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Assessing the conservation status of Mediterranean coastal ponds: Checklist, ecological and functional diversity of diatom communities

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ABSTRACT

Coastal ponds are peculiar and threatened habitats, considered biodiversity hotspots. In many ecosystems ponds can be a valuable resource for the conservation of freshwater biodiversity. The Castelporziano Presidential Estate is a Mediterranean protected area hosting numerous ponds which are suffering severe droughts and a general decline in number and hydroperiod length. Hydroperiod changes can be particularly relevant for biodiversity inhabiting ponds, in particular diatom communities. Here, for the first time, we evaluate the conservation status of Mediterranean ponds inside the Castelporziano Reserve. The aims of this research are to i) investigate the influence of environmental variables on diatom communities of ponds; (ii) analyse diatom community structure in function of species richness and competitive interactions; (iii) highlight the potential and effectiveness of diatom ecological guilds in supporting the interpretation of the ecological status of threatened small waterbodies. Overall, 122 diatom taxa in the 29 ponds were described. *Nitzschia* (18 species) was the most species-rich genus, followed by *Gomphonema* (14) and *Navicula* (13). 17 species were included in the Red List of endangered species. Intra-pond distance and anthropogenic impacts didn't influence diatom assemblages. On the contrary, diatom communities were influenced by altitude and the distance from the sea. Furthermore, temporary ponds showed the lowest values for Shannon, evenness and Simpson indexes Considering Beta-diversity, both Bray-Curtis and Jaccard dissimilarity matrices highlighted a large dissimilarity between sampling sites. Inter-variability between sites and intra-variability within the sites resulted both very low. Our results highlight that diatom species are not influenced by hydroperiod length. Moreover, a structured diatom community emerged, where segregation processes predominating over aggregation ones. Finally, the average water quality of the ponds, calculated using the EPI-D index, was described as "good". This study represents the most complete checklist of diatom communities inside coastal ponds of Castelporziano, providing valuable insights into the effects of natural and anthropogenic influences on ponds conservation and management activities. However, spatial-temporal changes and anthropogenic impacts should be deeply investigated.

1. Introduction

Coastal ponds are peculiar and important habitats with a worldwide distribution (Hassall, 2014). The term "pond" is the common name used to refer to small (<5 ha) water bodies which are shallow (<5 m), with <30% emergent vegetation (Olmo et al., 2022; Richardson et al., 2022). Moreover, ponds represent the most abundant freshwater ecosystems on Earth (Meerhoff and Beklioğlu, 2024). Despite their small size compared to other waterbodies, ponds constitute biodiversity hotspots, both in terms of community composition and functional traits, and are fundamental habitats for a wide range of rare and protected species (Fehlinger et al., 2023). Furthermore, they play a significant role in the provision of ecosystem services (Krivtsov et al., 2022; Fu et al., 2018). Hydroperiod, the amount of time that a pond experiences the wet phase, i.e. when the pond is filled with water, is a crucial environmental variable for these ecosystems (Coccia et al., 2024). Considering the hydroperiod, ponds can be distinguished between temporary and permanent ponds (Seminara et al., 2015). The definition of "pond" is actually challenging scientists, often overlapping with the definitions of other waterbodies: for example, as described by Richardson et al. (2022), at an international level, there is no consensus on how to discriminate among ponds and wetlands. The functional and

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morphological differences between ponds and wetlands in this research were taken from Richardson et al. (2022) and Tinelli et al. (2012): both are considered small (<5 ha) and shallow (<5 m depth) water bodies but distinguished using the 30% coverage in emergent vegetation threshold (ponds <30%; wetlands >30%). The term “temporary” refers to the hydroperiod of these ecosystems (i.e., the length of aquatic phase) (Fernández-Zamudio et al., 2016). Temporary ponds are characterized by a dry phase which occur, regularly, during spring and summer, when temperatures and scarce precipitations can reduce partially or totally the water supply (Boix et al., 2016). Temporary ponds contain a self-regulating hydrology (Florencio et al., 2010). They often occur in shallow depressions with an impermeable ground and have a relatively small catchment area. But they can be also found in karstic areas, where preferential groundwater flows originate outside of their catchment area, which can result in water level rise (Zacharias and Zamparas, 2010). Differently, permanent ponds are characterized by the presence of water throughout the year, without suffering a dry phase during the summer season (Obrador et al., 2018). Permanent coastal ponds provide diverse ecosystem services, including water quality improvement and supply, food provisioning, and biodiversity support, despite significant pressure from multiple anthropogenic stressors and the impacts of ongoing global change (Fehlinger et al., 2023). Usually, temporary ponds support groups that clearly differ from those of permanent ponds, and, in some cases, species of great conservation importance (Zacharias and Zamparas, 2010). Moreover, the influence of marine ecosystems proximity can be an important factor influencing coastal ponds, which are often subjected to strong alterations (Della Bella and Mancini, 2009). In the Mediterranean region, coastal ponds are threatened ecosystems, despite receiving protection through the EU Habitats Directive (92/43/CEE) (Coccia et al., 2024). Generally, ponds are highly threatened by human activities and anthropogenic pollution, affecting biota at different levels (Gallitelli et al., 2024; Martínez-Megías and Rico, 2022; Manoj and Padhy, 2015). Climate change presents a particular challenge for coastal ponds, influencing hydroperiod length and shifting seasonal weather patterns (Segura and Palomar, 2023).

Hydroperiod changes can be particularly relevant for biodiversity inhabiting ponds, in particular diatom communities (Blanco et al., 2020). Diatoms are unicellular brown algae (Soininen and Teittinen, 2019), showing a global distribution, characterized by the “frustule” (cell wall), photosynthetic pigments, and storage capacity of specific compounds (oil and chrysolaminarin) (Taurozzi et al., 2023a). They are both planktonic and benthic and populate all types of aquatic environments (i.e., fresh, brackish, and marine waters) (ISPRA, 2014c; Stevenson, 2014). In addition, diatoms are fundamental for supporting global life forms, being primary producers and an important source of oxygen (Letáková et al., 2018). Furthermore, diatoms show remarkable ecological importance (Soininen and Teittinen, 2019). Through their photosynthetic activity, the group contributes to about 30–35% of the Earth’s global primary production, carbon fixation and oxygen release into the atmosphere. (Serôdio and Lavaud, 2022). Diatom communities also have great importance as bioindicators for the water quality and are required by legislation, such as the Water Framework Directive (Marcheggiani et al., 2019). Traditionally, diatoms have been used as bioindicators for evaluate the ecological status of different aquatic environments due to some important features like ubiquity and sensitivity to environmental alteration (Salmaso et al., 2019). Indeed, there are species that might be considered biological indicators, and their occurrence and abundance can be used in the assessment of water quality (Costa and Schneck, 2022; Salomoni et al., 2011).

In many ecosystems ponds can be a valuable resource for the conservation of freshwater biodiversity, often containing specialized flora and fauna which are not represented in other habitats (Bazzanti, 2015): an example is provided byThe Castelporziano Presidential Estate, which could be considered a “natural island” inside the Municipality of Rome: this ecosystem is hosting numerous ponds which are suffering severe droughts and a general decline in number and hydroperiod length (Cerini et al., 2020). The Reserve is inaccessible by citizens, protected by law enforcement and fenced: this high level of protection makes it an extremely suitable environment for community monitoring and analysis given the almost total absence of external anthropogenic influences. In fact, the Reserve represents a good example of pristine un-fragmented and biodiversity-rich ecosystem (Moretti and Salvati, 2014). Ponds in the CastelPorziano Presidential Estate show an historical generation, generated from 1920 with the reclamation of marshy areas. All this great reclamation work has thus led to a rapid landscape change and a notable reduction in floristic richness and fauna that had been preserved for centuries. Moreover, during the last years, there was a clear decrease in these environments in number and in extension due to the lowering of the groundwater, climatic variations and presumable external pumping (Tinelli et al., 2012). To date, ponds inside the Reserve are subject to a Monitoring program to evaluate the real ecological importance of these environments.

In this sense, this research aims to evaluate the conservation status of Mediterranean ponds inside a protected Reserve. To do that, we also provided a complete overview about diatom communities inhabiting the natural coastal ponds of the Castelporziano Presidential Estate. To date, little is still known about the diatom communities of coastal freshwater ponds in the Mediterranean region. Moreover, while a certain importance is given to monitoring the hydroperiod fluctuations of ponds inside the Reserve, low attention is given to the biotic compounds of the ponds. To the best of our knowledge, this is the first research exploring diatom richness, ecological characteristics and distribution inside the natural ponds of the Castelporziano Presidential Estate. The main aims of this research are to:

i. Provide the most complete checklist of diatom communities inhabiting the ponds of the Castelporziano Reserve;
ii. Investigate the influence of environmental variables on diatom communities of ponds included in a Protected area;
iii. Analyse diatom community structure in function of species richness and competitive interactions;
iv. Highlight the potential and effectiveness of diatom ecological guilds in supporting the interpretation of the ecological status of threatened small waterbodies;
v. Evaluate the effectiveness of Protected areas in ponds conservation activities, focusing on the presence of species included in the IUCN Red Lists.

