Zooplankton dynamics and water quality of the reservoirs from the Alqueva Irrigation System

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.
O Presidente do Júri,

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Dissertação submetida a Faculdade de Ciências da Universidade do Porto, para a obtenção do grau de Mestre em Ecologia e Ambiente, da responsabilidade do Departamento de Biologia.

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“ReDEFine: a multi-scale and multi-tiered toolbox for assessing ecosystem quality of freshwater REservoirs: briDging the gaps of the watEr Framework dIrEctive approach”

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Resumo

As construções de barragens causam uma enorme mudança ecológica no ecossistema de um rio. Além de ser uma barreira física, existe uma alteração de um ambiente natural lótico para um meio artificial de caraterísticas lênticas a montante da barragem. Uma das comunidades que se aproveita destas novas condições é o zooplâncton. Através da avaliação da estrutura da comunidade deste grupo de organismos, é possível inferir sobre os impactes nestes ecossistemas aquáticos. Neste sentido, o objetivo do presente trabalho foi avaliar a dinâmica zooplanctónica como elemento na avaliação da qualidade da água, usando como local de estudo as albufeiras do Sistema Global de Rega de Alqueva. Foram selecionadas oito albufeiras e mensalmente, ao longo de um ano, foram avaliados parâmetros físicos e químicos na água, e analisada a comunidade zooplanctónica. Os resultados dos parâmetros físicos e químicos quantificados, exceto a clorofila *a*, não ultrapassaram os limites estipulados pela DQA para a classificação do bom potencial ecológico para este tipo de massas de água. No entanto, a condutividade (~600 µS/cm) e o potencial redox, negativo durante todo o ano, evidenciaram a pobre qualidade da água de todas as albufeiras estudadas. As albufeiras classificaram-se como mesotróficas, tendo em conta a concentração de clorofila *a*, exceto a albufeira de Roxo, que se classificou com o estado eutrófico. A densidade do zooplâncton foi baixa durante todo o ano (<50 ind/L, exceto em Serpa), mas apresentou um pico no fim do Verão ou no Outono. A comunidade zooplanctónica apresentou uma sazonalidade clara e semelhante em todas as albufeiras, com a exceção da albufeira do Roxo. O final da Primavera foi marcada pelo declínio de *Daphnia longispina*, acompanhado por um aumento de pequenos cladóceros e *Diaphanosoma mongolianum*. As amostras de verão apresentaram uma dominância de géneros caraterizados como “high efficiency bacteria feeders” (*Ceriodaphnia* sp. e *D. mongolianum*), destacando a importância do bacterioplâncton neste período do ano. A albufeira do Roxo no entanto apresentou uma dominância de *Bosmina longirostris* durante todo o ano. No Outono, foi observado o potencial ecológico mais baixo em todas as albufeiras, devido à concentração de clorofila *a* (>15 µg/L) e aos baixos valores de transparência observados que se refletiu na dominância de ciclopóides. O declínio de grandes cladóceros e de aumento de “high efficiency bacteria feeders” no Verão, a falta de macrofiltradores, a dominância de omnívoros, e náuplios de copépodes durante o ano de amostragem mostraram um aumento da deterioração da qualidade da água das albufeiras do Sistema Global de Rega de Alqueva. Apesar da boa classificação pelos critérios da DQA, a comunidade estrutural e funcional do zooplâncton refletiu um estado eutrófico para estas albufeiras. Foram ainda observadas duas espécies exóticas, *Daphnia parvula* (exceto na albufeira de Odivelas) e *Bosmina coregoni* (exceto na albufeira do Pisão) justificando assim a necessidade de uma análise mais aprofundada, especialmente *B. coregoni*, visto que já foi descrita por diversos autores como invasora para outras albufeiras mediterrâneas. Neste sentido, a análise da dinâmica do zooplâncton foi uma ferramenta importante na classificação da qualidade da água das albufeiras estudadas, o que justifica a sua adição na DQA.

Palavras-chave: Cladocera, Copepoda, grupos funcionais, albufeiras mediterrânicas, Diretiva Quadro da Água, qualidade da água.

Abstract

The construction of dams causes a huge ecological change in the ecosystem of a river. In addition to being a physical barrier, there is the conversion of a lotic natural environment to an artificial lentic medium upstream of the dam. One of the communities that take advantage of these new conditions is the zooplankton. Through the assessment of its community structure at a given moment, it is possible to infer the impacts on these aquatic ecosystems. In this regard, our aim is to assess the zooplanktonic dynamics as an element for water quality assessment, by using the reservoirs from the Alqueva Irrigation System as study cases. Eight reservoirs from this system were selected and, throughout one sampling year, several physical and chemical parameters were evaluated monthly, as well as the zooplanktonic community. All quantified parameters except chlorophyll *a* never surpassed the stipulated thresholds defined by the WFD for this type of waterbody. However, conductivity (~600 µS/cm) and the negative redox potential throughout the year showed the poor water quality of all studied reservoirs. These reservoirs were classified as mesotrophic, according to chlorophyll *a* concentrations, except the Roxo reservoir which was classified as eutrophic. Zooplankton density was low throughout the year (<50 ind/L, except in Serpa) but showed a peak in late summer or autumn. The zooplankton community presented a clear similar seasonality in all the reservoirs, except in the Roxo reservoir. The end of spring was marked by the decline of *Daphnia longispina*, with a shift to small cladocerans and *Diaphanosoma mongolianum*. The summer samples had a dominance of high efficiency bacteria feeders (*Ceriodaphnia* sp. and *D. mongolianum*), underlining the importance of bacterioplankton in this period. In the autumn, the lowest ecological potential due to high chlorophyll a concentration (>15 µg/L) and low transparency values were observed, which reflected a dominance of cyclopoids. The extensive decline of large cladocerans and dominance of high efficiency bacteria feeders in the summer, the lack of macrofiltrators, the dominance of omnivores, and copepod nauplii throughout the sampling year show a deterioration of the water quality of the reservoirs in the Alqueva Irrigation System. Despite the good classification by WFD criteria, the structural and functional community of zooplankton reflected the eutrophic status of these reservoirs. Two exotic species, *Daphnia parvula* (except in Odivelas) and *Bosmina coregoni* (except in Pisão), were found in all of the sampled reservoirs, justifying an in-depth analysis, especially *B. coregoni* which was already described by several authors as invasive in other Mediterranean reservoirs. With all of this in mind, the assessement of zooplankton dynamics was an important tool in classifying the water quality of the studied reservoirs, which justifies its addition to the WFD.

Keywords: Cladocera, Copepoda, functional groups, Mediterranean reservoirs, Water Framework Directive, water quality.

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List of Abbreviations

WFD	Water Framework Directive
EDIA	Empresa de Desenvolvimento e Infra-estruturas do Alqueva
IPMA	Instituto Português do Mar e da Atmosfera
INAG	Instituto da Água
CDOC	Colored Dissolved Organic Carbon
Chl *a*	Chlorophyll *a*
TSI	Trophic State Index
OV	Omnivore
MF	Macrofiltrator
HEBF	High efficiency bacteria feeder
LEBF	Low efficiency bacteria feeder
SNIRH	Sistema Nacional de Informação de Recursos Hídricos
EQR	Ecological Quality Ratio
NTU	Nephelometric Turbidity Units
FEDER	Fundo Europeu de Desenvolvimento Regional

Introduction

Dams represented a significant milestone in water management and energy production, since their sudden emergence in the 50-70s. They allowed for better flood control, more stable water supply, recreation and production of renewable energy (Shiklomanov, 1998; Nestmann & Stelzer, 2007). But although dams have satiated the needs of an ever resource demanding society, they came with a fair share of environmental impacts (McGully, 2001). The alteration of a river's flow regime leads to geomorphologic changes downstream as a result of altered sediment allocation (Kondolf, 1997) and to the establishment of a lentic ecosystem, a reservoir upstream of the dam. This newly formed ecosystem is alien to most of the native species and facilitates the establishment and dispersal of exotic species (Havel, Lee & Vander Zandem, 2005). Moreover, the altered flood pattern prevents the development of a stable riparian community (New & Xie, 2008). In these artificial lentic ecosystems, the slower water current and longer water residence time favor the colonization of these waters by new communities of phytoplankton and zooplankton (Czerniawski & Domaga, 2014). On the other hand, these water bodies are subject to the accumulation of pollutants and inorganic nutrients, which in time could negate the dam's original purpose (Elçi, 2008; Palma *et al.*, 2014b). Therefore, it is of utmost importance to establish physical, chemical and biological standards for these lentic ecosystems in order to monitor them and prevent eutrophication processes or reduced water quality.

In 2000, the European Commission established the Water Framework Directive (WFD), to set the standard for water quality management in the European Union, not only for reservoirs but for all inland, transitional waterbodies, and coastal waters. The main purpose of the WFD was to establish a framework to be followed by the member states to ensure that all waterbodies reach a good or high ecological status (European Commission, 2000). The original deadline of 2015 was only fulfilled by 53% of the surface waterbodies and the European Commission extended it to 2027 or possibly beyond that (European Commission, 2012). This delay could be a result of some misconceptions and lack of clarity of the WFD (Voulvoulis, Arpon & Giakoumis, 2017). Despite that, it is still considered one of the most important and ambitious pieces of legislation in water management issued at the European level (Giupponi, 2007). It promoted a shift from the typical fragmented policies, that acted separately and focused on water quality for domestic, agricultural, recreational and industrial uses, to a more holistic, interdisciplinary and integrated approach, with an added significance of the biological elements (Solimini, Ptacnik & Cardoso, 2009). These biological elements (phytoplankton, macroalgae, macrophytes, macrobenthic invertebrates and fish) whose monitoring, combined with physical, chemical and hydromorphological variables, allows for the assessment of those most impacted by anthropogenic pressures (European Commission, 2000). The final realizing step is the mitigation of these pressures through monitoring programs, in order to reach the desired good ecological status (Voulvoulis *et al.*, 2017). A waterbody achieves a good ecological status when it barely deviates from reference conditions i.e. “the expected ecological quality in the absence of anthropogenic influence” (Søndergaard *et al.*, 2005).

In the case of reservoirs (deemed as heavily modified waterbodies), their assessment should be similar to a lake (Solimini *et al.*, 2006). The main difference resides in the goal, since, for these artificial ecosystems, one strives to reach a good ecological potential instead of a good ecological status. While ecological status always refers to a pristine or only slightly altered system as reference, ecological potential is the best possible conditions for a waterbody, given its hydromorphological changes (caused by river impoundment, for example), and the mitigation of these changes and anthropogenic pressures (Borja & Elliott, 2007; Poikane *et al.*, 2015). Accordingly, the end product of a reservoir or any waterbody under monitoring should be an ecosystem with functioning similar to its reference conditions. All *taxa* in such ecosystem (structure), as well as their relation (function), should be taken into account to ensure maximum success in the restoration process (Society for Ecological Restoration International Science & Policy Working Group, 2004). For that, the most explanatory intervenors in this ecosystem should be assessed and the framework does not discourage the addition of new variables (Jeppesen *et al.*, 2011). With that in mind, many european researchers questioned the absence of zooplankton from this framework (Caroni & Irvine, 2010; Davidson *et al.*, 2011; Jeppesen *et al.*, 2011; Jensen *et al.*, 2012; Ejsmont-Karabin & Karabin, 2013; Haberman & Haldna, 2014; García-Chicote, Armengol & Rojo, 2018). This polyphyletic group is easy and cheap to assess and a well-known indicator of trophic state and water quality in lentic ecosystems (Brito, Maia-Barbosa & Pinto-Coelho, 2011; Gazonato Neto *et al.*, 2014). This ability derives from their position in the lentic food web, being influenced by bottom-up effects, mainly dictated by the phytoplankton community structure and abundance (Sommer *et al.*, 1986; Stemberger & Miller, 1998) and top-down effects, like predation by planktivorous fish (Brooks & Dodson, 1965; Drenner & Hambright, 2002; Nicolle *et al.*, 2011).

