DYNAMICS OF ZOOPLANKTON COMMUNITIES IN THE ALQUEVA RESERVOIR

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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 à 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”

RESUMO

A construção da Barragem do Alqueva levou à criação da Albufeira do Alqueva, o maior reservatório artificial de água na Europa ocidental. Apesar do seu tamanho e importância para os ecossistemas circundantes e para a economia local, a albufeira encontra-se presentemente sob um processo de eutrofização. Os organismos zooplanctónicos têm sido apresentados como promissores bioindicadores para avaliação da qualidade da água, uma vez que estes organismos representam um dos principais elos das teias tróficas aquáticas, alimentando-se de fitoplâncton e a serem predados por peixes, contribuindo assim no controlo da qualidade da água. Apesar já ter sido demonstrada a sua utilidade enquanto indicador de qualidade, o zooplâncton não está, atualmente, inserido nos elementos de qualidade biológica sugeridos pela Diretiva Quadro de Água (DQA), uma omissão que tem gerado críticas por diversos autores. Assim, este estudo pretende analisar a abundância e a dinâmica do zooplâncton na albufeira do Alqueva, pretendendo com esta avaliação classificar o estado ecológico atual do ecossistema aquático. Para este efeito, foram realizadas amostragens mensais em nove locais na albufeira durante o período de aproximadamente um ano (Fevereiro 2017 a Janeiro 2018). Diversos parâmetros físicos e químicos foram quantificados de acordo com o descrito pela DQA; sendo o parâmetro biológico utilizado a concentração de clorofila *a* e o Índice de Estádio Trófico. Os resultados obtidos apontam para a existência de uma dinâmica sazonal e espacial nos parâmetros abióticos da albufeira. A análise permitiu concluir que sete dos nove locais, de acordo com os parâmetros da DQA, apresentaram bom potencial ecológico, enquanto dois (Elvas e Juromenha) foram classificados apenas com medíocre potencial ecológico. A análise realizada aos parâmetros de zooplâncton revelou uma dominância de omnívoros (Cyclopoida) durante o ano, e de *taxa* filtradores de bactérias de baixa eficiência (*Daphnia, Bosmina, Alona*) na primavera e outono e filtradores de bactérias de alta eficiência (*Ceriodaphnia, Diaphanosoma*, *Alona*) no verão. A sucessão de cladóceros apresentou uma dinâmica de lagos temperados apenas em três pontos (Amieira, Álamos, Loureiro). A comunidade zooplanctónica mostrou corresponderem com o espectável de zonas mais fundas confinadas. A informação obtida através de uma análise zooplanctónica permite a elaboração de um argumento para a sua inclusão nos critérios de qualidade biológicos da DQA.

PALAVRAS-CHAVE: Qualidade da água, Diretiva Quadro de Água (DQA), estado trófico, sucessão zooplâncton, albufeira, sistema eutrófico, parâmetros físico-químicos, clorofila *a*

ABSTRACT

The construction of the Alqueva Dam led to the creation of the Alqueva reservoir, the largest reservoir in Western Europe. Despite its large size and key role in the surrounding ecosystems and local economy, the reservoir is currently under a process of eutrophication. Zooplanktonic organisms have been proved to be promising bioindicators for water quality. These organisms represent one of the key levels in aquatic trophic chains, as they graze on phytoplankton and are in turn predated by fish, as such contributing for the quantity control of available nutrients in an aquatic system. Despite their usefulness as an indicator having already been proved, zooplankton are currently not a part of the biological quality elements suggested by the Water Frame Work Directive (WFD), an omission that has garnered criticism from several authors. As such, this study intends on analysing the abundance and dynamics of these organisms in the Alqueva reservoir, aiming to classify the current ecological state of the water body. For this effect, monthly samples were performed in nine sites within the reservoir over the period of one year (from February 2017 to January 2018), with the goal of studying the spatial and seasonal variation on dynamics and distribution of the zooplankton community. Aside from the biological component, several physical and chemical parameters were quantified in accordance to what is recommended by the WFD, as well as the concentration of chlorophyll *a* and the Trophic State Index for the various sites. The attained results point towards the existence of a seasonal and spatial dynamic to the abiotic parameters of the reservoir. The analysis permitted to conclude that, of the nine sites, in accordance with the WFD four sites were of good quality, with two of them (Elvas and Juromenha) being of considerably worse quality. The analysis performed to the zooplankton revealed a dominance of omnivores (Cyclopoida) during the year, as well as of Low-Efficiency Bacterial Feeders (*Daphnia*, *Bosmina, Alona*) in spring and autumn, and High-Efficiency Bacterial Feeders (*Ceriodaphnia, Diaphanosoma, Chydorus*) in summer. The cladoceran succession only occurred in three sites (Amieira, Álamos, and Loureiro). The communities were found to be distributed as expected from a eutrophic reservoir in accordance to Geller and Müller (1981). The unique information garnered from the use of zooplankton dynamics allows for an argument to be made for their inclusion as a Biological Quality Element in the WFD.

KEYWORDS: Water quality, Water Framework Directive (WFD), trophic state, zooplankton succession, reservoir, eutrophic system, physical and chemical parameters, chlorophyll *a*

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ABBREVIATIONS

Abs - Absorbance

Avg – Average

BQE – Biological Quality Element

CDOC – Coloured Dissolved Organic Carbon

Chl a – Chlorophyll a

DOC – Dissolved Organic Carbon

EDIA – Empresa de Desenvolvimento e Infraestruturas do Alqueva, S.A.

EFMA – Empreendimento de Fins Múltiplos de Alqueva

EQR – Ecological Quality Ratio

EU – European Union

HEBF – High-Efficiency Bacteria-Feeders

INAG – Instituto Nacional da Água

LEBF – Low-Efficiency Bacteria-Feeders

Min – Minimum

Max - Maximum

NTU(s) – Nephelometric Turbidity Unit(s)

ORP – Oxidation Reduction Potential

[P] – Phosphorus concentration

TSI – Trophic State Index

WFD – Water Framework Directive

1. INTRODUCTION

Water is the most important resource available on Earth. Not only is it an integral part to the growth and advancement of human society, but it is widely attributed that the existence of water in all three physical states (solid as ice, gas as water vapour and, perhaps more importantly, as a liquid) is a key factor for the existence of life on the planet (Moss 2010). Water's availability and distribution has been a key component in the existence of certain ecosystems as well as for the establishment of human communities (Moss 2010).

In nature, water is mostly found in the form of salt water (97.2%), having a high degree of concentrated salts in its composition (Shiklomanov and Rodda 2004). On the other hand, freshwater has much lower amount of salts and is present in both lotic systems (running waters) like rivers, and lentic systems (absence of flow) like lakes and reservoirs. For humans, and most land organisms, only freshwater is available for consumption (Hoekstra and Mekonnen 2011). Despite being highly important, freshwater is a finite resource (Hoekstra and Mekonnen 2011). However, pressure led by the advancement of human society, both in terms of population increase and for technological and economic growth, has progressively threatened the quality of freshwater resources. Furthermore, it is important to note that these changes not only impact on the resources for direct human usage, but also in the quality of freshwater and in a loss of biodiversity (Gleick 1998).

The degradation of freshwater ecosystems, and its associated loss of biodiversity, has seen an increase over the last few decades (Ayyad 2003). There exist several ways in which biodiversity may be altered: pollution, flow alterations, the introduction of invasive species and overexploitation of aquatic ecosystems (Sala et al. 2000). These factors can lead to alterations not only in the overall species richness (the total number of species present) but also in the functions upheld by the different species within the system (Sala et al. 2000). These factors contribute to increased eutrophication, a dysregulation of nutrients and minerals (specifically phosphorus and nitrates) in the water, leading to an excessive growth of plants, algae, and phytoplankton, negatively impacting the system's balance. In some cases, the systems can even reach levels of hypertrophication, a more severe form of eutrophication (Carlson 1977). With this knowledge and in order to make an effort in diminishing the current degradation of water courses, as well as to take measures for the restoration of water bodies to their previous states, several projects have been headlined and designed (Pretty and Shah 1997; Lazarova et al. 2001). Among them, perhaps the most relevant is the European Union's Water Framework Directive.

Published in the 23rd of October 2000, and put in place in the 22nd of December of the same year, the Water Framework Directive (WFD) consists on the establishment and upkeep of a series of guidelines and rules in order to promote better care and protection of water bodies (lakes, rivers, reservoirs, etc.) in the European Union member states. The main goal of this directive is the establishment of a framework for the protection of the European waters, intending on achieving a "good ecological state" in all surface and subterranean bodies of water by the year 2015, however, as not all water bodies were yet in accordance by that year, the deadline has been pushed forward to 2021 (Voulvoulis et al. 2017). The ecological status of an ecosystem is defined by how the dynamics and characteristics of its biological communities and physicochemical parameters compare to previously established reference values. A good system will have its parameters within the range of reference values, while a system in a bad ecological state will have its values for those same parameters far removed from the reference interval (Jeppesen et al. 2009).

In Portugal, the WFD was officially recognized into national legislation in December 29th 2005 (by the DL 58/200) and further cemented on March 30th of the following year (DL 77/2006). The Portuguese documentation makes use of specific reference values and different metrics depending on the type of water body: ranging from both lotic systems like rivers, to lentic systems like lakes and reservoirs. Reservoirs themselves can be classified either as Northern or Southern (Instituto da Água 2009). To allow the correct evaluation and interpretation of the results, the WFD takes into account different types of parameters: biological, physical and chemical, and hydromorphological (European Commission 2009). For reservoirs, these parameters include, among others, total phosphorus, temperature, dissolved oxygen, pH, as well as the composition of the phytoplankton communities and their biomass (Instituto da Água 2009). To properly assess these elements, the WFD recommends using Ecological Quality Ratios (EQRs). The EQR for a specific parameter can be calculated as the ratio between an observed value and that parameter's reference value for that type of water body and locale. This EQR calculated value is then used to classify the water body according to a pre-defined colour scale, with five levels of quality: Excellent (blue), Good (green), Average (yellow), Mediocre (orange) or Bad (red) (Instituto da Água 2009). The results attained from this analysis are used in order to assess the ecological status of the waterbody of the aquatic ecosystem in study (Figure 1).

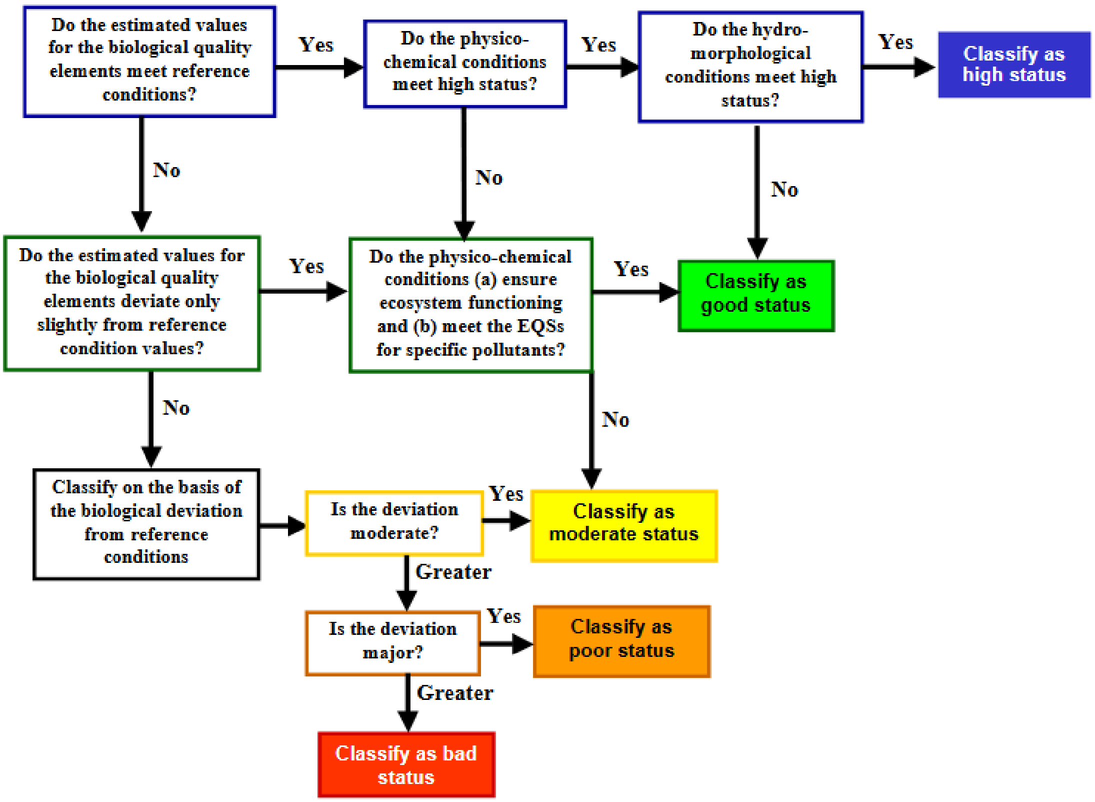


Figure 1 - The relative roles of biological, hydromorphological and physical and chemical quality elements for the classification of an aquatic ecosystem's Ecological Status (European Commission 2009).

