Vertical distribution of benthic diatoms in a large reservoir (Alqueva, Southern Portugal) during thermal stratification

Maria Helena Novais [a,b,*], Alexandra Marchã Penha [a,b], Eduardo A. Morales [a,b], Miguel Potes [a], Rui Salgado [a,c], Manuela Morais [a,b]

[a] Institute of Earth Sciences - ICT, University of Évora, Rua Romão Ramalho n°. 59, 7000-671 Évora, Portugal
[b] Water Laboratory, University of Évora, P.I.T.E. Rua da Barba Rala No. 1, 7005-345 Évora, Portugal
[c] Department of Physics, ECT, University of Évora, Rua Romão Ramalho n° 59, 7000-671 Évora, Portugal

HIGHLIGHTS

- Diatoms in lentic systems are poorly studied and their vertical dynamics is often neglected.
- Meteorological, water physico-chemical and biological characterizations were carried out.
- Diatom assemblages attached to artificial substrates were studied in the ALEX campaign.
- Vertical gradient of diatom assemblages, diatom guilds, diversity and SPI indices was detected.
- Living diatoms were observed at 30 m, far below the photic zone.

GRAPHICAL ABSTRACT
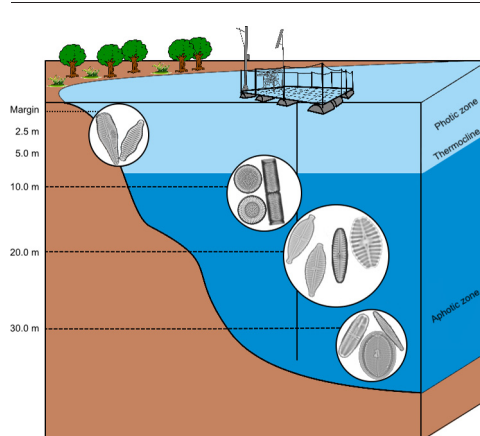
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ABSTRACT

Freshwater diatom communities are known to respond to a wide range of environmental factors, however, the depth gradient is usually neglected and few studies are available, especially in large reservoirs. During the ALqueva hydro-meteorological EXperiment (ALEX) field campaign, diatom communities were studied in the margins and in three platforms (from the surface to the bottom of the reservoir) located in the limnetic zone of the Alqueva reservoir, one of the largest artificial lakes in western Europe. A detailed meteorological and physico-chemical characterization of the reservoir was carried out from June to September in Summer 2014, when the reservoir was stratified, to relate these variables with diatom assemblages. Despite the large dimensions of the reservoir, no differences in the water physico-chemical characteristics and diatom descriptors were detected among platforms. Small changes in diatom assemblages, ecological guilds, taxa richness and Shannon diversity index were observed between sampling campaigns. Nevertheless, differences in diatoms were detected along a depth gradient, both in terms of diatom assemblages and ecological guilds. Taxa richness, Shannon diversity index, Pielou's evenness and Specific Pollution sensitivity Index (SPI) also differed with depth, with the lowest values of all indices detected at surface samples, increasing with depth, reaching the highest values at 20 m for taxa richness, Shannon diversity and Pielou's evenness indices.

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* Corresponding author.
E-mail address: hnovais@uevora.pt (M.H. Novais).

1. Introduction

Water resources in the Mediterranean Region are limited, fragile and threatened, and there is an urgent need for their sustainable management, which can only be achieved by understanding and predicting the complex interactions between climate, hydrology, ecosystem processes, water quality and biodiversity (Ferragina, 2010; Hamdy, 2001).

Lakes and reservoirs, due to their vulnerability, have been considered sentinels of environmental changes caused by anthropogenic effects such as intensive agriculture, livestock raising, dam construction, global warming and eutrophication (Hsieh et al., 2011), considered by Sutton et al. (2013) as one major threat to the ecological integrity of lentic systems. The determination of the trophic condition is therefore an important step in the ecological assessment of a reservoir because of the powerful predictive statements that can be made describing abiotic and biotic relationships for a given trophic state (Karadžić et al., 2010). The assessment of the trophic status of lentic systems is commonly based on water chemistry and phytoplankton attributes such as biomass. But fish and macrophytes are also often monitored because of the importance of fish as suppliers of significant ecosystem services and macrophytes as providers of habitat for invertebrates and fish (DeNicola and Kelly, 2014). Despite their important role in primary productivity, nutrient cycling and food web interactions (Vadeboncoeur and Steinman, 2002), there are only a few published studies incorporating benthic diatoms as a means to assess lake ecological status (e.g. Rimet et al., 2015). However, some countries are already incorporating littoral diatoms for the assessment of lake ecological status within a European regulatory context related to the application of the Water Framework Directive (WFD; The European Parliament & European Council, 2000), as described by Kelly et al. (2014). Until now, research on lake primary production has focused much more on planktic than on benthic algae, with phytoplankton studies outnumbering periphyton studies by an order of magnitude (Cantonati and Lowe, 2014; Poikane et al., 2016). In turn, more attention has been given to stream than to lentic systems phytobenthos, with twice the number of papers focusing on the former, which has implications for monitoring and the application of the European Union (EU) Water Framework Directive (WFD; The European Parliament & European Council, 2000; Poikane et al., 2016). In 2014, only 1/3 of EU member states had phytobenthos assessment methods for lentic systems, whereas almost all (22 of 27, including Portugal) had methods for streams (DeNicola and Kelly, 2014; Poikane et al., 2016). DeNicola and Kelly (2014) questioned whether a dedicated periphyton assessment of lentic systems was justified and if the decision not to include periphyton could lead to instances where important information is overlooked. The conceptual model presented in their review consists on an attempt to provide a guide for developing quantitative periphyton-based approaches for the assessment of the biological condition of lentic systems.

The few existing lacustrine periphyton studies focus on littoral zones (e.g. Vadeboncoeur et al., 2014; Rimet et al., 2015; Faria et al., 2017), probably due to the physical heterogeneity that increases the spatial variation in periphyton biomass and productivity, especially where macrophytes provide a temporally variable and structurally complex substratum (Vadeboncoeur et al., 2014). However, studies including periphyton from the limnetic lacustrine zones are rare (e.g. Stevenson and Stoermer, 1981; Hoagland and Peterson, 1990; Flower et al., 2004; Cantonati et al., 2009), even though the importance of a thorough knowledge of the biological depth-distribution patterns and their environmental controls is well-recognized as a valuable tool for an ecologically sensible management of reservoirs and natural lakes (Cantonati et al., 2009).

Light and nutrients are considered the most frequently reported environmental controls of phytobenthos in lakes (Cantonati et al., 2009). Yang and Flower (2012) suggested that light influences benthic diatom abundance, whereas substrate type has a higher influence on species composition in the case of oligotrophic lakes. Also, the results presented by Vadeboncoeur et al. (2014) indicate that light is the primary structuring mechanism for epipelon in lakes of all sizes, whilst productivity patterns point to an added role of wave disturbance in limiting biomass. In the case of epiphyton, wind disturbance acts as a structuring force in its dynamics (Faria et al., 2017). These findings concur with the previous work of Stevenson and Stoermer (1981) that distinguished three zones (shallow, mid-depth and deep) in Lake Michigan, based on diatom community composition, structure and abundance. These attributes were shaped by substrate disturbance, wave action in the shallow zone, and by low light levels in the deep zone. Also, Cantonati et al. (2009) identified the same three zones in Lake Tovel, defined for diatoms and total epilithic phytobenthos (using physiological, functional and community organization parameters) and having water-level fluctuations, photosynthetically active radiation (PAR), silica and nitrates as the main environmental controls. Among biotic regulating factors, zooplankton grazing plays an important role in epiphyton dynamics in a large shallow lake (Faria et al., 2017); also Higgins et al. (2014) identified the important effect of large-bodied zooplankton and dreissenid mussels (grazers of phytoplankton) on total ecosystem production (TEP) and autotrophic structure (the partitioning of TEP between phytoplankton and periphyton) across gradients in lake size and total phosphorus concentration.

As a contribution to the knowledge of benthic diatoms in large reservoirs, the present work explores the influence of water physicochemistry on diatoms in the margins and in a stratified depth gradient in the limnetic zone of the Alqueva reservoir, southern Portugal. Data were collected within the framework of the ALqueva hydro meteorological Experiment (ALEX 2014), an integrated field campaign with measurements of chemical, physical and biological parameters at different experimental sites in the reservoir and in its surrounding area, from June to September 2014, with the main purpose of studying lake-atmosphere interactions. Data obtained during the ALEX 2014 are available via http://www.alex2014.cge.uevora.pt/data/ (Salgado et al., 2018). These data have already been used in different studies, including the heat, vapour and CO_2 fluxes in the water–air interface (Potes et al., 2017), the formation and development of a lake breeze system at a large reservoir (Iakunin et al., 2018), and the effects of an inland water body on the atmospheric electrical field (Lopes et al., 2016; Nicoll et al., 2018).

Given the vertical zonation of diatoms observed in European and US natural lakes, we hypothesize that similar dynamics can occur in Alqueva reservoir, a deep and relatively young system. To do so, spatial and temporal variations are explored for biotic and abiotic variables, and benthic diatoms are analysed in terms of assemblages, ecological guilds, and the Specific Pollution Sensitivity (SPI) and diversity indices.

2. Methodology

2.1. Study area

The ALEX field campaign took place in the Alqueva Reservoir, Guadiana basin (SW Iberian Peninsula) from June to September 2014. The construction of the dam started in 1995 and the dam floodgates were closed in 2002. The reservoir, an 83 km section along the main course of the Guadiana River, is one of the largest western European reservoirs, with a surface of 25,000 ha when completely filled, a capacity to store 4500 million m^3 of water, a catchment area of 55,000 km^2 and maximum depth of >70 m.

The Guadiana basin is characterized by a Mediterranean climate (Csa according to the Köppen climate classification). Summer periods are dry and hot, with high solar radiation at the surface and high potential evapotranspiration, whilst winter periods are colder and relatively harsh.

