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RESEARCH ARTICLE

Feeding inhibition tests as a tool for seston quality evaluation in lentic ecosystems: salinization impact

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Abstract – Environmental disturbance on freshwater ecosystems significantly impacts all levels of the trophic web. Salinity in lentic freshwater ecosystems, as a consequence of climatic changes, is rising concern in the scientific community. These alterations affect water quality, the composition and diversity of the aquatic communities. This study aimed to assess the seston quality of two lentic ecosystems, Crestuma reservoir and lake Vela, and evaluate the effects of salinity on food performance of *Daphnia magna* and *Daphnia longispina* (isolated from the referred ecosystems). Feeding inhibition tests were used to conduct these two evaluations. Physical and chemical water classification revealed a poor or good ecological potential of the two lentic ecosystems, according to WFD approach. To evaluate seston quality, filtered and unfiltered water samples from the lentic ecosystems were used as treatments. Although only summer samples from Crestuma presented good water quality, the results from the feeding inhibition tests showed that seston from lake Vela presented more nutritional quality. Concerning the assay to evaluate the salinity effect on *Daphnia* spp., a significant reduction in the filtration rate was observed. The NOEC value was 0.7 g/L NaCl and 0.8 g/L NaCl for *D. magna* and *D. longispina C and V*, respectively. The LOEC value was also distinct between species, being 1.0 g/L and 0.9 g/L, respectively. This work allowed to verify that feeding inhibition tests are not sensitive enough for assess the quality of natural waters. However, these tests are economically viable and have high sensitivity to evaluate NaCl effects on *Daphnia* spp.

Keywords: *Daphnia* spp / lentic ecosystems / feeding rate / salinity / seston quality

1 Introduction

Lentic freshwater ecosystems are water bodies that can provide habitat for several groups of organisms and correspond to an essential natural water source. Aquatic ecosystems biodiversity is changing across the globe (Sala *et al.*, 2000; Dudgeon *et al.*, 2006) as a response to different anthropogenic threats and geologic characteristics. Freshwater ecosystems are especially vulnerable since they are intensively explored and subjected to human-induced impacts (*e.g.* industrial chemicals, dams for energy production). Several studies have already demonstrated that the occurrence of natural or anthropogenic disturbance can alter the ecosystems microhabitat characteristics and, consequently, affect the biota communities and the trophic web (Figueiredo et al., 2006; Nõges *et al.*, 2016; Hintz *et al.*, 2017). On lentic freshwater ecosystems, the habitat characteristics depend on the range variation of biotic and abiotic factors. These parameters are responsible for the plankton community fluctuation and distribution (Antunes *et al.*, 2003; Figueiredo *et al.*, 2006; Choi *et al.*, 2014). The occurrence of such modifications on these communities can cause significant impacts on the entire ecosystem, namely eutrophication processes, where the primary consumers (zooplankton population) become unable to filter and control the significant increase of phytoplankton and a degradation of water quality occurs as last consequence (Gamito *et al.*, 2017).

Zooplankton community plays an important role in freshwater ecosystems since it includes organisms with high capacity of modifying the structure of planktonic food webs, due to their predatory and grazing behavior. Zooplanktonic

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primary consumers, such as cladocerans, can filter particles from seston, including bacteria and algae (Leonard and Paerl, 2005; Marinho *et al.*, 2018). Moreover, various studies had already verified the key role of this organisms in controlling the growth of phytoplankton communities and cyanobacteria blooms (Lampert *et al.*, 1986; Muylaert *et al.*, 2006; George *et al.*, 2015). On the other hand, these organisms have a limited food resource selection capacity and, consequently, the quality and quantity of food present in seston are determinant for the performance of their life history (Müller-Navarra and Lampert, 1996; Hülsmann, 2001; Marinho *et al.*, 2018). It is known that the concentration and composition of the phytoplankton community are essential to the zooplanktonic community since different phytoplankton species show different nutritional quality (von Ruckert and Giani, 2008; Choi *et al.*, 2014). However, abiotic factors, such as temperature, pH, nutrients, and salinity are also important since they may influence the grazing, life history and survival parameters of phytoplankton and zooplankton species (Elser *et al.*, 2001; George *et al.*, 2015; Loureiro *et al.*, 2015).

In the last decades, the salinity values (abiotic stress) increase in freshwater ecosystems, has been an issue of rising concern in the scientific community (Nielsen *et al.*, 2003; Herbert *et al.*, 2015; Canedo-Arguelles *et al.*, 2016). Namely, freshwater ecosystems situated at coastal zones and subjects to different pressures of climatic changes, such as increasing salinity and temperature (Gonçalves *et al.*, 2007; Venâncio *et al.*, 2018). There are several causes of salinization on coastal freshwater ecosystems such as decrease on precipitation levels and sea level rise, both consequences of global climate changes (Schallenberg *et al.*, 2003; Jeppesen *et al.*, 2015). These alterations affect the water quality and, consequently, the plankton communities, which must adapt to saline stress in order to survive (Gonçalves *et al.*, 2007). Several authors have already reported a significant loss of biodiversity in freshwater ecosystems as a consequence of salinity increase (Ramdani *et al.*, 2001; Schallenberg *et al.*, 2003; Jeppesen *et al.*, 2015).

