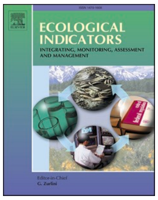
Ecological Indicators 183 (2026) 114655

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind



Flying above fragility: Remote sensing and field samplings unveil microcrustacean patterns in ephemeral ponds

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ARTICLE INFO

Keywords:
Drone census
Ephemeral habitats
Crustaceans
Diversity index
Pond size

ABSTRACT

Temporary ponds (TPs) are ephemeral freshwater habitats that undergo seasonal drying, creating harsh and highly dynamic environments. Microcrustaceans are key biological components of TPs since they play a crucial role in ecosystem dynamics. The main objective of this study is to test an innovative approach that combines field sampling with modern remote sensing technologies to: (i) investigate the temporal variation of microcrustacean communities and the influence of hydroperiod length and pond area in three coastal temporary ponds (TpA, TpB, TpC) and (ii) assess the hydroperiod length using unmanned aerial vehicles (UAVs). Overall, eight microcrustacean families were identified. In TpA, five families were recorded, whereas six families were documented in both TpB and TpC exhibiting diverse feeding strategies. Our observations suggest that the presence and relative abundance of taxa in the ponds significantly changed over time. We observed statistically significant similarities in TpB and TpC communities, with a Jaccard similarity coefficient of 0.833 in January, whereas the comparison between these communities and TpA did not show the same level of similarity. We also found a positive correlation between pond size and Shannon diversity index (Spearman: rho = 0.607, $p < 0.01$), indicating that an increase in pond area corresponded to greater microcrustacean diversity. Furthermore, our analyses revealed that temporal variability plays a more prominent role than spatial heterogeneity (transect and subtransect) in explaining the observed biodiversity patterns. Our findings highlight the effectiveness of analyzing temporary environments through a multi-methodological approach that can be replicated over time and internationally adopted.

1. Introduction

Temporary ponds (TPs) represent one of the most endangered habitats in the Mediterranean region, (Dražina et al., 2022). Despite variations in their definitions, these biotopes share common characteristics: they are typically small (<5 ha) and shallow (5 m) water bodies, predominantly freshwater but occasionally brackish, that retain water for at least three months annually (Céréghino et al., 2008; Richardson et al., 2022). They generally occur in endorheic depressions with restricted hydroperiods (the period when the pond is filled with water) that allow the settlement of a characteristic aquatic or semi-aquatic flora and fauna (Taurozzi et al., 2024b). These unique obligatory species are adapted to thriving under time stress and extreme environmental conditions (Zacharias et al., 2007). Indeed, the hydrological regime is the main driver shaping the composition and structure of aquatic communities in these ecosystems (Pérez-Bilbao and Garrido, 2015).

Among the key biological components of TPs, zooplankton plays a crucial role in the ecosystem dynamics, contributing to nutrient cycling, energy transfer and top-down regulation of phytoplankton communities (Taurozzi et al., 2024a; Effler et al., 2015; Harris et al., 2004). Due to

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https://doi.org/10.1016/j.ecolind.2026.114655
Received 5 June 2025; Received in revised form 21 November 2025; Accepted 20 January 2026

their short life cycles and sensitivity to environmental changes (such as hydroperiod alteration and increasing temperatures), zooplankton communities serve as effective indicators of water quality and ecosystem health (Coccia et al., 2024; Zokan and Drake, 2015). Specifically, the composition of species and their variations in functional traits have been analyzed in many studies to obtain information on the water quality status (Sodré and Bozelli, 2019). In freshwater environments, zooplankton is primarily composed of rotifers, cladocerans and copepods, with significant diversity observed across these groups (Gavrilko et al., 2024; Umi et al., 2024). The latter two belong to the microcrustacean group, which exhibits a wide range of adaptations to astatic ponds (Seminara et al., 2008). Indeed, they are capable of surviving both predictable and unpredictable fluctuations in hydroperiods, adopting diverse strategies to withstand desiccation and re-establish populations upon waterbody replenishment. These adaptations include the production of resting eggs through mechanisms of diapause, as well as dispersion and colonization during favorable flooding periods (Brock et al., 2003; Havel et al., 2000).

The length of the hydroperiod shapes the community structure of microcrustaceans and other invertebrates that depend on dormant stages for survival (Williams, 2006). Climatic warming, leading to earlier drying events, can drastically alter rainfall and drought patterns, resulting in profound seasonal shifts in species abundance and decreases in species diversity (Celewicz and Gołdyn, 2021; Erwin, 2009). Consequently, TPs have been suffering from severe droughts worldwide in the recent centuries, in addition to severe alterations in their hydrological and salinity regimes due to global climate change: they have been also classified as endangered across the globe (Dimitriou et al., 2006).

Traditional field surveys provide essential biological insights, but they are often time-consuming, costly and spatially limited (Rocchini et al., 2019). The European Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC) have underscored the need for faster, cost-effective and reliable assessment methodologies to evaluate the status of aquatic environments (Heiskanen et al., 2016). To enhance the effectiveness of monitoring small-scale ephemeral ecological systems, recent studies have tested a more automated and precise approach involving the application of unmanned aerial vehicles (UAVs) in Mediterranean regions (Campanale et al., 2023; Scalici et al., 2021). Drones allow for the observation of natural phenomena at a very fine spatio-temporal scale while significantly reducing costs compared to traditional methods (López and Mulero-Pázmány, 2019). However, so far, only one article has focused on the importance of monitoring the hydroperiod of TPs using drones (Scalici et al., 2021).

In the Lazio region, previous studies have documented rich and dynamic zooplankton communities in TPs, although most research has been conducted within the Natural Reserve of Castelporziano (Seminara et al., 2008; Vagaggini et al., 2002). This highlights the importance of broadening the dataset in order to cover a more diverse range of environmental conditions, with particular attention to coastal ecosystems, which represent some of the environments most affected by global climate change (Lincoln et al., 2022).

A proper evaluation of these wetlands requires a deep understanding of their ecosystem's organization, structure and functioning, as well as the composition of their aquatic communities. The zooplankton community plays a crucial role in monitoring studies, as it enables the quick detection of ecosystem changes by facilitating the development of management strategies for these biotopes (Pérez-Bilbao et al., 2015). Recent research continues to highlight the significance of functional traits in understanding ecological interactions and community structure, emphasizing their influence on nutrient cycles and food web dynamics (de Lima et al., 2022). From this perspective, adopting a functional approach can enhance our knowledge of how environmental factors impact natural communities. Functional diversity focuses on the functional traits of species, which are key ecological characteristics (Violle et al., 2007; Hooper et al., 2005), to establish a direct connection between community structure (Obertegger and Flaim, 2018; Petchey et al., 2007) and ecosystem functioning (Flynn et al., 2011; Díaz and Cabido, 2001).

This study aims to increase the knowledge of these small freshwater biotopes at a regional level, since they are a particularly endangered component of biodiversity in the Mediterranean area. In particular, the main objective of this study is to test an innovative approach that combines the efforts of field operators with the latest remote sensing technologies.

The key objectives of this study can be summarized in three main points:

(i) To analyze, through an ecological-functional approach, the temporal variation of microcrustacean communities in three coastal temporary ponds.
(ii) To assess the hydroperiod length using unmanned aerial vehicles (UAVs) based remote-sensing techniques.
(iii) To investigate the influence of hydroperiod length and pond area on microcrustacean communities.