In particular, we hypothesize that:

i. Diatoms are little affected by direct anthropogenic influences, while environmental parameters like distance from the sea and proximity to the routes can mostly affect diatom communities;
ii. Diatom communities are structured following segregation patterns in permanent ponds and aggregation patterns in temporary ponds;
iii. Motile diatoms are dominant in temporary ponds;
iv. The status of Natural Reserve influences positively the water quality and promote the presence of endangered species.

This study could pose an important step to the knowledge of diatom communities in a Mediterranean protected area, without human disturbances and threats influencing ecological dynamics. Therefore, to monitor diatom community dynamics and understanding their evolution in the future, all-year monitoring campaigns are necessary.

2. Materials and methods

2.1. Study area

The Castelporziano Presidential Estate, a nature reserve along the Tyrrhenian coast near Rome, covers an area of about 6000 ha. In 1999 the Presidential Estate of Castelporziano was established as a State nature Reserve (Recanatesi, 2015), becoming one of the residences of the Italian Republic President (Castracani et al., 2010). The area is characterized by a highest altitude of 80 m a.s.l. (De Nicola et al., 2014). Castelporziano shows most of the typical ecosystems of the Mediterranean biome and is characterized by different habitats: a large area of uncontaminated beach, sandy dunes with pioneer and colonizing species, ancient dunes consolidated with ponds behind the dunes and vast oak forests. Moreover, the Reserve could be considered a biodiversity hotspot, hosting more than 1000 plant species and more than 2900 animal species (Tinelli et al., 2012).

Currently, 169 coastal ponds have been recorded in the Estate (Tinelli et al., 2012). The ponds inside the Castelporziano Reserve represent one of the last well-preserved biotopes of the coastal environment, generated the outcrop of the aquifer and direct precipitations. From a hydrological point of view, it is possible to distinguish two areas characterized by ponds fed mainly by groundwater: one in the northern part of the Estate (Ortaccio, Malpasso, Ponte Ruffo) and the other including the coastal interdune depressions and the Tor Paterno wetlands; the entire central part of the Estate is instead mainly characterized by ponds fed by rainwater The ponds of Castelporziano develop mainly on the Ancient Dune lands characterized by strong variations in the water level which can cause flooding in the winter-spring period and complete drying in the summer. These pools are made up of natural depressions in which the collection and permanence of water are favored by localized soil layers with low permeability (Tinelli et al., 2012).

Based on the presence of water in the reservoir, 118 coastal ponds have been identified as "temporary" (70%), 41 as "permanent" (24%) and 10 as "wetlands" (6%). Moreover, 148 ponds were recorded as natural (88%) and as 21 artificial (12%).

2.2. Sampling activities and data analysis

Sampling activities were performed in February 2023. Overall, 29 ponds were sampled: 11 temporary ponds (TP), 14 permanent ponds (PP) and 4 wetlands (WP) (Fig. 1). Considering the partition of the Reserve, divided into 14 zones, 3 ponds for each zone (1 temporary, 1 permanent, 1 wetland) were selected, where available, to include samples from all the zones of the Reserve. Epiphytic diatoms were sampled and prepared following ISPRA (2014a; 2014b): using a commercial toothbrush the surface of underwater vegetation was scratched (at least, a surface of 1 m^2) from different zones to cover as much of the available surface of the ponds. Then, each toothbrush was immersed in a 50 ml Falcon containing 70% diluted ethanol and 20 ml of distilled water. Samples were transported to the laboratory where oxidation of diatoms was carried out following the standardized protocol by ISPRA (2014a, 2014b): after homogenization of the samples, 7 ml was withdrawn from each sample, placed in a 15-ml centrifuge tube and centrifuged at 1500 rpm in an "Eppendorf 5810 R" centrifuge for 10 min, the supernatant was removed, and the remaining sample (about 0.5 ml) was placed in a 25-ml beaker. We proceeded through the oxidation method using hydrogen peroxide: 20 ml of hydrogen peroxide was added to the centrifuged sample, and the beaker was placed on a heating plate at 95 °C, waiting for all the substance organic to be oxidized (3–5 h). The beaker was removed from the plate when approximately 2–3 ml of substance remained, the contents were transferred to a centrifuge tube with distilled water, centrifuged at 1500 rpm, and the supernatant was

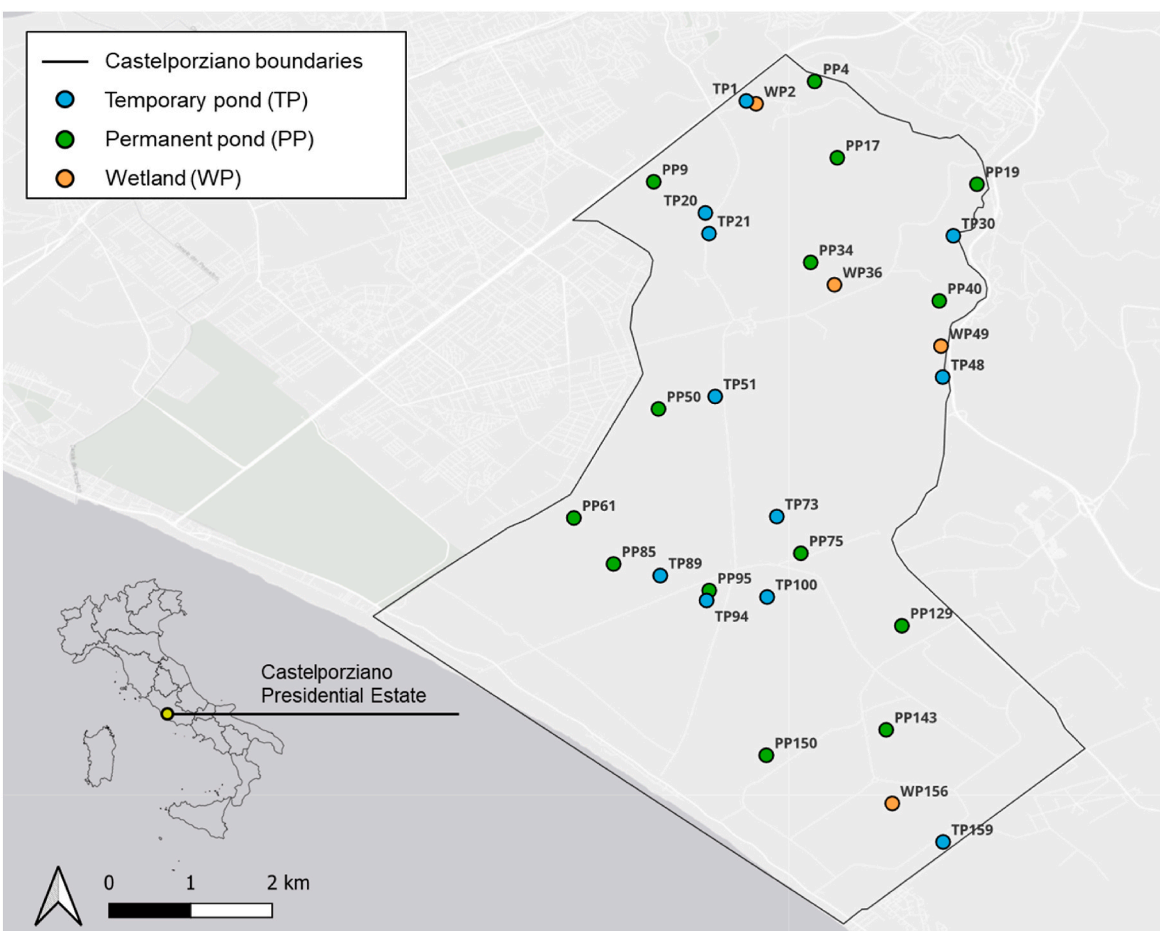


Fig. 1. Map of the Castelporziano Presidential Estate showing sampling sites.

removed. After having resuspended the contents with distilled water, two more decanting cycles were carried out and once all traces of hydrogen peroxide were eliminated, 2 ml of ethanol for the conservation of the samples was added. After the samples were oxidized, the permanent slides were prepared for the recognition of diatom species using Naphrax (high resin power of refraction) to fix the coverslip. For the identification of diatom species, the morphological analysis was conducted with a Leica microscope with 100 × magnification in immersion and using taxonomic guides (Ector and Hlúbiková, 2010; Taylor et al., 2007; Krammer et al., 2002; Krammer and Lange-bertalot, 1991b; Krammer and Lange-bertalot, 1991a; Krammer and Lange-bertalot, 1988; Krammer and Lange-bertalot, 1986). For each slide, the analysis was considered completed when identification at the species level of at least 400 valves was achieved.

