Evaluation of Aguieira Reservoir Water Quality: insights into parameters in addition to the Water Framework Directive

Ivo Filipe Magalhães Pinto
Mestrado em Ecologia e Ambiente
Departamento de Biologia
Ano 2018/2019

Orientador
Sara Cristina Ferreira Marques Antunes, Professora Auxiliar Convidada, Faculdade de Ciências da Universidade do Porto e Investigadora Auxiliar do CIIMAR (Centro Interdisciplinar de Investigação Marinha e Ambiental)

Coorientador
Olga Maria Oliveira da Silva Lage, Professora Auxiliar, Faculdade de Ciências da Universidade do Porto e Investigadora do CIIMAR (Centro Interdisciplinar de Investigação Marinha e Ambiental)

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

Porto, _____/_____/_____

Dissertação submetida à Faculdade de Ciências da Universidade do Porto, para a obtenção do grau de Mestre em Ecologia e Ambiente, da responsabilidade do Departamento de Biologia, desenvolvida no âmbito do **projeto POCI-01-0145-FEDER-029368**, co-financiado pelo COMPETE 2020, Portugal 2020 e a União Europeia através do ERDF, e pela FCT através de fundos nacionais.

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

A presente tese foi desenvolvida sob a orientação científica da Doutora Sara Cristina Ferreira Marques Antunes, Professora Auxiliar Convidada do Departamento de Biologia da FCUP e Investigadora Auxiliar do CIIMAR (Centro Interdisciplinar de Investigação Marinha e Ambiental); e coorientação científica da Doutora Olga Maria Oliveira da Silva Lage, Professora Auxiliar do Departamento de Biologia da FCUP e Investigadora do CIIMAR (Centro Interdisciplinar de Investigação Marinha e Ambiental)

COMPETE 2020 FCT Fundação para a Ciência e a Tecnologia Cofinanciado por: PORTUGAL 2020 UNIÃO EUROPEIA Fundo Europeu de Desenvolvimento Regional REPÚBLICA PORTUGUESA

Agradecimentos

Em primeiro lugar gostaria de agradecer à minha orientadora, a professora Sara Antunes. A minha jornada no lab 1.14 não começou agora e estou extremamente agradecido por ter acreditado novamente que eu seria capaz de realizar este enorme trabalho e em cada etapa ter-me desafiado a fazer mais e mais sem desistir. Sempre demonstrou uma enorme paciência, confiança, compreensão e disponibilidade quando precisei. Foi, sem dúvida alguma, a pessoas mais importante e fundamental ao longo desde trabalho, o meu sincero obrigado.

Da mesma forma que não podia deixar de agradecer também à minha coorientadora, a professora Olga Lage, que apesar de ainda não termos realizado a parte científica do trabalho da sua área me ajudou imenso a clarear ideias, pensamentos e transmiti-los de forma muito mais clara, objetiva e científica, sem a sua ajuda esta dissertação teria sido bem mais difícil de realizar.

Obrigado também ao professor Nuno Formigo, pelo acompanhamento ao longo deste mestrado, por todos os debates de ecologia quer nas suas aulas quer no seu gabinete. Pela companhia, boa disposição e todos os ensinamentos durante as várias saídas de campo ao longo do mestrado, especialmente durante este último ano da minha dissertação. Pelas suas visitas ao laboratório e sempre se preocupava comigo e brincávamos com o que eu estava a fazer tornando o trabalho em algo divertido e fácil.

À Conceição, por me ter ajudado e ensinado a tomar conta e a ter paciência com as nossas *Daphnia*'s.

Ao Fábio, por me ter acompanhado nas saídas de campo e me ter ajudado de enumeras formas no laboratório especialmente nesta fase final com os resultados.

À Sandra, por dividirmos o minúsculo espaço que tínhamos no início e que cada vez está maior, por me ter ensinado o que era o mundo do fito e do zoo, por todos os momentos de arrumação que aquele laboratório sofreu e por toda a companhia e preocupação que teve para comigo desde o início.

Ao Sérgio, pela grande ajuda na elaboração dos mapas até chegarmos ao mapa final.

Por fim e não menos importante, à Sara, pela ajuda na organização do trabalho antes e depois das saídas e por todos os debates de como colocar os resultados e fazer a sua interpretação.

O meu sincero obrigado a todos vós do lab 1.14, os que passaram e os que entraram agora, obrigado por me terem recebido novamente tão bem e por todos os momentos que passamos juntos, de outra forma este trabalho teria sido bem mais difícil de chegar ao fim.

Aos meus meninos que o MEA me deu, Paulo, Zé, Chico, Bruna e Rita, entramos nisto juntos e saímos juntos. Obrigado por todos os momentos que passamos ao longo destes últimos anos, as rizadas até chorar, os stresses sem fim, os jantares pela noite dentro, os desabafos e confissões sem noção do local onde estávamos e com quem estávamos, pela companhia, boleias, ajudas e todos os nossos segredos. Foram os primeiros amigos a saber e isso teve uma enorme importância. Obrigado por me fazerem ver que apesar de a vida não ser um mar de rosas existi sempre coisas boas na nossa vida pelo que valem a pena lutar.

Ao Filipe, por tudo aquilo que ele representa para mim desde que nos conhecemos. Por toda a ajuda que conseguiu dar, por todo o apoio, por todo o carinho, por me tirar daqui quando precisava, pelos jantares, cafés, caminhadas, noitadas e mais e mais. Não tenho palavras para agradecer e dizer tudo o que quero. O meu obrigado.

Aos macacos, os meus meninos mais velhos, não preciso dizer nomes que vocês sabem bem quem são, obrigado por fazerem parte da minha vida ao longo de muitos e muitos anos. Por me verem crescer e me incentivarem a querer ser mais e que cada obstáculo é apenas uma pedra no caminho e que no fim irei criar aquilo que quero e que me satisfaz.

A minha mãe por todo o esforço que fez ao longo destes anos para que eu conseguisse chegar aqui sem nunca me prendar. Ao meu pai, por me ter transmitido o gosto por esta área e o que ela significa para o homem. A minha irmã, cunhado e ao meu sobrinho, obrigado por todos os momentos felizes e que venham muitos mais. Nunca terei palavras e forma de vos agradecer a todos tudo o que fizeram e fazem por mim. Obrigado por tudo.

A todos aqueles que não foram mencionados e que, de alguma forma, contribuíram para a realização deste trabalho. Muito obrigado!

“Juntos no mesmo barco rumo ao infinito”

GAPC

Resumo

Para avaliar o potencial ecológico de massas de água artificiais, a Diretiva Quadro Água usa parâmetros específicos, mas ignora as funções ecológicas do ecossistema. Este trabalho teve como objetivo avaliar o funcionamento de uma albufeira utilizando ferramentas convencionais e ecotoxicológicas para avaliar a qualidade da água, indo além da abordagem da Diretiva Quadro Água. Foram definidos quatro locais de amostragem na albufeira de Aguieira (centro de Portugal) e parâmetros físicos, químicos e biológicos, impostos pela Diretiva Quadro Água, foram quantificados em dois períodos, outono de 2018 e primavera de 2019. Os resultados obtidos mostraram que no outono os locais A1, A2 e A4 apresentaram um potencial ecológico de moderado e o local A3 um mau potencial ecológico. Na primavera os locais A1, A3 e A4 apresentaram um potencial ecológico de moderado e o local A2 um bom potencial ecológico. Estes estudos foram complementados por várias análises. O macrozooplâncton foi analisado e um conjunto de ensaios ecotoxicológicos (inibição do crescimento de *Raphidocelis subcapitata* e *Spirodela polyrhiza* e taxa de alimentação de *Daphnia longispina* e *Daphnia magna*) foram realizados usando amostras de água em três condições (sem filtração - NF, filtrada por poros de 1,2 µm - F1, e filtrado por 0,22 µm de poro - F2). A comunidade zooplanctónica (abundância relativa e grupos funcionais) mostrou ser um bom indicador na avaliação da qualidade da massa de água desta albufeira, estando esta fortemente relacionada com o estado trófico do ecossistema. Relativamente aos bioensaios, a inibição de crescimento da *S. polyrhiza* e da *R. subcapitata* mostraram ser sensíveis à massa de água desta albufeira apenas na amostragem do outono. Os bioensaios da avaliação na taxa de alimentação da *D. longispina* e *D. magna* mostraram alguma sensibilidade da avaliação da qualidade da massa de água da Aguieira, fornecendo informação adicional na avaliação dessa massa de água. Dessa forma, foi possível mostrar que a incorporação da dinâmica de zooplâncton e de alguns bioensaios na Diretiva Quadro Água são uma mais valia na avaliação do potencial ecológico das albufeiras.

Palavras-chave

Ferramentas ecotoxicológicas, Potencial ecológico, Diretiva Quadro Água, Águas naturais, ecossistema lêntico

Abstract

To assess the ecological potential of artificial water bodies, the Water Framework Directive uses specific parameters, but ignores the ecological functions of the ecosystem. This work aims to evaluate the functioning of a reservoir using conventional and ecotoxicological tools in order to evaluate the water quality, going thus beyond the Water Framework Directive approach. Four sampling sites were defined in Aguieira (centre of Portugal) reservoir and physical, chemical and biological parameters, imposed by the Water Framework Directive were quantified in two periods, autumn 2018 and spring 2019. The results obtained showed that in autumn sites A1, A2 and A4 presented moderate ecological potential and site A3 a bad ecological potential. In spring, sites A1, A3 and A4 presented moderate ecological potential and site A2 a good ecological potential. These studies were complemented by various analyses. The macrozooplankton was analysed and a set of ecotoxicological assays (growth inhibition of *Raphidocelis subcapitata* and *Spirodela polyrhiza* and feeding rate of *Daphnia longispina* and *Daphnia magna*) were performed using sampled water in three conditions (Unfiltered water - NF, filtered with 1.2 µm pore – F1, and filtered with 0.22 µm pore – F2). The zooplankton community (relative abundance and functional groups) showed to be a good indicator in the evaluation of the water quality of this reservoir, being strongly related to the trophic state of the ecosystem. For bioassays, the growth inhibition of *S. polyrhiza* and *R. subcapitata* showed to be sensitive to the water body of this reservoir only in the autumn sampling. The feeding rate assays of *D. longispina* and *D. magna* showed some sensitivity to Aguieira water body improving its evaluation. Thus, it was possible to show that the incorporation of zooplankton dynamics and some bioassays into the Water Framework Directive are assets in the assessment of the ecological potential of the reservoirs.

Keywords

Ecotoxicological tools, Ecological potential, Water Framework Directive, Natural waters, Lentic Ecosystem

Table of contents

Figure Index

for autumn) stands for significant differences between treatments (Pairwise t test, $p<0.05$) in each sampling period.

Table Index

List of abbreviations

AGI	Algae Group Index
Au	Autumn
BOD5	Biochemical Oxygen Demand
CDOC	Colored Dissolved Organic Carbon
Chl *a*	Chlorophyll *a*
Chl *b*	Chlorophyll *b*
COD	Chemical Oxygen Demand
DL	Detected Limit
EQR	Ecological Quality Ratio
EQO	Ecological Quality Objective
EP	Ecological Potential
Sp	Spring
TSS	Total Suspended Solids
VSS	Volatile Suspended Solids
WFD	Water Frame Directive

1. Introduction

Reservoirs are artificial water bodies created by human activities (INAG, 2009a), such as the construction of a barrier (dam) in a river which breaks the connectivity of the lotic ecosystem (União Europeia, 2000). These changes modify the ecological processes upstream of the dam, like the nutrient cycle and the increase of organic matter accumulation (Ackermann et al., 1973), changing the structure of biological communities and the functioning of the pre-existing ecosystem (Ward & Stanford, 1995; Simões et al., 2015). The hydrological complexity of these systems favours the upstream dam accumulation of nutrients (*e.g.* phosphorus and nitrogen), which leads to the occurrence of eutrophication processes causing an abnormal growth of the primary producers that can compromise the quality and balance of the aquatic ecosystem (Herschy, 2012).

The creation of these artificial structures creates reservoirs of freshwater, an important ecosystem which serve as water reserves for irrigation, consumption, recreation, tourism, among other activities. Over the past few years we have acknowledged a growing concern with the decreasing of the drinking water quality and availability (Johnson et al., 2001; Dudgeon et al., 2006). The decrease in water quality is mostly of anthropic origin due to intense agriculture, industrial production, domestic and urban waste, untreated and treated waste waters since the ETARs has no ability to remove many harmful compounds (World Water Assessment Programme, 2009). In order to minimize the reported impacts on these ecosystems, it is extremely important to understand their overall composition, structure and dynamics. Thus, it would be urgent to implement measures to ensure the protection of aquatic resources and assure water quality in these ecosystems (*e.g.* more specific treatments, population awareness and greater oversight).

Water Framework Directive 2000/60/EC (WFD) is an european water legislation released in 2000 by the European Union with the aim of standardizing the forms of monitoring and of management of the water bodies in all member states (União Europeia, 2000). The great objectives of WFD are to determine and define the “ecological status” of a natural water body (ex: rivers, transitional or coastal waters) or the “ecological potential” of an artificial water body (ex: reservoirs).

In order to assess the ecological potential of an artificial water body (reservoir), the WFD is based on specific physical, chemical, biological and hydromorphological parameters. The results achieved allow to classify the water body in Ecological Quality Ratio (EQR) in a scale with five-classes: bad, poor, moderate, good or excellent. Relatively to the physical and chemical parameters imposed by this directive, only four

specific parameters, pH, oxygen, total phosphorus (P) and nitrate (NO_3^-), have established reference values between the Good and Moderate classes to these water body. Regarding the biological parameters, only the analysis of the phytoplankton community is used in the characterization of reservoirs, being evaluated based on the Algae Group Index (AGI), % biovolume of Cyanobacteria, total biovolume and chlorophyll *a* concentration. The hydromorphological elements used are the hydrological regime and the morphological conditions, which guarantee the abiotic support for the biological communities that may occur (INAG, 2009a). In order to evaluate reservoirs, the WFD established a specific typology based on some characteristics such as geographical location and river course. This allows to represent three types of reservoir in Portugal: northern reservoirs (hydroelectric power plants in cold water, located in the northern region, in mountainous areas); southern reservoirs (irrigation / hot water supply, located in the southern region); and main reservoirs (located in the main courses of the rivers Douro, Tejo and Guadiana) (Pádua et al., 2005; INAG, 2009a).

