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Implications of Anthropic Activities in the Catchment Area of a Temporary Mediterranean Wetland Complex in the South of Spain

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Abstract: The Lagunas de Campillos Natural Reserve and adjacent ponds are fundamentally surrounded by regularly fertilized crop fields and livestock industry, producing leachates which can be found in the ponds. The interest in this Site of European Importance and the RAMSAR wetland complex lies in the habitats within it, which are included in the Directive on Habitats of Community Interest. It is essential to determine the trophic status of the ponds and the quality of these habitats, as well as whether corrective measures need to be established in order to maintain a good environmental status. To characterize and compare the ponds, different parameters were measured, such as conductivity, pH, nutrient concentration, Chl-a concentration, phytoplankton composition, phytoplankton abundance (<20 µm), and the quantification of heterotrophic microorganisms indicating contamination of the aquifers. The obtained results showed that all ponds, except a mesotrophic pond, are eutrophic or even hypertrophic, with high levels of total nitrogen (>8 mg L^{-1}), total phosphorous (>165 µg L^{-1}), and chlorophyll-a concentration. These findings explain the high densities of phytoplankton observed, with the predominant presence of small cells (<3.6 µm ESD). In addition, concentrations of heterotrophs and coliforms are, in some ponds, higher than expected. Eutrophication hinders ecological functions and ecosystem services, which finally affects biodiversity and human wellbeing. Five of the six analyzed ponds are within various protection figures for their essential importance to local and migrating avifauna. Therefore, ponds' status analysis and the implementation of measures for maintaining ecosystem services and trophic state are fundamental for the sustainable management of the studied area.

Keywords: eutrophication; Mediterranean wetlands; land uses; spatial scales; anthropocene; trophic status

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1. Introduction

Anthropogenic activities have been identified as key factors in wetland degradation [1]. Among these activities, those implemented in wetland watersheds are of the utmost importance for the structure and functioning of wetland communities, as well as for the conservation of these aquatic ecosystems [2].

In Mediterranean climates, the actions that occur in wetland drainage basins have a greater impact than those that occur in cold temperate wetlands, due to their greater catchment area and the higher catchment area/pond area ratio [3]. Therefore, the characteristics of the regional landscape should be considered to explain the functioning of wetlands [3,4]. Among the anthropogenic impacts that significantly affect wetland conservation, it is necessary to consider the changes in the land uses, wetland drainages, plowing, and wetland basin cultivation, as well as the nutrient enrichment caused by the input of fertilizers from the wetland catchment [2]. The latter process leads to the eutrophication of aquatic ecosystems, which has been defined as cultural eutrophication, as it is derived from human activities [5,6].

In the Mediterranean region, temporary wetlands are important components of the Mediterranean landscape, due to their importance in regulating water flows and providing a habitat for a wide diversity of plants and animals, being particularly sensitive to anthropogenic pressures [3]. Eutrophication generates changes in wetland communities and environmental conditions, affecting biodiversity and ecosystem function, and thus it must be studied. In this sense, the trophic state is considered as a global indicator of water quality that evaluates the contribution of nutrients to wetlands in relation to their primary production. This allows us to estimate the status of a wetland and detect possible contamination across a large spatial (catchment area) and temporal (over several hydrologic years) scale.

In Andalusia, eutrophication is a key problem in aquatic ecosystems, with the majority of wetlands being eutrophic as a consequence of diffuse pollution [2]. In this context, the high sensitivity of temporary wetlands to pollution makes the evaluation of eutrophication a key aspect in the management of these ecosystems. Therefore, the aim of the present study was to determine, for the first time, the eutrophication that might negatively affect six temporary wetlands and propose, if necessary, measures to improve their conservation. This wetland complex provides a natural laboratory for comparing the effects of human pressure on the trophic state of wetlands.

2. Materials and Methods

2.1. Study Area and Sampling Sites

The Campillos wetland complex, located in Andalusia (Southern Spain), is composed of several endorheic steppe ponds located on a plateau with little undulation on the land and has been intensely transformed by crops, dominated by olive trees and cereal and sunflower fields. This wetland complex consists of fifteen ponds [7]; five of them are included in the Natural Reserve of Lagunas de Campillos, also declared as Special Protection Areas (SPAs) [8], being the remaining ponds without the legal status of protection.

Samples were taken during the hydrological cycle of 2016–2017 (December 2016 to August 2017) with a monthly periodicity. Due to the reduced rainfall during the hydrological cycle, the hydroperiod of the ponds was greatly reduced, and only six of them were able to store water (Camuñas, Capacete, Cerero, Marcela, Redonda and Salada; Figure 1). The number of samplings from each wetland was determined according to the presence of the water sheet.

2.2. Environmental and Biological Data

At each sampling pond, conductivity (μS cm^{-1}), temperature (°C), and pH were measured with a Hanna multiparameter sensor HI 9829 (Hanna Instruments, Woonsocket, RI, USA). For the saline classification of the ponds, the mean conductivity values were converted to salinity using the equation proposed for Andalusian saline wetlands [9].

An integrated water sample, taking into account the depths of the ponds, was taken following a transect from the border to the center of each pond. Three samples were taken, in the border, in the center of the pond and halfway between both points. From this, samples for nutrient concentrations were taken and placed into sterile polyethylene vials, to analyze total nutrients (nitrogen and phosphorus) and dissolved nutrients (ammonia, nitrate, nitrite and phosphate). The samples for dissolved nutrient analysis were previously filtered with Whatman GF/F. After that, samples for nutrient analysis were immediately frozen (−20 °C). In the laboratory, total nitrogen (TN) was analyzed using the ultraviolet method for digested unfiltered water [10]. Total phosphorus (TP) was measured after the digestion of unfiltered water with potassium persulfate [10]. Ammonium (NH_4^+) was measured using the phenate method [11], nitrates (NO_3^-) were analyzed using the ultraviolet spectrophotometric screening method [10], and nitrites (NO_2^-) were determined with the sulfanilamide method [11]. Dissolved inorganic nitrogen (DIN) was calculated as the sum of NH_4^+, NO_3^-, and NO_2^-. Lastly, dissolved inorganic phosphorus (DIP) was determined by using the molybdenum blue method [12].