Four different ecological variables were considered: a) Altitude; b) Distance from the sea (m); c) Distance from the outside (m); d) Distance from the nearest internal road (m).

The diatomic species were then classified into the three ecological guilds proposed by Passy (2007): “High profile”, “Low profile” and “Motile”:

a) High profile: species of tall stature, including erect, filamentous, branched, chain-forming, tube-forming, stalked, and colonial centrics; species favored by a high presence of nutrients and low disturbance levels;
b) Low profile: species of short stature, including prostrate (adhering to the substrate with the entire valve surface), adnate (apically attached but parallel to the substrate), erect (apically attached but perpendicular to the substrate), solitary centrics, and slow-moving species; species present in nutrient-poor habitats with high disturbance levels;
c) Motile: Species whose distribution increases with increasing nutrients and decreases with increasing disturbance. These species can move to more favorable habitats in case of need.

2.3. Statistical analysis

Normality, homogeneity of variance and independence were tested before performing statistical analysis. Given all the assumptions checked, we only performed parametric tests in our analyses.

To test out first hypothesis, an ANOVA test was performed to determine differences in means in the number of taxa between TP, PP and WP.

Bray-Curtis dissimilarity matrices were set for each environmental variable and diatom abundances; then, Mantel tests were performed to evaluate significant relationships between species Bray-Curtis dissimilarity matrices and environmental variables matrices.

Linear bivariate models were performed between significative variables from Mantel test and the six most abundant diatom species to evaluate the influence of significant environmental variables on the most occurring species. Then, we performed a redundancy analysis (RDA) to model the effects of environmental variables on diatom communities.

To test our second hypothesis, alpha diversity was calculated through diversity indexes. Diversity indexes were calculated to test differences in the species community assemblages. Specifically, the diversity indexes calculated, were:

a) Shannon diversity index (H);
b) Simpson index (D);
c) Pielou’s evenness (J);
d) Number of species (N)

B-diversity analysis, i.e. the comparison of dissimilarity in community composition between each pair of sites, was calculated using Bray-Curtis dissimilarity and Jaccard dissimilarity. Given the aim to reflect ecological distances between diatom communities inter-ponds, Bray-Curtis coefficient and Jaccard index were selected as robust linear (proportional) measures of ecological distance (Faith et al., 1987) and then used to compare dissimilarity matrices. The Bray-Curtis dissimilarity is based on occurrence data (abundance), while the Jaccard distance is based on presence/absence data (does not include abundance information). We used both these approaches to better investigate ecological distances between ponds. Then, Mantel tests were performed to test our hypothesis and evaluate significant relationships between species Bray-Curtis and Jaccard dissimilarity matrices and environmental variables matrices. Principal coordinates analysis for both Bray-Curtis and Jaccard dissimilarity matrices was performed to identify the main components explaining the observed diversity. Cluster dendrogram was performed to identify similarities in ponds matrices. The use of cluster dendrograms graphically help to present the information concerning which ponds are grouped together at various levels of (dis)similarity.

Multivariate multiple linear model was used to show correlations between the four indices considered and the environmental variables.

The co-occurrence module was used to identify the presence of deterministic assembly rules in diatom communities. Co-occurrence was performed following Taurozzi et al. (2024).

To test our third hypothesis, multivariate multiple linear model was used to show correlations between the abundance of taxa belonging to a specific guild and the environmental variables.

One-way ANOVA test was performed to determine differences in means in the number of guilds for each sample between TP, PP and WP Tukey’s pairwise post hoc test was performed to highlight significative differences between guilds. A chi-square test was carried out to analyse the differences in the distribution of the number of taxa of a specific guild in relation to the different types of pond. Effects size was calculated following Cramer’s V as described in Kim (2017). Cramér’s V is an effect size measurement for the chi-square test of independence. It measures how strongly two categorical fields are associated. Cramer’s V ranges from 0 to 1, where 0 indicates no relationship and 1 indicates perfect association. Here we calculated Cramer’s V to investigate the relationship between habitat type (TP, PP, WP) and diatom guilds.

Finally, the Eutrophication/Pollution Index-Diatom based (EPI-D) was calculated following Dell’Uomo and Torrisi (2011) to test the hypothesis of the effectiveness of the Reserve as instrument to preserve pond’s water quality. The EPI-D index was calculated using the “diathor” package in R.

Statistical tests were considered significant when p-value was <0.05. All statistical analysis were computed in Ecosim 5.0 software (Gotelli and Entsminger, 2000), PAST 4.02 software (Hammer et al., 2001) and R software (2021).

3. Results

Overall, the analysis of the epiphytic diatoms from the Castelporziano Presidential Estate resulted in the description of 122 diatom species (Table S1) in the 29 ponds analyzed. *Nitzschia* (18 species) was the most species-rich genus, followed by *Gomphonema* (14 species) and *Navicula* (13 species). The most frequent diatom species were *Gomphonema parvulum* and *Eunotia minor*. Taxa found in 80% or more of all samples were *Eunotia minor* and *Gomphonema parvulum*, while *Gomphonema zellense*, *Nitzschia palea*, *Nitzschia frustulum* exceeded 60%. In particular, were identified 9 species with an abundance higher than 3% respect to the total number of individuals (Table 1). Overall, the highest number of taxa (29) was documented at site PP19, and the lowest number (4) at site TP50. The highest number of taxa for TP was found at site TP1 (23), and the lowest number at site TP73 (9); The highest number of taxa for PP was found at site PP19 (29) and the lowest number at site PP50 (4) and PP129 (5); The highest number of taxa for WP was found at site WP36 (29) and the lowest number at site WP2 (13).

One-way ANOVA test didn’t show significant differences between

Table 1
Values of minimum (Min), median, and maximum (Max) of the species with the highest number of individuals (>3%) respect to the total number of individuals.

Diatom species	TP			PP			WP			TOT
	Min	Median	Max	Min	Median	Max	Min	Median	Max	
Achnanthidium eutrophilum	0	0	2	0	4	179	0	1	15	382
Eunotia bilunaris	0	0	156	0	0	141	0	7	287	850
Eunotia minor	0	46	242	0	26	142	25	70	118	1617
Eunotia pectinalis	0	0	92	0	0	73	0	4	106	362
Gomphonema parvulum	0	9	281	0	100	292	7	90	265	2759
Gomphonema zellense	0	2	17	0	8	89	0	2	34	318
Nitzschia frustulum	0	4	126	0	3	40	0	7	22	380
Nitzschia palea	0	12	359	0	10	136	0	3	12	986
Pinnularia subcapitata	0	1	42	0	0	222	0	0	4	340

TP, PP and WP means ($F = 2.915$, $p > 0.05$). Therefore, the results highlighted that colonization of diatoms was independent of pond type and hydroperiod.

From the Mantel test, “Distance from the sea” distance matrix had a significative relationship with the species Bray-Curtis dissimilarity matrix (Mantel statistic R: 0.132, $p < 0.05$). Moreover, “Altitude” distance matrix had a significative relationship with the species Bray-Curtis dissimilarity matrix (Mantel statistic R: 0.113, $p < 0.05$) (Fig. 2).

Considering significative relationships, correlations performed between “Distance from the sea” and the six most abundant species revealed a significant relationship only for *Eunotia bilunaris* (Fig. 3).

RDA analysis highlighted that our full model is statistically significant ($F = 1.61$, $p = 0.009$), and two of the four variables included in this model are significant as well (Altitude, $F = 1.78$, $p = 0.04$; Distance from the sea, $F = 2.39$, $p = 0.008$). The first canonical axis resulting from the RDA is also statistically significant ($F = 2.75$, $p = 0.047$) (Fig. 4).

The first two axes (RDA1 and RDA2) accounted for 15.93% of the variation of 25 diatom species (Fig. 2). The first axis (RDA1) was more important, explaining 9.03% variation, and was most strongly positively correlated with the variables “Altitude” and “Distance from the sea”. The second axis (RDA2) explained 6.88% of variance and was most strongly positively correlated with “Distance from the roads” and negatively correlated with “Distance from outside”.