Three fixed platforms located in the limnetic zone of the reservoir were selected to carry out this study: Montante, Mourão and Alcarrache (Fig. 1). Montante and Mourão platforms are located in clear cut
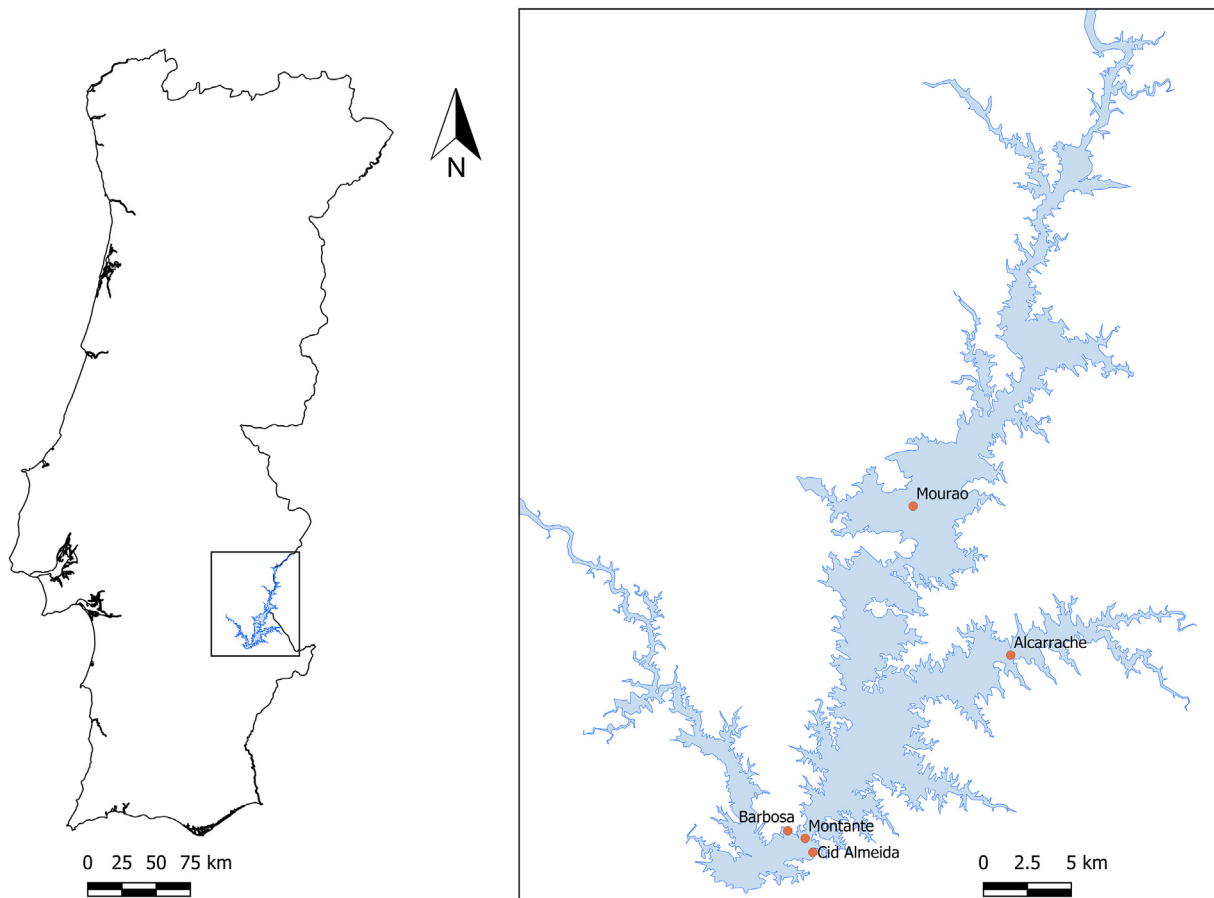
Fig. 1. Map of Portugal with the Alqueva reservoir and a detail with the location of the three sampling platforms, including the meteorological stations Barbosa and Cid Almeida.

surroundings and deep areas of the reservoir, with >60 m and 50 m depth, respectively; whilst Alcarrache platform is located in a forested surrounding and a ca. 20 m deep area.

Along this period, meteorological measurements were carried out in Montante platform and in Cid Almeida and Barbosa, two meteorological stations located on the shore nearby Montante platform (Fig. 1). The water physico-chemical characterization was based in in situ measurements and in laboratory analysis of water samples monthly collected from the three fixed platforms (Fig. 1). Biological characterization started in July 2014 and comprised monthly sampling of littoral benthic diatoms collected from the margins (in the nearest sites, accessible by car, of the platforms) and in diatoms collected from artificial substrates placed along a depth gradient from the surface to the bottom of the reservoir in Alcarrache platform and to 30 m in the Montante and Mourão platforms.

2.2. Meteorological, physico-chemical and biological characterization

The Montante platform was the main ALEX meteorological experimental site, equipped with an eddy-covariance system (IRGASON from Campbell Scientific), which directly measures atmospheric pressure, air temperature, water vapour and carbon dioxide concentrations, 3D wind components, and computes, among other quantities related to turbulence, the momentum flux, sensible and latent heat fluxes, carbon dioxide flux. The platform also had an albedometer (CM7B from Kipp & Zonen) and a pyrradiometer (8111 from Philipp Schenk) to assess downwelling and upwelling short and longwave radiative fluxes. On the lake shore, nearby the Montante platform, two standard meteorological stations were installed, known as Barbosa and Cid Almeida. It

includes wind speed and direction (Wind Sonic from Gill Instruments), air temperature and relative humidity (CS 215 from Campbell Scientific) and precipitation (ARG 100 STD from Institute of Hydrology). Data were acquired at 1 s sampling rate and values for 1-minute intervals were recorded.

To characterize the water column, vertical profiles of temperature (T, °C), dissolved oxygen (DO, mg L^{-1} and % of O$_2$ saturation), pH, oxidation-reduction potential (ORP, V) and Electrical Conductivity (EC, µS cm^{-1}) were taken in situ using a TROLL 9500 PROFILER XP multi parametric probe. Simultaneously, an integrated sample was collected for phytoplanktic chlorophyll *a* (Chl *a*) determination and water samples for physico-chemical analyses were collected at three depths (surface, metalimnion and bottom) using a Van Dorn bottle (3 L capacity) and kept cool, in the dark until laboratory processing. Water transparency was measured using a 20 cm black and white Secchi disk. Profiles of downwelling spectral irradiance were made in the water column, until 3 m depth, using the device developed by Potes et al. (2017). With these profiles the water spectral extinction coefficients were obtained using the formulation from Preisendorfer (1959). In the laboratory, standard methods for water chemical analyses were applied according to Apha (1995) and comprised the following parameters: alkalinity (Alk, mg HCO$_3^-$ L^{-1}), ammonium (NH$_4$, µg NH$_4$-N L^{-1}), biochemical oxygen demand after 5 days (BOD$_5$, mg O$_2$ L^{-1}), chemical oxygen demand (COD, mg O$_2$ L^{-1}), Kjeldahl nitrogen (KN, µg N L^{-1}), nitrates (NO$_3$, µg NO$_3$-N L^{-1}), nitrites (NO$_2$, µg NO$_2$-N L^{-1}), phosphates (PO$_4$, µg PO$_4$-P L^{-1}), silica (Si, mg Si L^{-1}), total hardness (TH, mg CaCO$_3$ L^{-1}), total nitrogen (TN, µg N L^{-1}), total phosphorus (TP, µg P L^{-1}), total suspended solids (TSS, mg L^{-1}) and turbidity (TU, NTU). Additionally, in the bottom samples, iron (Fe, µg Fe L^{-1}) and manganese

(Mn, µg Mn L^{-1}) were also analysed. Samples for Chl *a* analysis were filtered using Whatman GF:C glass fiber filters, chlorophyll *a* was extracted for 24 h in 8 mL acetone (90%) at 4 °C in the dark, measured spectrophotometrically and corrected for degradation products using the equations by Lorenzen (1967).

Littoral diatoms were sampled from stones in the margins following the European norms for sampling in lakes (King et al., 2006), which consisted in brushing the upper surface of 5 stones collected at ~40 cm depth. In each platform, 3 untreated ceramic tiles (dimension 7.5 cm × 5 cm) were attached at discrete depths (surface ca. 20 cm, 2.5 m, 5 m, 10 m, 20 m in the three platforms, and also at 30 m in Montante and Mourão) to a rope tied to the platform (this procedure is more cost-effective than the sampling by scuba divers, commonly used in this kind of studies). Artificial substrates were selected to include live diatoms in the analysis (dead frustules commonly deposited on hard substrates in lentic system are washed from these tiles) and intends also to eliminate differences in diatom assemblages caused by the substrate type, considered by Yang and Flower (2012) as one of the main factors influencing benthic diatom composition in oligotrophic lakes. Diatoms were collected by brushing the tiles with a toothbrush, washing them with distilled water (to avoid contamination with planktic diatoms present in the reservoir water) and preserving them with a formaldehyde solution (4% v/v) immediately after sampling. At lab arrival an aliquot of fresh sample (not preserved) was checked to verify if diatoms were alive. All diatom samples were treated by oxidation using hot hydrogen peroxide (35%) and dilute HCl (37%), in order to obtain a suspension of clean frustules. Permanent slides were mounted with Naphrax®. Diatoms were identified to specific or subspecific level using light microscopy (LM) (Leica DMLB with 100× oil immersion objective, N.A. 1.40). At least 400 valves were identified and counted from each slide to estimate the relative abundance of each taxon (Inag, 2008). The identification was based on reference floras (e.g. Krammer and Lange-Bertalot, 1986, 1988, 1991a, 1991b; Hofmann et al., 2011), as well as on recent bibliographic sources, including the series 'Diatoms of Europe', 'Iconographia Diatomologica', 'Bibliotheca Diatomologica' and relevant taxonomic papers, such as Reichardt (1997), Van de Vijver et al. (2011) and Novais et al. (2011, 2015). Furthermore, the samples were identified and counted by a single analyst, in order to decrease possible identification error effects, which can be the main source of variability according to Prygiel et al. (2002) and Kahlert et al. (2009).