Bioindicators can be found among the biological quality elements suggested by the WFD; these being organisms whose presence, composition and dynamics can be used to ascertain knowledge about the ecological status of an ecosystem. Depending on the nature of the ecosystem that is being analysed, different bioindicators can be used (e.g., phytoplankton, macroinvertebrates, fish species) (Parlamento Europeu 2008; Instituto da Água 2009). Currently, for highly modified water bodies, such as reservoirs, only phytoplankton are accepted as biological quality elements, through the analysis of the communities' composition, abundance, bloom rate, and intensity as well as biomass (European Commission 2009). Despite their importance in ecosystems, zooplankton is not recommended as a biological quality element (BQE) for lentic systems according to the metrics proposed by the WFD.

However, zooplankton is even cited on the WFD as a “supportive/interpretative parameter” of fish that are “often/typically measured or sampled at the same time” (European Commission Environment 2000). Caroni and Irvine (2010) have presented a theory for their omission, arguing that it’s a political decision, motivated by a financial component, but currently, the official reason for the absence remains unclear (Jeppesen et al. 2011).

Zooplankton can be defined as aquatic heterotrophic organisms of small size that occur in the water column. These organisms can be found in lotic or lentic ecosystems, in salt, and in freshwater. These organisms’ worth as bioindicators can be easily understood by knowing their role in lentic systems: zooplankton are predated by fish and other predatory aquatic species, supporting the upper levels of the food chain, but equally important, is their role in controlling the phytoplankton by feeding on them (Abrantes et al. 2006). Zooplankton is composed by three different groups of organisms: Rotifera (a class) and by two suborders of crustaceans: Cladocerans and Copepod (Wetzel and Boavida 1993). For this study, the focus was placed on Copepods and Cladocerans.

Cladocerans are members of the Cladocera order, featuring the well-known genus *Daphnia*, as well as others such as *Bosmina* or *Diaphanosoma*, and other small crustaceans. These organisms are ubiquitous in aquatic ecosystems, and particularly in freshwater ecosystems (Amoros 1984). Almost all cladocerans are herbivorous and feed on phytoplankton and suspended particles, however, certain genus feed on smaller herbivorous zooplankton (Moss 2010). The reproduction and survival of cladocerans can be conditioned by certain outside elements such as pH, contamination, and temperature (Wetzel and Boavida 1993). Cladocerans reproduce parthenogenically but when subjected to unfavorable conditions, such as low quality food and very high temperature, they can reproduce sexually and produce a higher number of offspring (Wetzel and Boavida 1993). In temperate lakes and reservoirs, cladocerans are known to undergo a process of seasonal succession in the transitory period between spring and summer. Large cladocerans such as *Daphnia* and *Diaphanosoma* are known to decrease, prompting an increase in smaller cladocerans like *Bosmina* and *Ceriodaphnia.* This process can occur due to several factors, namely in response to the increase in fish predation, and cyanobacterial blooms, factors that favour smaller cladocerans over larger ones (Amsinck et al. 2005).

Copepods, a sub-class of small crustaceans (with three orders: Cyclopoida, Harpacticoida, and Calanoida (Alonso 1996)), are also ubiquitous in both lentic and lotic ecosystems, often being the dominant group of zooplankton. Copepods, who reproduce sexually, are known to have larger growths in spring and autumn (Wetzel 2001), and are the largest forms of zooplankton communities. Regarding their ecological functions in the aquatic ecosystem, Calanoida tends to be macrofiltrators, feeding on small particles, while Cyclopoida are omnivores, feeding on smaller zooplankton, on phytoplankton and other organic detritus (Geller and Müller 1981; Moss 2010).

Indeed Geller and Müller (1981) have proposed a classification system for zooplanktonic crustaceans in accordance to their filter mesh, predicting their feeding habits and by placing them in different functional groups. These groups are macrofiltrators and two types of plankton that feed on bacteria, separated in accordance with their efficiency by Low-Efficiency Bacteria Feeders (LEBF) and High-Efficiency Bacteria Feeders (HEBF). The authors proposed that depending on a given system's trophic state, the composition of the zooplankton community, in terms of functional groups, could follow a different pattern (Figure 2).

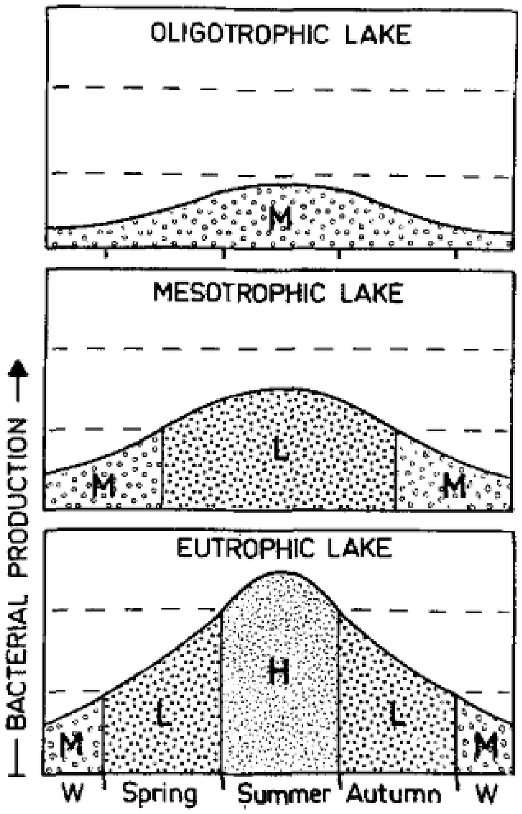


Figure 2 - Predicted seasonal succession of planktonic crustaceans according to Geller and Muller (1981) for different types of temperate systems according to their trophic state. M - Macrofiltrators, L - Low-Efficiency Bacteria Feeders, H- High Efficiency Bacteria Feeders.

According to the literature, several authors have already used the dynamics of zooplanktonic communities as indicators of water quality successfully (Timms and Moss 1984; Jeppesen et al. 2011). However, while there are authors, such as Erik Jeppensen and his collaborators (2011) and Caroni and Irvine (2010) that criticize the WFD for not including Zooplankton as a BQE, they do refer that, at the regional level, there still exists a lack of information and a need for further studies to be performed (Jeppesen et al. 2011). It is in order to address the lack of information that the present study intends to:

- Characterize the dynamics of the zooplankton communities within the Alqueva reservoir in the south of Portugal, in order to assess water quality
- Evaluate the water quality of the Alqueva reservoir using the parameters established in the WFD.

To achieve this objective, a few more specific goals were established:

- To study the seasonal variation of the zooplankton communities and the physical and chemical parameters, by monitoring across a full year.
- To assess the spatial distribution of the zooplankton communities and the variation of the physical and chemical parameters, across the reservoir.

2. MATERIAL AND METHODS

2.1 STUDY SITE

This study was conducted in the Alqueva reservoir, located within the Alentejo region, in the south of Portugal, being a part of the EFMA (*Empreendimento de Fins Múltiplos de Alqueva)*. The Alqueva reservoir is the largest artificial lake in Western Europe, occupying a total area of 25 000 ha (EDIA 2017). It can store up to 4150 hm^3 of water at a time, being supplied by the Guadiana River, with source in Spain. It is the basis for the Alqueva Global Irrigation System, located near the border that separates Portugal and Spain (EDIA 2017). After its inception in 2004, the water reservoir has been used for irrigation, electricity production and for public consumption (EDIA 2017).

The Guadiana River basin has been under an increasingly severe pressure from the continued use of its aquatic resources, particularly due to the construction of several dams across its length. One of these structures, the Alqueva dam, which formed the reservoir of the same name, has increased the total of water retained, not reaching the estuary and the coastal area, to an estimated 13,000 hm^3 per year (Dias et al. 2004). The Alqueva reservoir is subject to Mediterranean climate, being characteristically semi-arid whilst also possessing periods where the climate is fully arid (from July to August) as well as periods where it is instead of a temperate-humid variety (from November to January). By the attributes stated, it was classified by the WFD's Portuguese criteria as a Southern Water Body (Instituto da Água 2009). The area associated with the Portuguese portion of the Guadiana river basin is characterized by a large variation in the climate not only between seasons but also inter-annually (Chícharo et al. 2006).

Nine sites (Elvas, Juromenha, Monsaraz, Mourão, Estrela, Alqueva, Amieira, Álamos and Loureiro) within the Alqueva reservoir were monitored in this study (Figure 2). These sites were chosen to attain an understanding of the spatial dynamics of the zooplankters, covering a vast area of the Alqueva reservoir. Five sites are located in piers within the Alqueva reservoir (Monsaraz, Mourão, Estrela, Alqueva and Amieira), whilst the other two (Álamos II and Loureiro) are located in reservoirs to the east of the Alqueva reservoir (Figure 3). The sixth site, named "Alqueva", is located near the dam. The sites that are furthest from the dam being "Elvas", located within the Guadiana River, and "Juromenha", within the transitory area between the Guadiana River and the reservoir (Figure 3).

