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Biodiversity and Possible Bio-Indicators of Mediterranean Temporary Ponds in Southern Apulia, Italy

Leonardo Beccarisi [1], Vincenzo Zuccarello [2], Rita Accogli [2] and Genuario Belmonte [2,*]

[1] Ecological Consultancy, 73013 Galatina, Italy
[2] Department of Biological and Environmental Sciences and Technologies, Campus Ecotekne, University of Salento, 73100 Lecce, Italy
* Correspondence: genuario.belmonte@unisalento.it

Abstract: Mediterranean Temporary Ponds (MTPs) represent a priority habitat according to Directive 92/43/EEC (Natura 2000 code: 3170*). These are very shallow water habitats only seasonally flooded, with a flora mainly composed of Mediterranean therophytic and geophytic species. Its extreme seasonality and small size make this habitat highly vulnerable and hard to manage. In recent Italian monitoring campaigns, the conservation status of MTP 3170* was considered inadequate. In Apulia, where the habitat is considered as "the most vulnerable type", 73 sites were censused, with a total coverage of about 10,000 m^2. The present work refers to the monitoring for three years of a total of 16 habitat 3170* sites, with the aim of better describing faunal indicator species for this priority habitat. A total of 158 taxa of flora and 103 of fauna were identified from 54 floristic and 44 faunistic samplings in total, with a robust updating of the listed biodiversity. For the first time a group of faunal species is proposed as an indicator of the habitat MTP 3170*. The conservation status, assessed on the basis of structural and functional criteria, gave a satisfactory status for seven sites and an unsatisfactory one (variously rated as inadequate or bad) for nine.

Keywords: biodiversity; bioindication value; EU habitat directive; European Union (EU); habitat 3170; habitat characterisation; species richness

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1. Introduction

The increasing attention of limnologists has been focused on small temporary water habitats [1]. Temporary waters show a high aquatic biodiversity [2–7] and a noteworthy role in the provision of socio-economic benefits [8,9]. The data generated by the first studies also contributed to the proposal of the so-called Pond Manifesto [10] essentially addressed to the awareness, the understanding, and the conservation of ponds in a changing European landscape and climate.

This growing interest in temporary ponds also led the scientific community to promote and share knowledge on the biological and ecological characteristics of these environments [11–15].

Currently in Europe, there are two main laws that regulate the protection of ponds: the Water Framework Directive 2000/60/EC, and the Habitat Directive 1992/43/EC. In particular, the second document considers as priority habitat the so-called Mediterranean Temporary Ponds (MTPs), whose habitat code is 3170*, and the official interpretation manual for the identification of EU habitats [16] supplies a list of floristic alliances and species useful to identify them.

At the present time, the absence of fish is the only recognised faunal feature in the biological community of temporary ponds, and this absence is associated with (or favours) the presence of a rich aquatic fauna of invertebrates, generally not thriving in the presence of fishes [17]. Despite their high biodiversity, the official manuals do not include the faunal component among the criteria for habitat identification; accordingly, the use of plant assemblages currently remains the unique instrument useful to recognise the 'priority' habitat status of MTPs.

This notwithstanding, faunistic research on habitat type 3170* exist, but are generally focused on restricted faunal groups of the pond community (e.g., Amphibia, Insecta, Acari, Crustacea). In some cases, the interaction of such faunal groups with environmental features has been studied [6,18]. Few studies have been focused on the evaluation of the whole bio-community of temporary ponds [2,6,19–23].

Most of this research showed that the species richness of macro-invertebrates appears to increase with the hydroperiod (flooding time) of the pond [24–30]. Conversely, if micro-invertebrates (planktonic Crustacea) are considered, the species richness increase is not (or is inversely) correlated with the hydroperiod [31,32].

The extended review of [7] of Sardinia temporary fresh waters reports a rich dataset from a multiyear study of 100 sites (not all MTP 3170*). Vascular flora was represented by 350 species (with 81 typical of MTPs), and macrofauna was represented by 228 species (mostly Insecta), plus 3 Amphibia.

The paper of Boix et al. [33], also considering micro-invertebrates, listed 394 genera of animals for temporary ponds of the Mediterranean biogeographic area.

This "neglected habitat" [7], consequently, has demonstrated a high variability and community diversification in space and time, even in restricted geographic areas, with adjacent ponds only rarely sharing the same species composition. Diaz-Paniagua et al. [34], working on a multiyear scale on 21 temporary ponds of Southern Spain, found that although a small number of species were characteristics of many sites, an ensemble of temporary ponds did not differ in species richness from large permanent ponds. Ponds in each geographical area were heterogeneous and showed a different surface extension and flooding duration, even at a long temporal scale due to interannual differences in rainfall. For the same pond, the hydroperiod will change over time, and consequently the dominant species; thus, ponds may be optimal habitats for different species in different years [35], and these changes favour the increased richness of the whole community, if the long-term is considered. This result was also confirmed by [36] who found that the richness of species assemblages of temporary ponds can even increase by 60%, if the encysted (resting) part of biodiversity is also considered. The biodiversity of temporary ponds, finally, has to be considered over large space units and time spans, to have a correct perception of species possibly playing a role in the same habitat.

Limnological research recently carried out in Apulia (South-Eastern Italy) highlighted that most of the surface inland waters of the region have a temporary hydroperiod, and following the Habitat Directive, about 18% of them were catalogued as MTPs [37]. Further detailed studies at a local scale were carried out on a multiyear scale in inland waters in the central area of the region (National Park "Alta Murgia", [38]). Here, we present the results from a limnological survey over three years of 16 MTPs located in South-Eastern Apulia, in order to individuate the common characteristics of their biological communities. For several cases (ponds), fauna and flora samples of the past (2007–2012) were also accurately re-analysed to obtain a reliable characterisation of the selected ponds, also based on a long time of data collection. The main objective of the study was the biological characterisation of MTP 3170* through the assessment of biological communities (vascular plants and aquatic micro-fauna) in a geographic area relatively homogenous from the climatic and the biogeographic points of view.

2. Material and Methods

2.1. Study Area

This research was realised in the area of South-East Apulia, consisting of about 10,000 km^2 in the central area of the Mediterranean region (South-Eastern Italy) (Figure 1). This Apulian sub-area is a karstic territory (see the geologic interpretation in [39]) with a maximum elevation of 600 m a.s.l. and a typical absence of surface hydrography. The 16 fishless ponds monitored in the present study (Figure 1; Table 1) lie in an altitude range of 0–457 m a.s.l. With reference to the hydroperiod, all ponds are of the type "strictly temporary" (ST) [6], where water may be present for a few months, not necessarily every year.