Since zooplankton feeds on phytoplankton, it has the important function of controlling their densities, potentially mitigating the effects of eutrophication process (Gliwicz, 1990). This capability may be limited by the increase in abundance of planktivorous or filter feeding fish and absence of macrophytes that provide refugia (Angeler *et al.*, 2003), or the introduction of exotic species (Pace, Findlay & Fischer, 1998; Walsh, Carpenter & Vander Zanden, 2016). This results in a trophic cascade mediated by top-down effects (Carpenter *et al.*, 2001). The cascades can be used to our advantage through biomanipulation, by removal of planktivorous fish and/or complementing with the addition of *Daphnia* or macrophytes for refugia (Ha *et al.*, 2013), which in turn increases the effectiveness of the zooplankton community in allaying the impacts of eutrophication. This may provide a possible trajectory of lake restoration or to increase the ecological potential of a reservoir (Triest, Stiers & Van Onsem, 2016). Further cementing its usefulness for the WFD is its ability as indicators of possible habitat conditions and planktivorous fish abundance, establishing reference conditions and defining recovery targets, through the sampling of cladoceran remains in the sediment (Jeppesen *et al.*, 2003; Caroni & Irvine, 2010; Bennion *et al.*, 2011).

In its own right, monitoring the zooplankton structure might prove an useful tool to control invasions by other zooplanktonic species, that could slightly change the community (Riccardi *et al.*, 2004) or negatively impact the ecosystem and its services (Walsh *et al.*, 2016). Moreover, through the analysis of certain functional traits and groups of zooplankton, it is possible to easily determine a waterbody's functional state, an approach which the WFD doesn't emphasize (Caroni & Irvine, 2010). Traits based on morphology include body size and biomass, which may denote food availability (Jensen *et al.*, 2012; García-Comas *et al.*, 2016) or/and size-selective predation (Moss *et al.*, 2003). Zooplankton to phytoplankton ratios (either biomass or biovolume) can be used to measure the effectiveness of zooplankton in controlling phytoplankton abundance (Moss *et al.*, 2003; Jeppesen *et al.*, 2011). In defining the trophic progression and quality of a waterbody, these traits are by far the most used (Jeppesen *et al.*, 2011; Ejsmont-Karabin & Karabin, 2013; Haberman & Haldna, 2014) and would provide essential information to add to the WFD (Moss *et al.*, 2003). For example, the size of the defensive appendages related to predation pressure, as in the case of antennules and mucrones in *Bosmina* (Sakamoto & Hanazato, 2008) and tail spine and head spine in *Daphnia* (Caramujo & Boavida, 2000a; Dzialowski *et al.*, 2003), and fecundity, as an indicator of food quality and abundance (Devetter & Sed'a, 2003; Caroni & Irvine, 2010) are other potentially useful indicators.

Geller and Müller (1981) developed a zooplankton functional community classification based on its ability to efficiently filter bacteria, dividing them in macrofiltrators, low efficiency bacteria feeders and high efficiency bacteria feeders. The extent of this capability is based on the filtration apparatus's mesh-size, as zooplankters with coarse mesh-sizes are incapable of capturing small particles and ones with thinner mesh-sizes can more efficiently feed on bacteria. These authors also established a pattern between the succession of the defined ecological groups and the trophic state of a lake, since bacterial production is more abundant when trophic state increases (Sommer *et al.*, 1986). Barnett and colleagues (2007) (complemented by Benedetti *et al.*, 2015 and Rizo *et al.*, 2017) grouped species by functional similarity as well, but went beyond morphological traits, adding other important physiological and behavioral features such as feeding type, habitat preferences and predator avoidance behavior. This grouping could help understand certain mechanisms in aquatic ecosystems, but as Barnett and colleagues (2007) stated, more studies are needed to understand its potential. On the other hand, Geller and Müller's more simplistic classification could have some practical use (Jensen *et al.*, 2012), but it is yet to be fully explored. With all of these issues in mind, both the functional traits of the community and its structure, through the application of these ratios, should duly reflect the trophic state and water quality, as well as provide a background of the other elements of a lentic ecosystem.

Therefore, the main scope of this study is to assess the mesozooplankton community succession, along one year in order to discriminate the seasonal variation, in a group of reservoirs from Alqueva Irrigation System's reservoirs, both in the Sado and Guadiana rivers basins. This assessment was conducted to evaluate zooplankton's capability as a bioindicator of a reservoir functional status or as a water quality tool. Additionally, a WFD approach in terms of physical and chemical parameters was conducted in each reservoir. Furthermore, since the zooplankton communities of the southern Portuguese reservoirs are relatively unknown (save for Monteiro, 1988 and references herein; Caramujo & Boavida, 2000b; Baião & Boavida, 2005, for rotifers), this study will also be their first record in these reservoirs.

Materials and Methods

Study area

The Alqueva Irrigation System, which comprises reservoirs of the Sado and Guadiana rivers and various tributaries to the main rivers' basin, is located in Alentejo region, spanning the districts of Beja and Évora (EDIA, 2017). This region is largely occupied by agriculture, mainly olive groves, cereal crops, and pastures for livestock rearing. Since this region is very prone to intense droughts due to its Mediterranean temperate climate, water availability for these activities is difficult to manage (Santo *et al.*, 2005), coupled with the growing desertification of this region (Branco *et al.*, 2014). The advent of dams came as a new hope for this population-receding, yet highly productive region to ensure water availability throughout the year (FEDER, 2007). By increasing water availability, these dams concomitantly allowed for a shift from traditional to intensive and super-intensive olive groves. This type of cultivation contributed to land degradation and contamination due to the intense use of fertilizers and heavy machinery associated (Pires & Neves, 2013; Palma *et al.*, 2014a). Occasionally there are patches of *Montado* of holm oak (*Quercus ilex*) and cork oak (*Quercus suber*), which may also serve as pasture for livestock. Aside from farming practices, there are also recreational activities associated to dams, such as fishing and boating. As a consequence of all cited landscape uses, these reservoirs have accumulated high amounts of nutrients and organic matter, harmful pesticides and heavy metals (Palma *et al.*, 2014a b). Additionally, there have been inputs from urban/agricultural activities in Spain, discharges of untreated or inefficiently treated domestic wastewaters, untreated wastewaters from pig and cattle breeding farms and mining leaching (Silva *et al.*, 2011; Palma *et al.*, 2014b) that came to this system in the Guadiana river flow. As a result, these reservoirs were reported as eutrophic ecosystems with occasional cyanobacterial blooms (Morais *et al.*, 2007; Valério *et al.*, 2008; Palma *et al.*, 2014b). The construction of dams also modified the landscape, through the destruction of the riparian vegetation and the appearance of extensive areas that, during part of the year, are deprived of water and vegetation, contributing to habitat fragmentation (EDIA, 2004; Figueiredo *et al.*, 2005) Moreover, the construction of adductors further aggravated this fragmentation (NEMUS, 2008). The effect of habitat fragmentation was well perceived in the native ichtyofauna, avifauna and mammals (Filipe *et al.*, 2004; Figueiredo *et al.*, 2005; Oliveira, 2013). These reservoirs also bolster a diverse exotic fish fauna (EDIA, 2018), which further threaten the native fauna (Elvira, 1995; Leunda, 2010), but also may represent a significant predation pressure on zooplankton, namely zooplanktivorous species such as the bleak (*Alburnus alburnus*) (Politou, Economidis & Sinis, 1993), mosquitofish (*Gambusia holbrooki*) (Castro & Gonçalves, 2011) and pumpkinseed sunfish (*Lepomis gibbosus*) (Braband *et al.*, 1986; Castro & Gonçalves, 2011).

Eight reservoirs from this system were selected (Fig. 1). Each reservoir contains a sampling point near the dam, with the exception of the Pedrogão reservoir, which contains an additional point in the inlet area, due to its particular characteristics, with constant water disturbance from discharge/pumping cycle from electrical production in Alqueva dam's tailrace, located in this area. Details regarding morphological and hydrological characteristics of the selected reservoirs are summarized in table 1.

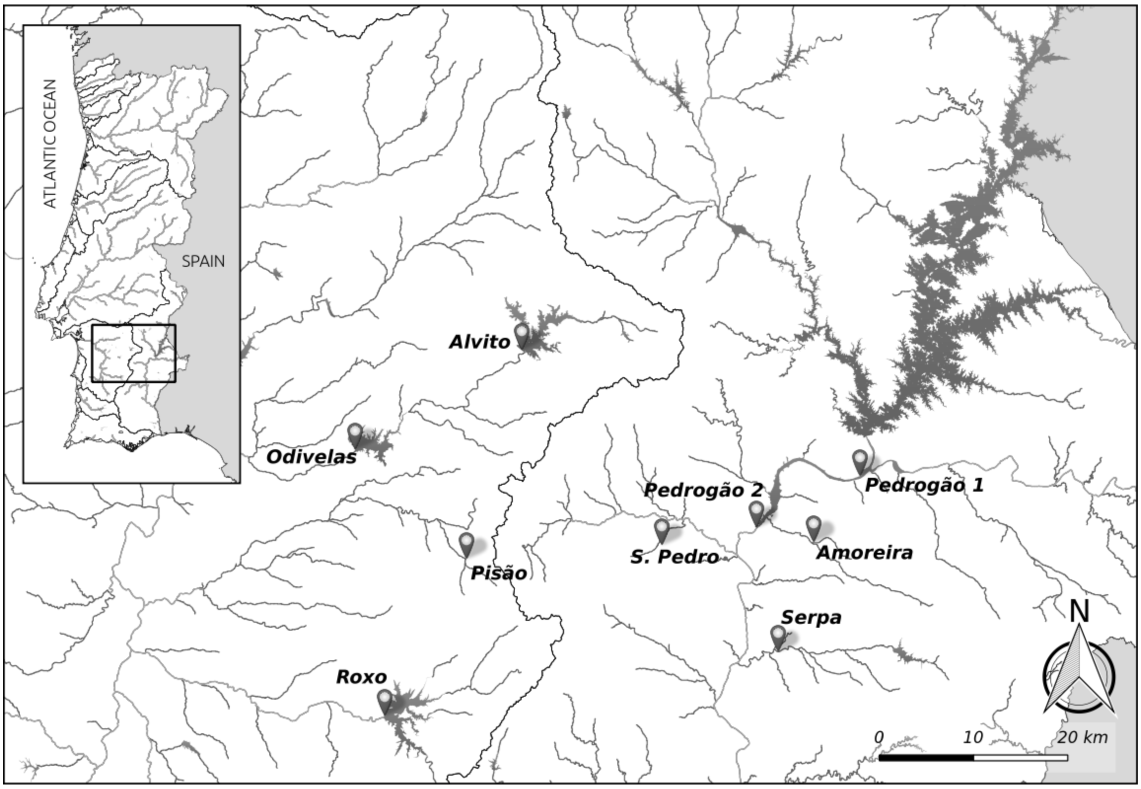

Fig. 1 – Reservoirs of the Alqueva Irrigation System, with the sampling sites: Alvito (38º16'54.21''N, 7º54'50.11''W), Odivelas (38°11'12.49"N, 8° 6'56.42"W), Pisão (38°4'55.93"N, 7°58'52.90"W), Roxo (37°55'55.70"N, 8°4'49.16"W), Pedrogão 1 (38°9'34.70"N, 7°30'13.46"W), Pedrogão 2 (38°6'39.30"N, 7°37'47.68"W), S. Pedro (38°5'40.46"N, 7°44'38.90"W), Amoreira (38°5'49.30"N, 7°33'38.21"W) and Serpa (37°59'30.59"N, 7°36'15.63"W).

The selected reservoirs are very similar from a biogeographical standpoint since they are all inserted in the Mediterranean Region. This region is characterized by an accentuated continentality, with dry summers, rainy winters and more than 75% of precipitation condensed between October and March (Chazarra *et al.*, 2011). The year of 2017 in particular was extremely hot and dry, even in the autumn (IPMA, 2017). This coupled with the high water demand, has implications on the water level. According to Sistema Nacional de Informação de Recursos Hídricos (SNIRH, https://snirh.apambiente.pt/), the water volume in the Alvito, Odivelas and Roxo reservoirs, in the Sado river basin, had a very similar tendency: the volume never exceeded 40%, remaining more or less constant (in the case of Roxo, it was much worse, <20%) and increasing only in January 2018. In the reservoirs of the Guadiana basin, Enxoé (which is near Serpa), Alqueva and Lucefécit, water level varied differently: enough water in February that was being consumed without being replenished in the summer months; autumn and winter with little water but it is replenished in January-February. These reservoirs are not part of the study but serve as a proxy for the sampled reservoirs in the Guadiana river basin in regards to water level variation, since no data is available specifically for them. Similarly, we will assume that the Pisão reservoir had a similar water level variation to the other reservoirs from the Sado river basin.