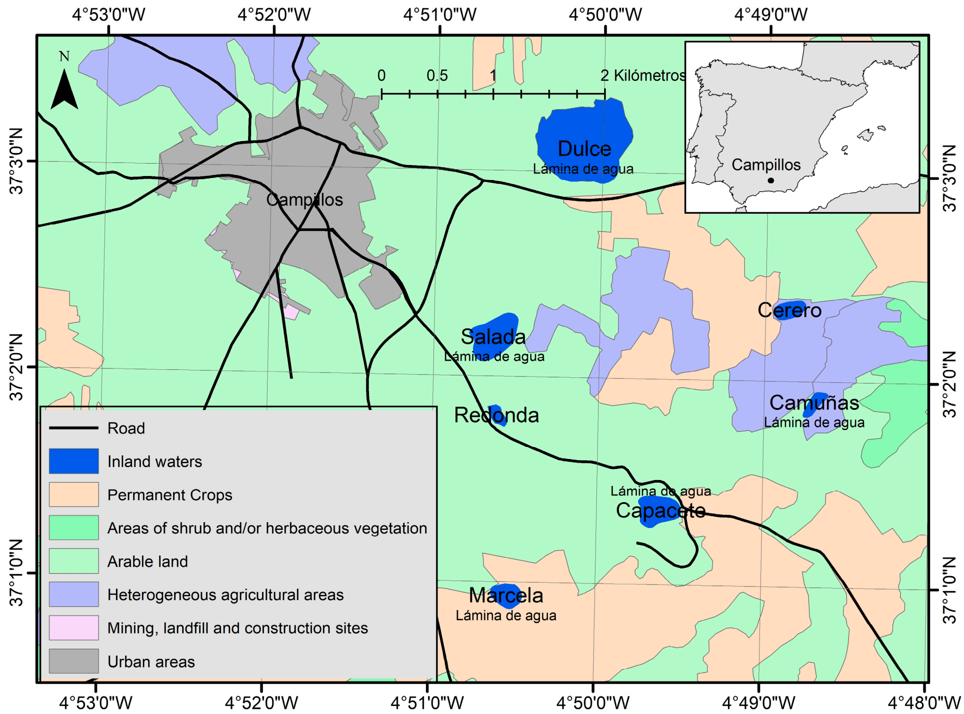

Figure 1. Geographic location of the six temporary wetlands studied. Salada, Redonda, Capacete, Camuñas and Cerero ponds are located within the Nature Reserve and SPA (ES6170015) Lagunas de Campillos. The Dulce pond was dry and therefore excluded from the sampling.

An integrated water sample for the evaluation of the biotic variables was taken following the previously mentioned methodology. Several biotic variables were evaluated: (i) total chlorophyll-a concentration (Chl-a) and relative contribution of phytoplankton groups; (ii) phytoplankton abundance (<20 µm Equivalent Spherical Diameter (ESD) and (iii) bacterial concentrations (abundances). Chl-a and phytoplankton composition were estimated with a submersible FluoroProbe (bbe Moldaenke GmbH, Schwentinental, Germany), which discriminates among diatoms and dinoflagellates, blue-green algae, and cryptophytes and green algae [13]. Abundance of phytoplankton (<20 µm ESD) was measured with a Beckdon Dickinson Acurri C6 flow cytometer, counting at least 10,000 events. The enumeration of heterotrophic cultivable microorganisms was carried out by adding 0.1 mL of serial dilution of the integrative water samples on Tripticase soy agar (Oxoid Ltd., Wade Road, Basingstoke, UK) plates. These plates were cultured at 22 °C for 48 h (ISO 6222:1999). Total coliforms and enterococci concentrations were determined by water filtration through sterile nitrocellulose filters (47 mm diameter, 0.45 µm pore size; Millipore Corp., Bedford, MA, USA). Membranes were incubated in Chromocult coliform agar (Merck, Darmstadt, Germany) at 37 °C for 24 h to determine total coliforms and *Escherichia coli* (ISO 9308-1:2000), or in m-Enterococcus agar (Merck, Rahway, NJ, USA) at 37 °C for 48 h to determine total enterococci (ISO 7899-2:2001). After incubation, colonies were counted on each medium, and bacterial concentrations (colony forming units, CFU mL^{-1}, or CFU 100 mL^{-1}) were determined.

The trophic state of each pond was obtained through the Trophic State Indexes (TSI) proposed by Carlson [14] and Kratzer and Brezonik [15], using chlorophyll-a (Chl-a), and TP and TN concentrations, respectively. The three previous values of the respective TSI indexes were combined in an integrated Trophic Level Index (TLI; [16]):

$$TLI = [TSI(Chla) + TSI\ (TP) + TSI\ (TN)]/3 \quad (1)$$

2.3. Data Analysis

The relationships between species and environmental variables were analyzed using a multivariate analysis. To select the most suitable statistical analysis, a Detrended Correspondence Analysis (DCA) was carried out, indicating a gradient length >3. Accordingly, a Canonical Correspondence Analysis (CCA) was selected as an adequate statistical analysis [17]. CCA analysis allows for inferring the linear combination of environmental variables assuming a unimodal species-environment relationship [18,19]. CCA results were plotted using the two first CCA axes, to show the greatest amount of variation in CCA ordination. The statistical significance ($p < 0.05$) of the axes was assessed using a Monte Carlo permutation test (999 permutations). Prior to this analysis, and when the normality of the data (species and environmental variables) was not fulfilled, a $\log10(x + 1)$ transformation was applied to reduce the influence of different scales of measured variables. The multivariate analysis was performed using PC-ORD version 4.0 software.