Table 1. List of the sampled ponds, along with their respective codes, names, and the main environmental features. Morphological groups are indicated by colours: green = dolines, yellow = cupular pools, pink = waterlogged soils. Geographic coordinates in the WGS84 datum.

Site Acronym	Site Name	Apulian Code ([37])	Latitude (WGS84)	Longitude (WGS84)	Altitude (m a.s.l.)	Morphological Type	Brief Description	Pond Area (m^2)
BEL	Masseria Bellimento	PU058	17°55′21.2″	40°11′46.7″	0	Dolines	System of two dolines 15 m apart.	1720
DTO	groove beside Padule Rotondo (Bosco don Tommaso)	PU233	18°16′49.6″	39°58′39.8″	113	Dolines	A doline crossed centrally by an artificial canal and surrounded by cultivated fields.	3103
FEL	Felline	PU156	18°7′16.3″	39°55′52.9″	44	Cupular pools	System of many small limestone cupular pools.	212
FER	Laccu Feretru	PU059	18°11′7.1″	40°13′0.6″	66	Dolines	A doline surrounded by cultivated fields and a stone quarry.	899
IAC	Iacorizzo	PU234	17°49′4.0″	40°23′11.7″	63	Dolines	A doline with a long to permanent hydroperiod, surrounded by cultivated fields also subject to flooding	2368
LEU	Leuca: vaschette su calcare	PU252, PU253, PU254	18°21′10.3″	39°48′19.4″	50	Cupular pools	A spread system of limestone cupular pools, within a maximum distance of 1.3 km.	480
MAA	Madonna del Lago A	PU126	18°10′3.6″	40°16′41.5″	57	Dolines	A pond with a relatively long flood phase.	287
MAN	Bosco Mangiato	PU042	17°14′43.0″	40°44′26.6″	427	Dolines	A pond in a wooded context.	345
PAL	Masseria I Pali	PU124, PU125	18°12′49.5″	39°50′54.1″	46	Cupular pools	Two limestone cupular pool systems, 70 m apart.	270
PMA	Padula Mancina	PU020	18°18′37.0″	39°59′8.6″	102	Dolines	A late hydroperiod pond, surrounded by earlier-drying flooded meadows.	8165
POS	Posticeddu	PU227	17°50′26.9″	40°41′24.3″	9	Waterlogged soils	A seasonally bogged coastal site.	1076
SEM	Masseria Semeraro	PU231	17°3′46.7″	40°36′45.8″	137	Cupular pools	Small limestone cupular pools, in shrubby landscape.	22
SPE	Lo Specchione	PU235	17°53′4.5″	40°29′40.1″	72	Dolines	A doline surrounded by cultivated fields.	753
SPL	Lago Splendore	PU218	16°22′57.5″	40°45′54.5″	457	Dolines	A pond in a wooded context.	2145
SUR	Suriani 3	PU100	18°14′16.7″	40°4′31.3″	110	Waterlogged soils	Site is part of a wetland made up of marshes and artificial canals, surrounded by cultivated fields.	71,043
ZE1	Paduli 1, strada Silva (Zello)	PU103	18°14′5.2″	40°3′38.5″	116	Waterlogged soils	Area subject to flooding between cultivated fields.	4367

The average annual temperature of the geographic area (the Salento peninsula) was 19.3 °C in the considered period, ranging between extremes of −0.3 °C and 43.2 °C, and rainfall averaged at 450.3 mm/year, ranging between 337 and 579 mm/year according to different measuring stations (source: Weather Data ARPA Puglia, URL http://www.webgis.arpa.puglia.it/meteo/index.php (accessed on 28 July 2024). These features determine a typical Mediterranean climate [40]. Different habitat types included in the Habitat Directive characterised the landscape of the studied area. In particular, 3170* MTPs were rare (recently enumerated as 57 by the Regione Puglia Administrative Body, also on data from [6,41,42], in a total of 73 sites in the entire Apulian territory).

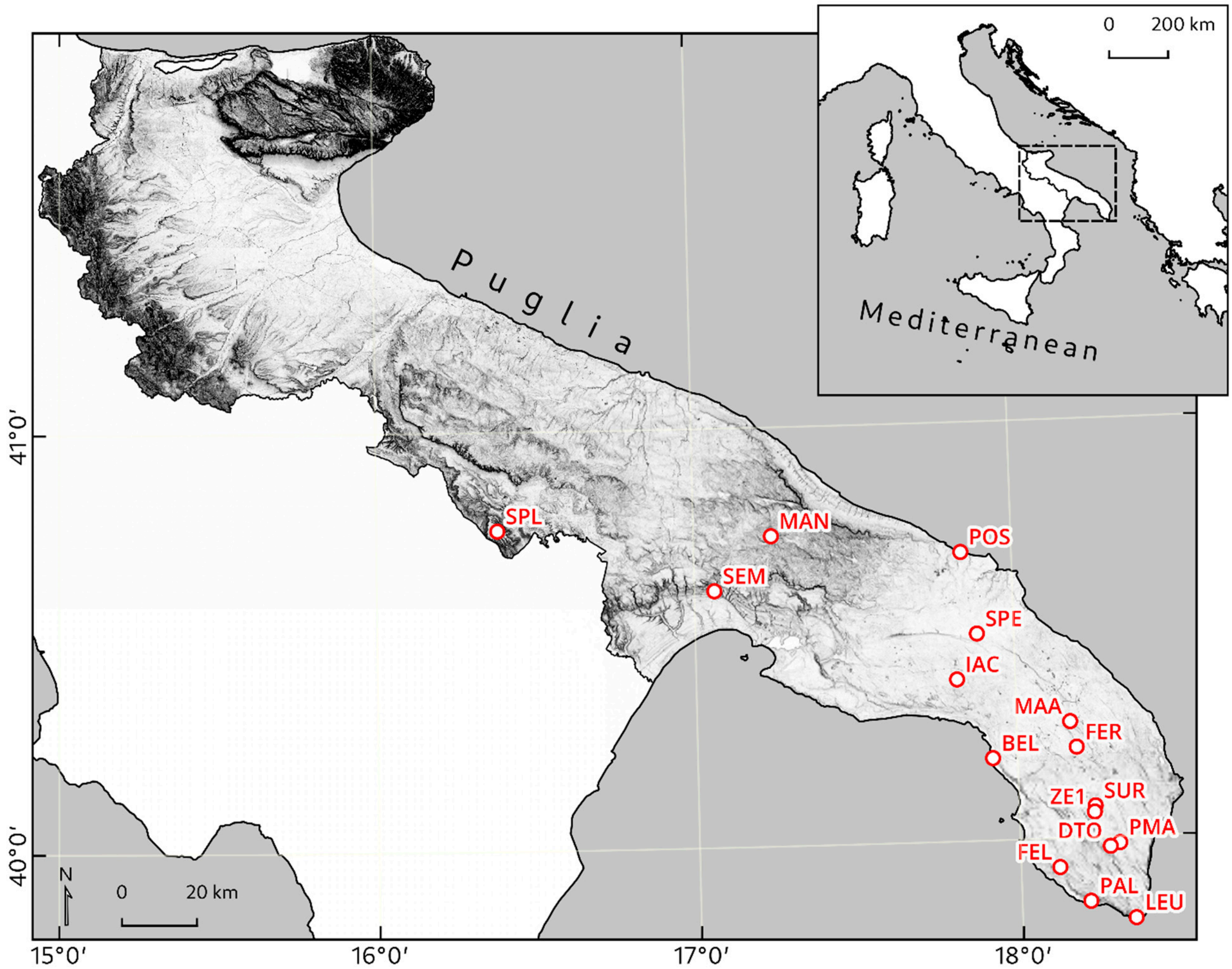

Figure 1. Location of the studied sites.

