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The phytoplankton-zooplankton nexus and bioindicator value in reservoirs:
a case study in meso-oligotrophic systems in NW Portugal



Universidade do Algarve

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a case study in meso-oligotrophic systems in NW Portugal

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Resumo

A água doce é um recurso valioso e finito, crucial para a biodiversidade e a saúde humana, além de ser fundamental para muitas atividades humanas, como agricultura e indústria. No entanto, as atividades humanas representam uma grande pressão sobre os corpos de água e são uma importante fonte de degradação da qualidade da água em diversos rios, lagos e albufeiras em todo o mundo. A poluição da água e o uso imprudente dos recursos de água doce ameaçam os ecossistemas aquáticos em todo o mundo e podem levar à perda de biodiversidade.

As albufeiras são ecossistemas artificiais que são criados após a construção de uma barragem, semelhantes a lagos naturais, que têm um grande impacto no ambiente e na saúde humana, resultando numa diminuição geral na qualidade da água a montante e a jusante da barragem. Estes impactos podem variar dependendo da localização e da gestão da barragem. Transformar um rio numa albufeira é um procedimento drástico que pode levar à formação de um ecossistema totalmente novo. A construção de uma barragem afeta a comunidade aquática e influencia a biodiversidade do sistema artificial. As albufeiras são mais vulneráveis à eutrofização em comparação com os rios, porque não têm a capacidade de autorrenovação que estes possuem.

Para avaliar a qualidade da água em albufeiras, vários descritores físicos, químicos e biológicos são utilizados. A Diretiva Quadro da Água (DQA) é uma legislação-chave da União Europeia que aborda a gestão dos recursos hídricos e exige que os Estados-Membros protejam e restaurem todas as águas interiores superficiais, transicionais, costeiras e subterrâneas para alcançar o "Bom Estado" até 2021 ou 2027. A avaliação do estado da água conforme a DQA é baseada em critérios químicos, físicos, hidromorfológicos e biológicos. Para as albufeiras, as comunidades de fitoplâncton têm sido consideradas os indicadores biológicos mais robustos e são frequentemente utilizadas para avaliar o estado da água. No entanto, alguns cientistas sugerem que o zooplâncton também deve ser considerado na avaliação destas águas fortemente modificadas, pois são importantes reguladores do fitoplâncton e desempenham um papel chave na dinâmica do ecossistema aquático. Considerar as comunidades de zooplâncton como um parâmetro de suporte ou interpretativo, em complemento às comunidades de fitoplâncton, pode fornecer uma visão mais integrada do estado do ecossistema aquático em albufeiras e melhorar a eficácia da sua administração.

Assim, o objetivo deste trabalho foi avaliar a variação sazonal e espacial da comunidade fitoplanctônica em quatro albufeiras da região do Minho em Portugal (Andorinhas, Caniçada, Touvedo e Venda Nova), bem como o seu valor bioindicador, associando-o à comunidade de zooplâncton residente. Ao relacionar os dois níveis tróficos, esperamos obter mais informações sobre a saúde ambiental destes sistemas, cuja produtividade é limitada por baixos níveis de fósforo (que limita a produtividade primária) e cálcio (que limita a herbivoria de grandes crustáceos zooplanctônicos).

As albufeiras escolhidas para este estudo estão sujeitas a distúrbios antropogênicos muito baixos (além de sua artificialidade), estando inseridas numa zona florestal. Além disso, são sistemas caracterizados por baixa mineralização da água, sendo considerados oligo-mesotróficos de acordo com a OCDE (1982). Em cada albufeira, foram escolhidos de dois a três pontos de amostragem ao longo da sua extensão. O período de amostragem foi realizado entre novembro de 2021 e julho de 2022, usando uma embarcação sem motor para aceder as áreas centrais das albufeiras. Foram medidos vários parâmetros físicos e químicos *in situ*, como temperatura, pH, condutividade, oxigênio dissolvido e transparência da água. Além disso, foram colhidas amostras de água para posterior análise laboratorial da biomassa fitoplanctônica (clorofila *a*), fósforo total, turbidez e sólidos suspensos. Para a comunidade de zooplâncton, as amostras foram recolhidas com uma rede de plâncton, e preservadas em álcool antes da identificação e enumeração dos organismos. As comunidades de fitoplâncton foram recolhidas na subsuperfície (amostra de água) e identificadas por meio de microscopia invertida. Diversos índices foram calculados para avaliar a diversidade e a composição de ambas as comunidades, complementados com técnicas de análise multivariável como Análise de Componentes Principais (PCA), Análise de Coordenadas Principais (PCoA) e Análise de Redundância Baseada em Distância (db-RDA) para relacionar os dados ambientais com as comunidades. As análises estatísticas foram realizadas no software R, e diversos pacotes estatísticos foram utilizados para a análise dos dados.

Os resultados mostram que a qualidade da água nas albufeiras escolhidas foi considerada boa ao longo do estudo. A análise dos parâmetros físico-químicos e biológicos indicou flutuações sazonais e variações espaciais na qualidade da água. A profundidade das albufeiras mostrou ter influência na composição de ambas as comunidades, com reservatórios menos profundos a apresentar maior ressuspensão de nutrientes. Quer o zooplâncton quer o fitoplâncton responderam

às mudanças ambientais sazonais, e ambos revelaram diferenças claras na composição das comunidades entre as albufeiras amostradas; em contraste, as diferenças espaciais intra-albufeira foram praticamente imperceptíveis. Regra geral, as diferenças nas comunidades zooplanctónicas revelaram uma associação mais forte aos gradientes ambientais (profundidade e produtividade) do que as comunidades fitoplanctónicas. As comunidades de zooplâncton nas albufeiras variam sazonalmente, com diferentes espécies predominantes em diferentes épocas do ano. O aumento da temperatura no verão pode favorecer zooplâncton menor, como *Bosmina* spp. e *Ceriodaphnia* sp., que não conseguem controlar efetivamente a biomassa do fitoplâncton, resultando em águas mais turvas. Além disso, a presença de peixes planctívoros pode ter tido um impacto significativo nas populações de zooplâncton, eliminando filtradores de grandes dimensões, como *Daphnia* spp., sendo a comunidade substituída por zooplantontes de pequenas dimensões, como, por exemplo, *Bosmina*, com menos capacidade de regulação do fitoplâncton. Quanto ao fitoplâncton, a comunidade foi influenciada pelo nível de nutrientes e profundidade da albufeira. Diatomáceas foram predominantes em albufeiras menos profundas, enquanto Chlorococcales coloniais dominaram albufeiras mais profundas. A presença de dinoflagelados também foi observada, principalmente no verão em todas as albufeiras. No geral de ambas as comunidades, as albufeiras Caniçada e Venda Nova foram muito próximas, quase indistinguíveis, mas claramente separadas das Andorinhas (a albufeira mais pequena e menos profunda), enquanto Touvedo ocupava uma posição intermédia entre estes dois extremos, embora tendencialmente mais próximo das Andorinhas.

Em conclusão, este estudo destaca a importância de considerar tanto o zooplâncton quanto o fitoplâncton como bioindicadores complementares para avaliar a saúde geral do ecossistema aquático. A análise integrada destas comunidades pode fornecer uma compreensão mais abrangente e representativa do estado real dos corpos de água e dos fatores ambientais que influenciam o seu desenvolvimento. Além disso, a monitorização do zooplâncton pode fornecer informações valiosas sobre o controle trófico no ecossistema e no controle da transparência da água. Com base nesses resultados, é recomendável a inclusão do zooplâncton como um dos indicadores biológicos na avaliação do status ecológico da água, a fim de melhorar os esforços de conservação e monitorização da qualidade da água.

Abstract

Water pollution and uncontrolled use of freshwater resources threaten aquatic ecosystems worldwide. The European Water Framework Directive (WFD) was developed to protect and improve the quality of European waters, but its implementation revealed numerous problems, such as the artificiality of used metrics, the absence of pristine conditions to be used as reference, and the lack of robust biological descriptors (often limited to phytoplankton). The aim of this work was to evaluate the seasonal and spatial variation of the phytoplankton community in four reservoirs of Minho region in Portugal (Andorinhas, Caniçada, Touvedo and Venda Nova), as well as its bioindicator value, linking it to the resident zooplankton community. By relating the two trophic levels, we expected to get further insight on the environmental health of these systems, whose productivity is constrained by low levels of phosphorus (which limits primary productivity) and calcium (which limits herbivory by large crustacean zooplankters). Results showed variation between reservoirs, with a particular contrast between deep and shallow systems, along with some degree of seasonal fluctuations. All reservoirs have been classified as having good ecological potential based on the physical and chemical information collected. However, the comparison of these with the dynamics seen in zooplankton communities allowed us to conclude that this biological component is more susceptible to subtle changes in the ecosystem when compared to the phytoplankton community. As a result, our work shows that the use of more than one biological descriptor gives a better understanding of the ecological potential of these semi-artificial systems, by elucidating abiotic and biotic regulation mechanisms.

Keywords: Water pollution, aquatic ecosystems, European Water Framework, reservoir, phytoplankton, zooplankton

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LIST OF ABBREVIATIONS

P	Phosphorus
N	Nitrogen
TSI	Trophic State Index
DO	Dissolved oxygen,
WFD	Water Framework Directive
IGA	Algae Group Index
SS	Suspended solids
TP	Total phosphorus
Chl <i>a</i>	Chlorophyll <i>a</i>
H'	Shannon's index
J	Pielou index
NMARSP	New Mediterranean Phytoplankton Assessment Index
EQR	Ecological quality ratio

1. Introduction

1.1 Human pressures in freshwater ecosystems

Freshwater is a valuable and finite resource, crucial for biodiversity and human health, as well as many human activities, such as agriculture and industry. On the other hand, human activities represent a large pressure on water bodies and are considered an important source of degradation of the water quality in various rivers, lakes and reservoirs all over the globe (Katsiapi et al., 2011). Water pollution and reckless use of freshwater resources threaten aquatic ecosystems worldwide and can lead to biodiversity loss (Bartram & Pedley, 1996). Between 1970 and 2016, the Living Planet index has estimated around 84% decline in freshwater species abundance, which demonstrates the vulnerability of freshwater ecosystems (Schmutz & Sendzimir, 2018; WWF, 2020).

Nowadays, one of the threats that degrades water quality of lakes and reservoirs globally is eutrophication, a consequence of organic pollution from numerous sources, like domestic sewage, agricultural run-off, and agro-industrial effluents (Katsiapi et al., 2012) with loads of nutrients such as nitrogen and phosphorus that enhance algal growth (algal blooms) along with favourable conditions like high temperature, high pH and stable water column (Padedda et al., 2017). Algal blooms have the potential to cause significant changes in aquatic ecosystems, especially given that they are mostly made up of potentially toxic cyanobacteria. Cyanobacteria blooms can also lead to a rise in water turbidity and pH (Visser et al., 2016) as well as the production of taste and odour compounds, which decreases ecosystem services like recreational value and water potability (INAG 2009). Thus, studying the ecology of planktonic microalgae is important for a proper management and monitorization of freshwater resources (Padedda et al., 2017).

Another global issue for water resource management is water scarcity (Carpenter et al., 2011). In recent decades, temperature and rainfall patterns have changed substantially, and predictions show a bigger change in the future due to climate change (Benestad et al., 2022). Regions like the Mediterranean are predicted to be considerably affected by global warming (IPCC, 2014). A decrease between 25-30% in precipitation and enhanced evaporation are foreseen to occur in the Mediterranean region by the end of the twenty-first century (Erol & Randhir, 2012). This type of change can represent a risk for the function and biodiversity of freshwater systems, causing

river intermittency, prolonged drought periods, or even make some water bodies disappear (Magand et al., 2020; Skoulikidis et al., 2011). Allowing that change with the constant demand for water quantity and quality, it is urgent that the management and monitoring of freshwater are rigorously assessed, providing a better and more responsible approach to its usage (Katsiapi et al., 2011). Water storage in reservoirs and ponds is one of the most frequent approaches to secure a safe water supply (Carpenter et al., 2011). However, monitoring and forecasting tools are needed to prevent water quality degradation when stored in such artificial or semi-artificial systems.

1.2 Reservoirs as artificial ecosystems

Reservoirs are heavily modified lentic water bodies that are created after the construction of a dam, becoming an artificial ecosystem comparable to natural lakes (Almeida et al., 2020). Dams are created to respond to various society needs, such as electricity from hydropower generation, water storage for drinking, industrial uses, irrigation, river regulation and flood control (Schmutz & Sendzimir, 2018). Due to climate change challenges and the consequent necessity to reduce the use of carbon as an energy base (IPCC, 2014), fossil fuels are being replaced with renewable energy technologies, to walk towards a low-carbon future. One of those renewable energy sources is hydropower, which might represent a bigger part in electricity generation in the future. According to the Renewables 2021 global status report, global hydropower had a 24% increase in 2020, essentially by China, representing 16.8% of the world's total electricity generation. In Europe, 41.7% of renewable electricity is from hydropower, making a total of 11.4% of total electricity generation in Europe (IEA, 2021).

Dams, however, can be the origin of many negative impacts on environmental and human health, resulting in an overall decrease in water quality upstream and downstream (Schmutz & Moog, 2018). The impacts of dams and reservoirs can vary depending on the location, and even the design and the way a dam is managed has influence (McCartney, 2009). Despite this context-dependence, the profound landscape change affects the aquatic community and influences the biodiversity of the artificial system. Transforming a river into a reservoir is a drastic procedure that can represent the formation of an entirely new ecosystem. Consequently, the manifestation of any environmental impacts is often related to the change of the hydrology and morphology of the river (Schmutz & Moog, 2018) that may result in effects such as fragmentation, flow alteration,

increased evaporative loss, decreased water quality, high sediment load, biomass reduction and enhanced hydraulic residence times (Carpenter et al., 2011). According to changes in dynamical, physical, chemical and biological properties, reservoirs can be separated longitudinally into three distinct zones (Figure 1.1): riverine, transitional, and lacustrine (Wetzel, 2001). With a narrow and shallow geomorphology typical of rivers, the riverine zone is the part of the system that is closest to the origin. It is powerful enough to carry the smaller sediments and organic particles carried by the river, despite the river flow's velocity starting to slow down at this zone. The velocity of the river drops near the transition zone, allowing for sedimentation. This circumstance will improve light penetration in water, which will increase primary production. The properties of the lacustrine zone are comparable to those of a lake system. Higher rates of primary production in the water column (pelagic zone) are possible due to low sedimentation and increased light penetration (Thornton et al., 1990).

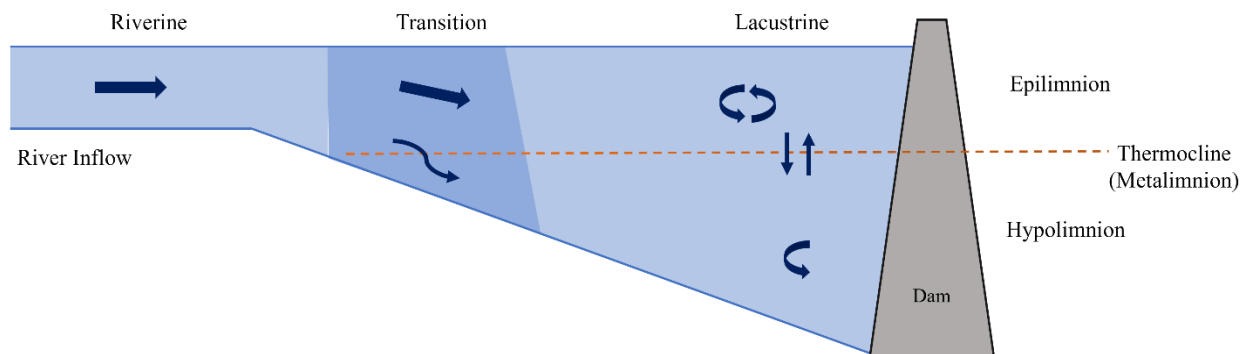


Figure 1.1 - Longitudinal and vertical zonation in reservoirs (adapted from Thornton et al., 1990; Ruhl et al., 2014; Almeida, 2015)

Vertical stratification, which results from variations in temperature and light intensity from the surface to the bottom, can also happen in deeper reservoirs. A thermocline, or abrupt change in temperature affecting the water density, can form a few meters below the surface and divide the water column into layers with different physical, chemical and biological properties, in addition to temperature. The epilimnion, a layer closer to the surface and above the thermocline, is characterized by a greater temperature, a higher concentration of dissolved oxygen, and a higher light intensity. The warming and cooling of the surface and the wind ensure that the water in the

epilimnion is thoroughly mixed. The hypolimnion, the layer below the thermocline, is deeper and has a reduced oxygen concentration as well as nearly no light penetration. Only the water cooling and dam discharges can be the cause for the minimal mixing of the water in this layer. Due to changes in temperature, there can occasionally be some mixing of the waters from the epilimnion and hypolimnion (Farley, 2012). Seasonally, de-stratification occurs (also known as lake turnover), releasing organic and inorganic nutrients that were trapped in the hypolimnion; in some systems, this can have drastic consequences in the water quality (e.g., decreased oxygen content and transparency, increased nutrient levels and turbidity) with concomitant impacts on the dynamics of local biotic communities (Crockford et al., 2015).

Another important factor in reservoirs is the hydraulic residence time, which is associated with the period water is retained in the reservoir; the water is kept there for longer periods of time, which results in increased nutrient content and decreased oxygenation, and this can promote eutrophication. When compared to rivers, reservoirs are more vulnerable to eutrophication, because reservoirs lack the capacity to self-purification (water renewal) that running waters have (Navarro et al., 2009; Wetzel, 2001). Phosphorus (P) and nitrogen (N) tend to accumulate in reservoirs due to increased water retention, which is worsened by the surrounding land usage and human activities that are source of organic or inorganic P and N forms (agriculture, livestock production, urban and industrial sewage, etc.).