2.3. Statistical analysis

2.3.1. Environmental parameters

The water physico-chemical parameters were log-transformed prior to the analysis and appropriate tests for normality were conducted (Shapiro–Wilk normality test, $p < 0.05$). These data were first tested for correlations among 19 parameters, by means of Pearson correlations using STATISTICA 8.0 (StatSoft Inc, 2001), to avoid multicollinearity in the selection of the variables to include in the following multivariate analysis. Afterwards, a Principal Component Analysis (PCA) (Goodall, 1954) was conducted in order to detect patterns and explore the relationships between environmental variables among the sites, depths and campaigns using CANOCO v. 4.5 (ter Braak and Šmilauer, 2002). Later, a one-way Analysis of Variance (one-way ANOVA) or a Kruskal-Wallis One Way Analysis of Variance on Ranks (Kruskal-Wallis test) were carried out, depending on whether the data followed the normality assumption. As post hoc and Pairwise Multiple Comparison analysis, both Dunn's and Holm-Sidak methods (Dunn, 1961; Holm, 1979) were performed, to test the preliminary results obtained with the PCA, i.e., to verify which physico-chemical parameters vary with depth and whether there are differences between platforms and sampling dates, using SigmaPlot 12.0 (Systat Software Inc., Chicago, IL).

2.3.2. Diatoms

Only diatom taxa reaching an abundance of >1% in at least one sample were included in the statistical analyses (123 diatom taxa in total).

A non-metric multidimensional scaling (nMDS) ordination technique was applied to the species data, based on a matrix with 123 diatom taxa x 56 samples, log-transformed (nMDS; Bray-Curtis distance; minimum stress 0.01; Kruskal fit scheme). In nMDS, site points are arranged in the best possible way so that closer points correspond to sites similar in their species composition, and points far apart are more dissimilar. Afterwards, statistical differences in diatom assemblages between platforms, sampling dates and depths (taking into account the preliminary nMDS results) were tested by the non-parametric Analysis of Similarity (ANOSIM; Bray-Curtis distance measure, 999 permutations) based on species abundances. ANOSIM is a randomization-based method of multivariate analysis and it is mainly used to test significant differences between two or more groups (defined a priori), based on any distance measure (Clarke and Ainsworth, 1993). R is scaled to lie between −1 and +l, a value of zero (or small negative or positive values near to zero) representing the null hypothesis of no differences among a set of samples, and its magnitude depends on the amount by which samples differ (Clarke 1993). The percentage breakdown of average dissimilarity between the platforms, sampling campaigns and depths was determined by means of SIMPER analysis, using the Bray-Curtis dissimilarity index (Clarke and Gorley, 2001). Both nMDS, SIMPER and ANOSIM analyses were performed using PRIMER Version 5.2.0 (Clarke and Gorley, 2001).

The indicator value (IndVal) method was used to identify the key species at each depth (Dufrêne and Legendre, 1997) using PC-Ord 6.0 (MjM Software Ltd., 217–219 Hamstel Road, Southend on Sea Essex SS2 4LB). This method provides IndVals for each species, based on the combination of information on the specificity and fidelity of occurrence of a taxon in a group. The statistical significance of the species IndVal was evaluated using Monte Carlo random permutation tests.

Ecological guilds were assigned to all taxa identified following the classification of Passy (2007), Berthon et al. (2011) and Rimet and Bouchez (2012): low-profile, high-profile, motile and planktic guilds. For each sample, taxa richness (S), Shannon diversity index (H′) and the Pielou's evenness index (J′) were determined. The Specific Pollution Sensitivity Index (SPI) was calculated from diatom abundances (Coste in CEMAGREF, 1982), using the OMNIDIA v. 5.5 software (Lecointe et al., 1993). This index is based on the Zelinka and Marvan (1961) formula, i.e., assigning each taxon a pair of individual values, pollution tolerance (S) and stenoecy degree (V), and the initial results ranging from 1 to 5 are transformed on a note to 20, in which higher values correspond to lower pollution. SPI was selected because it is the only diatom index based on the autecological parameters of virtually all known taxa, it has been developed for assessing the quality of running waters and has been recommended as a reference index for several Iberian basins (Gomà et al., 2005; Blanco et al., 2008). One-way ANOVA or a Kruskal-Wallis test were carried out, depending on whether the data met the normality and the equal variance requirements, followed by post hoc analysis, to test the abovementioned descriptors for differences between sampling sites, campaigns and depth gradient, using SigmaPlot 12.0 (Systat Software Inc., Chicago, IL).

Spearman rank order correlations were calculated to explore possible correlations between diatoms (expressed as taxa richness, Shannon diversity and Pielou's evenness indices, number of genera, SPI and ecological guilds) and water physico-chemical parameters (selected among those that varied with depth), using STATISTICA 8.0 (StatSoft Inc, 2001).

3. Results

3.1. Meteorological characterization

Table 1 shows the average air temperature, accumulated precipitation and wind speed recorded at Barbosa weather station (Fig. 1).

Table 1
Temperature, wind speed and precipitation values recorded at Barbosa weather station during the ALEX field campaign. Monthly and eight days before the field campaigns averages, maximum and minimum of air temperature at two meters height (ºC) and wind speed at three meters height (ms^{-1}); and accumulated precipitation (mm) for the same time periods.

Campaigns	Average temp. (°C)	Average temp. 8 days (°C)	Minimum temp. (°C)	Minimum temp. 8 days (°C)	Maximum temp. (°C)	Maximum temp. 8 days (°C)	Average wind speed (m s^{-1})	Average wind speed 8 days (m s^{-1})	Maximum wind speed (m s^{-1})	Maximum wind speed 8 days (m s^{-1})	Accumulated precipitation (mm)	Accumulated precipitation 8 days (mm)
25 June	20.71	20.36	15.62	17.51	29.22	28.30	2.42	2.53	8.28	9.56	13.33	13.33
24 July	24.27	24.09	16.83	17.00	32.67	31.99	2.34	2.44	8.51	8.49	3.98	0.00
27 August	24.64	24.80	16.77	16.29	33.78	34.31	2.83	2.38	9.41	8.78	0.00	0.00
25 September	21.44	20.33	17.57	16.37	28.58	26.24	2.11	1.90	8.45	8.13	56.32	13.93

Data are shown only for Barbosa, since similar values were measured in the Montante platform and the Cid Almeida weather station. The mentioned table presents monthly values as well as values from eight days prior to the field campaigns. In July and August, average temperatures were higher, with lower or no precipitation. In the eight days before the field campaigns of June and September record, lower temperatures occurred and a similar amount of rain (around 13 mm) fell on both months. Comparing these values with the temperature and precipitation Normals (1981–2010 normals – www.ipma.pt), we found that temperature and precipitation values agreed with the averages, with exception of the accumulated precipitation in September, which was 90% higher (average value was 29.5 mm in 1981–2010 period for Beja climatological station). The wind speed was low in average, with the highest values recorded in August (monthly average 2.83 m s^{-1}; maximum 9.41 m s^{-1}). However, wind speed varies throughout the day, with low values during the night and morning (<2.5 m s^{-1}) increasing at the end of the afternoon, with the maritime breeze influence (reaching values above 4 m s^{-1}) (Fig. 2).

3.2. Water physico-chemical characterization

Descriptive statistics of the environmental parameters recorded from the three platforms and depths are presented in Table 2.

From the in situ parameters, strong positive correlations were only detected among T and pH; whilst negative strong correlations between in situ parameters and nutrients were only found for ORP and NH$_4$. Strong negative correlations were not detected among the nutrients, but strong positive correlations were found for NH$_4$ with KN and TN; and PO$_4$ strongly correlated with TP. Among the other descriptors strong negative correlations were detected between Si and T, pH and
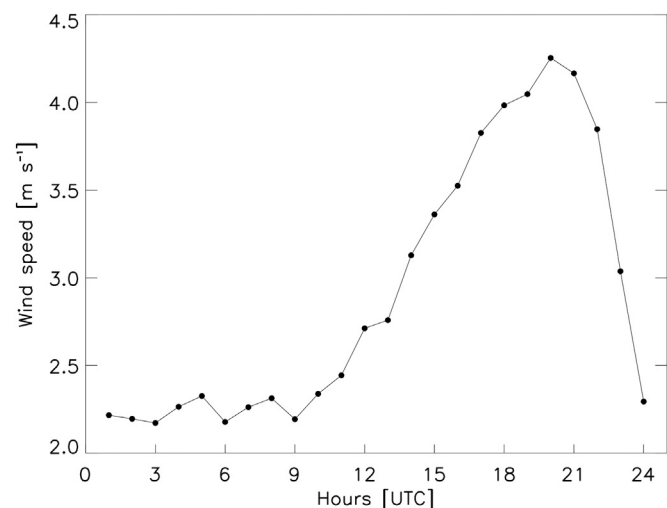
Fig. 2. Hourly averages of the wind speed (m s^{-1}) during the ALEX experiment at the Montante platform.

DO, and TU was highly correlated with TSS (Table A.1 with the correlations is presented as Appendix).

The strong correlations detected between TSS and TU; NH$_4$, KN and TN; PO$_4$ and TP, allowed the identification of the parameters that could be excluded from the PCA analysis, given their possible multicollinearity. Therefore, from the above-mentioned parameters, only TSS, TN and TP were part of the 15 parameters included in the PCA.

The PCA conducted to explore the variation of physico-chemical variables (Fig. 3) showed that the first PCA axis accounted for 36.7% variance and corresponded to the nutrient gradient (mainly TP and TN), revealing a depth gradient. Surface and metalimnion samples were separated from bottom samples, with higher concentrations of TP in the latter, especially in Mourão and Alcarrache platforms during August and September (in the positive side of the axis). By opposition to higher ORP and DO values in surface and metalimnion samples, collected in June and July (in the negative side of the axis). Complementarily, a Kruskal-Wallis test proved: 1) no differences between sampling campaigns (H = 1.814 with 3 degrees of freedom, $p = 0.612$); 2) the differences in depth (H = 31.369 with 2 degrees of freedom, $p < 0.001$), with Dunn's Method separating surface and metalimnion from bottom samples, but no distinction between the former two ($p > 0.05$). A One-way ANOVA or a Kruskal-Wallis test were further used to verify which parameters differed between surface, metalimnion and bottom samples. Differences with depth were detected for: T (F = 230.818, $p < 0.001$), EC (F = 5.340, $p = 0.009$) and Si (F = 95.710, $p < 0.001$), with Holm-Sidak method separating surface, metalimnion and bottom samples for T and Si ($p < 0.001$) and surface from bottom samples for EC ($p = 0.008$); pH (H = 31.282 with 2 degrees of freedom, $p = 0.001$), DO (H = 22.294 with 2 degrees of freedom, $p < 0.001$), PO$_4$ (H = 9.332 with 2 degrees of freedom, $p = 0.009$), TP (H = 8.747 with 2 degrees of freedom, $p = 0.013$), NO$_3$ (H = 9.505 with 2 degrees of freedom, $p = 0.009$) and TSS (H = 8.429 with 2 degrees of freedom, $p = 0.015$) with, Dunn's Method distinguishing surface and metalimnion from bottom samples ($p < 0.05$) for pH and DO; surface from bottom samples for PO$_4$, TP and TSS; bottom and metalimnion from surface samples ($p < 0.05$) for NO$_3$.