3. Results

The abiotic conditions of the six sampled ponds showed little differences in terms of temperature and pH. However, significant differences were observed for conductivity and derived salinity values (Table 1). According to the salinity estimation and typologies proposed by Hammer [20], the Campillos ponds showed different salinities throughout the studied period, with Salada being hypersaline; Redonda, Cerero, and Capacete being hyposaline; and Marcela and Camuñas being subsaline. In addition to the highest salinity, the Salada pond also showed the highest TN concentration, while the highest TP and DIP were observed in the Capacete pond. Nevertheless, this high phosphorus concentration did not lead to an especially high phytoplankton biomass (Chl-a concentration). The TN/TP and DIN/DIP ratios were, in all ponds, higher than or close to the Redfield ratio for optimal phytoplankton growth (N/P = 16). Thus, although in mesotrophic and eutrophic ecosystems nutrients do not limit phytoplankton growth, the high ratio could indicate a relative limitation by phosphorus in all ponds.

Considering the concentration of nutrients (TP and TN) and the concentration of Chl-a, the trophic state of the wetlands was calculated (Table 2). The obtained results indicate a high trophic level of all the studied ponds, ranging from mesotrophic (Redonda) to hypertrophic (Salada).

Figure 2 shows the temporal evolution of Chl-a and the different phytoplankton groups in the studied ponds throughout the whole sampling period. According to the trophic level, Chl-a concentration was always highest in the Salada pond, and lowest in the Redonda pond. The low chlorophyll value in the Capacete pond can be explained as a consequence of the fact that this wetland presented the lowest TN/TP ratio of all the ponds studied. This value is close to the phosphorus limitation value (TN/TP ~ 33) for algal growth [21]. The high Chl-a concentration in Salada is mainly due to a high concentration of green algae, diatoms, and dinoflagellates. On the other hand, cyanobacteria and cryptophyta showed the lowest concentration in the Salada pond and reached the highest concentration in the Cerero, Camuñas, and Capacete ponds.

Table 1. Annual mean values of abiotic variables ± standard deviation. T (Temperature), Cond. (Conductivity), TN (Total Nitrogen), TP (Total Phosphorus), DIN (Dissolved Inorganic Nitrogen), DIP (Dissolved Inorganic Phosphorus), and Chl-a (Total Chlorophyll-a concentration). *n* indicates the number of samples collected in each pond.

	Salada (n = 4)	**Redonda (n = 4)**	**Capacete (n = 4)**	**Camuñas (n = 3)**	**Cerero (n = 4)**	**Marcela (n = 1)**
T (°C)	13.74 ± 3.29	14.30 ± 3.27	14.88 ± 2.63	15.44 ± 3.86	15.37 ± 3.42	17.91
pH	8.71 ± 0.28	8.59 ± 0.69	8.85 ± 0.26	8.57 ± 0.77	8.76 ± 1.06	7.86
Cond. ($mS{\cdot}cm^{-1}$)	75.85 ± 11.32	15.39 ± 5.07	5.03 ± 2.02	2.12 ± 0.97	8.61 ± 2.43	0.70
TN ($mg\ L^{-1}$)	17.64 ± 4.18	8.24 ± 0.90	10.24 ± 2.24	11.52 ± 4.01	11.88 ± 3.05	8.48
TP ($\mu g\ L^{-1}$)	253.84 ± 166.70	52.30 ± 18.82	337.33 ± 220.25	208.69 ± 28.81	168.88 ± 102.55	177.22
DIN ($mg\ L^{-1}$)	2.14 ± 0.44	0.71 ± 0.17	1.48 ± 1.51	2.66 ± 3.70	1.56 ± 1.59	0.61
DIP ($\mu g\ L^{-1}$)	9.65 ± 7.70	11.70 ± 10.51	172.21 ± 240.86	60.00 ± 66.37	21.28 ± 15.35	23.33
Chl-a ($\mu g\ L^{-1}$)	92.90 ± 65.66	4.07 ± 3.14	8.60 ± 5.65	12.89 ± 0.96	16.79 ± 8.61	9.71
TN/TP	81.97 ± 27.78	182.44 ± 100.93	36.64 ± 14.10	56.33 ± 22.04	88.20 ± 55.11	47.85
DIN/DIP	288.16 ± 122.46	98.87 ± 64.92	15.39 ± 9.18	33.02 ± 15.36	66.01 ± 19.32	26.15

Table 2. Trophic states for each pond analyzed, obtained with the Trophic State Index (TSI) proposed by Carlson [14] and Kratzer and Brezonik [15]. TSI (Chl-a) and TSI (TP) were obtained according to [14], TSI (TN) according to [15], and TLI (Trophic Level Index) according to Equation (1).