The positioning of 25 diatom species in relation to environmental variables is shown in Fig. 4. Several diatoms including *Achnanthidium saprophilum*, *Hantzschia amphioxys, Navicula cryptocephala, Nitzschia phyllepta, Pinnularia gibba*, are positively correlated with “Altitude”. Likewise, several diatoms preferred positively correlated values of “Distance from the sea”, including *Nitzschia linearis* and *Navicula capitatoradiata*. Among Gomphonemaceae and Nitzschiaceae, *Gomphonema minutum*, *Gomphonema pumilum*, *Nitzschia frustulum*, *Nitzschia palea*, preferred negatively correlated values of “Distance from the outside”.

The highest values of diversity (Shannon-Wiener diversity index) and evenness (Pielou’s evenness index) were determined at sites PP19 and TP30 and the lowest values at site TP73; The highest value for species richness and Simpson index was determined at site PP19, while the lowest value for species richness was determined at site PP50 and the lowest value for Simpson index at site TP51 (Fig. 5; Table S2).

Multivariate multiple linear model didn’t show correlations between the four indices considered and altitude, distance from the sea, distance from the outside and distance from internal roads as independent variables (Number of species, $F = 2.5$, $p > 0.05$; Shannon, $F = 1.7$, $p > 0.05$; Simpson, $F = 1.22$, $p > 0.05$; Evenness, $F = 1.03$, $p > 0.05$).

Β-diversity analysis, i.e. the comparison of dissimilarity in community composition between each pair of sites, calculated using Bray-Curtis dissimilarity and Jaccard dissimilarity, are presented in Table S3. The contribution of each species to Bray-Curtis dissimilarity between sites in the Castelporziano Presidential Estate was shown in Fig. 6.

Cluster dendrogram and principal coordinates analysis for both Bray-Curtis and Jaccard dissimilarity matrices are presented in Fig. 7.

From co-occurrence analysis, a general accordance between the V-ratio results and the C-score values emerged: nonrandom community

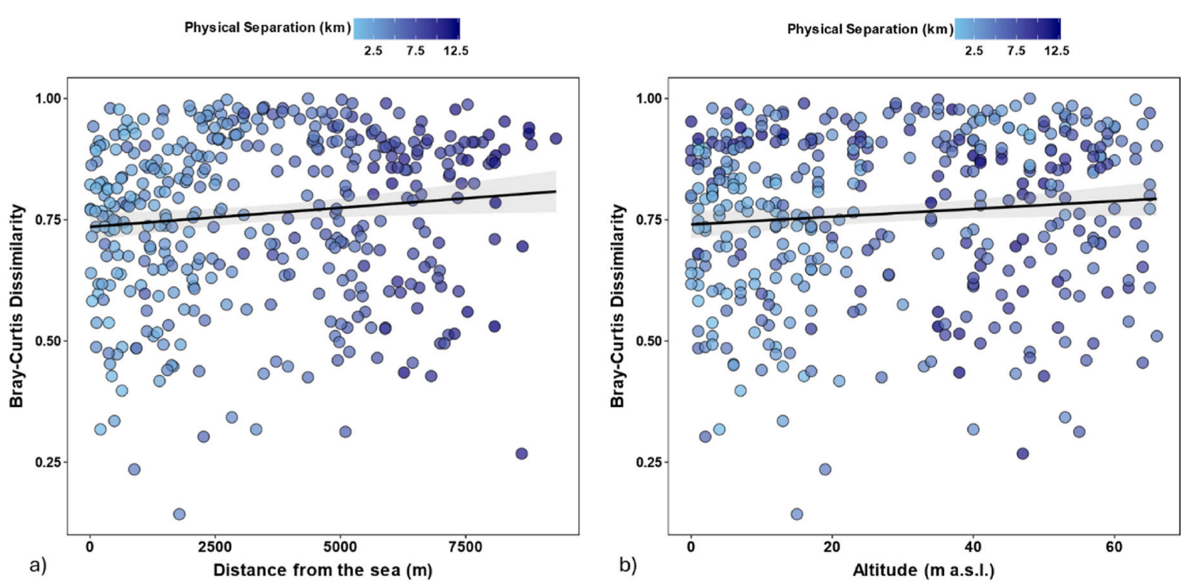


Fig. 2. a) Linear bivariate model for the distance matrix “Distance from the sea” and Bray-Curtis dissimilarity matrix; b) Linear bivariate model for the distance matrix “Altitude” and Bray-Curtis dissimilarity matrix. Physical geographical separation of the sampling sites was considered. In contrast, the species Bray-Curtis dissimilarity matrix did not have a significant relationship with the geographic separation of the samples (Mantel statistic R: 0.08, $p > 0.05$), with the distance from the outside (Mantel statistic R: −0.031, $p > 0.05$) and with the distance from internal roads (Mantel statistic R: 0.43, $p > 0.05$).

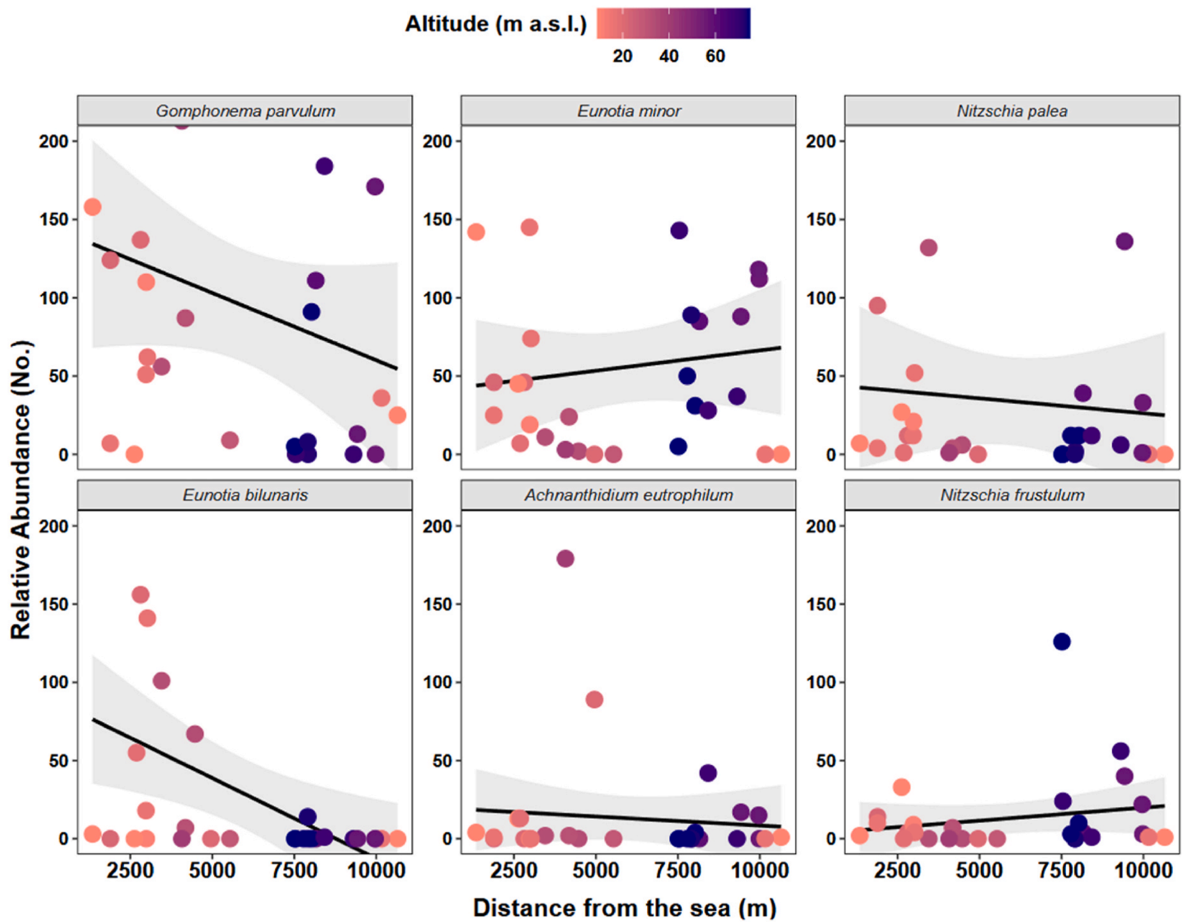


Fig. 3. Correlation between "distance from the sea" vs "abundance" for the six most abundant species, with "altitude" as supplementary variable.