2. Materials and methods

2.1. Study area

Three anthropogenic TPs were selected from two study areas at the opposite extremities of the Lazio region (central Italy), based on the georeferenced TPs from Scalici et al. (2021). The first was located in a private land in Montalto Marina (VT), while the second and third ponds were in Borgo Isonzo (LT) (Fig. 1). All TPs were located in areas subject to agricultural land use and are characterized by mostly clay substrates. Furthermore, both areas are subject to local-specific human-induced environmental stressors.

The Montalto Marina pond (TpA) was located in the province of Viterbo (42°17′54”N, 11°37′43″E), near the Tyrrhenian coast. It covered approximately 3 ha and was surrounded by phytocoenosis dominated by the common reed (*Phragmites australis*). The pond was less than 200 m from the coast; therefore, potential seawater influence was considered in the discussion. However, no direct salinity measurements were performed due to financial constraints and lack of suitable instrumentation. Furthermore, this pond acts as an “attractor” for the local avifauna (anatids, herons and waders) and is surrounded by hunting blinds. It is regularly frequented by hunters throughout the hunting season, as evidenced by the presence of numerous spent cartridges, that become more evident during the drying season.

The Borgo Isonzo ponds (TpB and TpC), in the province of Latina (41°26′50”N, 12°54′06″E and 41°26′47”N, 12°54′14″E), were 74 km from Rome and covered approximately 1 ha each. Located in a steno-Mediterranean environment, they were affected by slash-and-burn agriculture, a practice used to enrich the soil with organic matter and eliminate parasites.

When water was present, these TPs hosted species typical of ephemeral flooded habitats, including vascular plants, amphibians, many microorganisms and macroinvertebrates (Florencio et al., 2014). However, during summer evaporation, they became dominated by species from the surrounding agricultural landscape (i.e., the terrestrial matrix), making it difficult to assign the recorded taxa to one habitat or the other. Furthermore, as in TpA, human impact can be assessed by the scattered waste found in situ (personal observation).

2.2. Sampling activities

Samplings were conducted approximately every three weeks from October 2020 until the ponds dried up, corresponding to the last sampling dates in May for TpA, April for TpB and March for TpC. Consequently, no samples were collected after this period, as the ponds were completely dry. This drying-out during the summer months is a known

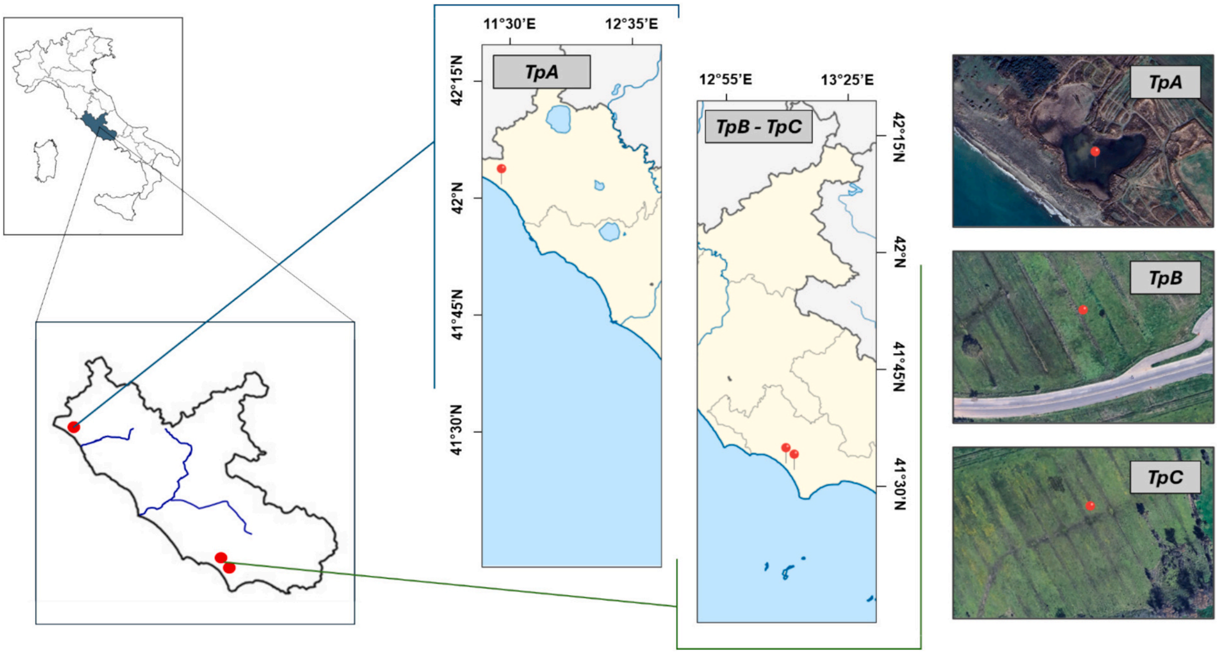

Fig. 1. Location of sampling sites in the Latium region, Italy. The inset map (top left) shows the location of Latium within Italy. The central panels display the geographical distribution of the three sampling sites (TpA, TpB and TpC) within Latium, with red dots indicating their precise locations. Detailed aerial views of each sampling site are shown in the right panels, highlighting the specific characteristics of each sampling site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

characteristic of Mediterranean-climate temporary ponds, which typically remain inundated during autumn, winter and spring and then enter a dry phase in late spring/summer (Olmo et al., 2022; Zacharias et al., 2007). During the entire sampling campaign, a total of 100 water samples were collected. For data analysis, samplings were coded as follows: S1 = 20/10/2020; S2 = 28/11/2020; S3 = 11/12/2020; S4 = 08/01/2021; S5 = 29/01/2021; S6 = 19/02/2021; S7 = 12/03/2021; S8 = 02/04/2021; S9 = 24/04/2021; S10 = 14/05/2021.

2.2.1. *Pond sampling*

In TpA, three transects (A, B, C) and nine sub-transects (A1-A2-A3, B1-B2-B3, C1-C2-C3) were established to facilitate the collection and subsequent analysis of water samples (Fig. 2). This subdivision allowed for a more detailed characterization of microcrustacean communities:

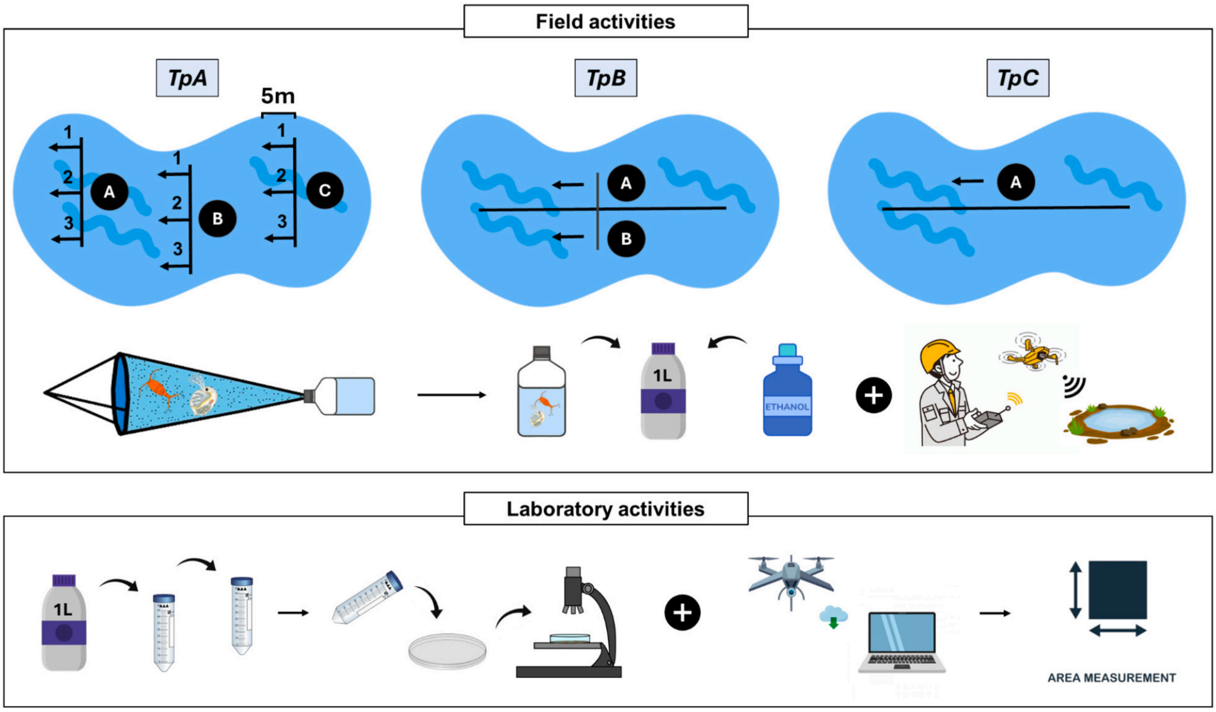

Fig. 2. Schematic representation of field (top) and laboratory (bottom) activities. The field activities include plankton sampling with a net, sample preservation and drone-based environmental imaging. The laboratory activities encompass sample processing, microscopic analysis and digital image analysis for area measurements.

water collection was performed from different microhabitats, considering the pond's zonation from one side to the other and its distance from the sea. At each marked point, the operator moved five meters toward the sea (covering a total of 45 m), oscillating an Apstein net to capture microcrustaceans. The net consisted of a stainless-steel upper ring (13 × 38 cm diameter), a conical mesh (100 cm in length) with a standard zooplankton mesh size (80 μm) and a PVC collecting cup. The contents of the collecting cup were then transferred into a 1000 ml rectangular wide-neck polyethylene (PE) bottle and an equal volume (1:1 ratio) of 96° ethanol was added to preserve the collected samples.

In Latina, two temporary ponds were selected: the first was a single pond (TpB), while the second (=TpC) showed a more dynamic morphology, with multiple small and interconnected shallow ponds. In the first area, in order to adopt the same sampling design by confronting the same areas, each transect was divided into two sub-transects (20 m each, marked by stakes placed in situ) to cover the same distance previously sampled in TpA (45 m) and to standardize the sampling methods as much as possible across the areas of all the three ponds. In contrast, in the second area, the smaller size of the habitat led to the establishment of a single transect of approximately 40 m, also marked by stakes placed in situ (= TpC). Sampling followed the same procedure as in TpA, with the net oscillated both vertically and horizontally, ensuring comparable data collection across sites (except for the single distance of the sub-transects, 5 m in TpA and 20 m in TpB).

2.2.2. Drone survey, photogrammetric processing and accuracy assessment

Throughout the entire sampling campaign, aerial photographs were acquired using the DJI Mavic Pro drone to characterize the terrestrial matrix surrounding the selected habitats and to detect variations in their surface area during the hydroperiod.

Flight plans were designed and executed with DroneDeploy software (v. 2.145.0), which was used to define an autonomous flight path over each study site (Fig. S1). A planned flight altitude of 40 m above ground level (AGL) was used; however, the actual flight altitudes averaged between 58.2 m and 61.4 m across the sites. The “Terrain Awareness” feature was enabled to ensure the drone maintained a consistent AGL over variable topography. This produced a consistent Ground Sampling Distance (GSD) across all sites, averaging 1.52 cm/pixel. The flight plans were configured with a frontal overlap of 75% and a side overlap of 70% and the camera was set at a 90° angle, capturing images in JPEG format.

The collected images were processed in Agisoft Metashape Professional (v. 2.0.3), a specialized software for photogrammetric processing of digital images (Ahmed et al., 2021; Ahmed and Mahmood, 2022). The DJI Mavic Pro's camera has a 1/2.3-in. CMOS sensor, capturing 12.35-megapixel images (4000 × 3000 pixels) with a focal length of 4.386 mm and a pixel size of 1.58 μm. Image alignment was run at the ‘Highest’ accuracy setting, and the camera model was self-calibrated during this step. Following alignment, a dense point cloud was generated using the ‘Ultra High' quality setting with ‘Mild’ depth filtering. Finally, a high-resolution orthomosaic for each site was generated using the ‘Mosaic’ blending mode with the corresponding DEM as the projection surface and hole filling enabled to create a seamless final map.

The final orthomosaics were used to measure changes in pond surface area over the hydroperiod. The measurements were derived using Agisoft Metashape's “Draw Polygon” tool to manually delineate the pond boundaries. To validate the reliability of this approach, a two-part accuracy assessment was performed. First, the geometric quality of the underlying photogrammetric models was confirmed. As the models were georeferenced using the drone's onboard GPS without Ground Control Points (GCPs), the final absolute georeferencing accuracy had a total error ranging from 1.49 m to 1.81 m. However, the internal consistency of the models was very high, with final RMS reprojection errors ranging from 0.423 to 0.879 pixels, confirming that the orthomosaics are geometrically sound and free of significant internal distortion.

Second, to quantify possible subjectivity in manual delineation, a recognized source of uncertainty in remote sensing analysis (Congalton and Green, 2019), we simulated a repeated measurement analysis in which the same operator re-delineated ten representative polygons on different days. The mean difference between delineations was 0.87% ± 0.23% (SD), corresponding to a mean area uncertainty of approximately 0.35 m^2 for 400 m^2 features. This variation is consistent with the expected pixel-level positional uncertainty (≈1.5 cm px^{-1}) and therefore negligible for the scale of our analysis. Together, these assessments confirm that the area measurements derived from the orthomosaics are precise and reproducible.

2.3. Laboratory activities

In the laboratory, the collected samples were analyzed under a stereomicroscope (Nikon C-LEDS with 4.0× objective). A simplified sorting method was first applied, with morphologically similar individuals extracted by pipette and grouped in the same Falcon tube. This approach facilitated and enhanced the efficiency of identification by grouping species with a certain degree of taxonomic affinity. To estimate taxon abundance, the contents of the Falcon tubes were transferred to Petri dishes, enabling both qualitative and quantitative characterization of the zooplankton community. Individuals were counted directly and when the number of individuals was high, subsampling was conducted by using a Sedgwick-Rafter counting chamber. Before every subsampling, each water sample was thoroughly mixed to ensure homogeneity and for each sample, 1 mL were taken and the total abundance estimated proportionally to the original volume. Specifically, 1 mL was extracted from the Falcon tube, and the total abundance was estimated by calculating the proportion between the counted individuals in the subsample and the total volume. Additionally, when taxa were present in very high numbers, the software ImageJ was used to facilitate counting. Indeed, the manual counting plugin enabled both the quantification of individuals and the recording of the different taxa observed. Microcrustaceans were identified to the family level using standard taxonomic keys and reference guides for freshwater organisms (e.g., Ghetti and McKenzie, 1981; Stella., 1982; Cottarelli and Mura, 1983; Margaritora, 1983), as well as the online checklist of Italian fauna, which also provides large-scale species distribution data (Ministry of Environment, Directorate General for Nature and Sea Protection PNM (https://www.faunaitalia.it/checklist/). This taxonomic resolution was considered sufficient to address the study objectives and to capture the main ecological patterns. Feeding strategies of the microcrustacean taxa were assigned based on these reference guides, which provide ecological and functional trait information for each family.