The use of the WFD parameters for assess the water quality of artificial water bodies allows to determine the ecological potential of these ecosystems and to compare between artificial water bodies in order to improve the efficiency of its management. However, the WFD focuses on the excessive evaluation of physical and chemical parameters, leading to a deficit in biological parameters. Indeed, the actual evaluation does not consider the functioning of the ecosystem, different temporal scales and responses at the individual organism level. Information about an indicator organism can shed light on the effect of stress on the ecosystem and provide warning signals. These disadvantages and a scarce bibliographical information about the ecosystem have led several authors to suggest other types of WFD approaches such as the assessment of the composition, abundance and age structure of the fish fauna present in the ecosystem. However, these parameters are under development in the second phase of the intercalibration exercise of WFD (Pádua et al., 2005; INAG, 2009a). On the other hand, several authors defended the analysis of the composition of other communities such as zooplankton, an important trophic level to assess water quality of lentic ecosystems (Caroni & Irvine, 2010; Jeppesen et al., 2011; Ejsmont-Karabin & Karabin, 2013; Haberman & Haldna, 2014; García-Chicote et al., 2018). The composition of the bacterioplankton was also proposed as a sensitive bioindicator of the health of aquatic ecosystems (De Figueiredo et al., 2007; Llirós et al., 2014). More recently the use of bioassays, with standard and autochthonous species from different trophic levels were proposed since are more simple and quick tools to assess the water quality (Palma et al., 2010, 2016; Pérez et al., 2010; Mkandawire et al., 2014; Kungolos et al., 2015).

Given the perception of the importance of the use of different biological tools, this work aimed to complement the assessment of Aguieira water quality in supplement to the conventional WFD parameters. Additionally, we intended to evaluate the sensitivities of other ecological parameters and tools for water quality assessment in a more integrative approach. In order to achieve this goal, the following specific parameters and tools were also evaluated: zooplanktonic community dynamics and the functionality of the ecosystem through different bioassays (*Raphidocelis subcapitata*, *Daphnia* spp. and *Spirodela polyrhiza*).

2. Material and Methods

2.1. Study Area

The Aguieira reservoir is located in the centre of Portugal, at Coimbra district (Fig. 1), integrated in the municipalities of Carregal do Sal, Mortágua, Penacova, Santa Comba Dão, Tábua and Tondela. This reservoir is inserted in the intermediate section of the Mondego river, at the confluence of two secondary rivers, Dão and Criz. The Aguieira dam, occupying an area of about 2000 ha, started operating in 1981 with the purposes of energy production, irrigation and water storage (APA, 2007; Presidencia do Concelho de Ministros, 2007; INAG, 2011). The climate of this region is strongly influenced by the Mediterranean conditions being characterized by mild/cold winters and hot summers (APA, 2007; Geraldes et al., 2016a). In its vicinity there are food, textile, wood and cork industries. The surrounding landscape is dominated by eucalyptus, acacias, pines, agricultural soils, moors and bushes (Pedroso et al., 2007; Geraldes & Silva-Santos, 2011).

For the accomplishment of the present work, four sampling sites were selected in the Aguieira reservoir. These sites are located along the bank of the reservoir and were selected based on the accessibility and in order to perceive the different impacts on the reservoir from the two rivers (Fig. 1).

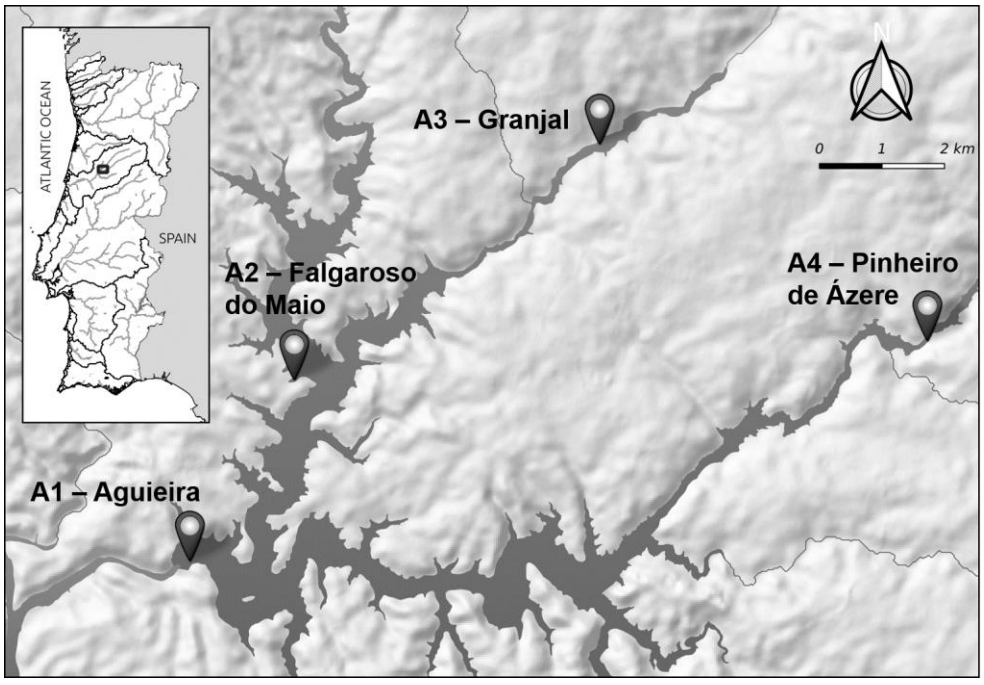

Figure 1 Map with the location of the sampling sites in the Aguieira reservoir, Coimbra. A1 – Aguieira: 40.341095 -8.194060; A2 – Falgaroso do Maio: 40.367190 -8.174523; A3 – Granjal: 40.400969 -8.116986; A4 – Pinheiro de Ázere: 40.372849 -8.055293.

2.2. Sampling Procedure

Two sampling periods were carried out to conduct this study (autumn-Au -and spring-Sp). *In situ*, and with the aid of a multiparameter probe (Multi 3630 IDS SET F) some general physical and chemical parameters were measured sub superficially: pH, oxygen (mg/L and %), conductivity (µS/cm) and temperature (°C). In addition, the transparency (m) was measured using a Secchi disk.

At each site, several water samples were collected and transported to the laboratory under thermal conditions (at 4 °C and in the dark) for further analysis. The samples included 1.5 L collected in glass amber bottles conserved with 1.20 g/L of $Na_2S_2O_3$ for pesticides quantification, 1 L in glass bottles for others priority substances, 120 mL in plastic bottles acidified to pH<2 with HNO_3 for metals, and 120 mL in plastic bottles for nutrient analysis. Additionally, 500 mL of water were also collected in each sampling site and stored with 5 mL lugol for later quantification of the phytoplankton community, according to the INAG protocol (2009b). For conducting several laboratory bioassays and water physical and chemical parameters determination, 10 L of water were collected, in plastic bottles.

For the sampling of macrozooplankton communities, a hand net (150 µm mesh) was used. In each site three trawls were conducted to collect these communities. The zooplankton samples were stored in plastic bottles and preserved with 96 % ethanol for later laboratory analysis.

2.3. Laboratory Procedure

2.3.1. Physical and chemical parameters

In the laboratory, water samples were used to determine the biochemical oxygen demand (BOD_5) (APHA, 1989), chemical oxygen demand (COD) (ISO 15705), turbidity (Brower et al., 1997), alkalinity (NF EN ISO 9963-1), hardness, nitrites (NO_2^-) (NF EN ISO 10304-1), nitrates (NO_3^-) (NF EN ISO 10304-1), total nitrogen (N_{total}) (NF EN 25663), ammoniacal nitrogen (NH_4^+), phosphates (PO_4), total phosphorus (P_{total}) (NF EN ISO 17294-2), and a set of priority substances defined in Directive 2008/105/CE of the Parlamento Europeu (2008). In addition, 17α-ethynylestradiol (SPE – LC/MS/MS (Neg.)) and diazepam (SPE – LC/MS/MS (Pos.)) were also quantified in order to perceive the impacts from anthropogenic activities in the aquatic ecosystem.

Moreover, a water sample was filtered through a Whatman GF/C filter (47 mm diameter and 1.2 µm pore) and the filtrated was used for determination of dissolved organic carbon (DOC) determined indirectly through the colour of the water (CDOC - Coloured Dissolved Organic Carbon) (Williamson et al., 1999). Three filters with the seston of each sample was used to determine the total suspended solids (TSS) and the volatile suspended solids (VSS) contents in the water samples (APHA, 1989).

2.3.2. Biological parameters

2.3.2.1. Phytoplankton

The protocol followed for the phytoplankton communities characterization was according to INAG (2009b). Each sample (500 mL) was placed in a sedimentation beaker and, after standing for one week, the supernatant was decanted and the pellet collected to falcon tubes (final volume <5 mL). The analysis of phytoplankton was performed in an optical microscope (Leica DM LB) in a Neubauer chamber and each sample was quantified in three replicates, counting at least 800 cells per replicate. Identification of the specimens was done using photographs, guides and specific identification keys; Baker (2012), Bellinger & Sigee (2015), and Błędzki & Rybak (2016).

The WFD proposes four indicators for assessing the EQR for phytoplankton based on composition and abundance - Algae Group Index (AGI) and Cyanobacteria biovolume %; and biomass - chlorophyll *a* concentration and total biovolume (INAG, 2009b). For the latter parameter the protocol was adjusted according to other bibliographic information (*e.g.* Olenina et al., 2006). The phytoplankton community was expressed in EQR, relative abundance based in the groups formed for AGI. A group of others was defined when genus did not fit into either group of AGI.

2.3.2.2. Zooplankton

For the quantification of the zooplankton community in each sample a magnifying glass and a counting plate were used. For the identification of the Cladocera specimens the identification was done to the species level, while the organisms of Copepoda were only identified to the order. The ostracods and the nauplii found were also counted. The identification was made using identification guides as Alonso (1996), Amoros (1984) and Witty (2004). The results of the zooplankton community were expressed in relative abundance and based on the different functional groups. For this purpose, the organisms

were grouped according to their food strategies (low efficiency, high efficiency and macrofilters) defined by Geller & Müller (1981). A group of organisms denominated by omnivores was added considering their general feeding capacity (*e.g.* Reid & Williamson (2010)). In addition, diversity index (Shannon-Weaver – H') and equitability index (Simpson - E) were calculated.

2.3.3. Bioassays

2.3.3.1. Culture maintenance

The monoculture of the microalgae *Raphidocelis subcapitata* was maintained in Woods Hole MBL medium (Stein, 1973). Culture in the exponential growth phase was renewed approximately every 7 days for a new medium (Pereira et al., 2009).

Cultures of the Cladocera water flea *Daphnia longispina* and *Daphnia magna* were continuously kept in the laboratory conditions for successive generations. Cultures were renewed on alternate days and were maintained in synthetic water medium "ASTM hard water" (ASTM, 1980), supplemented with a standard organic additive, *Ascophyllum nodosum* extract (Baird et al., 1988). Daphniids were fed with *R. subcapitata* at a rate of $1.5x10^5$ cells/mL/day for *D. longispina* and $3.0x10^5$ cells/mL/day for *D. magna*. All neonates used in the bioassays were aged 4 and 5 days old, born between the 3rd and 5th broods.

The floating aquatic plant *Spirodela polyrhiza* were grown and maintained in Steinberg medium (ISO 20079, 2005). The culture was renewed every week.

All cultures were maintained in a culture chamber (Incubator TC 445 S, Lovibond® Water Testing) under controlled conditions of 16hL:8hD photoperiod and a temperature of 20±2 ºC. More detailed rearing procedures can be found in Nunes et al. (2014a).

2.3.3.2. Natural water conditions

For conducting the bioassays to evaluate the water quality of Aguieira reservoir, three different water treatments were performed (NF, F1 and F2). Unfiltered water (NF) stands for natural water collected from the reservoir, with all components present at the sample

site. Water filtered through a Whatman GF/C filter of 47 mm diameter with 1.2 µm porosity (F1), in order to remove all suspended particles, phytoplankton and zooplankton communities. F2 depicted as water filtered through a sterile filter system with a porosity of 0.22 µm, to allow only the passage of dissolved compounds of the natural water sample.

2.3.3.3. *Raphidocelis subcapitata* growth inhibition assay

The growth inhibition assays with *R. subcapitata* followed the OECD (2006a) guidelines. The assay was performed in 24-well microplates, and MBL medium was used as a negative control. The natural water samples were evaluated under the three treatments, and a blank without algae addition was also measured. The assay was prepared with three replicates with algae addition at an initial concentration of 10^4 cells/mL (0.9 mL of the water sample plus 0.1 mL of algae that stands for an absorbance of 0.01 at λ=440 nm). The assays were initiated after placing the 24-well microplates in a climatic chamber (Incubator TC 445 S, Lovibond® Water Testing) at 24±2 ºC with permanent light (≈7000 lux). Every day the algal cultures were resuspended to avoid cell sedimentation using a micropipette. At the end of the assay (72 hours) the absorbance in each well was measured at λ=440 nm (UV-1600PC Spectrophotometer). The results were expressed in yield, calculated as the biomass (measured in absorbance) at the end of the test minus the starting biomass for each single vessel of controls and treatments, according to the protocol of OECD (2006a).

2.3.3.4. *Daphnia longispina* and *Daphnia magna* feeding rate assay

The feeding rate assessment of *D. longispina* and *D. magna* was conducted according to McWilliam & Baird (2002). For conducting this assay, 6-well microplates were used, where ASTM was used as a control and natural water in the different treatment were tested (*see* section 2.3.3.2.). For each water treatment a control (with water sample plus *R. subcapitata*), and five replicates with the same number of organisms (between three to five) plus *R. subcapitata* were prepared. Each replicate was conducted in 12.5 mL of each water treatment, and *R. subcapitata* was added at a concentration of $1.5x10^5$ cells/mL to *D. longispina* and $3.0x10^5$ cells/mL to *D. magna* assays. The absorbance of each well was measured at λ=440 nm (UV-1600PC Spectrophotometer), prior to the addition of the organisms. The assays were initiated after the addition of the organisms

in each replicate. The microplates were placed in a climatic chamber (Incubator TC 445 S, Lovibond® Water Testing) for 24 hours at 20±2 ºC in total darkness, to avoid algal growth. After this period the absorbances at λ=440 nm were measured, and the results were expressed in feeding rate according to Allen et al. (1995) equation.

2.3.3.5. *Spirodela polyrhiza* growth inhibition assay

In order to carry out the growth inhibition assay of *S. polyrhiza*, the protocols of OECD (2006b) and Nunes et al. (2014b) were followed. This assay was conducted in glass vials and the Steinberg medium was used as control and the three natural water treatments (*see* section 2.3.3.2.) were tested. For each treatment, 4 replicates were prepared in a final volume of 100 mL for each replicate. At the beginning of the assay, 9 fronds were added to each replicate and capped with perforated parafilm to allow gas exchange. The vials were placed in a climatic chamber (Incubator TC 445 S, Lovibond® Water Testing) for 7 days at 24±2 °C with permanent (≈7000 lux). At the end of the assay the final number of fronds was counted and the results were expressed in yield, calculated as the number of fronds at the end of the test minus the starting number of fronds for each single vessel of controls and treatments (OECD, 2006b). Additionally, a specimen with 4 to 5 fronds of each replicate was collected for quantification of chlorophyll *a*, *b*, *a+b* and carotenoids according to Lichtenthaler (1987). Pigments extraction was performed in 1 mL of 96 % ethanol overnight, at −4 °C. In the day after, samples were thoroughly vortexed for about 30 s and centrifuged for 5 min at 4 000 rpm at 4 °C. The supernatants were used to quantify the different pigments, through spectrophotometry by measuring absorbances of the extracts at wavelengths of 470, 648.6 and 644.2 nm (UV-1600PC Spectrophotometer). The results were expressed in order to evaluate the photosynthetic performance of the organisms exposed, as well as the concentration of the different pigments.