Cladocera is the most important primary group of consumers in lentic ecosystems, and has been widely used to evaluate the impact of environmental changes due to their key position in the trophic web and sensibility to different stressors (Jeppesen *et al.*, 2011; Loureiro *et al.*, 2015; Venâncio *et al.*, 2018). Aladin (1991) and Bezirci *et al.* (2012) verified that cladocera organisms presented a high sensitivity to osmotic stress and therefore are an ideal bio-indicator to assess the effects of saline stress. Among cladocera, *Daphnia* species are already described as tolerant to salinity (Gonçalves *et al.*, 2007). *Daphnia magna* Straus (1820) is a well-known standard laboratory species, and several studies already demonstrated the effect of salinity in these organisms, specifically, a significant decrease on growth, survival and life cycle parameters of this species (Arnér and Koivisto, 1993; Martínez-Jerónimo and Martínez-Jerónimo, 2007; Ghazy *et al.*, 2009). Nevertheless, there is still a lack of information on how the salinity affects other parameters in zooplankton species, namely the effect in feeding rates. Feeding inhibition tests have been used to assess effects of chemical compounds, pesticides, metal oxides, and cyanotoxins, on food performance of *Daphnia* spp. (McWilliam and Baird, 2002; Barata *et al.*, 2007; Lopes *et al.*, 2014). Barata *et al.* (2008) has considered these tests as cost-effective and sensitive

Fig. 1. Location of studied sites. Cresturna reservoir in Oporto, north of Portugal, and Lake Vela in Figueira da Foz in centre of Portugal.

comparing to standardized *D. magna* acute and chronic assays. Therefore, these tests can potentially be used as an important tool to assess the seston quality of freshwater ecosystems or evaluate the effects of different stresses (*e.g.* salinity).

According to this background, there is still a lack of knowledge on the effects of salinity on food performance of freshwater organisms. Alterations in the grazing behavior can potentially lead to rising of eutrophication processes and cause several damages in freshwater ecosystems, as well as other damages in these ecosystems. Therefore, this study defined two main objectives: (i) assess the seston quality, using feeding inhibition tests, of two lentic freshwater ecosystems (Crestuma reservoir and lake Vela) in two distinct periods (end of summer – worst case scenario and spring – best case scenario); (ii) evaluate the effects of salinity in the feeding rate of *Daphnia* spp.. The feeding inhibition tests were performed with two *Daphnia* species: *Daphnia magna*, standard species commonly used on ecotoxicological tests, and *Daphnia longispina* O.F. Müller, (1776), an autochthonous species isolated from the two Portuguese freshwater ecosystems studied.

2 Material and methods

2.1 Natural lentic ecosystems

Two Portuguese lentic freshwater ecosystems, subjects to different anthropic pressures (Crestuma-Lever reservoir and lake Vela – Fig. 1), were chosen to assess the seston quality and to the sampling of *D. longispina,* an autochthonous species. Crestuma-Lever reservoir and lake Vela are located relatively close to the Atlantic sea (Fig. 1) and there is the possibility of being under salinization processes in the near future, affecting the ecosystem quality. Furthermore, both ecosystems are under intensive anthropic pressures such as agriculture and recreative activities. Indeed, these two coastal water bodies are of great importance to understand the impacts of the salinization occurrence in already modified ecosystems.

Crestuma-Lever reservoir belongs to the Douro river hydrographic basin and is located in Vila Nova de Gaia and Gondomar municipalities (Oporto district, north of Portugal – Fig. 1). In the Portuguese territory, this river is sectioned by 10 dams originating artificial reservoirs. Crestuma reservoir was constructed in 1985 and is situated on the final stretch of the Douro river. Consequently, any alteration throughout the hydrographic basin will affect the water quality of this reservoir. Since its formation, it is considered an artificial mesotrophic water mass (POACL, 2004). Crestuma-Lever reservoir is classified with multiple uses (Regulatory Decree 2/88 of January 20) namely usage for consumption, irrigation, recreation activities and wastewater discharge.

Lake Vela is located in Figueira da Foz municipality (Coimbra district, centre of Portugal – Fig. 1) and although being the largest lake of a large system of interconnected reservoirs on the surrounding area it is a relatively small water mass (Antunes *et al.*, 2003; Castro *et al.*, 2005), classified as eutrophic since 1960 (Nauwerck, 1960). Several studies already demonstrated that this lake shows high levels of nitrates and phosphates, characteristics of eutrophic ecosystems, mainly due to the regular nutrient inputs from intense agricultural and livestock activities in the adjacent areas (Abrantes *et al.*, 2006; Figueiredo *et al.*, 2006; Castro and Gonçalves, 2007). Lake Vela is also characterized by a high turbid water (Secchi disk: summer 0.1 m to 1.0 m, and in spring 0.2 m to 1.4 m), without a spring clear water phase, and algal blooms are frequently observed in the warmer seasons (Abrantes *et al.*, 2009a, 2009b).

2.1.1 Sampling procedures

D. longispina organisms were collected at each site, to conduct individual laboratory cultures, using a plankton net with a 150 μm mesh. Samples were stored in plastic bottles and transported to the laboratory for taxa identification and initiate individual cultures.