Fig. 4. Redundancy analysis (RDA) ordination showing environmental variables correlated with RDA1 and RDA2. Sampling sites are shown in bold. Diatom species are shown with abbreviations (AE = *Achnathidium eutrophilum*; AS = *Achnanthidium saprophilum*; AX = *Achnanthidium exiguum*; EM = *Eolimna minima*; EP = *Eunotia pectinalis*; GI = *Gomphonema italicum*; GM = *Gomphonema minutum*; GP = *Gomphonema pumilum*; GZ = *Gomphonema zellense*; HA = *Hantzschia abundans*; HX = *Hantzschia amphioxys*; HV = *Halamphora veneta*; NC = *Navicula cryptocephala*; NF = *Nitzschia frustulum*; NL = *Nitzschia linearis*; NP = *Nitzschia palea*; NH = *Nitzschia phyllepta*; NT = *Navicula capitatoradiata*; PB = *Pinnularia brebissonii*; PG = *Pinnularia gibba*; PS = *Pinnularia subcapitata*; PV = *Pinnularia viridiformis*; SA = *Stauroneis anceps*; SB = *Surirella brightwellii*).

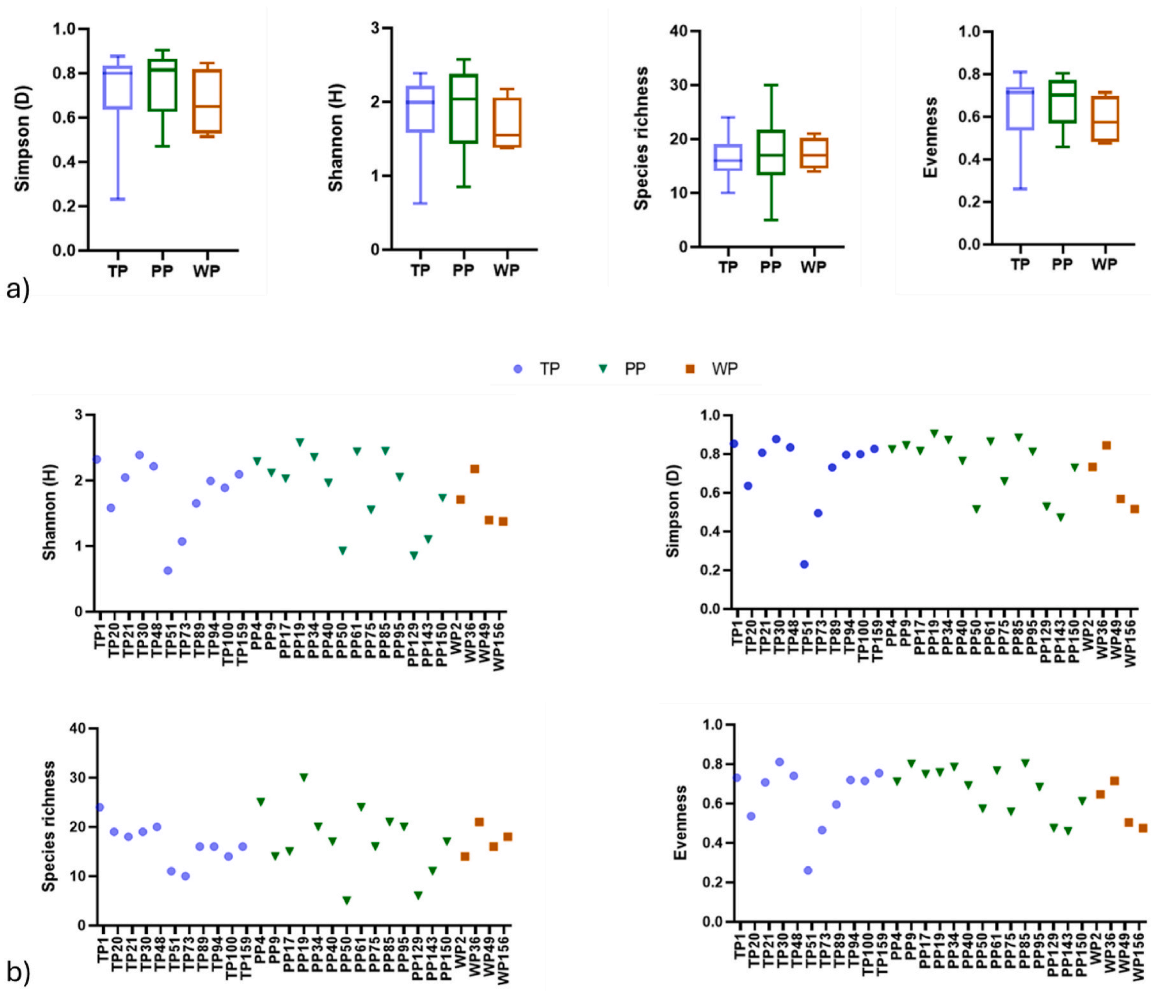


Fig. 5. a) boxplot showing the distribution of the four diversity indexes values considered; b) Values calculated for the indexes: Shannon (H), Simpson (D), Species richness (Number of species) and Pielou's evenness, considering all the sites sampled (PP = Permanent Ponds; TP = Temporary ponds; WP = Wetland Ponds).

structures were described both by the *ff* and *fe* models. The C-score and V-ratio values are reported in Table 2. Considering the overall dataset, in the *ff* model the C-score highlighted a competitive structured community. This competitive structure was highlighted also by the C-score using the *fe* algorithm, where C-score values resulted higher than expected by chance for TP and WP. Moreover, the V-ratio tests excluding empty sites, evinced a non-random structured community based on competition.

27 among the 122 different species identified belong to the "High profile" guild, 19 to the "Low profile" guild and 76 to the "Motile" guild (Fig. 8). Multivariate multiple linear model didn't show any correlation between the three guilds considered (intended as number of taxa belonging to a guild) and "altitude", "distance from the sea", "distance from the outside" and "distance from internal roads" as independent variables ($p < 0.05$).

Fig. 9 reports the values of the EPI-D global index, calculated for each sampling sites. EPI-D gradually increases following distance from the outside and the water quality passes from class IV (Bad) to class I (Excellent). However, the data never indicate very bad quality, not even in the ponds nearest to the boundaries of the Reserve.

4. Discussions

This study represents the first research focused on the analysis of diatom communities in three different waterbodies (temporary ponds, permanent ponds, wetlands) inside a protected Mediterranean ecosystem. To the best of our knowledge, this is the first and most complete study about diatom communities in different pond types inside the Castelporziano Presidential Estate. The Reserve provides protected habitats to investigate diatom communities, away from direct anthropogenic pollution and disturbances. Here we demonstrated that Castelporziano represents a biodiversity hotspot, and a natural "island" inserted in an urban context. Furthermore, we have demonstrated that diatoms can be considered excellent bioindicators of both temporary and permanent waters. The results of the present study highlight three aspects until today poorly treated: 1) the community dynamics of diatoms in three different types of small waterbodies; 2) the influence of environmental spatial and altitudinal variables on diatom communities; 3) the co-occurrence patterns of freshwater diatoms.

Following Bazzanti (2015), both temporary and permanent ponds should be included into the research to assess the conservation value of groups of small water bodies in a region. Here, diatom colonization was observed in all the sampling sites considered. The most frequent diatom species were characteristic of waters with a medium-high trophic level, populating well oxygenated waters (ISPRA, 2014c). These results are consistent with the general ecological condition of small waterbodies inside the Reserve, characterized by large eutrophication phenomena (Tinelli et al., 2012). Moreover, some of the diatom species found are also included in the Red List of endangered species from Lange-Bertalot (1996) (in brackets the red list category and the number of individuals found): *Encyonema caespitosum* (Rare, 6); *Eunotia flexuosa* (Endangered, 145); *Eunotia minor* (Vulnerable, 1617); *Eunotia pectinalis* (Endangered,

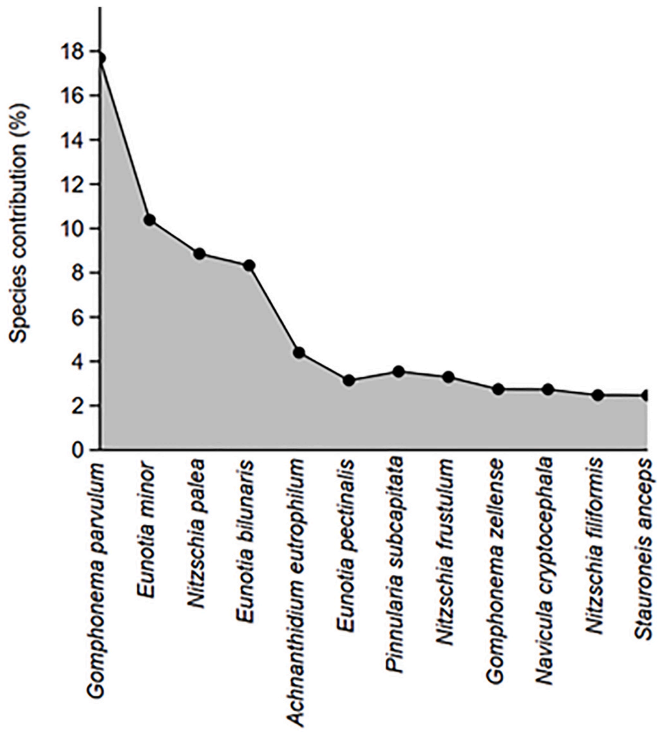


Fig. 6. Contribution of each species to Bray-Curtis dissimilarity between sites in the Castelporziano Presidential Estate.