Table 1 – Morphological and hydrological characteristics of each reservoir studied. Sources: EDIA.pt; sniamb.apambiente.pt (Atlas do Ambiente).

Basin	Reservoir (area ha)	Geology	End of dam construction	Full storage level (m)	Total capacity ($\times 10^6$ m^3)	Main Uses
SADO	Alvito (1563)	Schist, greywacke and metavolcanic rock	1977	197.5	132.5	Water storage; Electricity
	Odivelas (930)	Sandstone, gabbro and quartz-porphyry	1972	103	96.0	Irrigation; Electricity
	Roxo (1423)	Schist, greywacke, metavolcanic rock, gravel, sandstone	1967	136	96.3	Water storage; Irrigation; Electricity
	Pisão (202)	Sandstone, gabbro and quartz-porphyry	2007	155	8.2	Irrigation; Electricity
GUADIANA	Pedrogão (1097)	Schist, greywacke and metavolcanic rock	2003	84.8	106.0	Irrigation; Electricity
	Amoreira (153)	Gravel and granite	2009	135	10.7	Irrigation
	S. Pedro (184)	Schist, greywacke and quartz-diorite	2014	142.5	10.8	Irrigation
	Serpa (148)	Carbonate rock and diorite	2009	124	10.2	Irrigation; Electricity

Water sampling and analysis

Monthly, from February 2017 to January 2018, physical and chemical parameters were measured *in situ*, at the depth of 2 m, using a mutiparameter probe: pH, temperature (ºC), dissolved oxygen (mg/L), redox potential (mV) and conductivity (µS/cm). Water transparency (m) was also determined using a Secchi disk and turbidity (NTUs) was measured with a portable turbidimeter. Additionally, water samples were collected in each reservoir at a depth of 2 m, using a van Dorn bottle for further laboratory assays. In the laboratory, chlorophyll *a* (chl a; Lorenzen, 1967), total phosphorus (Ebina, Tsuitsui & Shirai, 1983; APHA, AWWA & WPCF, 1989) and dissolved carbon concentrations (indirectly through CDOC; by spectrophotometry, at 320 nm; Williamson *et al.*, 1999) were determined following standard procedures. The water hardness (Calcium concentration, in mg/L) was determined through the calmagite colorimetric method using the HI720 Checker® Handheld Colorimeter kit.

Zooplankton sampling and identification

Zooplankton samples were also collected monthly. A pumping system was used to extract 200 L of reservoir water, at a depth of 2 m, which went through a filter of 55 µm mesh size. Pumping systems have proved to be a good alternative to traditional nets since they usually collect more zooplankton while providing the same consistency (Masson *et al.*, 2004). In conjunction with that, this mesh size is ideal to contain more zooplankton than other mesh sizes (especially copepod nauplii), allowing a more accurate estimate of planktonic density (Makabe, Tanimura & Fukuchi, 2012). This method however is not recommended for the sampling of rotifers and protozoa, constituting part of the reason these groups were not assessed in this study (Rahkola, Karjalainen & Viljanen, 1994).

After *in situ* procedures, the zooplankton samples were preserved in 96% ethanol. The assessment of the zooplankton abundance and diversity was conducted using a subsampling method. An aliquot of each zooplankton sample was transferred to a counting chamber and zooplankton was identified and counted on a stereomicroscope. This procedure was repeated until at least half the taxa observed, in each sample, have an abundance recorded of at least 100 individuals (Mack *et al.*, 2012). Cladoceran individuals were identified down to the species level, whenever possible, with specific identification keys (Amoros, 1984; Alonso, 1996; Witty, 2004; Błędzki & Rybak, 2016). Organisms belonging to the subclass Copepoda were identified only to the order level (Witty, 2004).

Statistical and functional analysis

Spearman correlation (rs) was used for all the physical, chemical and biological parameters, since most parameters were not normally distributed. Only the statistically and ecologically significant correlations ($p<0.05$) were shown.

Water quality was assessed following the procedures in INAG (2009) for a WFD approach. Of the assessed physical and chemical parameters in this study, the ones calibrated for southern Portuguese reservoirs were dissolved oxygen concentration, pH and total phosphorus concentration. Chl *a* concentration is the only biological parameter calibrated for southern Portuguese reservoirs. Using chl *a* concentration, EQRs (Ecological Quality Ratios) are calculated based on the following equation:

$$EQR = \frac{\frac{1}{Observed\ value}}{\frac{1}{Reference\ value}} \ (1.6)$$

After normalization of the EQRs, if that value is greater than 0.6, the ecological potential for phytoplankton will be considered good or superior. A normalized EQR below 0.6 will classify the water body as moderate or inferior. In addition to this approach, Carlson's (1977) Trophic State Index for chl *a* was used to give an idea regarding the trophic state of the sampled reservoirs.

The Shannon-Weaver diversity index (H') was used to determine changes in the diversity/heterogeneity of a given sample and was calculated with the following equation:

$$H' = -\sum_{i=1}^{s} pi \ln pi$$

where s is the number of species and pi = n_i/N, n_i being the number of individuals of species i and N the total number of individuals in the sample. Additionally, Simpson evenness was calculated following the formula:

$$E = \frac{D2}{s}$$

in which D2 represents Simpson's Dominance (1/∑pi^2) and s the number of species. This diversity measure indicates whether individuals are evenly split among all *taxa* or if one or few species dominate (Morris *et al.*, 2014).

The ratio of large cladoceran density to total cladoceran density was used as a means to understand the intensity of fish predation in a reservoir (Moss *et al.*, 2003). In addition to this, the observed zooplankton *taxa* were divided according to Geller & Müller's (1981) ecological groups (table 2) and the result was compared with the seasonal patterns established by the same authors for different trophic states. Additional information portraying other *taxa* not present in Geller & Müller's study were found in literature (DeMott & Kerfoot, 1982; Porter, Feig & Vetter, 1983). Cyclopoida, while not present in the authors' classification, were added to this classification as omnivores (Adrian & Frost, 1993), as well as Harpacticoida (Dahms & Qian, 2004). Due to the lack of information regarding the filter mesh size of Chydoridae, outside *Chydorus sphaericus*, and Macrothricidae, they were considered high efficiency bacteria feeders.

Table 2 – List of *taxa* grouped according to Geller & Müller's (1981) classification.

Omnivores (OV)	**Macrofiltrators (MF)**	**Low efficiency bacteria feeders (LEBF)**	**High efficiency bacteria feeders (HEBF)**
		*Daphnia longispina**	*Diaphanosoma**
Cyclopoida	Calanoida	*D. parvula**	*Ceriodaphnia*
Harpacticoida	*Sida crystallina**	*D. pulex**	Chydoridae
		Bosminidae	Macrothricidae

*Large cladocerans according to Moss et al. (2003). Since *Ceriodaphnia* were not identified to the species level, they were considered small cladocerans.

Results

Physical and Chemical Parameters

Figure 2 presents the results of temperature (°C) and dissolved oxygen (mg/L) measured *in situ* in all sampling sites along the period of the study. Overall, temperature and dissolved oxygen followed opposite tendencies in all the sampling sites: when an increase of temperature values occurs (summer months), the dissolved oxygen concentration decreases (Fig. 2). Spearman correlation supported this observation, since a significant and negative correlation was observed (rs=-0.515; p<0.0001). The absolute minimum value of dissolved oxygen was recorded in Serpa May (6.67 mg/L), however it was above the limit for good ecological potential for reservoirs of the south (≥ 5 mg O_2/L; INAG, 2009).

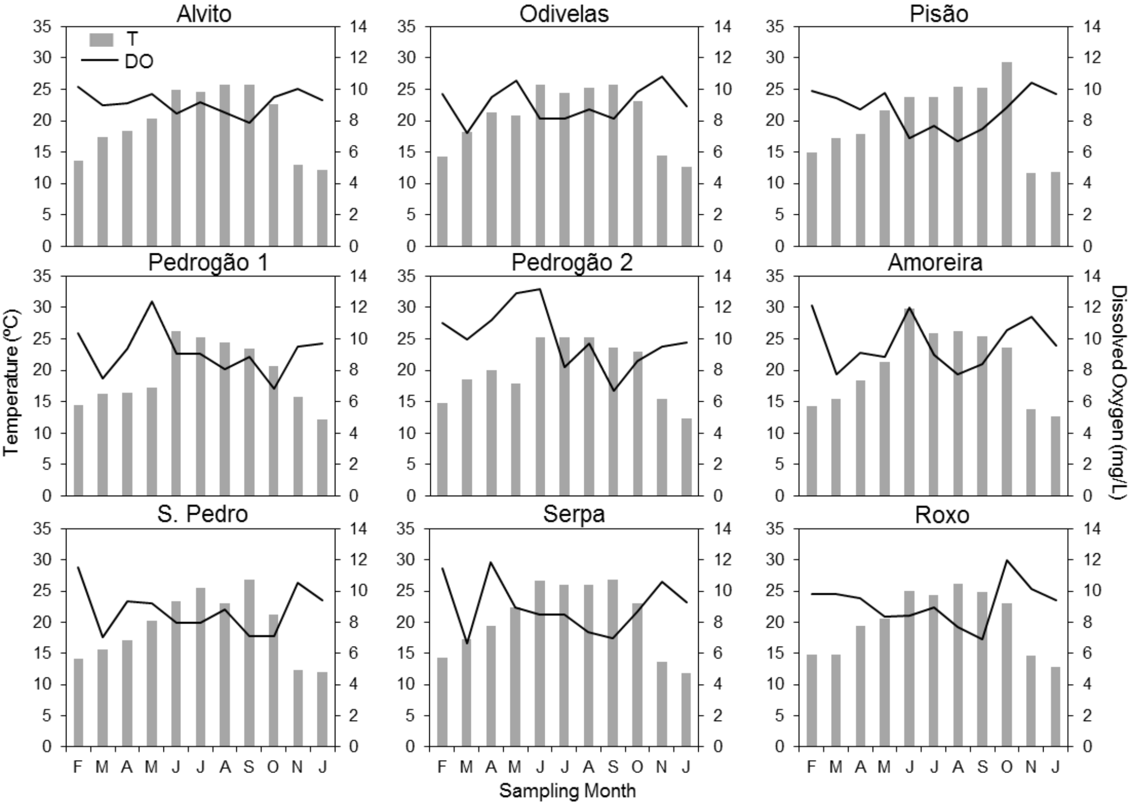

Fig. 2 – Monthly results of temperature (°C) and dissolved oxygen concentration (mg/L) for each studied reservoir along the sampling period.

Figure 3 presents the results of CDOC and redox potential of water. No correlation was observed between these parameters (p=0.325). CDOC values seem to be high in the spring, namely in Serpa reservoir, however a decrease was observed in May, in all sampling sites. In the reservoirs in the Guadiana Basin (Pedrogão 1, Pedrogão 2, Amoreira, S. Pedro and Serpa), the CDOC values showed a stabilization in the winter months (Fig. 3). The trend observed for redox potential showed an increase of values in the hotter months (rs=0.345; p<0.001).