2.4. Statistical analyses

The statistical analysis aimed at assessing the: (i) temporal variation in the taxonomic diversity of microcrustacean communities; (ii) similarity between the three study areas; (iii) correlation between the surface areas of all three temporary ponds and the diversity of the microcrustacean communities and (iv) influence of spatial and temporal factors on diversity.

The identification of taxa to the lowest taxonomic level and the classification of their trophic niches allowed us to analyze, through a qualitative-quantitative approach, the temporal variation in ecological and taxonomic diversity of microcrustacean communities collected in each pond throughout the hydroperiod. In particular, Kruskal-Wallis tests were performed to determine whether significant differences existed for the same families across different samplings. To account for multiple testing, results were checked with a post-hoc pairwise comparison with Bonferroni correction.

Subsequently, the similarity between the microcrustacean communities in the three ponds were quantified using the Jaccard similarity coefficient (Seminara et al., 2008). Jaccard index was selected as a robust linear (proportional) measure of ecological distance (Faith and Belbin, 1986) and then used to compare dissimilarity matrices. The

Jaccard similarity coefficient was calculated using *vegdist* function in vegan package and *dist* function in proxy package (Meyer et al., 2021; Oksanen et al., 2019). The ponds were compared based on the sample dates, which were the same for all three habitats.

The taxonomic α diversity of microcrustacean communities was quantified using the Shannon-Wiener index (H′), using *diversity* function in *vegan* package (Oksanen et al., 2019). This index was calculated for each pond by combining the data collected from each sub-transect on the various sampling dates. As changes in pond area over time may have directly influenced space and resources (i.e. niche) availability, we assessed if this was directly correlated with the microcrustacean community diversity observed using Spearman correlation test (as the data was not normally distributed). This analysis was carried out by aggregating the data from all three temporary ponds. The first measurement of TpA (S1 = 20/10/2020) was excluded from the analysis because it derived from a human-induced flooding event. Since it did not correspond to a natural hydrological phase of the pond, it was deemed ecologically non-representative and unsuitable for inclusion in the correlation analysis. Finally, to investigate how temporal and spatial variables influenced the community dynamics, a Generalized Linear Model (GLM) was developed to analyze the level of biodiversity in the transects and sub-transects in relation to the sampling dates. Spatial characteristics of each pond influenced the sampling design that could be carried out, which significantly limited the number of sub-sampling locations at TpB and TpC, limiting our ability to infer any spatial variability. As such this analysis was restricted to TpA. The Shannon-Wiener diversity index (H′) was re-calculated for each sub-transect on each sampling day, and to minimize deviance (model fit), the index values were transformed to an exponential scale. Several models were tested with various combinations of variables, and the best model was selected using the Akaike Information Criteria (AIC).

The GLM was developed using the ‘*stats*’ package (R Core Team, 2024), and the model was validated using the functions from the ‘*DHARMa*’ package (Hartig, 2024).

All analyses were conducted by using R software (version 4.4.2) (R Core Team, 2024).

3. Results

3.1. Taxonomic diversity

Eight families were identified within the samples. In TpA, five families were recorded (Daphniidae, Bosminidae, Podocopida, Cyprididae and Cyclopidae), whereas six families were documented in TpB and TpC (Daphniidae, Chydoridae, Podocopida, Cyclopidae, Diaptomidae and Harpacticoida), exhibiting several feeding strategies.

Our observations suggested that the presence and relative abundance of taxa in the ponds significantly changed over time. Certain families underwent substantial quantitative fluctuations (e.g., Daphniidae in TpA, Chydoridae in TpB and Podocopida in TpC), whereas others remained relatively stable (e.g., Cyprididae in TpA and Harpacticoida in TpB and TpC).

Specifically, in TpA, the proportion of individuals across all taxonomic groups significantly changed over time. In contrast, no significant variations were observed in TpB and TpC (Fig. 3 and Table S1).

3.2. Functional diversity

The studied taxa exhibited a high variability in the feeding strategies (Table S2). In TpA, saprophages and omnivores were present from October to May, whereas in TpB, all trophic niches (filter feeders, saprophages, omnivores and grazers) were present from December to late April. In TpC, saprophages and filter feeders exhibited significant fluctuations throughout the entire sampling period (Fig. 4).

The microcrustacean assemblages in TpB and TpC showed the highest similarity (Jaccard similarity = 0.83, maximal value). Moreover, the microcrustacean community of TpB was more similar to the one of TpA (Jaccard similarity = 0.50, maximal value) than the community of TpC was to that of TpA (Jaccard similarity = 0.43, maximal value), even though TpB and TpC were geographically close (< 200 m linear distance). (Table S3).

3.3. Drone-based photogrammetry analysis

After an initial artificial flooding in October (excluded from the correlation analysis)), TpA reached a flooding peak in December and January, before gradually decreasing from February onwards. In contrast, the sizes of TpB and TpC increased during the winter period, then both decreased significantly in February and reached a new flooding peak in March (Fig. 5).

Specifically, in TpA, the month in which the pond reached its flooding peak was December (17,338.4 m^2), while May was the month with the lowest water level, making impossible to quantify the exact value since the water was concentrated in only a few areas. In TpB and TpC, the maximum flooding size was recorded at the end of January (TpB: 185,935 m^2 and TpC: 534,289 m^2), while the minimum was

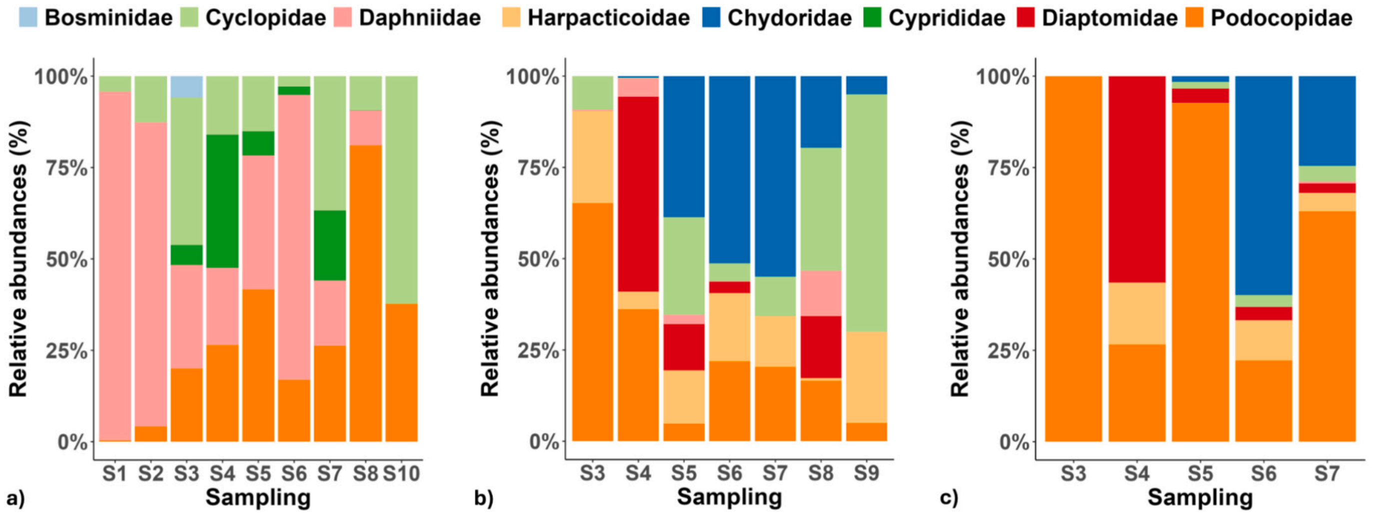

Fig. 3. Temporal fluctuations in the proportion of individuals belonging to the families found in a) TpA, b) TpB and c) TpC. S1 = 20/10/2020; S2 = 28/11/2020; S3 = 11/12/2020; S4 = 08/01/2021; S5 = 29/01/2021; S6 = 19/02/2021; S7 = 12/03/2021; S8 = 02/04/2021; S9 = 24/04/2021; S10 = 14/05/2021.