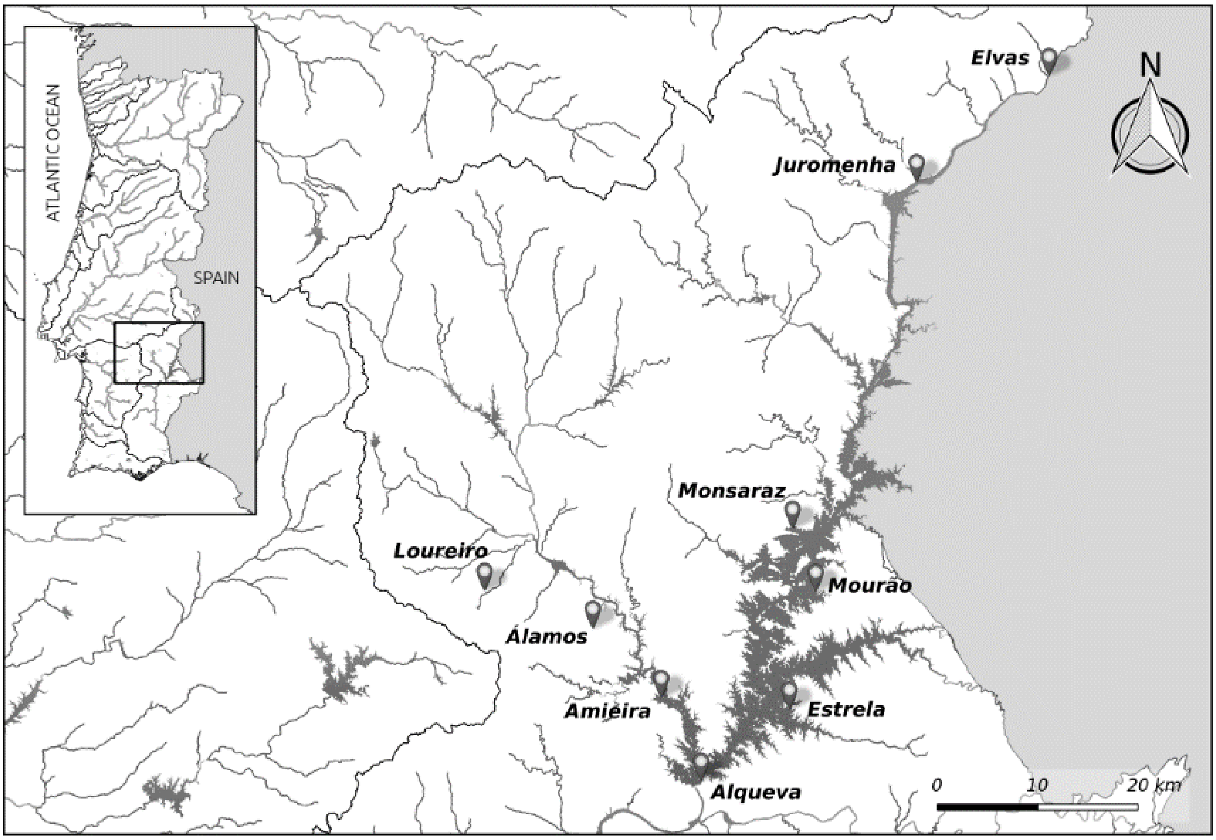


Figure 3 - Location of the nine sampling sites within the EFMA: Elvas (38°49'53.83"N, 7°5'4.39"W), Juromenha (38°44'15.47"N, 7°14'15.39"W), Monsaraz (38°25'37.83"N, 7°22'58.47"W), Mourão (38°22'13.14"N, 7°21'23.31"W), Estrela (38°15'54.19"N, 7°23'17.99"W), Alqueva (38°12'6.74"N, 7°29'20.01"W), Amieira (38°16'34.44"N, 7°32'2.74"W), Álamos (38°20'20.88"N, 7°36'42.02"W), Loureiro (38°22'24.19"N, 7°44'1.94"W) (Sérgio Ribeiro, 2018).

2.2 SAMPLING PROCEDURE

The sampling was conducted over approximately a year, with samples being collected every month between February 2017 and January 2018. In each site, the transparency of the water was measured with a Secchi disk. To measure the physical and chemical *in situ* parameters at a depth of 2 m a Van Dorn bottle was used to collect the water. After the water collection the physical and chemical water characterization was conducted with an AQUALYTIC AL15 multiparametric probe in order to determine several parameters suggested by the WFD: pH, temperature (°C), oxidation-reduction potential (mV), conductivity (µS/cm) and dissolved oxygen (mg/L and %), turbidity (NTUs) were also calculated, through the usage of a HANNA INSTRUMENTS HI 93703C turbidimeter. Additional, water samples were collected in each site in order to determine further physical and chemical parameters in the laboratory: Coloured Dissolved Organic Carbon (CDOC, m^{-1}), phosphates (mg/L), chlorophyll *a* (mg/L) and calcium concentrations (mg/L).

In order to collect the zooplankton samples at a depth of 2 m a fuel pump was used to extract the water samples. In each site, a single sampling was conducted with 200 L of water being pumped through a 55 µm net, in order to filter and collect the zooplankton communities. Only a single filtration was done for each site, and the samples were preserved in 70% EtOH until later identification in the laboratory.

2.3 LABORATORIAL PROCEDURE

In the laboratory, water samples were quantified for CDOC, concentration of phosphorus (mg/L), chlorophyll *a* (mg/L) and calcium (mg/L). The CDOC was quantified through the standard method described by the American Society for Testing and Materials (1992). The concentration of chlorophyll *a* was quantified through the method described by Lorenzen (1967). The concentration of calcium was determined by using an HI720 Checker Handheld Colorimeter, and the concentration of phosphorus through the APHA, AWWA, and WPCF standard method (2012). Attaining the absorbance of phosphates on the samples through the usage of a spectrophotometer, the concentration of phosphorus was calculated through the conversion of the absorbance in the water samples, using the following equation:

$$[P] = \frac{(Abs - 0.0135)}{0.09913}$$

The zooplankton samples were analysed in aliquots of 10 mL for each sample with recourse to counting chambers under a magnifying glass. For the quantification of the individuals per sample, successive chambers were counted in order to fulfill n=400, or otherwise n=100, depending on the abundance. Successive chambers were counted until over half the identified species fulfilled the above stated condition, or until the entirety of the sample was analysed. The identification was done to the species, genus or family level with recourse to identification guides, namely, cladocerans through Amoros's (1984) and other branchiopods through Alonso's (1996).

2.4 DATA ANALYSIS

The analysis of the acquired results was divided based on the nature of the data: physical and chemical support elements, trophic state and ecological result, and biological elements (zooplankton community).

The physical and chemical support parameters were studied according to the results for each sampling site along the sampling period, and the ecological potential of the water bodies were calculated according to the Water Framework Directive. Moreover, the chlorophyll *a* concentration during the sampling period was also analysed. Furthermore, using the available data, the Ecological Quality Ratio (EQR) and the Trophic State Index (TSI) were calculated using the chlorophyll *a* concentration. The equation used for the calculation of the TSI value, as described by Carlson (1977), was the following:

$$TSI(Chl\ a) = 10 \times \left(6 - \frac{2.04 - 0.68 \ln(Chl\ a)}{\ln(2)}\right)$$

In regards to the zooplankton communities: the diversity and evenness values were assessed with the Shannon-Weiner diversity index and the Simpson evenness index. The abundance and richness per site and across the year were also analysed.

In order to analyse the zooplankton communities in terms of their function in the aquatic ecosystem, the identified zooplankton were grouped in four functional groups: Omnivores, Macrofiltrators, High-Efficiency Bacteria Feeders – HEBF and Low-Efficiency Bacteria Feeders – LEBF, according to bacteria feeding efficiency, as described by Geller and Mueller (1981). The relative abundance of large cladocerans was also calculated, in an attempt to ascertain the succession using the following formula:

$$\frac{Large\ Cladocerans\ (Diaphanosoma\ sp. + Daphnia\ sp. + Sida\ sp.)}{Total\ Cladocerans}$$

3. RESULTS

3.1 PHYSICAL AND CHEMICAL SUPPORT ELEMENTS

Figure 4 presents the attained data for Temperature (ºC) and dissolved oxygen (mg/L). The temperature values varied across the year, with higher temperatures displayed in the summer, between ≈20ºC and ≈27ºC, with the highest values being registered at June, July, August or September depending on the site (Figure 4). The temperatures were much colder during the winter sampling period, ranging from ≈10ºC to ≈15ºC (Figure 4). The large variation between summer and winter points to the existence of a pronounced seasonality for this parameter. In terms of the dissolved oxygen levels (Figure 4), there was also a high variation. February registered the highest overall values for all sites (11.41 mg/L in Alqueva to 16.12 mg/L in Monsaraz). March exhibited a drop in dissolved oxygen for all sites, maintaining the same lower values during summer. All sites exhibited an increase in their dissolved oxygen between October and November of ≈2 to ≈3 mg/L (Figure 4). In accordance with the WFD, for a southern reservoir in Portugal to be considered of good quality it must exhibit at least 5 mg of dissolved oxygen per litre (Instituto da Água 2009). All of the sites in analysis, can be considered to be of good quality as all of the values are in agreement with the stipulated limits (Figure 4).

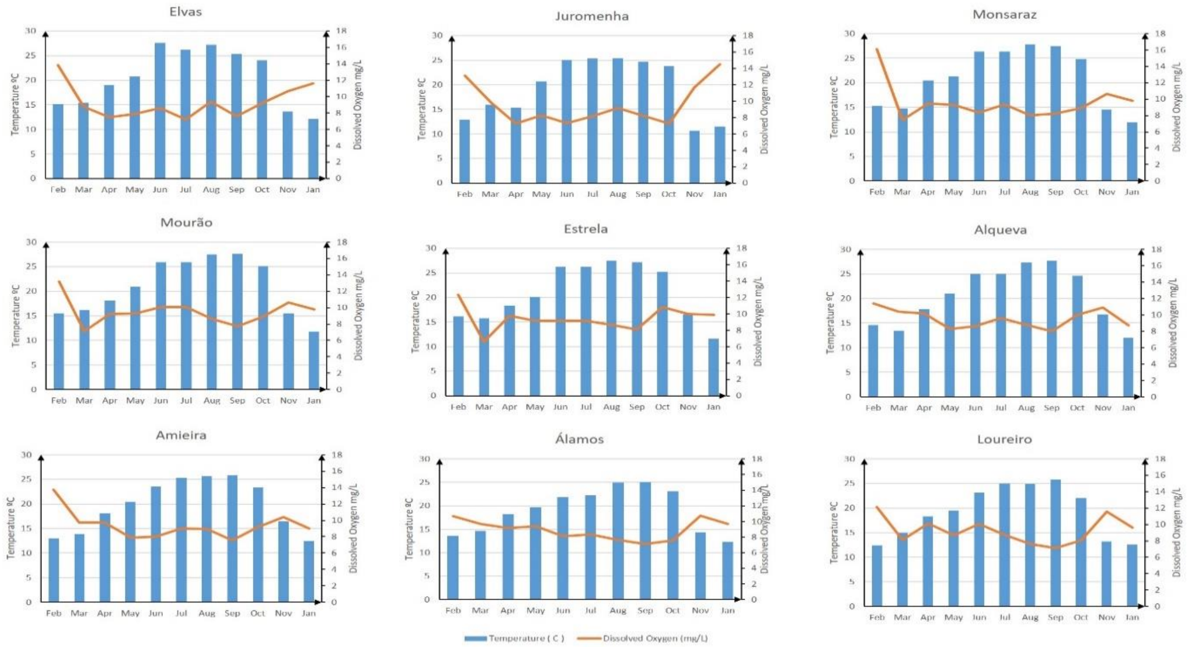


Figure 4 - Results of temperature (ºC) and dissolved oxygen (mg/L) across the sampling sites during the sampling period.

Figure 5 represents the results for the pH values as well as the conductivity (µS/cm) for all nine sites. Nearly all the nine sampling sites alternated periods of neutral and alkaline pH (≈6.8 to ≈8.7) across the year, with the summer months, as well as October and November, having the highest pH levels (>8.5). Elvas was the exception, with the pH values always below 8, also registering the most acidic sample out of all, with 6.11 in June (Figure 5). Despite the evidenced seasonality of the results, all the sites are within the values established by the WFD for this parameter (between 6 and 9) in highly modified water bodies (Instituto da Água 2009) for a good ecological potential water classification. For conductivity, the highest values recorded were in Elvas and Juromenha (more distant from the dam), having values in the range of ≈500 µS/cm to ≈770 µS/cm, while the remaining sites all remained at the range of ≈400 µS/cm to ≈500 µS/cm at the most. Elvas and Juromenha exhibited higher values during the winter months, while Monsaraz, Mourão, Estrela, and Amieira had higher values in July (≈530 µS/cm) to ≈560 µS/cm). pH and conductivity values were shown to mirror each other, as the highest points for one parameter, coincided with a low point for other (Figure 5).