2.4. Statistical Analysis

The relative abundance of the taxa identified for the phytoplankton and zooplankton communities was determined. The diversity index (Shannon-Weaver (H´)) and equitability index (Simpson (E)) were calculated for zooplankton communities. For the phytoplankton community, an additional analyse in terms of relative abundance based on the groups formed by the algae group index (AGI) was also performed. A group of

others was defined when genus did not fit into either group of AGI. The zooplankton community was functionally analysed and grouped according to the feeding strategies defined by Geller & Müller (1981). An omnivorous group was also defined considering the general feeding capacity (e.g. Reid & Williamson (2010)).

The Ecological Quality Ratio (EQR) is an indicator of the current deviation from the reference conditions of a water body. For the phytoplankton characterization, the EQR calculation was performed following the equations presented in INAG (2009a). The conjugation of the EQRs for Algae Group Index (AGI), Cyanobacteria biovolume %, chlorophyll *a* concentration and total biovolume, reflects the ecological potential of that body of water, which may be: Excellent or Good (≥0.6), Moderate (≥0.4 and <0.6), Mediocre (≥0.2 and <0.4) or Bad (<0.2).

The results of bioassays with *R. subcapitata* and *S. polyrhiza* are expressed in yield (OECD, 2006b, 2006a) as opposed to bioassays with *D. longispina* and *D. magna* which are expressed in feeding rate (McWilliam & Baird, 2002). For all bioassays, a one-way ANOVA was conducted to test the differences in water treatments in each season. Previously, data were tested for normality by the Shapiro-Wilk test and for homogeneity of variances by the Levene test, as normality and homogeneity of data are conditions for unidirectional ANOVA application. When one-way ANOVA detected significant differences ($p < 0.05$), a Tukey test was applied to discriminate differences between treatments. All the statistical analysis were done using vegan v.25-4 package of R software (Oksanen et al., 2019). When the results did not meet the ANOVA assumption, the data were analysed nonparametrically using Kruskal – Wallis and, when significant differences were found ($p < 0.05$), a Pairwise t test was applied to discriminate significant differences between treatments. Statistical analyses were performed using the stats v.3.5.1 package from R software (R Core Team, 2018). All the data were plotted using package ggplot2 v3.1.0 of the same software (Wickham, 2016).

3. Results and Discussion

3.1. Physical and chemical parameters

A series of physical and chemical parameters were measured in four sampling sites (A1-A4) in Autumn 2018 and Spring 2019, in Aguieira reservoir which are reported in Table 1. Based on the values obtained, the ecological potential (EP) for these four sites was calculated (Table 1), and a good EP was observed for sites 1, 2 and 4 for both sampling periods, while site 3 only presented an EP of moderate. This latter result is due to the high concentration of total phosphorus (P_{total}) recorded in both sampling periods (INAG, 2009a). The increase of fertilizers and manure in local agriculture, and the increase of untreated water discharges (urban and agro-industrial) were the main drivers for the accumulation of nutrients such as phosphorus and nitrogen in the water body (Table 1) (APA, 2007).

Table 1 Results of the general physical and chemical parameters (Sampling sites: A1, A2, A3 and A4; Sampling periods: Au - autumn and Sp - spring)., reference values according to INAG (2009a)); a Good/Moderate EP in northern reservoirs established by WFD were presented; DL – Detected Limit. Bold values stand for values outside the established thresholds.

			A1		A2		A3		A4	
	INAG (2009a)	DL	Au	Sp	Au	Sp	Au	Sp	Au	Sp
pH	6-9		8.40	9.20	7.62	8.99	8.10	8.31	7.40	9.15
O_2 (mg/L)	>5		8.79	11.90	7.97	12.40	9.89	11.30	7.36	12.20
O_2 (%)	60-120		106.3	119.4	94.4	124.9	112.0	112.1	89.0	125.2
P_{total} (mg/L)	≤ 0.05	0.01	<0.01	0.01	0.03	0.01	**0.22**	**0.09**	0.03	0.02
NO_3^- (mg/L)	≤ 25	0.5	1.3	2.8	<0.5	3.3	<0.5	4.0	2.4	1.2
Cond (µS/cm)			86.0	83.1	96.6	89.2	143.2	112.0	87.4	78.2
Temp (°C)			24,5	14.4	23.2	15.0	21.0	15.2	24.5	15.5
Secchi (m)			>2.0	2.5	>2.5	2.5	Bottom	2.0	>2.5	1.5
Turb (m^{-1})			0.018	0.072	0.036	0.069	0.115	0.087	0.038	0.074
BOD_5 (mg/L)			0.81	0.55	0.77	0.86	3.88	0.42	0.48	1.12
CDOC (m^{-1})			0.059	0.100	0.082	0.230	0.151	0.148	0.043	0.243
TSS (µg/L)			8.24	13.45	10.09	19.05	312.50	11.08	10.75	17.50
VSS (µg/L)			4.17	11.07	6.02	14.60	62.50	9.08	7.42	13.50
COD (mg/L)		5	10	53	18	51	90	45	<5	50
Alk (°F)		0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Hardness (°f)		0.5	22.1	1.6	1.7	1.7	1.8	1.9	1.8	1.6
NO_2^- (mg/L)		0.01	<0.01	0.04	0.02	0.07	<0.01	0.04	0.01	0.02
NH_4^+ (mg/L)		0.05	<0.05	0.07	<0.05	<0.05	<0.05	0.06	<0.05	0.09
N_{total} (mg/L)		0.5	<0.5	<0.5	<0.5	<0.5	6.3	<0.5	<0.5	0.7
Ecological Potential			**Good**	**Good**	**Good**	**Good**	**Moderate**	**Moderate**	**Good**	**Good**

Aguieira reservoir is being characterized as an eutrophic water body in the last decades (APA, 2007; De Figueiredo et al., 2007; Geraldes & Silva-Santos, 2011; INAG, 2011; Vasconcelos et al., 2011), and this classification has been attributed due to the high concentration of P_{total} observed (APA, 2007; INAG, 2011). Indeed, this site also presented the highest differences of physical and chemical parameters of the four sites, with higher values of conductivity, turbidity, TSS, VSS, BOD_5 and nitrogen (N), especially in autumn (Table 1). The other measured parameters are within the limit values for a good ecological potential, and even those that do not have reference values follow the general trend that has been observed for this reservoir, with an eutrophic state (INAG, 2011).

Concerning some specific pollutants and priority substances the results are shown in Table 2. Only the metals were carried out in both seasons, being the remaining compounds only measured in the autumn (the worst-case scenario, after the hot season). The specific pollutants, that were possible to quantify, presented higher concentrations in the autumn sampling, except for copper and zinc in A1. A2 presented the highest metal concentrations in both samples, except for arsenic, which presented the highest concentrations at the sites farthest from the dam. However, when compared to the limit values imposed by the legislation ((INAG, 2009a) and the Annex I of Legislative Decree No. 218/2015 (Ministério do Ambiente do Ordenamento do Território e Energia, 2015)), the here-obtained results are always below these limits, *i.e.*, reflecting a good / excellent (G/E) EP for all the sampling sites. According to data from “Serviço Nacional de Informação de Recursos Hídricos (SNIRH)” (no data) monitoring network, for the operating stations located in this reservoir during the period from 2001 to 2013, specific pollutants were always low. Concerning the priority substances, the same pattern, for compounds quantified in both seasons, was also observed, with higher concentration in autumn sampling with some exceptions namely in A1 site. When comparing the results obtained with the limits imposed ((INAG, 2009a) and the Annex I of Legislative Decree No. 218/2015 (Ministério do Ambiente do Ordenamento do Território e Energia, 2015)), all substances monitored were in the range of the reference values for a good/excellent EP for the Aguieira reservoir. Moreover, when the values obtained for the several priority substances (*e.g*. arsenic, mercury, lead, anthracene and aldrin) were compared to the SNIRH (no data) data, the results were similar .

The two pharmaceutical drugs, indicators of anthropogenic activities and not included in the WFD pollutant list of priority substances, Diazepam and Ethinylestradiol, were also quantified (Table 2). However, they could not be quantified because since they were below to the detection limit of the equipment.

Table 2 Results of the specific pollutants and priority substances according INAG (2009a) and with Annex I of Legislative Decree No. 218 (Ministério do Ambiente do Ordenamento do Território e Energia, 2015). In the case of cadmium, reference values vary according to five classes of water hardness (Class 1: <40 mg $CaCO_3$ /L; Class 2: 40 mg to <50 mg $CaCO_3$ /L; Class 3: 50 mg to <100 mg $CaCO_3$ /L; Class 4: 100 mg to <200 mg $CaCO_3$ /L and Class 5: ≥ 200 mg $CaCO_3$ /L)). (Sampling sites: A1, A2, A3 and A4; Sampling periods: Au - autumn and Sp – spring; DL –Detected Limit).

				A1		A2		A3		A4	
	INAG (2009a)	DL 218/2015	DL	Au	Sp	Au	Sp	Au	Sp	Au	Sp
Calcium (mg/L)			1	63.0	3.8	4.5	3.8	4.5	5.1	4.9	4.1
Magnesium (mg/L)			0.5	16.0	1.5	1.6	1.8	1.7	1.6	1.4	1.3
Iron (µg/L)			1	36	45	220	57	360	110	190	71
Manganese (µg/L)			0.05	3.26	4.34	14.2	4.78	65.7	12.8	29.2	6.04
Arsenic (µg/L)	50		0.01	1.77	0.93	2.13	0.93	2.95	1.48	3.34	2.2
Cadmium (µg/L)		≤0.45 (Class 1) 0.45 (Class 2) 0.6 (Class 3) 0.9 (Class 4) 1.5 (Class 5)	0.01	0.07	0.02	0.02	0.01	0.06	0.01	0.01	0.03
Copper (µg/L)	100		0.15	0.74	2.37	2.87	0.98	2.19	0.99	2.42	0.85
Mercury (µg/L)		0.07	0.01	<0.01	0.02	0.04	0.02	0.02	0.02	0.02	0.02
Nickel (µg/L)		34	0.2	<0.2	0.6	1.1	0.6	0.9	0.4	0.8	0.3
Lead (µg/L)		14	0.1	1.0	0.5	1.0	0.2	0.7	0.2	0.4	0.6
Zinc (µg/L)	500		0.9	1.4	6.7	16.0	12.8	14.2	6.7	11.0	6.8
Anthracene (µg/L)		0.1	0.01	<0.02		<0.01		<0.02		<0.01	
Benzo (a) pyrene (µg/L)		0.27	0.005	<0.01		<0.005		<0.01		<0.005	
Fluoranthene (µg/L)		0.12	0.01	<0.02		<0.01		<0.02		<0.01	
Naphthalene (µg/L)		130	0.05	<0.10		<0.05		<0.10		<0.05	
Aldrin (µg/L)		Not applicable	0.01	<0.01		<0.01		<0.01		<0.01	
β-endosulfan (µg/L)			0.01	<0.01		<0.01		<0.01		<0.01	
Dieldrin (µg/L)		Not applicable	0.01	<0.01		<0.01		<0.01		<0.01	
Endosulfan (total) (µg/L)		0.01	0.02	<0.02		<0.02		<0.02		<0.02	

α-Endosulfan (µg/L)			0.02	<0.02		<0.02		<0.02		<0.02	
Endrin (µg/L)		Not applicable	0.01	<0.01		<0.01		<0.01		<0.01	
HCH Alpha (µg/L)			0.005	<0.005		<0.005		<0.005		<0.005	
HCH Beta (µg/L)			0.01	<0.01		<0.01		<0.01		<0.01	
HCH, gamma – Lindane (µg/L)			0.001	<0.001		<0.001		<0.001		<0.001	
HCH Epsilon (µg/L)			0.001	<0.001		<0.001		<0.001		<0.001	
Hexachloro-1,3-butadiene (µg/L)		0.6	0.02	<0.02		<0.02		<0.02		<0.02	
Hexachlorobenzene (HCB) (µg/L)		0.05	0.005	<0.005		<0.005		<0.005		<0.005	
Isodrin (µg/L)		Not applicable	0.01	<0.01		<0.01		<0.01		<0.01	
Chlorfenvinphos (µg/L)		0.3	0.02	<0.02		<0.02		<0.02		<0.02	
Chlorpyrifos-ethyl (µg/L)		0.1	0.005	<0.005		<0.005		<0.005		<0.005	
Dichlorvos (µg/L)	0.001	$7x10^{-4}$	0.005	<0.005		<0.005		<0.005		<0.005	
Atrazine (µg/L)		2	0.005	<0.005		<0.005		<0.005		<0.005	
Atrazine-desethyl (µg/L)			0.005	<0.005		<0.005		<0.005		<0.005	
Desethyl-terbuthylazine (µg/L)	Without EQO		0.005	<0.005		<0.005		<0.005		<0.005	
Irgarol (Cybutryne) (µg/L)		0.016	0.005	<0.01		<0.01		<0.01		<0.01	
Terbuthylazine (µg/L)	Without EQO		0.005	<0.005		<0.005		<0.005		<0.005	
Terbutryn (µg/L)		0.34	0.005	<0.005		<0.005		<0.005		<0.005	
Alachlor (µg/L)		0.7	0.005	<0.005		<0.005		<0.005		<0.005	
Diuron (µg/L)		1.8	0.005	<0.005		<0.005		<0.005		<0.005	
Isoproturon (µg/L)		1	0.005	<0.005		<0.005		<0.005		<0.005	
Linuron (µg/L)	1.0		0.005	<0.005		<0.005		<0.005		<0.005	
Cypermethrin (µg/L)		$6x10^{-4}$	0.08	<0.08		<0.08		<0.08		<0.08	
Tebuconazole (µg/L)			0.005	<0.005		<0.005		<0.005		0,019	

Aclonifen (µg/L)		0.12	0.04	<0.04		<0.04		<0.04		<0.04	
Bifenox (µg/L)		0.04	0.08	<0.08		<0.08		<0.08		<0.08	
Dicofol (µg/L)		Not applicable	0.05	<0.05		<0.05		<0.05		<0.05	
Quinoxyfen (µg/L)		2.7	0.05	<0.05		<0.05		<0.05		<0.05	
Chloroalkanes C10-C13 (µg/L)		1.4	0.15	<0.30		<0.30		<0.30		<0.30	
Pentachlorophenol (PCP) (µg/L)		1	0.02	<0.04		<0.04		<0.04		<0.04	
Tributyltin cation (µg/L)		0.0015	0.00005	<0.00005		<0.00005		<0.00005		<0.00005	
Diazepam (µg/L)			0.05	<0.05		<0.05		<0.05		<0.05	
Ethinylestradiol (µg/L)			0.02	<0.02		<0.02		<0.02		<0.02	
Ecological Potential				**G/E**	**G/E**	**G/E**	**G/E**	**G/E**	**G/E**	**G/E**	**G/E**

3.2. Phytoplankton community

Through the analysis of the phytoplankton community (Table 3) a good (Sp A2 and A3), moderate (Au A1, A2, A4 and Sp A1, A4) or bad (Au A3) EP values were obtained. These results highlight that the autumn sampling, done after a very hot summer, was conducted at low levels of water.