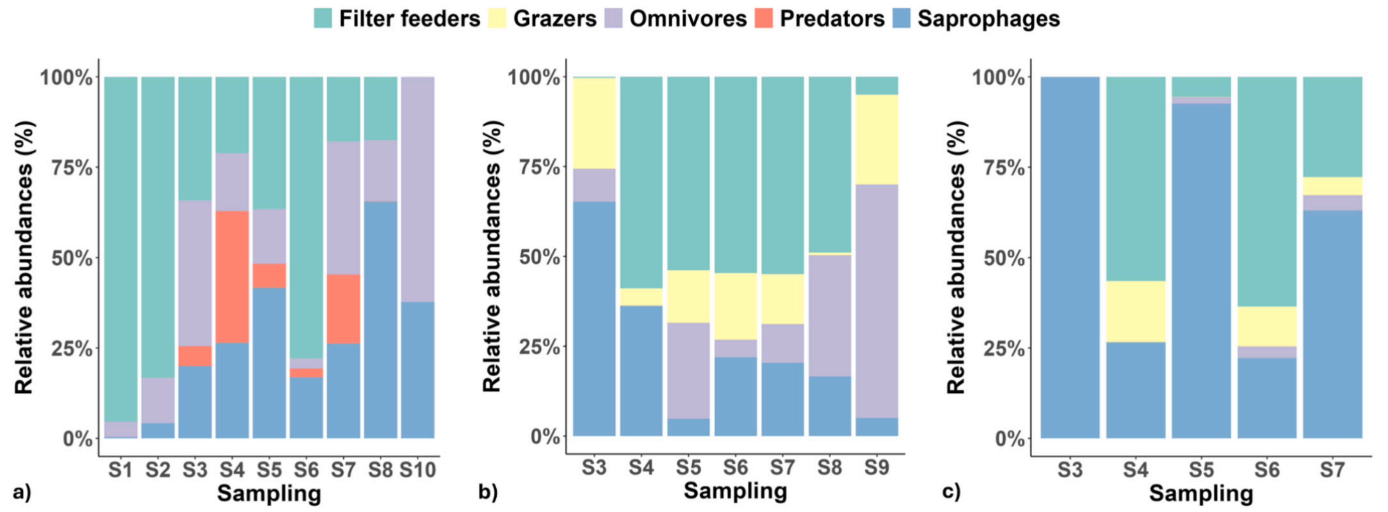

Fig. 4. Temporal variability and relative abundances in the proportion of individuals divided by trophic niche in a) TpA, b) TpB and c) TpC. S1 = 20/10/2020; S2 = 28/11/2020; S3 = 11/12/2020; S4 = 08/01/2021; S5 = 29/01/2021; S6 = 19/02/2021; S7 = 12/03/2021; S8 = 02/04/2021; S9 = 24/04/2021; S10 = 14/05/2021.

recorded in TpB at the end of April (15,755 m^2) and in TpC in March (277,679 m^2) (Table S4).

3.4. Microcrustacean communities and dimensional variation of ponds

We found a positive correlation between pond size and Shannon diversity index (Spearman: rho = 0.607, $p < 0.01$), indicating that the increase in the size of the temporary ponds corresponds to an increase in the biodiversity of the microcrustacean communities (Fig. 6).

The GLM analysis was restricted to TpA due to the limited spatial and temporal variability in the samples collected at TpB and TpC. he variability of the microcrustacean assemblage observed in TpA was primarily explained by the temporal (Time) and temporal-spatial (Time + Sub-Transect) variables (Fig. 7 and 7b and Table 1), as indicated by the selected model (Table 2).

Even though the p-value for the spatial variables is not significant, the low ΔAIC value (Table 2) suggests that the spatial variables might still play a role. This potential effect might not have been clearly detected due to limitations in the sampling design.

4. Discussions

4.1. Ecological-functional dynamics of microcrustacean communities

The three TPs analyzed, located in Viterbo and Latina, exhibited distinct ecological characteristics, despite sharing a predominantly clay substrate and agricultural surroundings. Another environmental difference between the two areas is the closeness to the sea, which could be another factor that can hypothetically influence the ecological-functional dynamics of microcrustacean communities. The microcrustacean community in TpA could be affected by an increase in the pond's salinity, with salt-tolerant species thriving while sensitive species may decline. In terms of anthropogenic threats, no potentially harmful infrastructure was observed in the Viterbo site, while tertiary roads were identified as a potential disturbance in the Latina area. Additionally, pollution from the numerous spent cartridges, as well as slash-and-burn agriculture practices in the Latina area, could act as further stressors. More resilient species can survive, while others disappear, altering the pond's ecological balance.

Contrary to the findings of Crosetti and Margaritora (1987), the temporary pond with the highest species richness in our research was TpB, with a total of six families. Previous studies have reported that larger ponds with longer hydroperiods support greater taxonomic diversity, likely due to the increased availability and complexity of microhabitats (Oertli et al., 2002). According to this hypothesis, we would have expected the TpA, with its larger surface area (3 ha) and longer hydroperiod (7 months), to harbor the highest species richness. Unexpectedly, TpB, a smaller pond of about 1 ha and with a shorter hydroperiod of 5 months, showed the highest richness (Scheffer et al., 2006). This is interesting because usually ponds characterized by shorter hydroperiods are generally smaller, decreasing the likelihood of propagule dispersal to settle in this type of ponds (Incagnone et al., 2015). As stated in the study of Seminara et al. (2015) conducted in the Lazio region too, some species were only found in ponds with short hydroperiod (< 6 months) suggesting they could be considered good bioindicators of basin hydroperiod. Another explanation could be caused by "early species appearance" that can shape the following assemblages through mechanism of predation or competitive exclusion resulting in microcrustacean communities with a lower number of species (Florencio et al., 2013). This latter explanation indicates that hydroperiod alone may not fully explain microcrustacean taxon richness and communities' dynamics, suggesting that additional factors, such as intrinsic community interactions, should be investigated further.