The transition of biomass production from the benthic compartment (riverbed) to the pelagic compartment (water column), that occurs when a dam is built, is another significant ecological change. In contrast to rivers, where decomposing or photosynthetic microbial biofilms cover the riverbed, plankton, which are microscopic organisms that drift in the water column, become the foundation of the food web in reservoirs. Key processes that are closely related to plankton dynamics, and in particular with the interaction between zooplankton, phytoplankton, and fish, include the regulation of water transparency and top-down and bottom-up mechanisms in the food web (Machado, 2022).

The Trophic State Index (TSI) is a common tool for classifying water bodies (Carlson, 1977). Based on nutrient enrichment and its consequences on water quality, this index divides the water bodies into four categories: oligotrophic, mesotrophic, eutrophic, and hypertrophic. Oligotrophic features include clear waters, a high level of dissolved oxygen in the hypolimnion, high

transparency and light penetration, a reduced concentration of potentially limiting nutrients and low primary productivity. Mesotrophic conditions provide relatively clear waterways, moderate nutrient supply, and medium primary productivity. Eutrophic conditions include an abundance of nutrients, high primary productivity, poor transparency and light penetration, and a low concentration of dissolved oxygen in the hypolimnion. Hypertrophic conditions include an exceptionally high nutrient input, very eutrophic waters, and low transparency. According to Carlson (1977), TSI can be determined using various standard measures to assess water quality, such as water transparency, using data gathered using Secchi disk, content of chlorophyll *a*, and total phosphorus concentration (Table 1.1).

Table 1.1 - Trophic State Index classification (adapted from Carlson & Simpson, 1996)

Trophic State	Transparency (Secchi disk – m)	Concentration of Chl <i>a</i> (µg/L)	Total phosphorus (µg/L)
Oligotrophic	>8	<0.95	<6
Mesotrophic	2-4	2.6-7.3	12-24
Eutrophic	1-2	7.3-20	24-48
Hypertrophic	<1	56-155	96-192

see also: <https://www.nalms.org/secchidipin/monitoring-methods/trophic-state-equations/>

1.3 Plankton in freshwaters and associated trophic interactions

Phytoplankton are photoautotrophic microorganisms that act as primary producers in any aquatic ecosystem, be they prokaryotes, such as cyanobacteria, or eukaryotes like diatoms or green algae (Singh & Ahluwalia, 2013). The productivity of an aquatic ecosystem is related to phytoplankton density (Naselli-Flores, 2000), as they transfer energy to consumers (e.g., zooplankton and fish), creating an essential channel in the aquatic food web (Carpenter et al., 2009) (Figure 1.2). Abiotic factors directly impact the production of phytoplankton, as they are highly sensitive to environmental changes such as temperature, turbidity, dissolved oxygen, and pH (Reynolds, 2006; Rugema et al., 2019). As so, they are viewed as good indicators of water quality and trophic status due to their fast response to environmental variations and degradation of water quality. Temperature is an important factor regulating phytoplankton, as well as a variety of other

activities in aquatic ecosystem. Alteration of productivity is associated to variation in temperature and photic conditions (Darchambeau et al., 2014). Light represents an important factor for phytoplankton growth as a driver of photosynthetic activity (Grobbelaar, 1989; Marzetz et al., 2020); waters with high turbidity have a lower light availability, which can lead to a decrease in phytoplankton growth and density (Wootton and Power, 1993). Several other abiotic factors, such as dissolved oxygen (DO), pH, nitrogen (N), and phosphorus (P) are also known to have an effect in phytoplankton growth and density (Carpenter et al., 2009). The density of phytoplankton is particularly related to the nutrients available in the water, especially nitrate and phosphate. As stated by Bellinger et al. (2015), a high nutrient load (such as phosphorus), an increase in temperature and favourable light conditions encourage the rapid expansion of the phytoplankton community.

Zooplankton are organisms that live in the water column and rely on tides and currents for their transportation. Their diet is mainly composed of phytoplankton, being responsible for the link between primary producers and consumers, as a food source for other invertebrates and fish (Sterner & Schulz, 1998). Zooplankton communities are typically represented by sensitive organisms that can react to environmental changes with a significant and rapid response (Neto et al., 2014). For basic research and for the application as a management tool, zooplankton has been considered as a key node in the food web (Figure 1.2), and measures trying to promote water quality through top-down interactions in the system (e.g., manipulating planktivorous fish) have been heavily considered (Carpenter et al., 1985; Mehner et al., 2008). For this reason, top-down mechanisms are considered essential for the control and dynamic of zooplankton when placed in a natural ecosystem (Sellami et al., 2010). Large zooplankters, like *Daphnia*, can be reduced from an ecosystem by planktivorous fish creating a top-down pressure (Couture & Watzin, 2008; Petzoldt et al., 2009). When zooplanktivorous fish are present in the ecosystem, large-bodied zooplankton like *Daphnia* are taken over by small-bodied zooplankton like *Bosmina* (Brooks & Dodson, 1965). Brooks and Dodson (1965) detected that there is a relationship between the body size and the filtering efficiency of zooplankton, so the bigger the animal, the better its capacity of filtration and the spectrum of particle size it can capture, leading to a cleaner water column. So, not only phyto- and zooplankton modulate energy transfer along the food web – serving as the basis for supporting fish populations, especially larvae and juveniles (Dettmers & Stein, 1992; Brett & Goldman, 1997) – but they also play a key role in the dynamics of water transparency.

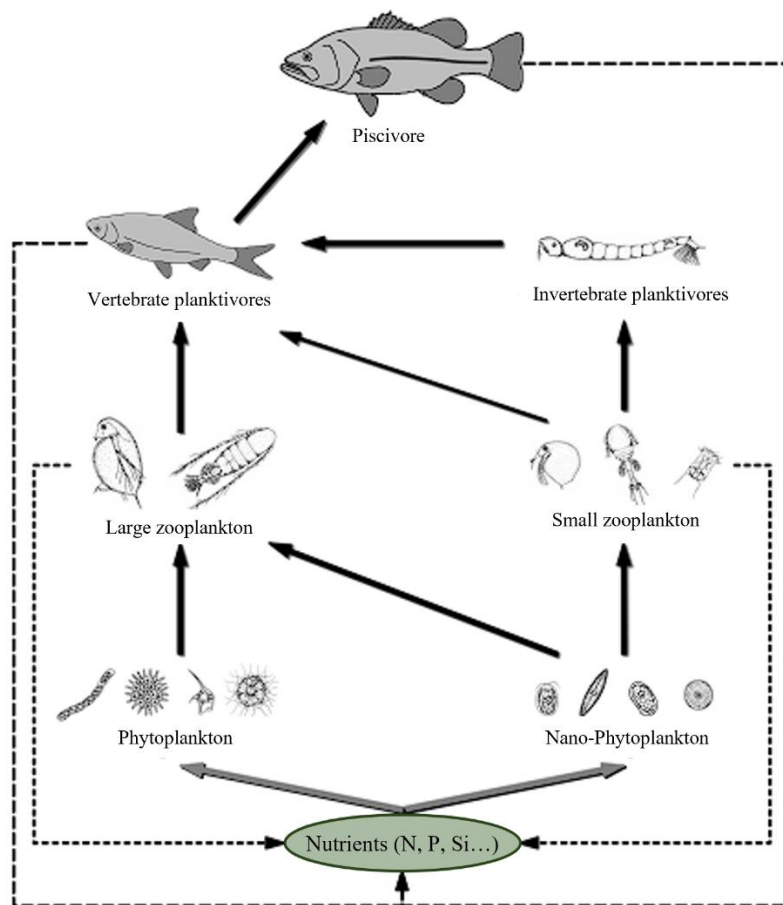


Figure 1.2 - Simplified conceptual model of a pelagic food web in a lentic ecosystem. The thickness of the arrows represents the intensity of the interaction, and the dashed arrows correspond to the recycling of nutrients (Taken from Castro, 2007).

The biomass and composition of both phyto- and zooplankton represented in the aquatic ecosystem, as well as their distribution, can be an indication of the environmental situation of that ecosystem. As such, these communities have large potential as bioindicators, and monitoring programs based on planktonic communities are a potentially powerful tool for water quality assessment and environmental management. Indeed, both phyto- and zooplankton communities have been used as biological descriptors in biomonitoring programs to assess water quality (Caroni & Irvine, 2010; Jeppesen et al., 2011; García-Chicote et al., 2018; Almeida et al., 2020).

1.4 Water quality assessment in Europe

The Water Framework Directive (WFD) is one of the key pieces of legislation that the European Union has put into place regarding the management of water resources. The European Commission established this directive in 2000, and all signatory Member States are required to safeguard, restore, and preserve all water resources with the goal of achieving "Good Status" for all inland surface, transitional, coastal, and ground waters by 2021 or 2027 (European Commission, 2005). Numerous problems were revealed over the years when the assessment of this directive was implemented for reservoirs, such as the absence of pristine or unpolluted reservoirs to be used as reference. In addition, the assessment scheme of reservoirs was repeatedly elaborated, considering that their ecological properties are intermediate between rivers and lakes but distinct from both (Navarro et al., 2009). Intercalibration exercises were used to calibrate the metrics to be used to efficiently classify European water bodies, which required grouping waterbodies which shared similar features (in types). For artificial and heavily modified water bodies, three types of reservoir typologies were defined in Portugal: Northern reservoirs (cold waters, used for hydroelectric power and residence time <7 months), Southern reservoirs (warmer waters, used for irrigation/water supply and residence >7 months), and Main Course reservoirs (cover a large drainage area, very low residence time usually less than 10 days).

The WFD assessment scheme is to use established criteria of chemical, physical, hydromorphological and biological descriptors to estimate the ecological status of water bodies by defining a pre-set of guidelines for each descriptor. For reservoirs, phytoplankton communities have been considered the most robust biological descriptor (and in some cases, the only descriptor available), but are mostly an indicator of productivity or trophic status. Metrics based on other important descriptors, such as fish and macrophytes, have also been developed, but historical background data is incomparably smaller than for phytoplankton. Phytoplankton-based assessment according to the WFD in Portugal is based on a multimetric index (the Algae Group Index, IGA) coupled with general estimates of biovolume of cyanobacteria, total biovolume and chlorophyll *a* concentration. Physical and chemical (pH, dissolved oxygen, total phosphorus and nitrate) and hydromorphological parameters (morphological features and hydrological regime) are used to support biological evaluation (Mazur et al., 2017).

The implementation of the WFD bioassessment and classification scheme allows establishing the ecological potential of ecosystems like reservoirs, improving the efficacy of its management. However, and unlike for lotic ecosystems, it lacks a more integrated view of the biological communities that there reside. Many scientists have recommended the use of zooplankton as a “supportive/interpretative parameter” of fish (Jeppesen et al., 2011). With that suggestion in mind, some ecologists emphasize the need to consider zooplankton community in the WFD bioassessment scheme in reservoirs (Caroni & Irvine, 2010; Jeppesen et al., 2011; García-Chicote et al., 2018; Almeida et al., 2020).

Zooplankton comprehend organisms with brief life cycles (from few days to few weeks) (Lampert and Sommer, 1997), which modulate a temporal variation in the ecosystem. The identification of zooplankton is relatively simple and cost-effective and zooplanktonic communities are usually described as a good indicators of water quality in lentic ecosystems like reservoirs (Sellami et al., 2010; Neto et al., 2014; Almeida et al., 2020). This capacity comes from their key position in the pelagic food web, since a shift in zooplankton communities composition can alter the strength of top-down control on phytoplankton communities, dictating the capacity of an aquatic ecosystem to uptake carbon dioxide and influencing the volume of processed nutrients (Brooks & Dodson, 1965; Carpenter et al., 2009). Jeppesen et al. (2011) has verified a reduction of zooplankton richness with increasing levels of phosphorus, which is a nutrient frequently associated with eutrophication. As such, decreased levels of calanoid to cyclopoid copepods and cladocerans are often correlated to eutrophic environments (Gannon & Stemberger 1978; Tundisi 1988). The decrease of zooplankton biomass can be related to the increase in abundance of planktivorous fish (Brooks & Dodson, 1965), or the introduction of invasive species (Walsh et al., 2016). To measure the capacity of zooplankton of regulating phytoplankton biomass, zooplankton to phytoplankton ratios (either biovolume or biomass) can also be applied (Jeppesen et al., 2011; García-Chicote et al., 2018) and possibly provide a better understanding of the ecosystem, becoming an essential information to add to the WFD (Moss et al., 2003).

1.5 Objective

The aim of this work was to evaluate the seasonal and spatial variation and bioindicator value of the phytoplankton community in four reservoirs of the Minho region (Andorinhas, Caniçada, Touvedo and Venda Nova), linking it to the zooplankton communities. Following the guidelines of the Water Framework Directive to assess the water quality of the reservoirs, standard evaluation parameters were used (IGA - Algae Group Index, biovolume of cyanobacteria, total biovolume and chlorophyll *a* concentration), supported by complementary physical and chemical data. Zooplankton data was collected to support the biotic context of phytoplankton and to check whether it can provide additional information on ecosystem health, allowing a better understanding of the ecological potential of these semi-artificial systems.

The obtained data from this work is connected to previous data collected during the last year (Machado, 2022) for the REDEfine project. The four reservoirs chosen for this work (Andorinhas, Caniçada, Touvedo and Venda Nova, following Machado, 2022), are all located in the north region of Portugal, being characterized by having a low mineralization and nutrient load, along with large hydrologic fluctuations related to their usage for hydroelectric power and water supply for human consumption. The selected systems differ in extension, depth, and morphology. Distinct sampling points were selected along each reservoir to encompass spatial heterogeneity.

2. Materials and methods

2.1 Study area

Four reservoirs were selected for this study: Andorinhas (river Ave), Caniçada (river Cávado), Touvedo (river Lima) and Venda Nova (river Rabagão) (Table 2.1). In each reservoir, two to three sampling points were selected along the extension of the reservoir (Table 2.2). The chosen reservoirs for this study were located at the northern-Atlantic area of Portugal, in the Hydrographic Region of Rivers Minho and Lima (RH1) and Rivers Cávado, Ave and Leça (RH2) (Figure 2.1). These reservoirs are characterized by low mineralization (Table 2.3) and (relatively) low nutrient content, differing in terms of depth, extension, and morphology. According to the WFD, the different Member States were divided into Geographic Intercalibration Groups (GIG) that share various types of common water in order to conduct the Intercalibration Exercise. Portugal joined the GIG for the Mediterranean. The Minho region demonstrates Cantabrian mixed woods, Iberian Mediterranean sclerophyllous and mixed forests, the Köppen–Geiger climate classification classified as Csb (warm-summer Mediterranean) (H. E. Beck et al., 2018; Santos et al., 2023).

Table 2.1 - Features of the reservoirs under study (Andorinhas, Caniçada, Touvedo and Venda Nova) and respective river basins (SNIRH 2022)

Features	Andorinhas	Caniçada	Touvedo	Venda Nova
Location	Travassos (Braga district)	Vieira do Minho e Terras de Bouro (Braga district)	Ponte da Barca and Arcos de Valdevez (Viana do Castelo district)	Montalegre (Vila Real district) and Vieira do Minho (Braga district)
Hydrographic basin	Ave river	Cávado river	Lima river	Cávado River
Dam wall height (m)	25	76	53	97
Total capacity (Dam ³)	1 200	159 300	15 500	94 500
Usable capacity (Dam ³)	No information	147 900	4 000	93 000
Dead volume (Dam ³)	No information	11 400	11 500	1 500
Operation type	Energy production and recreation	Energy production and recreation	Energy production, defense of floods	Energy production and recreation
Annual average temperature (°C)	13	13	11	10
Average annual rainfall (mm)	2 104	2 729	2 466	2 086

Table 2.2 - Geographic coordinates and altitude of the sampling points of each reservoir under study (Andorinhas, Caniçada, Touvedo and Venda Nova).