Table 3 Results obtained for the EQR of phytoplankton community. Reference values for calculation of EP and EQR normalized (below in parentheses) for each parameter for good/moderate EP according (INAG, 2009a), (Sampling sites: A1, A2, A3 and A4; Sampling periods: Au - autumn and Sp - spring).

		A1		A2		A3		A4	
	Reference values (EQR)	**Au**	**Sp**	**Au**	**Sp**	**Au**	**Sp**	**Au**	**Sp**
Chl *a* (mg/m^3)	2 (0.21)	6.03 (0.33)	29.24 (0.07)	11.22 (0.18)	34.02 (0.06)	1202.48 (0.002)	11.29 (0.18)	3.82 (0.53)	31.00 (0.06)
Biovolume Total (mm^3/L)	0.36 (0.19)	10.77 (0.03)	62.99 (0.02)	67.92 (0.01)	115.17 (0.003)	268.49 (0.001)	165.43 (0.002)	13.46 (0.03)	35.39 (0.01)
% Biovolume Cyanobacteria	0 (0.91)	0.29 (1.00)	1.24 (0.99)	1.36 (0.99)	1.02 (0.99)	91.82 (0.08)	1.56 (0.98)	24.80 (0.75)	5.61 (0.94)
AGI	0.1 (0.97)	86.65 (0.79)	20.34 (0.95)	97.36 (0.76)	5.14 (0.99)	166.07 (0.59)	8.63 (0.98)	37.79 (0.91)	36.67 (0.91)
EQR	**0.6**	**0.56**	**0.52**	**0.49**	**0.73**	**0.11**	**0.78**	**0.47**	**0.47**
Ecological Potential		**Moderate**	**Moderate**	**Moderate**	**Good**	**Bad**	**Good**	**Moderate**	**Moderate**

The bad EP classification recorded in the autumn in A3 sample (Table 3) must be due to a cyanobacterial bloom of *Microcystis* (EQR for % biovolume of Cyanobacteria ≈ 92%), that led to the worst EQR value (0.11) registered. Moreover, in autumn, Chl *a* concentration in site A3 exceeded 1000 mg/m^3 and Cyanobacteria biovolume accounted for over 90 % of the total biovolume which are reflected in the AGI index (> 150). This index assigns weights and compares groups of algae characteristics of eutrophic zones and groups associated with less productive environments (Catalan et al., 2003). In spring, however, this site showed a good EP, with a reduction of Chl *a* content to ≈ 11 mg/m^3 and a <2 % Cyanobacteria biovolume, resulting in a lower AGI value (≈ 9).

Overall, sites A1, A2 and A4 followed a similar pattern, with the highest values of Chl *a* concentration of ≈ 30 mg/m^3, higher total biovolume and lower % of cyanobacterial biovolume in the spring season (Table 3). AGI tendency to decrease in spring with the exception of A4, reflected the decrease of phytoplankton abundance typical of eutrophic zone groups. Bellinger and Sigee (2015) already demonstrated that high nutrient availability, temperature increase and light conditions that occur in spring and summer favour growth and diversity of the phytoplankton community.

Since the 1990s, Aguieira reservoir has been characterized by high frequency of cyanobacterial blooms (Vasconcelos et al., 1996, 2011; APA, 2007), with the genera *Microcystis*, *Anabaena*, *Gomphosphaeria* and *Synechococcus* being the most frequently

recorded in this reservoir (Figueiredo et al., 2012). The accumulation of nutrients in the water body (*e.g.* phosphorus and nitrogen), also recorded in this study specifically in A3 sample (Table 1), promotes an excessive growth of phytoplanktonic organisms, namely, Cyanobacteria (APA, 2007). The increase of nutrients in this aquatic ecosystems (Table 1 and 2) with a consequent high concentrations of Chl *a* in almost all sites (Table 3) induces an high trophic state, which is situated between the mesotrophic and the eutrophic states as describe in INAG (2011). Moreover, eutrophic reservoirs promote the development of macro-chlorophytes and Cyanobacteria which are unpalatable for zooplankton community as well as the occurrence of toxins, breaking the links in the food chains, which become less complex and diverse (APA, 2007). Furthermore, the cyanobacterial overgrowth in freshwater ecosystems may release to the water harmful toxins that can be consumed by humans, and other aquatic and terrestrial organisms with noxious consequences (Graça et al., 2002; Figueiredo et al., 2012).

Figure 2 shows the relative abundance of AGI groups obtained in the present study. In the autumn sampling, a high difference between sites was recorded. In A1 and A2, colonial Chlorococcales were the dominant group (≈ 95%), while in A3 Cyanobacteria was the most abundant group (≈ 98%). A4 showed a similar abundance of the two previous groups (≈ 45% and ≈ 50%, respectively) which represents almost 95% of the phytoplankton in the sample. In the spring sampling, an increase of Cyanobacteria group in all sites was observed, representing more than 60% of the phytoplankton recorded. Colonial Bacillariophyceae and Others (standing for species not represented in AGI) also presented higher abundances in all sites. The genera *Chroococcus*, *Synechocystis* and *Synechococcus*, which are not included in the AGI calculation, have now been included in this analysis, resulting in a high abundance of the cyanobacterial group.

Indeed, Oliveira & Monteiro (1992) already observed the existence of a spatial phytoplankton gradient in Aguieira reservoir. The high concentration of Cyanobacteria in Dão river (Santa Comba Dão) is usually four times higher than the observed near to the dam, probably due to the agricultural and urban activities. Moreover, the Plano de Ordenamento da Albufeira da Aguieira (APA, 2007) also states that the results of phytoplankton analysis showed a phytoplankton gradient, with values of 29.3% of Cyanobacteria near de dam, 49.4% in Tábua (Mondego river) and 93.8% in the Santa Comba Dão (Dão river). Our data corroborates the results previous mentioned from the 90's regarding A3 location (very close to Santa Comba Dão) and A4 location (near Tábua).

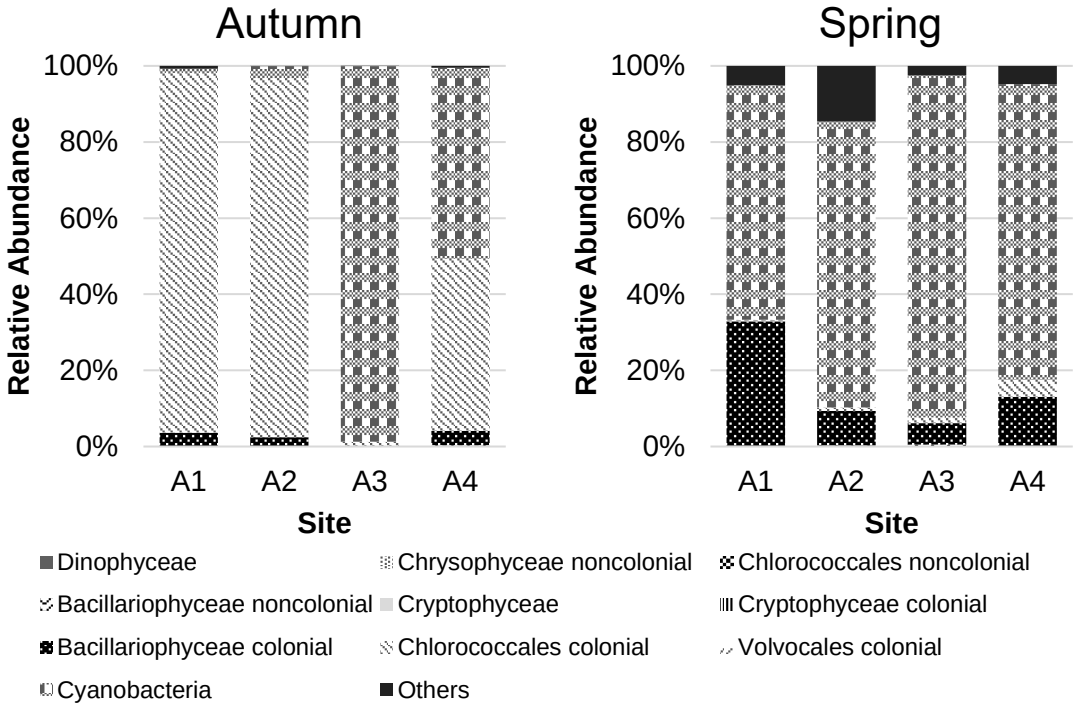

Figure 2 Results of relative abundance (%) of groups of AGI for phytoplankton communities for each sampling site in the two seasons. Each texture represents the different phytoplankton groups.

3.3. Zooplankton community

Figure 3 shows the relative abundances of zooplankton for the four sites in both sampling seasons. In autumn the highest abundant organisms were *Diaphanosoma*, *Ceriodaphnia*, Calanoida and Cyclopoida, followed by *Chydorus* and *Bosmina*, although these presented different proportions between sites. Site A3 showed a more distinct zooplankton community with a high abundance of *Ceriodaphnia quadrangular*. In spring season, *Daphnia* group was dominant in almost sites, except for A2 where Calanoida was the dominant group. The groups Calanoida, Cyclopoida and the genus *Bosmina* showed a high abundance in this season. Regarding the richness values (Fig. 3), an increase was recorded for all sites in spring season. A2 and A3 showed the highest richness in both seasons.

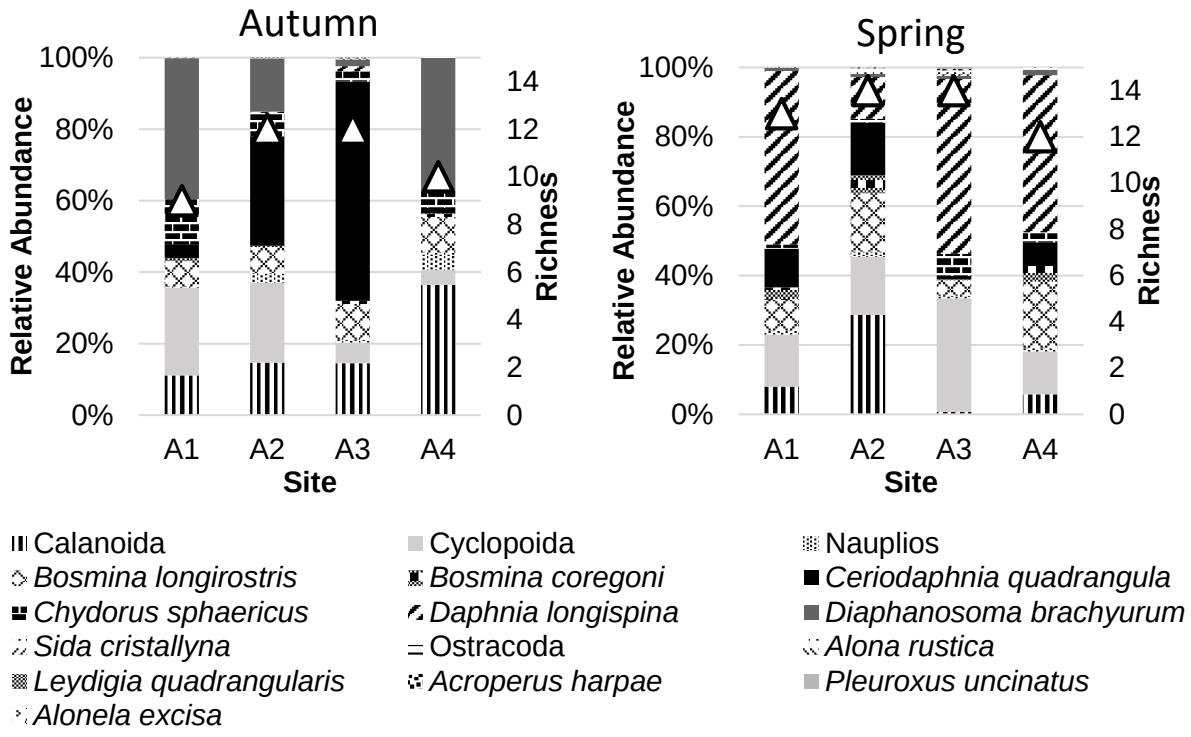

Figure 3 Results of relative abundance (%) and specific richness (Δ) of zooplankton communities for each sampling sites in the two seasons. Each texture represents the different zooplankton groups.

Zooplanktonic community can change according to the phytoplankton community and other nutrients available (Jeppesen et al., 2000, 2011; Geraldes & Silva-Santos, 2011). Indeed, several authors already demonstrated that the zooplanktonic community can be related to the trophic state of the water body (Geraldes & Silva-Santos, 2011; Haberman & Haldna, 2014; Pociecha et al., 2018). Aguieira is classified as a eutrophic reservoir, so typical species can occur, such as the case of the genus *Bosmina*. In fact, *Bosmina*, is a tolerant species described for eutrophic ecosystems (Beaver et al., 1999; Jensen et al., 2013), which represents about 10% of the species recorded in our samples. Also, copepods such as cyclopoids are indicators of eutrophic environments (Beaver et al., 1999), and these organisms may have eating habits ranging from large particles to filamentous algae (Fryer, 1957; Beaver et al., 1999) which are characteristics of seston in eutrophic conditions. In contrast, the macrofiltration herbivores, such *Daphnia* spp.*,* tend to decrease in abundance in eutrophic conditions. Geraldes & Silva-Santos (2013) and Geraldes et al. (2016b, 2017) observed that the genus *Daphnia* appears in high abundance when Cyanobacteria are in lower abundance in Aguieira reservoir. According to Geller & Müller (1981), the genus *Daphnia*, a low efficient filter feeding organism, presents a peak of abundance in spring and a lower peak in autumn in eutrophic temperate lakes. It is also in the spring, when all phytoplankton groups start to increase mainly due to the high light period, that more available food occurs for

zooplankton. However, a high relative abundance of Cyanobacteria group in all sites was observed (Fig. 2).