2.2. Field Surveys

Each temporary pond was labelled with a 3-letter acronym. Furthermore, each pond was assigned an alphanumeric code starting with PU (for Puglia) and followed by a number indicating its position in the list of the author's dataset. For each pond, geographical coordinates and altitude were indicated (Table 1). Each pond was inserted into one of three different morphological types (according to [4,5,41]): cupular pools (CPs), dolines (Ds), and waterlogged soils (WSs) (Figure 2). CPs are small incaves mostly in rock matrix (also called rock pools), having an extension ranging around 1 m^2, with very reduced catchment areas and depths of only a few centimetres. Ds are soil depressions created by karstic dissolution and/or subsidence, characterised by thick soil layers and a water depth of <50 cm. WSs occur on impermeable (clayey–silty) substrates with low hydraulic conductivity, in slight depressions of the land surface, mostly isolated from underground water and often situated in the clearings of woods.

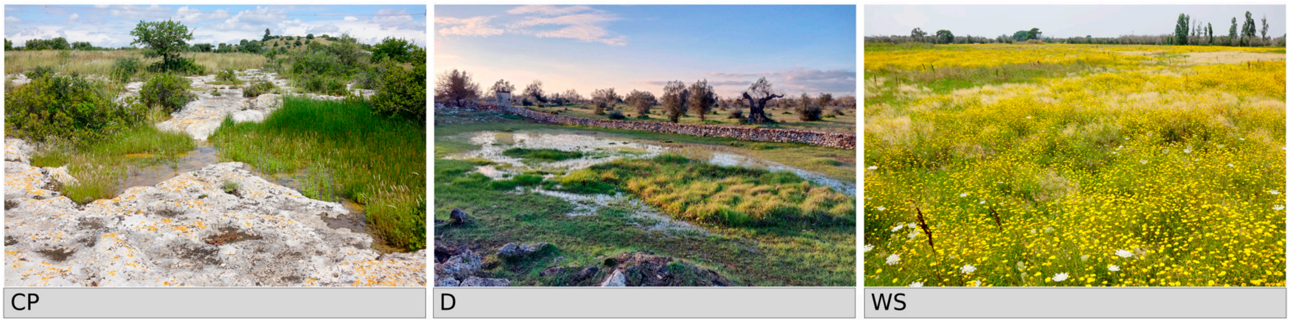

Figure 2. Morphological types of 3170* MTPs: cupular pools (CPs), dolines (Ds), and waterlogged soils (WSs).

The faunistic field surveys were a total of 38 between 25 November 2021 and 23 April 2022, and between 29 December 2022 and 7 July 2023. The floristic surveys were a total of 48 between 24 February 2022 and 26 October 2022, and between 5 April 2023 and 11 November 2023. The surveys comprised the measurement of some abiotic parameters of water (temperature, conductivity, Dissolved Oxygen, pH, measured in situ by means a multiprobe Hanna instrument, HI98194-95-96). In many cases, however, it was not possible to obtain abiotic data because of the scarcity of water. Thus, the cupular pools of LEU (3 sites) and FEL (1 site) were not considered (the water available was very minimal), and a total of 16 abiotic profiles were recorded (in DTO and PMA, replicated at different dates) (see Table S1). Also, because of this lack of multiple abiotic profiles for each pond, the study has been focused on the correlation between floristic and faunistic components.

Maximum depths have been measured directly if less than 1 m. The comparison of the water level at maximum and minimum (even dry) periods, in each pond, allowed us to establish also the water depths exceeding 1 m. The water hardness and concentration of Nitrates, Nitrites, Ammonium, and Phosphates were measured in the laboratory using samples of pond water preserved in darkness at 4 °C, soon after their collection, by a mono-parametric checker based on specific colorimetric devices (Hanna instruments, Multiparameter Photometer HI83399).

2.3. Floristic Data

Floristic data were collected through the plot method, which consists of positioning rectangular frames of 1 m^2 on the soil and performing the identification of vascular species contained within the frame. Each plot was positioned in an area with homogeneous vegetation, preliminarily selected on the basis of MTP species. For each site, the number of plots executed was 1–8 accordingly to the site area's extensiveness. The survey points were given a geographic position using a GPS with an average error of ±2 m.

Plant specimens collected during each survey were determined in the lab under a stereomicroscope and using the analytical guides of Pignatti [43–46] and Tutin et al. [47–51]. The nomenclature used was that of anArchive for Botanical Data (http://www.anarchive.it, accessed on 25 June 2024) [52]. The collected plant specimens were dried and preserved in the *Herbarium lupiense* of the Botanical Garden of the University of Salento (LEC).

As part of the present monitoring, typical species (sensu [53]) were taken from the list of [54] and according to the indications of [16,53,55]. A habitat 3170* was identified on the basis of the number and abundance of typical species found.

Information on non-indigenous species (NISs) of Apulian flora were extracted from [56]. NISs were classified as invasive or naturalised. Naturalised NISs are established alien plants that occur with self-maintaining populations without direct human intervention, whereas invasive NISs are, like the naturalised, alien plants that occur with self-maintaining populations without direct human intervention, but produce also fertile offspring that can be found at considerable distances from the parents, thus being able to spread over a large area [57]. Information about the conservation interest of each species was obtained from [58–62].