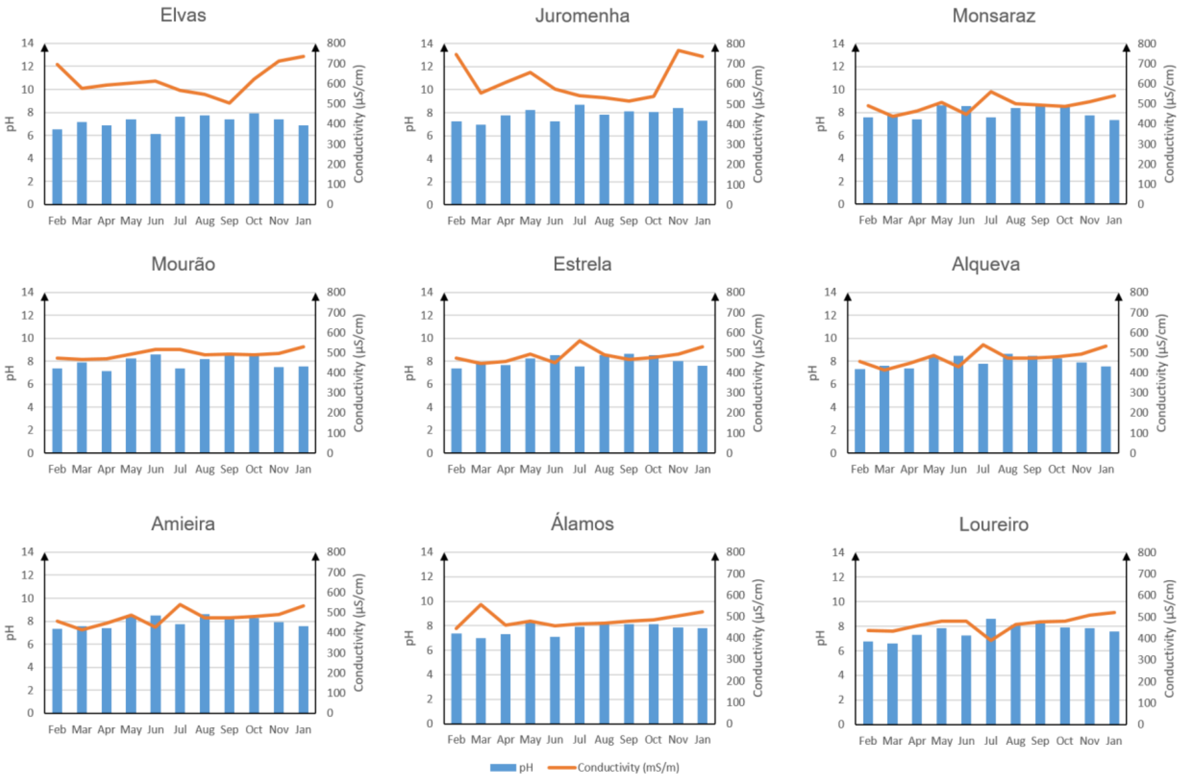


Figure 5 - Results of pH and conductivity (µS/cm) across the sampling sites during the year.

Figure 6 focuses on the results for transparency (m) and for turbidity (NTUs) in the sampling sites across the sampling period. Focusing on the transparency, a large difference between sites was observed, namely Elvas and Juromenha comparatively to the other seven sites (Figure 6). For these samples, at no point did the Secchi disk remain visible at a depth higher than 2 m. The values of transparency for the other sites are much higher, being superior to 2 m on all samples (exception being September and October for Monsaraz, Mourão and Estrela). Turbidity results (Figure 6) showed the same dissonance between Elvas and Juromenha and the other sites. Elvas and Juromenha presented the highest levels of turbidity with Elvas having the highest recorded turbidity at 38.66 NTUs in November. For the other sites, the turbidity levels were much smaller, often being even zero, or below 1 NTU. There was a marked increase in turbidity for these sites in the same months as the transparency dropped (Figure 6). Turbidity levels were shown to be lower for sites farthest away from the river and closest to the dam (Figure 6).

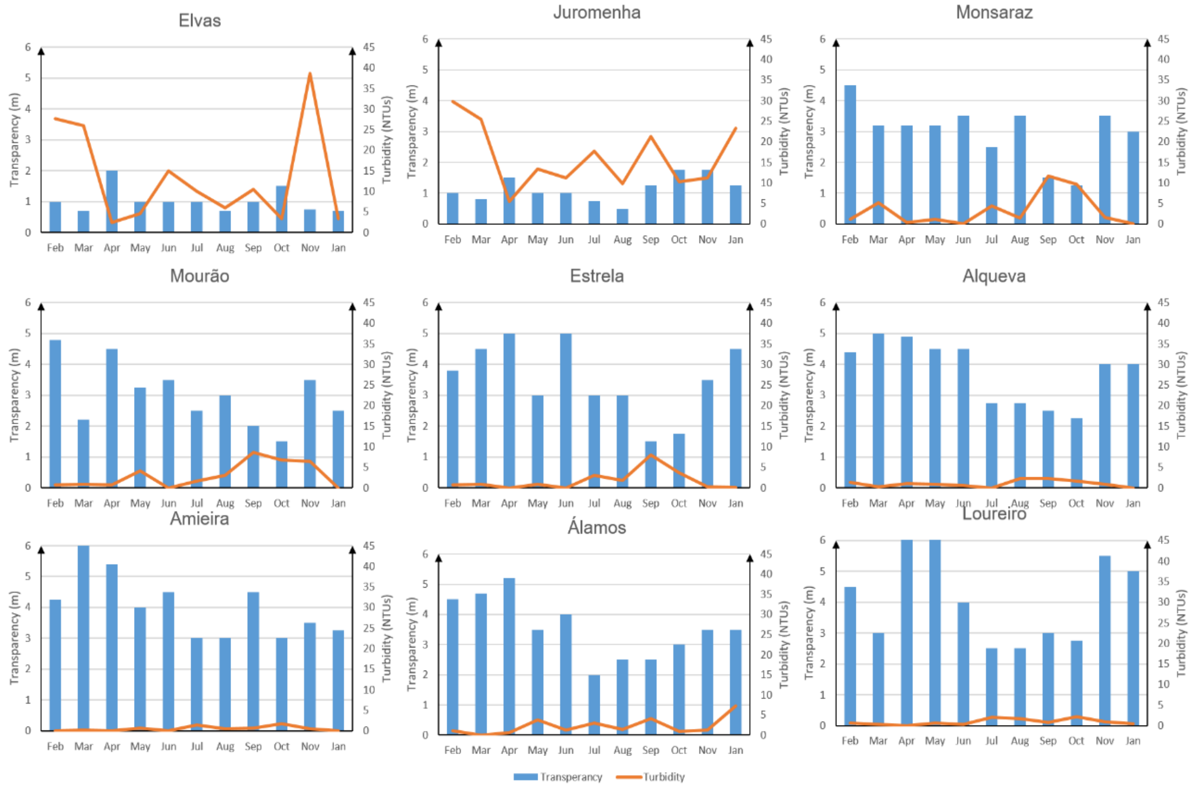


Figure 6 - Recorded values for transparency (m) and turbidity (NTUs) across the sampling sites during the year.

Figure 7 shows the results for CDOC (m^{-1}) and for oxidation-reduction potential. In regard to CDOC (Figure 7), the data for Elvas (January) and Juromenha (July) is incomplete, as these samples were lost. Despite this, the two sites furthest from the dam once again stand out by registering larger values compared to what occurred for the other sites, with Juromenha's June sample of 0.254 m^{-1} being the highest value registered. The remaining sites have very small values across the year, with nearly all being under 0.05 m^{-1}. There was not a clear seasonality to be seen in the sites, as the registered highs and lows occurred in an assortment of different months, depending on the site. In regard to the redox potential, all sites registered negative values for the entire year (Figure 7). There was a clear seasonal variation, as almost all sites featured less negative values in summer and more negative ones for winter (Figure 7). March, with the exception of Elvas and Mourão, registered the lowest values for all sites.

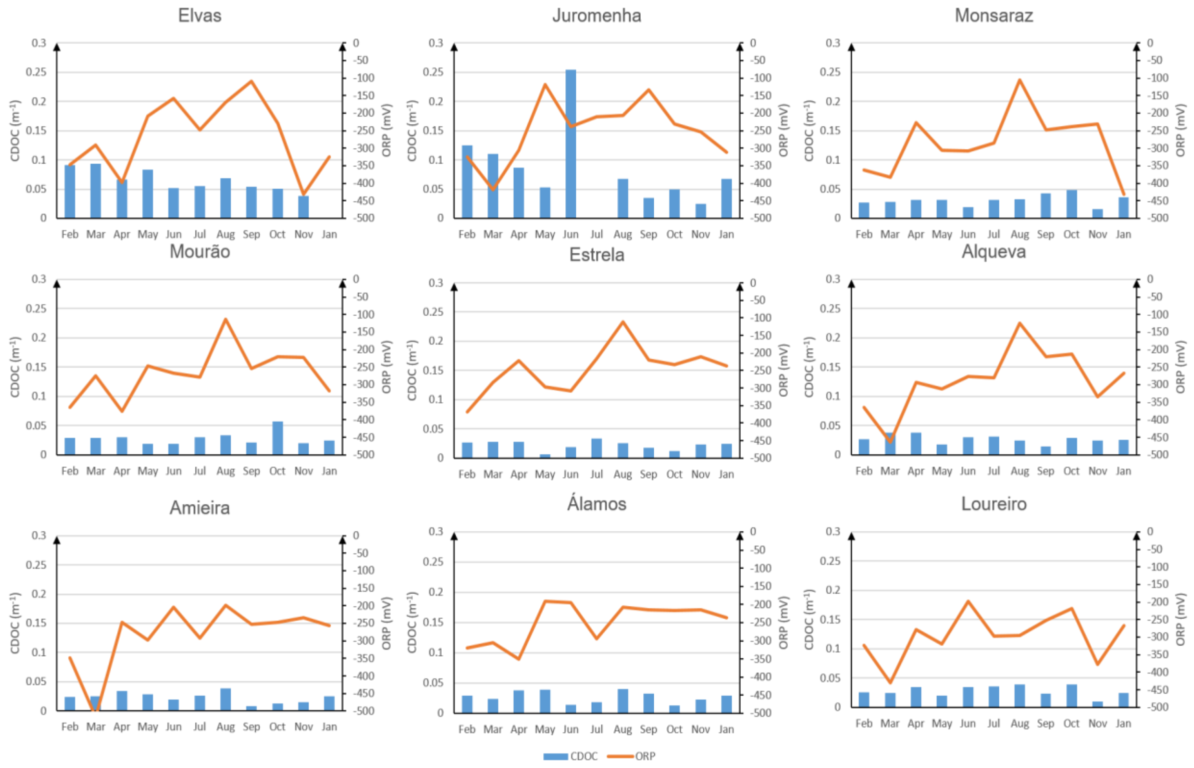


Figure 7 - Results of CDOC (m^{-1}) and Oxidation-Reduction Potential (mV) across the sampling sites during the year.

Figure 8 shows the results for calcium (mg/L) and phosphorus (mg/L) concentrations. Looking first at the calcium levels, once again Elvas and Juromenha stand apart from the other reservoirs. However, in this particular parameter, they are joined by Monsaraz, the site that is closest to these two. All these sites feature a clear peak of calcium concentration in March of ≈2 mg/L. The phosphorus concentration recorded was extremely low for nearly the entire sampling, and in a lot of these samples, the value was below the detection limit (Figure 8). Moreover, in Estrela and Álamos reservoirs all the samples were above the detection limit. Looking at the criteria set by the INAG for southern Portuguese highly modified water bodies, the required condition to be met for a good ecological potential in terms of phosphorus is to have a value below that of 0.07 mg P/L (Instituto da Água 2009). According to this criteria, the only sites that remained consistently of good quality were Estrela, Álamos, Alqueva, and Loureiro.