362); *Fragilaria biceps* (Danger of unknown magnitude, 1); *Frustulia crassinervia* (Vulnerable, 2); *Gomphonema zellense* (Danger of unknown magnitude, 318); *Kobayasia okadae* (Seriously endangered, 7); *Kobayasiella subtillissima* (Starkly endangered, 2); *Navicula longicephala* (Extremely rare, 11); *Pinnularia divergens* (Vulnerable, 5); *Pinnularia marchica* (Danger of unknown magnitude, 1); *Pinnularia pseudogibba* (Danger of unknown magnitude, 3); *Stauroneis anceps* (Vulnerable, 253); *Stauroneis gracilior* (Danger of unknown magnitude, 9); *Stauroneis phoenicenteron* (Vulnerable, 39); *Surirella tenera* (Vulnerable, 4). There is nowadays agreement that Red Lists for microalgae are fundamental for conservation activities: they should be used primarily to guide protection of their habitats, and only secondarily to achieve protection of some taxa (Cantonati et al., 2022a,b). Our study revealed that ponds and wetlands show a higher percentages of both endangered Red List species and species very rarely found, also if referring to the German region (Lange-Bertalot, 1996). However, these occurrences are significantly smaller compared to other taxa. Anyway, our findings support the suitability of ponds and wetlands from Castelporziano Presidential Estate as habitats for threatened diatom species. Conservation activities are fundamental to preserve these habitats and their unique and threatened diatom species. Fortunately, over the last decade, many projects and programs like The European Pond Conservation Network have made important progress to promote awareness, understanding and conservation of ponds in Europe (EPCN, 2008). Protection passes through conservation actions aimed at communicating the values of these ecosystems: improving policy and legislation, increasing research and monitoring plans and promoting communication, protection and enhancement of the resource can be of inestimable value for the conservation of these ecosystems (EPCN, 2008).

Our results highlight that diatom species are not influenced by coastal ponds hydroperiod. This is in accordance with Blanco et al. (2020), where it was highlighted that floristic uniqueness does not change with hydroperiod. These results represent the first evidence in literature of the scarce influence of hydroperiod length on diatom communities (Celewicz and Gołdyn, 2021). Our hypothesis is that, given the last description of ponds dating back to about 30 years ago (Tinelli et al., 2012), most of the permanent ponds transformed into temporary ponds, maybe due to environmental factors like longer and hotter summer seasons (Bonaldo et al., 2023) and lowering of the groundwater (Tinelli et al., 2012). However, further studies are necessary to investigate any changes in ponds ecology and environments since the last census. The other geographical variables considered didn't show to influence diatom communities. Anthropogenic pollution, expressed here as "Distance from the outside", was reported to be an important factor influencing freshwater biodiversity and community assemblages (Prakash and Verma, 2022). On the contrary, we hypothesize that in the Castelporziano Estate ponds are isolated from anthropogenic impacts and diatom communities result less impacted. Moreover, although the presence of internal roads is noticeable, the daily car traffic inside the Reserve can be considered treasurable and does not influence diatom community structure. Finally, a relationship between diatom communities was not evinced considering the intra-pond distance inside the Reserve. This can be explained considering the historical presence of the ponds inside the reserve and the many diatom dispersion roads: these two factors coupled, could have led to a stabilization of similar diatom communities inside the reserve, like an island. Moreover, our fust hypothesis was confirmed by the results: "Distance from the sea" and "altitude" variables appeared to influence diatom communities. In particular, the salt wedge and sea aerosol are reported to influence freshwater diatom communities (Miyazaki et al., 2020). Similarly, the altitudinal gradient, although very limited (0–80 m a.s.l.), resulted to influence diatom communities. As proposed by Teittinen et al. (2016), the relationship between altitude and diatom communities can be explained by a variety of ecological variables that covary with elevation, and independently or jointly influence diatom distributions. Here, the correlation between altitude and distance from the sea could be identified as the reason for the observed similarity between matrices. Although our research lacks information about physio-chemical variables like salinity and electrical conductivity, we can hypothesize that diatom community assemblages, in accordance with Virta et al. (2020) follow the salinity gradient.

Considering Alfa-diversity and the diversity indices considered, the lowest values for the Shannon Index were calculated for temporary ponds. However, contrary to Novais et al. (2014) and Collinson et al. (1995), no significant differences for taxa richness (S) between temporary and permanent ponds emerged. Ecologically, communities inhabiting permanent and temporary ponds show different taxonomic and functional diversity (Coccia et al., 2024). However, as described above, we hypothesize that ponds which were described as "permanent" 30 years ago, can be considered as "temporary" nowadays. In fact, during the last surveys in summer 2023, most of the permanent ponds sampled were completely dry. As described by Taurozzi and Scalici (2024), pond's hydroperiod should be monitored for at least 15 years to be characterized as permanent or temporary: however, our findings and observations could support the hypothesis that all the ponds inside the Reserve are shifting to temporary hydroperiods. Given the lack of studies about diatom communities inhabiting temporary and permanent ponds and the novelty of our work, it could be difficult to compare our results with another research. For this reason, this research could be considered a starting point and then we encourage to perform further studies comparing diatom communities from permanent and temporary ponds. The interaction between diatom richness and the qualitative variable (temporary/permanent) was proposed by Tornés et al. (2021), where durations of 50–100 days were estimated as ideal period for showing higher alpha diversity, richness, and evenness, while non-flow periods caused their decrease. This is in accordance with our results and the sampling period: January–March represents a stable season after summer droughts where diatoms can recover and proliferate, leading to community stabilization. Significant differences in diatom community richness between temporary and permanent ponds can be observed in the first stages after drought periods. Following Teittinen et al., 2016,