From a functional-ecological perspective, the most abundant and earliest-appearing taxonomic group in TpA was represented by daphnids, consistent with findings by Stella (1988) and predictions from the Plankton Ecology Group (PEG) model (Sommer et al., 2012). The PEG model emphasizes the interplay of abiotic factors, nutrient availability and grazing pressure in shaping plankton community dynamics (Sommer et al., 2012). Zooplankton development, in turn, is driven by the availability of trophic resources and predation, particularly by fish (Brysiewicz et al., 2017). Typically, a phytoplankton bloom is followed, after a lag period, by a sharp decline in biomass due to both food scarcity and increased predation, which together regulate zooplankton abundance (Lampert, 2006). The succession of cladocerans was also partially influenced by variation in aquatic vegetation cover, as shown in two studies conducted in permanent coastal water bodies (Hann and Zrum, 1997; Paterson, 1993). Moreover, Seminara et al. (2015) observed that cladocerans exhibited slower developmental rates than copepods, particularly during the initial pond-filling stages, reaching comparable densities only after approximately 15 days. In contrast, in TpA, daphnids (filter feeders) were initially more abundant than cyclopoids (omnivores), although they exhibited more pronounced temporal fluctuations. As the ecosystem matured, ostracods also emerged, exhibiting not only saprophagous behavior but also peculiar trophic strategies. Among them, *Heterocypris incongruens*, recorded in this study, has previously

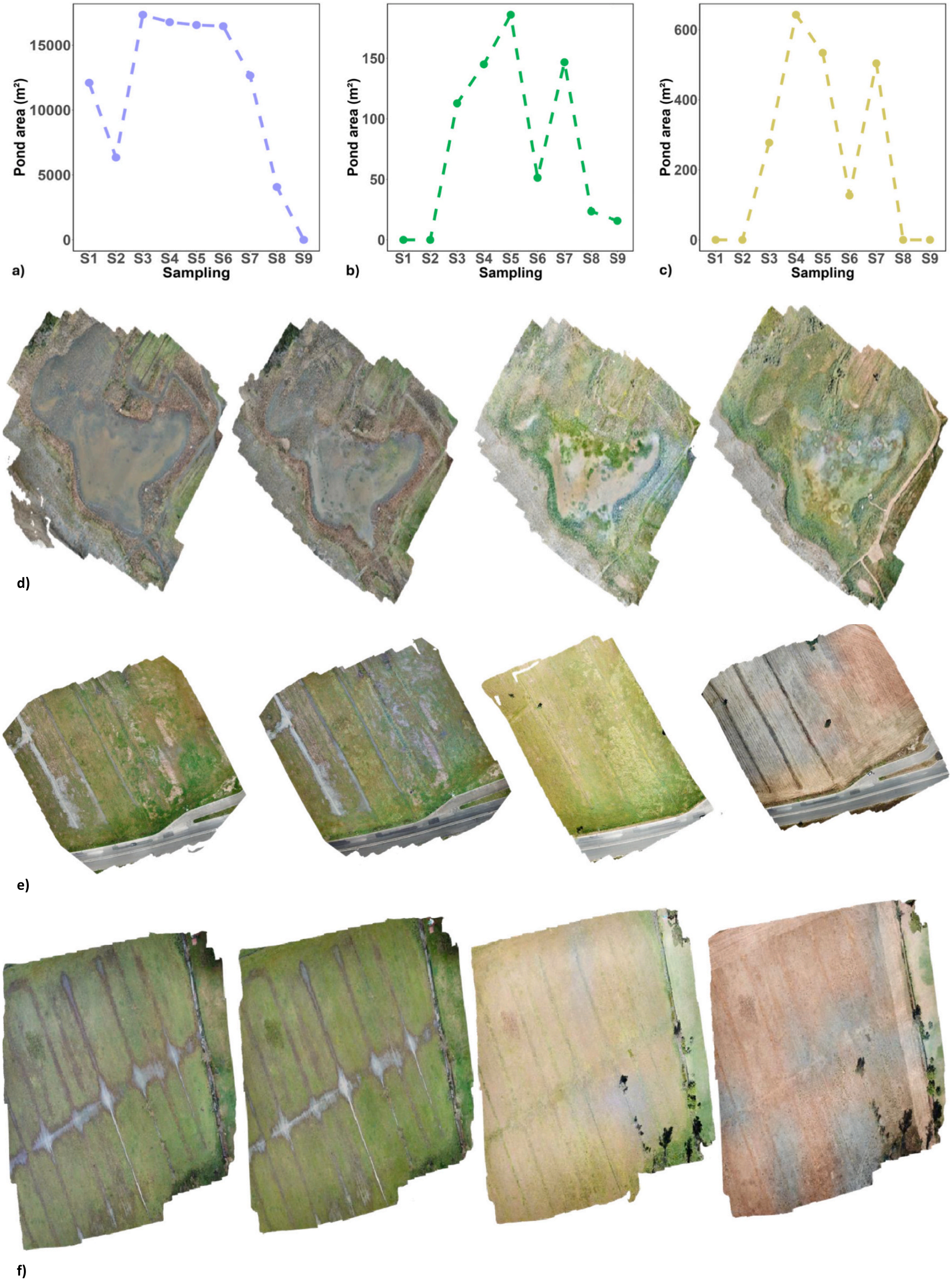

Fig. 5. Graph showing the change in the areas of the ponds from October to May. The y-axis represents the pond area in m^2, while the x-axis shows the dates on which aerial photos of the ponds were taken. a) TpA, b) TpB and c) TpC. Evolution of d) TpA, e) TpB, f) TpC; from left to right: orthophoto from January, March, May and July. S1 = 20/10/2020; S2 = 28/11/2020; S3 = 11/12/2020; S4 = 08/01/2021; S5 = 29/01/2021; S6 = 19/02/2021; S7 = 12/03/2021; S8 = 02/04/2021; S9 = 24/04/2021.

been reported to engage in unexpected trophic interactions in freshwater environments of northern Italy (Ottonello and Romano, 2011). This species generally feeds on small invertebrates, bird and amphibian carcasses (Gusakov et al., 2021; Juarez-Franco et al., 2009), showing a preference for amphibian larvae, particularly those of the common toad (*Bufo bufo*), whose occurrence was recorded.

The final phase of the hydroperiod was characterized by low biodiversity, consistent with the findings of Vagaggini et al. (2002), with only cyclopoids (omnivores) and podocopids (saprophages) remaining. In the Latina ponds, podocopids appeared immediately after the first rain

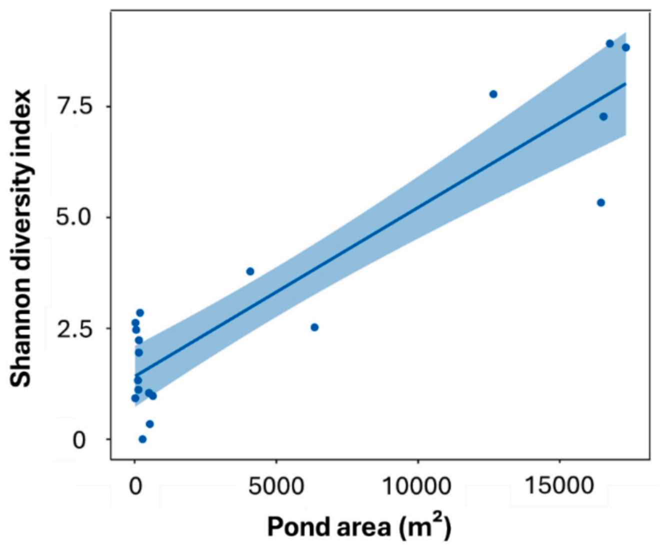

Fig. 6. Correlation between the areas (m^2) of the three temporary ponds and the Shannon diversity index (H′).

events, rapidly reaching high densities before gradually declining as the community matured and was colonized by new taxa. Daphnids and chydorids (both filter feeders) showed contrasting growth trends, likely due to their occupation of different microhabitats; chydorids in particular are commonly associated with the presence of macrophytes. Furthermore, as also reported by VagagB36gini et al. (2002), the pre-drying phase was characterized by the dominance of small-sized cladocerans such as chydorids. Although graphical analyses revealed clear temporal shifts in microcrustacean assemblages across all three ponds, statistically significant quantitative variations over time were only observed for the taxonomic groups in TpA. This may be due to the more pronounced oscillations in taxonomic group abundances in TpA compared to the more gradual and stabilized trends observed in TpB and TpC. TpA may have supported stronger interspecific interactions, exhibited a more complex microhabitat structure, or been influenced by additional environmental stressors affecting community dynamics. Given their spatial proximity, the microcrustacean assemblages in the two Latina ponds were more similar to each other than to those observed in TpA. According to Seminara et al. (2008), during the colder months, abiotic factors such as temperature, dissolved oxygen and water volume tend to become more uniform across ponds, leading to increased homogeneity in community structure. Indeed, several taxa, such as daphnids, Podocopidae ostracods and cyclopoids, were shared among all three ponds. Nevertheless, distinct taxa were also found: TpA hosted unique species such as bosminids and *Heterocypris incongruens,* while TpB and TpC were characterized by the presence of chydorids (typically associated with macrophyte-rich habitats), diaptomids (recognized as valuable bioindicators) and harpacticoids.