Ponds	**TSI (Chl-a)**	**TSI (TP)**	**TSI (TN)**	**TLI**	**Classification**
Salada	76.99	84.03	80.51	80.51	Hypertrophic
Redonda	46.55	61.24	53.89	53.89	Mesotrophic
Capacete	51.68	88.13	69.90	69.90	Eutrophic
Camuñas	55.28	81.20	68.24	68.24	Eutrophic
Cerero	58.25	78.15	68.20	68.20	Eutrophic
Marcela	52.87	78.84	65.86	65.86	Eutrophic

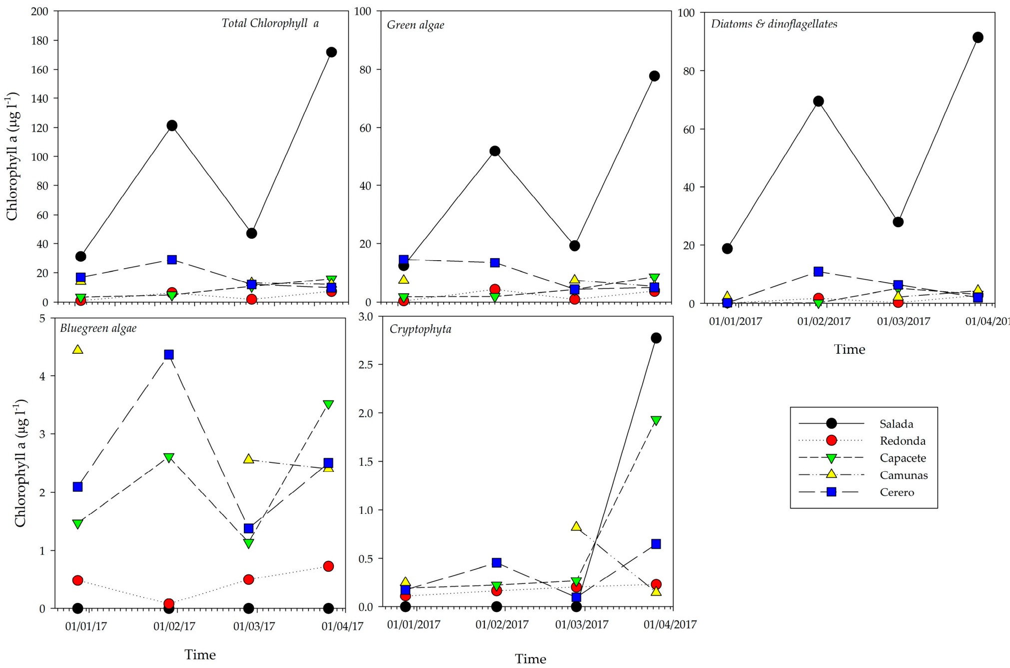

Figure 2. Temporal (26 December 2016–26 March 2017) evolution of chlorophyll-a and the different phytoplankton groups in the studied ponds throughout the sampling period.

Phytoplankton abundance showed a high variability (Figure 3). Three ponds (Camuñas, Capacete, and Salada) reached their highest abundance in the last sampling month

(March), while Cerero presented the highest abundance in the first sampling month (December). Redonda was the pond with the lowest abundance of phytoplankton (<20 µm) throughout the entire sampling period, which might be due to its mesotrophic character. In terms of biovolume, Salada always showed the highest value, especially during the last sampling month, coinciding with the highest Chl-a concentration (Figure 2).

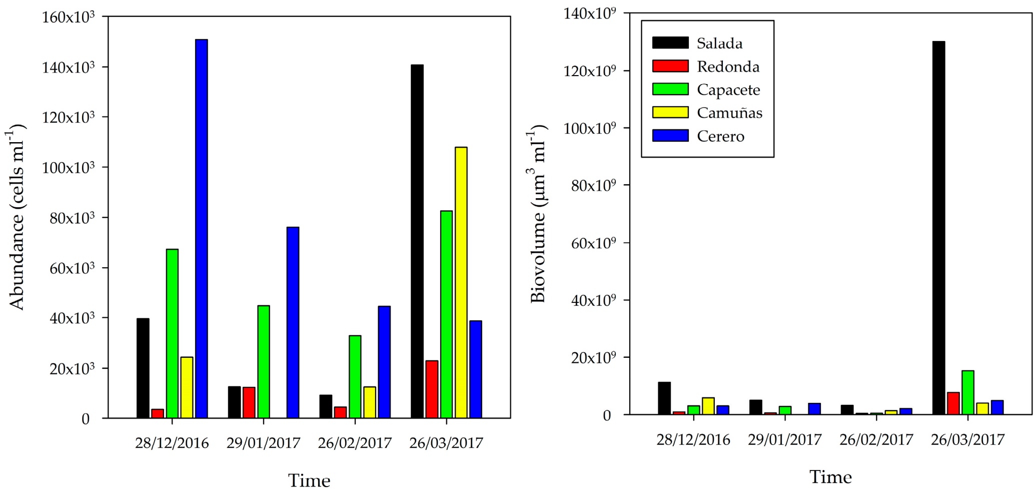

Figure 3. Abundance (cells mL^{-1}) and biovolume (μm^3 mL^{-1}) of phytoplankton in the studied ponds.

Phytoplankton size spectra are shown in Figure 4. The results revealed the typical high abundance of small cells (<3.6 µm ESD) with a clear decrease in large cells in all ponds, except in the Salada pond on 26 March 2017, where larger cells (>5.3 µm ESD) reached and maintained abundances around 100,000 cells mL^{-1}, which explains the extremely high values of phytoplankton biovolume. The remaining ponds never reached 100,000 cells mL^{-1} and always decreased after a doming at similar size classes (Figure 3).