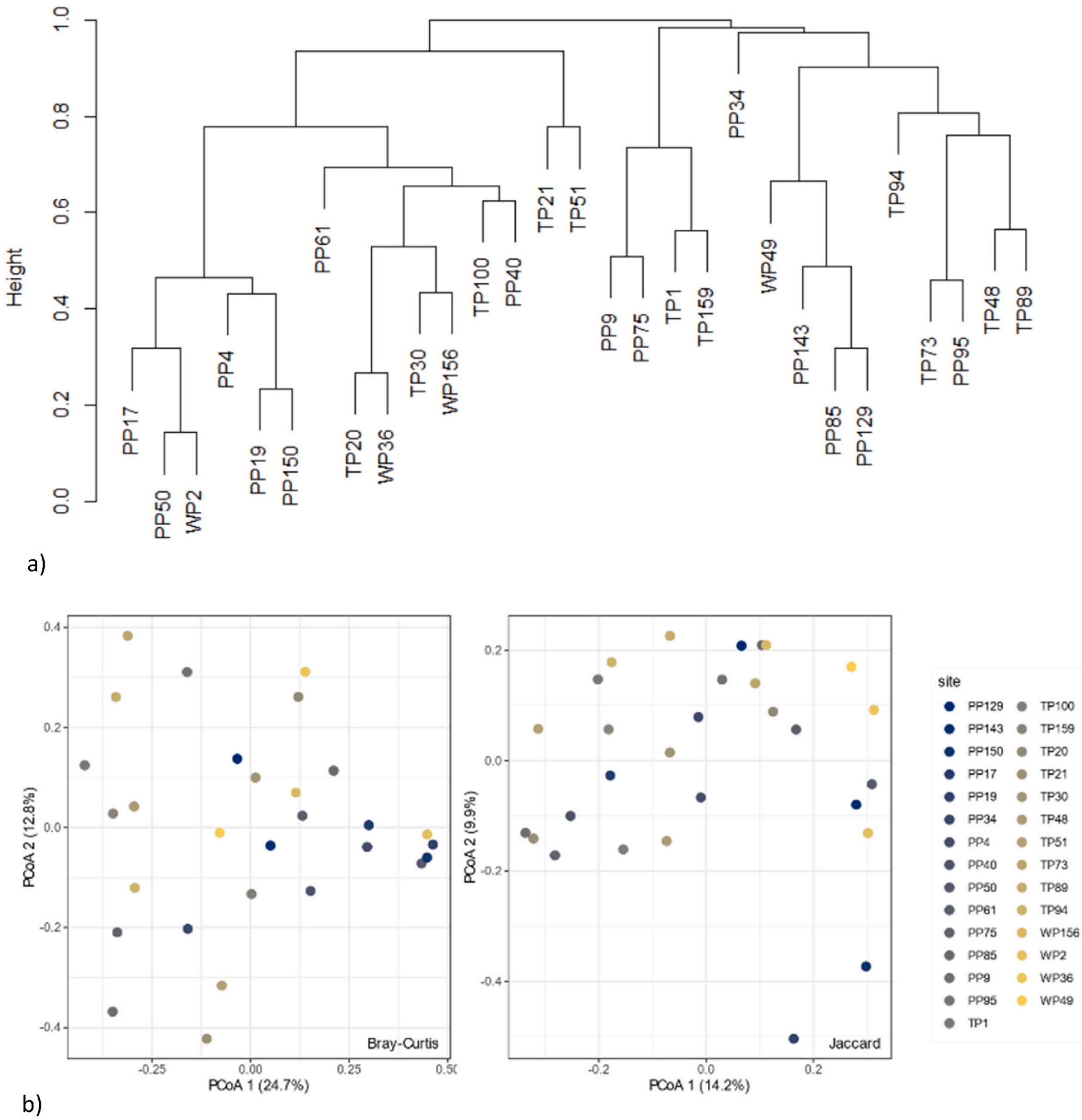


Fig. 7. a) Cluster dendrogram of the sites considered; b) Principal coordinates analysis for both Bray-Curtis and Jaccard dissimilarity matrices.

Table 2
C-score and V-ratio values and relative p-values calculated for the four different microhabitats (*ff* = fixed-fixed algorithm; *fe* = fixed-equiprobable algorithm; TP = Temporary Pond; PP = Permanent Pond; WP = Wetland Pond; p = p-value; obs = observed; exp. = expected).

		TP	PP	WP
ff	C-score p (obs > exp)	2.31229 <0.01	115.62640 <0.01	0.85947 <0.05
fe	C-score p (obs < exp)	2.31229 0.937	115.62640 <0.01	0.85947 0.9968
fe	V-ratio p (obs > exp)	1.57394 0.10	3.51565 <0.01	0.81679 0.4986

Table 3
Tukey's post hoc test values: Tukey's Q below the diagonal, p (same) above the diagonal.

	H	L	M
H	–	3.105E-06	1.167E-06
L	7.451	–	1.582E-10
M	7.787	15.24	–

diatom species richness showed no apparent trends in relation to elevation. This is in accordance with our results. However, it is possible that the small altitudinal gradient was not enough to highlight significative correlations. Considering evenness and Simpson index, the highest values emerged for permanent ponds and wetlands, while temporary ponds showed the lowest values in some cases. This is in accordance with Tornés et al. (2021) and Carneiro et al. (2024). Temporary ponds, with a prolonged dry phase, showed a dominance of some species over others. The dominance was evinced for pioneer species, which represent the first colonizers after dry periods. Our results are in accordance with Taurozzi et al. (2024), where more than 300 days were demonstrated to be necessary for reaching diatom community stability. Temporary ponds in the Castelporziano Estate can be impacted from droughts also for several months (Tinelli et al., 2012) and during the wet period (winter season), there may not be enough time for the community to fully regenerate. Anyway, Alfa-diversity in all the ponds type

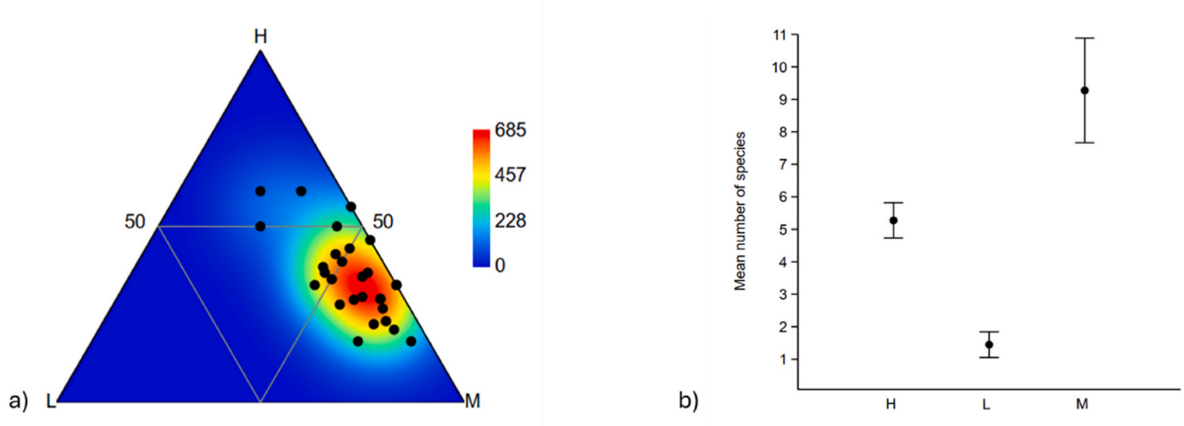


Fig. 8. a) Density plot for distribution of the species (number of occurrences) in the three guilds considered; b) Average mean values for the number of species belonging to the three different guilds (H = High profile; L = Low profile; M = Motile). One-way ANOVA test highlighted a significant difference between sample means (F = 58.06, $p < 0.05$). Tukey's pairwise highlighted strong differences between M and L (Table 3). The chi-square test applied to the number of taxa for each guild and habitat type showed significant results ($\chi^2 = 27.654$, $p < 0.05$), highlighting the dependence between this two variables. From the effect size calculated, a low dependence between the two variables (Cramer's V = 0.188) emerged.

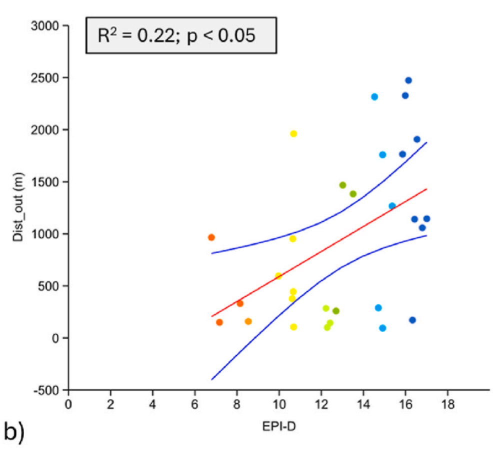
Average values: PP 11,71 TP 13,76 WP 15,08

WP2	WP36	WP49	WP156
14,71	16,55	12,28	16,8

TP1	TP20	TP21	TP30	TP48	TP51	TP73	TP89	TP94	TP100	TP159
16,33	17,01	13,51	12,22	14,91	6,79	13,02	15,86	16,14	14,91	10,69

PP4	PP9	PP17	PP19	PP34	PP40	PP50	PP61	PP75	PP85	PP95	PP129	PP143	PP150
8,54	12,7	10,65	7,17	10,69	10,67	10,62	8,15	16,43	14,53	15,99	9,97	12,42	15,37

a)



Numerical values	Classes	Quality
20 ≥ EPI-D > 15.5	I	Excellent
15.5 ≥ EPI-D > 14.5	I – II	Excellent – good
14.5 ≥ EPI-D > 12.5	II	Good
12.5 ≥ EPI-D > 11.5	II – III	Good – mediocre
11.5 ≥ EPI-D > 9.5	III	Mediocre
9.5 ≥ EPI-D > 8.5	III – IV	Mediocre – bad
8.5 ≥ EPI-D > 6.5	IV	Bad
6.5 ≥ EPI-D > 5.5	IV – V	Bad – Very bad
5.5 ≥ EPI-D > 1	V	Very bad

c)

Fig. 9. a) EPI-D values for each sampling site and average values for PP (Permanent Ponds), TP (Temporary ponds) and WP (Wetland Ponds); b) linear bivariate model for EPI-D values and distance from the outside of the Reserve (Dist_out (m)); c) EPI-D table showing the numerical values (0–20) and relative water quality classes.

appeared to be not related to environmental and geographical variables, while pond's hydroperiod seems to be the main driving force influencing diatom community structure. Considering Beta-diversity, both Bray-Curtis and Jaccard dissimilarity matrices highlighted a large dissimilarity between sampling sites. Inter-variability between sites and intra-variability within the sites was very high, as confirmed by the cluster dendrogram. These results can suggest that temporary, permanent ponds and wetlands inside the reserve don't share similar ecological features, reflecting it in a different diatom community composition. From cluster analysis, also WP result distant from each other, highlighting differences from an ecological pint of view. As described above, there is a heterogeneity in pond and wetland definitions in literature (Richardson et al., 2022). Following Søndergaard et al. (2005), ponds have closer contact with the adjacent terrestrial environment and a relatively greater littoral zone; moreover, they are potentially more isolated from other wetlands and have a more insular nature. We hypothesize that, although the geographical proximity between ponds and wetlands, each of these ecosystems should be intended as an "island" inside the Reserve, where the freshwater ecosystems and diatom communities inhabiting them are differently influenced and structured. Influence sources should be investigated in further analysis, important to identify the main ecological drivers for the observed differences.