2.4. Habitat MTP 3170* Data

During the field surveys, the pressures (realised factors) affecting the conservation of the habitat were also detected. The classification system of the Natura 2000 Network monitoring was followed [63], attributing to each pressure category an importance value on a three-class ordinal scale (low, medium, high). The mapping of the area occupied by the habitat or species was also supported by the visual interpretation [64] of AIMA 2022 orthophotographs. The assessment of the conservation status was based on the combination of three criteria: structural, functional, and areal. Helm et al. [65] drove the structural criterion, distinguishing between characteristic and derived diversity. In detail, the criterion is based on the calculation of the index of Favourable Conservation Status (FCISi) = log ((number of MTP 3170 typical species + 1)/(other species + 1)). The functional criterion was based on pressure categories. The recognition of at least one category with high importance provides a negative contribution to the assessment of the conservation status. The areal criterion was based on the comparison between the coverage of habitat 3170* with previous monitoring data, if available. Any reduction in coverage provides a negative contribution to the final assessment of conservation status.

The conservation status of the habitat 3170* for each site was assessed on a three-values ordinal scale: favourable, inadequate, bad [66]. The final score for each site/habitat was considered as "Favourable" in the case of a majority of positive values; "Bad" in case of a totality of negative values, and "Inadequate" in the case of a majority of negative values. The codes used to indicate the land use in each site were taken from the European Environmental Agency [10].

2.5. Aquatic Faunal Data

Aquatic invertebrates were collected using a zooplankton net (mouth diameter, 24 cm; mesh size, 125 μm) horizontally towed from side to side in each pond in order to sample the open waters. Samples were fixed in situ in ethanol (final concentration, 76–85%), and all taxa were identified in the laboratory at species level using a stereoscope or a microscope depending on their size. Calanoida were identified according to the keys by [67–71]. Cladocera were identified following the guides of [72,73]. Refs. [71,74] were used to determine Cyclopoida; ref. [75] for Ostracoda; ref. [76] for Anostraca and Notostraca; and ref. [77] for Isopoda. Considering the difficulty in evaluating the quantitative data of taxa in ponds where the water volume might vary in few days, quantitative data are not judged as useful and only the presence–absence of each taxon has been recorded.

2.6. Data Analysis

The software used for data analysis was Gingko [78]. Quantitative analyses were performed following an approach based on Fuzzy Set Theory [79] in order to describe floristic groups by assigning a membership function (degree of belonging) ranging continuously between 1 and 0 for each site. In this way, a site belongs to every group according to its value of the membership function, in contrast to the classical Set Theory [79].

Fuzzy groups of floristically similar sites, named floristic types, were defined on the basis of presence–absence flora data by fuzzy c-means clustering [80], using the chord distance function [78] between pairs of objects. The m parameter of the method was set to 1.4. The tabulated results correspond to the degree of belonging of each site (on the rows), expressed in the interval [0.0, 1.0], to a specific floristic type (on the columns). The degree of belonging of each species to every floristic type was calculated as the average of the degrees of belonging of the sites where such a species was present. The obtained value showed the link between the species and a specific floristic type [81] (Feoli and Zuccarello, 1988). This method was applied to both the flora and the fauna datasets. In the case of fauna, the degree of belonging of each taxon to a specific floristic type is an expression of the bioindication value of each species for the specific environmental conditions of the floristic type.

The co-occurrence of flora and fauna taxa was also analysed through the calculation of the cosine of the angle based on presence–absence data collected.

3. Results

3.1. Abiotic Characteristics of the Water

The environmental features (available data) and the water variables (years 2021–23) showed an oscillation of environmental parameters, with a heterogeneous characterisation of the habitat (considered as represented by 13 sites and 16 measurements) different according to each site (Table S1).

The mean concentration of pH over all the ponds was 7.69 $\pm$ 0.69 (mean $\pm$ 1.0 SD); Dissolved Oxygen was 161.9 $\pm$ 93.4%; salinity was 0.22 $\pm$ 0.20‰; and temperature was 13.10 $\pm$ 2.30 °C. Among chemicals, Phosphorus (total, mg/L) varied from 0.01 to >1.15; the amount of Nitrates (mg/L) was 0.1 $\pm$ 0.2; Nitrites (mg/L) was 7.4 $\pm$ 13.4; and Ammonium NH_3 (mg/L) was 0.3 $\pm$ 0.5. Oscillations of chemicals in the water were also registered in the same ponds, if these were the recipient of repeated visits (as in the case of Bosco Don Tommaso—DTO and Padula Mancina—PMA). In these cases, however, the variation in parameters (seasonal difference) was not higher than the variation among different sites (see Table S1).

3.2. Flora

The total number of floristic taxa, including those uncertainly determined, is 158 (Table S2). The most frequent species (found in six or more sites) resulted to be *Mentha pulegium*, *Lythrum hyssopifolia*, *Polygon subspathaceus*, *Symphyotrichum squamatum*, *Juncus bufonius*, and *Ranunculus sardous.* Thirty-six species of the list are typical of the habitat MTP 3170*. Furthermore, there are 17 species of conservation interest and 4 NISs, of which 2 are naturalised (*Abutilon theophrasti*, *Amaranthus albus*) and 2 are invasive (*Symphyotrichum squamatum*, *Xanthium orientale* subsp. *italicum*). The sites showed an extreme variability in taxa richness, with the cupular pools of LEU having 5 taxa and the dolines of PMA having 53 taxa.

3.3. Aquatic Fauna

A total of 103 taxa have been found in the 16 selected ponds. Some of the sites (total, 8) were sampled at more than one date. Bosco Don Tommaso (DTO) and Padula Mancina (PMA) were investigated with fauna collections on six and five dates, respectively, and supplied the maximum taxa richness (32 and 34, respectively) (Table S3).

The most important zoological group was that of Crustacea, present with a total of 77 taxa, followed by Insecta (13) and Rotifera (7). The faunistic investigation added 56 Crustacea taxa to the list already available for Apulian 3170* MTPs obtained from the published literature ([37,38,82]). A total of 21 Crustacea taxa already listed in past investigations, on the contrary, were not found in the present survey.

Apart from the "heterogeneous" groups (specimens not identified at the level of species, e.g., Insecta families), the more frequent taxa (found in at least 6 of the 16 sites) were the Cyclopoida *Diacyclops bicuspidatus* (10 sites), *D. nanus* (8), and *Megacyclops viridis* (8), then the Cladocera *Chydorus sphaericus* (8), *Alona nuragica* (6), and *Pleuroxus leturneauxi* (6) and Rotifera *Keratella* sp. (6).

3.4. Habitat MTP 3170 Conservation Status*

The assessment of the conservation status of habitat 3170* at each site is provided in Table 2. The status was ascertained as "favourable" for only 7 out of 16 sites. The structural criterion was the most negative among those considered, and had a negative judgement for 12 out of 16 sites. It was possible to apply the areal criterion only to those sites for which accurate historical habitat coverage data are available. Finally, the functional criterion individuated the most frequent code of pressures as PA05 (abandonment of management/use of grasslands and other agricultural and agroforestry systems = cessation of grazing, mowing, and traditional agriculture).