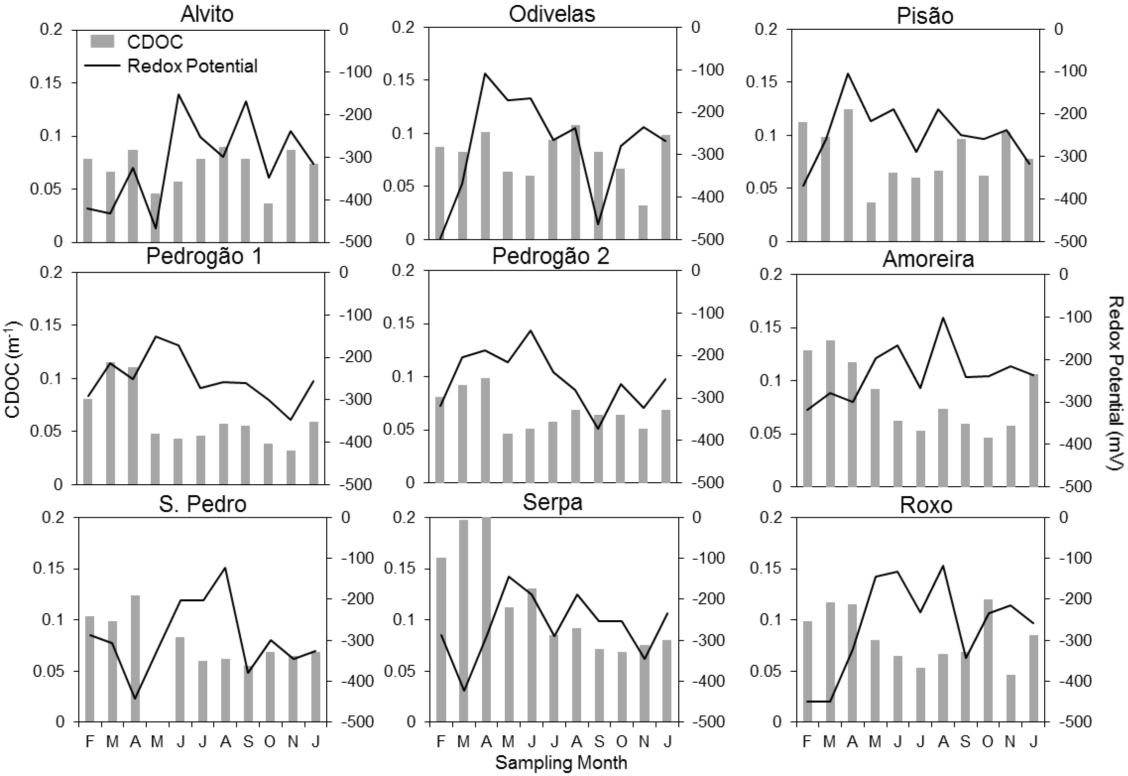

Fig. 3 – Monthly results of CDOC (m^{-1}) and redox potential (mV) for each studied reservoir along the sampling period.

Figure 4 shows the pH and conductivity values recorded along the sampling period for all sites. Overall, conductivity values remained constant throughout the sampling period in all reservoirs. The Roxo reservoir stands out by starting with higher conductivity, but however, from April, a decrease was recorded to similar values to the other reservoirs (Fig. 4). For the most part, the pH values were relatively high, classifying the waters as alkaline. The pH values follow a global trend with a slight increase along the first months of the sampling period (colder months), followed by an increase in hotter months. The variation of pH values was within the range stipulated for the classification of good ecological potential for southern reservoirs (6 < pH < 9; INAG, 2009).

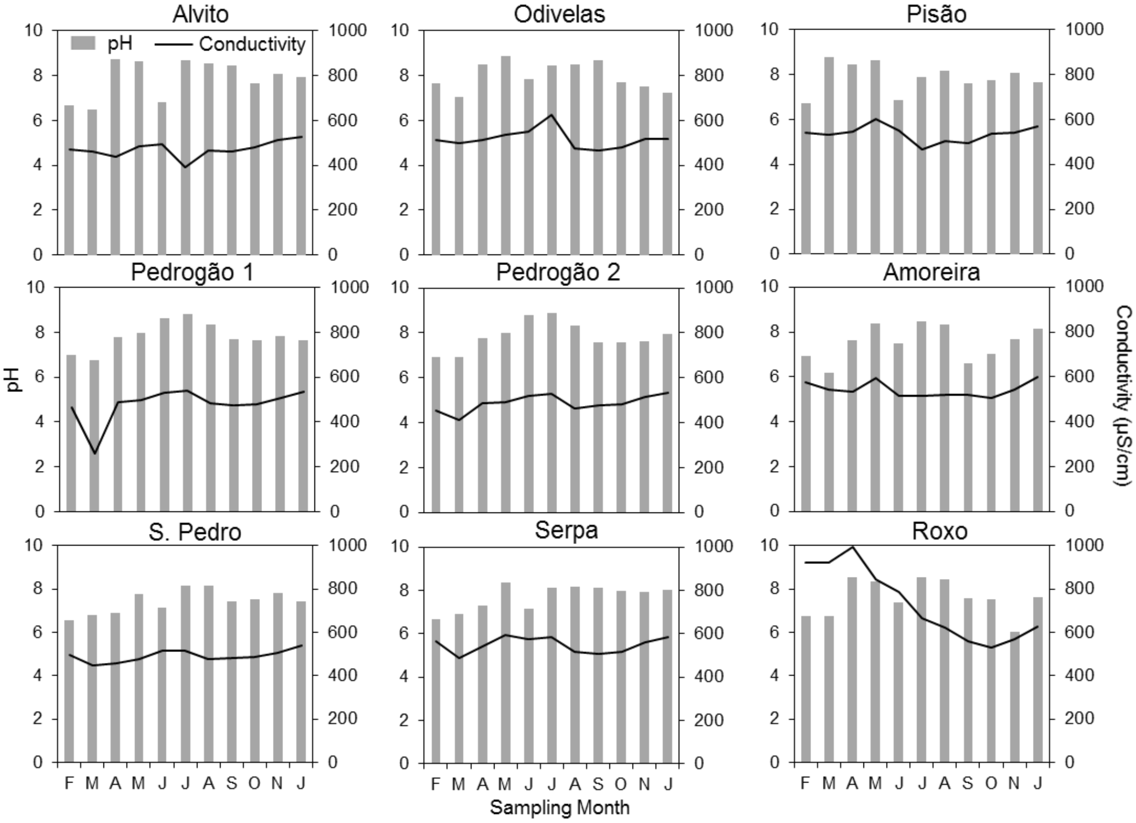

Fig. 4 – Monthly results of pH and conductivity (µS/cm) for each studied reservoir along the sampling period.

Transparency and turbidity data are displayed in figure 5 and they followed an opposite trend (rs=-0,628; p<0.001) along the sampling period for all the sites. In the summer months, water presented higher turbidity values than was observed in spring or winter seasons. The highest values of turbidity were recorded in August in Alvito and Pisão, while lowest transparency was observed in June in Pisão. Overall, Pisão, Amoreira, Serpa and Roxo reservoirs have the least transparent waters throughout the sampling year with similar values along the sampling period.

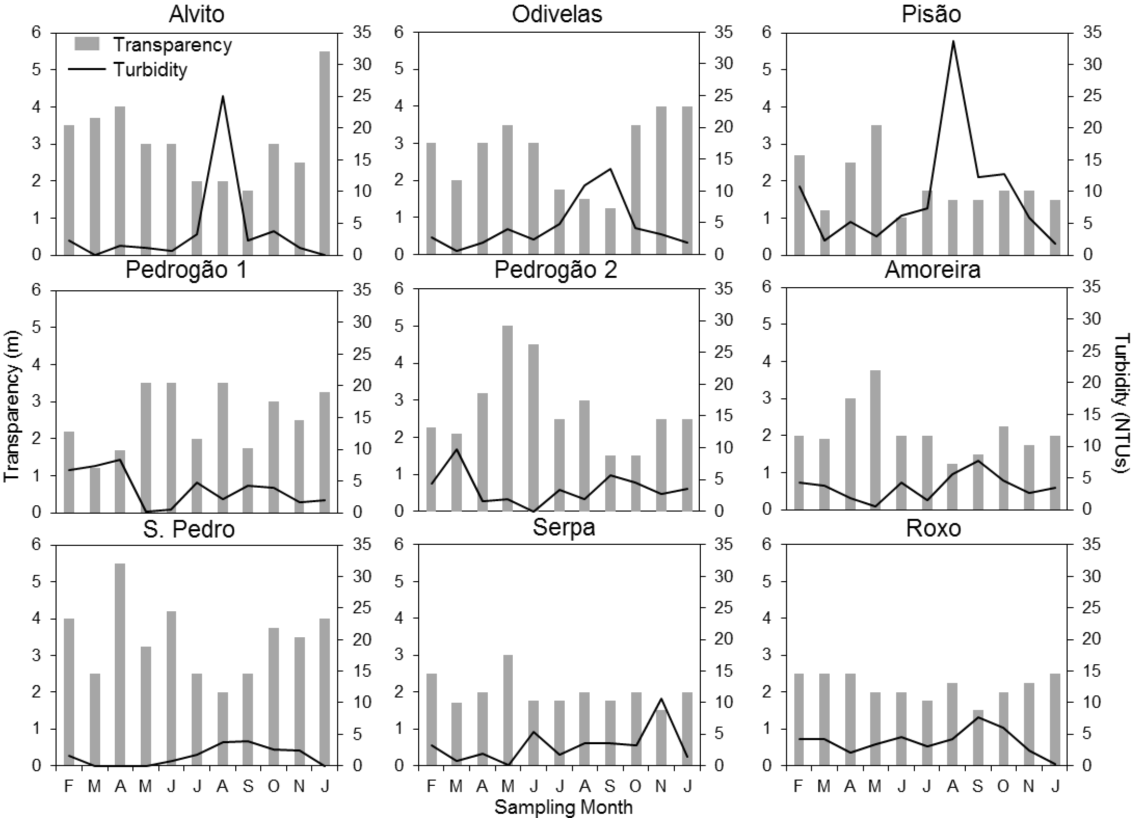

Fig. 5 – Monthly results of transparency (m) and turbidity (NTUs) for each studied reservoir along the sampling period.

Results of total phosphorus and calcium concentrations are shown in figure 6. Detected values of total phosphorus concentration were only observed in the spring and winter months. In the Odivelas, Pisão, Pedrogão 2 sampling sites, it was also detectable in the summer. Overall, the highest values were observed in the spring season with occasional peaks in Pisão (August), Amoreira (November) and Serpa (February). Nevertheless, phosphorus concentrations never exceeded the limit for good ecological potential (≤ 0.07 mg P/L; INAG, 2009). In the case of calcium concentration, the highest values recorded were in February or March. After this peak, a decrease of calcium concentrations values was observed. Calcium concentration values stayed constant throughout summer and a slight increase in autumn was observed.

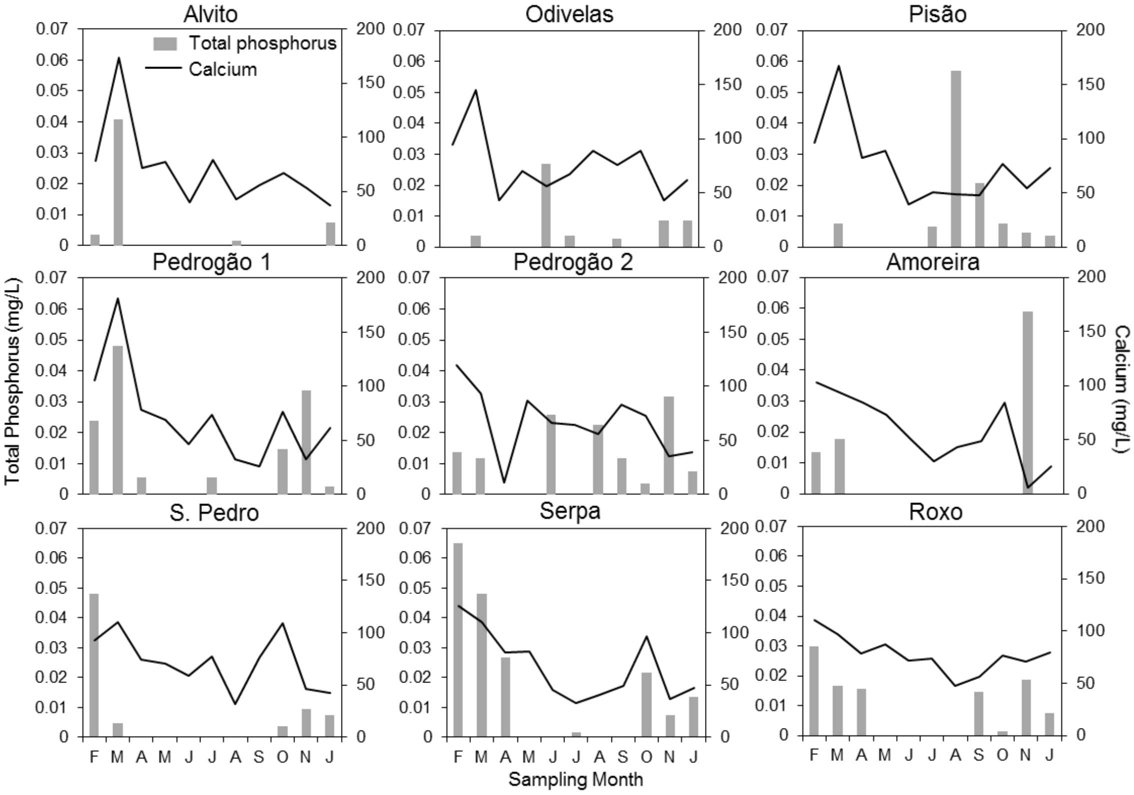

Fig. 6 – Monthly results of total phosphorus concentration (mg/L) and calcium (mg/L) for each studied reservoir along the sampling period. The months without total phosphorus values stand for below detection limit of the equipment.