The second axis explained 28.9% variance, reflecting a temporal gradient, correlated with NO$_3$, EC, TSS and TH. Higher values of TSS and EC were detected in surface and metalimnion samples collected in August and September (negative side of the axis), whilst in the positive side of the axis higher concentrations of NO$_3$ were detected in the beginning of the Summer (June and July) in bottom samples.

In the PCA ordination plot no clear separation of samples collected in different platforms was detected, neither in the further Kruskal-Wallis test. (H = 1.584 with 2 degrees of freedom, $p = 0.453$).

The previous results show no differences between sites but reveal a depth gradient for the parameters measured in situ, such as T, pH, EC and DO; therefore, vertical profiles for these parameters (additionally, ORP was also included) are presented for Mourão platform (Fig. 4), as representative of the three platforms. The vertical profiles show the stratification of the reservoir during the whole period of the campaign (from June to September), thus allowing its classification as warm monomictic, with the thermocline depth around 10 m in Mourão in

Table 2
Physical-chemical characterization of the water column in Montante, Mourão and Alcarrache platforms, median (interquartile range) values are presented.

Variable	Alqueva-Montante			Alqueva-Mourão			Alcarrache	
	Surface	Metalimnion	Bottom	Surface	Metalimnion	Bottom	Surface	Bottom
T (°C)	24.65 (23.51–25.71)	20.02 (19.24–20.54)	13.64 (13.32–13.96)	24.40 (23.62–25.62)	23.12 (22.99–23.42)	14.19 (13.88–14.57)	25.68 (24.78–26.88)	14.75 (14.35–15.31)
pH	9.19 (8.99–9.44)	8.03 (7.85–8.27)	7.50 (7.41–7.55)	8.97 (8.90–9,09)	8.85 (8,75–8,93)	7.32 (7,17–7,51)	9.03 (8.92–9.12)	7.25 (7.17–7.34)
DO (% O_2 sat)	101.18 (98.64–124.75)	89.54 (69.47–107.41)	22.91 (18.05–43.40)	101.84 (98.20–107.90)	98.12 (89.21–104.92)	15.44 (7.58–29.19)	90.26 (83.41–100.44)	10.72 (8.55–19.63)
EC (μS cm^{-1})	387.5 (375.8–399.7)	385.8 (375.5–394.0)	369.4 (357.7–382.3)	422.1 (404.2–445.2)	390.6 (371.6–413.8)	377.7 (351.3–493.0)	387.0 (375.2–399.0)	368.8 (355.2–383.1)
ORP (V)	0.090 (−0.003–0.090)	0.110 (−0.015–0.113)	0.110 (−0.128–0.123)	0.110 (0.045–0.115)	0.100 (0.020–0.110)	0.065 (−0.150–0.098)	0.125 (0.025–0.140)	−0.080 (−0.373–0.088)
Alk (mg L^{-1})	98.50 (95.50–100.75)	100.50 (93.25–101.00)	98.00 (89.25–111.25)	98.50 (95.00–101.50)	100.00 (97.00–107.25)	100.00 (96.25–110.50)	100.50 (97.75–105.75)	104.50 (102.00–109.00)
NH_4 (μg N L^{-1})	23 (10–37)	31 (14–43)	47 (16–153)	43 (29–99)	35 (17–116)	353 (203–519)	39 (29–97)	395 (192–620)
BOD_5 (mg L^{-1})	1.00 (0.25–1.75)	2.00 (1.25–3.50)	1.00 (0–2.00)	0 (0–0.25)	0 (0–0.25)	0 (0–0.25)	0 (0–0.25)	1.00 (1.00–1.25)
COD (mg L^{-1})	12.00 (6.00–15.00)	10.50 (7–16.25)	9.00 (3.00–14.25)	15.00 (8.50–21.25)	10.00 (4.50–15.50)	6.50 (0.75–12.75)	12.5 (7.5–17.5)	15.5 (11.25–19.5)
KN (μg L^{-1})	0 (0–450)	0 (0–450)	600 (150–600)	0 (0–150)	0 (0–150)	0 (0–700)	0	0 (0–1125)
NO_3 (μg N L^{-1})	50 (20–60)	150 (140–180)	280 (120–350)	4.5 (0–14.1)	60 (22–95)	109 (55–282)	65 (30–168)	130 (30–443)
NO_2 (μg N L^{-1})	0.5 (0.1–11.6)	2 (1–5)	0.5 (0.3–1.1)	1 (0–2)	2 (1–3)	3 (2–3)	0 (0–0.2)	5 (1–9)
PO_4 (μg P L^{-1})	2 (0–11)	8 (2–42)	20 (8–26)	0 (0–9)	0 (0–15)	101 (52–146)	0 (0–9)	13 (9–72)
Si (μg L^{-1})	150 (100–200)	400 (250–550)	1150 (1025–1200)	250 (175–325)	300 (300–400)	1450 (1225–1600)	100	1050 (950–1175)
TH (mg L^{-1})	162.5 (151.8–169.5)	157.5 (150.3–176.0)	174.5 (153.5–186.5)	155.0 (148.8–163.5)	160.0 (146.8–173.8)	142.5 (125.5–154.5)	166.5 (148.8–189.0)	156.5 (149.8–164.8)
TP (μg P L^{-1})	25 (13–30)	50 (18–60)	50 (43–125)	15 (10–43)	20 (10–73)	180 (133–203)	30 (8–68)	80 (68–143)
TN (μg N L^{-1})	84 (57–477)	244 (157–630)	765 (440–1055)	21 (7–174)	74 (23–261)	416 (132–1213)	15 (7–38)	135 (9–1333)
TSS (mg L^{-1})	3.25 (2.25–4.63)	1.85 (1.55–2.00)	1.00 (1.00–2.50)	4.50 (3.50–5.00)	2.25 (2.00–3.00)	1.75 (1.13–2.20)	3.25 (2.93–3.75)	1.85 (1.03–7.88)
TU (NTU)	2.91 (2.26–3.64)	2.09 (1.87–2.27)	2.43 (2.14–12.02)	3.45 (2.75–4.14)	1.81 (1.58–2.03)	1.95 (1.87–2.02)	3.57 (2.965–4.175)	17.16 (9.84–24.48)
Fe (μg L^{-1})	–	–	70 (70–350)	–	–	215 (80–333)	–	275 (245–365)
Mn (μg L^{-1})	–	–	0 (0–210)	–	–	220 (125–305)	–	510 (398–610)
Chl a (μg L^{-1})		7.45 (5.80–9.48)			13.10 (8.23–17.63)			7.85 (5.40–11.03)

June, decreasing to ca. 8.5 m in July and August, and increasing again in September to ca. 14 m. The T of epilimnetic mixed waters reached 27.3 °C in the 2014 summer hottest periods (August), in contrast with the hypolimnetic waters that maintained the T between 13 and 14 °C. Therefore, there was a difference of >10 °C between the two layers, which remained constant over the monitoring period, despite the slight warming of the hypolimnion through time. Water pH values are higher in the epilimnion (8.8–9.3) than in the hypolimnion (7.0–7.8) due to photosynthesis, which is also shown in the oxygen oversaturation of surface waters (particularly evident in July, with 121.8% O_2 sat). However, the hypolimnion does not present anoxia in June (49.1% O_2 sat at the bottom) neither in September (22.5% O_2 sat at the bottom), most probably due to the precipitation events in the days prior to the sampling (Table 1). Anoxia (0–10% O_2 sat) occurs at 43 m in July and higher in the water column (35 m depth) in August (Fig. 4). The analysis of the ORP shows positive values from June to August.

During the study period, Secchi disk water transparency decreased in Montante, from 3 m in July to 1.6 m in September, whilst the light extinction coefficient (k) increased from 0.495 m^{-1} to 0.905 m^{-1}. The mean lower depth limit of the euphotic zone (1% of surface PAR) was 6.7 m in Montante. Whilst in Alcarrache and Mourão, lowest transparency values were detected in August, the peak of the Summer (1.0 m

and 1.2 m, respectively), with high k values (0.695 m^{-1} in Alcarrache and 1.21 m^{-1} in Mourão).