4.2. Hydroperiod assessment using UAV-based remote sensing

Drone-based photogrammetric analysis provided a detailed and dynamic view of the area variations of the temporary ponds during the study period, from October to May, allowing for the quantification of hydroperiod length and its fluctuations.

TpA pond exhibited distinctive hydrological regime. After an initial artificial flooding in October, a decrease in the area was observed.

Table 1
Results of the GLM showing a significant effect of the Temporal variable on the Shannon diversity index of TpA. The spatial variable (Sub-transect) was not statistically significant; however, the model including both variables had the lowest AIC value. Therefore, the spatial variable was also retained in the GLM.

Variable	Chi-sq	Df	*P*-value
Temporal (Time)	164.82	9	< 0.001
Spatial (Sub-transect)	4.78	2	0.09165

Table 2
Model selection based on the Akaike Information Criterion (AIC). As shown in the table, the best-fitting model — i.e., the one with the lowest AIC — is the one which includes both the 'Time' and 'Sub-transect' variables.

Covariate formula	AIC	ΔAIC
Time + Subtransect	126.7448	0
Time	128.0020	1.257214
Time + Transect + Subtransect	129.9286	3.183788
Time + Transect	131.1783	4.433508
Time + Transect * Subtransect	135.8202	9.075379
Null model	208.9267	82.181922
Subtransect	211.0698	84.325011
Transect	212.5128	85.767977

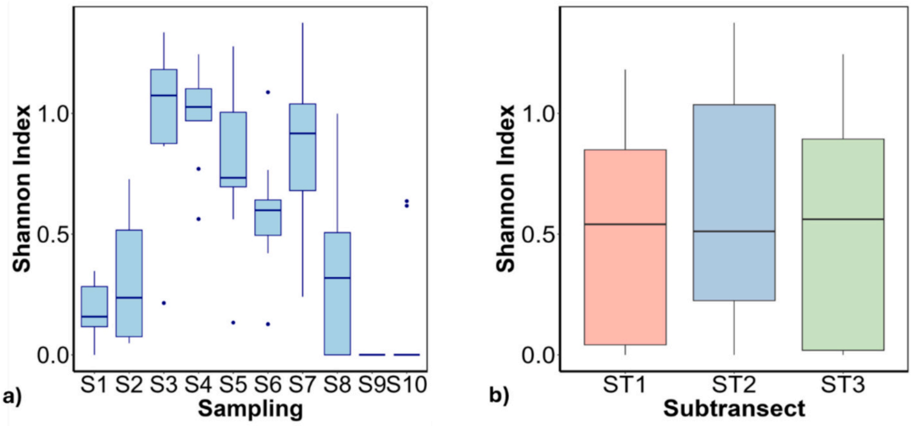

Fig. 7. a) Boxplot showing the variability in microcrustacean community diversity, calculated for each sub-transect of TpA on every sampling day. For the last pond area measurement (S10), it was not possible to quantify the exact amount of water present in situ (as it was too scarce); however, individuals belonging to the main target taxa of interest were still found, as shown by the corresponding Shannon index (H′) values on the y-axis. b) Boxplot showing the spatial covariate 'Sub-transect' (also included in the GLM model) and the increase in Shannon diversity index (H'), particularly in the sub-transects belonging to sector '2'. S1 = 20/10/2020; S2 = 28/11/2020; S3 = 11/12/2020; S4 = 08/01/2021; S5 = 29/01/2021; S6 = 19/02/2021; S7 = 12/03/2021; S8 = 02/04/2021; S9 = 24/04/2021; S10 = 14/05/2021. ST1 = Subtransect 1; ST2 = Subtransect 2; ST3 = Subtransect 3.

Subsequently, a flooding peak occurred in December and January, reaching a maximum area of 17,338.4 m^2 in December. From February onwards, the area of TpA underwent a gradual reduction, culminating in May with a water level so low that precise quantification was impossible, indicating an extreme reduction in the aquatic area. This pattern suggests that TpA is influenced by specific hydrological factors, potentially related to the initial artificial flooding and evaporation or infiltration dynamics.

Unlike TpA, TpB and TpC showed an increase in area during the winter period, with a maximum flooding peak at the end of January (185,935 m^2 for TpB and 534,289 m^2 for TpC). However, both experienced a significant reduction in the area in February, followed by a new flooding peak in March. Subsequently, the area decreased again, reaching the minimum at the end of April for TpB (15,755 m^2) and in March for TpC (277,679 m^2). These fluctuations indicate a dynamic response to winter and spring rainfall, with subsequent water loss likely due to evaporation and infiltration.

The use of drone-derived orthophotos (Fig. 5d, e, f) allowed for the direct visualization of pond area evolution over time (Taurozzi and Scalici, 2024; Laliberte and Rango, 2009). The images clearly show the variations in water coverage, confirming the quantitative data obtained from the photogrammetric analysis. The ability to monitor these variations with high spatial and temporal resolution is a significant advantage of using drones, providing valuable data for understanding the hydrological dynamics of these temporary ponds (Anderson and Gaston, 2013). Drone-based methods made it possible to precisely identify small variations in the water's extent over time, which is especially useful in small or ephemeral ponds characterized by hydrological fluctuations (Vélez-Nicolás et al., 2021). Accurate pond boundary delineation allows the production of orthophotos and digital surface models, and the results of repeated surveys can reveal information on the seasonal changes in pond size. This approach was also valuable in other studies focused on the nature reserve management of a wetland area (Marzialetti et al., 2024).

The ability to accurately monitor the hydroperiod and the subsequent area variation of temporary ponds is crucial for understanding their ecological functioning and for assessing the impacts of climate change and other environmental stressors (Van den Broeck et al., 2015). These ecosystems are particularly vulnerable to changes in precipitation patterns and temperature, which can alter their hydroperiod and lead to habitat loss (Zacharias and Zamparas, 2010). Therefore, the use of remote sensing technologies, such as drones, is essential for monitoring the conservation status and climate change impacts on these important and vulnerable environments (Gardelle et al., 2010). The data obtained from these studies can be used to inform conservation management strategies and to develop effective mitigation measures.

In summary, the drone-based analysis allowed for the precise quantification of pond area variations, revealing distinct hydrological patterns between the Viterbo and Latina ponds. These data are fundamental for understanding the hydroperiod of these ponds and for assessing the impact of environmental and anthropogenic factors on their hydrological dynamics. The ability to monitor these variations with high spatial and temporal resolution is a significant advantage of using drones.

4.3. *Influence of hydroperiod length and pond area on microcrustacean communities*

Although relatively few studies have addressed the temporal succession of microcrustacean communities, several authors have reported a positive correlation between species richness and pond size, particularly in large water bodies with extended hydroperiod (Martins et al., 2019; Crosetti and Margaritora, 1987). Notably, Seminara et al. (2008), observed a clear correlation between fluctuations in cladoceran density and changes in pond surface area within the Castelporziano Reserve.