The values of chl *a* concentration are displayed in figure 7. All reservoirs showed a similar trend of chl *a* concentration along the year with the lowest values recorded in winter and spring, with a high increase in hot months (June and August-October). Roxo had the highest chl *a* concentration in November. Serpa stands out by having high concentrations of chl *a* in the spring.

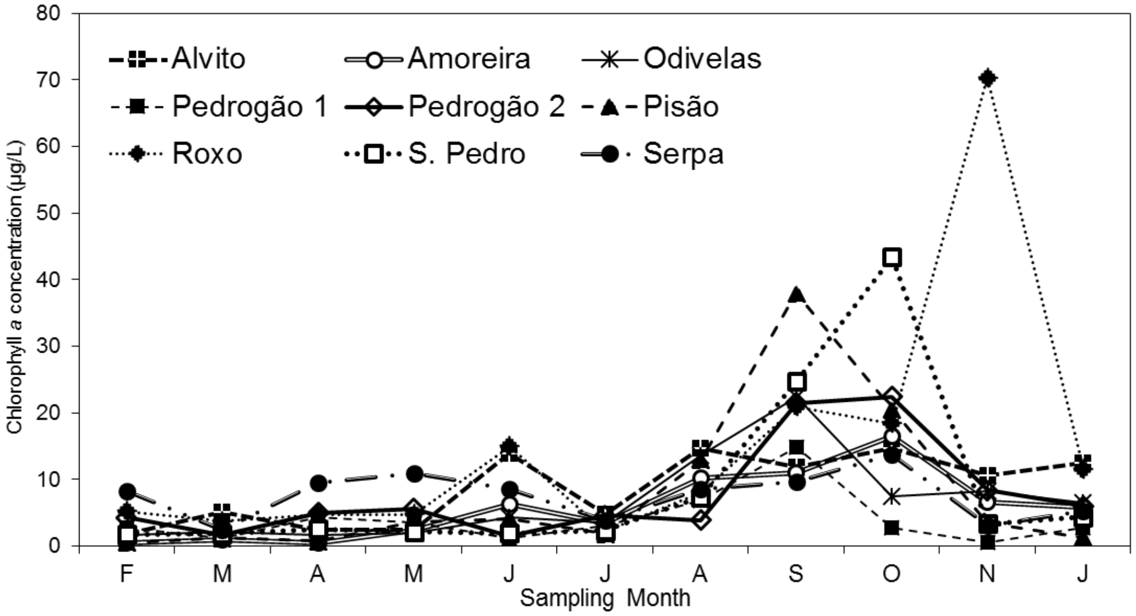

Fig. 7 – Monthly results of chlorophyll *a* concentration (µg/L) for each studied reservoir along the sampling period.

Of the evaluated parameters (pH, dissolved oxygen and total phosphorus; Figs. 2, 4 and 6), all were within the stipulated limits for good ecological potential for Portuguese heavily modified waterbodies (INAG, 2009). Chl *a* was also used to evaluate ecological potential. Table 3 shows the monthly values of normalized EQRs based on chl *a*. Overall, the studied reservoirs had moderate or inferior ecological potential in August, September (all reservoirs) and/or October, coinciding with the chl *a* highest value (Fig. 7). The reservoirs with most months of moderate or inferior ecological potential were Alvito, Roxo and Serpa.

To evaluate trophic state of each reservoir, Carlson's Trophic State Index (1977) for chl *a* was used (table 3). According to this index, most of the reservoirs are considered mesotrophic. Roxo is the only exception and should be considered eutrophic.

Table 3 – Range and Annual Mean (± standard deviation) of Trophic State Index (TSI) for chlorophyll *a* (Chl *a*) and monthly ecological quality ratios for each reservoir. For the trophic state index (TSI) values, grey cells represent mesotrophic conditions (between 40 and 50) and black cells represent eutrophic conditions (above 50); as for EQRs values, green cells represent good or superior ecological potential (above 0.6) and yellow cells moderate or inferior.

	TSI Chl *a*		Monthly EQRs										
	Range	Mean	F	M	A	M	J	J	A	S	O	N	J
Alvito	36.8 - 57.0	49.3 ± 8.0	0.92	0.83	0.65	0.83	0.33	0.66	0.31	0.38	0.31	0.43	0.37
Odivelas	35.3 - 61.1	45.6 ± 8.8	0.99	1.00	0.88	0.90	0.68	0.72	0.34	0.20	0.60	0.56	0.62
Pisão	24.9 - 66.3	42.5 ± 13.4	1.94	1.59	1.14	0.72	0.69	0.93	0.35	0.12	0.22	0.73	1.13
Pedrogão 1	25.1 - 57.0	40.7 ± 8.6	0.79	0.68	1.14	0.72	1.14	0.76	0.61	0.31	0.79	1.91	0.79
Pedrogão 2	34.7 - 61.1	47.3 ± 8.4	0.68	0.66	0.97	0.64	1.03	0.67	0.70	0.21	0.20	0.55	0.63
Amoreira	21.9 - 58.1	42.4 ± 12.7	2.46	2.37	1.40	0.83	0.62	0.73	0.45	0.41	0.28	0.62	0.63
S. Pedro	36.1 - 67.6	44.9 ± 10.8	0.96	0.81	0.93	0.87	0.92	0.84	0.60	0.19	0.11	0.73	0.68
Serpa	39.6 - 56.3	49.3 ± 5.4	0.55	0.48	0.82	0.42	0.54	0.70	0.53	0.47	0.33	0.75	0.65
Roxo	38.2 - 72.3	52.3 ± 9.7	0.65	0.67	0.71	0.66	0.30	0.87	0.57	0.22	0.25	0.06	0.39

Biological element: Zooplankton

A total of 18 zooplankton *taxa* (nauplii not included) were identified in the reservoirs of the Alqueva Irrigation System. It is important to note that copepod nauplii densities were not accounted for in the community succession. However, they were the most abundant zooplankters in almost all of the samples (ranging from 0.105 and 257.75 ind/L and 6% to 88% of relative density) and followed a very similar trend to cyclopoids (r_s=0.788; $p<0.0001$).

Zooplankton density ranged from 0.33 to 469.95 ind/L and followed an overall similar seasonality in all reservoirs (Fig. 8). For the most part, zooplankton density was low in all reservoirs throughout the year (<50 ind/L), except in Serpa. At the end of the summer season (August-September) the zooplankton density abruptly increased, namely in Pisão and Roxo. Serpa reservoir was the only exception, having the highest densities recorded in the spring season. A significant correlation with chl a concentration was recorded, and a similar pattern was observed (r_s=0.371; $p<0.001$). Taxa richness ranged from 4 to 10 species per month. Occasional peaks in-between the seasons were recorded, due essentially to the appearance of *Daphnia parvula* and some “pelagic visitors”, such as Chydoridae and Macrothricidae *taxa*.

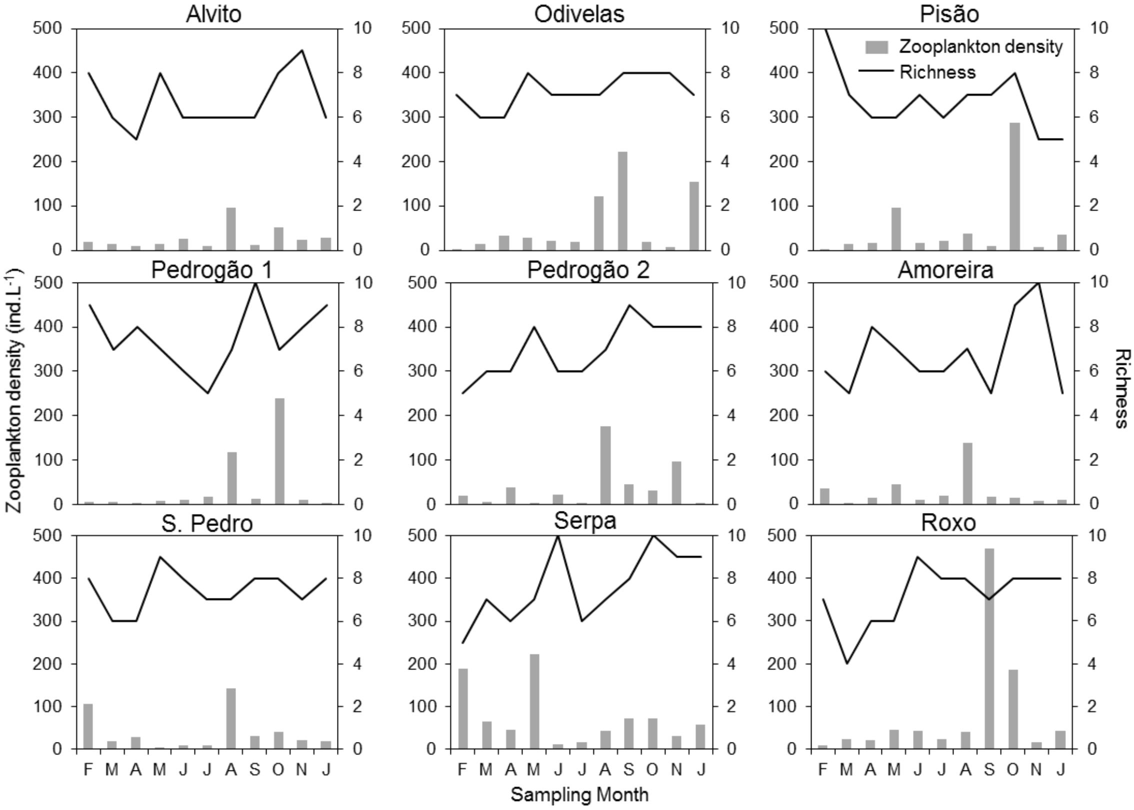

Fig. 8 – Zooplankton density and Richness values throughout the sampling period in each studied reservoir.

Figure 9 showed the relative abundance of the zooplankton community throughout the sampling period. The most abundant zooplankters were cyclopoid copepods along the sampling period for all reservoirs. The most abundant cladocerans recorded were *Bosmina longirostris*, followed by *Ceriodaphnia* spp. and then *Daphnia longispina*, namely in the spring season. In almost all the reservoirs, the first two taxa were replaced by *D. longispina* in this period. In the summer months, this cladoceran became undetectable. *B. longirostris* and *Ceriodaphnia* spp. were the dominant cladocerans. *Diaphanosoma mongolianum* and *Chydorus sphaericus* appear in noticeable quantities and co-exist with the remaining *taxa* in all reservoirs. From this point forward, *D. longispina* increase with an abundance peak in the autumn. Rarer *taxa*, like *Sida crystallina* and daphnids from *D. pulex* group, appeared almost exclusively in the spring and winter months. *Bosmina coregoni* also was almost exclusive of these months, but only in the case of Alvito and Odivelas. However, in the remaining reservoirs, this *taxa* also occurs in August and autumn months, but the highest abundance was observed in the colder period. It was not present at all in the Pisão reservoir. Contrary to the other *taxa*, *Daphnia parvula* does not have a clear seasonality. This zooplankter was detected in all reservoirs, except in the Odivelas reservoir.