3.3. Diatoms

A total of 237 specific and infraspecific diatom taxa were identified in the dataset (56 samples), from which only 123 were present with a relative abundance above 1% in at least one sample (presented as Appendix in Table A.2). These taxa were comprised in 37 genera, from which *Nitzschia* (20 taxa), *Navicula* (12 taxa), *Gomphonema* (11 taxa) and *Achnanthidium* (10 taxa) were the most specious. The most abundant species (relative abundance >20%) occurring in >50% of all samples were: *Cyclotella ocellata* Pantocsek (96.4% of samples), *Navicula cryptotenelloides* Lange-Bertalot (94.6%), *Achnanthidium minutissimum* (Kützing) Czarnecki (92.9%), *Navicula cryptotenella* Lange-Bertalot (80.4%), *Achnanthidium caravelense* Novais & Ector (76.8%), *Amphora pediculus* (Kützing) Grunow (69.6%), *Fragilaria recapitellata* Lange-Bertalot & Metzeltin (69.6%) and *Fragilaria radians* (Kützing) D.M. Williams & Round (64.3%). Other taxa, such as *Achnanthidium lineare* W. Smith (66.1% of samples), *Puncticulata radiosa* (Lemmermann) Håkansson (64.3%), *Navicula capitatoradiata* Germain (62.5%), *Nitzschia inconspicua* Grunow (60.7%), *Fragilaria tenera* (W.Smith) Lange-Bertalot
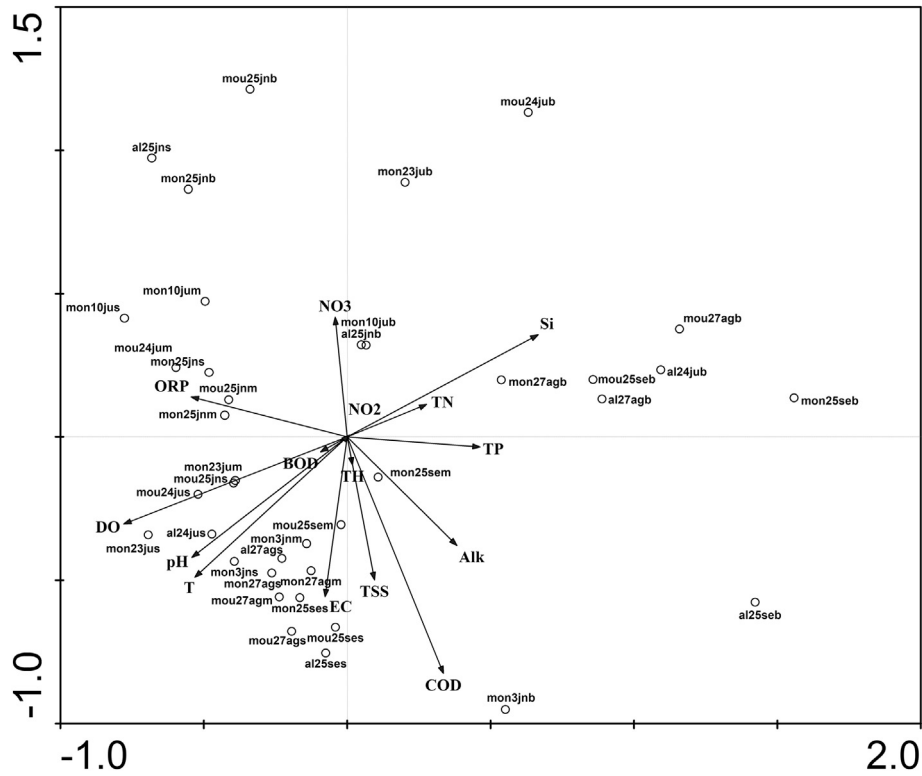
Fig. 3. PCA plot for the first two components, showing the distribution of sites according to the water physico-chemical gradients.

(57.1%), *Nitzschia dissipata* (Kützing) Grunow (51.8%), *Actinocyclus normanii* (Gregory ex Greville) Hustedt (50%), *Cyclostephanos dubius* (Fricke) Round (50%) and *Encyonopsis minuta* Krammer & E. Reichardt (50%) were present in >50% of the samples, but in low abundance (relative abundance always <20%). On the other hand, there are taxa such as *Cocconeis lineata* Ehrenberg (42.1% of relative abundance), *Achnanthidium nanum* (F. Meister) Novais & Jüttner (25%), *Eolimna minima* (Grunow) Lange-Bertalot (24.9%) and *Achnanthidium* aff. *kranzii* (Lange-Bertalot) Round & Bukhtiyarova (21.2%) that are abundant

(>20% relative abundance in at least one sample), but not widely distributed in the dataset.

The nMDS analysis (2D ordination plots presented in Figs. 5, 6 and 7) presented a stress level (indicator of the adequacy of the representation) of 0.18, which is below the limit for a potentially useful 2-D representation (0.2). This analysis revealed that the samples are not grouped by platform, despite the large dimensions of the reservoir (ordination plot in Fig. 5). These results were further confirmed by the ANOSIM analysis, with no differences detected between the three platforms
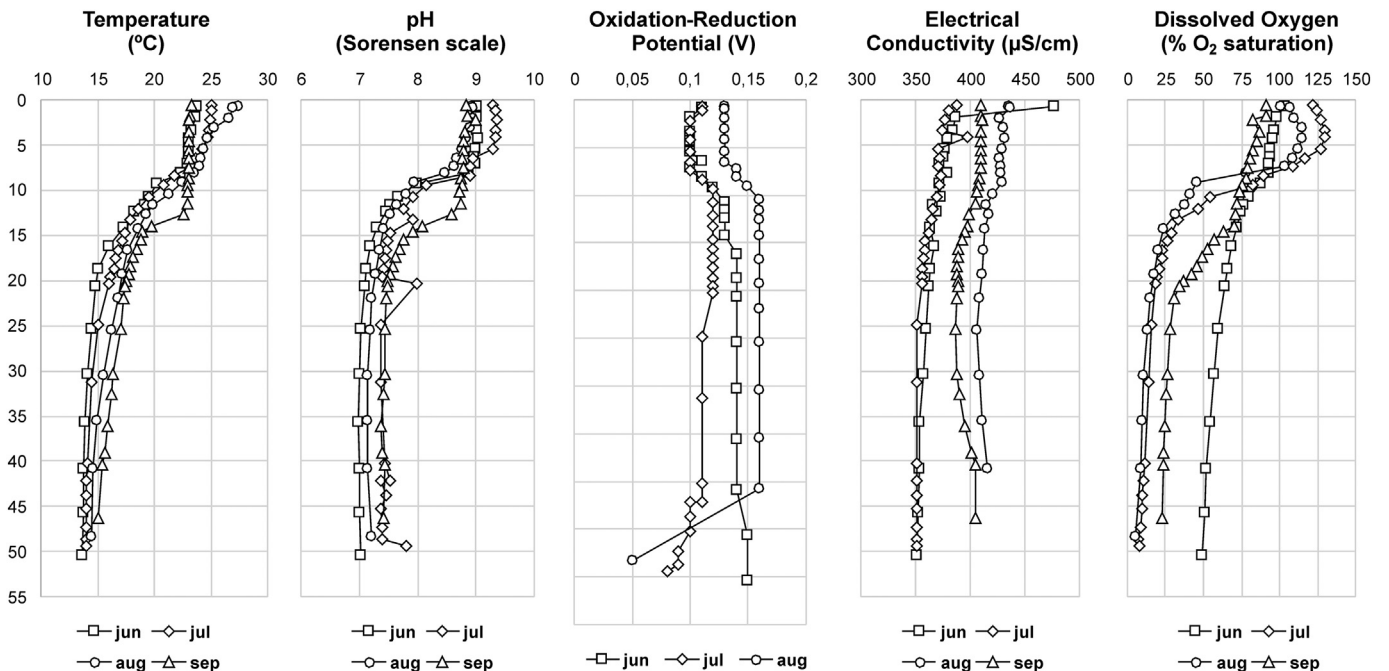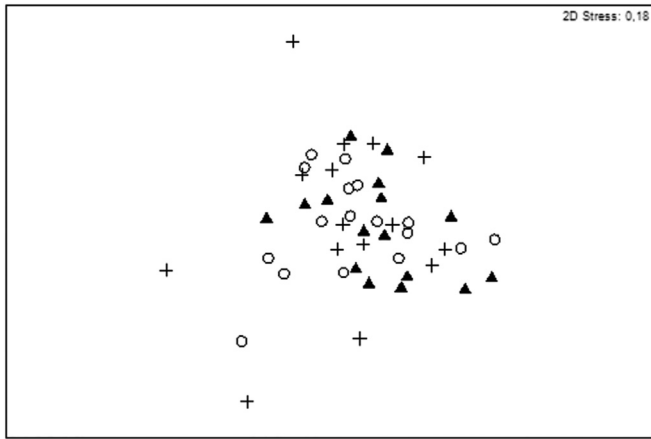

**Fig. 4.** Vertical profiles of in situ physical-chemical parameters in Mourão platform.

Fig. 5. 2D Non-Metric Multidimensional Scaling (nMDS) ordination plot of sites based on diatom assemblages. Black triangle: Montante; white circle: Mourão; cross: Alcarrache.

Fig. 7. 2D Non-Metric Multidimensional Scaling ordination plot of sampling depths based on diatom assemblages. Inverted triangle: Surface; square: 2.5 m; diamond: 5 m; circle: 10 m; cross: 20 m; asterisk: 30 m.

(Global $R = 0.123$, $p < 0.001$). Nevertheless, diatom assemblages in Montante and Mourão are more similar ($R = 0.09$, $p < 0.05$) than in Montante/Alcarrache ($R = 0.131$, $p < 0.05$) and in Mourão/Alcarrache ($R = 0.147$, $p < 0.05$) (Table 3). Also, the SIMPER analysis revealed a lower average dissimilarity between Montante and Mourão platforms (61.25%), presented in Table 3. The nMDS analysis revealed that the samples are also not grouped by month, with the three samplings randomly represented (ordination plot in Fig. 6). The ANOSIM analysis did not detect differences between the sampling dates (Global $R = 0.158$, $p < 0.001$), either; even though there is some evidence of small shifts in diatom assemblages, given that the campaigns in July/August ($R = 0.073$, $p < 0.05$; 60.69% average dissimilarity) are more similar than in August/September ($R = 0.123$, $p < 0.001$; 64.46% average dissimilarity) and July/September ($R = 0.279$, $p < 0.001$; 65.31% average dissimilarity) (Table 3). On the other hand, samples are grouped according to the depth (Fig. 7). These results were further confirmed by the ANOSIM analysis (presented in Table 3), revealing differences among diatom assemblages collected at different depths (Global $R = 0.336$, $p < 0.001$). Littoral samples collected from the margin were significantly different from the platform samples, independently of the depth (Table 3). There was a gradual shift in diatom assemblages with depth, revealed by the low R values between adjacent depths, and complemented by the lower average dissimilarity percentages. Diatom assemblages were more different with higher distances in depths, revealed by the higher R values and average dissimilarities (Table 3), except for the comparisons with the 30 m depth, probably because of
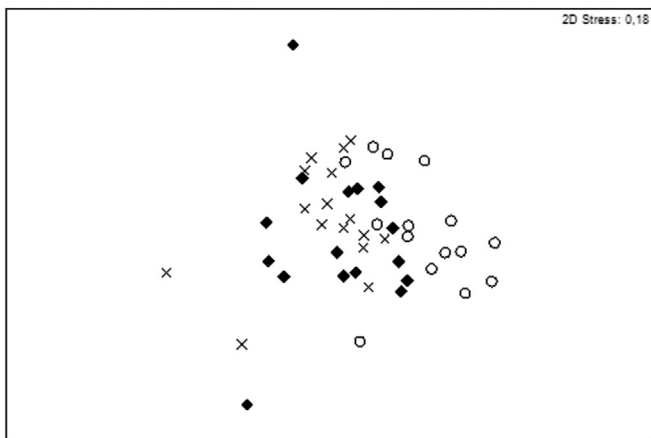
Fig. 6. 2D Non-Metric Multidimensional Scaling (nMDS) ordination plot of sampling months based on diatom assemblages. x: July; black diamond: August; white circle: September.
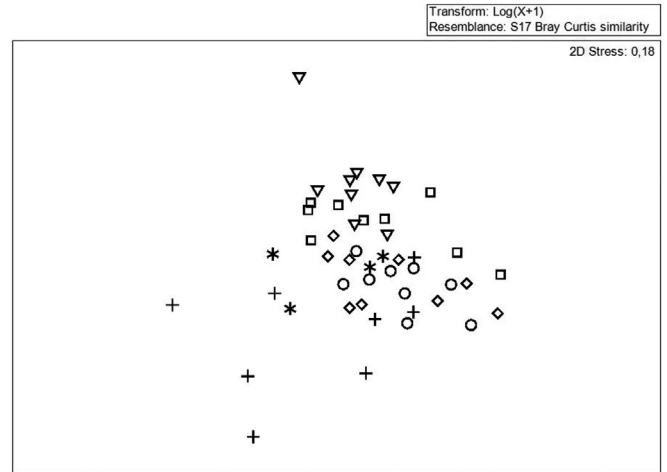
the lower number of samples that were possible to collect (some tiles detached from the rope and were impossible to recover).