These findings led to an investigation into the primary factors driving increased biodiversity in TpA, considering both spatial (transect and sub-transect) and temporal (sampling date) variables. Temporal variation emerged as the most significant factor, although spatial variables also contributed, though to a lesser extent. Specifically, the sub-transect 'ST2' (located centrally within the pond boundaries) exhibited higher biodiversity compared to sub-transects 'ST1' and 'ST3'. This observation aligns with the findings of Riis et al. (2000), who demonstrated that macrophyte presence — by influencing pH and conductivity through photosynthetic activity — plays a key role in creating suitable microhabitats for microcrustacean colonization. Future research should further investigate the drivers of biodiversity in these systems, particularly regarding the floristic component and the potential influence of marine stressors such as aerosol and salt wedge intrusion.

Although our case study is restricted to only three temporary ponds within the Lazio region, the observed patterns could also be observed in other astatic ponds of the Mediterranean regions such as those in Portugal (Caramujo and Boavida, 2010), Spain (Florencio et al., 2016) and Southern Italy (Bagella and Caria, 2013), where a correlation between microcrustacean community turnover and hydrological length has been reported suggesting that similar ecological mechanisms may operate across different regions. Nevertheless, local factors such as soil type and surrounding land use can influence community composition. Therefore, further studies across multiple sites and regions would help validate the applicability of these findings and refine multidisciplinary monitoring strategies for temporary ponds of the Mediterranean areas.

In the Latina area, the presence of taxa highly vulnerable to habitat destruction emphasizes the critical importance of conserving temporary aquatic environments. Specifically, individuals of the Southern smooth newt (*Lissotriton vulgaris meridionalis*) were observed in February, highlighting the ecological value of these ephemeral habitats. In the same month, another remarkable species was recorded: *Lepidurus apus,* a classic inhabitant of astatic ponds often referred to as a 'living fossil' as it has retained its ancestral morphology since the Triassic period. This species is strictly confined to temporary water bodies free from predatory fish and crayfish (Pérez-Bote, 2004). Its presence in the Latina area is particularly unexpected, given the annual practice of stubble burning. Further investigations are needed to understand how L. *apus* persists under such conditions and to assess the broader implications for conservation in managed agro-ecosystems.

Finally, the discovery a red swamp crayfish (*Procambarus clarkii*) carapace in TpC during June provides evidence that temporary ponds can serve as important connectivity zones between permanent aquatic habitats. These ephemeral environments may act as ecological steppingstones facilitating the migration and dispersal of both native and non-native species (Fehlinger et al., 2023; Scalici et al., 2021).

5. Limits of research and future perspectives

Despite the strict protocols available in the literature and followed in this study, the sampling design adopted here has some limitations that should be acknowledged. First, we did not investigate the soil component in detail due to the lack of specific instrumentation. Further studies are therefore needed to understand how soil composition and surrounding land use may influence the structure and distribution of microcrustacean communities. Moreover, the ponds, due to their different sizes and the strong variability associated with several factors (seasonality, rainfall, groundwater level), were sampled using slightly different designs and approaches. We aimed to maintain overall comparability among ponds, while reducing the number of samples where necessary. A limitation of the present study is the lack of direct salinity measurements, despite the proximity of the pond to the sea (<200 m), which could influence local environmental conditions and the microcrustacean community. Future studies should include systematic salinity monitoring across different sampling periods to better quantify the effects of seawater intrusion and strengthen the understanding of community responses to marine influence. In addition, some methodological limitations related to UAV-based mapping should be acknowledged. Adverse weather conditions, such as wind, rain, or low light, can reduce image quality and affect the precision of photogrammetric outputs. Furthermore, the delineation of small or irregular pond boundaries may introduce uncertainties in estimating pond area and hydroperiod duration. These factors should be considered when interpreting the results and future applications could benefit from integrating UAV surveys with complementary ground-based measures to improve accuracy under challenging environmental conditions. Finally, some limitations regarding taxonomic resolution should be acknowledged. Since the main objective of this study was to analyze the spatial-temporal variation of microcrustacean communities and to relate their turnover to the dimensional changes of temporary ponds, achieving species-level identification was not essential for our purposes. Therefore, taxonomic resolution was intentionally limited to the most informative level achievable using standard freshwater identification guides and a stereomicroscope, without requiring specialist taxonomic expertise. Future studies focusing on species-level analyses could provide additional insights into ecological and functional patterns that were beyond the scope of the present work.

6. Conclusions

Although our study is limited to a relatively small geographical area, the findings contribute to the development of more effective, multidisciplinary monitoring strategies, with broader implications for conservation and management efforts in temporary ponds of the Mediterranean regions.

This study highlights the need to analyze temporary environments through a multi-methodological approach that can be replicated over time and, ideally, adopted at a national level. In this context, the innovative application of UAV-based remote sensing emerged as a valuable tool for monitoring the hydrological dynamics of temporary ponds. Drones offer several advantages over traditional ground-based methods, including high spatial and temporal resolution, the ability to access otherwise unreachable areas and cost-effectiveness, especially for long-term ecological monitoring. To properly assess the effectiveness of drones as a sampling tool, further studies are needed, including long-term monitoring, to allow comparisons between ponds with similar characteristics, such as soil type, surrounding land use and hydroperiod.

A substantial number of temporary ponds were recorded, forming an ecological corridor along the coast and enabling the dispersal of taxa, eggs, or aestivating stages of species typical of these habitats through the activity of birds, mammals, or atmospheric agents. Unfortunately, pollution and other anthropogenic activities also promote the spread of tolerant species with high colonization potential, leading to a decline in the more specialized communities inhabiting these ponds. Although the intensity of human disturbances, such as the presence of garbage, was not quantified in this study, such impacts could potentially affect pond communities and therefore warrant further investigation in future research.

TPs contribute significantly to regional biodiversity, exhibiting a disproportionately high species richness relative to their small surface area. For this reason, TPs are considered true biodiversity hotspots and serve as effective tools for assessing the impact of anthropogenic activities on the environment. These biotopes are particularly sensitive to the effects of climate change, which destabilizes their hydroperiod and reduces the number of ponds across the landscape. In addition, direct destruction caused by human activities is often the result of a lack of recognition of these habitats as aquatic ecosystems with highly diverse biotic communities, whose life cycles are intimately tied to their temporary nature. To prevent further loss of biodiversity in temporary waters and to safeguard biodiversity at both regional and national scales, the development of an urgent land management plan is imperative, one aimed at preserving and, where possible, promoting the formation of new temporary aquatic ecosystems.

CRediT authorship contribution statement

Livia Benedini: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Giulia Cesarini:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization. **Davide Taurozzi:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Virginia Iorio-Merlo:** Writing – review & editing, Visualization, Investigation, Formal analysis, Data curation. **Francesco Simone Mensa:** Writing – review & editing, Visualization, Investigation, Formal analysis, Data curation. **Massimiliano Scalici:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Funding

This research was supported by the Grant of Excellence Departments, MIUR-Italy (ARTICOLO1, COMMI 314–337 LEGGE 232/2016). This research was supported by NBFC at the University of Roma Tre.

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.

Acknowledgements

The authors acknowledge the support of NBFC to University of Roma Tre, funded by the Italian Ministry of University and Research, PNRR, Missione 4 Componente 2, “Dalla ricerca all'impresa”, Investimento 1.4, Project CN00000033.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2026.114655.

Data availability

Data will be made available on request.

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