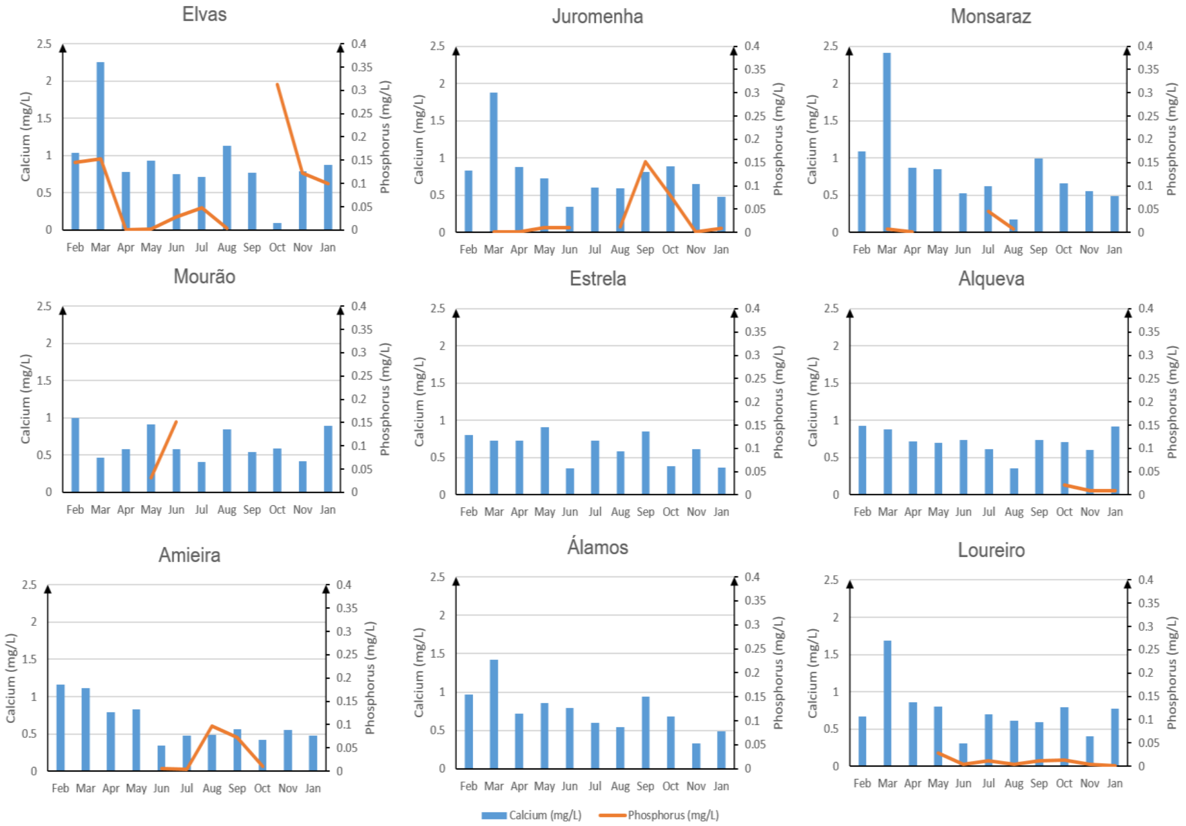

Elvas
Juromenha
Monsaraz
Mourão
Estrela
Alqueva
Amieira
Álamos
Loureiro

Figure 8 - Results of Calcium (mg/L) and Phosphorus (mg/L) concentration.

3.2 TROPHIC STATE AND ECOLOGICAL RESULTS

In order to calculate the trophic status and to analyse the ecological status of the ecosystem, the concentration of chlorophyll *a* (Chl *a*) was measured (Figure 9). For all sites, the concentration of chlorophyll *a* increased as the year progressed, reaching the highest values, for all sites, between August and October. However, as with what had occurred with the physical and chemical parameters, the values for Elvas and Juromenha are quite different from the other sites, having much higher values in the October sampling of Elvas achieving 101.26 mg/L of chl *a*. While Juromenha did not register as high of a peak as Elvas did in October, it did feature a higher average amount of Chl *a* across the year (33.76 mg/L in comparison with 31.80 mg/L for Elvas), recording high values across the entire year, with the maximum value being recorded in September (81.95 mg/L). Aside from those two sites, Monsaraz also had high values of chl *a* concentration for September and October (27.74 mg/L and 42.41 mg/L respectively) (Figure 9).

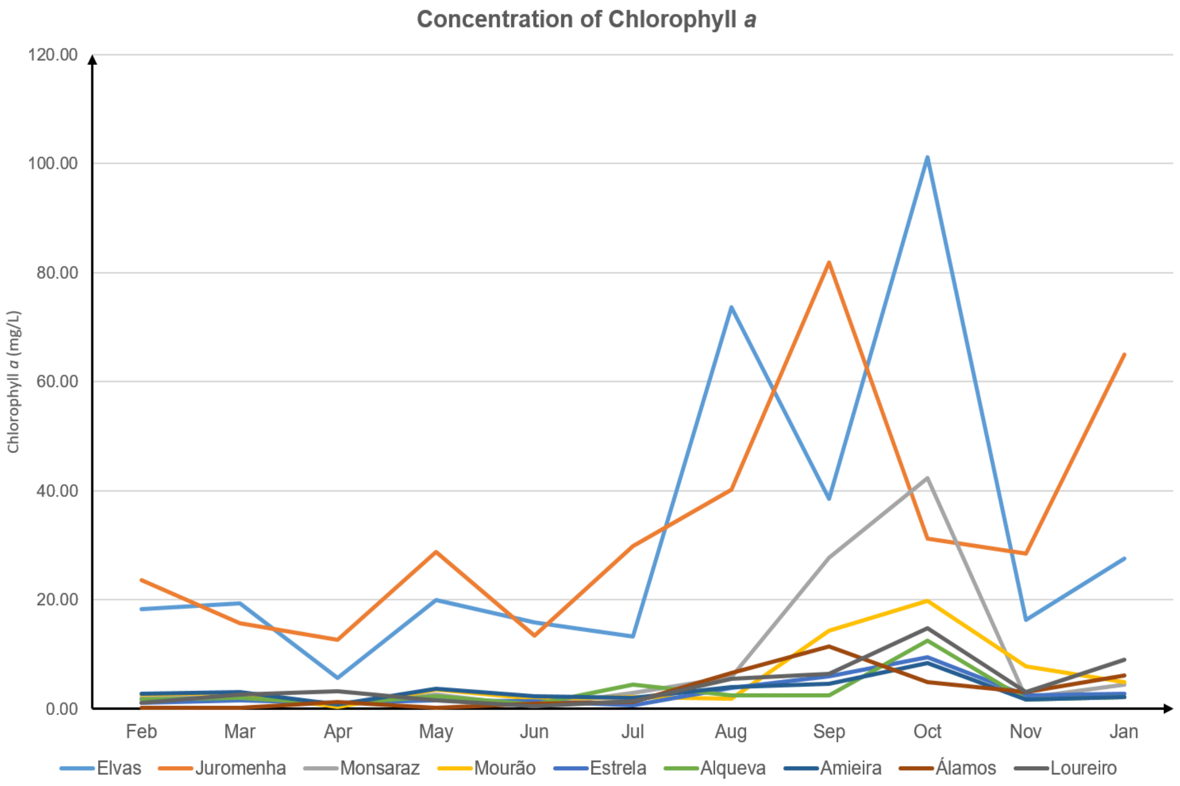
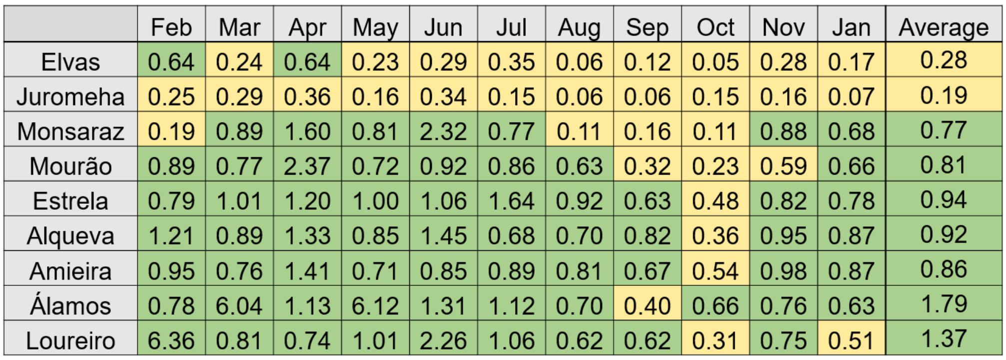


Figure 9 - Variation of chlorophyll a concentration (mg/L) across the year for each sampling site.

Through the methodology described above, the Chl *a* data was converted into two metrics: EQR for the evaluation of the ecological quality of the water (Table 1), and the TSI to determine the system's trophic status (Table 2). As the INAG (2009) states, in order for a southern heavily modified water body to be considered of good quality, its annual average EQR for chlorophyll *a* concentration should be above 0.6. Under these conditions, Elvas (0.42 EQR) and Juromenha (0.06 EQR) are currently in mediocre ecological state, as they do not fulfill the required annual average (Table 1); Elvas, while having an annual average below the required level, did register values above 0.6 in February (0.64), April (0.64) and August (1.60). Juromenha, however, did not register a single EQR above 0.6 (Table 2). All of the other sites besides these two registered a minimum EQR below 0.6, however, their yearly average was above that threshold (Table 1). As such, all sites besides Elvas and Juromenha were of good quality.

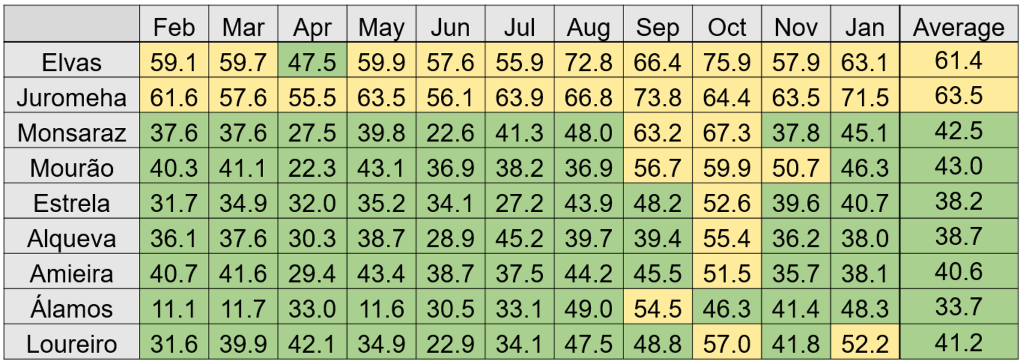
Table 1 - Monthly variation of EQR (for [chl a]) for the nine sampling sites. Green denotes good EQR (>0.6), while yellow indicated mediocre EQR (<0.6).