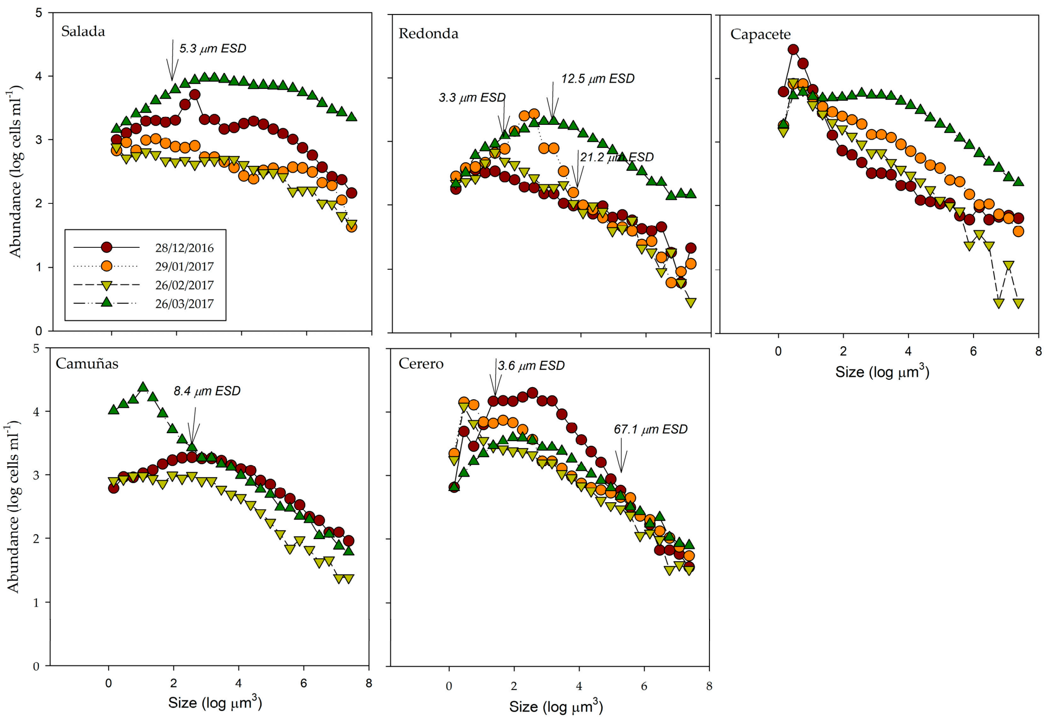

Figure 4. Phytoplankton size spectra in the studied ponds. ESD = Equivalent Spherical Diameter. Camuñas was dry on 29 January 2017.

Figure 5 shows the bacterial concentration (total coliforms, *E. coli*, enterococci, and heterotrophic bacteria) in the studied ponds. Total coliform abundance was highest in Capacete, and lowest in Salada and Redonda, and slightly higher abundances were found at the Cerero pond. Enterococci concentration was highest at Marcela, and absent at Salada, Redonda, and Cerero, and it showed sporadic peaks at Capacete in February and Camuñas in March. *Eschericia coli* was absent at Salada and Redonda, and it showed low concentrations at Marcela and Camuñas. At Capacete and Cerero, high *E. coli* concentrations (510–440 CFU 100 mL^{-1}) were observed until March, which decreased to values lower than 20 CFU 100 mL^{-1}. Heterotrophic bacteria were highest at Marcela, while the remaining ponds showed lower abundances. Again, the Salada and Redonda ponds showed the lowest concentration, while Cerero, Capacete, and Camuñas reached values between 300 and 1600 CFU mL^{-1}.

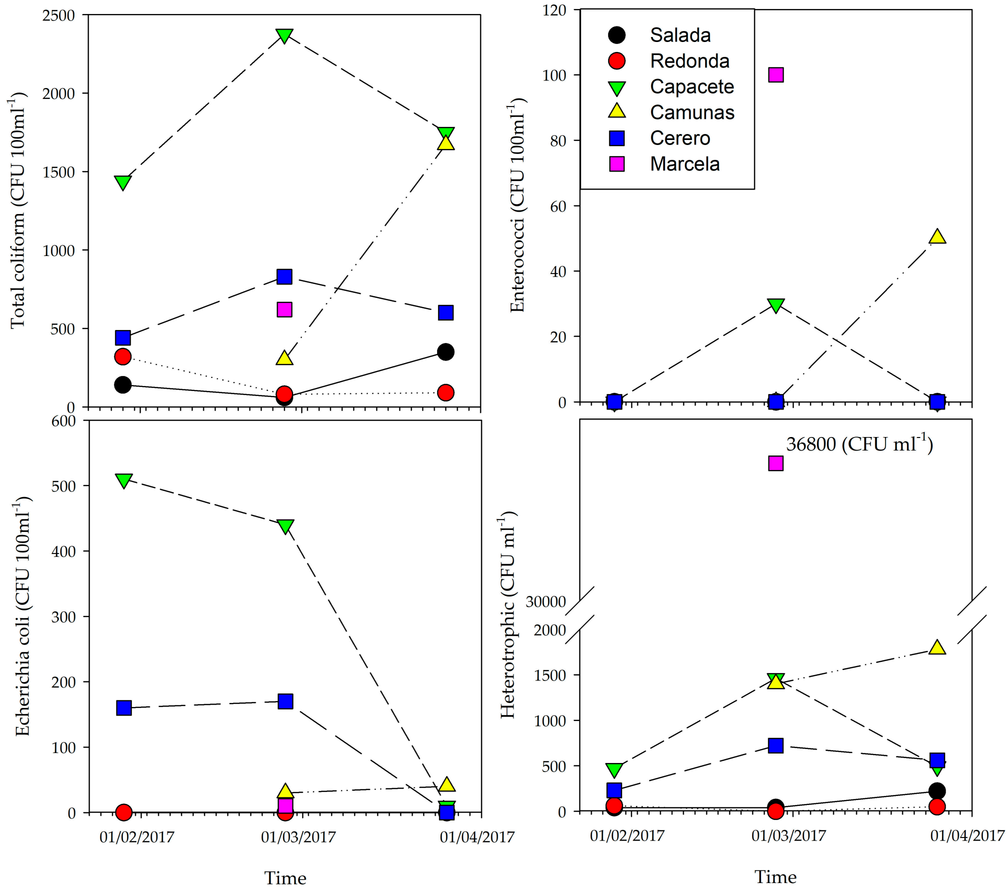

Figure 5. Bacterial concentration in the sampled ponds. CFU indicates colony forming units.