In order to evaluate the seston quality in a natural worst-case (summer) and best-case scenario (spring), the water sampling at Crestuma reservoir and Vela lake occurred at two distinct seasons, at the end of the summer and beginning of spring. Water temperature (°C), conductivity (μS/cm), dissolved oxygen (mg/L and %) and pH were determined *in situ* using a multi-parameter probe (WTW Multi 350i/SET). Water samples (5 L) were collected to characterize the water quality in nutrient content and conduct the laboratory tests.

2.2 Laboratory procedures

2.2.1 Water physical and chemical analysis

Water samples were processed according to the physical and chemical parameters established by the Water Frame Directive (WFD) for artificial and heavily modified surface water bodies. To evaluate total phosphorus and nitrates, the water was initially mineralized with potassium peroxodisulfate ($K_2S_2O_8$). Total phosphorus was determined using the methodology described by APHA (1989). Samples were read on the spectrophotometer at 690 nm and total phosphorus was quantified according to a standard calibration curve. Nitrates were determined by an adaptation of the cadmium reduction method. A photometric test (1.1477) was performed using a Spectroquant Multy Colorimeter according to standard procedures.

2.2.2 *Daphnia* spp. culture maintenance

At the laboratory, cladoceran taxa were identified with specific dichotomic keys (Amoros, 1984) through a binocular stereoscope and *Daphnia longispina* isolated for conducted individual cultures.

D. magna monoclonal cultures were cultured from a pre-established culture (clone A, *sensu* Baird *et al.*, 1989) and kept over several generations at the laboratory under controlled conditions.

Monoclonal *D. longispina* populations were maintained for several generations under laboratory conditions. For culture maintenance, a synthetic hard water medium "ASTM hard water" was used (ASTM, 1980). The medium was supplemented with a standard organic additive (*Ascophyllum nodosum* (Linnaeus) Le Jolis extract) since ASTM is a poor nutrient medium (Baird *et al.*, 1988). The organisms were fed with *Raphidocelis subcapitata* (Korshikov) Nygaard, Komárek, J. Kristiansen & O.M. Skulberg (freshwater microalgae formerly known as *Pseudokirchneriella subcapitata* (Korshikov) F.Hindák) with a ratio of 3.0×10^5 and 1.5×10^5 cells mL^{-1} day^{-1} for *D. magna* and *D. longispina*, respectively. Microalgae cultures were kept in Woods Hole MBL medium (Stein, 1973) under controlled conditions of temperature (20 ± 2 °C) and photoperiod ($16h^L$:$8h^D$). After 7 days growing, cultures reached exponential phase (OECD, 2006) and were harvested. Cultures were then centrifugated at 3900 rpm for 5 min and the supernatant was discarded, and the pellet resuspended in ASTM medium. The obtained suspension was diluted in a 1:10 proportion and the absorbance was measured at $\lambda = 440$ nm on a spectrophotometer. A standard volume of food was added to the cultures depending on algae cell concentrations, which was calculated based on the correlation of the absorbance measured values (maintained between 0.400 and 0.900).

2.3 Feeding inhibition tests

The feeding inhibition tests were performed according to the methodology described by McWilliam and Baird, 2002. Two different assays were design based on the aims of this study, *D. magna* and *D. longispina* (from Crestuma reservoir – C and lake Vela – V) were used as test organisms in both assays. All treatments had 5 replicates, with 5 organisms each, and a blank control to account for the potential algae growth during the test period. Neonates with 4 or 5 days old born between the 3rd to 5th broods were used for all the tests.

Table 1. Physical and chemical parameters measured in the water samples from Crestuma reservoir and lake Vela for the two sampling periods. Comparison between the thereshold values for these parameters for Good Ecological Potential (GEP) in northern reservoirs established by WFD. Bold values stand for values outside the established thresholds.

Parameters	Limit to GEP Northern reservoirs	Crestuma reservoir		Lake Vela	
		Summer	Spring	Summer	Spring
Dissolved oxygen (mg/L)	≥5	6.29	**3.20**	**1.35**	**2.02**
Oxygen saturation rate (%)	60–120	77.9	**35.1**	**19.0**	**22.5**
pH	6–9	7.48	**5.48**	7.47	**5.68**
Nitrates (mg/L)	≤25	3.0	1.4	2.1	0.8
Total phosphorus (mg/L)	≤0.05	0.04	0.03	**385.34**	**0.20**
Temperature (°C)	–	24.8	19.2	21.7	20.5
Conductivity (µS/cm)	–	276	211	700	473

The treatments volume used on both assays was 100 mL of medium and *R. subcapitata* was added to the vessels with a ratio of 3.0×10^5 cells mL^{-1} day ^{1} for *D. magna* and 1.5×10^5 cells mL^{-1} day^{-1} for *D. longispina* (C and V). The absorbance of the medium was measured at $\lambda = 440$ nm ($AbsFI_0$) with a spectrophotometer and after this measurement, 5 organisms were added to each vessel. Afterward, the assay was initiated, with all the vessels placed in a climate chamber for 24 h at 20 °C, and in total darkness, to avoid algae growth. After the assay period (24 h), the absorbance of the medium of each vessel was measured and registered ($AbsFI_{24}$). Feeding rate was calculated according to the following equation, given by Allen *et al.* (1995):

$$F = ((V * (AbsFI_0 - AbsFI_{24}))/t)/n$$

where V corresponds to the assay volume used (mL), t stands for the assay period (h) and n the number of organisms per vessel/replicate.