Sampling location	Latitude (°)	Longitude (°)	Altitude (m)
Andorinhas 1 (An1)	41.5704705° N	8.1802222° W	188
Andorinhas 2 (An2)	41.5694077° N	8.1946498° W	189
Caniçada 1 (Cn1)	41.6815235° N	8.1671595° W	146
Caniçada 2 (Cn2)	41.6805356° N	8.1802964° W	146
Caniçada 3 (Cn3)	41.6805356° N	8.1802964° W	146
Touvedo 1 (To1)	41.828944° N	8.3099828° W	50
Touvedo 2 (To2)	41.8257577° N	8.3215059° W	49
Touvedo 3 (To3)	41.8141995° N	8.3404054° W	48
Venda Nova 1 (Vn1)	41.699677° N	7.9412327° W	691
Venda Nova 2 (Vn2)	41.6895684° N	7.9568585° W	691
Venda Nova 3 (Vn3)	41.6766019° N	7.965966° W	691

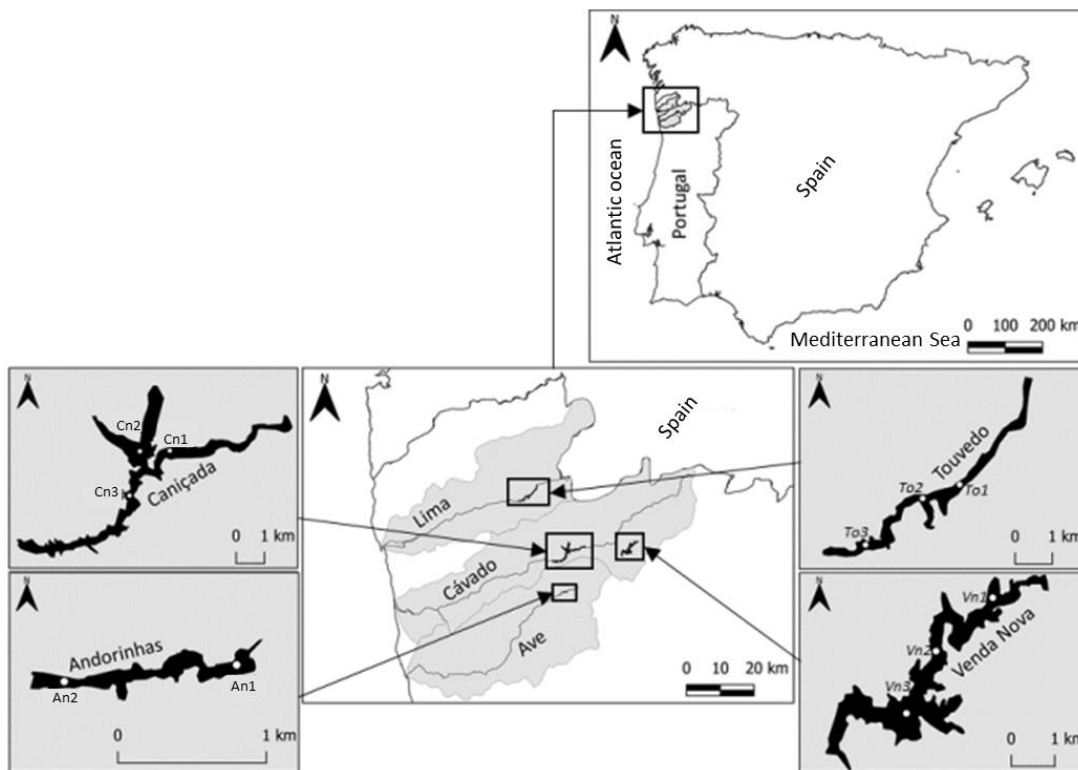


Figure 2.1 - Geographic location of the studied reservoirs, and the sampling points in each reservoir (Figure adapted from Machado, 2022)

Table 2.3 - Mineral characteristics of the waters of the reservoirs under study (Andorinhas, Caniçada, Touvedo and Venda Nova) in February 2022

Features	Andorinhas	Caniçada	Touvedo	Venda Nova
Water hardness (°F)	1	0.9	0.7	0.6
Calcium (mg Ca ²⁺ L ⁻¹)	3.1	2.7	2	1.6
Magnesium (mg Mg ²⁺ L ⁻¹)	0.61	< 0.5	< 0.5	< 0.5
Bicarbonates (mg HCO ₃ ⁻ L ⁻¹)	< 6.0	< 6.0	< 6.0	< 6.0

2.2 Sampling procedure and *in situ* analysis

Three sampling points were defined along the main axis of each reservoir due to the potential spatial heterogeneity in these ecosystems (Figure 2.1, Table 2.2); the only exception was Andorinhas (two points), due to its smaller extension. Sampling points were numbered from upstream (e.g., Cn1) to downstream (e.g., Cn3). The points were always sampled in the most central and deepest zone at each location, to ensure a better representation of the pelagic communities. All the locations were under influence of a dam (i.e., lentic ecosystem).

Sampling campaigns were carried out between November 2021 and July 2022, using a kayak to access the central areas of the reservoirs. Various physical and chemical parameters were measured *in situ*, using a multiparametric probe (WTW Multi 3630 IDS): temperature (°C), which is a strong seasonal variable that can influence other variables and interfere with the distribution and abundance of organisms; pH, which controls the chemical status and availability of inorganic components; conductivity ($\mu\text{S cm}^{-1}$), which is an indicator of the amount of ions present in the water; dissolved oxygen (% and mg L⁻¹), which provides information about the biochemical reactions and biological processes (e.g., oxidations, respiratory and photosynthetic activity, etc.) taking place in the aquatic ecosystem. Depth at each site was measured with a portable sonar. The transparency of water was quantified using the Secchi disk to evaluate the penetration of light in the water, which is an essential parameter for phytoplankton growth. Water was also collected from each site (± 4.5 L) for laboratory analysis of phytoplanktonic biomass (chlorophyll *a*), total phosphorus, turbidity, and suspended solids. These samples were preserved in a thermal box and in the dark during transport to the laboratory.

For the sampling of zooplanktonic communities, several oblique trawls were done with a plankton net of 153 μm mesh size and 20 cm in diameter (Figure 2.2 a). This network porosity avoids reflux effects caused by partial occlusion of the network mesh, thus allowing a better filtration efficiency. However, larger mesh nets are unable to effectively sample nauplii and rotifers (Mack et al., 2012); this mesh size (153 μm) represents a compromise between representativity and efficiency, following Saunders et al. (1999) and Mack et al. (2012). The number of hauls performed at each location depended on the zooplanktonic density at each sampling time, while the length of the trawl was defined as a function of the depth of the site. Typically, one or two 25 m hauls were carried out at the deepest locations and multiple 4-10 m hauls at shallower locations. Zooplankton samples were collected in a beaker, washed with local water to recover all the organisms, and later concentrated with a small sieve of 100 μm porosity. This method allowed to concentrate the samples, following a conservation process in 70% alcohol after a brief period of fixation in 95% alcohol (Black & Dodson, 2003)

Phytoplankton communities were sampled in the subsurface (0.5 m depth), by collecting water samples (500 mL) using a pumping system (Figure 2.2 b) into a glass vessel. Samples were fixed with neutralized Lugol's iodine solution (2-3 mL), stored in the dark and brought to the laboratory to be decanted.



Figure 2.2 - (a) Zooplankton sampling with a plankton net of 153 μm ; (b) Pumping system for phytoplankton sampling.

2.3 Laboratory analysis - chlorophyll *a* and physico-chemical variables

The following parameters were analysed in the water samples brought from the field: suspended solids, turbidity, total phosphorus, and chlorophyll *a* concentration.

Suspended solids (SS) or seston are a measure of particulate matter (particulate organic and inorganic matter, clay, plankton) in water and therefore are related to turbidity and water transparency. The water samples were vacuum filtered through pre-weighed fiberglass filters (47 mm in diameter and 1.2 µm pore size) (Figure 2.3) to retain the particulate matter, which was then quantified by gravimetry (APHA, 2000), according to the expression:

$$SS = \frac{1000 \times (W_{final} - W_{initial})}{V}$$

where SS is the content of suspended solids (mg L⁻¹), W final is the weight of the dry filter with residue (g), W initial is the weight of the filter (g), and V is the volume of filtered water (L). The factor of 1000 in the equation converts the weights (in g) to mg.

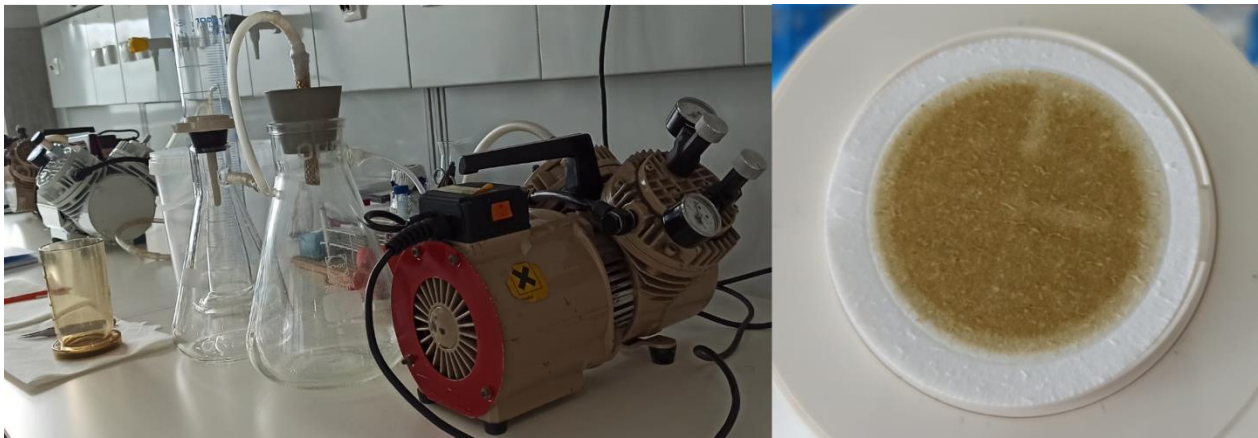


Figure 2.3 – Vacuum filtration (left) of water samples through fiberglass filters with 47 mm in diameter and 1.2 µm in porosity (right).

Turbidity is an optical property of water clarity and directly modulates the penetration of light into the water column that can influence the capacity of photosynthesis. This property mainly depends on the concentration of suspended solids and dissolved chemical substances, as well as the presence of planktonic microorganisms (especially photosynthetic organisms such as

phytoplankton). The higher the concentration of these elements, the lower the light transmission in the column of water since there are more losses by absorption and dispersion. Turbidity was determined indirectly through the absorption coefficient at 450 nm (ϵ_{450}), using spectrophotometry (Brower et al., 1997):

$$\epsilon_{450} = \frac{2.30 \times Abs_{450}}{l}$$

where ϵ_{450} is expressed in m^{-1} , Abs_{450} is the absorbance read at 450 nm and l is the optical path of cuvette (m).

Phosphorus occurs in natural aquatic ecosystems in the form of phosphate that is found both in solution and in particles, as well as aquatic organisms. It is a limiting nutrient for the primary productivity of a body of water and essential for the growth of organisms, and its quantification is fundamental for the functional characterization of lentic ecosystems. A previous step of mineralization of the samples with potassium persulfate, $K_2S_2O_8$ (Ebina et al., 1983) was used to oxidize all forms of phosphorus to soluble orthophosphate, which was then quantified by the stannous chloride method (APHA, 2000). Total phosphorus (TP) was calculated by interpolation from a calibration curve using potassium dihydrogen phosphate (KH_2PO_4) as a standard, according to the expression:

$$[TP] = \frac{Abs_{690}}{0.89}$$

where $[TP]$ is total phosphorus concentration ($mg L^{-1}$) and Abs_{690} is the absorbance read at 690 nm.

Chlorophyll *a* (Chl *a*) is a photosynthetic pigment present in all microalgae and cyanobacteria, so its quantification represents an approximate estimate of the algal biomass of aquatic ecosystems. After filtration in vacuum through glass fiber filters (47 mm in diameter and 1.2 μm pore size) (Strickland & Parsons, 1957), chl *a* was extracted from the filtered residue with neutralized acetone at 90% and determined spectrophotometrically by the difference in the absorbance of the extracts before (Abs) and after (Abs^a) acidification with 0.1 M HCl, according to the monochromatic method of Lorenzen (1967). Absorbance measures at 665 nm prior and after acidification were corrected for turbidity, by subtracting the corresponding absorbance at 750 nm. The chl *a* content ($\mu g L^{-1}$) was calculated according to the equation:

$$\text{Chl } a = \frac{26.7 \times (E665_0 - E665_a) \times v}{V \times l}$$

where $E665_0$ and $E665_a$ correspond to the corrected absorbance at 665 nm, v is the volume of acetone used (mL), V is the volume of water filtered (L) and l the optical path of the cuvette (cm). The factor 26.7 derives from the absorption coefficient of chl a at 665 nm.

2.4 Laboratory analysis – zooplankton and phytoplankton

For zooplankton identification, each preserved sample was washed and concentrated with a sieve (to remove excess of ethanol), and diluted with running water to a known volume, which was adjusted to the density of organisms. Whenever necessary, a drop of liquid soap was added to the diluted sample to break the surface tension and prevent agglomeration of specimens on the water surface. The identification and enumeration of the specimens was carried out in an appropriate counting chamber (with a capacity of 5-10 mL and a grid to facilitate counting), in which an aliquot of known volume of sample was placed. The identification effort was adjusted for the different taxa, according to their abundance, with a minimum of 400 individuals (in total) being counted per sample. The zooplankton samples were identified with a Leica S APO stereoscope and, when necessary, a Leica DM500 optical microscope was also used. Only juvenile or adult zooplanktonic crustaceans were identified and enumerated. Given that a relatively large mesh (153 μm) was chosen to guarantee greater filtration efficiency, it was considered that the sample was not representative of the organisms of small dimensions (rotifers and copepod nauplii) (Mack et al., 2012). Copepoda were identified up to the order (Calanoida, Cyclopoida and Harpacticoida), while Cladocera were identified to the lowest taxonomic level possible, typically the genus, according to the identification key of Amorós (1984) and Alonso (1996).

The total density of each taxon (ind m^{-3}) was based on the relationship between the volume of sample inspected (v_i , in mL), the total sample volume (v_s , in mL), and the volume of water collected with the plankton net (V , in L), according to the following expression:

$$\text{Density of taxon } A \text{ (ind m}^{-3}\text{)} = \frac{(\textit{n}^{\circ} \textit{ of individuals counted} \times \frac{v_s}{v_i})}{V} \times 1000$$

The phytoplankton identification was determined through inverted microscopy, following the method of Utermöhl (1958). For enumeration and identification, Lugol-preserved samples were allowed to settle in sedimentation chambers and observed at 400x magnification with a Leica DMIL inverted microscope. Considering the density of all samples, the volume sedimented was between 10 and 3 mL. For the analysis of the composition and abundance of the phytoplankton community, individuals and colonies were counted until reaching at least 400 specimens in random counting fields and identified to genus level, whenever possible, according to Baker et al. (2012), Streble & Krauter (1988) and Carter-Lund & Lund (1995). The number of algae and cyanobacteria counted by taxa was converted into concentration per unit volume of the sample using the enumeration of specimens in the sample, according to the equation:

$$N = X \times \frac{A \times d}{a \times v}$$

where N is the density of cells from each taxa per total volume of the sample (cell L⁻¹), X is the average number of cells per random counting field, the chamber area is represented by A, v is the volume of the sample sedimented in the chamber, a is the count field area and d is the sample dilution or concentration factor, when necessary. In general, phytoplanktonic density was expressed in cells per litre (cell L⁻¹).

2.5 Metrics of the zooplankton community

After compiling the density data matrix by date, reservoir and point of sampling, several metrics of zooplankton communities were calculated. Diversity was calculated with Shannon's index (H'), according to the expression:

$$H' = - \sum_{i=1}^s p_i \ln(p_i)$$

where S represents richness (number of taxa) and p_i the relative abundance of each taxon (i.e., the number of individuals of taxon i divided by the total number of individuals in the sample).

Evenness was determined with the Pielou index (J), which is comprised in a range between 0 and 1, where 1 represents the maximum evenness (J = 1; abundance of organisms is evenly

distributed across all taxa present in the sample). This index was calculated according to the expression:

$$J = \frac{H'}{H'máx}$$

where H' is the Shannon index and H'max = ln(S) which represents the maximum richness (S).

Two additional metrics were estimated: the Calanoida/Cyclopoida ratio, which provides an overview of the relative dominance of herbivore vs. omnivorous copepods, and the proportion of large cladocerans. Both indices work as indicators of herbivory and control over phytoplankton by important groups of zooplankters (Machado 2022).

The Calanoida/Cyclopoida ratio was calculated according to the expression:

$$\frac{\text{Total Calanoida density (ind m}^{-3}\text{)}}{\text{Total Cyclopoida density (ind m}^{-3}\text{)}}$$

The proportion of large cladocerans (*Daphnia pulicaria*, *Daphnia longispina* and *Sida crystallina*) is also a measure of the intensity of predation by planktivorous fish in the ecosystem (Moss et al., 2003), and was calculated by the expression:

$$\frac{\text{Density of large cladoceran species (ind m}^{-3}\text{)}}{\text{Total cladoceran density (ind m}^{-3}\text{)}}$$

2.6 Metrics of the phytoplankton community

After compiling the density data matrix by date, reservoir and point of sampling, several metrics of phytoplankton communities were calculated. The data obtained was used directly to calculate the Algal Group Index (IGA), which is a multimetric index (INAG, 2009) that gives distinct weights to specific groups of microalgae (Table 2.5). To acquire the metric % of Biovolume of Cyanobacteria, the species of Chroococcales should be excluded, except for genera *Microcystis* and *Woronichinia*. The following expression was used to calculate IGA (see taxa abbreviations on Table 2.4):

$$IGA = \frac{1 + 0.1 \times Cr + Cc + 2 \times (Dc + Chc) + 3 \times Vc + 4 \times Cia}{1 + 2 \times (D + Cnc) + Chnc + Dnc}$$

Table 2.4 - Constitution of the Algal Group Index (IGA)

Algae Group	Acronym
Dinophyceae	D
non-colonial Chrysophyceae	Cnc
non-colonial Chlorococcales	Chnc
non-colonial Bacillariophyceae	Dnc
Cryptophyceae	Cr
Colonial Chrysophyceae	Cc
Colonial Bacillariophyceae	Dc
Colonial Chlorococcales	Chc
Colonial Volvocales	Vc
Cyanobacteria	Cia

Quality assessment using the phytoplankton quality element is carried out by applying the NMARSP index (New Mediterranean Phytoplankton Assessment Index in reservoirs). NMARSP integrates four metrics (Table 2.5): two for biomass (chlorophyll *a* and total biovolume) and two for composition and abundance (biovolume of cyanobacteria and the Algal group Index). The NMARSP index is obtained by first determining a normalized ecological quality ratio (EQR_{norm}, which represents degree of deviation from a pre-defined reference status) for each metric (based on the reference value in Table 2.5, and following APA, 2021) and then calculating the arithmetic mean between the normalized EQR of the Biomass and Composition and Abundance components. The following expression was used to calculate NMARSP index:

$$NMARSP = \frac{\left(\frac{EQR_{norm}(Chla) + EQR_{norm}(Tbv)}{2} \right) + \left(\frac{EQR_{norm}(IGA) + EQR_{norm}(Cyanbv)}{2} \right)}{2}$$

Where EQR_{norm} (Chl *a*) is normalized EQR of chlorophyll *a* concentration; EQR_{norm} (Tbv) is Normalized EQR of Total Biovolume; EQR_{norm} (IGA) is normalized EQR of the Algal Group Index and EQR_{norm} (Cyanbv) is Normalized EQR of Total Biovolume of Cyanobacteria.