The Canonical Correlation Analysis (CCA) results (Table 3) produced three significant axes (Monte Carlo test, $p < 0.05$), explaining 77.9% of the total variance. CCA 1 (51.3%) was negatively correlated with conductivity. CCA 2 was negatively correlated with temperature and positively correlated with TN, DIP, and TP. The CCA ordination diagram (Figure 6) indicates that the first axis represents a gradient of mineralization, separating samples according to their conductivity. Samples with higher values of conductivity appear in negative coordinates, while samples in positive coordinates are associated with less mineralized waters. Thus, they are positively correlated with the presence of heterotrophs and enterococci (Table 4). The second axis divides the samples according to nutrient content gradient and temperature. The samples with the largest content of nutrients appear in the positive coordinates, and they are correlated with the presence of green algae, diatoms, and total phytoplankton concentration.

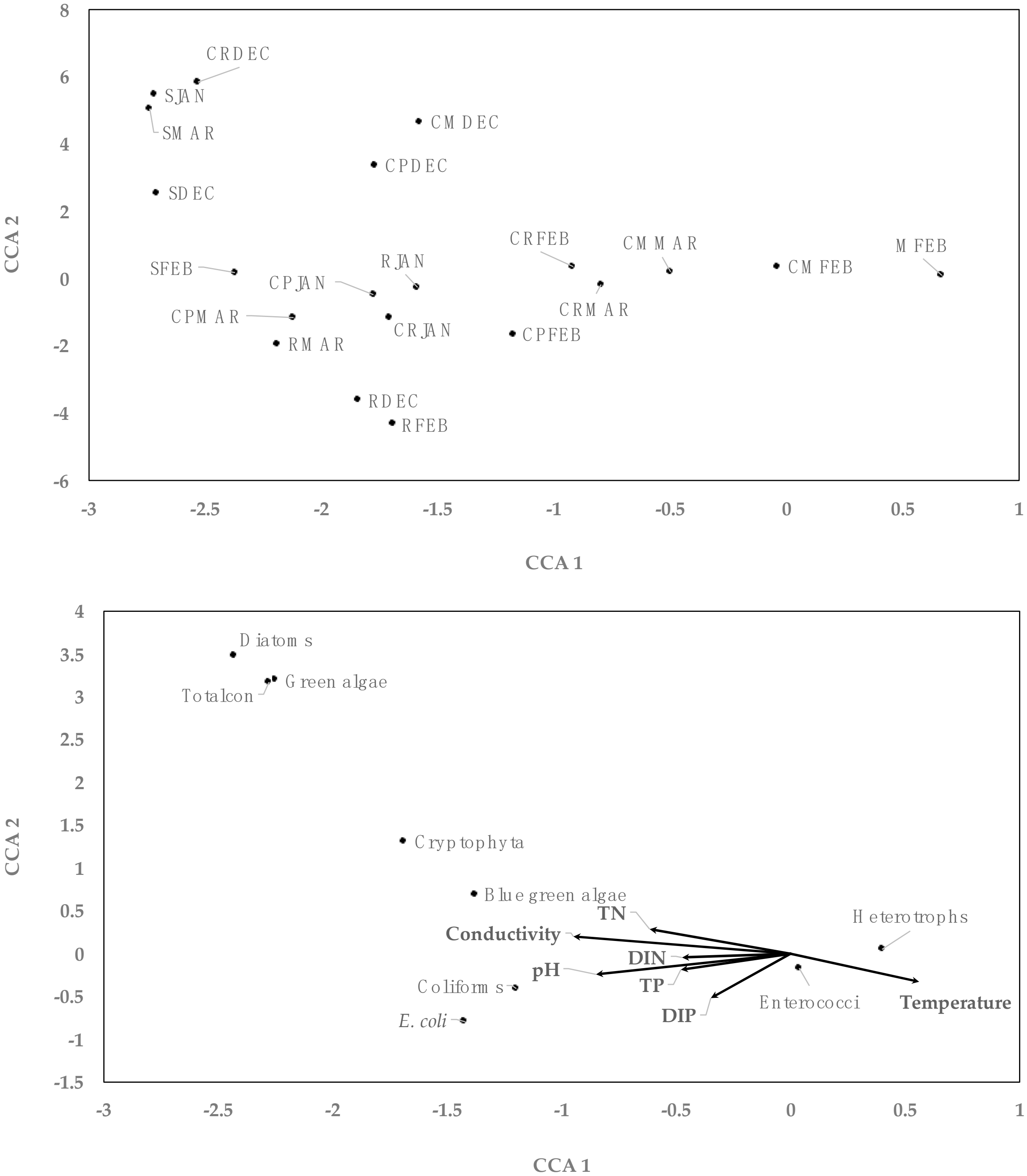

Figure 6. Canonical Correspondence Analysis (CCA) ordination diagram. The temporal and spatial sample sites (upper panel) are named with the first letters of the wetland (S = Salada; CP = Capacete; R = Redonda; M = Marcela; CM = Camuñas; CR = Cerero) and the last three letters with the abbreviation of the sampling month (DEC, JAN, FEB, MAR). Totalcon (lower panel) refers to total chlorophyll a concentration (µg L^{-1}).