The genus *Diaphanosoma* also showed high abundance in the autumn sampling (Figure 3). These organisms are more abundant in hot summer months (Geraldes et al. (2016a), and, although our samplings were collected in autumn, the recorded temperature was still high (Table 1), which may explain the high abundance of this organism. The genus *Ceriodaphnia* also showed high abundance, especially at A2 and A3 sites in the autumn sampling. This genus is strongly related to the P_{total} concentration. In fact, an increase of small cladocerans occurred when an increase of phosphorus concentration in water was observed in lakes in Denmark (Jeppesen et al., 2000, 2011). The here-obtained results showed the same trend with an increase of small Cladocera in A2 and A3 (Figure 3) coincident with the highest P_{total} values, (Table 1). In the identified *taxa*, an occurrence of a non-native species of the Portuguese water bodies was recorded. *Bosmina coregoni* was observed in all sites in both seasons. Indeed this is a non-native species, recently described for Portuguese water bodies (Geraldes & Alonso, 2014). *B. coregoni* tends to present the maximum abundance in winter as observed in the work of Geraldes & Silva-Santos (2011) and Geraldes et al. (2016a) in Aguieira reservoir. However, in the present study the occurrence of this species was recorded in both seasons for all sites, namely in site A4.

Jeppesen et al. (2011) in studies in Denmark, Estonia and UK, observed always a decrease in zooplankton richness, especially in Cladocera group, with an increase of P_{total} concentration. This means that, under eutrophic conditions, the richness of zooplankton tends to be lower. This situation was also recorded in the here-presented data in autumn, when the concentration of P_{total} was higher and the lowest number of microzooplankton species was observed, and the high trophic state was recorded. In two reservoirs, Poções and Camalaú, in the Paraíba River basin, northeastern Brazil, Azevedo et al. (2015) positively related hypereutrophic conditions to high richness values of zooplankton. However, this was mainly due to the higher number of rotifer species observed, which have a wider trophic plasticity and high tolerance to these conditions. Indeed, Moss et al. (2003), related the higher abundance of larger zooplankton species (*e.g.* Cladocera) with better trophic conditions, which is in agreement with our results.

Diversity and equitability Indexes values for zooplankton community are shown in Table 4. High diversity values for all sites in both seasons (> 1) were observed. Site A2 presented the highest diversity values for both seasons (Au=1.78 and Sp=1.89). Regarding equitability, were obtained low values (< 0.44), representing low equitability.

Site A2 showed the highest equitability values for both seasons (> 0.40). Site A3 showed less diversity and equitability in both seasons (Table 4). In fact, site A3 had some regularly cyanobacterial blooms, which can release toxins to the water body affecting higher trophic levels. On the other hand, this site also showed high concentrations of P_{total}. As mentioned earlier, eutrophic conditions lead to the proliferation of phytoplankton with poor quality for zooplankton diet, making the zooplanktonic community simple and less diverse. Situation observed in our results in A3 site (the worst EP recorded - Tables 1, 3), especially in the autumn, when the lowest zooplankton diversity was recorded (Table 4 and Fig. 3).

Table 4 Diversity and Equity Indexes: Shannon-Weaver (H' - diversity) and Simpson (E- equitability) indexes for the zooplankton community for each sampling site and season: Au - autumn and Sp - spring.

	A1		A2		A3		A4	
	Au	**Sp**	**Au**	**Sp**	**Au**	**Sp**	**Au**	**Sp**
H'	1.59	1.57	1.78	1.89	1.32	1.32	1.52	1.67
E	0.44	0.26	0.42	0.40	0.20	0.19	0.35	0.31

The relative abundance of zooplankton functional groups is shown in Figure 4. In the autumn period, a high abundance of high efficiency filter feeding organisms (*e.g. Ceriodaphnia*, *Chydorus*, and *Diaphanosoma*) was recorded. Indeed, these organisms are described as dominant in warm months, usually expected in summer months (Geller & Müller, 1981; Jensen et al., 2013). Actually, in the autumn sampling period, high water temperature values were recorded (Table1) with high light intensity, variables responsible for the high abundance of these organisms. In the spring season, a replacement of zooplankton functional groups was observed with an increase of the low efficiency filter feeding organisms (*e.g. Bosmina* and *Daphnia*) in detriment of those previously observed in higher abundance (high efficiency filter feeding capacity). These results were expected once these organisms tend to have higher abundance in months when the temperature starts to rise and the days start to get longer (Geller & Müller, 1981; Jensen et al., 2013). In the present study, the abundances of low efficiency filter feeding organisms are essentially due to the high abundance of *Daphnia* spp. that have been observed in all sites of Aguieira reservoir. Geraldes & Silva-Santos (2011) in the same reservoir, observed, in September 2010, high abundances of *Chydorus*, *Diaphanosoma* and *Ceriodaphnia*, three organisms with high efficiency filter feeding, which are in accordance with our results obtained in the autumn sampling. In the same study in March 2010, higher abundances of two low efficiency filter feeding organisms, *Bosmina* and *Daphnia,* were observed. Indeed, these are the same genera recorded in the present study with higher abundances in spring season.

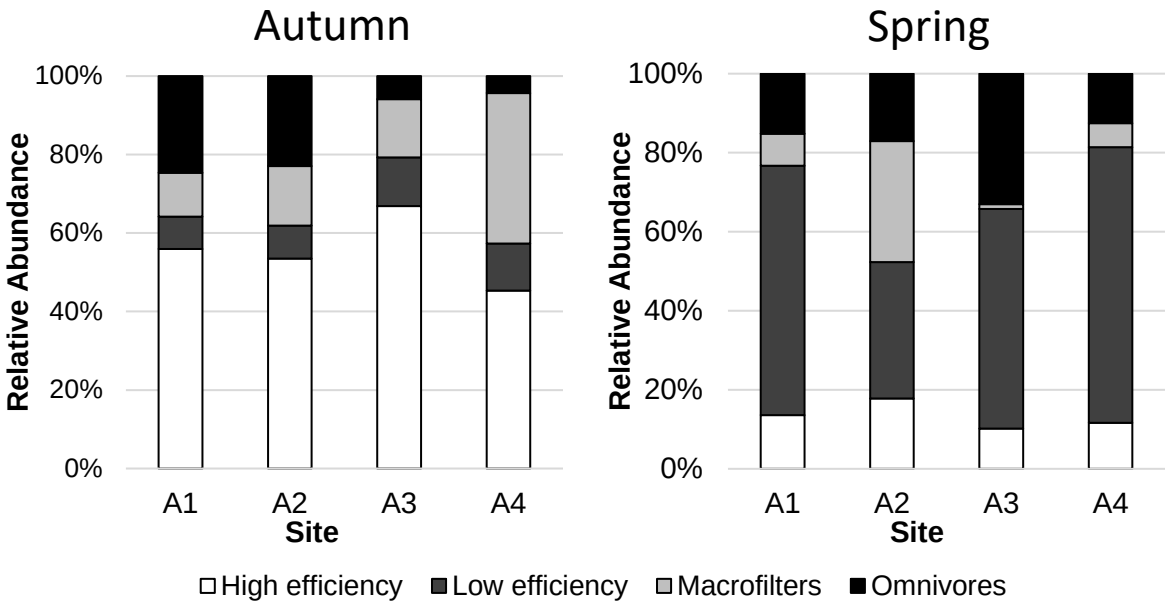

Figure 4 Results of relative abundance (%) of zooplankton functional groups for each sampling site in the two seasons taking into account filtering and feeding capacities according to Geller e Müller (1981) and Reid & Williamson (2010).

3.4. Bioassays

Figure 5 shows the results of the growth inhibition assay of the alga *Raphidocelis subcapitata*, for the two sampling periods. In autumn samples, a significant increase in growth of *R. subcapitata* (Table A1) in F2 water (Aguieira water filtered with 0.22 µm where all suspended material and organisms were removed) for all sites was recorded. Although the lowest increase of growth was obtained for A3 water, this was not significant (Table A1) when compared to the control group. Indeed, site A3 was already classified with the worst ecological potential, according to WFD metrics, for physical, chemical and the biological parameter (phytoplankton community). Moreover, a bloom of Cyanobacteria was observed at this site in autumn period (Table 3). For the other water treatments, NF and F1 (NF = unfiltered water of the reservoir; F1 = the 1.2 µm filtrated water in which suspended material, phytoplankton and zooplankton were removed), a significant decrease (Table A1) of algae growth was observed for all sites.

In spring water samples, a significant decrease (Table A1) of algal growth was recorded for all water treatments and sites (Fig. 5). Note that algae growth was always higher in spring samples. Pérez et al. (2010) in unfiltered natural waters from Alqueva reservoir, always observed lower algae growth rates throughout the year comparing to the control group. When looking at their results in the same period of our results, we observe the same pattern, with higher growth rates in spring season and lower growth rates in autumn (Fig. 5). Pérez et al. (2010) linked the presence of herbicides such as

atrazine, simazine, terbutylazine, and metholachlor (used in agriculture practices) to the low growth rates recorded. However, in the present study, these pesticides were detected in low concentrations according to INAG (2009a) and the Annex I of Legislative Decree No. 218/2015 (Ministry of Environment for Spatial Planning and Energy, 2015).

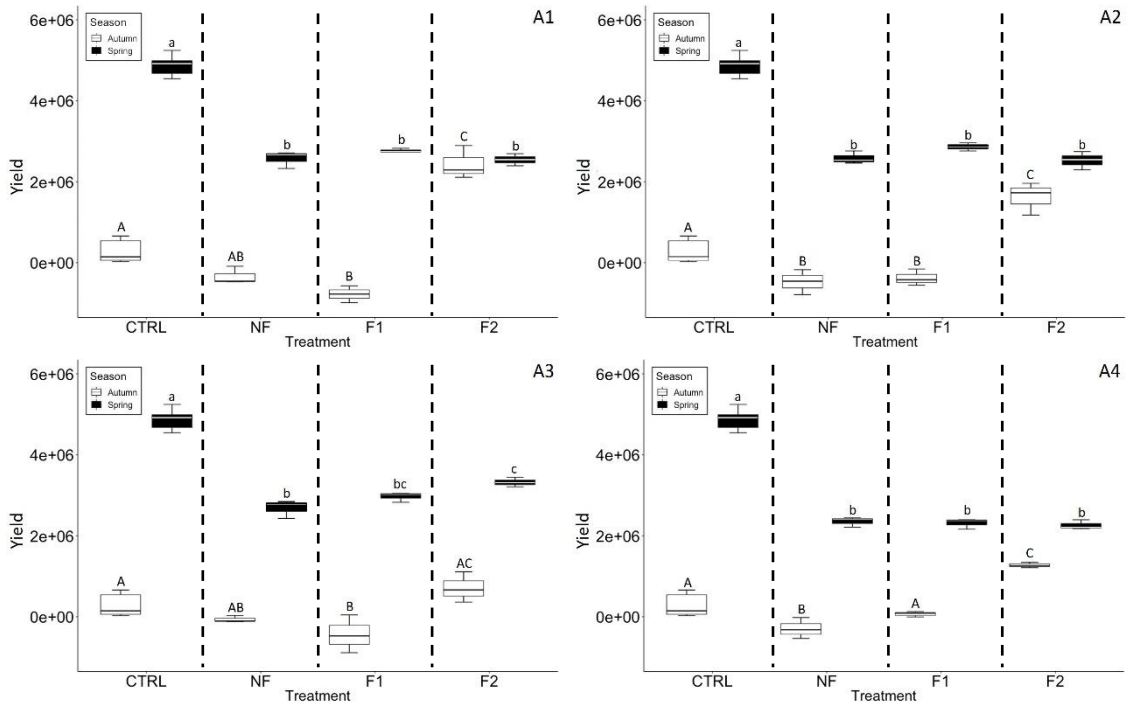

Figure 5 Results of growth inhibition assay of *R. subcapitata* when exposed to different natural water conditions from the Aguieira reservoir (CTRL – Control treatment; NF – Unfiltered water; F1 – filtered with 1.2 µm and F2 – filtered with 0.22 µm). Sampling sites: A1, A2, A3 and A4. Different letters (a, b, c for spring; A, B, C for autumn) stands for significant differences between treatments (Tukey test, $p<0.05$) in each sampling period.

The results of *Daphnia longispina* feeding rate assay are shown in Figure 6. In autumn samples, a significant decrease of the feeding rate (Table A1) was observed for all water treatments from sites A3 and A4, and for NF and F1 waters from A1 and A2, respectively. This lower feeding rate in NF may reflect that phytoplankton community was not palatable for *D. longispina*. Indeed, the zooplankton community appeared in low abundance in autumn (Fig. 3 and Table 4). In the spring sampling, the results of the feeding rate assay for *D. longispina* were similar to the control group, with few exceptions. All water treatments from site A2 showed a significant decrease in the feeding rate (Table A1). However, in A1, A3 and A4 a significant decrease of the feeding rate (Table A1) was only observed for the F1 water treatment.

In the *Daphnia magna* autumn assays (Fig. 6), a significant decrease of the feeding rate (Table A1) was observed for NF water for all sites, and for all treatments in site A3. Regarding the spring samples, similar results were observed in almost water treatments and sites. This assay demonstrated sensitivity for the assessment of natural waters conditions, being more noticeable in the autumn samples. Orlowicz (2012) in Old

Gurham Reservoir, observed that the presence of phytoplankton with poor quality for *Daphnia* diet such as Cyanobacteria and large filamentous or colonial algae, may lead to lower feeding rates. Furthermore, it is known that *Daphnia* prefers smaller organisms such *Chlorella* and *Raphidocelis* and organic detritus including protists and bacteria in its diet (Ebert, 2005).