	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Jan	Average
Elvas	0.64	0.24	0.64	0.23	0.29	0.35	0.06	0.12	0.05	0.28	0.17	0.28
Jurometa	0.25	0.29	0.36	0.16	0.34	0.15	0.06	0.06	0.15	0.16	0.07	0.19
Monsaraz	0.19	0.89	1.60	0.81	2.32	0.77	0.11	0.16	0.11	0.88	0.68	0.77
Mourão	0.89	0.77	2.37	0.72	0.92	0.86	0.63	0.32	0.23	0.59	0.66	0.81
Estrela	0.79	1.01	1.20	1.00	1.06	1.64	0.92	0.63	0.48	0.82	0.78	0.94
Alqueva	1.21	0.89	1.33	0.85	1.45	0.68	0.70	0.82	0.36	0.95	0.87	0.92
Amieira	0.95	0.76	1.41	0.71	0.85	0.89	0.81	0.67	0.54	0.98	0.87	0.86
Álamos	0.78	6.04	1.13	6.12	1.31	1.12	0.70	0.40	0.66	0.76	0.63	1.79
Loureiro	6.36	0.81	0.74	1.01	2.26	1.06	0.62	0.62	0.31	0.75	0.51	1.37

In terms of the Trophic State Index (Table 2), as Carlson (1977) established, a system is oligotrophic if its TSI results are below 40, mesotrophic if between 40 and 50, and eutrophic if above 50. Without exception, all sites passed through periods of eutrophication, as all registered a maximum value above 50. Elvas (August) and Juromenha (September and January) even registered values above 70, which are even above eutrophic, entering into hypertrophy. The two sites farthest away from the dam as such stand out comparatively: Elvas was mesotrophic in April, but eutrophic for the rest of year, having a eutrophic yearly average. Juromenha was eutrophic for the entire year. All the other sites registered a mesotrophic yearly average. As was expected, the higher values for Chl *a* and the lowest values for EQRs were registered in the same months for each locale (Table 2).

Table 2 - Monthly variation of TSI (according to [chl a]) for the nine sampling sites. Green denotes a mesotrophic TSI (<50), while yellow indicates a eutrophic TSI (>50).



	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Jan	Average
Elvas	59.1	59.7	47.5	59.9	57.6	55.9	72.8	66.4	75.9	57.9	63.1	61.4
Juromeha	61.6	57.6	55.5	63.5	56.1	63.9	66.8	73.8	64.4	63.5	71.5	63.5
Monsaraz	37.6	37.6	27.5	39.8	22.6	41.3	48.0	63.2	67.3	37.8	45.1	42.5
Mourão	40.3	41.1	22.3	43.1	36.9	38.2	36.9	56.7	59.9	50.7	46.3	43.0
Estrela	31.7	34.9	32.0	35.2	34.1	27.2	43.9	48.2	52.6	39.6	40.7	38.2
Alqueva	36.1	37.6	30.3	38.7	28.9	45.2	39.7	39.4	55.4	36.2	38.0	38.7
Amieira	40.7	41.6	29.4	43.4	38.7	37.5	44.2	45.5	51.5	35.7	38.1	40.6
Álamos	11.1	11.7	33.0	11.6	30.5	33.1	49.0	54.5	46.3	41.4	48.3	33.7
Loureiro	31.6	39.9	42.1	34.9	22.9	34.1	47.5	48.8	57.0	41.8	52.2	41.2

3.3 BIOLOGICAL ELEMENTS - ZOOPLANKTON

Figure 10 represents the total abundance values of zooplankton for the nine sites across the sampling period. The zooplankton community's abundance was highly variable across the entire year for the different sites (Figure 10). All sites had periods with very low zooplankton abundance (Figure 10). The lowest recorded abundance was for the March sample of Elvas (200 ind/L), with low abundance also being registered for Monsaraz in the same month. Other sites showed an increase of zooplankton abundance in different months. Elvas in June showed a high abundance (55950 ind/L), but had very poor abundance across the year. The sites closest to the Alqueva Dam appeared to showcase similar abundance, with higher volume for September and October (Figure 10). Alqueva registered high abundances at September and October, while Amieira and Álamos showed similar progressions and September as the highest abundance values recorded.

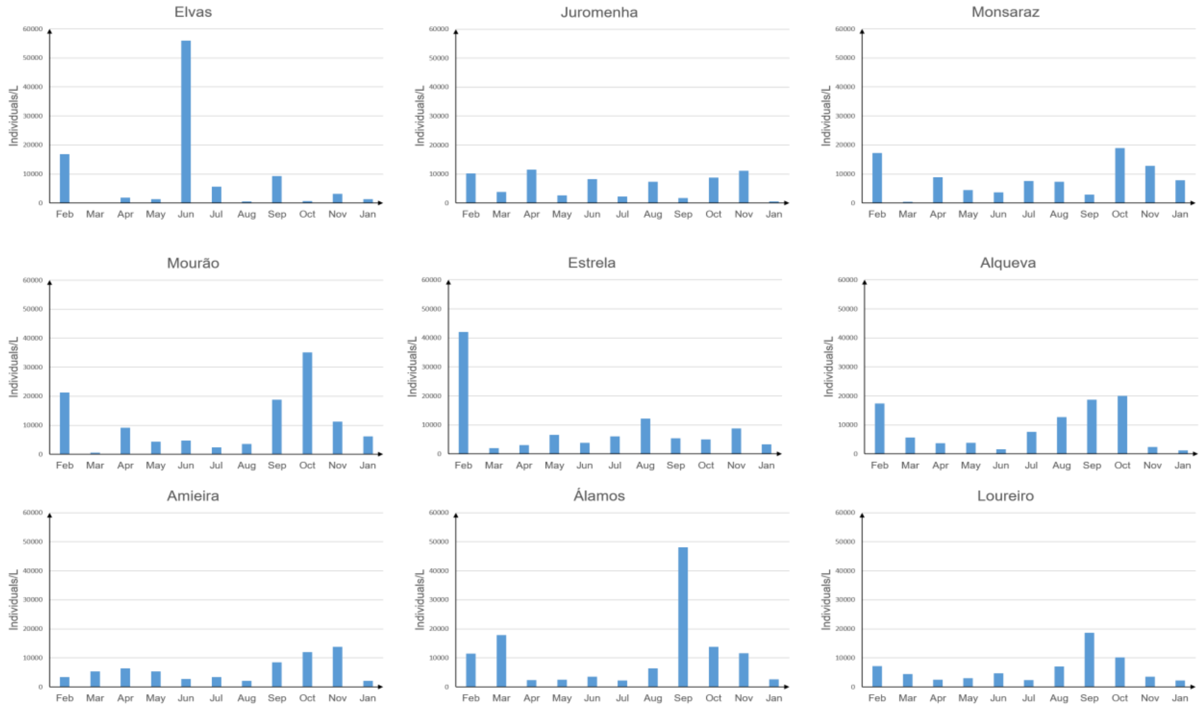

Elvas
Juromenha
Monsaraz
Mourão
Estrela
Alqueva
Amieira
Álamos
Loureiro

Figure 10 - Results for the abundance of zooplankton communities (ind/L) in the nine sites across the sampling period.

Figure 11 displays the relative abundance of each *taxa* across the nine sampling sites. The most common form of zooplankton found across the entire year was Cyclopoida. These copepods were found on all sites, and for every month, often being the dominant species, especially for Elvas and Juromenha sites, with the exception being the month of March (Figure 11). *Daphnia longispina* were also present in all sites, but comparatively less abundant in the sites closest to the dam. *D. longispina*'s abundance was much less pronounced on most sites in the summer months. *Diaphanosoma mongolianum* individuals were present in all sites, however they were a lot less pronounced in Elvas and Juromenha, while being omnipresent in the other sites, particularly from April onwards. *Ceriodaphnia sp.* were present in all sites, but were relatively more abundant in summer for most sites. The presence of *Bosmina longirostris* was a constant during the year, but was only ever dominant in a handful of samples, with Juromenha in particular. The other registered copepod, Calanoida, was present for the summer samples of some sites, sometimes supplanting Cyclopoida as the most numerous (Figure 11). *Sida crystalina* was registered in all sites at one point, but their numbers were much more expressive within the sites closest to the dam, comparatively to the ones more influenced by the river. Moreover, the occasional presence of other species of *Bosmina*, *B. coregoni*, an exotic species, was recorded, not previously encountered for this site. These species were mostly found in Monsaraz (Figure 11). There were also registered occurrences of *Alona sp.* as well as *Chydorus sphaericus*, never the less, they were only ever encountered in small quantities.

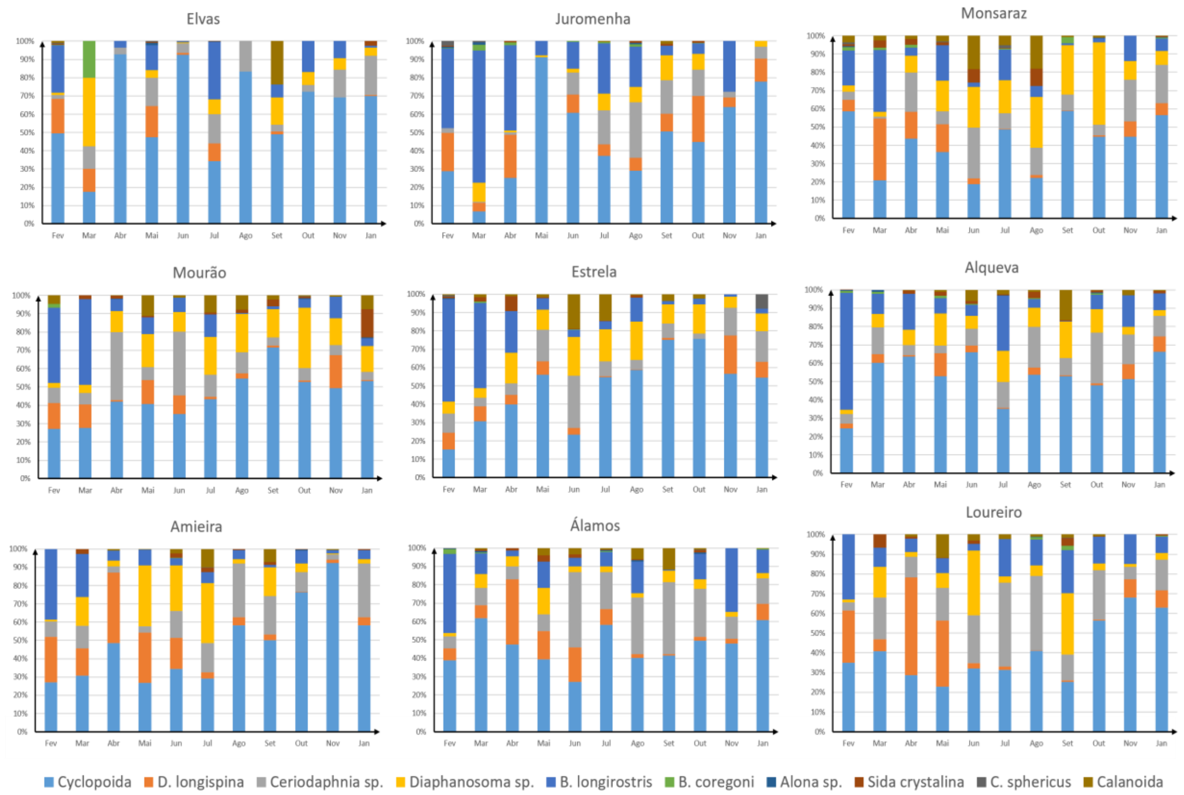


Figure 11 - Results for the relative abundance of the zooplankton taxa across the year for the nine sampling sites.

Figure 12 represents the results for the diversity (Shannon-Weiner) and evenness (Simpson) indexes. The analysis of both indexes present a further complement to what could be observed from the relative abundance of the *taxa* (Figure 11). Overall, Elvas showed the lowest diversity and evenness, however, Juromenha's May sample was the least diverse of all (over 95% of the individuals were Cyclopoida). Most of the sites seemed to maintain a generally similar evenness and diversity across the whole year, however, Elvas stood apart from the others with a high fluctuation during the first half of the year, however, there was also a marked drop for both Juromenha and Amieira in May and November respectively (Figure 12). Monsaraz proved to be the site with the highest values for both indexes, followed by Mourão. Diversity and evenness behaved the same way for all of the samplings.