2.3.1 Seston quality assay

To perform the feeding inhibition tests to assess the seston nutritional quality of Crestuma reservoir and lake Vela filtered and unfiltered water, from each site, were used as treatments. These two treatments allowed to evaluate the effect of the natural seston in the feeding rate of the organisms. For the filtered treatment, water samples were filtrated through a glass microfiber filter with a 1.2 µm porosity, 47 mm diameter (Whatman GF/C filter), using a vacuum pump. The unfiltered treatment consisted on water samples used directly without filtrations or another handling. ASTM hard water medium was used as control treatment.

2.3.2 Salinity assay

To evaluate the effect of increasing salinity on the food performance of *Daphnia* spp., nonlethal concentrations of sodium chloride (NaCl) were used. The concentrations tests on *D. magna* were selected based on EC_{50} or LC_{50}, described on Gonçalves *et al.* (2007) and Martínez-Jerónimo and Martínez-Jerónimo (2007), and ranged from 0.7 g/L to 3.3 g/L of NaCl, using a dilution factor of 1.35. For *D. longispina* the tested concentrations were selected according to EC_{50} and LC_{50} values already reported by Gonçalves *et al.* (2007), Leitão *et al.* (2013) and Loureiro *et al.* (2015), and ranged from 0.7 g/L to 1.0 g/L of NaCl, using a dilution factor of 1.1. ASTM hard water medium was used as control treatment.

2.4 Statistical analysis

A one-way ANOVA was done to test the differences between the treatments of each feeding test (seston quality and salinity). Data was tested for normality by the Shapiro-Wilk test and for homogeneity of variances by the Levene's test, since data normality and homogeneity are conditions for the one-way ANOVA application. When ANOVA was statistically significant ($p < 0.05$), a Dunnett's test was applied to discriminate which treatment or concentrations were significantly different from the control group. In the salinity assay, this procedure allowed the determination of the standard no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) values. All the statistical analysis was done using the SPSS 25 software package for Windows (IBM® SPSS® Statistics, New York, USA).

3 Results

3.1 Aquatic ecosystem characterization: physical and chemical parameters

The water quality parameters used to classify heavily modified and artificial superficial water bodies can be applied to the category of natural surface water which most closely resembles the water body in question (INAG, 2009). Therefore, for lakes and lagoons, reservoirs are the most closely resemble water body. Table 1 presents the range of values for general physical and chemical parameters proposed in Water Framework Directive (WFD) obtained in the two sampling periods, for the two lentic ecosystems studied, and the respective comparison to the maximum thresholds values established for the "Good Ecological Potential" (GEP) for northern heavily modified and artificial water bodies.

Dissolved oxygen (mg/L and %) shows values below the minimum of 5 mg/L, with exception of the water sample from Crestuma reservoir in summer. The two sampling sites showed neutral pH values at the end of summer and acidic pH values in spring, the latter below the range of 6–7 required for the classification of Good Ecological Potential. Nitrates

