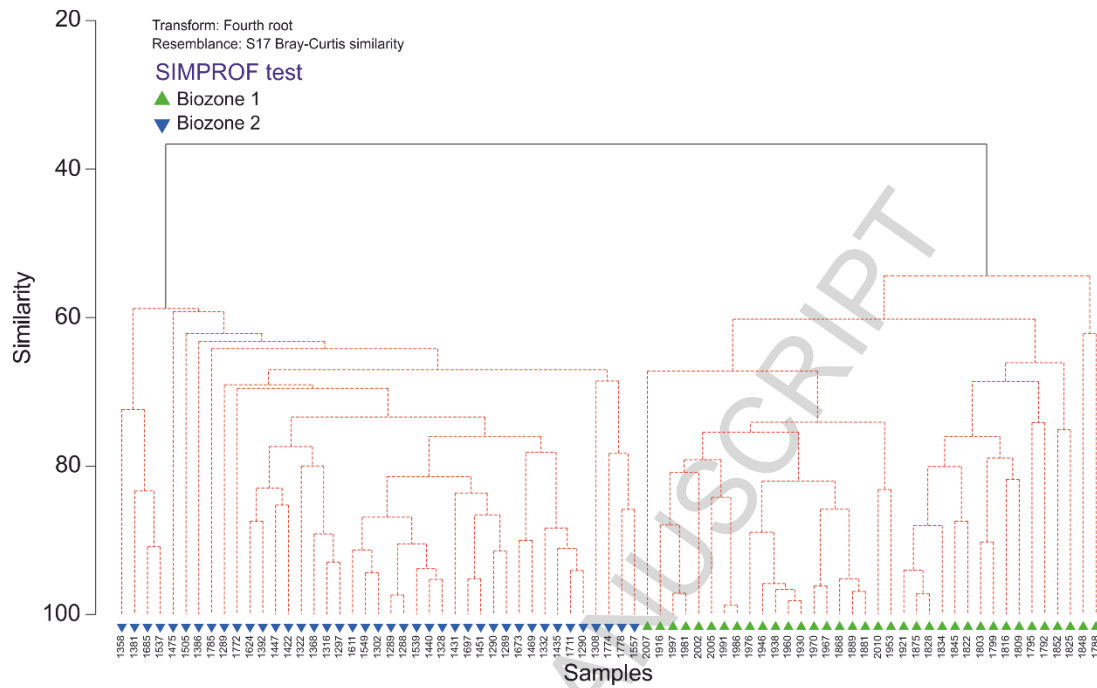


Figure 4 – Biostratigraphic zone resulting from: Cluster analyses of chironomid data according to their similarity. Similarity profile (SIMPROF) permutation tests were used to test for significant differences in the hierarchical cluster structure (i.e., the red dotted lines) at the 99% level.

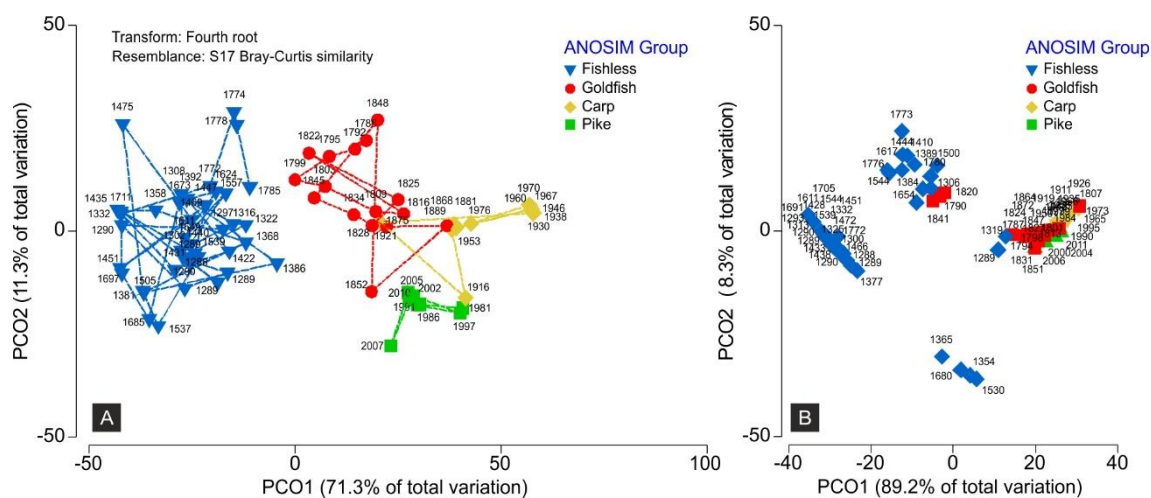


Figure 5 –Principal Coordinate Ordination (PCO) of first and second axes relating the introductions to A) chironomid assemblages B) chironomid feeding group composition