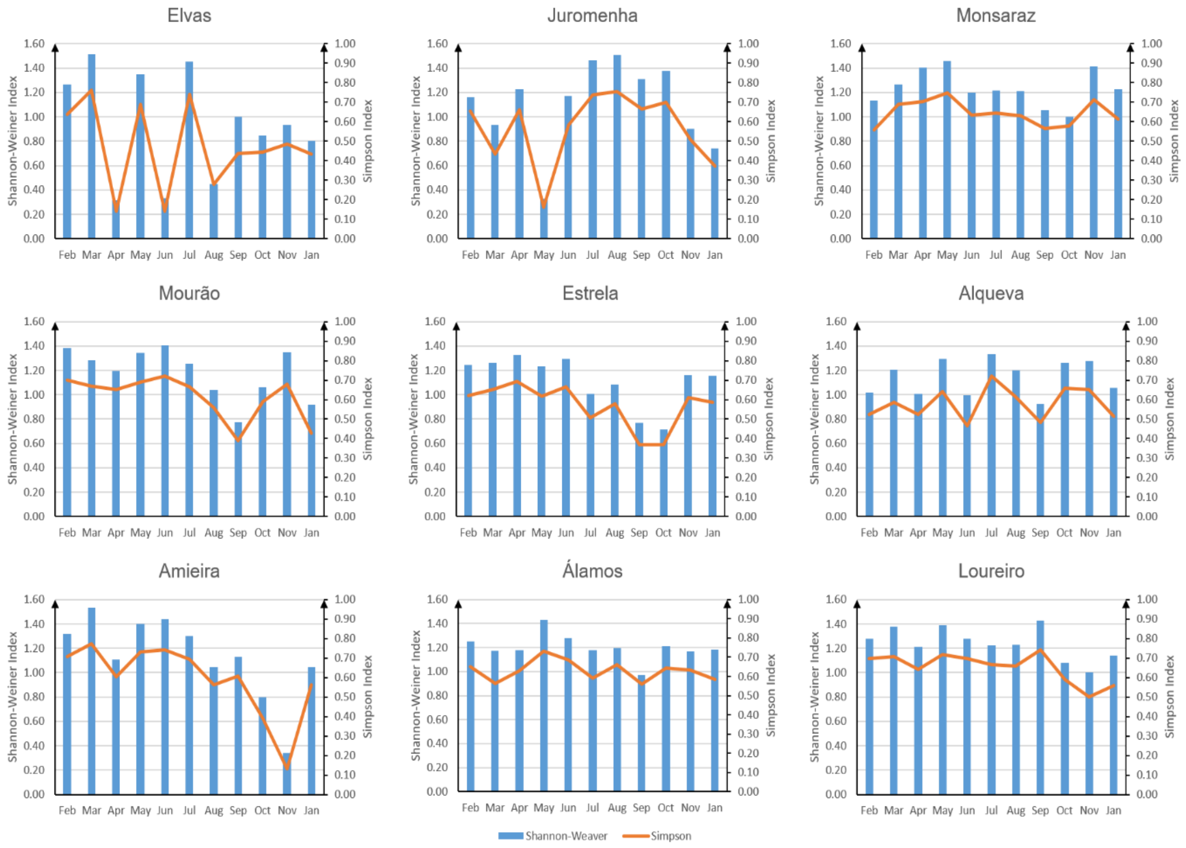


Figure 12 - Results for evenness through Shannon-Weiner index and Simpson index.

For a more compressive understanding of functional dynamics, the observed zooplankters were sorted by their functional feeding groups as suggested by Geller and Müller (1981) (Table 3).

Table 3 - Identified zooplankton taxa sorted by their feeding functional group, according to Geller and Müller (1981)

Functional Group	***Taxa***
Macrofiltrators	Calanoida *Sida crystalina*
Low-Efficiency Bacteria Feeders (LEBF)	*Daphnia longispina* *Bosmina longirostris* *Bosmina coregoni* *Alona sp.*
High-Efficiency Bacteria Feeders (HEBF)	*Ceriodaphnia sp.* *Diaphanosoma mongolianum* *Chydorus sphaericus*
Omnivores	Cyclopoida

Besides being sorted by their functional groups, the zooplanktonic dynamic was also observed under the lens of the ratio of large cladocerans (*Daphnia longispina*, *Sida crystalina* and *Diaphanosoma mongolianum*) and total cladocerans (Figure 13). Regarding the functional groups, Omnivores were often the most abundant group, expected given the dominance of Cyclopoida when looking at taxa relative abundance (Figure 11). Omnivores were not always the most dominant group (e.g.: Juromenha in March), but were always present and always relevant. The Bacteria-Feeders were also present for all the samples, with the Low-Efficiency taxa (LEBF) showcasing generally higher numbers in comparison with their High-Efficiency counterparts, particularly during the colder months (Figure 13). On the other hand, High-Efficiency Bacteria-Feeders (HEBF) organisms were more dominant during summer time, although there were exceptions, such as the sample for Elvas in March, which was shown before to include very low total abundance (Figure 10). Finally, Macrofiltrators were the least represented group, mostly only being represented for the summer months for all sites, with Juromenha in particular boasting almost no Macrofiltrators on its samples. As for the cladoceran succession, three sites evidenced a succession process closer to the normal (large cladocerans becoming less prevalent across the end of spring/start of summer), Amieira, Álamos, and Loureiro, with the decrease in the number of large cladocerans in the summer months. Even then, Álamos appears to be the one in the best condition. Monsaraz had a decrease in the ratio of large cladocerans in July, but rise again in August, and only dropped significantly once more in January. Juromenha had low levels of large cladocerans for the whole year, while Mourão saw the increase across the year. Elvas had an erratic ratio, with rises and drops alternating wildly, not evidencing a real succession. Monsaraz and Estrela presented a steady rise of the ratio from February until October, thus not having a normal succession. Alqueva had two peaks in May and September but dropped between these two months. October, in particular, had very low large cladocerans in proportion to smaller ones for several sites, namely: Juromenha, Estrela and Mourão.

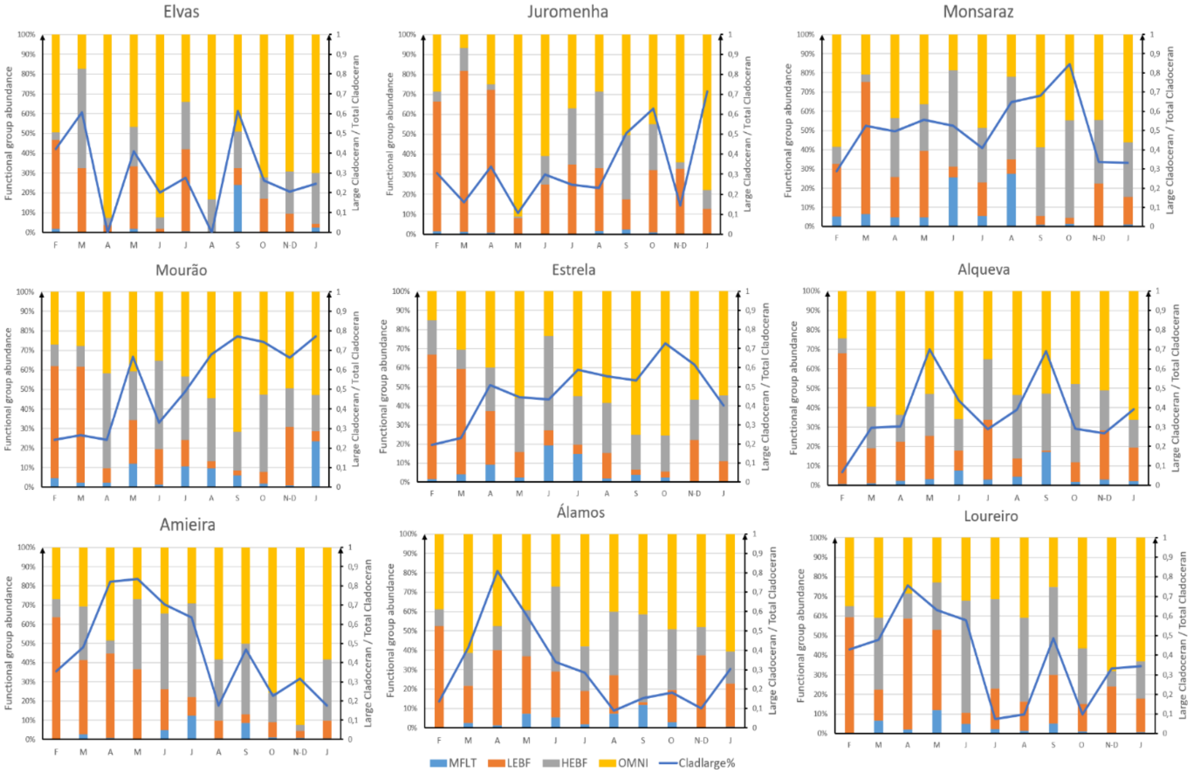


Figure 13 - Results of the relative abundance of the zooplankton species according to functional feeding group (represented by different colours, see table 2), and the ratio of large cladocerans and the total amount of individuals (trend line) across the sampling period.

4. DISCUSSION

The Alqueva reservoir has been the object of several studies across the last few years. Despite the fact that this waterbody has been intensely monitored since its inception, the successive studies done to measure its water quality have always presented it to be under eutrophication and below the standards for the Water Framework Directive (Fontes 2010; Potes et al. 2010; Palma et al. 2014). Several studies already studied the zooplanktonic communities of Alqueva reservoir, showcasing a community that was mostly composed of copepods and cladocerans, with higher zooplankton densities in autumn and less densities during periods of more intense river flow (Chícharo et al. 1998, 2006; Muha et al. 2012). As previously stated, due to zooplankton's characteristics and role within the food chains in these types of ecosystems, the analysis of their dynamics in a given system becomes highly useful for water quality evaluation (Jeppesen et al. 2011).

The WFD proposes for the analysis of highly modified water bodies in the South of Portugal, the usage of several physical and chemical metrics (Instituto da Água 2009). Of the several metrics in study, dissolved oxygen, pH, and total phosphorus include reference values for a system to be of good quality (Instituto da Água 2009). All of the nine sites in analysis were of good quality for the entire year in terms of dissolved oxygen (≥ 5mg/L) (Figure 4) and pH (>6 and <9) (Figure 5). Studies have shown, however, that for eutrophic system's, the level of oxygen in the water is expected to decrease with the rising of the temperatures for the summer (Navarro et al. 2009), something which occurred for all sites (Figure 4). In addition, in terms of total phosphorus the reference value for a good ecological potential (< 0.07 mg/L) was only observed for the entire year in Estrela, Alqueva, Álamos and Loureiro (Figure 8); while the other sites had periods where they were over the limit for good potential (Figure 8). Palma et al. (2009) have shown a link between low rainfall and lower amounts of phosphorus. The weather for the region of the study is characteristically very dry, with 2017 being the driest year on record for the months of September (IPMA 2017a) and October (IPMA 2017b) in the last 87 years, which may explain the very low amount of phosphorus attained from the samples. By only taking into account these above referred parameters: Elvas, Juromenha, Monsaraz, Mourão and Amieira are all below the good ecological potential threshold. However, Elvas and Juromenha are in worse condition than the other three. In terms of good ecological potential, it was present only for Estrela, Alqueva, Álamos and Loureiro.