Table 2.5 - Reference values of the NMARSP index in northern reservoirs, according to APA (2021) (left). Border values are presented for the quality classes of the index NMARSP, expressed in EQR, for the reservoirs (right).

Metrics	Reference value	Good
Chlorophyll a (mg m ⁻³)	1.70	7.90
Total biovolume (mm ³ L ⁻¹)	1.20	2.80
Cyanobacterial biovolume (mm ³ L ⁻¹)	0.02	0.80
IGA	2.00	37.60

Maximum	0.80
Good	0.60
Reasonable	0.40
Bad	0.20

For a more adequate and accurate comparison between samples and quantification of the contribution of different groups of phytoplankton to the primary production of a lentic system, the biovolume was calculated using the Rott (1981) method. In the literature, there are several references with biovolume values of the different taxa, and the geometric shapes associated with each species (Olenina et al., 2006). For colonial forms, the average number of cells must be estimated and multiplied by the average volume of 10 cells. The total biovolume corresponds to the total cell volume of phytoplankton species present in the sample.

Similar to zooplankton, the diversity of phytoplankton was calculated with Shannon's index (H'), according to the expression:

$$H' = - \sum_{i=1}^s p_i \ln(p_i)$$

where S represents richness (number of taxa) and p_i the relative abundance of each taxon (i.e., the number of individuals of taxon i divided by the total number of individuals in the sample).

Evenness was determined with the Pielou index (J), which is comprised in a range between 0 and 1, where 1 represents the maximum evenness ($J = 1$; abundance of organisms is evenly distributed across all taxa present in the sample). This index was calculated according to the expression:

$$J = \frac{H'}{H'_{\text{máx}}}$$

where H' is the Shannon index and $H'_{\max} = \ln(S)$ which represents the maximum richness (S).

2.7 Statistical analysis

Environmental data were analyzed with Principal Component Analysis (PCA) to examine the main environmental gradients. This type of analysis allows you to group data into few dimensions (unique vectors), taking into account their similarity, preserving most information in order to explain as much variation as possible. Prior to this analysis, the variables were standardized to accommodate the different scales. From this analysis, it was possible to evaluate the main variations (temporal and spatial) of environmental gradients.

Principal Coordinate Analysis (PCoA) was used to determine the primary sources of variation in the phytoplankton and zooplankton communities separately (while accounting for their densities). PCoA is a multivariate analysis technique that depicts the separation (in this case, ecological) of samples in a condensed spatial universe; its key benefit is the freedom in the selection of the separation measurement (Borcard et al., 2011). For community data, a distance matrix based on the Bray-Curtis dissimilarity square root is one of the preferred options among ecologists (Faith et al., 1987; Legendre & Legendre, 2012) because it does not consider the double zero problem (shared absence). The square root transformation and double Wisconsin transformation were used to normalize the zooplankton densities prior to analysis (Borcard et al., 2011). From this analysis, it was possible to evaluate the main variations (temporal and spatial) across phyto- and zooplankton communities (i.e., ecological gradients).

The relationship between environmental data and phytoplanktonic and zooplanktonic communities was assessed using distance-based Redundancy Analysis (db-RDA), a PCoA-based method that enables direct analysis of environmental gradients by superimposing them on the dissimilarity matrix between locations in a manner similar to multiple regression (Borcard et al., 2011). In order to prepare the data for analysis, they were transformed in a manner similar to that of PCoA (community data transformation and use of Bray-Curtis dissimilarity) and PCA (standardization of environmental variables). In order to avoid include duplicate or decreased variables contribution, the environmental variables to be included in the model were *a priori* selected using the function `ordiR2step` from the `vegan` package (Borcard et al., 2011). As a result, a more parsimonious model is created (good level of explanation with minimal predictors). The following explanatory factors (the most significant non-redundant environmental gradients) were

chosen to explain the differences across zooplankton communities: electrical conductivity, dissolved oxygen in mg L^{-1} , chlorophyll *a*, depth, total phosphorus, turbidity, transparency, and temperature.

All statistical analyses were carried out in R software, version 4.2.3 (R Core Team, 2023), using the graphical environment RStudio 2022.2.0.443 (RStudio Team, 2022). The programs "ggplot2" (Wickham, 2016), "ggord" (Beck, 2021), "ggpubr" (Kassambara, 2020), "vegan" (Oksanen et al., 2020).

3. Results

3.1 Physicochemical variables and chlorophyll *a* concentration

Higher depths were recorded in Caniçada and Venda Nova (> 20 m), followed by Touvedo (11-22 m), with Andorinhas registering the lowest values (4.5-12 m) (Table 3.1). In all reservoirs, depth increased towards downstream, with the site nearest to the dam displaying the highest value. Minor variations in depth were observed in all reservoirs through time, which were proportional to reservoir mean depth. The pH of the water always remained slightly acidic in all reservoirs, showing a more neutral profile in July, and again slightly acidic in November (Table 3.1). Electrical conductivity was higher in shallower reservoirs (Andorinhas and Touvedo) and lower in the deeper reservoirs (Caniçada and Venda Nova), generally increasing in the warmer months (Table 3.1).

Dissolved oxygen was close to maximum saturation (100%) in all sampling sites, having lower values in November and higher values in July (Table 3.1), which represented the coldest and warmest month, respectively. Apart from depth, no relevant differences within reservoirs were found, with most sites in the same reservoir following similar temporal patterns.

A clear seasonal pattern was observed in water temperature (Figure 3.1), which varied between 9.5°C and 26.2°C over the months sampled, having reached its maximum value in July (summer) in all reservoirs ($\geq 23^\circ\text{C}$). Deeper reservoirs (Caniçada and Venda Nova) displayed higher transparency, generally above 4 m, in contrast to shallow reservoirs (Andorinhas and Touvedo), where Secchi depth was generally under 4 m. Turbidity and suspended solids were higher in deeper reservoirs, and particularly variable in Touvedo, where it reached a maximum in November (Figure 3.1).

In some reservoirs, total phosphorus (TP) and chlorophyll *a* content (Chl *a*) were negatively associated, with TP decreasing as chl *a* content increased (Figure 3.1). Higher TP and chl *a* values were recorded in the shallow reservoirs, where the more nutrient-rich water from the bottom can be mixed with the surface water. In most cases, nutrient (TP) levels and phytoplankton biomass (chl *a*) were within acceptable levels in terms of ecological potential (*sensu* WFD), except for Touvedo (high TP in November and high chl *a* in July).

Table 3.1 - Physicochemical variables measured *in situ* at the different sampling points within each reservoir (Andorinhas, An1–2; Caniçada, Cn1–3; Touvedo, To1–3; Venda Nova, Vn1–3) and sampling months (nov21 to jul22): depth, pH, electrical conductivity (Cond.) and dissolved oxygen (O₂).

Measured parameters	Campaign	An1	An2	Cn1	Cn2	Cn3	To1	To2	To3	Vn1	Vn2	Vn3
Depth (m)	November	4.5	12	40	37	50	11	15	21	30	26	30
	March	5.0	12	37	40	52	12	14	22	27	25	58
	April	5.0	11	20	37	53	11	14	21	27	20	40
	June	4.5	12	38	43	46	11	15	21	30	35	54
	July	5.0	11	35	35	50	11	16	21	31	40	41
pH	November	5.9	6.4	5.9	5.9	5.9	6.1	6.4	6.4	6.1	5.9	6.1
	March	6.3	6.3	6.4	6.7	6.4	6.6	6.8	6.6	7.2	7.0	7.3
	April	6.6	6.7	6.2	6.6	6.6	6.6	7.0	6.7	7.1	6.6	6.8
	June	6.8	6.8	6.8	6.8	6.9	7.0	6.8	6.9	6.8	6.9	6.8
	July	6.9	6.7	7.6	7.7	6.8	7.1	7.2	7.1	7.1	7.2	7.1
Cond (μS cm ⁻¹)	November	28	30	18	18	19	31	31	31	20	20	19
	March	26	27	18	18	18	20	20	21	18	18	18
	April	29	29	18	18	18	22	22	22	18	18	18
	June	32	32	19	19	19	25	25	25	18	18	18
	July	50	49	26	25	30	42	42	41	30	27	50
O ₂ (mg L ⁻¹)	November	9	9	8	8	8	8	8	8	7	7	7
	March	12	11	12	12	11	11	11	11	13	12	11
	April	9	10	10	11	10	10	10	11	11	11	11
	June	9	9	8	9	9	9	9	9	10	10	10
	July	9	9	10	10	10	9	9	9	9	9	9
O ₂ (%)	November	89	90	86	86	85	85	84	85	79	76	75
	March	113	108	115	111	102	103	102	104	120	114	106
	April	100	101	101	109	101	102	103	114	112	112	111
	June	102	104	98	99	99	105	106	107	120	122	120
	July	116	111	121	119	119	118	117	116	114	115	112

A PCA applied to environmental data captured 64% of the total variation in the first two axes (Figure 3.2). The main environmental gradient consisted of transparency and depth increasing from the left (negative) to the right (positive) side of the diagram, contrary to phosphorus content, suspended solids, and turbidity. This gradient separated deeper reservoirs (Caniçada and Venda Nova, on the right) and shallower reservoirs (Andorinhas and Touvedo, on the left), showing that the latter were more productive (higher phosphorus content, chlorophyll *a* and turbidity). The second dimension of the PCA reflected a gradient of temperature, dissolved oxygen, and pH, being related to seasonal variation: effectively, the samples follow a consistent trend from November to July (scores go down- and leftwards) in all reservoirs. In Touvedo, November samples were segregated from the rest (due to higher turbidity and TP – see also Figure 3.1).

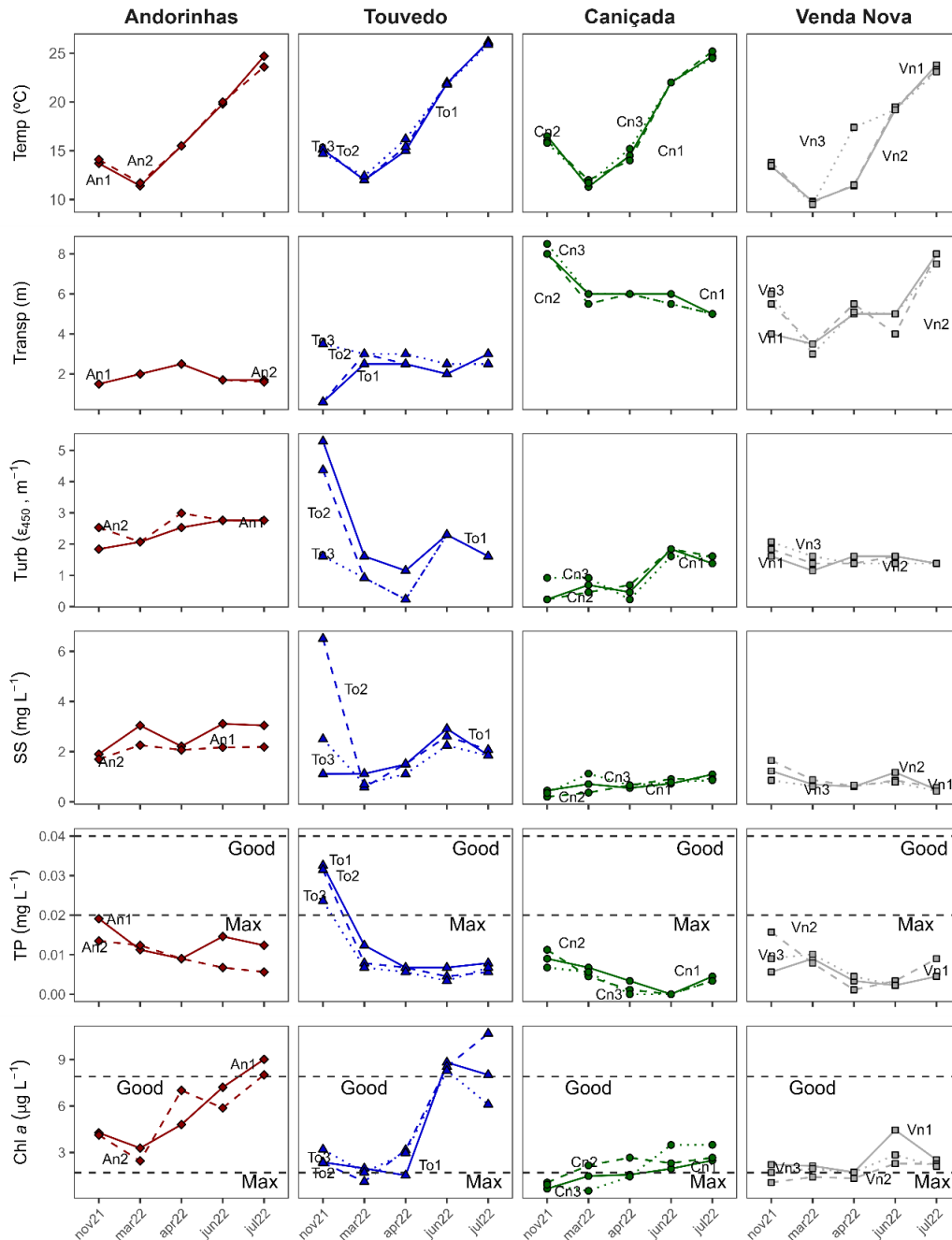


Figure 3.1 - Seasonal and spatial variation of temperature (Temp), transparency (Transp), turbidity (Turb), suspended solids (SS), total phosphorus (TP) (Max ≤ 0.02 and $0.02 > \text{Good} \geq 0.04$) and chlorophyll *a* content (Chl *a*) (Max ≤ 1.70 and $1.70 < \text{Good} \leq 7.90$) in each reservoir, with corresponding limits for ecological potential (sensu WFD) for TP and Chl *a*. Different lines represent sampling sites within each reservoir (Andorinhas, An1–2; Caniçada, Cn1–3; Touvedo, To1–To3; Venda Nova, Vn1–Vn3) and sampling months (nov21 to jul22).

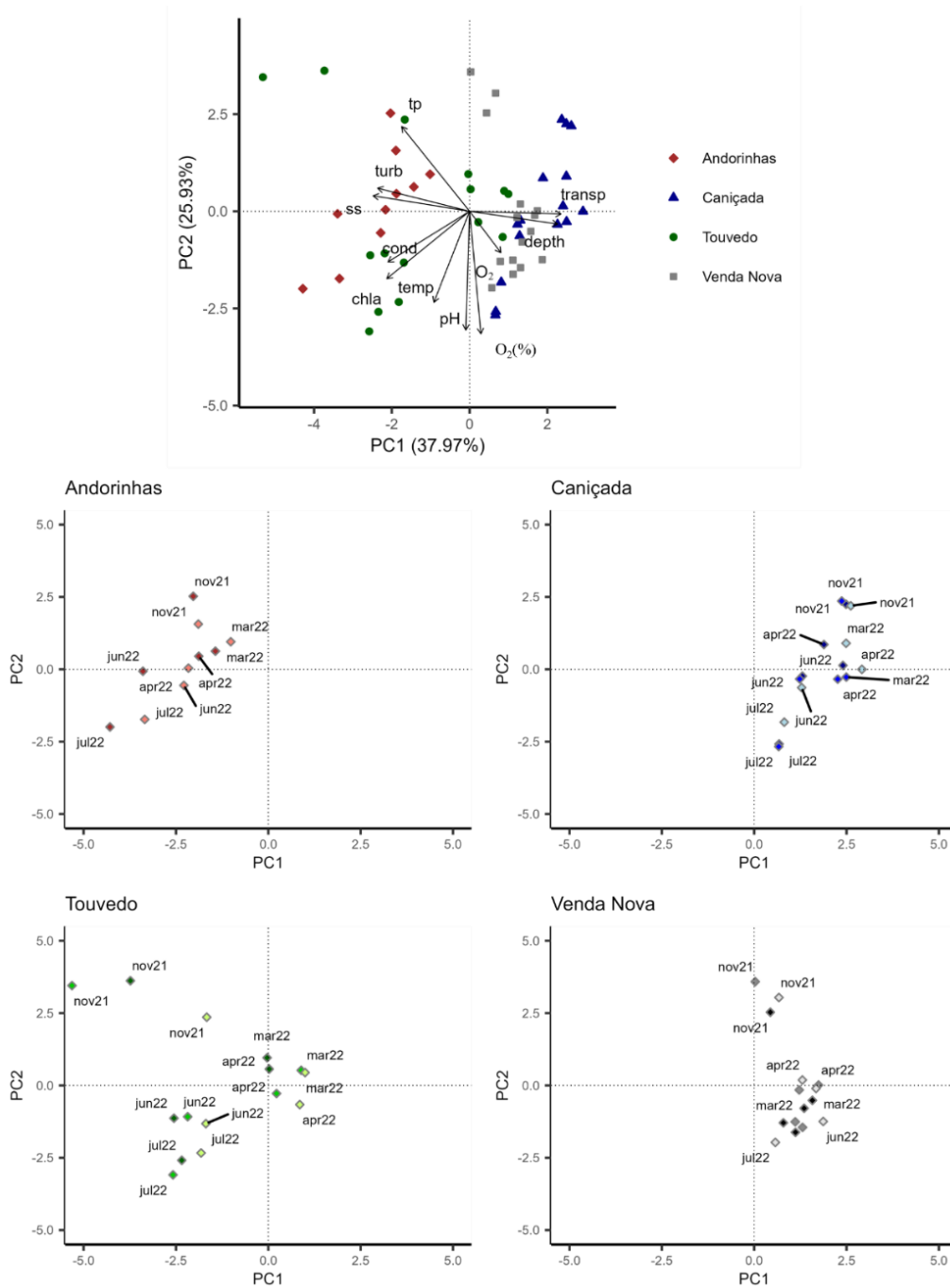


Figure 3.2 - PCA ordination based on the physicochemical parameters and chl *a*, showing environmental variables (arrows) and sample scores (geometric forms or symbols) in the upper panel or a more detailed insight of sample scores per reservoir (bottom panel). Each reservoir is represented with a different colour, while shades of colour represent the sampling sites in each reservoir, with the dark-to-light tones representing the upstream-downstream gradient.