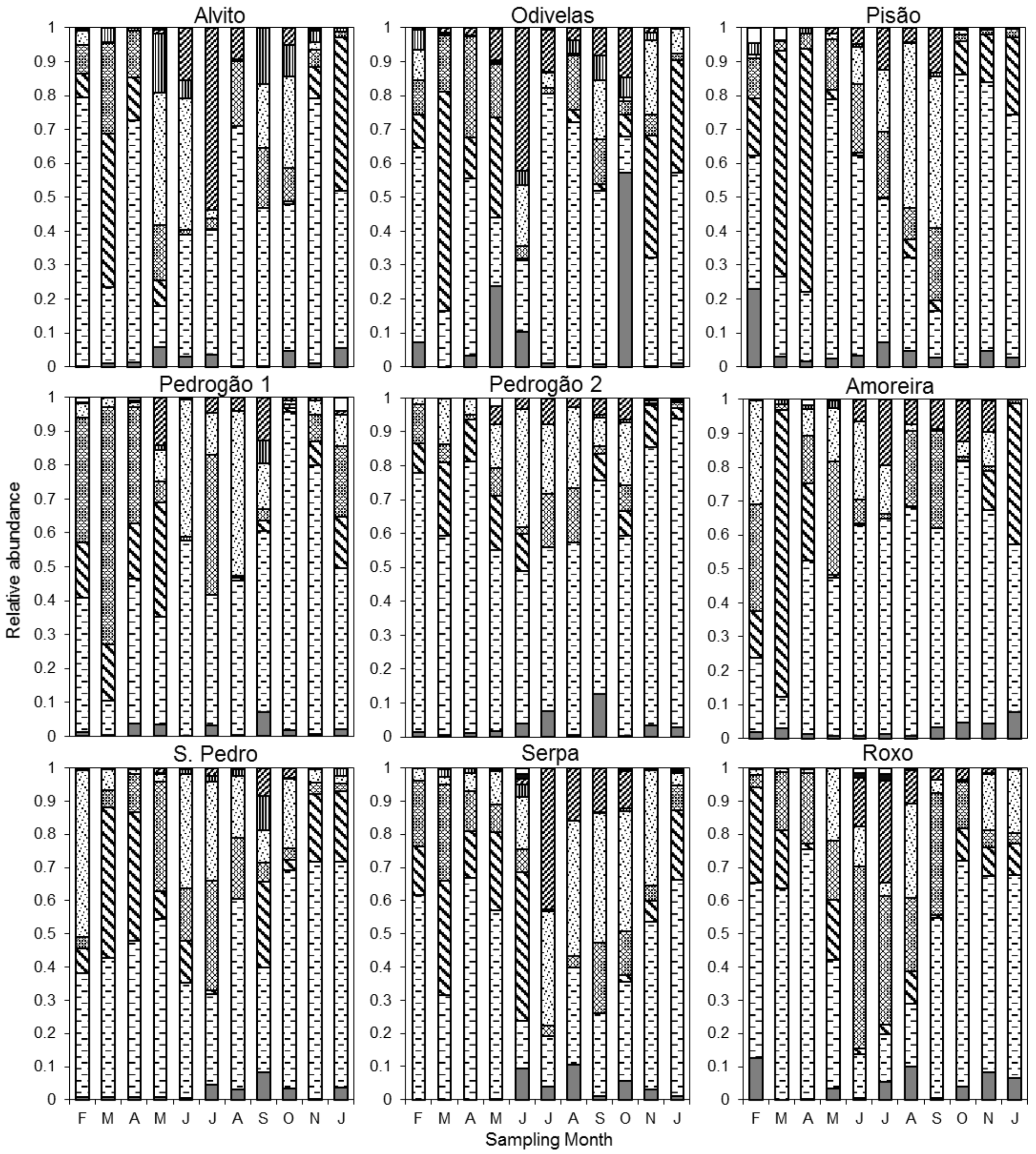

Fig. 9 – Succession of the zooplankton community of the reservoirs of the Alqueva Irrigation System: Calanoida (▒); Ciclopoida (⊟); *Daphnia longispina* (▧); *Bosmina longirostris* (▦); *Ceriodaphnia* spp. (⬚); *Chydorus sphaericus* (▥); *Diaphanosoma mongolianum* (▨); *Bosmina coregoni* (■); Other taxa (Harpacticoida, *D. parvula, D. pulex* group*, Alona* spp., *Alonella* spp., *Campocercus* spp., *Oxyurella tenuicaudis*, *Pleuroxus denticulatus*, *Macrothrix* spp. and *Sida crystallina*; □).

Diversity and evenness indices are presented in Figure 10. Shannon-Weaver diversity index ranged from 0.34 and 1.8 values, and Simpson evenness assumed values between 0.15 and 0.71, and a significant and positive correlation was observed (rs=0.684; p<0.0001). The trends observed were distinct between the reservoirs. Overall, all reservoirs, except Pedrogão 1 in October (due to high abundance of cyclopoids), had high diversity values (Figure 10). However, Amoreira, S. Pedro, Serpa and Roxo stand out by having more stable values of the Shannon-Weaver diversity index along the sampling period. In Amoreira March, this index (as well as evenness) was one of the lowest values recorded due to the dominance of the genus *Daphnia* (Figure 9). In Pisão, Amoreira and Roxo, the lowest values of Simpson evenness were observed in October, a month with moderate or inferior ecological potential to chl *a* concentration. However, an increase of these values was recorded in the next sampling (November). On the other hand, Pedrogão 2, S. Pedro and Serpa reservoirs present the lowest values of evenness in January.

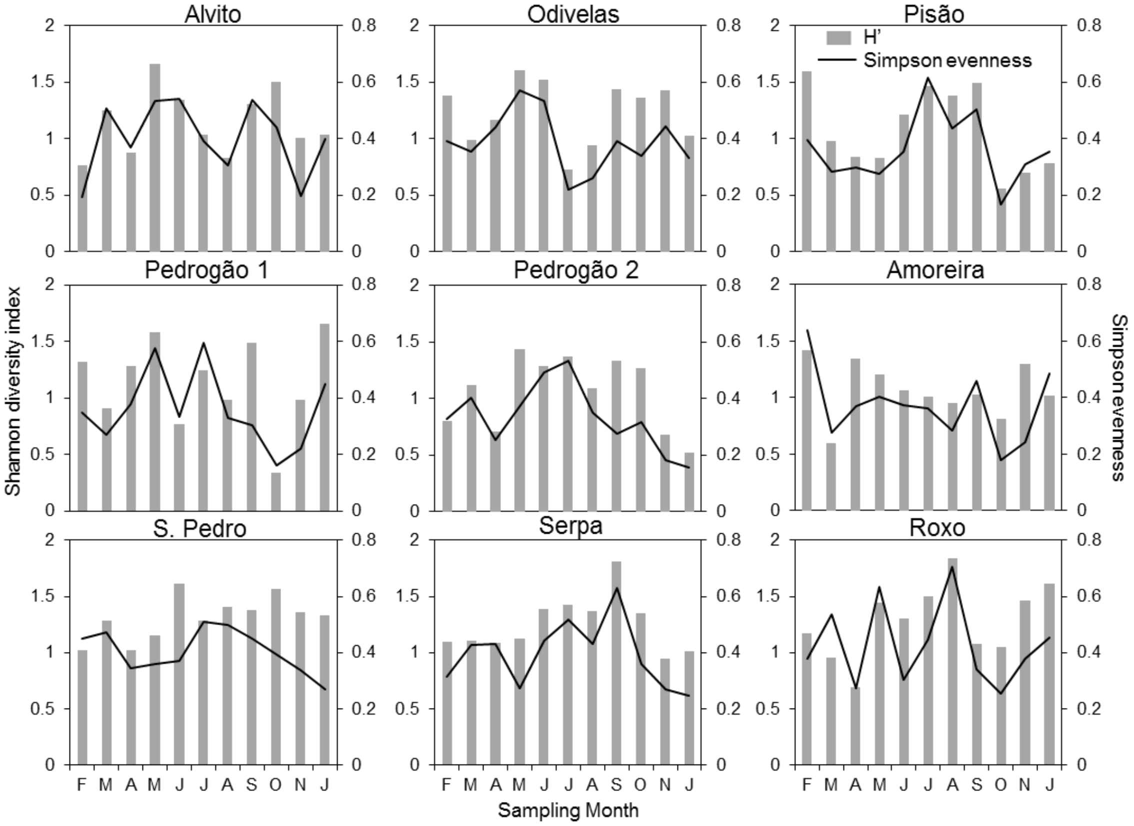

Fig. 10 – Shannon-Weaver diversity index and Simpson evenness throughout the sampling period in each of the studied reservoir.

The succession of functional groups formed by the identified zooplankton *taxa* is represented in figure 11. Overall, OV (almost solely composed by cyclopoids) were abundant throughout all year, while MF were the less represented. The former seems to occupy a smaller portion in the summer compared to other groups, except in Amoreira and Odivelas where OV presented a high relative abundance in the hotter months. LEBF were more present in the colder months (spring and winter), due to the high abundance of *Daphnia* recorded in this period in all reservoirs (Fig. 9). In some reservoirs (S. Pedro and Roxo), this group was also well represented in the hotter months (summer and autumn), namely due to the higher abundance of *Bosmina* (Fig. 9). HEBF dominated the community from June until November (Fig 11). Ratio of large cladoceran density by total cladoceran density was the highest in the colder months and the lowest in the hotter months, being negatively correlated with temperature values (rs=-0.382; p<0.0001).

Occasional peaks in the summer occur due to a dominance of *Diaphanosoma mongolianum* over other cladocerans. Roxo had the most constant and lowest values of this ratio throughout the year.

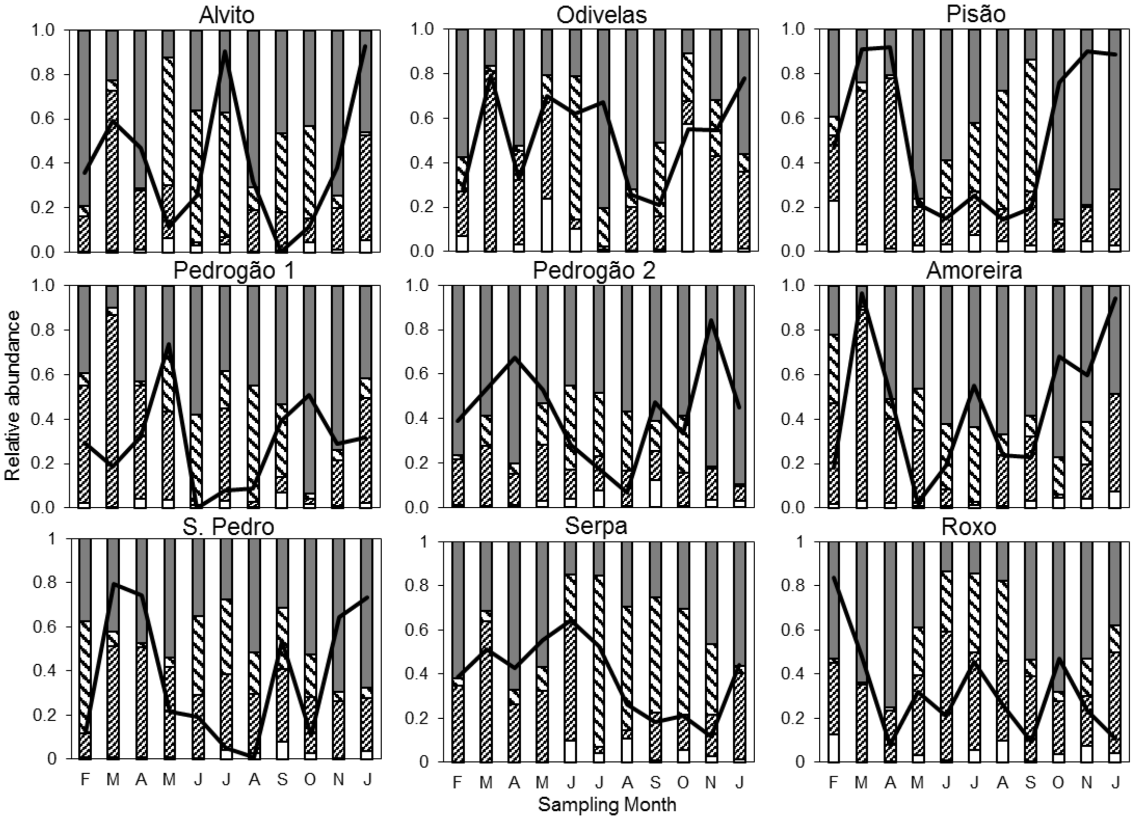

Fig. 11 – Succession of zooplankton functional groups (Geller & Müller, 1981) and ratio of large cladocerans to total cladocerans (─) of the reservoirs of the Alqueva Irrigation System: Macrofiltrators (☐); Low efficiency bacteria feeders (▨); High efficiency bacteria feeders (▧); Omnivores (■). Refer to table 2 for the classification attributed to each *taxa.*