Table 3. Summary of the Canonical Correspondence Analysis (CCA) results. Bold letters reflect significant correlations of environmental variables with the first 3 CCA axes (rcritical = 0.389 for 19 df). Correlations between environmental variables and canonical axes (Intraset correlations of ter Braak, 1986 [22]).

	CCA 1	**CCA 2**	**CCA 3**
Axis summary statistics			
Eigenvalue	0.540	0.239	0.041
p-value	0.001	0.001	0.001
% of variance explained	51.3	22.7	3.9
Pearson's Correlation			
Spp-Evnt	0.988	0.800	0.791
p-value	0.001	0.001	0.001
Temperature	0.343	**−0.500**	0.055
pH	−0.544	−0.212	**−0.576**
Conductivity	**−0.832**	0.207	0.141
TN	−0.277	**0.530**	−0.028
TP	0.125	**0.530**	−0.028
DIN	0.111	0.100	−0.092
DIP	0.456	**0.530**	−0.028

Table 4. Correlations of photoautotrophic and heterotrophic microbial organisms (Pearson's r) with the first 3 axes of the Canonical Correspondence Analysis (CCA). Statistically significant correlation coefficients are in bold letters (rcritical = 0.389 for 19 df). Totalconc indicates total concentration of chlorophyll a.

Species	**CCA 1**	**CCA 2**	**CCA 3**
Green algae	−0.483	**0.595**	−0.097
Blue green algae	0.286	−0.010	−0.345
Diatoms	−0.487	**0.523**	0.087
Cryptophyta	−0.062	0.154	0.091
Totalconc	−0.481	**0.562**	−0.103
Total Coliforms	0.364	−0.305	−0.064
E. coli	0.096	−0.244	−0.204
Enterococci	**0.679**	−0.109	−0.025
Heterotrophs	**0.622**	−0.060	−0.036

4. Discussion

The Campillos wetland complex constitutes a pool of ponds with the highest level of protection on a national and international scale. However, very few scientific studies have been carried out in this area, and most of them are based on the assessment of their biological diversity [23–25]. Thus, the assessment of the trophic state of this set of ponds was essential for a better understanding of this reserve.

4.1. Nutrient Concentration and Redfield Ratio

According to this study, the TN/TP and DIN/DIP ratios observed in all ponds are equal to or higher than Redfield number 16, which indicates the N/P proportion for optimal phytoplankton growth [26], showing relative nutrient limitation by phosphate. Phosphate deficiency can be explained by the ironhydroxyde–phosphate–sulfide system that retains phosphate in the sediment under oxygenic sediment interface [27], leading to phosphate limitation in epicontinental waters in the absence of anthropogenic phosphate input [28]. Anthropogenic (enhanced nutrient input driven by human activities) eutrophication of epicontinental waters detected in the 1970s was mainly due to phosphate input from detergents. Therefore, phosphate content in detergent was reduced by law at the EU level [29]. This measure enabled a worldwide reduction in anthropogenic phosphate spills in the last decade, especially in high-income countries [30]. Although relative limitation by phosphate occurs, the phosphate concentrations observed in the eutrophic and hypertrophic wetlands (TP > 177 mg L^{-1}; DIP > 9.65 mg L^{-1}) are far from being limiting for algal growth. In fact, only in the mesotrophic Redonda wetland, with the highest TN/TP-ratio (182.44 ± 100.93), lowest TN (8.24 ± 0.90 mg L^{-1}) and TP (52.30 ± 18.82 μgL^{-1}) values, and a low DIN (0.71 ± 0.17 mg L^{-1}) concentration, can algal growth be limited by nutrient availability, as suggested by the lowest Chl-a concentration (4.07 ± 3.14 μg L^{-1}) observed among all ponds. The adjacent RAMSAR salty lake Fuente de Piedra showed low N/P (<16) in December and January due to an increase in phosphate by almost two orders of magnitude (10–1000 μg at L^{-1}), which is related to runoff fertilization, as indicated by the filling of the salty lake [31]. From March to November, phosphate was around 8–10 μg at L^{-1}, which is similar to the concentration of the Salada pond in our study. Applying the eutrophication index and the N/P ratio to evaluate the trophic state of the Paldang Reservoir (Republic of Korea), Young-Chul [32] concluded that the mesotrophic and eutrophic sampling stations were also limited by phosphate, which, however, increased, particularly in summer, due to runoff events, domestic waste water, and industrial and agricultural effluents.

4.2. Phytoplankton Composition

The phytoplankton community in hypertrophic and hypersaline ponds (Salada) is usually characterized by simple phytoplankton communities dominated by few species [33]. In our study, the Salada pond showed the highest Chl-a concentrations (30–180 μg L^{-1}) during the whole sampling period, dominated by green algae (12–77 μg L^{-1}) and diatoms (18–91 μg L^{-1}), while cyanobacteria and cryptomonads were almost entirely absent. In fact, one effect of eutrophication is the drastic decrease in cyanobacteria [34]. The Chl-a concentration of the remaining wetlands was lower than 28 μg L^{-1}, and cyanobacteria and cryptomonads were present throughout the entire sampling period. Except for the hypertrophic and mesotrophic ponds, cyanobacteria concentrations were between 1.13 μg L^{-1} and 4.43 μg L^{-1}, indicating that cyanobacteria might be outcompeted by diatoms and green algae in the Salada pond and limited by phosphate in the mesotrophic Redonda pond. Calculating the evenness (E) of the mean relative contribution of the four phytoplankton groups to total Chl-a in each pond (E = D/D_{max}, [35] where $D = \frac{1}{\Sigma\left(\frac{Chl-a_i}{CHLT}\right)^2}$ $D_{max} = 4,\ Chl - a_i = Chlorophyll\ concentration\ of\ group\ (i),\ CHLT = Total\ Chlorophyll - a\ of\ the\ pond$), the lowest evenness was observed in the hypertrophic wetland (Salada, E = 0.52), followed by the mesotrophic wetland (Redonda, E = 0.61). The evenness in the mesotrophic ponds Cerero, Marcela, and Camuñas ranged between E = 0.63 and E = 0.69. Capacete had the highest evenness (E = 0.77). Thus, high nutrient availability (Salada) and nutrient limitation (Redonda) reduces evenness due to the proliferation of fast-growing algae and the limitation of N-fixing groups (cyanobacteria) if phosphate is the limiting factor [36].