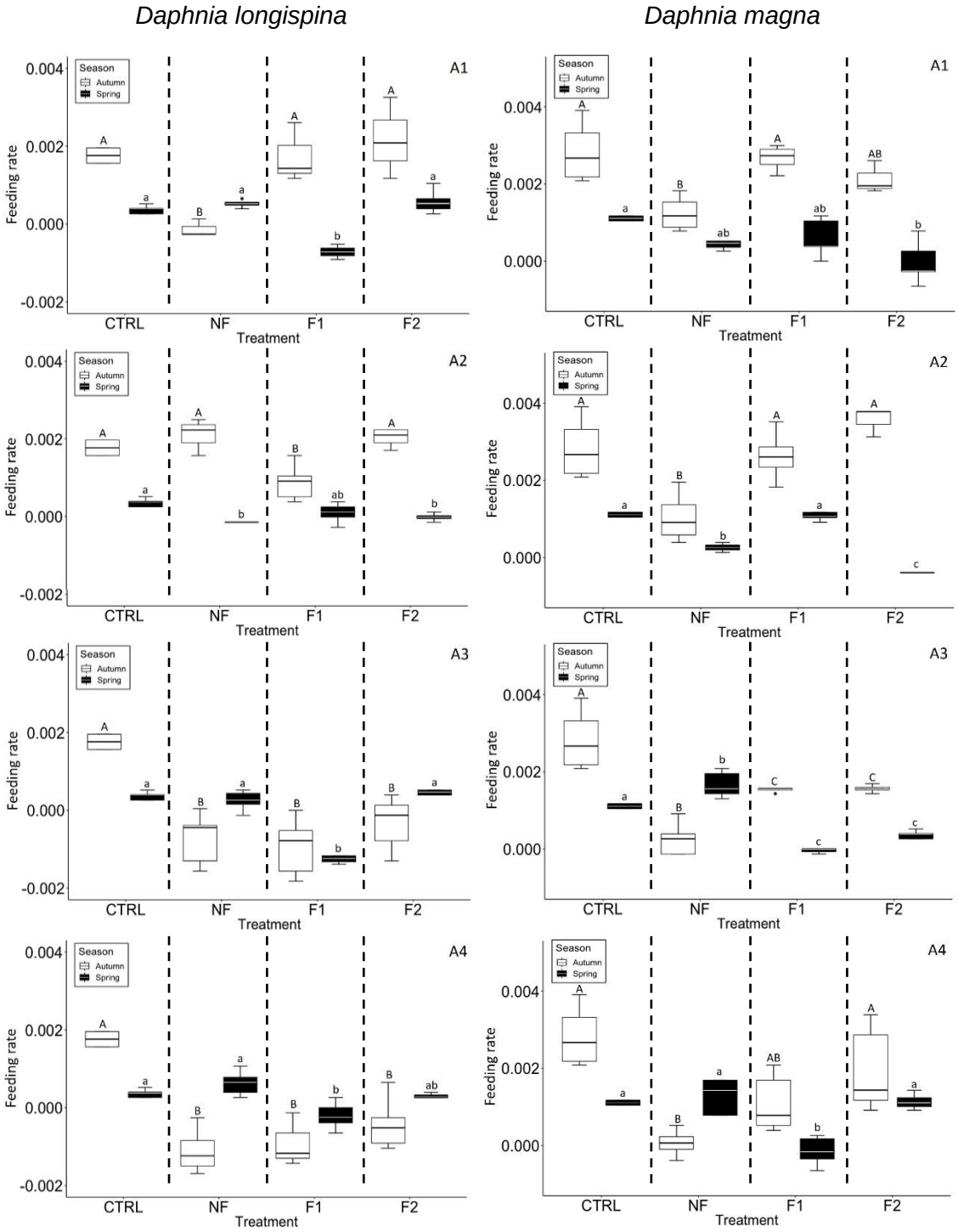

Figure 6 Results of feeding rate of *D. longispina* and *D. magna* when exposed to different natural water conditions from the Aguieira reservoir (CTRL – Control treatment; NF – Unfiltered water; F1 – filtered with 1.2 µm and F2 – filtered with 0.22 µm). Sampling sites: A1, A2, A3 and A4. Different letters (a, b, c for spring; A, B, C for autumn) stands for significant differences between treatments (Tukey test, $p<0.05$) in each sampling period.

Figure 7 shows the results of *Spirodela polyrhiza* growth inhibition assay. In autumn samples a significant decrease of the growth was observed in NF for all sites (Table A1). F2 treatment also induced a significant decrease of *S. polyrhiza* for waters from sites A2 and A4 (Table A1). In spring, a significant decrease of growth was also observed in all water treatments and sites, but without significant differences between water treatments (Table A1). In general, the autumn sampling presents the worst EP (three sites with Moderate EP and one with Bad EP) when compared to spring sampling (two sites with Good EP and two with Moderate EP) (Table 1, 2 and 3). However, the same tendency in both seasons was observe, with a significant decrease of growth in almost treatments and sites (Table A1), which means that the results of this assay do not reflect the difference observe in the final EP.

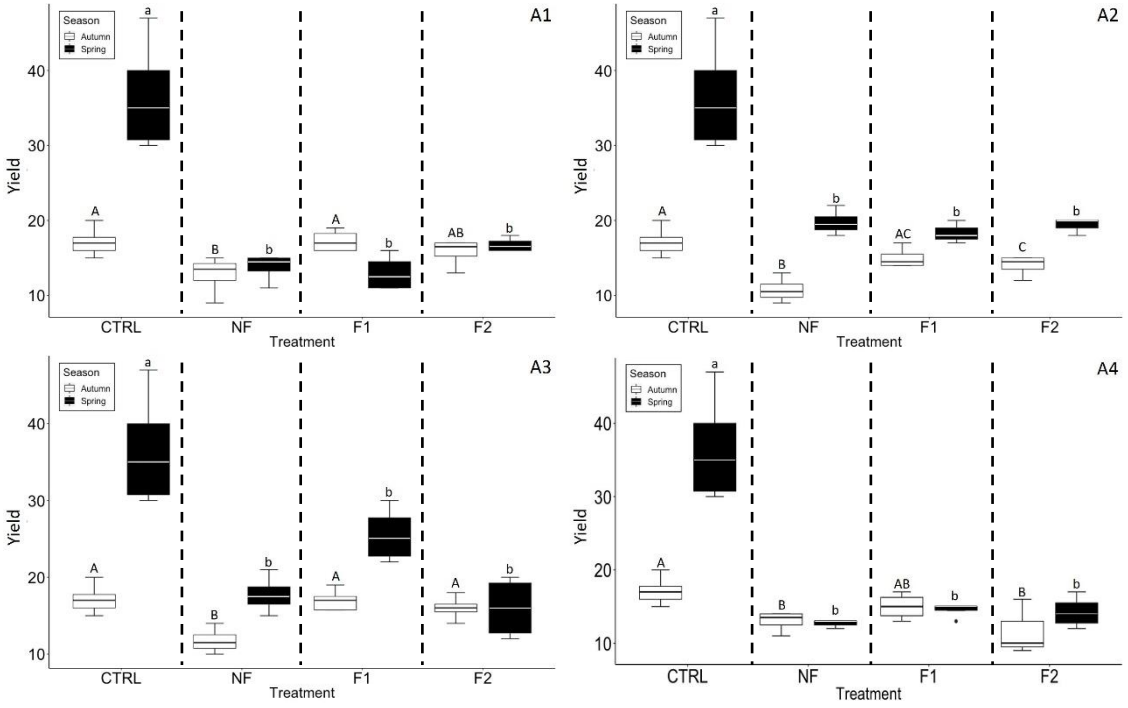

Figure 7 Results of growth inhibition assay of *S. polyrhiza* when exposed to different natural water conditions from the Aguieira reservoir (CTRL – Control treatment; NF – Unfiltered water; F1 – filtered with 1.2 µm and F2 – filtered with 0.22 µm). Sampling sites: A1, A2, A3 and A4. Different letters (a, b, c for spring; A, B, C for autumn) stands for significant differences between treatments (Tukey test, $p<0.05$) in each sampling period.

The decrease in growth may be due to the lack of nutrients in the natural waters, important parameter for development of macrophyte (Table 1). On the other hand, the possibility of toxins from Cyanobacteria species, such as the bloom of *Microcystis* observed in A3 in autumn, may have been the factor that conditionate the growth of *S. polyrhiza* (Mohamed, 2017). Romanowska-Duda et al. (2002) on the Sulejow reservoir in Poland, observe that the high toxic and cytotoxic effect of blue-green algal blooming, *Microcystis aeruginosa*, reduced the number of fronds about of 50%. Henry-Silva et al. (2008) observed in their work that low concentrations of nitrogen and phosphorus, as

well as temperatures below 15 °C or above 30 °C, induced a significant decrease on macrophyte growth. Junk & Piedade (1997) observed, in a study of ecology of aquatic macrophytes in the Amazon floodplain, that when nutrient concentrations were low the growth of macrophyte was slower.

According to several studies (Eaton et al., 1995; Yan & Zhou, 2011; Nunes et al., 2014b) changes in pigment composition such as Chl *a* + *b* and carotenoids may serve as an indicator of the general physiological status of photosynthetic organisms. The capacity of macrophytes for bioaccumulation and biotransformation of toxins may lead to decreased photosynthetic capacity and changes in plant pigment composition (Mohamed, 2017). In here-presented results (Fig. 8), a significant decrease in the content of photosynthetic pigments of *S. polyrhiza* exposed to natural waters was observed for all treatments and sites for both sampling periods (Table A1). However, no significant differences between water treatments were recorded (Table A1), which means that the photosynthetic performance of *S. polyrhiza* did not change due to the different water treatments.

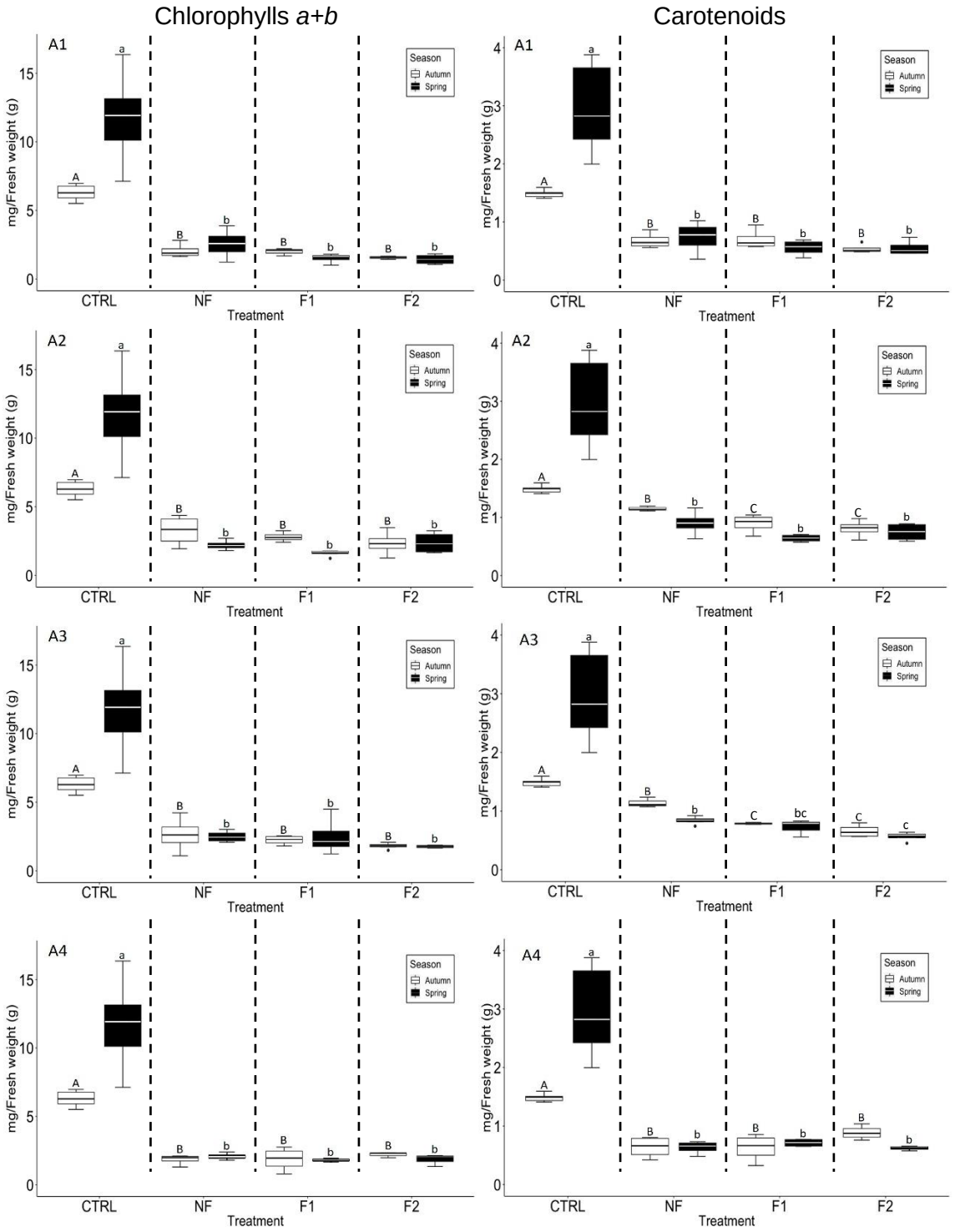

Figure 8 Results of chlorophylls *a+b* and carotenoid concentrations, of the *S. polyrhiza* after exposed of growth inhibition assay to different natural waters conditions (CTRL – Control treatment; NF – Unfiltered water; F1 – filtered with 1.2 µm and F2 – filtered with 0.22 µm). Sampling sites: A1, A2, A3 and A4. Different letters (a, b, c for spring; A, B, C for autumn) stands for significant differences between treatments (Pairwise t test, $p<0.05$) in each sampling period.

Zooplankton community is not part of the WFD biological assessment for water quality in reservoirs. However, this community has shown to be sensitive in all sites sampled and very important in the assessment of the quality of water bodies (Table 5). The natural dynamic of the structural and functional of the zooplankton community clearly

showed the existence of differences between sites and throughout the two seasons (Table 5). This evaluation also showed the tendency of this reservoir to eutrophic conditions, which implies poor quality of the water. In this way, the analysis of zooplankton community provides a more realistic scenario of water quality since respond to changes in water reservoir, while almost physical and chemical parameters only give momentary information. However, the zooplankton evaluation does not allow us to make a classification by ranges, such as provides the WFD approach. Therefore, this biological parameter proves to be fundamental in the assessment of the water quality of a reservoir in complement of WFD. Moreover, the combination of the three parameters: physical and chemical, phytoplankton and zooplankton, will permit a stronger evaluation of an artificial water body allowing a more accurate and realistic determination of the ecological potential.

R. subcapitata and *S. polyrhiza* bioassays only showed sensitive results in the autumn sampling (Table 5). Indeed, these bioassays should be repeated in other seasons and different water pressures, in order to better understand the information that they may provide. The photosynthetic pigments of *S. polyrhiza* exposed to natural waters did not show sensitive results in order to discriminate water conditions. *D. longispina* and *D. magna* assays showed a similar trend throughout the two sampling periods, although not showing a clear specific pattern, but evidencing an existence of sensitivity to the different treatments, especially in the autumn for *D. magna*.

The use of bioassays has several advantages like the use of standard species. However, some difficulties may be recognised since biological organisms can have different responses along the year due to biological clocks (Noordally & Millar, 2015). Although they are standard organisms several studies already demonstrated the variability of responses (Palma et al., 2010), fact that was also observe in this study (Fig. 5, 6, 7 and 8). Pereira et al. (2009) noted that uncontrollable variations in laboratory methods and the use of different cultures may explain unexpected results. Moreover, the fact of working with natural waters makes the interpretation of the results even more difficult, due to a diverse of confounding factors (*e.g.* competition, predation, toxin concentrations). Moreover, from the results obtained in the bioassays it is not possible to define a range of values about the ecological state of the water body. On the other hand, the use of species from different trophic levels to assess water quality, may provide more realistic scenarios and results, since different species have different water sensitivities. Burton & Macperson (1995) already demonstrated that species metabolic processes respond differently to the same stress, providing additional and

complementary information. In addition, sensitive bioassays provide faster data when compared to chemical compound determination and community characterization.

Table 5 Classification of ecological potential for each site in each sampling according to WFD. Results of the sensitivity of zooplankton and bioassays in the evaluation of Aguieira reservoir water body. Sampling sites: A1, A2, A3 and A4. (+ present sensitivity; + / - present some sensitivity; - no sensitivity).