Correlation analysis revealed some associations between measured abiotic and biotic variables (see full correlation matrix or correlogram in Supplementary Figure S1). Figure 3.3 illustrates the main significant associations found. Water transparency was negatively correlated with turbidity, suspended solids, and total phosphorus content, having a stronger and more evident association with suspended solids. Turbidity was positively correlated with total phosphorus content and chlorophyll *a*, and suspended solids also increased with increasing chlorophyll *a*. Chlorophyll *a* had no correlation with total phosphorus content and temperature (as well as suspended solids, as previously said). The only relevant correlations between plankton community metrics and environmental variables were the associations of zooplankton density with temperature and NMARSP with turbidity.

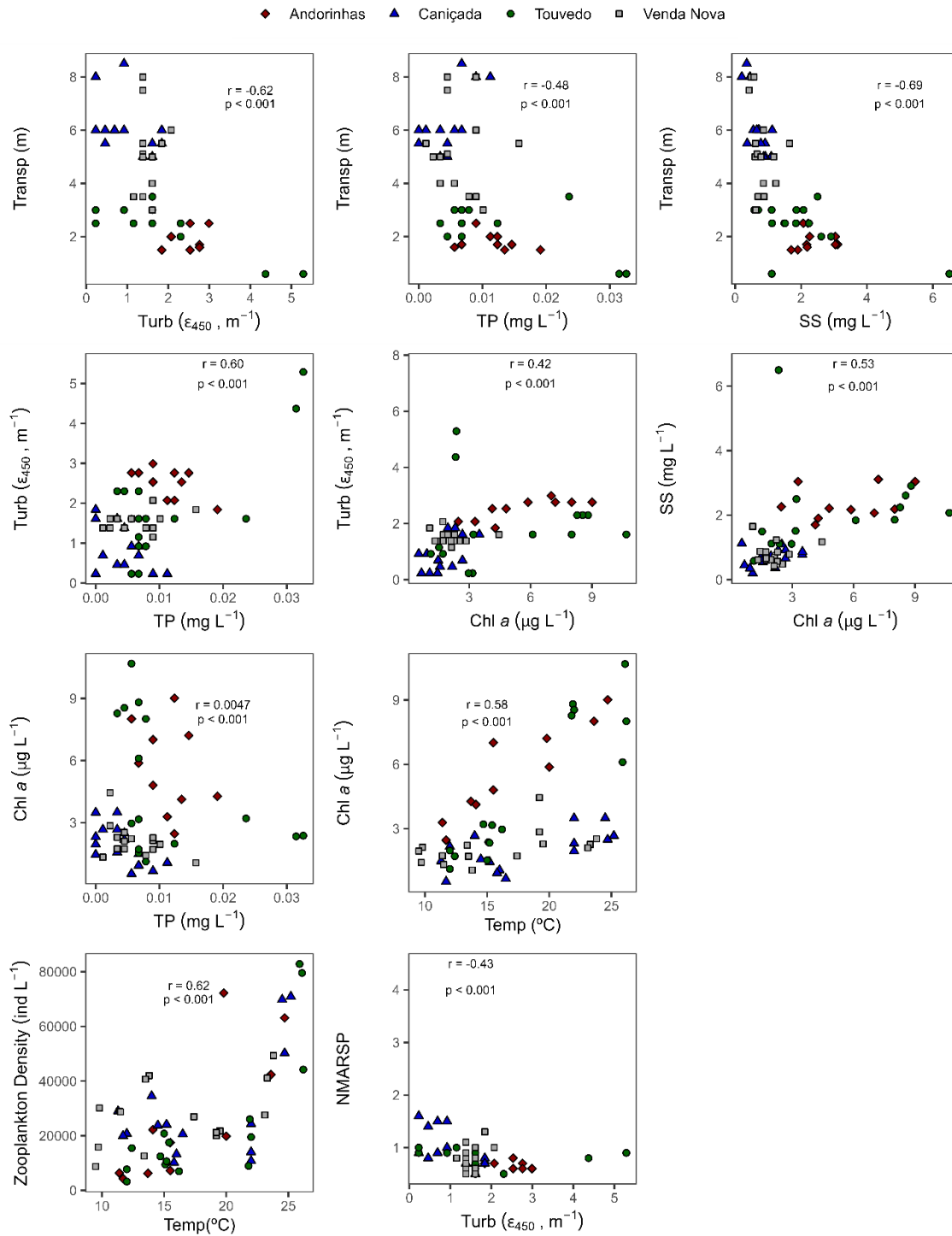


Figure 3.3 - Correlation (Pearson's r and respective significance, p) between the main biotic and abiotic variables measured in each reservoir throughout the sampling campaign. Each reservoir is represented with a different symbol and colour.

3.2 Zooplankton community

Zooplankton density (Figure 3.4) increased from early Spring towards the Summer, especially in Andorinhas and Touvedo, but was heterogeneous across reservoirs in November. In general, zooplankton density was comparable across sites within each reservoir. A low diversity was observed (mean $H' = 1.63$, mean $J' = 0.48$), which was consistent with the low number of taxa observed (≤ 8). In general, maximum diversity was attained in April; a similar bell-shaped pattern was observed in Caniçada and Venda Nova, with Shannon's diversity increasing throughout the Spring but decreasing during Summer.

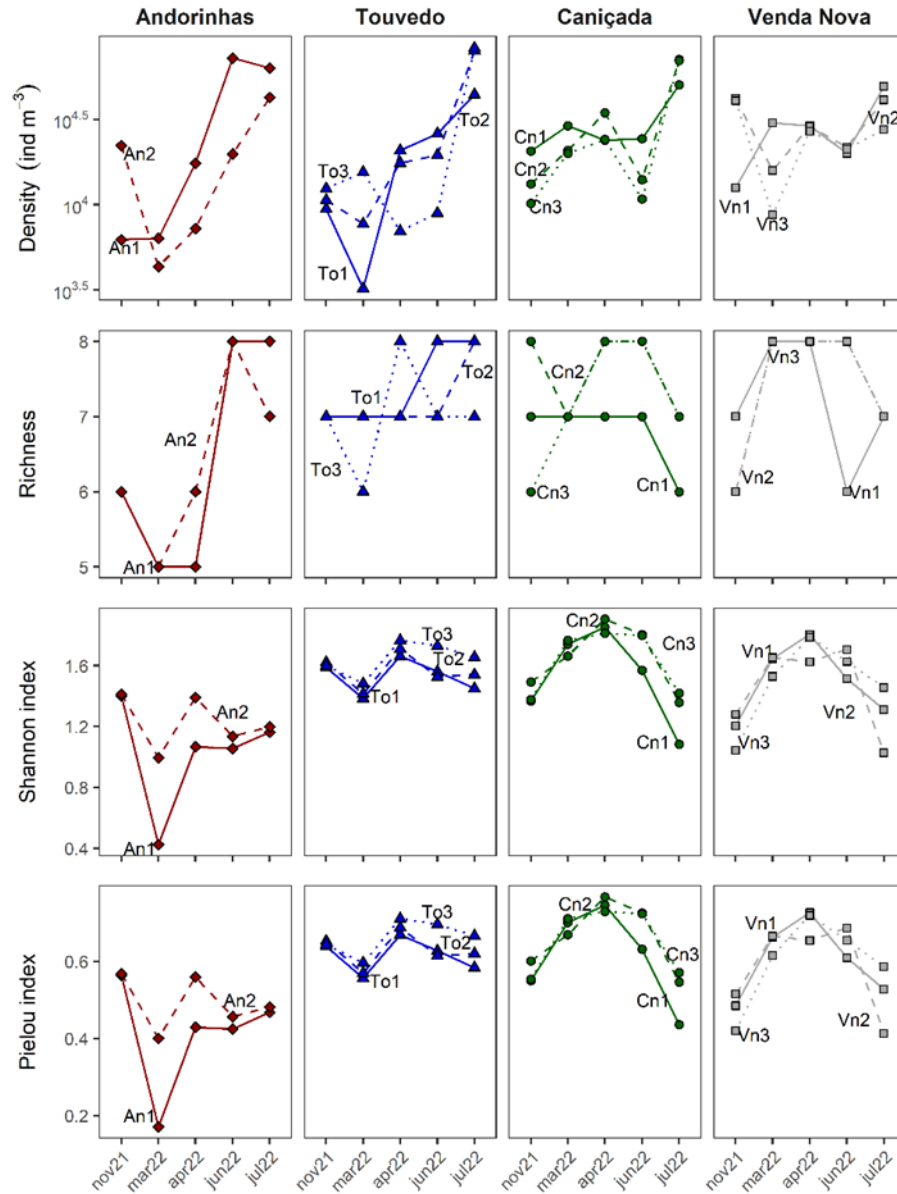


Figure 3.4 - Seasonal and spatial variation in total zooplankton density, richness, biodiversity (Shannon's H') and evenness (Pielou's equitability, J) in each reservoir. Different lines represent sampling sites within each reservoir (Andorinhas, An1–An2; Caniçada, Cn1–Cn3; Touvedo, To1–To3; Venda Nova, Vn1–Vn3) and sampling months (nov21 to jul22).

Seasonal and spatial variation in zooplanktonic taxa across reservoirs is shown in Figure 3.5. Calanoid copepods were present in all reservoirs throughout the whole sampling period; their relative proportion was generally stable throughout the study period, except in Andorinhas, which was dominated by Cyclopoida (which, in turn, were less important in Caniçada and Venda Nova).

Small cladocerans, mostly *B. longirostris* and *Ceriodaphnia* sp. were present in all reservoirs during the study; in Andorinhas, a marked seasonal trend was observed, with *Ceriodaphnia* dominating in November and July and *Bosmina* attaining high densities in late Spring (Apr and June). The species *H. gibberum* was present in all reservoirs except Andorinhas. Also, *D. brachyurum* appeared in the summer (June-July) in all reservoirs, demonstrating a stronger presence in Touvedo (June). Large cladocerans, represented by *D. longispina*, *D. pulicaria* and *S. crystallina*, displayed distinct patterns across reservoirs. The largest, *D. pulicaria*, was numerically important during spring (March and April) in deep reservoirs (Caniçada and Venda Nova), being replaced by *D. longispina* in late spring. On the contrary, *D. longispina* was important in Touvedo for most of the time, whereas in Andorinhas it was present only in November (and then absent for the rest of the study). *Syda cristallina* was usually present in low numbers, but important in terms of biomass (due to their large size) in November and July; this species did not occur on Andorinhas. *Daphnia parvula*, an exotic species, was present in Andorinhas for most of the time.

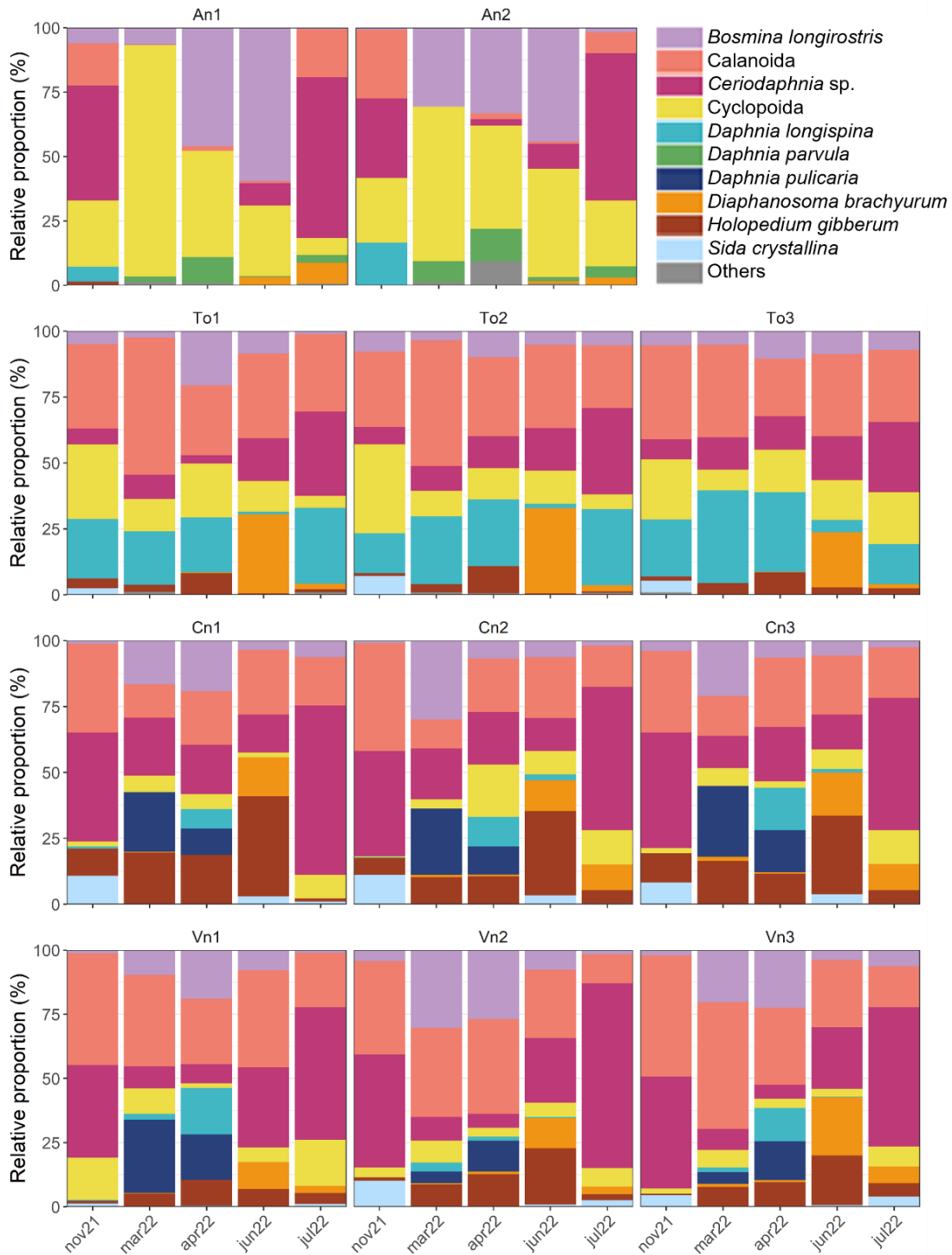


Figure 3.5 - Relative proportion of main zooplanktonic taxa in each reservoir (from top to bottom: An – Andorinhas, To – Touvedo, Cn – Caniçada, and Vn – Venda Nova) across sites (An1–An2, Cn1–Cn3, To1–To3, Vn1–Vn3) and sampling months (nov21 to jul22).

The ratio between Calanoida and Cyclopoida (Figure 3.6) was higher in the deepest reservoirs, and less subject to fluctuations in Andorinhas and Touvedo. An exceptionally high value of this ratio was observed in Cn2 in November because of a very reduced number of cyclopoid copepods. The ratio between large cladocerans and total cladocerans varied greatly throughout the study period, except in Andorinhas, which was very low most of the time (Figure 3.6). Whereas the proportion of large cladocerans in Touvedo was irregular, Caniçada and Venda Nova displayed a maximum in March-April (during the *Daphnia* dominance period) and a consistent decrease towards the summer.

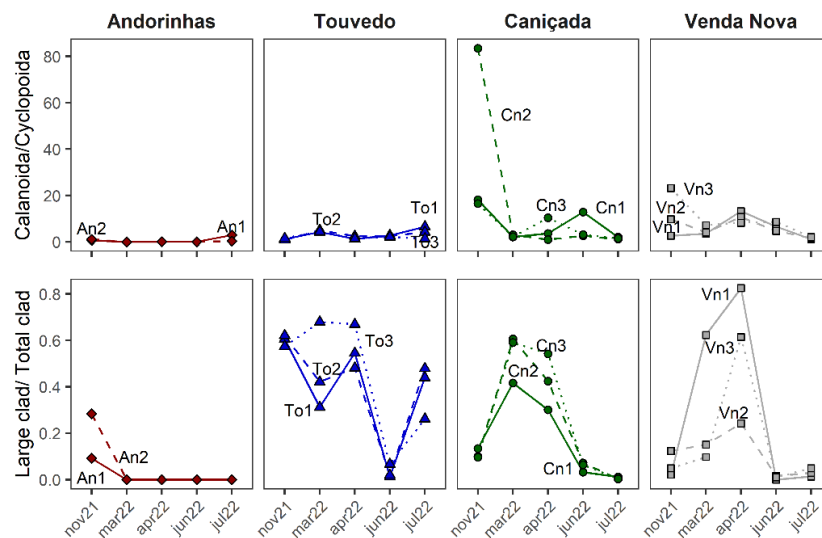


Figure 3.6 - Seasonal and spatial variation of zooplankton community metrics, namely Calanoida/Cyclopoida ratio (top panel) and proportion of large cladocerans (relative to total cladocerans; bottom panel) in each reservoir. Different lines represent sampling sites within each reservoir (Andorinhas, An1–An2; Caniçada, Cn1–Cn3; Touvedo, To1–To3; Venda Nova, Vn1–Vn3).