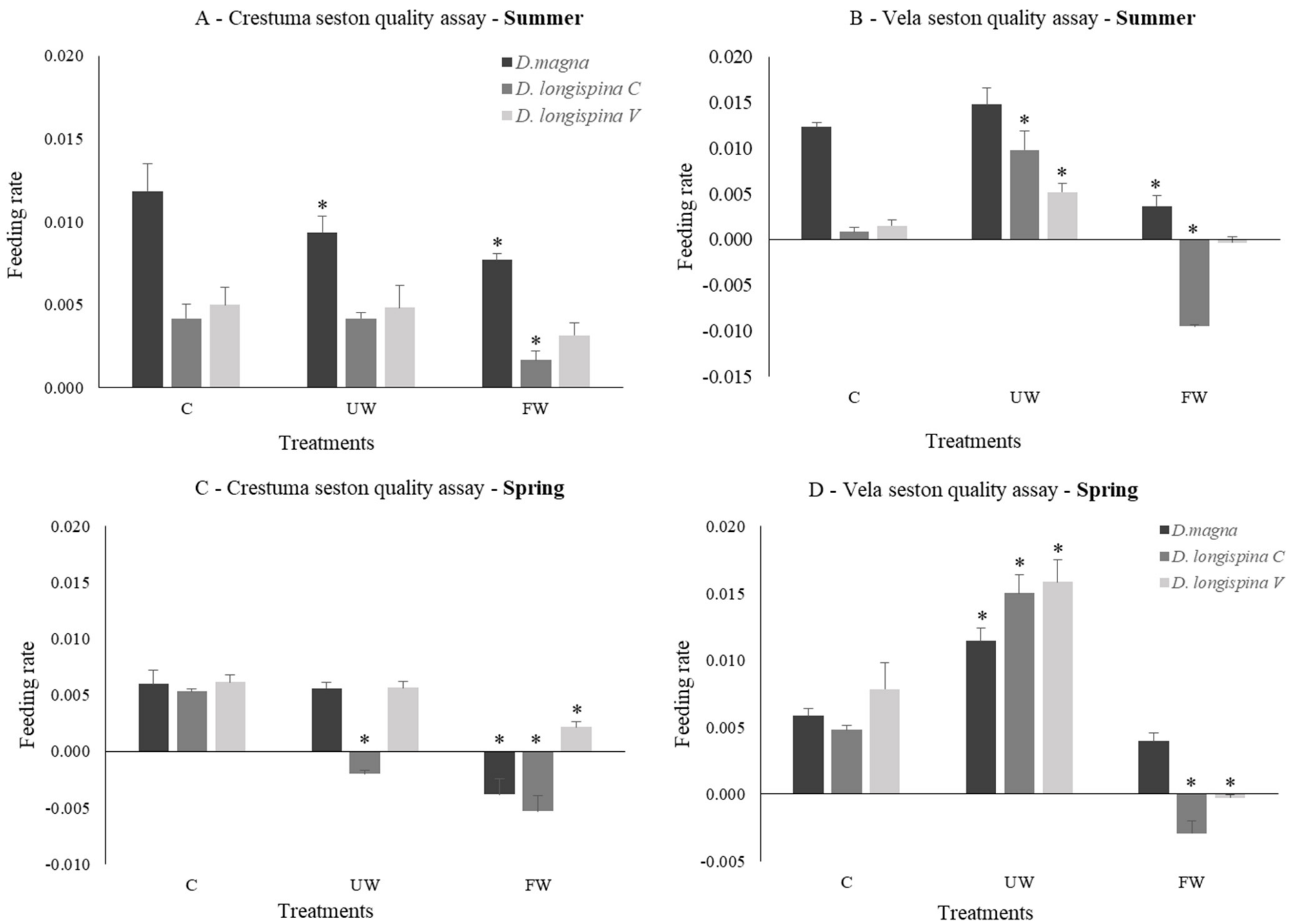

Fig. 2. Results of feeding inhibition rate (mean±SE) of *D. magna*, *D. longispina* C and *D. longispina* V when exposed to natural waters from Crestuma reservoir (A, C) and lake Vela (B, D) collected in summer and spring. C – control treatment with ASTM hard water medium; UW – Unfiltered water treatment; FW – Filtered water treatment. *stands for significant differences compared to control treatment (Dunnett's test).

concentrations showed for all water samples values below the maximum established (≤25 mg/L) for a GEP classification. For the concentration of the total phosphorous analysis, no seasonal variations at Crestuma reservoir were recorded and the values remained below of the maximum limit required for the GEP classification. However, for lake Vela, these values were always above the 0.05 mg P/L limit.

Overall, regarding the analyzed physical and chemical water parameters, only summer water samples from Crestuma reservoir were within the considered thresholds for the GEP classification. Contrarily, considering the majority of these parameters, neither summer nor spring's lake Vela water samples were within the limits, resulting in a poor ecological potential of these freshwater ecosystems.

3.2 Feeding inhibition tests

3.2.1 Seston quality assay

Figure 2 shows the results obtained on the feeding inhibition tests performed with the natural waters sampled from Crestuma reservoir and lake Vela at the end of summer and beginning of spring with the three *Daphnia* spp. populations: *D. magna*, *D. longispina* C and *D. longispina* V.

Regarding the results obtained on summer with *D. magna*, a significant decrease in the feeding rate was observed for the unfiltered and filtered water from Crestuma reservoir ($F_{[2, 11]} = 10.956$; $p = 0.004$) (Fig. 2A). While with water from lake Vela, a significant decrease in the feeding rate of *D. magna* was only observed on the filtered water treatment ($F_{[2, 13]} = 22.448$; $p < 0.001$) (Fig. 2B). On the assay with *D. longispina* isolated from Crestuma reservoir, a significant decrease in the feeding rate of *D. longispina* C after exposed to filtered water from Crestuma was observed ($F_{[2, 13]} = 4.420$; $p = 0.039$) (Fig. 2A). When comparing the treatments with filtered and unfiltered water from lake Vela with the control treatment, significant differences were recorded to *D. longispina* C ($F_{[2, 12]} = 60.194$; $p < 0.001$) (Fig. 2B). A significant increase in the feeding rate was observed when *D. longispina* C was exposed to unfiltered water, however, when *D. longispina* C was exposed to the filtered water from lake Vela, a significant feeding inhibition was recorded (Fig. 2B). When *D. longispina* isolated from the lake Vela (*D. longispina* V), was exposed to the natural waters from Crestuma reservoir, no significant differences in the feeding rate were registered ($F_{[2, 13]} = 0.136$; $p = 0.874$) (Fig. 2A). Contrarily, the assay performed with water from Vela showed a significant increase of the feeding rate in the *D. longispina* V exposed to the unfiltered water

($F_{[2, 14]} = 13.339$; $p = 0.001$) (Fig. 2B). However, when exposed to the filtered water treatment, *D. longispina* V showed feeding inhibition, even though no significant difference was detected (Fig. 2B).