Indicator value analysis undertaken to identify characteristic taxa for the margin and different depths of the reservoir revealed that *Gomphonema italicum* Kützing, *Navicula antonii* Lange-Bertalot and *Staurosira binodis* Lange-Bertalot were indicators of the littoral areas of the reservoir, whilst *Actinocyclus normanii*, *Aulacoseira ambigua* (Grunow) Simonsen, *Cyclostephanos dubius* and *Nitzschia subacicularis* Hustedt were indicators of 10 m depth; *Chamaepinnularia* sp., *Discostella pseudostelligera* (Hustedt) Houk & Klee, *Gomphonema lagenula* Kützing, *G. parvulum* (Kützing) Kützing, *G. pumilum* var. *rigidum* E. Reichardt & Lange-Bertalot, *Mayamaea permitis* (Hustedt) Bruder & Medlin and *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot were indicators of 20 m depth; and *Achnanthidium nanum*, *Cocconeis pseudolineata* (Geitler) Lange-Bertalot and *Gomphonema rhombicum* M. Schmidt were indicators of 30 m; and no indicator taxa were identified for the surface, 2.5 m and 5 m (Table 4). From these, there is no information available on the autecology of *Gomphonema italicum*, *G. lagenula*, *G. pumilum* var. *rigidum*, *G. rhombicum*, *Chamaepinnularia* sp. and *Achnanthidium nanum* (Van Dam et al., 1994, retrieved from the OMNIDIA v.5.5). For the other taxa, most are classified as alkaliphilous (*Staurosira binodis*, *Navicula antonii*, *Actinocyclus normanii*, *Aulacoseira ambigua*, *Nitzschia subacicularis*, *Mayamaea permitis*, *Planothidium frequentissimum* and *Cocconeis pseudolineata*), only *Cyclostephanos dubius* is alkalibiontic, and *Discostella pseudostelligera* and *Gomphonema parvulum* are circumneutral. The majority of the indicator taxa is nitrogen autotrophic, tolerating elevated concentrations of organically bound nitrogen, but *Nitzschia subacicularis* only tolerates very small concentrations of organically bound nitrogen, and *Gomphonema parvulum* and *Mayamaea permitis* are facultatively nitrogen-heterotrophic taxa, needing periodically elevated concentrations of organically bound nitrogen. The oxygen requirements of these taxa are more variable, since *Staurosira binodis* and *Nitzschia subacicularis* require continuously high oxygen levels (aprox. 100% O_2 sat), *Cyclostephanos dubius* fairly high (<75% O_2 sat), *Actinocyclus normanii*, *Aulacoseira ambigua*, *Discostella pseudostelligera* and *Planothidium frequentissimum* moderate (<50% O_2 sat) and *Gomphonema parvulum* and *Mayamaea permitis* low (>30% O_2 sat). Only *Staurosira binodis* is oligosaprobous, *Aulacoseira ambigua* and *Nitzschia subacicularis* are β-mesosaprobous, *Actinocyclus normanii*, *Cyclostephanos dubius*, *Discostella pseudostelligera* are α-mesosaprobous and *Gomphonema parvulum*, *Mayamaea permitis* and *Planothidium frequentissimum* are α-meso/polysaprobous. The great majority of these taxa are eutraphentic, except for the meso-eutraphentic *Staurosira binodis* and for *Nitzschia*

Table 3

Percentage breakdown of average dissimilarity between the platforms, sampling campaigns and depths, using SIMPER analysis. Statistical and global R values for the pairwise analysis of similarity (ANOSIM) tests are presented, ***$p < 0.001$ and *$p < 0.05$ were regarded as significant, n.s. = not significant.

Factors	Groups	Average similarity (%)	Average dissimilarity (%)	ANOSIM (statistical R)	ANOSIM (Global R)
Platform	Montante	40.83%	Mont/Alc = 64.25	R = 0.131*	Global R = 0.123***
	Alcarrache	35.74%	Mont/Mou = 61.25	R = 0.09*	
	Mourão	40.89%	Alc/Mou = 64.50	R = 0.147*	
Month	July	43.34%	Jul/Aug = 60.69	R = 0.073*	Global R = 0.158***
	August	38.32%	Jul/Sep = 65.31	R = 0.279***	
	September	37.77%	Aug/Sep = 64.46	R = 0.123*	
Depth	Littoral	45.11%	Mar/Surf = 65.17	R = 0.599***	Global R = 0.336***
			Mar/2.5 m = 65.88	R = 0.477***	
			Mar/5 m = 64.83	R = 0.454***	
			Mar/10 m = 66.85	R = 0.81***	
			Mar/20 m = 69.75	R = 0.393***	
			Mar/30 m = 62.22	R = 0.467*	
	Surface	49.06%	Surf/2.5 m = 56.46	R = 0.085 n.s.	
			Surf/5 m = 63.75	R = 0.48***	
			Surf/10 m = 63.29	R = 0.647***	
			Surf/20 m = 72.31	R = 0.499***	
			Surf/30 m = 59.35	R = 0.374*	
	2.5 m	41.28%	2.5 m/5 m = 59.07	R = 0.089 n.s.	
			2.5 m/10 m = 59.97	R = 0.36*	
			2.5 m/20 m = 71.09	R = 0.333*	
			2.5 m/30 m = 59.23	R = 0.062 n.s.	
	5 m	43.55%	5 m/10 m = 53.51	R = 0.027 n.s.	
			5 m/20 m = 66.99	R = 0.194*	
			5 m/30 m = 58.79	R = 0.163 n.s.	
	10 m	49.91%	10 m/20 m = 64.11	R = 0.237*	
			10 m/30 m = 56.16	R = 0.29*	
	20 m	30.04%	20 m/30 m = 63.61	R = −0.222 n.s.	
	30 m	42.23%			

subacicularis and *Planothidium frequentissimum* that can range from oligo to eutraphentic (Van Dam et al., 1994, retrieved from the OMNIDIA v.5.5). All the characteristic species of the 10 m depth are aquatic and euplanktonic, except *Nitzschia subacicularis*, for which this information is not available (Denys, 1991, retrieved from the OMNIDIA v.5.5).

Other community descriptors such as taxa richness, Shannon diversity and Pielou's evenness indices did not reveal differences between the three sampling sites, confirming our nMDS results for diatom assemblages (One-way ANOVA, with F = 2.919, $p = 0.063$ for taxa richness; Kruskal-Wallis test with H = 3.134 with 2 degrees of freedom, $p = 0.209$ for Shannon diversity index and H = 2.183 with 2 degrees of

Table 4

Indicator values for the characteristic taxa of littoral areas and different depths of the reservoir. Monte Carlo tests were used to assess the significance of each taxon as a group-specific indicator ($p < 0.05$). Fidelity (F) and Specificity (S) values are also presented. Highlighted in bold are species with high fidelity and specificity values identified as group-specific indicator by Monte Carlo tests.