	A1		A2		A3		A4	
	autumn	**spring**	**autumn**	**spring**	**autumn**	**spring**	**autumn**	**spring**
Physical and chemical parameters	Good	Good	Good	Good	Moderate	Moderate	Good	Good
Specific pollutants and priority substances	Good/ Excellent	Good/ Excellent	Good/ Excellent	Good/ Excellent	Good/ Excellent	Good/ Excellent	Good/ Excellent	Good/ Excellent
Biological parameters	Moderate	Moderate	Moderate	Good	Bad	Good	Moderate	Moderate
Ecological Potential	**Moderate**	**Moderate**	**Moderate**	**Good**	**Bad**	**Moderate**	**Moderate**	**Moderate**
Zooplankton	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
Bioassays								
R. subcapitata	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**
D. magna	**+**	**+ / -**	**+**	**+ / -**	**+**	**+ / -**	**+**	**+ / -**
D. longispina	**+ / -**	**+ / -**	**+ / -**	**+ / -**	**+ / -**	**+ / -**	**+ / -**	**+ / -**
S. polyrhiza	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**
Pigments of *S. polyrhiza*	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

+ the parameter presented sensitivity for the evaluation of the water body.
+ / - the parameter presented some sensitivity for the evaluation of the water body.
- the parameter did not present sensitivity for the evaluation of the water body.

4. Conclusion

Based on the WFD metrics, our results showed that the Aguieira reservoir was, in general, characterized by having a poor ecological potential, especially in autumn when it revealed the worst results (Table 5). These results were a consequence of a low quality of biological parameters, especially the phytoplankton, due to the occurrence of cyanobacterial blooms and the P_{total} concentration, recorded in levels higher than the limit imposed by law specially in site A3.

For the other ecological parameters and tools, not considered in the WFD metrics (Table 5), only the zooplankton community analysis showed sensitivity in the water body evaluation. The bioassays with *R. subcapitata* and *S. polyrhiza* presented sensitivity only in the autumn sampling, unlike the *D. longispina* and *D. magna* assays showed some sensitivity in both samples. The analysis of *S. polyrhiza* pigments did not discriminate the water quality of Aguieira reservoir.

Further research is needed in order to better understand the effects of waters from reservoirs have on the standard organisms in the bioassays. In *D. magna* and *D. longispina* assays, quantification of biomarkers such as oxidative stress biomarkers should be considered. In the *S. polyrhiza* assay, biomarkers quantification (*e.g.* malondialdehyde content, proline content), quantification of fresh weight as well as final dry weight might be important parameters for the assessment of water quality. Regarding the zooplankton community, the quantification of biomass by species may also be another important factor on the status of this community, as the analysis of others groups of zooplankton such rotifers. Furthermore, bacterioplankton should not be neglected as it is of great added importance in mineralization of organic matter and in zooplankton diet, which may explain the results obtained by the zooplankton composition of functional groups (Geller & Müller, 1981). Also important is the ichthyofauna composition, some of which are predators of large cladocerans leading to the existence of a top-down pressure in the trophic chain. Moreover, the quantification of specific biomarkers (*e.g.* enzymatic systems of detoxification; neurotransmission biomarkers) in local ichthyofauna can provide more information about the state and pressures on the ecosystem. Another point of view, and in order to complement the whole ecosystem assessment, the composition of the surrounding landscape should be analysed in order to understand the potential external contributions that may influence the quality of the water body.

5. References

Ackermann, W., G. White, E. Worthington, & J. Ivens, 1973. Man-Made Lakes: Their Problems and Environmental Effects. American Geophysical Union. Washington, DC.

Allen, Y., P. Calow, & D. J. Baird, 1995. A mechanistic model of contaminant-induced feeding inhibition in Daphnia magna. Environmental Toxicology and Chemistry Wiley Periodicals, Inc. 14: 1625–1630, http://doi.wiley.com/10.1002/etc.5620140923.

Alonso, M., 1996. Crustacea Branchiopoda. En: Fauna Iberica, vol. 7. , 486.

Amoros, C., 1984. Introduction pratique à la systématique des organismes des eaux continentales françaises : Crustacés cladocères. Bulletin Mensuel de la Société Linnéenne de Lyon. , 72–183.

APA, 2007. Plano de Ordenamento da Albufeira da Aguieira. Diário da República, 1.ª série - N.º 8. , 1–2, https://www.apambiente.pt/_zdata/Ordenamento/POA/Aguieira/Relatorio.pdf.

APHA, 1989. Standard Methods for the Examination of Water and Wastewater. American Public Health Association. Washington, https://www.mwa.co.th/download/file_upload/SMWW_1000-3000.pdf.

ASTM, 1980. Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians, Reports E 729-80. Philadelphia, USA.

Azevêdo, D. J. S., J. E. L. Barbosa, W. I. A. Gomes, D. E. Porto, J. C. Marques, & J. Molozzi, 2015. Diversity measures in macroinvertebrate and zooplankton communities related to the trophic status of subtropical reservoirs: Contradictory or complementary responses?. Ecological Indicators Elsevier 50: 135–149, https://www.sciencedirect.com/science/article/pii/S1470160X14004981.

Baird, D., A. Soares, A. Girling, I. Barber, M. Bradley, & P. Callow, 1988. The long-term maintenance of Daphnia magna Straus for use ecotoxicity tests: Problems and prospects. Em Lokke, H., H. Tyle, & F. Bron-Rasmussen (eds), Proceedings First European Conference on Ecotoxicology. Lyngby, Denmark: 144–148.

Baker, A. L., 2012. Phycokey -- an image based key to Algae (PS Protista), Cyanobacteria, and other aquatic objects. University of New Hampshire Center for Freshwater Biology. , http://cfb.unh.edu/phycokey/phycokey.htm 9.

Beaver, J. R., A. M. Miller-Lemke, & J. K. Acton, 1999. Midsummer zooplankton

assemblages in four types of wetlands in the Upper Midwest, USA. Hydrobiologia 380: 209–220, https://link.springer.com/content/pdf/10.1023%2FA%3A1003452118351.pdf.

Bellinger, E. G., & D. C. Sigee, 2015. Freshwater Algae: Identification, Enumeration and Use as Bioindicators John Wiley & Sons, Ltd, Chichester, UK.

Błędzki, L. A., & J. I. Rybak, 2016. Freshwater crustacean zooplankton of Europe. Freshwater Crustacean Zooplankton of Europe: Cladocera and Copepoda (Calanoida, Cyclopoida) Key to Species Identification, with Notes on Ecology, Distribution, Methods and Introduction to Data Analysis. .

Brower, J. E., J. H. Zar, & C. N. von Ende, 1997. Field and laboratory methods for general ecology. .

Burton, G. L., & C. Macperson, 1995. Sediment toxicity testing issue and methods Handbook of ecotoxicology. Boca Raton, Lewis publishers: 70–103.

Caroni, R., & K. Irvine, 2010. The Potential of Zoozplanlton Communities for Ecological Assessment of Lakes: Redundant Concept or Political Oversight?. Biology and Environment: Proceedings of the Royal Irish Academy. Royal Irish Academy, 35–53, https://www.jstor.org/stable/20799767.

Catalan, J., M. Ventura, A. Munné, & L. Godé, 2003. Desenvolupament d'un índex integral de qualitat ecológica i regionalització ambiental dels sistemes lacustres de Catalunya. Agència Catalana del Agua 177.

De Figueiredo, D. R., M. J. Pereira, A. Moura, L. Silva, S. BÃ¡rrios, F. Fonseca, I. Henriques, & A. Correia, 2007. Bacterial community composition over a dry winter in meso- and eutrophic Portuguese water bodies. FEMS Microbiology Ecology Oxford University Press 59: 638–650, https://academic.oup.com/femsec/article-lookup/doi/10.1111/j.1574-6941.2006.00241.x.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, M. L. J. Stiassny, & C. A. Sullivan, 2006. Freshwater biodiversity: Importance, threats, status and conservation challenges. Biological Reviews of the Cambridge Philosophical Society 81: 163–182.

Eaton, A. D., L. S. Clesceri, A. E. Greenberg, M. A. H. Franson, American Public Health Association., American Water Works Association., & Water Environment Federation., 1995. Standard methods for the examination of water and wastewater. American Public Health Association, http://agris.fao.org/agris-search/search.do?recordID=US19960076169.

Ebert, D., 2005. Ecology, Epidemiology, and Evolution of Parasitism in Daphnia Introduction to Daphnia biology. National Center for Biotechnology Information, Bethesda, MD.

Ejsmont-Karabin, J., & A. Karabin, 2013. The suitability of zooplankton as lake ecosystem indicators : Crustacean trophic state index. Polish Journal of Ecology 61: 561–573, https://www.researchgate.net/publication/287948342_The_suitability_of_zooplankton_as_lake_ecosystem_indicators_Crustacean_trophic_state_index.

Figueiredo, D., B. Castro, M. Pereira, & A. Correia, 2012. Bacterioplankton community composition in Portuguese water bodies under a severe summer drought. Community Ecology Akadémiai Kiadó 13: 185–193, http://www.akademiai.com/doi/abs/10.1556/ComEc.13.2012.2.8.

Fryer, G., 1957. The food of some freshwater cyclopoid copepods and its ecological significance. Journal of Animal ecology 26: 263–286.

García-Chicote, J., X. Armengol, & C. Rojo, 2018. Zooplankton abundance: A neglected key element in the evaluation of reservoir water quality. Limnologica Urban & Fischer 69: 46–54, https://www.sciencedirect.com/science/article/pii/S0075951117300464?via%3Dihub.

Geller, W., & H. Müller, 1981. The filtration apparatus of Cladocera: Filter mesh-sizes and their implications on food selectivity. Oecologia Springer-Verlag 49: 316–321, http://link.springer.com/10.1007/BF00347591.

Geraldes, A. M., & M. Alonso, 2014. Bosmina (Eubosmina) coregoni Baird, 1857 (Crustacea, Branchiopoda, Anomopoda): un nuevo invasor planctónico en la península ibérica. Graellsia 70: e015, http://graellsia.revistas.csic.es/index.php/graellsia/article/view/500/529.

Geraldes, A. M., R. Pasupuleti, & P. Silva-Santos, 2016a. Influence of Some Environmental Variables on the Zooplankton Community of Aguieira Reservoir (Iberian Peninsula, Portugal): Spatial and Temporal Trends. Asian Journal of Environment & Ecology 1: 30661, www.sciencedomain.orgcompletepeerreviewhistory:http://www.sciencedomain.org/review-history/17547.

Geraldes, A. M., & P. Silva-Santos, 2011. Monitorização da comunidade zooplanctónica da albufeira da Aguieira (bacia do Mondego, Portugal): que fatores a

influenciam?. Captar: Ciência e Ambiente para Todos 3: 12–23.

Geraldes, A. M., & P. Silva-Santos, 2013. Serão as comunidades zooplanctónicas boas indicadoras da degradação ambiental em albufeiras? - O caso da Albufeira da Aguieira. 10ª Conferência Nacional do Ambiente /XII Congresso Nacional do Ambiente. , https://bibliotecadigital.ipb.pt/bitstream/10198/9727/3/aveiroagueira.pdf.

Geraldes, A. M., P. Silva-Santos, & M. C. Crispim, 2017. Using zooplplankton community as indicator of seasonal changes in some water quality parameters in a mesoeutrophic reservoir. III Congresso Ibero-Americano de Empreendedorismo, Energia, Ambiente e Tecnologia Instituto Politécnico de Bragança, https://bibliotecadigital.ipb.pt/handle/10198/15877.

Geraldes, A. M., P. Silva-Santos, & R. Pasupuleti, 2016b. Zooplankton community structure in a deep reservoir: seasonal trends and structuring variables?. International Zoological Congress of “Grigore Antipa” Museum , https://bibliotecadigital.ipb.pt/handle/10198/16087.

Graça, M. A., M. Â. Pardal, J. C. Marques, & V. Vasconcelos, 2002. Toxic cyanobacteria in the Mondego basin reservoirs: an overiew Aquatic ecology of the Mondego River basin global importance of local experience. Imprensa da Universidade de Coimbra: 105–114.

Haberman, J., & M. Haldna, 2014. Indices of zooplankton community as valuable tools in assessing the trophic state and water quality of eutrophic lakes: Long term study of Lake Võrtsjärv. Journal of Limnology 73: 61–71, http://www.jlimnol.it/index.php/jlimnol/article/view/jlimnol.2014.828.

Henry-Silva, G. G., A. F. M. Camargo, & M. M. Pezzato, 2008. Growth of free-floating aquatic macrophytes in different concentrations of nutrients. Hydrobiologia 610: 153–160, http://link.springer.com/10.1007/s10750-008-9430-0.

Herschy, R. W., 2012. Ecological Threat to Lakes and Reservoirs. In: Bengtsson L., Herschy R.W., Fairbridge R.W. (eds) Encyclopedia of Lakes and Reservoirs. Encyclopedia of Earth Sciences Series. : 258–270.

INAG, I. P., 2009a. Critérios para a Classificação do Estado das Massas de Água Superfíciais - Rios e Albufeiras. , http://www.apambiente.pt/dqa/assets/critérios-classificação-rios-e-albufeiras.pdf.

INAG, I. P., 2009b. Manual para a avaliação da qualidade biológica da água em lagos e albufeiras segundo a Diretiva Quadro da Água. Protocolo de amostragem e

análise para o Fitoplâncton. , http://www.apambiente.pt/dqa/assets/protocolo-de-amostragem-e-análise-para-o-fitoplâncton.pdf.

INAG, I. P., 2011. Modelação Matemática da Qualidade da Água em Albufeiras com Planos de Ordenamento - lll - Albufeira da Aguieira. , https://www.apambiente.pt/_zdata/Ordenamento/ModQualAgua/III - Relatrio_Modelao_Aguieira.pdf.

ISO 20079, 2005. Water quality — Determination of the toxic effect of water constituents and waste water on duckweed (Lemna minor) — Duckweed growth inhibition test. .

Jensen, T. C., I. Dimante-Deimantovica, A. K. Schartau, & B. Walseng, 2013. Cladocerans respond to differences in trophic state in deeper nutrient poor lakes from Southern Norway. Hydrobiologia 715: 101–112.

Jeppesen, E., J. P. Jensen, M. Søndergaard, T. Lauridsen, & F. Landkildehus, 2000. Trophic structure, species richness and diversity in Danish lakes: changes along a phosphorus gradient. Freshwater Biology 45: 201–218.

Jeppesen, E., P. Nõges, T. A. Davidson, J. Haberman, T. Nõges, K. Blank, T. L. Lauridsen, M. Søndergaard, C. Sayer, R. Laugaste, L. S. Johansson, R. Bjerring, & S. L. Amsinck, 2011. Zooplankton as indicators in lakes: a scientific-based plea for including zooplankton in the ecological quality assessment of lakes according to the European Water Framework Directive (WFD). Hydrobiologia Springer Netherlands 676: 279–297, http://link.springer.com/10.1007/s10750-011-0831-0.