Regarding the assays performed with water collected in spring season, on *D. magna,* the filtered water treatment from Crestuma reservoir, caused a significant feeding inhibition ($F_{[2, 13]} = 23.636$; $p < 0.001$) (Fig. 2C). On the other hand, a significant increase in the feeding rate of this species was recorded when exposed to the unfiltered water from lake Vela ($F_{[2, 11]} = 22.246$; $p < 0.001$) (Fig. 2D). The feeding rate of *D. longispina* C was significantly inhibited when organisms were exposed to the majority of the natural waters tested, independently to the treatment (Fig. 2C, D). An exception was recorded in the treatment with unfiltered water from lake Vela when a significant increase in the feeding rate was verified (Fig. 2D). *D. longispina* C was significantly inhibited between the Crestuma treatment groups ($F_{[2, 13]} = 253.484$; $p < 0.001$) and between the Vela treatment groups ($F_{[2, 13]} = 74.745$; $p < 0.001$). Regarding the results obtained with *D. longispina* V, on the treatment with unfiltered water from lake Vela a significant increase in the feeding rate was observed ($F_{[2, 11]} = 16.157$; $p = 0.001$) (Fig. 2D). On the other hand, when this species was exposed to the filtered water, a significant feeding inhibition was verified. Regarding the filtered water from Crestuma reservoir, a significant decrease in the feeding rate of *D. longispina* V was observed ($F_{[2, 14]} = 15.088$; $p = 0.001$) (Fig. 2C).

3.2.2 Salinity assay

Figure 3 present the results of salinity effects in the feeding rate of *D. magna* and *D. longispina* (C and V). The results showed a significant decrease in the feeding rate for all the species studied: *D. magna* ($F_{[6, 29]} = 22.179$; $p < 0.001$), *D. longispina* C ($F_{[4, 23]} = 11.146$; $p < 0.001$) and *D. longispina* V ($F_{[4, 21]} = 23.053$; $p < 0.001$), along with the NaCl concentration tested. On the assay with *D. magna*, a LOEC was recorded at 1.0 g/L of NaCl (Fig. 3A). Regarding *D. longispina* species, a significant decrease in the feeding rate was observed from 0.9 g/L of NaCl (LOEC) (Fig. 3B). The NOEC value recorded was similarly between species, with 0.7 g/L of NaCl for *D. magna* and 0.8 g/L for the two *D. longispina* populations tested. The results here obtained showed that *D. magna* appears to be more tolerant to salinity compared to *D. longispina* species, although both species have been significantly affected by low NaCl concentrations (<1.0 g/L).

4 Discussion

Environmental changes in lentic freshwater ecosystems can have impacts at any level of the trophic web, altering the water quality and, as a consequence, the structure and diversity of the planktonic community. The nutritional quality and quantity of seston are essential for growth and reproduction of zooplankton organisms (Boersma *et al.*, 2001). Moreover, abiotic stresses, as salinity increase, can play an important role in the communities organization (Van Meter *et al.*, 2011). The results here presented showed that *Daphnia* spp. food performance was affected by the seston quality from water collected in sites with different anthropic impacts, as well the increase of salinity.

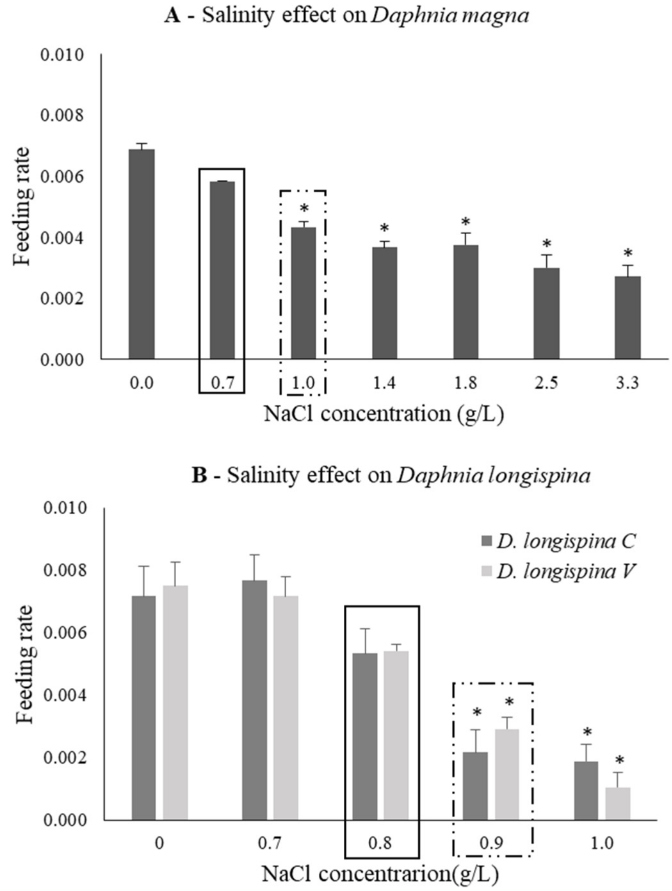

Fig. 3. Results of salinity effects in the feeding rate (mean±SE) of *Daphnia magna* and *Daphnia longispina* (C and V). *stands for significant differences compared to control treatment (Dunnett's test). NOEC (—) value; LOEC (—··—) value.