Code	Taxon name	Littoral F	S	IndVal	Surface F	S	IndVal	2.5 m F	S	IndVal	5 m F	S	IndVal	10 m F	S	IndVal	20 m F	S	IndVal	30 m F	S	IndVal
	Achnanthidium nanum (F.Meister) Novais & Jüttner	0	0	0	0	0	0	2	11	0	0	0	0	4	22	1	14	63	9	**80**	**75**	**60**
ANMN	*Actinocyclus normanii* (Gregory ex Greville) Hustedt	3	25	1	2	22	0	4	44	2	10	78	8	**43**	**89**	**38**	31	50	15	7	25	2
AAMB	*Aulacoseira ambigua* (Grunow) Simonsen	3	13	0	0	0	0	3	22	1	12	44	5	**49**	**78**	**38**	25	63	16	7	25	2
CHSP	*Chamaepinnularia* sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**100**	**25**	**25**	0	0	0
COPL	*Cocconeis pseudolineata* (Geitler) Lange-Bertalot	16	38	6	0	0	0	4	11	0	4	11	0	0	0	0	8	13	1	**69**	**50**	**35**
CDUB	*Cyclostephanos dubius* (Fricke) Round	2	25	0	2	11	0	2	22	0	12	56	7	**48**	**100**	**48**	14	75	10	21	75	15
DPST	*Discostella pseudostelligera* (Hustedt) Houk & Klee	0	0	0	0	0	0	6	11	1	3	11	0	6	11	1	**86**	**50**	**43**	0	0	0
GITA	*Gomphonema italicum* Kützing	**100**	**25**	**25**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GLGN	*Gomphonema lagenula* Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**100**	**25**	**25**	0	0	0
GPAR	*Gomphonema parvulum* (Kützing) Kützing	0	0	0	0	0	0	28	22	6	1	11	0	3	11	0	**69**	**50**	**34**	0	0	0
GPRI	*Gomphonema pumilum* var. *rigidum* E. Reichardt & Lange-Bertalot	0	0	0	0	0	0	0	0	0	12	22	3	0	0	0	**88**	**50**	**44**	0	0	0
GRHB	*Gomphonema rhombicum* M. Schmidt	13	25	3	0	0	0	24	11	3	0	0	0	5	22	1	0	0	0	**58**	**50**	**29**
MPMI	*Mayamaea permitis* (Hustedt) Bruder & Medlin	4	13	0	0	0	0	0	0	0	1	11	0	0	0	0	**95**	**50**	**48**	0	0	0
NANT	*Navicula antonii* Lange-Bertalot	**78**	**50**	**39**	0	0	0	0	0	0	9	11	1	5	22	1	8	13	1	0	0	0
NSUA	*Nitzschia subacicularis* Hustedt	2	13	0	0	0	0	7	22	1	24	22	5	**61**	**78**	**48**	6	13	1	0	0	0
PLFR	*Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot	4	50	2	1	11	0	0	11	0	6	67	4	12	56	7	**67**	**88**	**58**	10	75	8
SBND	*Staurosira binodis* Lange-Bertalot	**55**	**63**	**34**	9	33	3	4	11	0	21	22	5	11	22	2	0	0	0	0	0	0

freedom, $p = 0.336$ for Pielou's evenness index). The shift in diatom communities during the sampling campaign was also verified for these biological parameters (One-way ANOVA, F = 4.243, $p < 0.05$ for taxa richness and F = 7.123, $p < 0.05$ for Shannon diversity index; Kruskal-Wallis test, H = 7.081 with 2 degrees of freedom, $p = 0.029$ for Pielou's evenness index). Taxa richness and Shannon diversity index differ between July/September and August/September (Holm-Sidak method, $p < 0.05$), confirming the small shifts in diatom taxonomic composition detected by the ANOSIM results, and present the lowest values in September. Pielou's evenness index differs only between July/September (Dunn's Method, $p < 0.05$) and the lowest values were also detected in September (Table 5). Taxa richness changed with the depth gradient, according to the One-way ANOVA results (F = 5.269, $p < 0.001$), since surface samples differ from 5, 10 and 20 m samples (Holm-Sidak method, $p < 0.05$). Additionally, surface and littoral samples are also different (Holm-Sidak method, $p < 0.05$). Even though significant differences were obtained for the globality of Shannon diversity index data (littoral, surface and different depths) F = 2.742, and $p = 0.022$; further tests only detected significant differences between littoral and surface samples (Holm-Sidak method, $p < 0.05$). Lowest values of all parameters were detected at surface samples, increasing with depth, reaching the highest values at 20 m for taxa richness, Shannon diversity and Pielou's evenness indices. Comparable values with deeper samples were detected for littoral samples (Table 5).

Comparison of SPI index revealed that littoral samples SPI was lower than in upper layers of the limnetic zone of the reservoir, surface samples presented the highest SPI values and samples in the mid depth of the reservoir are more similar with each other (10 m and 20 m) (Table 5). These results were further validated by One-way ANOVA (F = 9.532, $p < 0.001$), with surface samples differing from littoral and samples collected at 5 m, 10 m and 20 m; samples collected at 2.5 m are also different from those at 10 m and 20 m (Holm-Sidak method, $p < 0.05$). SPI index varied during the campaign, with highest values in July, decreasing throughout the campaign (Table 5). These differences were further confirmed by the Kruskal-Wallis test (H = 8.855 with 2 degrees of freedom, $p = 0.012$, with July significantly different from September (Dunn's Method, $p < 0.05$). As expected no differences were detected between SPI index from different sites (Kruskal-Wallis test, H = 0.133 with 2 degrees of freedom, $p = 0.936$).

Diatom guilds showed a shift with depth, with surface and 2.5 m depth samples dominated by the low-profile guild (except for Montante surface and 2.5 m in August and September, respectively); whilst deeper samples were dominated by the planktic guild (Fig. 8), except for bottom samples in Alcarrache (20 m), that were dominated by the motile guild, especially in September (Fig. 8).

The high profile and motile guilds presented the lowest abundances in all sites and throughout the study, except for high profile in littoral samples collected in Montante in August. There was a shift in diatom guilds dominance in littoral samples, in July the low-profile dominated,

but the relative abundance of the high-profile guild increased throughout the campaign. An increase of the low-profile guild was revealed in Montante at 30 m and Mourão at 20 m and 30 m (Fig. 8). Despite data transformation, only the high-profile and motile guilds data passed the normality and equal variance tests. Therefore, differences on diatom guilds between platforms, sampling date and depth were tested by One-way ANOVA only for the motile and high-profile guilds, whilst for the remaining data a Kruskal-Wallis test was carried out. This analysis proved the results visible in Fig. 8, with the relative abundance of low-profile (H = 23.436 with 6 degrees of freedom, $p < 0.001$) and planktic (H = 25.252 with 6 degrees of freedom, $p < 0.001$) guilds differing with depth. Dunn's Method revealed that the low-profile guild at the surface of the reservoir differs from 5 m and 10 m samples ($p < 0.05$); and the planktic guild from littoral and surface samples differ from the 10 m depth ($p < 0.05$). The motile guild relative abundance from littoral samples showed differences from that of the surface samples (H = 13.922 with 6 degrees of freedom, $p = 0.031$), according to the Dunn's Method ($p < 0.05$). Differences between platforms were only detected for high-profile guild ($p = 0.031$), with Holm-Sidak method detecting changes between Montante and Mourão ($p < 0.05$). Throughout the summer, there was a shift in low-profile (H = 13.923 with 2 degrees of freedom, $p < 0.001$) and planktic diatom guilds (H = 9.337 with 2 degrees of freedom, $p = 0.009$), with the Dunn's Method separating July from September samples ($p < 0.05$).

Positive Spearman rank order correlations were detected between: 1) taxa richness (S) and depth, TP and Si; 2) H′ index, TP and Si; 3) J′ index and Si; 4) number of genera, depth and Si; 5) SPI, DO and T; and 6) the motile guild and Si. Whilst negative correlations were detected between: 1) S and T; 2) number of genera, pH and T; 3) SPI, depth, PO_4-P, NO_3-N and Si (Table 6).

4. Discussion

During this study, vertical profiles of T, pH and DO showed the stratification of the reservoir, starting in June and lasting until the end of the campaign, at the end of September. However, the depth of the thermocline changed throughout the experiment, from ca. 10 m in June, decreasing in July and August to ca. 8.5 m and deepening again in September to ca. 14 m (see Fig. 4). These changes in thermocline depth and thickness of epilimnetic waters can be related to the meteorological conditions during the experiment, such as the monthly average temperature, the average, minimum and maximum temperature 8 days before the sampling campaign and the maximum temperature, that increased in July and August and decreased again in September. Furthermore, this decrease in atmospheric temperature was associated with the precipitation events before the campaign in September, contributing to the decrease of the water T.

An interesting finding, observed in all vertical profiles, is the absence of any inflexion in the vertical pattern. This demonstrated the low

Table 5

Mean values ± standard deviation for the taxa richness (S), Shannon index of diversity (H′), Pielou's evenness index (J′) and Specific Pollution Sensitivity Index (SPI) for the depth gradient, sampling campaign and site.

		S	H′	J′	SPI
Depth gradient	Littoral	36.75 ± 10.22	3.76 ± 0.60	0.73 ± 0.06	14.04 ± 1.11
	Surface	18.78 ± 8.94	2.24 ± 0.83	0.53 ± 0.14	16.60 ± 1.81
	2.5 m	26.44 ± 8.50	2.64 ± 0.99	0.55 ± 0.17	14.38 ± 2.19
	5 m	34.00 ± 11.52	2.72 ± 1.41	0.52 ± 0.24	12.33 ± 1.56
	10 m	38.89 ± 10.68	2.77 ± 0.89	0.52 ± 0.14	11.90 ± 1.30
	20 m	40.75 ± 11.57	3.62 ± 0.93	0.68 ± 0.13	11.83 ± 1.67
	30 m	36.75 ± 4.27	3.18 ± 0.47	0.62 ± 0.11	13.83 ± 1.42
Sampling campaign	July	36.26 ± 10.75	3.34 ± 0.80	0.65 ± 0.11	14.70 ± 2.03
	August	35.21 ± 11.61	3.21 ± 0.90	0.63 ± 0.13	13.34 ± 1.97
	September	26.22 ± 12.04	2.26 ± 1.13	0.48 ± 0.20	12.58 ± 2.41
Sampling site	Montante	30.11 ± 10.09	2.77 ± 1.01	0.56 ± 0.17	13.41 ± 2.22
	Alcarrache	29.59 ± 13.04	2.75 ± 1.01	0.57 ± 0.16	13.76 ± 2.74
	Mourão	37.75 ± 11.99	3.29 ± 1.09	0.63 ± 0.17	13.52 ± 2.61

Fig. 8. Depth gradient of relative abundance of diatom guilds for the three platforms and sampling campaigns. Mont: Montante; Alc: Alcarrache; Mour: Mourão; Jul: July; Aug: August; Sep: September.

influence of a riverine inflow. Therefore, the Alqueva reservoir seems to present the typical characteristics of a lake system throughout the campaign.