The results of the present study highlight the average absence of co-occurrence patterns of the freshwater diatoms in ponds. Our second hypothesis was not confirmed: from the analysis emerges a structured community, based on segregation processes. The segregation patterns emerged could be explained considering both spatial and a temporal factor. Following Tinelli et al. (2012), ponds represent "a fundamental part of the Reserve ecosystem since many decades". In other words, ponds have been present inside the reserve for many years. However, considering permanent ponds and wetlands, this translates into a stabilization of the diatom community, where competition represented a fundamental driving force for the community assemblages over years; on the contrary, in temporary ponds, where seasonals drought reset diatom communities, in the first stages of re-colonization of the substrates inter-specific competition represent the driving force for community assembly. We hypothesize that environmental factors such as light seeking and position on the substrate represent important variables by which diatomic species compete. Moreover, following Taurozzi et al. (2024), the low availability of substrates and the reduced surface didn't allow the presence of different species, favoring competition and mutual exclusion. Generally, larger habitats are characterized by aggregation patterns, while smaller habitats favor competition, especially in algae (Marino et al., 2011).

The segregation patterns emerged from co-occurrence analysis could reflect the guild distribution. Most of the diatom species found belong to the "Motile" guild, followed by the "High profile" guild and the "Low profile" guild. Our results and the relative abundances of species for each guild are in accordance with the ecological conditions of the ponds as described in Tinelli et al. (2012). "Motile" guild includes the largest number of species that are tolerant of eutrophication and pollution situations (Peszek et al., 2021): the largest number of species belonging to the "Motile" guild complies with the ecological characteristics of the study area, which favors their development. Moreover, "Motile" species can move easily to search for the required ecological conditions (Heine-Fuster et al., 2021) With increasing nutrient and organic matter concentrations, increasing abundances of high and motile guild taxa and a subsequent decrease of low-profile taxa have been demonstrated (Gottschalk and Kahlert, 2012). We hypothesize that the high presence of motile species is correlated with the segregation patterns observed. Given the competitive structure described, "Motile" species can move away looking for more suitable surfaces were settled and live. Moreover, our results highlighted that diatom guilds are low dependent from habitat type (Cramer's V = 1.8): high profile and motile guilds were expected to show different frequencies among samples in TP and PP. However, contrary to our third hypothesis, the low association emerged could suggest that, inside the Reserve, TP and PP show similar ecological features that, although the differences in hydroperiod length, allow the presence of similar diatom communities from a functional point of view. Further studies are therefore needed to investigate the real influence of habitat type on diatom guilds.

Finally, the large use of diatoms in assessing water quality (Celekli et al., 2021) as described in the Water Framework Directive was also applied to our research. Applying the Eutrophication/Pollution Index-Diatom based (EPI-D) (Dell'Uomo and Torrisi, 2011), the general status of conservation of the ponds was described as "good", confirming our fourth hypothesis about the importance of Reserves to positively influence water quality. The highest value, corresponding to the "Excellent – good" class was reported for wetlands. However, inside the Reserve, the distinction between wetlands and ponds can be difficult to perform following these ecological features. Following Richardson et al. (2022), differences between these two water basins are included in ecological, morphological and physio-chemical features like surface area, maximum depth, emergent vegetation cover, pH. However, categorizing small waterbodies is particularly challenging. The observed differences in the water quality of the three types of ponds (PP, TP, WP) can be difficult to assess to a precise reason and further studies are needed to discriminate the main environmental and ecological characteristics influencing water quality. Positive correlation between anthropogenic activities and water quality is largely proved in literature. Urban development and road traffic were proved to be an important anthropogenic factor responsible for water quality degradation (Akhtar et al., 2021; Bakr et al., 2020; Song et al., 2020). In this study, the effect of anthropogenic disturbances was highlighted by differences in water quality related to distance from the Reserve boundaries; these results highlight the deleterious effects of urban development on freshwaters quality and the importance to implement conservation plans to protect these ecosystems.

5. Conclusions

Scientists, policy makers and water resource management are mainly focused on the study and management projects of lakes, wetlands and rivers. However, ponds represent hotspots for biogeochemistry and biodiversity, providing numerous ecosystem services for humans. Anthropogenic pressures like climate change, pollution (litter discharge, water contamination) and water catchment represent the main threats to ponds' conservation (Meerhoff and de los Ángeles González-Sagrario, 2022; Hill et al., 2018). However, as described in Taurozzi et al. (2023b), stranded litter could favor the settlement of diatoms, leading to an enrichment in biodiversity. Further investigations are therefore necessary and should be carried out to perform specific threat analysis, fundamental to evaluating the intensity of the human pressures on these small and threatened ecosystems (Battisti et al., 2016). In this context, the Castelporziano Presidential Estate can be considered as an "island", where biodiversity is less impacted from anthropogenic pressures. Given the lack of knowledge about the diatom fraction that colonizes vegetal substrates in Castelporziano ponds, this study is a valuable contribution to begin to fill this gap. This study represents the most complete report of diatom communities inside the Reserve, providing important information about the conservation status of ponds. Moreover, the conservation status of many ponds was investigated for the first time in a Protected area. Our study represents a starting point to investigate the evolution of diatom communities inside an inaccessible Reserve, with very low human influences. Castelporziano should be considered an ideal field laboratory, where periodical modifications in diatom community structure can be investigated and the environmental drivers easily identifiable. The checklist provided here can be used to monitor diatom communities' evolution over seasons and years; moreover, to the best of our knowledge, this is the first research investigating diatom communities' functional differences in three different ecosystems (temporary ponds, permanent ponds and wetlands), subject to different environmental influences. Overall, our results highlight that diatom communities show little shifts between different pond types and that salinity is the main factor influencing their assemblages. In summary, diatoms live in assemblages largely structured by interspecific competitive interactions. Accurate pond's diatom datasets are indispensable in generating policies on pond conservation and their appropriate land use, global climate change studies, and biodiversity conservation.

However, our research shows some limitations which make it incomplete and that could be addressed in further studies: for example, physical chemical parameters can give a complete overview of ecological drivers influencing diatom communities; seasonal sampling throughout the year could highlight diatom species turnover over time and space; recording and investigating how ponds' depth and surface area of sampling sites influence diatom communities could provide important indications to better understand some species occurrence.

This suggests that more attention should be paid to the diatom community patterns at relatively small spatial scales in water environment management practices. Therefore, further studies should be conducted to better understand diatom community dynamics, expanding the research to the other ponds of the Reserve. The main limit of this research is the absence of physical-chemical parameters available at the sampling points and many physiographic parameters. Future studies are therefore necessary to correctly define any physical-chemical parameter of ponds and to make a complete analysis. However, the results found could have ecological implications and generate a comprehensive dataset useful to monitoring spatial-temporal changes and anthropogenic impacts on the ponds inside the Reserve. Finally, it can provide valuable insights into the effects of natural and anthropogenic threats on ponds conservation and management activities. Finding conservation solutions can be very challenging for environmental managers (Battisti, 2018). Fencing the ponds could be a solution to maintain their ecology and avoid damage caused by ungulates such as wild boars (Carpio et al., 2014). At the same time, given the limited water availability within the estate, once the ponds are fenced, the water regime of the artificial springs should be implemented. Long-term monitoring of hydroperiod and diatom communities should be performed regularly, given the important ecological responses provided by these types of data (Cantonati et al., 2022a,b; Oertli et al., 2010). Reducing vehicle traffic inside the reserve could preserve ponds' biological uniqueness and reduce anthropogenic pollution. Considering the protected nature of the Reserve and the surrounding population density, management plans could involve citizens, improving their knowledge about these ecosystems, through guided tours (Krasny et al., 2014). Divergent/convergent thinking activities, communication among expert and non-expert members and multi-criteria decision making are considered fundamental tools to draw up management plans (Battisti, 2018). Promotion of awareness, understanding and conservation of ponds through free workshops could create a better link between scientists and practitioners (Oertli et al., 2010), with the shared aim of ponds protection.

CRediT authorship contribution statement

Davide Taurozzi: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Massimiliano Scalici:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition.

Consent to participate

Not applicable.

Ethics approval

Not applicable.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csr.2024.105359.

Data availability

Data will be made available on request.

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