According to the last data available on National Water Resources Information System (*Sistema Nacional de Informação de Recursos Hídricos* – SNIRH), from 2013, the water of Crestuma reservoir had a reasonable quality, which is confirmed by other authors through water quality index determination, physical and chemical water analysis and feeding inhibition tests (Bordalo *et al.*, 2006; da Silva, 2013). This study was conducted during an atypical summer, described as extremely hot and dry (annual average air temperature 1.1 °C higher than normal value, being the second hottest year since 1931) (IPMA, 2017), and a cold and rainy spring (IPMA, 2018). Given these observed conditions, alterations were expected in the water quality parameters when compared with previous years. The relation between temperature and dissolved oxygen (DO) in aquatic ecosystems is well known, with high temperatures associated with low DO levels, and low temperatures with high DO levels (Odum, 1996). However, this relation was not verified in this study, since the lowest DO (3.20 mg/L) value occurred when the lower values of temperature were recorded, in the spring season. This situation could be explained by lixiviations processes, causing high values of organic matter which leads to a decrease in DO levels. Concerning the water samples from lake Vela, it were verified low and similar DO levels (mg/L and %) and high turbidity water. Indeed, summer sampling took place after a massive fire in lake Vela area, which could explain the here-obtained results, since the leaching of the ashes causes an alteration in the nutrients dynamic and in water turbidity and, as consequence, a decrease of DO levels. Additionally, lake Vela has been classified as an eutrophic lake in the last decades (Abrantes *et al.*, 2009a) and one of the most serious effects of eutrophication is DO depletion (Foley *et al.*, 2012).

Nitrates and phosphates are important parameters for the water quality assessment since they control primary production and species composition, whereas the phosphorous is considered the most limiting nutrient in freshwater ecosystems (Xu *et al.*, 2010). These nutrients are considered the principals responsibles for the eutrophication process (Paerl, 2009). Therefore, both parameters are important indicators of pollution and trophic state of freshwater ecosystems. In Crestuma reservoir, nitrates concentration showed a substantial decrease in the last decade. In 2007 and 2011, according to SNIRH, the annual average was 5.6 mg NO_3/L and 5.9 mg NO_3/L, respectively. In a study carried out in 2013, the annual average was 0.034 mg NO_3/L (da Silva, 2013) and, in this work, the values measured were 3.0 mg NO_3/L (summer) and 1.4 mg NO_3/L (spring). These results are indicative that Crestuma reservoir is not susceptible to contamination by leaching, which is reinforced by the results of the total phosphorous recorded. For the total phosphorous parameter, Crestuma reservoir showed similar values in the two sampling seasons, 0.04 mg P/L in the summer and 0.03 mg P/L in the spring. These values are within the class 2 defined by Nisbet and Verneaux (1970), classifying these waters with low productivity levels. In this study, the values were slightly above the registered by SNIRH, in which the annual average in 2010 was 0.025 mg P/L. Lake Vela samples also presented low values of nitrates, below the limit established for GEP (see Tab. 1). Samples from summer presented values similar to the reported by Abrantes *et al.* (2006). In the spring season, nitrates concentration was also below the limit for GEP, contrary to the results obtained in the referred study. Regarding lake Vela results for total phosphorous, both summer and spring samples were much above the maximum limit required for the GEP. As previously mentioned, summer sampling took place after an intensive fire, which can partly explain the extremely high value obtained, 385.34 mg P/L, typical of pollutant waters (class 6) (Nisbet and Verneaux, 1970). Moreover, lake Vela is surrounded by agricultural fields and, as consequence, inputs of nutrients by leaching are expected. These nutrient inputs in combination with the rise of temperature in the warmer seasons promotes the frequently observed algal blooms, which consequently contributes to the permanent eutrophic status of this lake (Abrantes *et al.*, 2009a; 2010). This situation has been described in several lakes, in all world, over the last years, being a worldwide environmental problem (Ansari *et al.*, 2010; Cruz *et al.*, 2015; Du *et al.*, 2019). In the spring, total phosphorous values had a significant decrease, from 385.34 mg P/L to 0.2 mg P/L, however, it is still above of the limit maximum required for the GEP classification, being a eutrophic lake with high productivity (Nisbet and Verneaux, 1970). Once again, the nutrients concentration has a key role in the eutrophization process at lake Vela.

Conductivity (an indirect salinity measurement) was another parameter analyzed *in situ* and measures the quantity of dissolved ions in the water. In the Crestuma reservoir, the results for this parameter were similar between seasons and were very similar to the annual average registered in 2011 by SNIRH, which was 251 μS/cm. On lake Vela, this parameter was very high in both sampling season, which could be related to ashes from the fire, in the case of summer sampling, and the sea proximity classifying this lake as a coastal lagoon.

The analysis of the physical and chemical parameters under the WFD approach indicates that the two lentic freshwater ecosystems studied suffered some variations along the year. Although being under different anthropic pressures, both ecosystems showed similar results on the water quality parameters. Taking into account these parameters, with the exception of the summer water samples, from Crestuma reservoir, the two types of water (Crestuma and Vela) presented poor quality, regardless of the season. However, biological parameters should be associated with the physical and chemical parameters to evaluate the water quality, since these two parameters can provide distinct information (Martinez-Haro *et al.*, 2015).