The surface oxygen concentration is constantly regulated by the air-water fluxes, which tend to bring the surface concentration towards the saturation value (Capet et al., 2013). However, in July the surface waters

Table 6
Spearman rank order correlation coefficients between diatoms, expressed as taxa richness (S), Shannon diversity (H′) and Pielou's evenness (J′) indexes, number of genera, SPI and ecological guilds, depth and water physical-chemical parameters depth dependent *$p < 0.05$, in bold.

	pH	T	%OD	EC	Depth	PO_4	TP	NO_3	Si	TSS
S	**−0.34***	**−0.40***	−0.28	−0.28	**0.59***	0.34	**0.44***	0.20	**0.55***	−0.21
H′	−0.21	−0.22	−0.13	−0.28	**0.37***	0.40	0.42*	0.13	**0.59***	−0.10
J′	−0.12	−0.14	−0.05	−0.23	0.22	0.32	0.28	0.09	**0.50***	0.00
Number of genera	**−0.45***	**−0.49***	**−0.34***	−0.21	**0.64***	0.26	0.38	0.30	**0.58***	−0.41
SPI	**0.39***	**0.49***	**0.45***	−0.09	**−0.54***	**−0.47***	−0.37	**−0.48***	**−0.52***	0.37
Low-profile	0.25	**0.32***	**0.31***	−0.20	**−0.36***	−0.32	−0.17	−0.37	−0.32	0.18
High-profile	−0.04	0.16	−0.06	0.18	−0.10	0.39	0.27	−0.20	0.03	0.22
Motile	−0.13	−0.14	−0.14	−0.19	0.26	0.39	0.39	−0.10	**0.57***	−0.08
Planktic	−0.10	−0.26	−0.15	0.12	0.26	0.01	−0.09	0.52	0.04	−0.14

in the three sites were oversaturated with oxygen due to enhanced photosynthesis, revealed also by the higher pH in the epilimnion. Stratification should allow the settlement of bottom anoxic conditions by preventing the bottom oxygen-depleted waters from mixing with the surface ventilated waters (Boulton and Brock, 1999). However, the hypolimnion does not present anoxia in June and September, most probably due to the precipitation events in the days prior to the sampling, and in July and August, anoxia occurs deep in the water column (Fig. 4). Hence, the consumption of oxygen by respiration is not sufficiently high to deplete oxygen in the whole hypolimnion, contrary to what occurs in highly eutrophic reservoirs (Wetzel, 1993). The analysis of ORP (Fig. 4) shows positive values from June to August. This oxidized environment during the stratified period contrasts with other Mediterranean eutrophic reservoirs, where ORP is highly negative due to respiration (i.e. degradation of organic matter).

Horizontal gradients in physical, chemical and biological factors in reservoirs result from the combined influence of hydrodynamics and basin morphology (Kennedy and Walker, 1990), separating the nearshore (the littoral zone) from the offshore environment (Lewis, 2009). No significant differences were detected between sampling platforms for water physico-chemical variables and neither for diatoms (in terms of diatom communities, diversity indices and SPI). This spatial homogenization can be related with the absence of a riverine effect, detected on the smooth vertical profiles for T, pH, DO and EC, with no visible inflexions (Fig. 4). This absence of a riverine effect during summer can reflect the lower discharge from the Guadiana River (regulated upstream), and the null discharge from the other intermittent tributaries. This means that other factors, like the light extinction coefficient (k), nutrient and dissolved oxygen availability, and sedimentation are shaping the system organization, along the vertical axis. Kahlert et al. (2002), obtained opposite results for Lake Erken (Sweden), with horizontal variation accounting for 60–80% and 7–70% of the total variation in biomass and in nutrient status, respectively. Among the physicochemical variables, nutrients are one of the most important factors controlling reservoir water quality and productivity, and nutrient distribution and concentrations are affected by several processes, such as nutrient loading, sedimentation, flow, mixing and discharge (Kennedy and Walker, 1990; Zhenyao et al., 2013). No differences were detected in nutrient concentration between platforms. A depth gradient was observed, with higher values of PO_4 and TP detected in bottom samples than at the surface; and bottom and metalimnion presented higher NO_3 concentrations than surface samples. This can be related to the low contribution of external loads to the system, during summer.

The fact that there is no information about the autecology of several indicator species in Van Dam et al. (1994, retrieved from the OMNIDIA v.5.5) reveals the need to study more in detail these systems, combining a fine-grained taxonomy with a good environmental characterization. From these, *Achnanthidium nanum* was first described from high altitude lakes in the Swiss Alps and was later found in streams in the Centre and North of Portugal by Novais et al. (2015), characterized by soft waters, but with concentrations of nutrients within the same range as

in the present study, and also associated with *A. caravelense* and *Gomphonema rhombicum*. These taxa found at 30 m depth should be studied in more detail, since there is also not enough data to assess the Red List status (Lange-Bertalot and Steindorf, 1996, retrieved from the the OMNIDIA v.5.5) for *A. nanum* and *C. pseudolineata*, whilst *Gomphonema rhombicum* is evaluated as "in risk" for German watercourses by Hofmann et al. (2011).

The indicator species identified in this study are mostly alkaliphilous or alkalibiontic, reflecting the alkaline characteristic of the reservoir, revealed in the vertical profile of pH, with values >7 during the whole campaign and in all depths (Fig. 4). Interestingly, all indicator species of the 20 m depth have moderate (<50% O_2 sat.) or low (>30% O_2 sat.) oxygen requirements, reflecting the low oxygen availability at these depths (<50% O_2 sat.) from July to September, as seen in Fig. 4.

Littoral benthic diatoms differ from the limnetic zone in terms of diatom communities, diversity indices and SPI, with highest values for diversity indices detected in littoral zones. A depth gradient was detected for diatoms (diatom assemblages and ecological guilds) and diversity indices, as can be seen in the nMDS plot (Fig. 7) validated by the ANOSIM results in Table 3. This gradient is part of a continuum, but no zonation was detected, unlike the results obtained by Cantonati et al. (2009) and Stevenson and Stoermer (1981) who detected three zones in their studies in the oligotrophic lakes Tovel and Michigan. This zonation was characterized by disturbance due to wave and water fluctuation in the shallow zone, high stability with favourable growth conditions in terms of nutrients and light in the mid-depth zone and severe light limitation in the deep zone (Stevenson and Stoermer, 1981; Cantonati et al., 2009). Water level fluctuations were negligible, due to the large dimension of the reservoir and to the sampling carried out during stable weather conditions in summer. However, wave disturbance could be detected due to the increase of the wind speed at the end of the day (as seen in Fig. 2), playing an important role in shaping diatom communities of the upper layers and contributing to the differences between them and the communities present in the deeper layers of the reservoir. During the campaign the mean lower limit of the euphotic zone was 6.7 m in Montante, with the lowest value detected in Mourão in August (3.3 m), but this limit didn't have an apparent effect on diatom assemblages, given that living diatoms were present at 30 m depth. Richardson et al. (1983) stated that the adaptation of microalgae to very low photon flux densities may be related to a combination of structural, behavioural, physiological and biochemical factors, such as the lower amount of non-photosynthetic tissue when compared with macroalgae and vascular plants. Other possible adaptation to these harsh conditions may be the photosynthetic pigments composition related to light harvesting and photoprotection: chlorophylls and carotenoids. Diatoms are characterized by the presence of Chl *a* and Chl *c*, being Chl *c*1 and *c*2 the most abundant. The Chl *c* has a distinctive structure that allows changes in the absorption spectrum to produce a strong blue absorption band and a weak band in the red region (Kuczynska et al., 2015). This fact together with the higher penetration of blue/green/yellow light in waters of Alqueva reservoir than red light (Potes et al., 2017), allows diatoms to use light more efficiently, and can

contribute to explain their presence in deeper layers. Species present at low light depths can be supplementing their photosynthetic carbon assimilation with heterotrophic or photoheterotrophic use of organic carbon sources (Richardson et al., 1983). Stevenson and Stoermer (1981) also suggested that mobile taxa capable of heterotrophic nutrition could have gained competitive advantage in deeper habitats characterized by greater sediment accumulation.

In the Alqueva reservoir, diversity indices increased with depth (reaching a maximum at 20 m), similarly to the results obtained by Hostetter and Stoermer (1968), in a Summer campaign in Lake West Okoboji (Iowa, USA), who observed a consistent increase in diatom diversity. Cantonati et al. (2009) also stated that diversity increased with depth in the oligotrophic Lake Tovel (characterized by relevant seasonal water-level fluctuations) and Flower et al. (2004) found that diatom communities were more diverse and taxonomically complex in deeper water (ca. 20 m) samples from Lake Baikal. Whilst Stevenson and Stoermer (1981) present contrasting results, with a decrease in species diversity with depth. In the Alqueva reservoir, S and H′ were positively correlated with TP and Si, in agreement with the results of Cantonati et al. (2009), who also identified these environmental controls, together with water level fluctuations and PAR.

A depth-gradient was also observed for diatom guilds, with low-profile dominating the upper layers (surface and 2.5 m), and gradually being replaced by the planktic guild at 5 m and especially at 20 m. Exceptionally, the motile guild dominated the assemblages at the bottom of the Alcarrache platform (20 m), in accordance with the observations of Hoagland and Peterson (1990), who stated that the dominance of motile diatoms on substrata in deeper waters undoubtedly relates to the need of motility in a habitat subject to a constant rain of sediments. Furthermore, Admiraal and Peletier (1979) stated that heterotrophic diatoms are mainly found among pennate benthic forms, and proved that the growth rate in the dark of several motile *Nitzschia* and *Navicula* species was enhanced by organic substrates in the medium. The dominance at upper layers of low-profile guild, characterized by species adapted to high current velocities and low nutrient concentrations (Rimet and Bouchez, 2012), reflects the lower nutrient content at the surface of the reservoir (Table 2) and the wave disturbance due to the wind, especially at the end of the afternoon with the effect of the maritime breeze, as can be seen in Fig. 2. The 5 m and 10 m depths were dominated by the planktic guild, which includes taxa adapted to lentic conditions (Rimet and Bouchez, 2012), as is the case at these depths.

This is one of the few studies analysing the vertical variation of benthic diatoms in deeper layers in a large reservoir in the Mediterranean region. Our findings reveal a vertical succession of diatom assemblages (but no clear zonation was detected), with living diatoms present at 30 m in the Montante and Mourão platforms, and at the bottom in the Alcarrache platform. These preliminary results can lead to further studies, where physiological adaptations of diatoms to these conditions can be explored in detail, such as differences/changes in the photosynthetic apparatus. Furthermore, it would be interesting to verify if living diatoms are present in deeper areas of the reservoir, since it can reach >70 m, and if there are seasonal variations of deep diatom assemblages.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.12.251.

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