The composition of zooplankton communities was also evaluated through a redundancy analysis (db-RDA) represented globally in Figure 3.7. The physical and chemical variables explained a significant portion of the ecological distance among sites. This model explains 33% of the variation of similarity between zooplankton communities as a function of environmental variables and chl *a*. The first dimension of db-RDA can be interpreted as a spatial (inter-reservoir) gradient, being strongly related to overall trophic status (conductivity, phosphorus content, turbidity, chl *a*), which increased from right to left, in opposition to depth and transparency, which

increased from left to right. Deeper and more transparent reservoirs (Caniçada and Venda Nova) were characterized by lower trophic status when compared to shallower reservoirs (Touvedo and Andorinhas), and this gradient was associated with higher densities of cyclopoid copepods and small cladocerans (to the left) and *Holopedium gibberum* (to the right). Sample scores from Caniçada and Venda Nova were very close, almost indistinguishable, but clearly separated from Andorinhas; Touvedo occupied an intermediate position between these two extremes, although closer to Andorinhas. The second dimension of db-RDA can be interpreted as a seasonal variation, being strongly related to seasonal changes in temperature variation and dissolved oxygen (opposing gradients). In terms of the zooplanktonic community, the separation of sample scores is driven by species associated to warmer months (*D. brachyurum*, *Ceriodaphnia* sp., *S. cristallina*) – see June, July and November scores in the bottom half of the ordination diagram for all reservoirs (Figure 3.7, bottom panel). In opposition, Spring samples display higher values in the second ordination axis, which is especially noticeable in Caniçada and Venda Nova, and is associated with higher density of *D. pulicaria* in March and April samples.

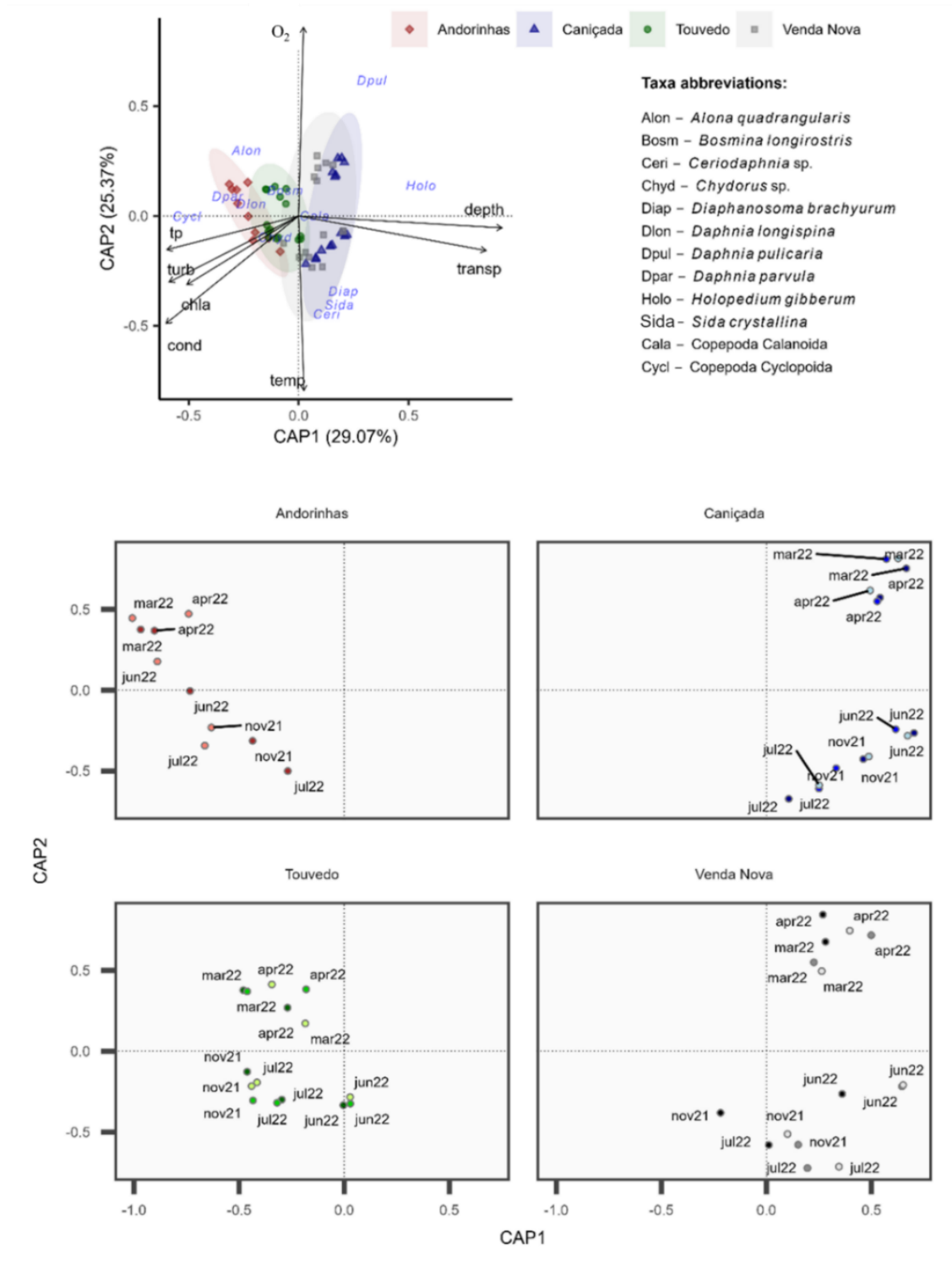


Figure 3.7 - db-RDA ordination based on the zooplanktonic communities (taxon scores in blue) constrained by the environmental context (physicochemical parameters and chl *a*, represented by arrows). Sample scores (represented by geometric forms or symbols) are shown along zooplankton taxa and environmental data (upper panel) and in more detail per reservoir (bottom panel). Each reservoir is represented with a different colour, while shades of colour represent the sampling sites in each reservoir, with the dark-to-light tones representing the upstream-downstream gradient.

3.3 Phytoplankton community and ecological potential

Phytoplankton density (Figure 3.8) had some fluctuations seasonally and spatially in all reservoirs but was overall higher during the Summer. The phytoplankton of the reservoirs under study was not very diversified (H' mean=1.63, J' mean= 0.48), as the number of taxa observed was frequently less than 14. Richness tended to increase throughout the season, peaking in the Summer, with the exception of Venda Nova, where richness was overall low with a maximum in April.

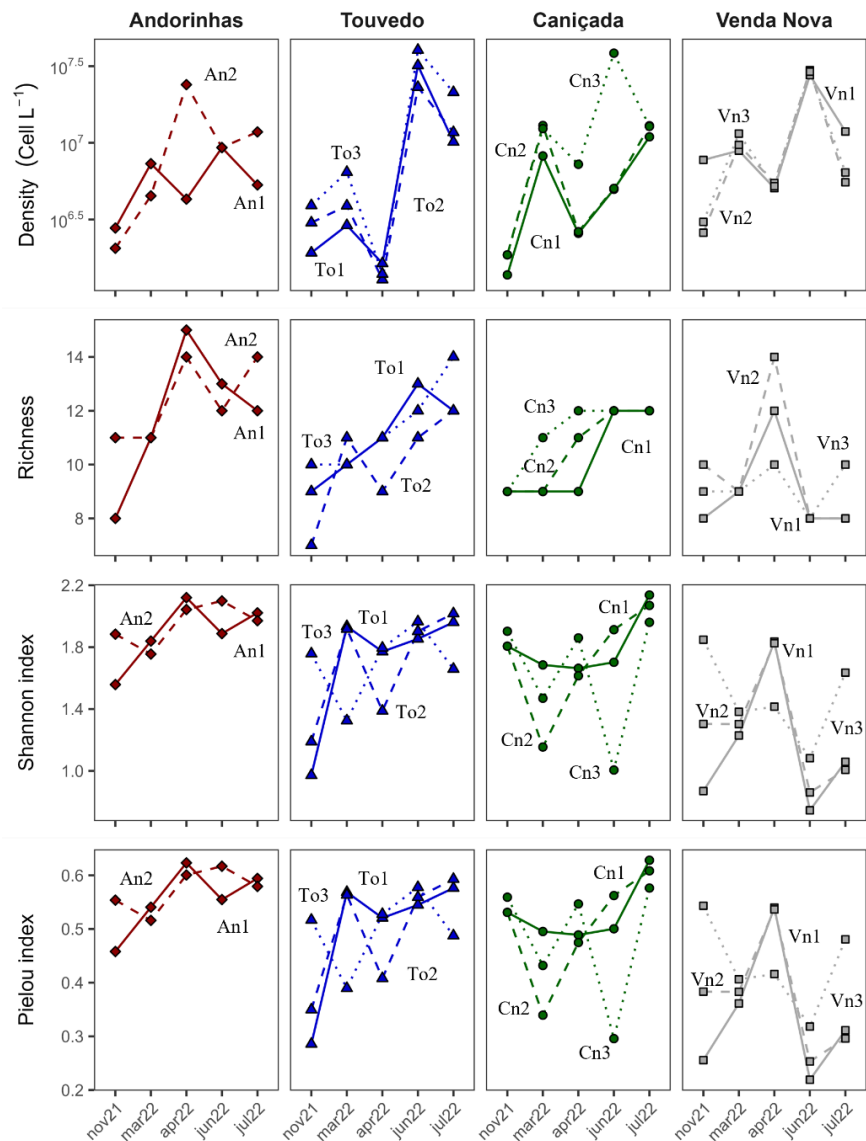


Figure 3.8 - Seasonal and spatial variation in total phytoplankton density, richness, biodiversity (Shannon's H') and evenness (Pielou's equitability, J') in each reservoir. Different lines represent sampling sites within each reservoir (Andorinhas, An1–An2; Caniçada, Cn1–Cn3; Touvedo, To1–To3; Venda Nova, Vn1–Vn3) sampling months (nov21 to jul22).

Seasonal and spatial variation of the composition of phytoplankton communities is represented in Figure 3.9. Bacillariophyceae (colonial and non-colonial) and Chlorococcales (colonial and non-colonial) were present in all reservoirs throughout this study, often dominating the community. Most reservoirs displayed a strong dominance of only two or three groups of algae, although this was less apparent in Andorinhas (in some months, dominance was shared across various groups). In general, the relative proportion of Bacillariophyceae was less pronounced in deeper reservoirs (Caniçada and Venda Nova) and stronger in shallower reservoirs (Touvedo and Andorinhas). On the contrary, Caniçada and Venda Nova were dominated by Chlorococcales (mostly colonial forms) and, in some periods, Colonial Chrysophyceae were also important. From April to July, Dinophyceae were observed in all reservoirs. Colonial Volvocales were abundant in Venda Nova in November. Little variation across sampling sites (within-reservoir variability) was observed.

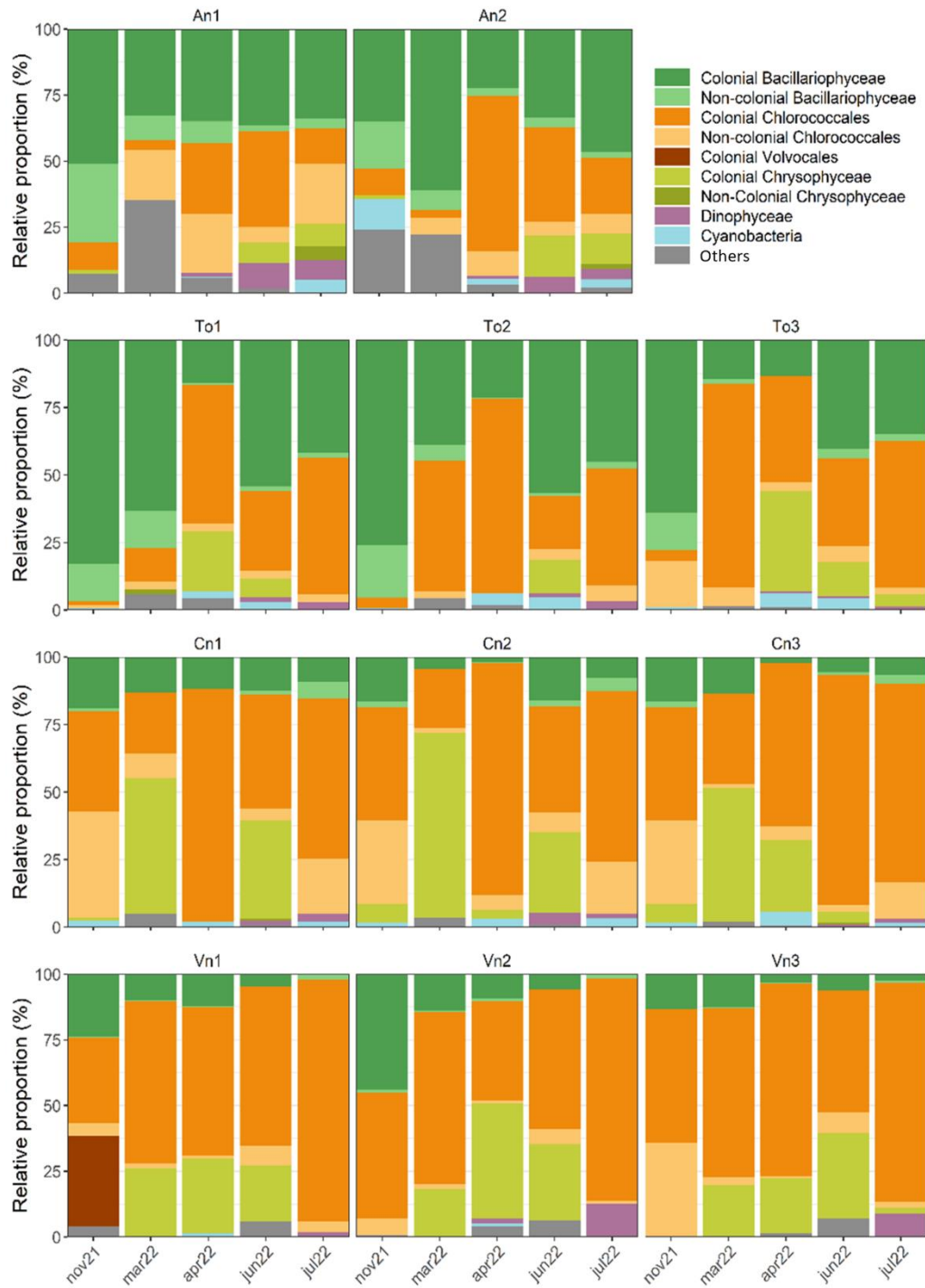


Figure 3.9 - Relative proportion of main phytoplanktonic taxa (IGA algal groups) in each reservoir (from top to bottom: An – Andorinhas, To – Touvedo, Cn – Caniçada, and Vn – Venda Nova) across sites (An1–An2, Cn1–Cn3, To1–To3, Vn1–Vn3) and sampling months (nov21 to jul22).

According to the WFD guidelines, for Northern Reservoirs of Portugal, the results obtained in this study show a slight variation in the EQR values among the studied reservoirs. All metrics values were between maximum and good in these reservoirs, having few cases below good (Figure 3.10), classifying all studied reservoirs with maximum or good ecological potential. The lowest value for Total Biovolume was $0.3 \text{ mm}^3 \text{ L}^{-1}$ for Caniçada in Autumn and spring (Cn1 and Cn2), while the highest was $19.9 \text{ mm}^3 \text{ L}^{-1}$ for Touvedo in Summer and $19.3 \text{ mm}^3 \text{ L}^{-1}$ for Andorinhas in the same period. The biovolume of cyanobacteria showed the highest value of $3.4 \text{ mm}^3 \text{ L}^{-1}$ for Touvedo in Summer. The highest values for IGA were registered in Caniçada 136.2 in Spring. Caniçada scored the highest NMARSP index value (1.2 in Autumn) and Touvedo had the lowest values (0.4 in Summer).

The composition of phytoplankton communities was also evaluated through a redundancy analysis (db-RDA) represented globally in Figure 3.11. The physical and chemical variables explained a lower portion of the ecological distance among sites (17%) compared to the zooplankton. The first dimension of db-RDA was related to overall trophic status (conductivity and phosphorus content), which increased from right to left, in opposition to transparency, which increased from left to right. This segregated Andorinhas scores (on the left, being more productive and less transparent) from Caniçada and Touvedo (on the right, being less productive and more transparent), with Touvedo occupying an intermediate position (albeit closer to Andorinhas). This trophic status was associated with higher densities of centric and pennate diatoms, as well *Aulacoseira* sp. in shallower reservoirs; on the opposite end, deeper and less productive reservoirs were associated with *Staurastrum* sp.