Table 2. Assessment of the conservation status of habitat MTP 3170 for each site (see Table 1 for the meaning of the colours). NA = missing value; PA04 = removal of small landscape features for agricultural land parcel consolidation (hedges, stone walls, rushes, open ditches, springs, solitary trees, etc.); PA05 = abandonment of management/use of grasslands and other agricultural and agroforestry systems (e.g., cessation of grazing, mowing, or traditional farming); PA17 = agricultural activities generating pollution in surface or ground waters (including marine)).

Site	Estimated Area (m^2)	Areal Criterion	FCSi	Structural Criterion	High Importance Pressure Category	Fun-ctional Criterion	Habitat 3170 Conservation Status
BEL	1299	NA	−0.1	–	PA04	–	Bad
DTO	310	NA	−0.5	–	PA05	–	Bad
FEL	23	+	−0.1	–		+	Favourable
FER	62	+	−0.5	–	PA05	–	Inadequate
IAC	7	NA	−0.4	–	PA04, PA17	–	Bad
LEU	480	+	NA	NA		NA	Favourable
MAA	86	+	−0.3	-		+	Favourable
MAN	173	NA	0.0	+		+	Favourable
PAL	216	–	−0.3	–		+	Inadequate
PMA	3280	NA	−0.4	–	PA05	–	Bad
POS	215	+	−0.2	–		+	Favourable
SEM	7	NA	0.4	+		+	Favourable
SPE	377	NA	0.0	–	PA17	–	Bad
SPL	1073	NA	−0.6	–	PA05	–	Bad
SUR	49,730	NA	−0.4	–	PA05	–	Bad
ZE1	2620	NA	0.2	+	PA05	–	Favourable

3.5. Flora–Fauna Relationships

The floral and faunal taxa richness of the sites appeared to be positively correlated (Pearson corr. coeff. = 0.6656, t = 3.3373, df = 14, p value = 0.00489) (Figure 3). There were no correlations between the species richness of each taxonomic group and the coverage of the habitat 3170*.

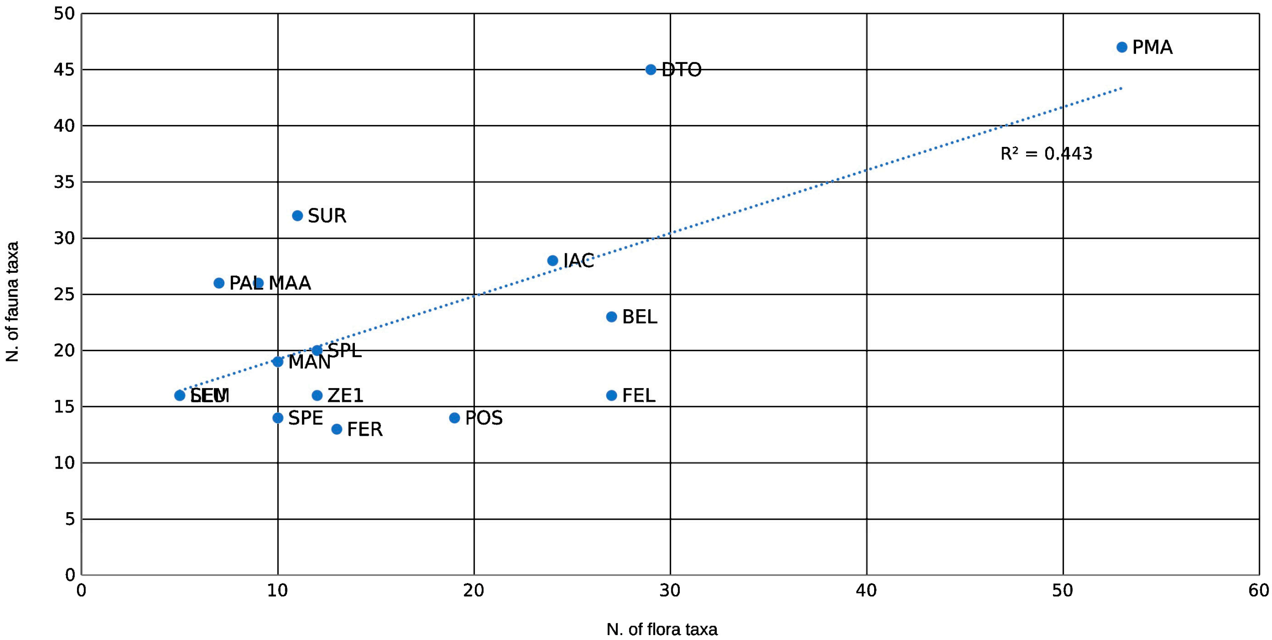

Figure 3. Relationship between the number of floristic and faunal taxa per site.

The fuzzy clustering of sites gave three floristic types, called g-1, g-2, and g-3 (Table 3). The type g-1 is mainly represented by cupular pools (CPs), and the type g-3 by dolines (Ds). The type g-2 equally showed characteristics of Ds and waterlogged soils (WSs) (Figure 4).