Discussion

Reservoirs from Alqueva Irrigation System have had a record of bad water quality in the recent years (Morais *et al.*, 2007; Silva *et al.*, 2011; Palma *et al.*, 2014b). However, according to the standards set by the WFD, this did not reflect in the sampling year (INAG, 2009). Dissolved oxygen concentrations (Fig. 2), pH (Fig. 4) and total phosphorus concentrations (while relatively high in some situations; Fig. 6), did not overcome the stipulated limits for good ecological potential. CDOC was also present in very low concentrations (<1 m^{-1}; Pace *et al.*, 2002) (Fig. 3), which is also common for large and deep lakes (Stemberger & Miller, 2003). Regarding phosphorus and CDOC, these values were to be expected due to the low values of precipitation recorded along the sampling period (IPMA, 2017), which did not allow the runoff of nutrients and allochthonous organic matter to the reservoirs (Palma *et al.*, 2014a). Furthermore, the low values of phosphorus, with the high temperatures, from June to the end of summer, indicate a stable stratification in this period. Such as other southern reservoirs, including the Alqueva reservoir (Monteiro, 1988; Caramujo & Boavida, 2000b; Palma *et al.*, 2014b), the studied reservoirs are classified as warm monomictic. The concentrations of calcium, as well as pH values, may seem high, but they are typical of the calcareous nature of the region. Regardless of the observed aerobic conditions, redox potential values were negative for the most part, indicating low water quality (Fig. 3). Nevertheless, the higher values of temperature (Fig. 2) and redox potential (Fig. 3) is indicative of an increase in oxidation reactions (i.e. higher metabolic activity), which tends to happen in the summer (Wetzel, 2001). Conductivity values were relatively high, assuming values observed in other Mediterranean waterbodies (Naselli-Flores & Barone, 1994; Feijoó *et al.*, 2008; Jeppesen *et al.*, 2015) (Fig. 4). This clearly displays a higher productivity in the reservoirs' basin (Kehayias & Doulka, 2014; Jeppesen *et al.*, 2015) and could be also due to the water level decline observed in the sampling year (Jeppesen *et al.*, 2015). In addition, the values of EQR (of [chl *a*]) clearly display a period of moderate or inferior ecological potential. The lowest EQRs were observed in the end of summer and autumn and, in the case of Alvito and Roxo, also in June and January (table 3). In this period, the lowest values of dissolved oxygen (Fig. 2), transparency (Fig. 5) and the highest concentrations of chl *a* (Fig. 7) were observed. In fact, the low values of transparency were likely caused by the intensification of phytoplankton density reflected in the results obtained in chl *a* concentration (Dokulil & Teubner, 2000). Serpa was the outlier in mesotrophic reservoirs, when comparing its chl *a* and monthly EQRs. In the spring, this reservoir had high concentration of chl *a* (Fig. 7), with values of EQRs that remained low throughout spring, summer and autumn (except in April and July; table 3). This high concentration of chl *a* may be due to the high total phosphorus concentrations (Fig. 6) recorded in these periods.

Zooplankton *taxa* richness and community composition described in this study were typical of Mediterranean mesotrophic and eutrophic reservoirs (Monteiro, 1988; Caramujo & Boavida, 2000b; Sellami *et al.*, 2010). The values recorded of the diversity index were within the range observed in another study of a similar aquatic system (Chalkia & Kehayias, 2013). The overall density values however were very low (Fig. 8). This could be attributed to the fish predation typical of warm waterbodies (Havens, 2002; Moustaka-gouni, Michaloudi & Sommer, 2014). This pressure is an important factor to the zooplankton community in Mediterranean lakes, namely larger zooplankton (Sellami *et al.*, 2010). Indeed, a high diversity of planktivorous and omnivorous fish was already described for the reservoirs of the Alqueva Irrigation System (EDIA, 2018). In the case of Serpa, in the spring, zooplankton densities were much higher when compared with other reservoirs (Fig. 8), probably due to the high phytoplankton abundance recorded (reflected in the high values of chl *a* concentration; Fig. 7). It could mean that high food availability was a buffer against predation pressure (Nicolle *et al.*, 2011).

Cyclopoids were dominant in many samples (Fig. 9) like small cladocerans (*Bosmina* and *Ceriodaphnia*) and nauplii. Several studies (Fryer, 1957; Kerfoot, 1977; Lynch, 1980; Roche, 1990; Soto & Hurlbert, 1991; Adrian & Frost, 1993; Ha & Hanazato, 2009) already describe the predator-prey interaction between cyclopoids and small cladocerans, namely *Bosmina* and *Ceriodaphnia* and copepod nauplii, as well as rotifers and protozooplankton. In fact, Monteiro (1988) reported the dominance of predatory cyclopoids in a southern eutrophic reservoir (Divor) and associated this circumstance with the high abundance of small cladocerans. Moreover, due to their versatile diet, they can also commit to herbivory (Adrian & Frost, 1993; Perbiche-Neves *et al.*, 2007; Brito *et al.*, 2011). In contrast, calanoids (the main representatives of MF in this study) were badly represented and were not dominant in the winter, contrary to Geller and Müller's (1981) description (Fig. 9 and 11). Calanoids are replaced by cladocerans and cyclopoids in eutrophic conditions (Jeppesen *et al.*, 2000; Pinto-Coelho *et al.*, 2005; Eskinazi-Sant'Anna *et al.*, 2013) and that was also confirmed in other mesotrophic and eutrophic reservoirs to the South of Tagus (Monteiro, 1988; Caramujo & Boavida, 2000b). Calanoids might have been affected by the fish predation on adult forms and food inadequacy (Soto & Hurlbert, 1991; Jeppesen *et al.*, 2000; Perbiche-Neves *et al.*, 2007). Regarding copepod nauplii, they were even more abundant than cyclopoids. Such a thing is associated with eutrophic waters, which favor the juvenile forms in zooplankton

(Haberman & Haldna, 2014), and with intense fish predation (Illyová & Pastuchová, 2012).

Regarding the cladoceran succession, in the winter or early spring for all reservoirs (except Pedrogão 1 and Roxo), it is possible to observe a dominance of *Daphnia* over other cladocerans (Fig. 9). In this period, the phytoplankton community was not limited by phosphorus, since this nutrient was available in high quantities (Fig. 6), resulting in phytoplankton with high nutritional value for zooplankton (Sommer *et al.*, 1986; Moustaka-gouni *et al.*, 2014). In these situations, large filter feeders, like *Daphnia*, may thrive, since they gather food more efficiently, gaining a competitive advantage over small cladocerans (Gilbert, 1988; Chen *et al.*, 2016) (Fig. 9). In all reservoirs, *Bosmina longirostris* did not seem as affected as, for example, *Ceriodaphnia* by the competitive superiority of *Daphnia* (Fig. 9). That may be due to lower nutritional requirements (Balseiro, Modenutti & Queimaliños, 1992; Greenwood *et al.*, 1999) and to the selective mechanism characteristic of *Bosmina* (DeMott & Kerfoot, 1982; Bleiwas & Stokes, 1985), which allow this zooplankter to coexist with *Daphnia*. Still, the dominance of *Bosmina longirostris* in Pedrogão 1 in the spring and throughout the year in Roxo is a matter of concern. This bosminid has been regarded by the scientific community as an indicator of eutrophic conditions in temperate (Jensen *et al.*, 2012; Ejsmont-Karabin & Karabin, 2013; Haberman & Haldna, 2014) and in Mediterranean lakes (Monteiro, 1988; Caramujo & Boavida, 2000b; Kehayias & Doulka, 2014). Moreover, the dominance of *B. longirostris* in Roxo is in accordance with the obtained TSI, which indicated eutrophic conditions for this water body (table 3). However, the situation of Pedrogão 1 in the spring might be due to the high turbulence observed in this site.

While *D. longispina* was the most important cladoceran in winter-early spring, *Bosmina longirostris and Ceriodaphnia* spp. were dominant in the summer and autumn (Fig. 9). *Diaphanosoma mongolianum* and *Chydorus sphaericus* also had a high contribution to the zooplankton community in these seasons (Fig. 9). The shift from *Daphnia* to small cladocerans, resulting in low relative abundances of the large cladoceran, is linked with the intensification of fish predation (Brooks & Dodson, 1965; Sommer *et al.*, 1986; Hansson *et al.*, 2004). Fish are visual predators, and large prey, such as *Daphnia longispina*, are preferable over smaller prey (Werner & Hall, 1974; Hansson *et al.*, 2007). The importance of *Daphnia* in the diet of zooplanktivorous and omnivorous fish was reported for various Portuguese waterbodies with Mediterranean influence (Braband *et al.*, 1986; Castro & Gonçalves, 2007). This predation pressure intensified when temperatures increased (Mehner & Thiel, 1999; Geraldes & Boavida, 2004), which coincided with the breeding season of fish in the studied reservoirs (middle of spring-start of summer; EDIA, 2018). Analyzing the tendency of the ratio of large cladocerans over total (Fig. 10), it is possible to identify this moment, as it is marked by the almost disappearance of *Daphnia*. Cyclopoid relative densities also decreased in the summer due to fish predation (Lazzaro *et al.*, 1992; Romo *et al.*, 2004). The increase of the large over total cladocerans ratio observed midsummer was due to the appearance of another large cladoceran, *Diaphanosoma mongolianum*, particularly in the Alvito, Odivelas and Amoreira reservoirs (Fig. 10). Different from *Daphnia*, this member of the Sididae family is adapted to fish predation. Their massive eyes, body transparency and highly developed swimming antennae decrease the probability of preying on these crustaceans (Korovchinsky, 1990; Rizo *et al.*, 2017). The fact that *Diaphanosoma* appears exclusively in the summer and autumn in all of the studied reservoirs (Fig. 9) was already commented by Monteiro (1988) for southern Portuguese reservoirs. The author stated that *Diaphanosoma* was only present when *Daphnia* was absent and associated this fact with competitive exclusivity by *Daphnia*. Orcutt Jr. (1992) assessed the competitive interactions between *Diaphanosoma* and *Daphnia*, and concluded that *Daphnia* had a competitive edge over *Diaphanosoma* in situations of high food concentration, corroborating this assumption. After the exponential growth of the young-of-the-year fish, *Daphnia longispina* rebounds in the autumn months (Fig. 9). This *Daphnia* increase may be a result of a reduction of young-of-the-year density due to piscivore predation (e. g. Vijverberg *et al.*, 1990). On the other hand, the low light availability could hinder the success of search and capture of prey by fish (Wright & Shapiro, 1990; Hartleb & Haney, 1998; Pinto-Coelho, 1998), since it was in this period that the lowest values of transparency (and high turbidity values) were recorded (Fig. 5). Furthermore, and as Castro (2007) stated in his study of the zooplankton dynamics in Lake Vela, we cannot neglect the possibility of other factors such as cyanobacteria to contribute to the midsummer decline of *Daphnia* (Lampert, 1987; Jarvis, Hart & Combrink, 1988; Gliwicz, 1990; Abrantes *et al.*, 2006; Hart & Wragg, 2009). In fact, the high abundance of colonial or filamentous cyanobacteria, especially in the summer and autumn, has been reported for the Alvito, Odivelas and Roxo reservoirs (Galvão *et al.*, 2008; Valério *et al.*, 2008). Still, the role of fish might be more important than the existence of grazing resistant cyanobacteria in influencing *Daphnia* seasonality (Vijverberg & Boersma, 1997; Jeppesen *et al.*, 2000).