Johnson, N., C. Revenga, & J. Echeverria, 2001. Managing Water for People and Nature. Science 292: 1071–1072, http://www.ncbi.nlm.nih.gov/pubmed/11352054.

Junk, J. W., & M. T. F. Piedade, 1997. Plant life in the flood- plain with special reference to herbaceous plants Em Junk, J. W. (ed), The Central Amazon Floodplain: Ecology of a Pulsing System. Springer-Verlag, Berlin: 147–185.

Kungolos, A., C. Emmanouil, V. Manakou, & E. Darakas, 2015. Assessment of vulnerable freshwater ecosystems and various aquatic effluents by means of ecotoxicological assays. Desalination and Water Treatment Taylor & Francis 54: 2120–2129, http://www.tandfonline.com/doi/full/10.1080/19443994.2014.933617.

Lichtenthaler, H. K., 1987. Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. Methods in Enzymology 148: 350–382.

Llirós, M., Ö. Inceoɬlu, T. García-Armisen, A. Anzil, B. Leporcq, L. M. Pigneur, L.

Viroux, F. Darchambeau, J. P. Descy, & P. Servais, 2014. Bacterial community composition in three freshwater reservoirs of different alkalinity and trophic status. PLoS ONE Public Library of Science 9: e116145, https://dx.plos.org/10.1371/journal.pone.0116145.

McWilliam, R. A., & D. J. Baird, 2002. Postexposure feeding depression: A new toxicity endpoint for use in laboratory studies with Daphnia magna. Environmental Toxicology and Chemistry 21: 1198–1205, http://doi.wiley.com/10.1002/etc.5620210612.

Ministério do Ambiente do Ordenamento do Território e Energia, 2015. Decreto-Lei n.º 218/2015 de 7 de outubro do Ministério do Ambiente e do Ordenamento do Território. Diário da República, 1ª série - Nº 196 196: 8667–8685, https://dre.pt/application/file/a/70476114.

Mkandawire, M., J. A. Teixeira Da Silva, & E. G. Dudel, 2014. The lemna bioassay: Contemporary issues as the most standardized plant bioassay for aquatic ecotoxicology. Critical Reviews in Environmental Science and Technology Taylor & Francis Group 44: 154–197, http://www.tandfonline.com/doi/abs/10.1080/10643389.2012.710451.

Mohamed, Z. A., 2017. Macrophytes-cyanobacteria allelopathic interactions and their implications for water resources management—A review. Limnologica. Elsevier GmbH, 122–132.

Moss, B., D. Stephen, C. Alvarez, E. Becares, W. Van De Bund, S. E. Collings, E. Van Donk, E. De Eyto, T. Feldmann, C. Fernández-Aláez, M. Fernández-Aláez, R. J. M. Franken, F. García-Criado, E. M. Gross, M. Gyllström, L.-A. Hansson, K. Irvine, A. Järvalt, J.-P. Jensen, E. Jeppesen, T. Kairesalo, R. Kornijów, T. Krause, H. Künnap, A. Laas, E. Lill, B. Lorens, H. Luup, M. Rosa Miracle, P. Nõges, T. Nõges, M. Nykänen, I. Ott, W. Peczula, E. T. H. M. Peeters, G. Phillips, S. Romo, V. Russell, J. Salujõe, M. Scheffer, K. Siewertsen, H. Smal, C. Tesch, H. Timm, L. Tuvikene, I. Tonno, T. Virro, E. Vicente, & D. Wilson, 2003. The determination of ecological status in shallow lakes - a tested system (ECOFRAME) for implementation of the European Water Framework Directive. Aquatic Conservation: Marine and Freshwater Ecosystems 13: 507–549, http://doi.wiley.com/10.1002/aqc.592.

Noordally, Z. B., & A. J. Millar, 2015. Clocks in algae. Biochemistry American Chemical Society 54: 171–183.

Nunes, B., S. C. Antunes, J. Santos, L. Martins, & B. B. Castro, 2014a. Toxic potential of paracetamol to freshwater organisms: A headache to environmental

regulators?. Ecotoxicology and Environmental Safety Academic Press 107: 178–185, https://www.sciencedirect.com/science/article/pii/S014765131400236X?via%3Dihub.

Nunes, B., G. Pinto, L. Martins, F. Gonçalves, & S. C. Antunes, 2014b. Biochemical and standard toxic effects of acetaminophen on the macrophyte species Lemna minor and Lemna gibba. Environmental Science and Pollution Research 21: 10815–10822.

OECD, 2006a. Guideline 201: Freshwater Alga and Cyanobacteria , Growth Inhibition Test. OECD guidelines for testing of chemicals. 1–26, http://www.nikkakyo.org/ontai/merumaga/Challenge/OECD/TG_201.pdf.

OECD, 2006b. Guideline 221: Lemna sp. Growth Inhibition Test. OECD Guidelines for the Testing of Chemicals OECD 22, https://www.oecd-ilibrary.org/docserver/9789264016194-en.pdf?expires=1537537029&id=id&accname=guest&checksum=28CD2078B328CB8190846462553AB22D.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R., R. B. O. Minchin, G. L. Simpson, M. Peter Solymos, H. H. Stevens, E. Szoecs, & H. Wagner, 2019. vegan: Community Ecology Package. R package. , https://cran.r-project.org/package=vegan.

Olenina, I., A. Andersson, N. Wasmund, & S. Busch, 2006. Biovolumes and size-classes of phytoplankton in the Baltic Sea Helsinki Commission Baltic Marine Environment Protection Commission. HELCOM Balt.Sea Environ. Proc. 106: 144 pp.

Oliveira, M. do R. L. de, & M. T. Monteiro, 1992. Blooms de Cyanophyceae na Albufeira de Aguieira : efeitos na qualidade da água e no zooplâncton. Instituto Nacional de Investigação das Pescas , http://servir.uevora.pt/SearchResultDetail.aspx?mfn=36677&DDB=BD1&pageformat=complete#.XVmszuhKjIU.

Orlowicz, E., 2012. Filtering efficiency and feeding mechanisms of Daphnia pulex on Microcystis aeruginosa and Nannochloropsis. .

Pádua, J., J. M. Bernardo, & M. H. Alves, 2005. Exercício De Intercalibração Em Massas De Água Fortemente Modificadas – Albufeiras , No Âmbito Da. 1–14, http://www.apambiente.pt/dqa/assets/exercício-de-intercalibração-em-albufeiras.pdf.

Palma, P., P. Alvarenga, V. Palma, C. Matos, R. M. Fernandes, A. Soares, & I. R. Barbosa, 2010. Evaluation of surface water quality using an ecotoxicological approach: A case study of the Alqueva Reservoir (Portugal). Environmental Science and Pollution

Research Springer-Verlag 17: 703–716, http://link.springer.com/10.1007/s11356-009-0143-3.

Palma, P., L. Ledo, & P. Alvarenga, 2016. Ecotoxicological endpoints, are they useful tools to support ecological status assessment in strongly modified water bodies?. Science of The Total Environment Elsevier 541: 119–129, https://www.sciencedirect.com/science/article/pii/S0048969715306744.

Parlamento Europeu, 2008. Directiva 2008/105/CE do Parlamento Europeu e do Conselho, de 16 de Dezembro de 2008 , relativa a normas de qualidade ambiental no domínio da política da água, que altera e subsequentemente revoga as Directivas 82/176/CEE, 83/513/CEE, 84/156/CEE, 84/491/C. Journal Oficial da União Europeia 46: 14.

Pedroso, N. M., T. Sales-Luís, & M. Santos-Reis, 2007. Use of Aguieira dam by Eurasian otters in Central Portugal. Folia Zoologica 56: 365–377.

Pereira, J. L., S. C. Antunes, B. B. Castro, C. R. Marques, A. M. M. Gonçalves, F. Gonçalves, & R. Pereira, 2009. Toxicity evaluation of three pesticides on non-target aquatic and soil organisms: Commercial formulation versus active ingredient. Ecotoxicology 18: 455–463.

Pérez, J. R., S. Loureiro, S. Menezes, P. Palma, R. M. Fernandes, I. R. Barbosa, & A. M. V. M. Soares, 2010. Assessment of water quality in the Alqueva Reservoir (Portugal) using bioassays. Environmental Science and Pollution Research Springer-Verlag 17: 688–702, http://link.springer.com/10.1007/s11356-009-0174-9.

Pociecha, A., I. Bielańska-Grajner, H. Kuciel, & A. Z. Wojtal, 2018. Is zooplankton an indicator of the water trophic level in dam reservoirs?. Oceanological and Hydrobiological Studies De Gruyter 47: 288–295, http://www.degruyter.com/view/j/ohs.2018.47.issue-3/ohs-2018-0027/ohs-2018-0027.xml.

Presidencia do Concelho de Ministros, 2007. Resolução do Conselho de Ministros n.º 186/2007. Diário da República, 1.ª série - N.º 246 .

R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reid, J. W., & C. E. Williamson, 2010. Copepoda Ecology and Classification of North American Freshwater Invertebrates. Elsevier: 829–899, https://linkinghub.elsevier.com/retrieve/pii/B9780123748553000212.

Romanowska-Duda, Z., J. Mankiewicz, M. Tarczyńska, Z. Walter, & M. Zalewski, 2002. The Effect of Toxic Cyanobacteria (Blue-Green Algae) on Water Plants and Animal Cells. Polish Journal of Environmental Studies. .

Simões, N. R., A. H. Nunes, J. D. Dias, F. A. Lansac-Tôha, L. F. M. Velho, & C. C. Bonecker, 2015. Impact of reservoirs on zooplankton diversity and implications for the conservation of natural aquatic environments. Hydrobiologia 758: 3–17.

SNIRH : Sistema Nacional de Informação de Recursos Hídricos, (sem data). SNIRH : Sistema Nacional de Informação de Recursos Hídricos. , https://snirh.apambiente.pt/index.php?idMain=.

Stein, J. R., 1973. Handbook of Phycological Methods – Culture Methods and Growth Measurements. Cambridge University Press, London, UK.

União Europeia, 2000. Directiva 2000/60/CE. Jornal Oficial das Comunidades Europeias 1–72, http://www.apambiente.pt/dqa/assets/01-2000_60_ce---directiva-quadro-da-água.pdf.

Vasconcelos, V. M., K. Sivonen, W. R. Evans, W. W. Carmichael, & M. Namikoshi, 1996. Hepatotoxic microcystin diversity in cyanobacterial blooms collected in Portuguese freshwaters. Water Research Pergamon 30: 2377–2384, https://www.sciencedirect.com/science/article/pii/0043135496001522.

Vasconcelos, V., J. Morais, & M. Vale, 2011. Microcystins and cyanobacteria trends in a 14 year monitoring of a temperate eutrophic reservoir (Aguieira, Portugal). Journal of Environmental Monitoring 13: 668–672, http://pubs.rsc.org/-/content/articlepdf/2011/em/c0em00671h.

Ward, J. V., & J. A. Stanford, 1995. The serial discontinuity concept: Extending the model to floodplain rivers. Regulated Rivers: Research & Management 10: 159–168.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Williamson, C. E., D. P. Morris, M. L. Pace, & O. G. Olson, 1999. Dissolved organic carbon and nutrients as regulators of lake ecosystems: Resurrection of a more integrated paradigm. Limnology and Oceanography 44: 795–803, http://doi.wiley.com/10.4319/lo.1999.44.3_part_2.0795.

Witty, L. M., 2004. Practical Guide to Identifying Freshwater Crustacean Zooplankton. .

World Water Assessment Programme, 2009. The United Nations World Water Development Report3:Water in a Changing World. Paris: UNESCO, and London: Earthscan.

Yan, S., & Q. Zhou, 2011. Toxic effects of Hydrilla verticillata exposed to toluene, ethylbenzene and xylene and safety assessment for protecting aquatic macrophytes. Chemosphere 85: 1088–1094, https://linkinghub.elsevier.com/retrieve/pii/S0045653511008769.

6. Appendix

Table A1 Results of statistic analyse of one-way ANOVA and Kruskal – Wallis ANOVA (*) for the bioassays, carotenoids and Chl *a+b*. Sampling sites: A1, A2, A3 and A4.

			d.f.	***F***	***P***
R. subcapitata	autumn	A1	3, 11	70.06	**< 0.001**
		A2	3, 11	29.68	**< 0.001**
		A3	3, 11	6.95	**0.007**
		A4	3, 11	25.07	**< 0.001**
	spring	A1	3, 15	185.40	**< 0.001**
		A2	3, 15	173.30	**< 0.001**
		A3	3, 15	131.50	**< 0.001**
		A4	3, 15	261.00	**< 0.001**
D. longispina	autumn	A1	3, 9	8.04	**0.007**
		A2	3, 12	9.87	**0.002**
		A3	3, 15	15.90	**< 0.001**
		A4	3, 14	23.40	**< 0.001**
	spring	A1	3, 14	40.80	**< 0.001**
		A2	3, 14	8.46	**0.002**
		A3	3, 14	105.80	**< 0.001**
		A4	3, 13	8.47	**0.002**
D. magna	autumn	A1	3, 11	6.34	**0.009**
		A2	3, 12	9.06	**0.002**
		A3	3, 13	21.53	**< 0.001**
		A4	3, 14	8.26	**0.002**
	spring	A1	3, 14	6.03	**0.007**
		A2	3, 12	259.10	**< 0.001**
		A3	3, 13	58.62	**< 0.001**
		A4	3, 14	18.95	**< 0.001**
S. polyrhiza	autumn	A1	3, 16	5.29	**0.010**
		A2	3, 16	14.02	**< 0.001**
		A3	3, 15	10.82	**< 0.001**
		A4	3, 15	6.59	**0.005**
	spring	A1	3, 16	42.04	**< 0.001**
		A2	3, 14	21.73	**< 0.001**
		A3	3, 16	21.58	**< 0.001**
		A4	3, 15	41.60	**< 0.001**
Carotenoids	autumn	A1	3, 16	98.47	**< 0.001**
		A2	3, 16	23.93	**< 0.001**
		A3	3, 16	33.80	**< 0.001**
		A4	3, 16	46.14	**< 0.001**
	spring *	A1	3, 16	14.10	**0.003**
		A2	3, 16	15.19	**0.002**
		A3	3, 15	15.30	**0.002**
		A4	3, 15	14.29	**0.003**
Chl *a+b*	autumn	A1	3, 16	93.20	**< 0.001**
		A2	3, 16	36.26	**< 0.001**
		A3	3, 16	55.93	**< 0.001**
		A4	3, 16	86.63	**< 0.001**
	spring *	A1	3, 16	15.01	**0.002**
		A2	3, 16	15.31	**0.002**
		A3	3, 16	15.49	**0.002**
		A4	3,14	13.21	**0.004**