4.3. Phytoplankton Size Structure

Except for the last sampling day, the highest phytoplankton abundance was observed at Capacete. However, in terms of biovolume, the Salada wetland showed the highest values on each sampling day, especially during the last day, when phytoplankton biovolume at the Salada wetland was almost one order of magnitude higher than at the previous samplings. This extremely high biovolume could be due to the very high abundance of cells > 5.3 μm ESD. In fact, the SAS of 26 March 2017 is atypical, showing fewer small cells than larger cells and an almost constant abundance of cells > 5.3 μm ESD, suggesting the lack of top-down control of phytoplankton > 5.3 μm ESD and an intense diatom bloom. Size Abundance Spectra (SAS) are frequently adjusted to a lineal log–log function, where the steeper the slope, the more oligotrophic the aquatic environment [37]. However, uneven SAS have been described for frequently perturbed aquatic ecosystems [38], and shallow, saline, and eutrophic ecosystems typically show a higher variability in SAS [39] and a more uneven biomass distribution [40]. The predominance of nanoplankton, in terms of abundance and biovolume, has been described in coastal, nutrient-rich lagoons [41], and it can be said that the SAS observed in the five wetlands indicate that all of them are perturbed aquatic ecosystems and bottom-up controlled.

4.4. Heterotrophic Bacteria

Total coliforms are mainly found in the intestine of humans and homeothermic animals, and they are released to the environment through feces [42]. Capacete shows by far the highest abundance of total coliform, which can be due to the proximity of an animal farm. Slurry input from a neighboring farm is a possible origin. With values >1400 CFU 100 mL^{-1}, total coliform abundance is very high. Nonetheless, the highest *E. coli* concentration is around (500 CFU 100 mL^{-1}), which is below the legal upper limit (900 CFU 100 mL^{-1} [43]) of epicontinental bathing water.

Heterotrophic bacteria and enterococci reached their highest abundance at Marcela, the pond with the lowest conductivity (salinity), which was dry except during the only sampled day (26 February 2017). Thus, the high values of heterotrophic bacteria might be due to the recent flooding of the pond. This also explains the low salinity of this lagoon on the sampling day.

4.5. Analysis at Ecosystem Level

The CCA analysis depicts two main axes. The first axis is related to salinity, with negative values for high salinity, as indicated by the negative values for the Salada wetland samplings, and positive values with low salinity, where Marcela and Camuñas are located. The second axis depicts the trophic level, with positive values for hypertrophic wetlands (Salada), and negative values for mesotrophic wetlands (Redonda). Enterococci and Heterotrophic bacteria were positively correlated with the first CCA, as they were more strongly affected by salinity, regardless of nutrient availability. In contrast, photoautotrophic organisms, such as diatoms and green algae, and total Chl-a concentration were obviously positively related to CCA 2, which shows the trophic state. In fact, Salada, as a hypertrophic and hypersaline wetland, had a greater influence on this CCA, and it determined both axes.

It is worth highlighting that the hypertrophic (Salada) and mesotrophic (Redonda) wetlands are close to each other (Figure 1). Without considering the specific land use around both wetlands, which could be similar due to their proximity, the difference might be due to the catchment area, located around the Salada pond, with an area of 135.91 ha [44], which is 4.8 times the catchment area of the Redonda wetland (28.19 ha [35]). The mesotrophic wetlands of Camuñas (59.24 ha) and Cerero (69.29 ha) have an intermediate catchment area. The larger the catchment area, the higher the nutrient inflow into the wetland due to fertilizer use in the surrounding agricultural activity. Capacete, with 206.7 ha, had the highest TP (337.33 $\pm$ 220.25) and DIP (172.21 $\pm$ 240.86) concentrations. The abovementioned Fuente de Piedra lake has the largest catchment area (152 km^2) and reached phosphate concentrations >500 µg L^{-1} when recharged in December–January. Thus, we suggest that fertilizer applied to the cropland is washed to the ponds, consequently increasing the phosphate input in the catchment area, being a clear example of anthropogenic eutrophication.

5. Conclusions

The present study allowed us to determine, for the first time, the trophic state of a set of wetlands of international importance located in Andalusia (Southern Spain). The obtained results show a high heterogeneity among wetlands located close to each other. Except for the Redonda wetland, with the smallest catchment area, the other wetlands are eutrophic, and the Salada wetland, with the largest catchment area, and intensive cultivated and fertilized land use, even reaches hypertrophic conditions. In contrast, the adjacent Redonda wetland, with a small catchment area, is less affected by anthropic activities. Furthermore, cattle raising activities also affect at least one of the wetlands (Capacete), increasing nutrient availability and raising potential problems for public health. The high trophic level of the studied wetlands encourages the establishment of restoration measures to improve their conservation. Among them, a lower use of fertilizers should be encouraged in crops established in their drainage basins, while also promoting the development of powerful border vegetation that helps to minimize the entry of nutrients into this set of wetlands. Despite the age of the data (6 years), the importance of publishing these results lies in the fact that they were taken in a cycle of extreme drought, a circumstance that will be increasingly recurrent in the Mediterranean climate as a result of climate change.

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