Concomitant to the intensification of fish predation, in the summer and autumn, a weak linkage between zooplankton densities and chl *a* concentration was observed. The most obvious example is the disparity in maximum zooplankton densities (Fig. 8) and maximum chl *a* concentrations (Fig. 7). Various authors have described this poor association between the small cladocerans dominant in the summer and autumn (e.g. *Bosmina* and *Ceriodaphnia*; Fig. 9) and phytoplankton (McQueen, Post & Mills, 1986; Jarvis *et al.*, 1988; Gliwicz, 1990; Jeppesen *et al.*, 2000). For effective phytoplankton control (especially large, inedible algae), large cladocerans like *Daphnia* are integral for water quality improvement in various temperate waterbodies (e.g. Sarnelle, 1992, 2007; Scharf, 2007). The decline of this cladoceran in the summer (Fig. 9) could be evidence of the poor water quality (high [chl *a*]) in the autumn in Pisão, S. Pedro and, more evidently, in Roxo (Fig. 7). In contrast, in the case of the Serpa reservoir, high abundances of *Daphnia longispina* in the spring and summer (Fig. 9) and also high overall zooplankton densities in the spring (Fig. 8) relative to the other reservoirs could have been a determining factor in the lower chl *a* concentration in the autumn (Fig. 7).

As the dominant phytoplankton is not efficiently grazed upon by small cladocerans, different sources of nutrition have to compliment zooplankton growth. For that, detritus and bacteria play an important role in the aquatic food web and that is especially important in eutrophic environments, observed in the summer and autumn (Vijverberg & Boersma, 1997; Perhar & Arhonditsis, 2009; Haberman & Haldna, 2014) (Fig. 7). Havens (2002) stated that the zooplankton biomass peak observed in subtropical lakes was associated with increase in chl *a*, but more importantly increase in bacterioplankton. Indeed, bacterioplankton increase usually accompany phytoplankton increase (Havens, Work & East, 2000). Our data aligns with Havens's observation, since the zooplankton community in the summer and autumn was dominated by HEBF (Fig. 11) with the increasing chl *a* concentrations (Fig. 7). Jarvis and team (1988) also associated the dominance of these zooplankters with an increasing importance of finer particles (bacteria included), in a South African reservoir. *Bosmina longirostris*, whilst being a LEBF, was also considerably present (Fig. 9). The lowest values of EQR were observed in the autumn and the zooplankton community displayed a peculiar transformation in this season. Firstly, the high seston quantity (high concentrations of chl *a*) seemed to affect the densities of zooplankton. In many reservoirs (except in Alvito, Odivelas and Serpa), peaks of zooplankton density in the autumn were observed only after and/or before high concentrations of chl *a* (Fig. 7). In addition, in practically all reservoirs (except Odivelas and Amoreira), a low value of Simpson evenness in the month after the lowest value of EQR was observed (Fig. 10) In Roxo, even though the lowest value was observed in November (table 3), low values were also observed in October (Fig. 9), following the first peak of chl *a* in September (Fig. 7). Odivelas and Amoreira were interesting cases, since they started to display a cyclopoid dominance in the summer (Fig. 9) evidenced by the low values of Simpson evenness in this season

(Fig. 10). In Odivelas, this dominance shifts to calanoids in October when water quality was good or superior but shifts once again to a dominance of cladocerans in November. In Amoreira, cyclopoid dominance continues throughout autumn and winter seasons. As stated by (Azevêdo *et al.*, 2015), eutrophic conditions can lead to dominance of more tolerant species, thus decreasing evenness. The low values of Simpson evenness are due to an overwhelming dominance of cyclopoids (Fig. 9), which are known to prevail in eutrophic conditions (Jeppesen *et al.*, 2000; Pinto-Coelho *et al.*, 2005). One hypothesis that might explain both these observations is the high abundance of cyanobacteria, that are reported to occupy a large fraction of phytoplankton in the autumn in these reservoirs (Galvão *et al.*, 2008; Valério *et al.*, 2008). In general, cyanobacteria are of low nutritional value and may produce toxins, which might explain the decreases in density (Lampert, 1987; Bernardi & Giussani, 1990; Ghadouani, Pinel-Alloul & Prepas, 2003). The dominance of cyclopoids in these conditions was already reported, which might explain this shift in the community in the studied reservoirs (Perbiche-Neves *et al.*, 2007). In contrast, field studies have demonstrated the decline of cladocerans following an increase of these algae (Abrantes *et al.*, 2006; Gołdyn & Kowalczewska-Madura, 2008).

Aside from food quality and fish predation, several authors have also demonstrated that the irregularity of the water level is an important variable to consider in Mediterranean reservoirs for zooplankton succession (Naselli-Flores & Barone, 1994; Caramujo & Boavida, 2000b; Fernández-Rosado & Lucena, 2001; Geraldes & Boavida, 2007; Alfonso *et al.*, 2010). In this study, the water level did not vary significantly along the sampling period. Pedrogão 1 however was considered a turbulent site, due to the constant water disturbance from discharge/pumping cycle from electrical production in Alqueva dam’s tailrace. This fact was not reflected in the values of transparency and turbidity, which were very similar between the two sampled sites in the Pedrogão reservoir (Fig. 5). However the zooplankton community differed in two aspects: Pedrogão 1 had an overall lower relative densities of copepods and a dominance of small cladocerans compared to the site near the dam (Pedrogão 2; Fig. 9). Copepods require more stable conditions to complete their life cycles and, therefore, are more affected by reservoir management practices (Velho *et al.*, 2001; Brito *et al.*, 2011). Unstable environments favor zooplankton with shorter generation times, like the small cladocerans in this study and *Diaphanosoma mongolianum* (Geraldes & Boavida, 2007). This might explain the dominance of *Bosmina longirostris* in the spring over *Daphnia longispina*, which requires a longer development period (Lynch, 1980).

Among the identified taxa, there were two species, *Daphnia parvula* and *Bosmina coregoni,* that are non-native to Portuguese waterbodies. Alonso (1996), in his checklist of zooplankton Iberian lakes, mentioned that *D. parvula* might have been recently introduced in these waterbodies. Studies focusing on Portuguese reservoirs (Monteiro, 1988; Caramujo & Boavida, 2000b) showed that this zooplankter is a part of the community in these waterbodies and is sometimes the most relevant cladoceran. This cladoceran was more abundant in Roxo (June and July) and Serpa (October) when *D. longispina* had low densities. A similar pattern was observed in a pioneer population of *D. parvula* in an Italian lake (Riccardi *et al.*, 2004). The presence of *Bosmina coregoni* in Iberian reservoirs was only made aware in a recent study and there's a possibility that it is invasive (Geraldes & Alonso, 2014). Similar to Geraldes and Alonso's (2014) study, *B. coregoni* was mostly present in the colder months in the present study. Nevertheless, it showcased low densities in almost all sites (<3 ind/L), except in S. Pedro in August (14.88 ind/L) and October (8.17 ind/L) and Roxo in September (6.45 ind/L) and January (14.75 ind/L). In spite of their minor representation in the assessed reservoirs, more studies are needed to understand the possibility of an invasion by both species and a more careful look into the specimens of *B. coregoni* is necessary to attribute a morphotype.

The tested functional groupings were the ones based on body size from ECOFRAME (Moss *et al.*, 2003) and Geller & Müller's (1981) classification using mesh size. Both of these groupings divided species or taxon by different traits, aiding in the study of seasonality of the community in different ways. Body size provided some insight on fish predation intensity as was intended by the ECOFRAME team with a slight dissimilarity. As the framework was designed for shallow lakes, this ratio is expected to be relatively high even when young-of-the-year densities are high as well, in lakes with good ecological status. In shallow lakes, macrophytes provide refugia throughout the year for large cladocerans (Angeler *et al.*, 2003), while, in deep lakes, only the littoral region might have some vegetation cover. It can be said that it was expected to observe a decrease in this ratio when fish predation intensifies in pelagic samples, such as in this study. But, as stated previously, the tendency in this ratio might be unclear due to an increase in the large cladoceran *Diaphanosoma* midsummer. Therefore, in the case of studies in the pelagic region of deep Mediterranean reservoirs, *Diaphanosoma* should be grouped together with small cladocerans, since it shares many characteristics with them (see discussion above). The other grouping aided in drawing the parallel between the bacterial production and the zooplankton community succession. Similar to Geller and Müller's (1981) findings, in the summer, the community shifts towards a dominance of HEBF (*Diaphanosoma* and *Ceriodaphnia*) as bacterial production increases, which was described by the authors for the eutrophic reservoirs. In the eutrophic reservoir of our study (Roxo), HEBF were never dominant, but they clearly reached the highest relative densities in summer and autumn (Fig. 11). The addition of OV to this classification was an important step due to the significant representation of cyclopoids in these reservoirs.

Curiously, the TSI based on chl *a*, which categorized the lake as mesotrophic (except Roxo) does not coincide with the zooplankton seasonality described by Geller and Müller (1981) for eutrophic lakes. Kehayias and Doulka (2014) also observed the shift from LEBF to HEBF in the summer (albeit different species) and a disappearance of calanoid species in a deep, Mediterranean lake. Moreover, they also observed a discrepancy between the TSI based on chl *a* and the zooplankton succession. Therefore, we can reach the same conclusion as them that all of the studied lakes showcase a tendency for a eutrophic state.

In sum, through the usage of these different indices and approaches, we were able to establish a more functional view of these reservoirs. The extensive midsummer decline of large cladocerans, the lack of MF, the dominance of OV, copepod nauplii throughout the sampling year and HEBF in the summer show a deterioration of the water quality of the reservoirs in the Alqueva Irrigation System. The absence of large cladocerans in the summer decreases the control of phytoplankton by zooplankton. This partially explains the decrease of the value of EQR throughout summer and the blooms at the end of this season.

Conclusion

An assessment of the zooplankton community succession of the reservoirs of the Alqueva Irrigation was conducted, coupled with a water quality appraisal following the WFD’s standards and using other important physical and chemical parameters. The reservoirs of the Alqueva Irrigation System were deemed mesotrophic or eutrophic, according to the trophic state index using chl *a* concentration as an EQR. However, all parameters except chl *a* never surpassed the stipulated thresholds defined by the WFD and did not reflect the effect of the current land use on the water quality, mostly likely due to the atypical conditions of the sampling year (lack of rain/runoff). The biological parameters (chl *a* concentration and zooplankton) however did reflect the poor state of these reservoirs, especially in the summer and autumn. The zooplankton functional and structural community succession clearly showed the reservoirs’ tendency for eutrophic conditions, regardless of the observed physical and chemical parameters.

Our results indicate that zooplankton would provide a great addition tool to the WFD, an opinion shared by other specialists. This community provided valuable complementary information and aided in understanding the water quality of these reservoirs. Most of it came from an analysis of the succession, coupled with the usage of functional groupings and ratios at our disposal. Regarding the functional groupings, they were straight-forward and accessible and may become valuable tools for future studies. With this in mind, in no way should an assessment of ichtyofauna and phytoplankton be neglected. Phytoplankton is directly related to the intense blooms in autumn, the biggest issue in these reservoirs, and fish indirectly contribute to it by removing large filter feeding cladocerans and decreasing the overall zooplankton abundance. Monitoring these two elements is vital to fill the gaps in the knowledge of the zooplankton community in the sampled reservoir, not to mention it is required by the WFD. Since it was established that bacterioplankton had an added importance to the diet of zooplankton in the summer, it should also be assessed. Moreover, it would be wise to test the functional indices in more samples and compliment with others, like the zooplankton biomass to phytoplankton biomass ratio and zooplankton fecundity.

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