In order to assess the seston quality and functioning of Crestuma reservoir and lake Vela, feeding inhibition tests were performed with *D. magna* and *D. longispina*, collected from both sites (*D. longispina* C a *D. longispina* V). The results of the seston quality assay presented are indicative that seston from lake Vela has more quality than the seston from Crestuma reservoir, besides the results of the physical and chemical parameters registed. When *Daphnia* spp. were exposed to the summer water samples from Crestuma, it was verified that the seston had nutritional quality only to *D. longispina* spp. On the other hand, when daphnids were exposed to the spring water samples from Crestuma, the results suggest that the seston quality was insufficient for *D. longispina* C, since it was the only species with a significant feeding inhibition in the unfiltered water. Therefore, the decreasing in the feeding rate of *D. longispina* C reinforced the hypothesis that feeding behavior is affected by the water quality. *D. magna* is considered as a more tolerant species than *D. longispina*, which could explain why this species was not affected by the poor quality of Crestuma water and seston in this season. Contrarly, the results presented by da Silva (2013) showed a significant increase in the feeding rate of *D. magna* in this season, however, the water quality in this period was also significantly better. As for *D. longispina* from lake Vela, this population is adapted to an eutrophic ecosystem (Nauwerck, 1960; Abrantes *et al.*, 2009a, 2010) that shows poor water quality and, as consequence, it is expected to be more tolerant to alterations in the water quality.

Concerning the assays with the summer and spring water samples from lake Vela, although the results observed in the physical and chemical water parameters, the seston showed nutritional quality for all *Daphnia* spp. tested. It was verified a significant increase in the feeding rate of these species in the unfiltered water treatment in the majority of the assays, independently of the season. It is known that phytoplankton species show different nutritional quality (von Ruckert and Giani, 2008) and therefore, not only the quantity but also composition of the phytoplanktonic community is essential to the performance of zooplanktonic community (Abrantes *et al.*, 2006). Taking this into account, it is possible that the phytoplanktonic community of lake Vela is more diverse and abundant than the phytoplanktonic community of Crestuma reservoir. Moreover, these results can be related to cyanobacteria presence in Crestuma water since these cannot only inhibit the filter capacity of some zooplankton species but also release toxic metabolites that remain in the water, even after filtration (Freitas *et al.*, 2014; Ger *et al.*, 2014). In this scenario, the treatment with filtered water will cause feeding inhibition since the organisms are at the same way exposed to toxins, and on the other hand, do not have sufficient food available to cope.

The assay performed to evaluate the effect of salinity on daphnids showed that *D. magna* and *D. longispina* (C and V) were affected by low NaCl concentrations. Indeed our results were above of described by Gonçalves *et al.* (2007) that reported a LOEC value of 5.0 g/L and a NOEC of 4.55 g/L when analyzing survival and life history parameters under salinity stress. In another study, the results showed that *D. magna* can grow under concentrations up to 6.0 g/L NaCl (Martínez-Jerónimo and Martínez-Jerónimo, 2007). These results showed that the feeding rate is affected at lower concentrations than the growth and survival, reinforcing that this species cannot survive with high salinities values (Ghazy *et al.*, 2009). For *D. longispina*, the same trend is observed, and the here presented results showed a LOEC of 0.9 g/L and a NOEC of 0.8 g/L of NaCl. A LOEC value of 2.07 g/L and a NOEC value of 1.88 g/L of NaCl were reported in another study for the majority of life history parameters for *D. longispina* (Gonçalves *et al.*, 2007). Comparing our results with the ones from other studies, daphnids feeding behavior seems to be a more sensitive parameter than other physiological parameters already mentioned. According to Villarroel *et al.* (2003) feeding behavior has the potential of being an important parameter to study in short-term tests using these organisms due to their high sensitivity to environmental stresses, reflected in their feeding rates. Moreover, the here presented results showed that feeding inhibition tests have proven to be a sensitive tool to evaluate the induced salinity stress, reinforce the results obtained in other studies that aimed to evaluate the effect of pesticides, metals and pharmaceuticals (Allen *et al.*, 1995; Agatz *et al.*, 2013; Silva *et al.*, 2015; Lari *et al.*, 2017; Araujo *et al.*, 2019), using this type of methodology. Barata *et al.* (2008) also demonstrated the effectiveness and ecological relevance of feeding bioassays to environmental risk assessment of other stresses such as toxic effluents (contaminant mixtures).

The LOEC value of the two species studied were similar, however *D. longispina* revealed more sensibility than *D. magna*, which is confirmed by other authors in studies with various environmental stresses (Koivisto, 1995; Bossuyt and Janssen, 2005; Muyssen *et al.*, 2005; Gonçalves *et al.*, 2007).

5 Conclusion

The results obtained in this study indicate that the feeding inhibition tests should be used in association with other parameters (physical and chemical) when evaluating the water quality of lentic freshwater ecosystems. In this study an environmental disturbance occurred in the lake Vela area (fire) with a high impact in the aquatic ecossystem, as revealed by physical and chemical water analysis. However, overall it was possible to verify that the seston from lake Vela had more nutritional quality than the seston from Crestuma reservoir, for *Daphnia* spp. In thoses assays, *D. magna* presented a more tolerant behavior towards poor water quality. Regarding the salinization impact, this study reinforces the results obtained in previous works, showing that *Daphnia* spp. is affected even by lower NaCl concentrations. With these assays it was possible verified that although *D. magna* being described as more tolerante than *D. longispina* when exposed to different disturbances, these two species appear to be equally sensitive to induced saline stress.

The use of two different species, a standard species in ecotoxicologic assays and a portuguese autochonous species, in both assays, has allowed a more realistic simulation of stress scenarious in the portuguese freshwater ecosystems. Furthermore, it was verified that feeding inhibition tests are a cost-effective tool, that can be used to evaluate the impact of abiotic stresses such as salinity.

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