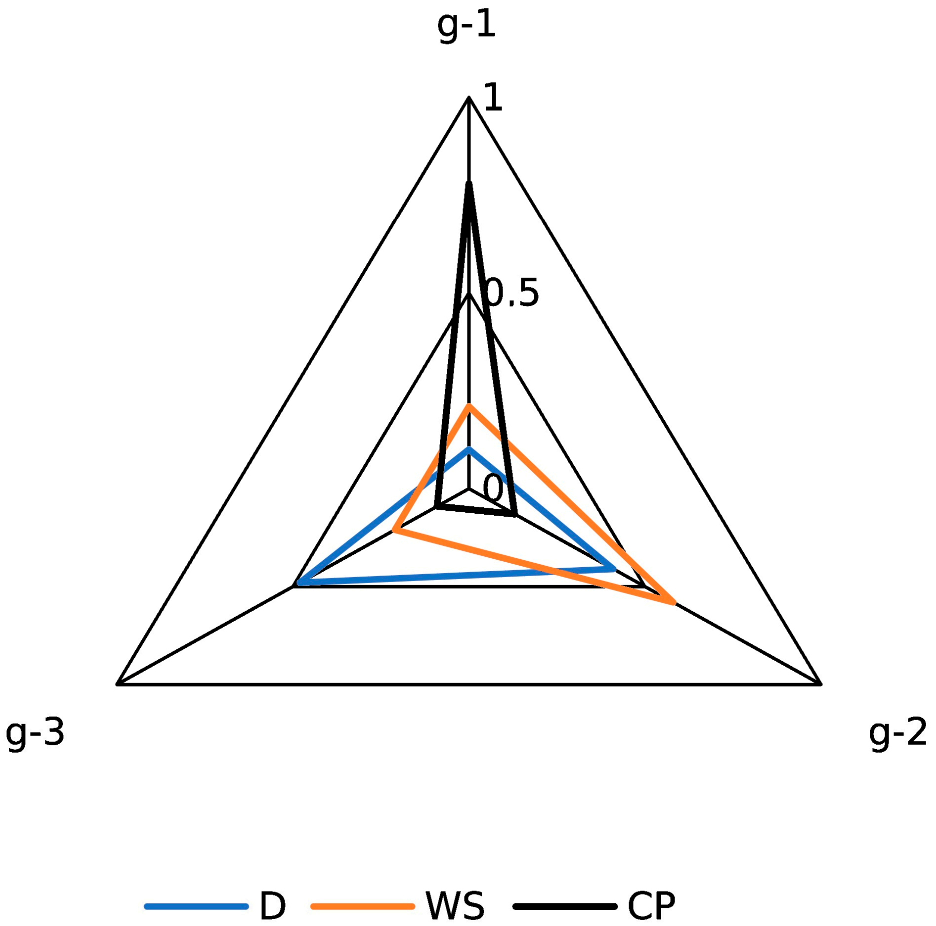

Figure 4. Average degrees of belonging of morphological types to the three floristic types (CPs = cupular pools; Ds = dolines; WSs = waterlogged soils).

Table 3. Degrees of belonging of plant species to the three floristic types (g-1, g-2, g-3). Colours classify species as typical of each morphological group (see Table 1 for correspondence of colours).

Species	g-1	g-2	g-3
Lythrum hyssopifolia L.	0.85	0.42	0.30
Mentha pulegium L.	0.41	0.83	0.77
Polypogon subspathaceus Req.	0.35	0.71	0.19
Juncus pygmaeus Rich. ex Thuill.	0.08	0.65	0.13
Lythrum borysthenicum (Schrank) Litv.	0.04	0.56	0.10
Ranunculus sardous Crantz	0.05	0.50	0.55
Juncus bufonius L.	0.42	0.55	0.14
Symphyotrichum squamatum (Spreng.) G.L. Nesom	0.41	0.40	0.52
Paspalum distichum L.	0.09	0.50	0.28
Lotus angustissimus L.	0.05	0.50	0.35
Poa infirma Kunth	0.47	0.01	0.01
Isolepis cernua (Vahl) Roem. et Schult.	0.08	0.46	0.13
Cynodon dactylon (L.) Pers.	0.03	0.09	0.44

The most important floristic taxa of each floristic type are deduced from the averages of the degrees of belonging to the sites (Figure 5). Assuming a minimum threshold of 0.4, the floristic type g-1 is characterised by *Lythrum hyssopifolia* which, among all the taxa of the same floristic type, showed the maximum level of belonging (0.85), followed by *Poa infirma*, *Juncus bufonius*, *Mentha pulegium*, and *Symphyotrichum squamatum*. Type g-2 is characterised by *Mentha pulegium* (0.83), *Polypogon subspataceus* (0.71), and *Juncus pygmaeus* (0.65). Type g-3 is characterised by *Mentha pulegium* (0.77), *Ranunculus sardous*, *Symphyotrichum squamatus*, and *Cynodo dactylon*.

Similarly, the most important faunal taxa of the floristic types were also individuated with the averages of degrees of belonging to the sites (Figure 6). Chironomidae (degree of belonging, 0.92) were the characterising animals of type g-1, together with other non-Crustacea–Artropoda (Culicidae, Isothomidae, Limnohalacaridae). The most important taxa of type g-2 resulted again to be Chironomidae, although with a lower degree of belonging (0.74), followed by a series of 7 micro-Crustacea species. The most important taxa of type g-3 were the Cyclopoida *Diacyclops bicuspidatus* (0.88) and *Megacyclops viridis* (0.70), with a higher degree of belonging to type g-3 than Chironomidae and Insecta in general.

The cosine of the angle calculated on the co-presences of floristic and faunistic taxa allows the individuation of the stable associations of taxa (Table 4). The selection of the most important species for each floristic type makes possible the individuation of such an association and its visualisation in diagrams (Figures 7–9).

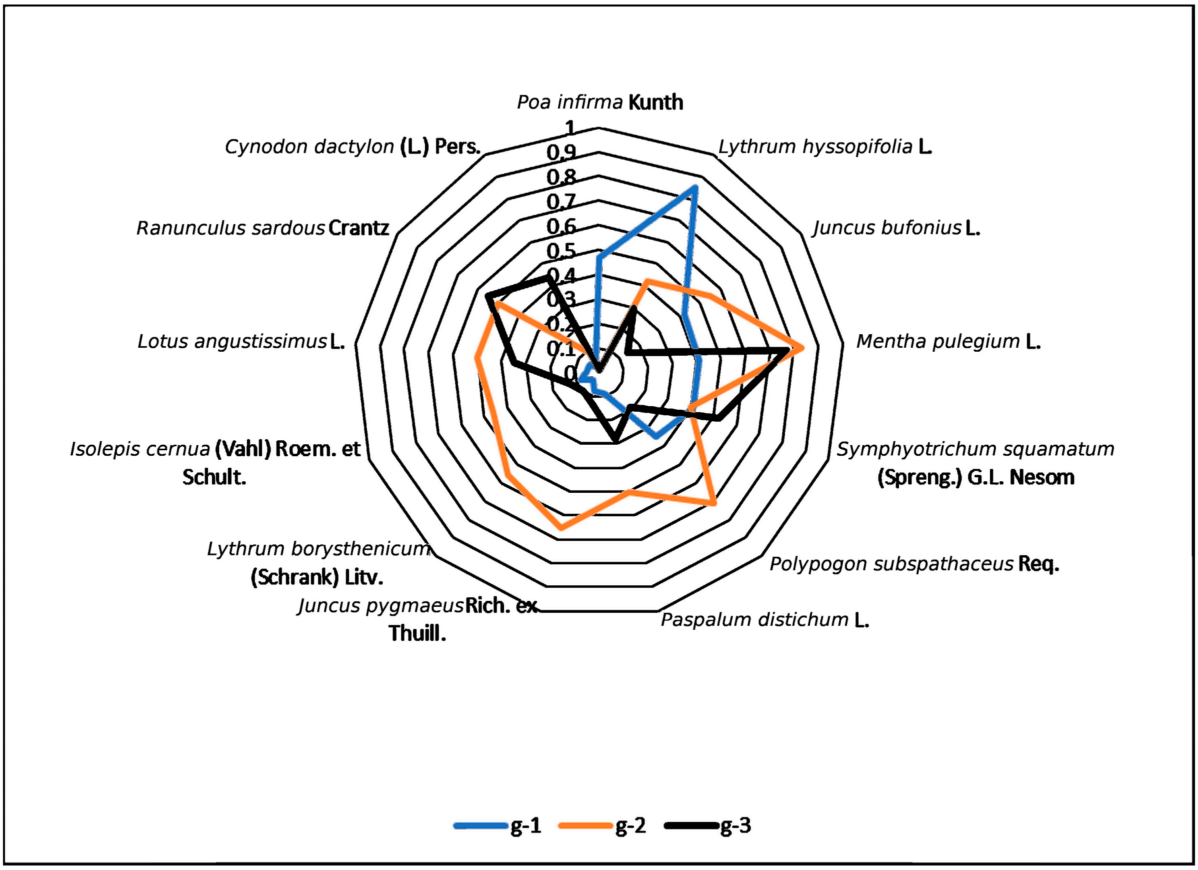

Figure 5. Degree of belonging of the most important plant species to the three floristic types.