The second dimension of db-RDA revealed seasonal variation, showing an association of some taxa (*Peridinium* sp., colonial Chlorococcales, Cyanobacteria) with the increase of temperature and decrease of dissolved oxygen (mostly June and July samples); opposing that, the density of *Scenedesmus* sp. increased (April and March, but also November samples). As observed in the PCA (for environmental variables) and the db-RDA (for zooplankton community), deeper reservoirs (Caniçada and Venda Nova) shared common dynamics and were distinct from the shallower reservoir (Andorinhas); in this case, Touvedo shared features with both types of reservoir, which were not this evident in the previous datasets (environmental and zooplankton).

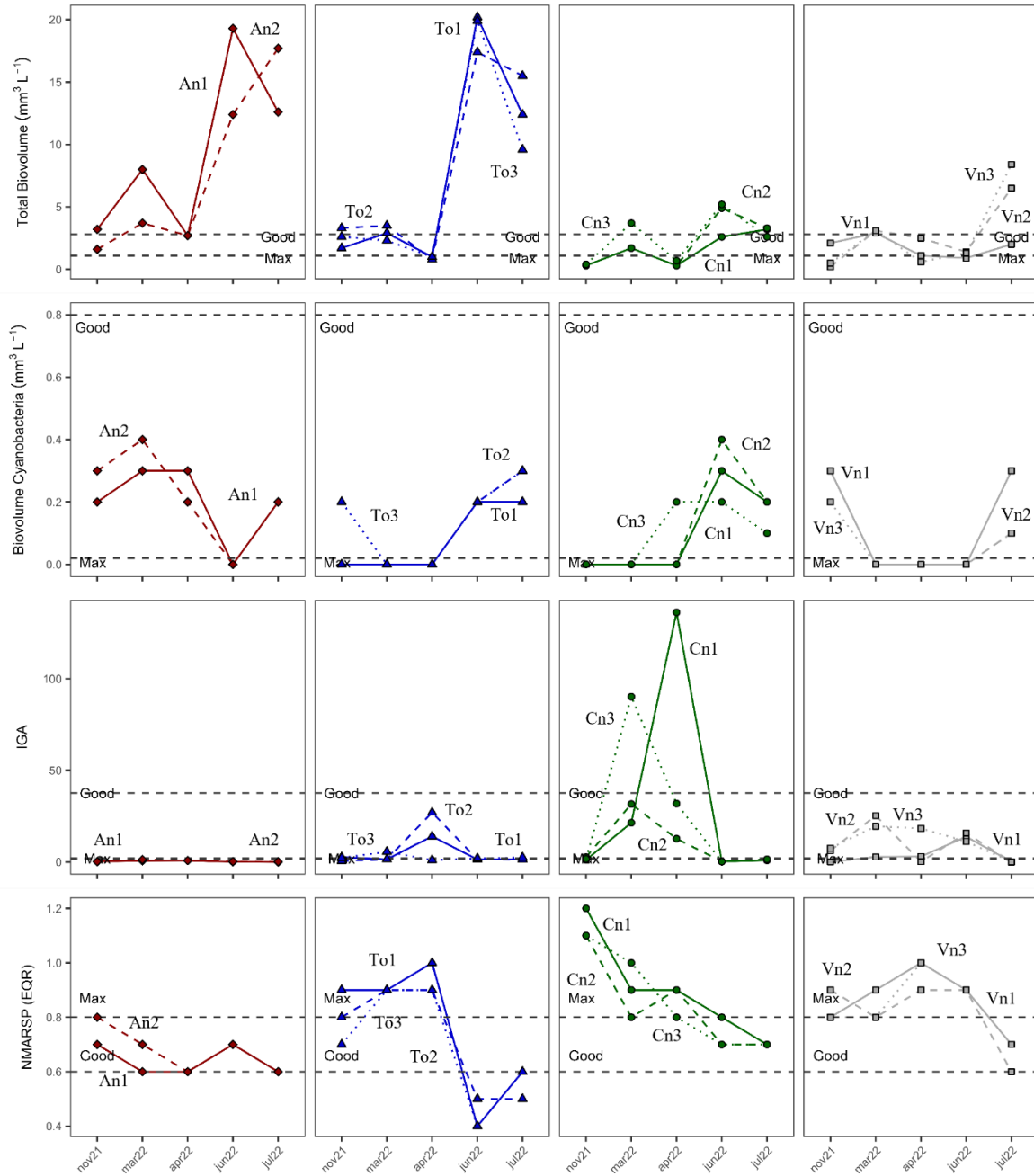


Figure 3.10 - Seasonal and spatial variation of the four phytoplankton composition metrics: Total biovolume ($\text{Max} \leq 1.20$ and $1.20 < \text{Good} \leq 2.80$); % Biovolume of cyanobacteria ($\text{Max} \leq 0.02$ and $0.02 < \text{Good} \leq 0.80$); IGA (Index group algae) ($\text{Max} \leq 2.00$ and $2.00 < \text{Good} \leq 37.60$) and NMARSP index ($\text{Max} \geq 0.80$ and $0.80 > \text{Good} \geq 0.60$). Different lines represent sampling sites within each reservoir (Andorinhas, An1–An2; Caniçada, Cn1–Cn3; Touvedo, To1–To3; Venda Nova, Vn1–Vn3) and sampling months (nov21 to jul22).

4. Discussion

The main objective of this work was to evaluate the seasonal and spatial variation of the phytoplankton community in four reservoirs of the Minho region, as well its bioindicator value, linking it to the zooplankton community. This study followed the previous work of Machado (2022), who had studied the zooplankton dynamics of these semi-artificial systems. The reservoirs chosen for this project are subjected to very low anthropogenic disturbance (other than its artificiality) and surrounded by forest cover in its margins (Cabecinha, et al., 2009a; Cabecinha, et al., 2009b) and are characterized for having low water mineralization. Therefore, they were anticipated to have acceptable water quality and exhibit little disturbance, except from those brought on by the dam and sporadic runoffs from the surrounding land. According to OECD (1982), these reservoirs are oligo-mesotrophic considering the TP ($\leq 0.02 \text{ mg L}^{-1}$, on average), the chl *a* ($3.4 \mu\text{g L}^{-1}$, on average) and transparency (4.1 m, on average).

By following the rules for each parameter, WFD suggests using the predetermined criteria of physical, chemical, hydromorphological and biological factors to evaluate the ecological status of a waterbody (Navarro et al., 2009). The WFD approach represents an advancement over currently available methods for aquatic environment monitoring and conservation. Since evaluating the waterbody's quality demands a multidisciplinary approach, a deeper comprehension of the ecosystem's structure and functions is also necessary. Nevertheless, the bioassessment scheme implemented in Portugal within the WFD only requires evaluating phytoplankton—the key primary producer in aquatic ecosystems—when it comes to reservoirs. WFD for water classification does not include consumers like fish (although work is ongoing, but metrics lack intercalibration) and zooplankton. However, zooplankton is a crucial primary consumer in every aquatic ecosystem and is extremely vulnerable to changes in the ecosystem (Azevêdo et al., 2015; Neto et al., 2014). With that in mind, some authors have discussed the possibility of the use of zooplankton as a biological quality element for the evaluation of the Ecological Potential of the water bodies proposed in the WFD (Caroni & Irvine, 2010; Jensen et al., 2013; Jeppesen et al., 2011) and they suggest its inclusion in the Biological Quality Elements, namely for “Heavily Modified Water bodies”. For this reason, our study addresses both communities and their potential complementary role as bioindicators.

4.1 Physicochemical parameters and chlorophyll *a*

Through multivariate analysis of the environmental data, it was possible to observe that waterbody quality fluctuated according to a seasonal pattern. Dissolved O₂ (in mg/L), which was inversely related to the rise in water temperature, was one of the most variable parameters, given that decreased O₂ concentrations are typically observed during warmer months (Çelekli & Öztürk, 2014). The peak in concentration of total phosphorus was verified in November, occurring simultaneously in all reservoirs, and being associated with the increase of rainfall registered in October (the season's first rains after a dry summer), leading to runoff events. The magnitude of runoff events and rainfall amount could be the main factors controlling phosphorus concentration in freshwater (Rodríguez-Blanco et al., 2012). In addition to this, the presence of senescent macrophytes was noticeable in November, especially in Touvedo. Aquatic macrophyte decomposition has a significant impact on the flow of energy and nutrient in aquatic ecosystems (Masifwa et al., 2004; Shilla et al., 2006). When organic matter is broken down, inorganic elements are also released, including nutrients that were chemically incorporated into plant tissues (Shilla et al., 2006). The concentration of nutrients in the water increases when nutrients are released (Godshalk & Barko, 1985; Howard-Williams & Allanson, 1981). The presence of decomposing macrophytes, in Touvedo, may have been a significant influence in the high levels of turbidity.

Depth was the main factor differentiating the Caniçada reservoir (average = 42 m) and Venda Nova (37 m) from the Touvedo (15 m) and Andorinhas (8 m) reservoirs. Depth may also be the main reason for the observed differences in phytoplankton biomass and zooplankton, as the lower depth of reservoirs such as Touvedo and Andorinhas facilitates the resuspension of nutrients present in the sediment. Because these reservoirs are smaller and shallower, nutrient exchange between the sediments and surrounding waters is facilitated by water mixing (Matias & Boavida, 2005). Moreover, the top-down control from planktivorous fish is more pronounced in shallow reservoirs (Jeppesen et al., 1997). Zooplankton, in order to avoid predators, migrates vertically into colder, darker, hypolimnetic waters during the day, a behaviour that has been observed to be valuable in deep reservoirs (Petzoldt et al., 2009; Ersoy et al., 2019). Since shallow lakes do not stratify for very long, they frequently lack a hypolimnetic refuge (Burks et al., 2002). As so, the fish preferable predation of large body herbivorous zooplankton, like *Daphnia* (Brooks & Dodson, 1965), interferes indirectly with controlling the biomass of phytoplankton (Jeppesen et al., 1997).

The comparison of the physical and chemical values in the analysed reservoirs with the WFD standards for the Good Ecological Potential reservoirs in the Northern Mediterranean allowed us to consider all our samples as Good. Environmental data evaluated by the Laboratory of Environmental and Applied Chemistry (LABELEC) considered that these reservoirs are references for Good Ecological Potential (Cabecinha, et al., 2009a), except for the Andorinhas reservoir, that was not covered by their study. Our results are in concordance with the data gathered throughout their sampling period (between 1996 and 2004) for pH, dissolved oxygen, total phosphorus, and chlorophyll *a* content. For the Andorinhas reservoir, our results are consistent with the data evaluated by Machado (2022) and Lima (2021), demonstrating that the water quality of these reservoirs has been overall good and constant over the previous few years.

The analysis of the physical and chemical parameters, both independently and in the context of the WFD, show that all the reservoirs were remarkably stable over the course of the sampled year, with no notable changes in the water quality or major disturbances brought on by changes in the dam's usage or the reservoirs themselves. Curiously, we observed *in situ* large fluctuations in water level, resulting from the interlinked hydroelectrical activity of these reservoirs and others in the same catchment, but these did not translate into measurable changes in their abiotic context or phytoplankton biomass. Nonetheless, there was some variation between reservoirs, seasonal fluctuations within reservoirs, but reduced spatial variation within each reservoir when it comes to physicochemical parameters and chlorophyll *a*. However, it is reasonable to deduce that certain modifications to the ecosystem's structure happened based on the phytoplankton and zooplankton community data.

4.2 Zooplankton

In terms of zooplankton communities, there were differences between reservoirs, some seasonal variations – particularly in the deeper reservoirs – but reduced spatial variation within reservoirs. The taxonomic richness and community composition were typical of zooplankton from Mediterranean reservoirs that are oligotrophic and mesotrophic (Sellami et al., 2010). As was observed for the environmental parameters, the depth of the reservoirs has an impact on the composition of the zooplankton groups. Deeper reservoir communities are primarily represented by larger cladocerans like *Daphnia longispina* and *Daphnia pulicaria* (present exclusively in

deeper reservoirs), as well as *Ceriodaphnia* sp., *Holopedium gibberum* and calanoid copepods. In turn, smaller-sized *Bosmina longirostris*, *Daphnia parvula* (present only in Andorinhas) and cyclopoid copepods make up the communities of the shallower reservoirs. *Daphnia longispina* was also important in Touvedo, demonstrating the intermediate position of this system between the deeper ones (Caniçada and Venda Nova) and the shallower and more productive Andorinhas.

Temperature has a significant impact on the seasonal succession of the zooplankton community (Geraldes & Boavida, 2004). In Summer, reservoirs are normally dominated by small zooplankters (Geraldes & Boavida, 2006) and our results confirm this with the dominance of *Ceriodaphnia* in almost all reservoirs in July, accompanied by the decrease of *Daphnia*, especially in deeper reservoirs. The summertime replacement of *Daphnia* by *Ceriodaphnia* could be linked to *Ceriodaphnia*'s superior ability to feed at temperatures over 20°C (Lynch, 1978). Nevertheless, zooplankton assemblage characteristics can also result from fish predation in addition to low disturbance and temperature, since large zooplankton are particularly vulnerable to fish predation, according to numerous studies (Caramujo et al., 1997; Lampert & Sommer, 1997; Sellami et al., 2010) and increasing predation pressure as temperature rises (Geraldes & Boavida, 2004). It is feasible to pinpoint this period, by examining the ratio of large cladocerans to the total cladocerans (Figure. 3.6), as it is distinguished by the practically complete extinction of *Daphnia* sp. from June to July in deeper reservoirs. As a warm-water species that is primarily restricted to mid- or late-summer in seasonal temperate lakes, *Diaphanosoma* was only seen throughout the summer (Hart, 2000), similarly to what was observed in Machado (2022) in the same reservoirs.

In some reservoirs, *Sida crystallina* was a very important component of the zooplanktonic community, which is uncommon in non-littoral environments. Macrophyte biomass typically has a high connection with *S. crystallina* (Choi et al., 2014; Fairchild, 1981). However, in this study, *S. crystallina* was found in reservoirs (Caniçada and Venda Nova) without macrophytes. *Sida* may also enter the pelagic zone as a result of wind agitation, the presence of different floating objects at different times of the year, such as autumn leaves that fall into the water (*Sida* had higher density during November), and other factors (Korovchinsky, 1986). The influence of human activity could also be the reason for the establishment of *Sida* in Caniçada, since pontoons, recreational water vehicles, and buoys remain in the water all year round throughout the entire reservoir. The presence of these objects can serve as shelter for *S. crystallina* as replacement of emergent macrophytes that

are the preferential habitat of this species. A similar explanation was provided by Machado (2022) to explain this abnormal dominance of *S. crystallina* in a pelagic environment without macrophytes.

The presence of *Holopedium gibberum* in the four reservoirs was also a distinctive feature of the studied waterbodies, which may be caused by the low water mineralization (Hessen et al., 1995; Jeziorski et al., 2014). Large cladocerans, such as *Daphnia*, have demanding requirements of water hardness due to their highly calcified carapace, which is supported by a dense network of phosphorus-rich cells (Beaton & Hebert, 1989). On the other hand, *Holopedium* has a gelatinous capsule instead of a carapace, and it is not dependent on calcium (Jeziorski & Yan, 2006) and phosphorus (Andersen & Hessen, 1991; McCarthy & Irvine, 2010) as other zooplankters. Allied to this, *Holopedium*'s resistance to macroinvertebrate predators may also contribute to its success in the reservoirs under study (Allan, 1973; Wissel et al., 2003). The consistent presence of *Holopedium* in all four reservoirs were also reported by Machado (2022).

Along with small cladocerans (*Bosmina* and *Ceriodaphnia*), the cyclopoids were found in all reservoirs, although they were more prevalent in Andorinhas, as also noted in Machado (2022). Numerous studies (Kerfoot & Peterson, 1980; Andrade & López, 2005; Sakamoto & Hanazato, 2008) have already discussed the interactions between cyclopoids and small cladocerans like *Bosmina* and *Ceriodaphnia*. According to some authors (Błędzki & Ellison, 2000; Geraldés & Boavida, 2006), *Ceriodaphnia* sp. and cyclopoids have adaptive advantages in disturbed conditions because of their feeding habits and r-strategy, thus having shorter generation times and a greater tolerance to large amounts of organic matter in the water. This supports the findings of other authors (Ejsmont-Karabin & Karabin, 2013; Karabin, 1985), which demonstrate that eutrophication resulted in a rise in the total biomass of Cyclopoida. The biomass of Cladocera decreases as trophic state increases, since species with small body sizes predominate in the assemblage. In contrast, calanoids were less present in Andorinhas reservoir but strongly represented in deeper reservoirs (Caniçada and Venda Nova). Calanoids can suppress cyclopoid populations by reducing the abundance of other cyclopoid prey (e.g., rotifers) (Soto & Hurlbert, 1991). Alongside with the low presence of vegetation like macrophytes on these reservoirs, this could explain the reduced population of cyclopoids compared to calanoids (Fairchild, 1981; Goulden, 1971).

Large cladocerans, and particularly *Daphnia* sp., are very important in zooplankton dynamics and in the clearance rate of the community, which is key in controlling phytoplankton and maintaining water transparency, which obviously links to the bioindicator role of these taxa. Among the observed taxa in our study, *D. pulicaria* is one of the largest species in lentic ecosystems with fish, but its abundance can change significantly from year to year (Einsle, 1988). In 2020/2021, *D. pulicaria* was absent from the studied reservoirs, as observed by Machado (2022), and our results show the presence of *D. pulicaria* only a year after this study. Large-bodied *D. pulicaria* may be the preferred food source of zooplanktivorous fish (Cerny & Bytel, 1991). A study on the distribution and abundance of *Daphnia pulicaria* in Lake Constance concluded that the species is present in some years, but non-existent or very nearly so in other years (Stich & Maier, 2007). Most likely, fish predation pressure has a significant role in controlling the growth of the population. In accordance with Stamou et al. (2021), changes in diverse functional groups, such as daphnids, bosminids, and chydorids should be closely monitored because they use a variety of feeding strategies (Geller & Müller, 1981) that reflect changes in other communities, thus offering essential data for the functioning of the food web (Jeppesen et al., 2011). Steps should be taken in this sense, and functional metrics of the zooplankton community should be pursued to satisfy the pleas of Jeppesen et al. (2011) and García-Chicote et al. (2018).