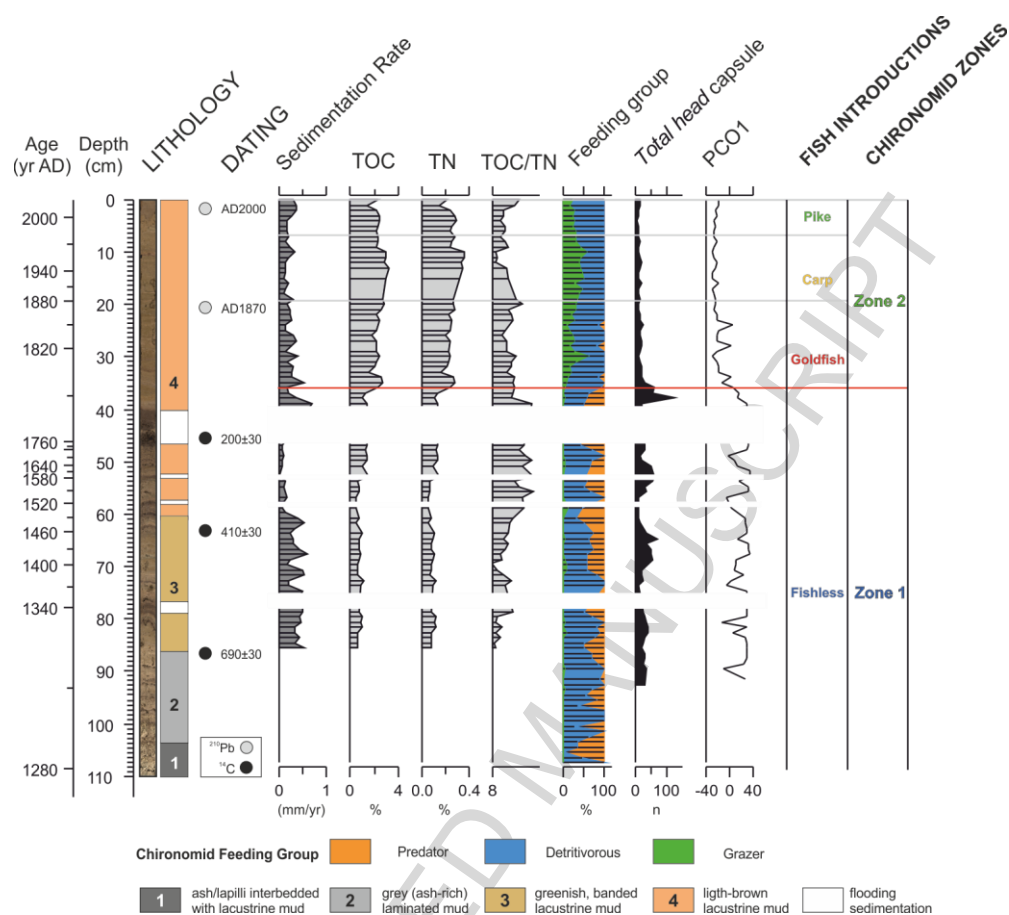


Figure 6 – Palaeolimnological data from Azul Lake. Horizontal white bands represent flood events removed from the analyses.

Highlights

The introduction of top predators markedly affects the chironomid assemblages of lacustrine ecosystems

Fish introduction drastically changed the composition of the chironomid assemblage via direct predation impact and cascading impact

Climate oscillations and anthropogenic activities might play a secondary role in shaping and structuring the biotic communities

Micropsectra group and Polypedilum nubifer-group, seem to be more sensible to the introduction of top predators

Chironomus plumosus-type, owing to their ecological warming requirements, seem to be more triggered by climate fluctuations.