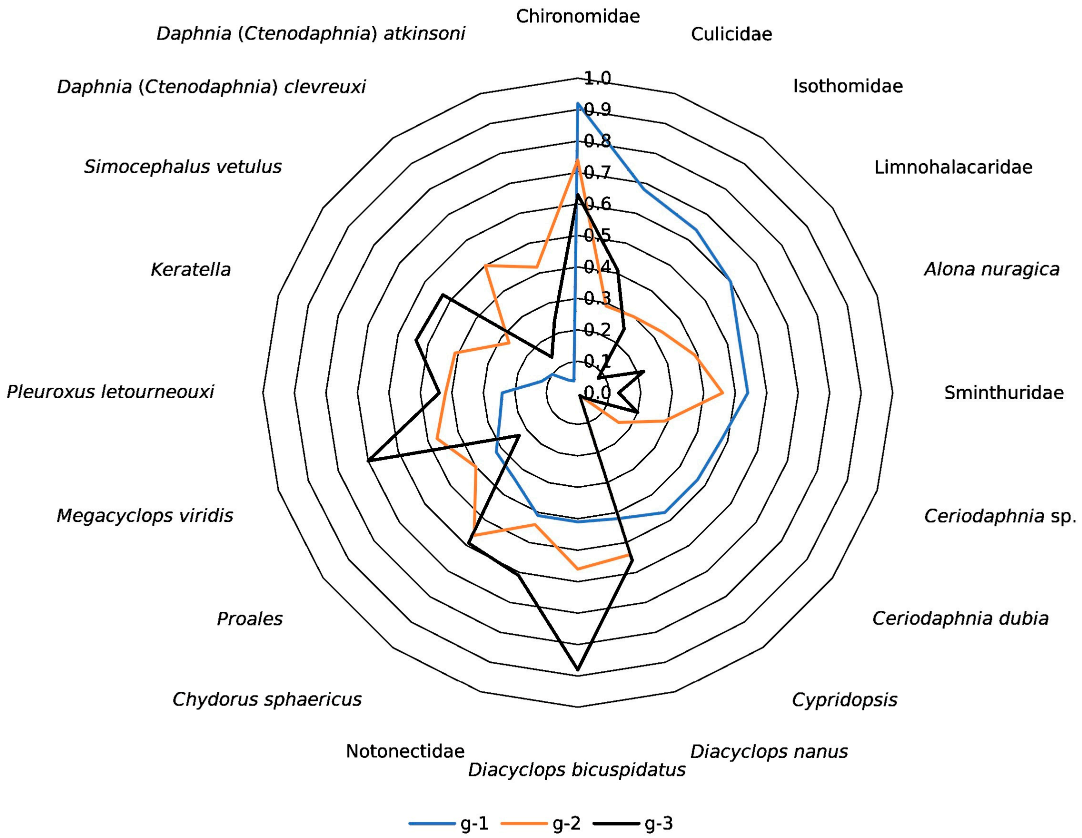

Figure 6. Degree of belonging of the most important faunal species to the three floristic types.

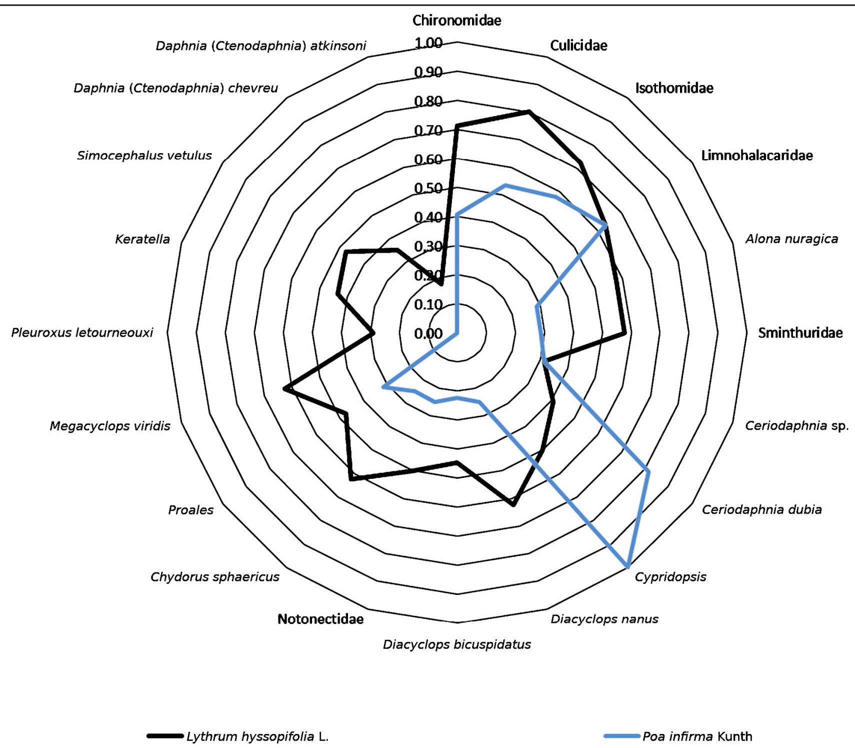

Figure 7. Fauna and plant species of floristic type g-1: similarities expressed as values of cosine of angle.