As stated above, the high pressure from planktivorous fish is a significant factor relating to the zooplankton community, having a significant impact on its abundance, biomass, community composition, and even the size structure, especially in low productive waters (Jeppesen et al., 2004; Bruce et al., 2010). The importance of zooplankton as an indicator of ecological conditions derives from their position in the food chain, placed between top-down regulators (fish) and bottom-up factors (phytoplankton). This gives them the opportunity to reveal the relative significance of top-down and bottom-up control and their effects on water clarity (Jeppesen et al., 2011).

4.3 Phytoplankton

The taxonomic richness and community composition of phytoplankton described in the study were characteristic of oligotrophic and mesotrophic Mediterranean reservoirs (Cabecinha et al., 2009a; Çelekli et al., 2014;). The depth of the reservoirs influenced the composition of these

communities, as also observed for environmental parameters and zooplankton. The depth of a water body can significantly influence the composition of phytoplankton communities. The distribution and abundance of phytoplankton species are often stratified in response to the vertical variations in light availability, temperature, nutrient concentrations, and water movement, all of which change with depth. Different phytoplankton species have varying adaptations to these vertical variations. Understanding how these factors interact is essential for studying and managing aquatic ecosystems effectively. In shallower reservoirs, colonial Bacillariophyceae were the dominant groups, while in deeper reservoirs the colonial Chlorococcales dominated. Moreover, in deeper reservoirs colonial Chrysophyceae were often present, with its main representative being *Dinobryon* sp. and with the main biovolume peaks occurring between spring and early summer. This was expected considering the preference of *Dinobryon* spp. for cold oligotrophic waters (Rawson, 1956; Çelekli & Kulköylüoğlu, 2007), since Caniçada and Venda Nova meet these requirements with temperature <20°C, Secchi depths > 5 m and low content of Chl *a* and TP between that period. As stated, these reservoirs were considered oligo-mesotrophic and the phytoplankton biomass and dominant taxonomic groups were comparable to a small Mediterranean lentic oligo-mesotrophic waterbody (Entrepeñas reservoir, Pareja, Spain) (Molina-Navarro et al., 2012, 2014).

According to some authors (Smol & Stoermer, 1999; Reynolds, 2006), diatoms are a good indicator of the trophic condition of the water. Andorinhas and Touvedo had higher density of diatoms during the study, as also found in other Mediterranean water bodies where this group was prominent (Moreno-Ostos et al., 2008; Hoyer et al., 2009; Molina-Navarro, Eugenio et al., 2012). One of the most significant phytoplankton variations between deeper and shallower reservoirs was the species mix and diatom abundance. The main diatom species in the shallower reservoirs were *Aulacoseira* sp. *Tabellaria* sp., whereas *Fragilaria* sp. and *Asterionella* sp. dominated the diatom community in deeper reservoirs. In oligo-mesotrophic or mesotrophic environments, *Tabellaria* sp. and *Asterionella* sp. are frequent and commonly found together (Eloranta, 1995; Negro et al., 2000), or they become the dominant species as nutrient supply increases (Olsén & Willén, 1980). This was the case in Touvedo, where *Tabellaria* sp. became dominant between November and March along with *Asterionella* sp. and *Aulacoseira* sp. (when the highest values of total phosphorus occurred).

Occasionally, dinoflagellates were also present in our samples. *Peridinium* sp. (Dinophyceae), being associated with summer epilimnia in mesotrophic lakes, appeared at the end of summer in all studied reservoirs (Reynolds et al., 2002; Padisák et al., 2009). Although the studied water bodies are considered oligo-mesotrophic, the shallower reservoirs were closer to a meso-eutrophic state, especially in the Summer. The dinoflagellate *Ceratium* sp. was only present in Andorinhas reservoir during the month of July, since this species is associated with summer epilimnia in eutrophic lakes, which was the case for Andorinhas in that month, with the lowest Secchi depth (< 2 m) and highest Chl *a* content (> 7.9 $\mu\text{g L}^{-1}$) (Reynolds et al., 2002; Padisák et al., 2009). The trophic state instability found in Andorinhas reservoir may be attributed to its small size, since smaller reservoirs are more susceptible to changes brought on by climatic variations and human activities, and, as a result, are more exposed to alterations in the phytoplankton community (Padisák et al., 2003). In fact, Andorinhas showed a much richer phytoplankton community throughout the study period when compared to larger and deeper reservoirs.

According to the WFD, the classification of the waterbody is carried out taking into account the Ecological Quality Ratio (EQR), for the specific biological elements of each mass of water, although, in the Portuguese case, this is limited to phytoplankton community in reservoirs (highly modified water bodies). Thus, and according to the EQR obtained for the biological indicator phytoplankton, the four reservoirs were classified as having Maximum or Good Ecological Potential throughout the study. Only Touvedo got classified as Reasonable in June, which could be due to the high values of chlorophyll *a* and total biovolume. Andorinhas reservoir had good ecological potential during the entire study. Caniçada reservoir registered the highest IGA values, and the reason for that could be the high density of Chlorococcales in the reservoir. Contributions from Dinophyceae and non-colonial phytoplankton groups are scored in IGA as promoters of good ecological potential, while cyanobacteria and colonial species are viewed as indicators of worse conditions. High biovolume proportions of non-toxic Chroococcales are therefore given the same negative weight as toxic filamentous cyanobacteria (Galvão et al., 2012). With this limitation in mind, the incorporation of additional metrics or descriptors, such as zooplankton, needs to be taken into consideration for a more precise assessment.

4.4 Phytoplankton-zooplankton nexus

The community of zooplankton responds efficiently to changes in the ecosystem physical and chemical characteristics as well as to cascades of trophic events, such as phytoplankton blooms, or fish community alterations (stocking, mass mortality). The abiotic changes, which are reflected seasonally, inevitably have an impact on species richness and density, and encourage changes in diversity and evenness as shown in our results. One of the key ecological processes in reservoir ecosystems is the top-down control of phytoplankton by planktivorous fish and zooplankton. This process helps to maintain the dynamic balance between the alternative stable states of clear water and phytoplankton-dominated turbid water (Scheffer et al., 1993). Water usage demands increase in summer (from agriculture, industry, and urban areas), leading to a higher water renewal (García-Chicote et al., 2018). This characteristic, along with the rise in temperature, may encourage the growth of smaller cladocerans, like *Bosmina* spp., with shorter life cycles. In summer, reservoirs have been shown to be dominated by smaller zooplankters (Geraldes & Boavida, 2006), which was also the case in our results with the dominance of *Bosmina longirostris* and *Ceriodaphnia* sp.. Such small species cannot control phytoplankton biomass nor keep the water transparent.

Between autumn and spring, when all reservoirs were classified with a good water quality, our analysis revealed low zooplankton abundances. Similar results were obtained in Vela lake (Abrantes et al., 2006) and in Torrão reservoir (Pinto et al., 2023). These results can also be explained by the decline in phytoplankton density and the resulting reduction in the availability of food. When thinking about top-down impacts, it is necessary to keep in mind that zooplankton can significantly alter phytoplankton populations (Naselli-Flores & Rossetti, 2010). This information is crucial when conducting a monitoring program and should be used to include these communities as complementary indicators of the overall ecosystem health.

5. Final remarks

The objective of the WFD was to improve the ecological status of European waterbodies. Phytoplankton, macrophytes, phytobenthos, benthic invertebrates, and fish are among the biological quality indicators used to classify the ecological potential; interestingly, zooplankton is not (García-Chicote et al., 2018). Due to its sensitivity and dynamic responses to the environment, phytoplankton is a key biological indicator that is of particular relevance for the assessment of water quality (Padisák et al., 2006). Additionally, it modifies its growth and composition in direct response to nutrient levels (Ptacnik et al., 2009). The organisms in this group are widely accessible, exhibit an amazing diversity in terms of environmental adaptation, and have rapid growth rates, for that reason they could be used as an early warning indicator of ecosystem changes (Salmaso et al., 2012). However, zooplankton have longer life cycles and can thus integrate environmental change over time, providing a more stable response; our results suggest this, as the zooplankton community was overall more strongly associated with environmental parameters than phytoplankton (see db-RDA). Zooplankton is useful as an indicator of ecological conditions because of their position in the food web, positioned between top-down regulators (fish) and bottom-up factors (phytoplankton), because it is able to demonstrate the relative importance of top-down and bottom-up control (Jeppesen et al., 2011).

Currently, it is accepted that bottom-up and top-down forces both simultaneously impact ecological communities (Brett & Goldman, 1997; Nicolle et al., 2011). The approach of looking at phytoplankton mostly reflects the focus on bottom-up control (nutrients modulate primary production) and the historical knowledge on saprobic classification systems and trophic status. As such, the WFD bioassessment scheme needs complementary indicators or biological descriptors that can also translate top-down forces, such as fish predation pressure. Consequently, studying both zooplankton and phytoplankton will enable us to acquire a more comprehensive, representative understanding of the actual state of water bodies and the environmental factors influencing their development (García-Chicote et al., 2018).

Our results demonstrated that a combination of abiotic and biotic variables in the four examined reservoirs influence the seasonal dynamics of both phyto- and zooplankton. Moreover, different phyto- and zooplankton communities were associated to contrasting systems (shallow reservoirs vs deeper reservoirs). Additionally, this work helped us comprehend the changes that

occurred in the reservoirs more thoroughly. Environmental data can only give information about a single static instant, whereas biological elements like phytoplankton and zooplankton dynamics integrate how the ecosystem evolved over time and which factors are associated to the observed community changes. By identifying both communities' composition, along with abiotic factors monitorization, a more accurate water quality assessment is possible to achieve.

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Annexes

Annex I

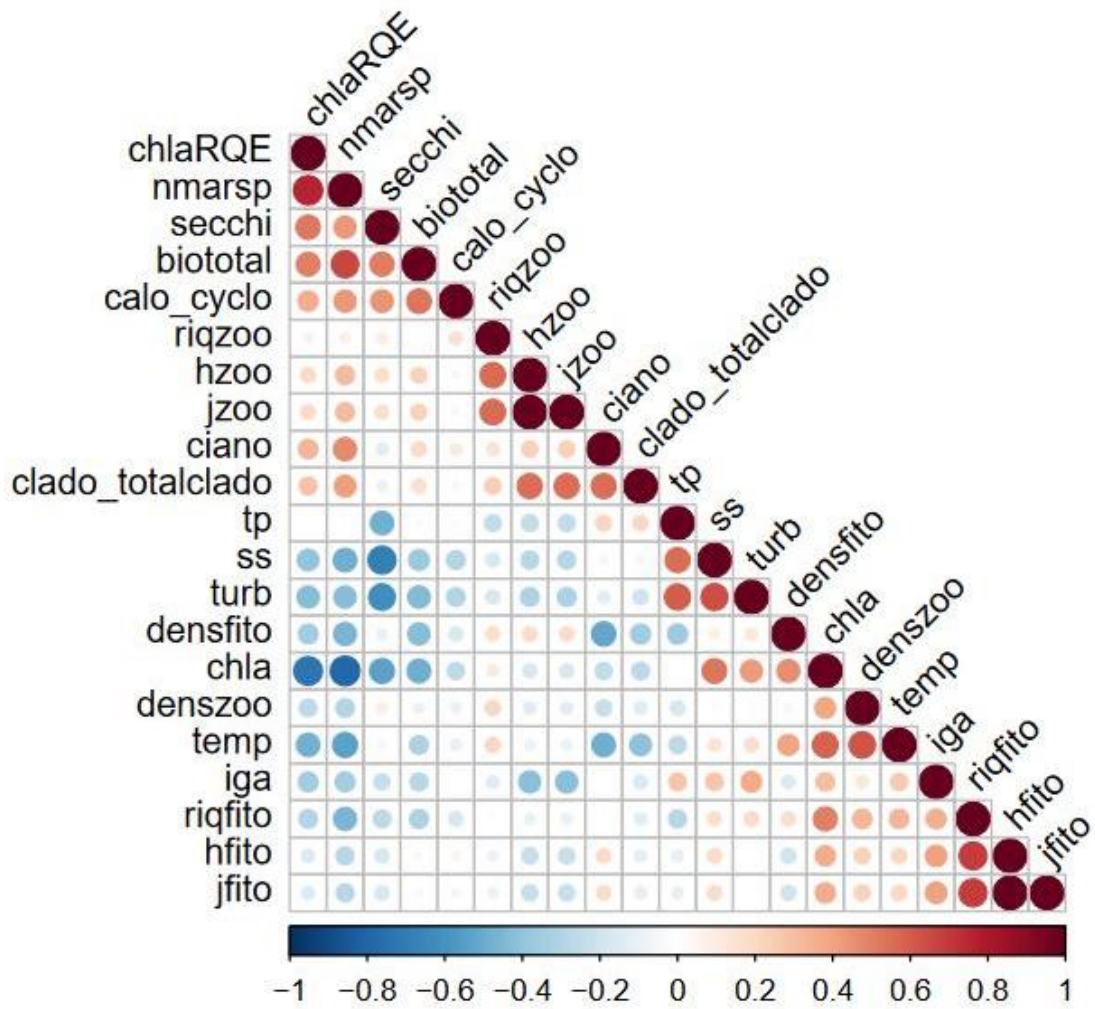


Figure S1- Full correlation matrix.

Annex II

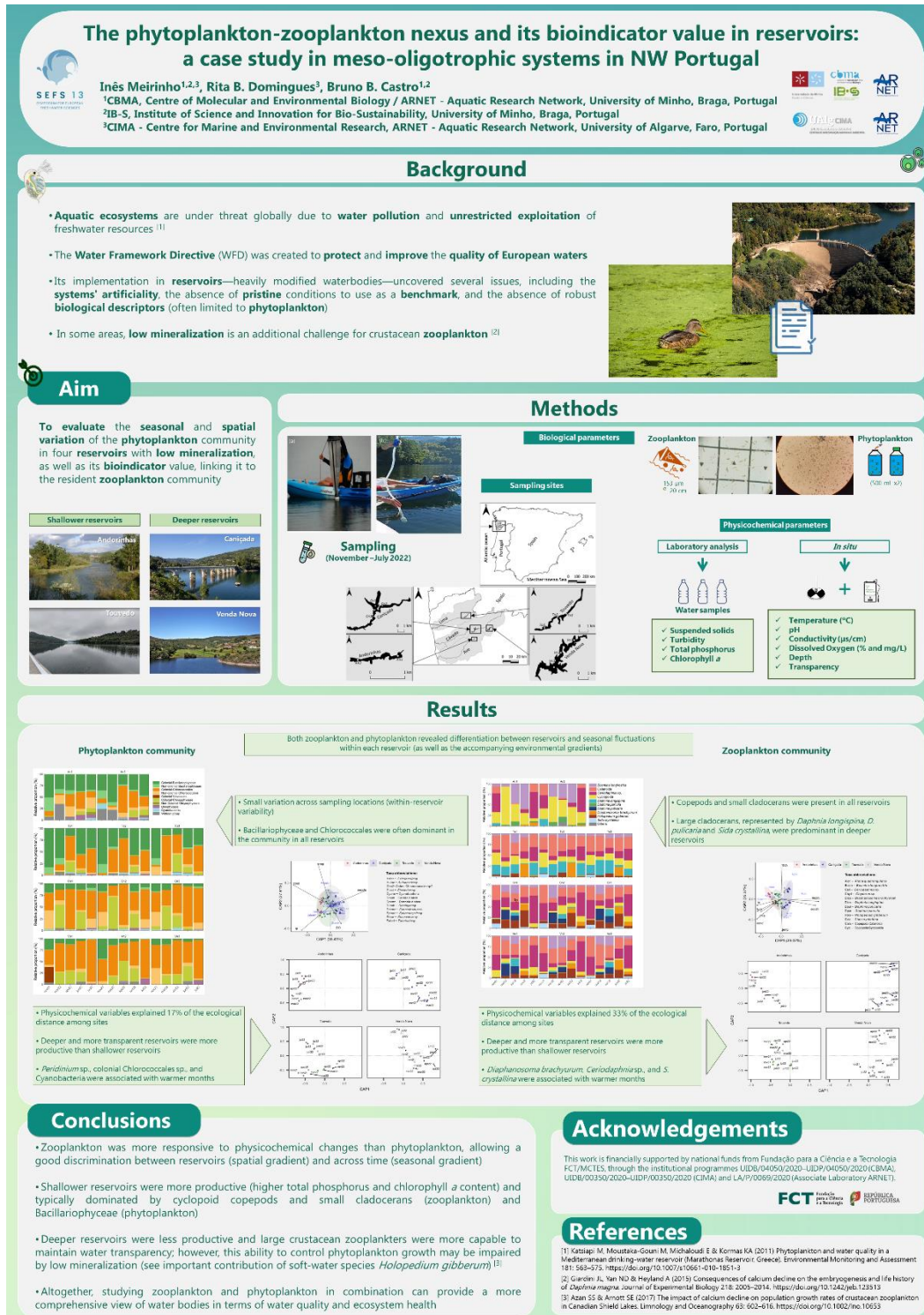


Figure S2 - This poster was created and presented at the Symposium for European Freshwater Sciences (SEFS 13), utilizing the data gathered during the course of this dissertation.