The WFD also references the use of other metrics to assess the quality of a water body, albeit without reference values. Among these: temperature, conductivity, transparency, turbidity, calcium and oxidation-reduction potential (Instituto da Água 2009). As previously discussed, analysis of these parameters points to the existence of seasonality within the sites, particularly for temperature, which is shown to be lower between November and March and higher between May and October (Figure 4). For the months of September (IPMA 2017a) and October (IPMA 2017b), Portugal has registered rising temperatures for both months. This may explain why, contrary to the expected seasonality, the water temperature for the Alqueva only registered a significant drop in November (Figure 4) instead of the expected more gradual drop starting in late September (IPMA 2017a). Observing the results for conductivity (Figure 5), transparency and turbidity (Figure 6) as well as CDOC (Figure 7) it is possible to observe how Elvas and Juromenha stand apart from the other seven sapling sites. In particular, these sites had lower transparency and higher turbidity (Figure 6). The Elvas site is located within the Guadiana River, while Juromenha is in close proximity to it, as such, these two sites are strongly affected by alterations caused by the river flow. Elvas and Juromenha are more turbulent comparatively to the sites in the inner reservoir. Elvas and Juromenha also registered higher monthly and yearly concentrations of CDOC (Figure 7) and calcium (Figure 8), meaning that these sites had both a higher amount of dissolved carbon in its water, as well as being harder. Finally, for all sites, oxidation-reduction potential (ORP) values recorded were always negative, something indicative of a system that tends to perform reduction over oxidation (Søndergaard 2009). A negative ORP and a tendency for reduction has been cited as an indicator of a system that is not only highly stratified, but also under eutrophication process (Søndergaard 2009).

When taking into account the biological WFD parameter analysed in this work, the concentration of Chl *a,* the above cited relationship between ORP and eutrophication for this system becomes less apparent. Through the calculation of Chl *a* TSI (Table 2), it is possible to observe that all sites had periods where they were mesotrophic, with one exception (Juromenha). The higher values for TSI for the sites that were not Elvas or Juromenha, were registered in September or October (Table 2). Aside from TSI, with the attained values for Chl *a* it is possible to calculate the Ecological Quality Ratio (EQR) of this biological parameter for each site. For this metric, seven out of the nine sites presented a good ecological potential. The two exceptions were Juromenha and Elvas. The EQR and TSI, as such, confirm what had been previously evidenced in the physical and chemical parameters. Elvas and Juromenha have mediocre potential status, while the other sites had generally good water quality. A study conducted by Palma et. al (2010) reached conclusions that coincide with this data, as it found that the Alqueva reservoir's water was in worse condition on the northern part of the reservoir. This study linked this occurrence to the toxicity from pesticides utilized in the farmlands surrounding the Guadiana river, as well as municipal waste (Palma et al. 2010). Several other studies have demonstrated a link between the water quality and the land use around it for agricultural fields and compounds (Yong and Chen 2002; Navarro et al. 2009; Silva et al. 2012). Aside from agricultural pressures, Juromenha is also under other forms of anthropogenic impact, namely tourism, which also occurs in Monsaraz, Mourão, Alqueva and Amieira. Tourism has been known to lead to a marked degradation of water courses (Garcia and Servera 2003). This factor is especially dire for Juromenha, as the marina on the Spanish coast has an accentuated impact on the area. Also of note is that, at the time of the construction of the Alqueva dam, this site was flooded, contributing to further degradation of an already polluted area (Melo and Janeiro 2005).

While it is possible to ascertain the quality of a water body with only the above cited metrics, it should be noted that an analysis of the zooplankton communities offers a wealth of new information that contributes to a better understanding and a more precise classification of the workings of an ecosystem (Caroni and Irvine 2010; Jeppesen et al. 2011). Looking into the attained samples, it is important to note the constitution of the communities, and their differences between sites and during the year (Figure 10, Figure 11). Elvas and Juromenha featured a much less diverse constitution community, having cyclopoids being the most numerous and most expressed taxa across the entire year (Figure 11). Cyclopoida tend to be particularly dominant in water bodies characterized by high instability (Geraldes and Boavida 2007), something that is in accordance with the mediocre quality indicated by the WFD-approved parameters for these two sites. Being omnivores, Cyclopoida also have a much more expansive niche than a lot of the other *taxa*, being as such, ubiquitous (Gliwicz 1977). Several studies have already shown that in eutrophic temperate lakes, there tends to be an increase in the numbers of Cyclopoida copepods (Betsill and van den Avyle 1994; Geraldes and Boavida 2007; Caroni and Irvine 2010). It also goes in accordance with the more intense pressure from the river flows in these sites, in comparison with the others (Chícharo et al. 2006). Cladoceran species of the genus *Bosmina* are also prevalent in eutrophic temperate lakes (Betsill and van den Avyle 1994), fact observed in the two studied sites under heavier eutrophication process (Elvas and Juromenha), but also in greater numbers in all sites during February. Of note for Bosmina is the presence of two species for this genus, the native and much more prevalent *B. longirostris*, but also the exotic *B. coregoni*, that was not recorded to have occurred in this reservoir in previous analyses (Muha et al. 2012). *Daphnia* spp. and *Diaphanosoma* spp. are also expected to appear in eutrophic temperate lakes, albeit in comparatively lower numbers to *Bosmina* spp. (Betsill and van den Avyle 1994). The remaining sites (Monsaraz, Mourão, Estrela, Alqueva, Amieira, Álamos and Loureiro), presented higher diversity and evenness ratios of zooplankton, with a higher number of cladocerans (Figure 12). These spatial differences can be explained by the different abiotic conditions recorded between sites, as variations in water level are known to affect the constitution and dynamics of the zooplankton assembly (Geraldes and Boavida 2007). A study by Abrantes et al. (2006) refers that the zooplankton community and the succession can be affected by high Mediterranean temperatures, leading to enhanced growth of Cyanobacteria in Spring and Summer. This same study claims that the constitution of the phytoplankton community can impact the succession of cladocerans, which could be a potential reason for this process not occurring as expected in all sites with the exception of Amieira, Álamos and Loureiro.

Comparing the results of the TSI to the ones attained by analysis of the zooplankton communities is a key step in understanding fully how the system ticks and evolves, as these communities are shaped by the level of nutrients and the system's trophic status (Geller and Müller 1981; Jeppesen et al. 2011). According to Geller and Müller's research (see adaptation for our results – Figure 2), for temperate lakes, mesotrophic systems are expected to be dominated mainly by Low-Efficiency Bacterial Feeders (LEBF) for most of the year, with macrofiltrators becoming more prevalent in the winter months. Eutrophic lakes, however, are expected to have HEBF more dominate in summer, with Macrofiltrators in winter, and LEBF the rest of the year. While all sites, with the exception of Elvas and Juromenha did feature a large amount of LEBF, the macrofiltrators, by far the least populous group, only surfaced in summer. The macrofiltrators attained in these samplings were Calanoida and *Sida crystalina*, which are known to be particularly sensitive to eutrophication, due to their filtration apparatus (Pinto-Coelho et al. 2005) which would explain why they were almost non-existent for Elvas and Juromenha. For all sites, the most dominant of the feeding groups during summer, excluding Omnivores, were the High-Efficiency Bacteria Feeders (HEBF) which encapsulate the taxa *Ceriodaphnia sp., Chydorus sp.* (smaller cladocerans) and *Diaphanosoma sp.* (larger cladocerans). The functional dynamics observed for all sites, points then to a distribution closest to the one Geller and Müller predicted to appear in eutrophic lakes, as opposed to a mesotrophic one (Figure 2). Geller and Müller (1981) postulated however, that the composition of filtering forms of zooplankton can be changed depending on the amount of bacteria present. Zooplankton communities can also have their structure changed depending on predation pressure as well as by the inhibition of filter-feeders by filamentous inedible algae (Gliwicz 1977). An influx of more cyanobacteria and the eutrophication of the system can also present a potential explanation for data that is non-conforming (Ger et al. 2014). A study by Ger et al. (2014) proposes that zooplankton grazing is affected by an affluence of Cyanobacteria, as they are known to be poor quality food, or even inedible for certain *taxa* of zooplankton. This status derives from the following three attributes: cyanobacteria are often organized in large colonies or filaments, thus becoming harder to predate and filter, clogging the filtering apparatus of certain larger species like *Daphnia* sp. (DeMott et al. 2001); cyanobacteria are lacking in long-chained polyunsaturated fatty acids (PUFAs) and sterols, these forms of phytoplankton impact zooplankton fitness, by not possessing these composites, key in regulating cell function in the zooplankters (Gulati and DeMott 1997); and finally, some of these organism are capable of producing toxic metabolites (neurotoxic or hepatotoxic) that are known to produce either sub-lethal, or even lethal effects on zooplankton (Leflaive and Ten-Hage 2007). As cyanobacteria are known to be numerous in eutrophic, and hypertrophic systems, it's a strong possibility that their presence may have impacted negatively the zooplankton communities of the Alqueva (DeMott et al. 2001; Potes et al. 2010), and could provide another potential explanation for the changes in the structure of the communities, and the lack of a real cladoceran succession for most sites. In terms of predation by fishes, studies conducted on the ichthyofauna of the reservoir also make note of the existence of several exotic fish species (EDIA 2019). These exotic fishes are shown to be active during the entirety of the year, especially in the northern part of the reservoir (Banha et al. 2017; EDIA 2019). These species may have had an impact in the zooplankton communities (Chícharo et al. 2006), as the sites closest to the top of the reservoir and the river, exhibited less diverse and less abundant samples (Figures 10, 11 and 12).

It is possible to determine in more detail the differences between the water quality of the sites when adding the data from the zooplankton to the WFD parameters: purely by the metrics established by the WFD, the northern part of the reservoir (Elvas and Juromenha) was of mediocre quality, but the remaining sites are of generally good quality. When adding the zooplankton analysis, it is clearer that the reservoir as a whole is in worse condition than those parameters indicate. The zooplankton communities are shown to be in accordance with studies for stratified and eutrophic lakes and reservoirs (Geller and Müller 1981; Morgado et al. 2006). The reservoir lacks a clear succession of cladocerans (besides the Amieira, Álamos and Loureiro) and is dominated by taxa such as Cyclopoida and *Bosmina longirostris*.

It should be noted once again that the constitution of the zooplankton communities tend to vary seasonally, and it is even possible, like in September and October of this study, that changes to the normal progression of the climate can impact the zooplankton (Wetzel and Boavida 1993). As such, it is important that an analysis of the zooplankton is to be made across a full year, and should not be contained to a single sampling-season. The monthly samplings of zooplankton would undoubtedly present a much higher financial cost to the operation, something that Caroni and Irvine (2010) debated as being a potential reason for their omission from the WFD. However, only with the full extent of knowledge provided from a complete yearly analysis of the zooplankton communities is it possible to ascertain the real ecological state of the ecosystem. Indeed, the information attained from these strategies for samplings would justify the financial costs of the operation (Jeppesen et al. 2011).

Overall, the proposed objectives for the present study were achieved: the seasonal and spatial distribution of the zooplankton were documented, and interpreted, allowing the case to be made for the inclusion of the characterization of their dynamics into the WFD approved parameters. The site within the river and the one closest to it, the two furthest from the dam (Elvas and Juromenha, respectively), have mediocre potential for their ecological quality. Moreover the remaining sites, while being of generally good water quality for most WFD parameters, were shown to have zooplankton communities characteristic of a eutrophic, and highly stratified ecosystem. When taking both WFD metrics and zooplankton into account: Only Álamos and Loureiro, the two sites from adjacent reservoirs, are shown to be closest to good quality. While the WFD parameters showcased a general consistency for all sites (excluding the outliers Elvas and Juromenha), the zooplankton analysis revealed a much more diverse constitution, making spatial differences within the reservoir more noticeable. In conclusion, the zooplankton analysis allowed for a stronger understanding of the ecological state of the water of Alqueva reservoir that was not possible with just the WFD mandated parameters.

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