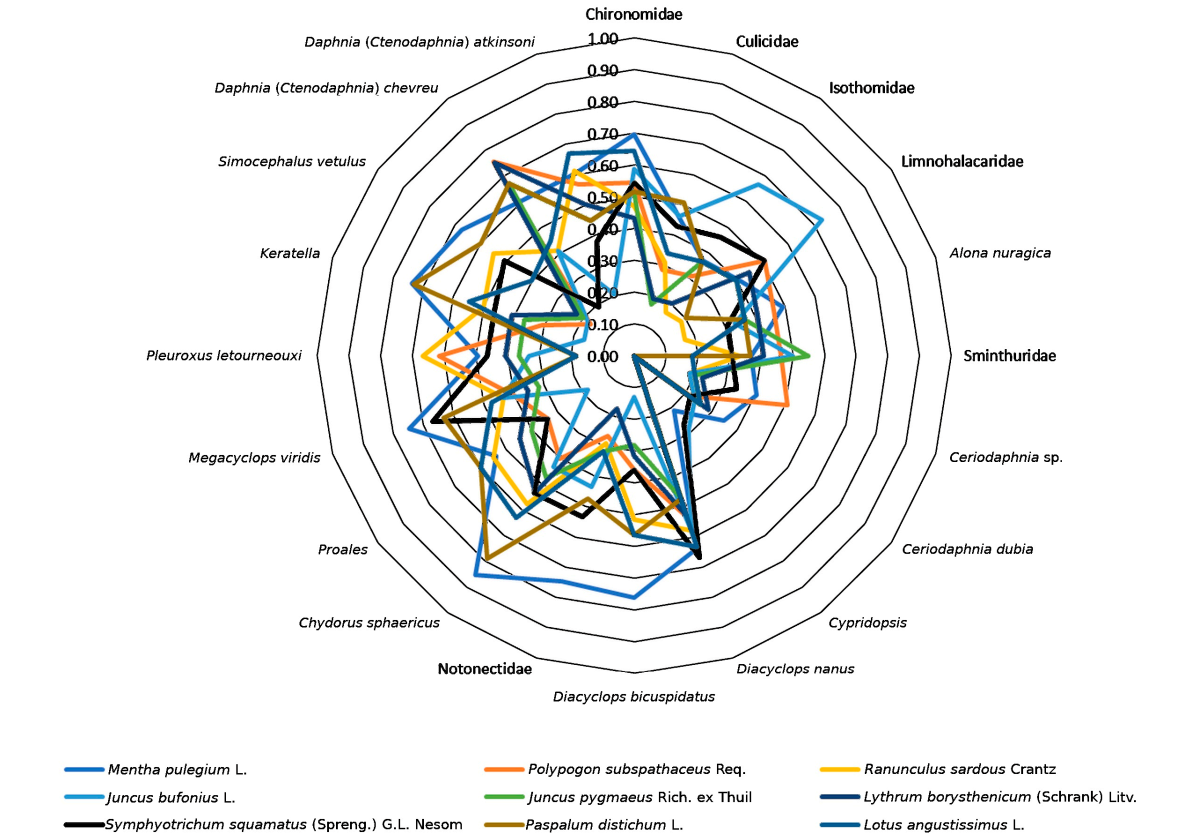

Figure 8. Fauna and plant species of floristic type g-2: similarities expressed as values of cosine of angle.

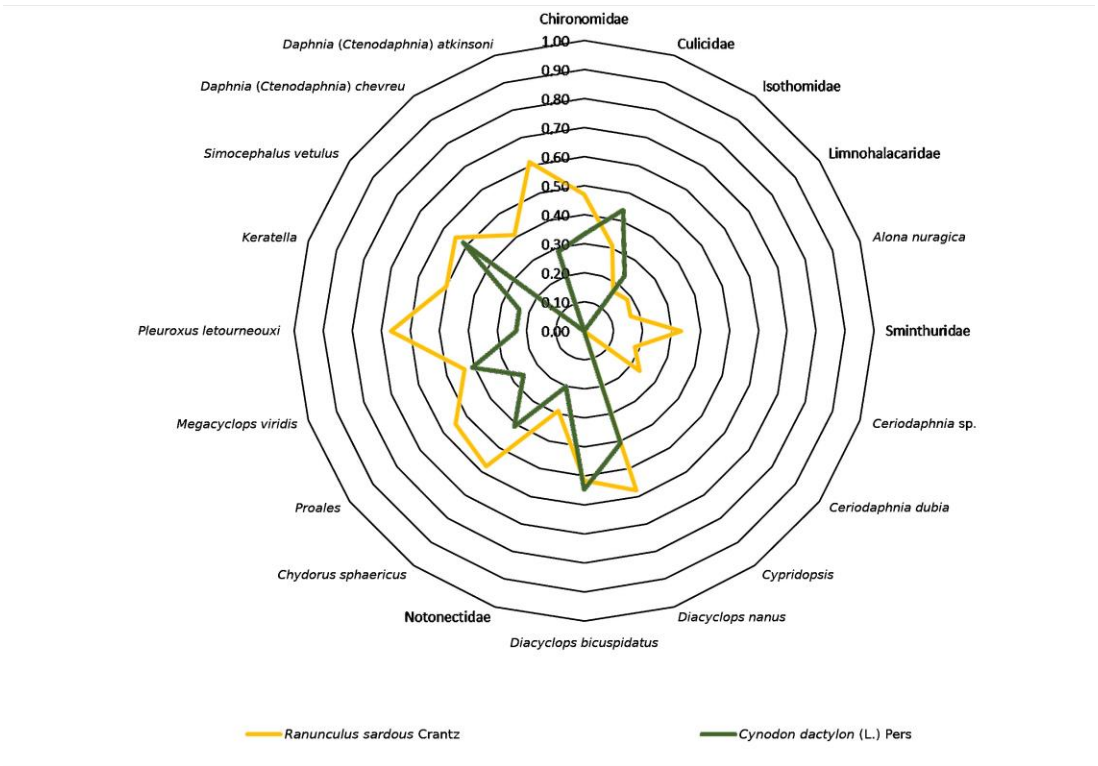

Figure 9. Fauna and plant species of floristic type g-3: similarities expressed as values of cosine of angle.

Table 4. Similarity (cosine of angle) based on co-presences of plant species and animal taxa. Maximum similarity between two species is indicated with the darkening of the cell colour (see Table 1 for the meaning of the colours).

Fauna\Flora	***Mentha puleg.* L.**	***Polypogon subspat.* Req.**	***Lythrum hyssopif.* L.**	***Ranunculus sardous* Crantz**	***Juncus bufonius* L.**	***Juncus pygm.* Rich. ex Thuil**	***Lythrum boryst.* (Schrank)**	***Isolepis cernua* (Vahl) Roem. et Schult.**	***Symphyotrich squam.* (Spreng.) G.L. Nesom**	***Paspalum distic.* L.**	***Lotus angust.* L.**	***Cynodon dactylon* (L.) Pers.**	***Poa infirma* Kunth**
Chironomidae	0.70	0.55	0.71	0.47	0.59	0.52	0.43	0.43	0.55	0.52	0.65	0.33	0.41
Culicidae	0.46	0.29	0.80	0.31	0.46	0.17	0.19	0.19	0.43	0.51	0.34	0.44	0.53
Isothomidae	0.37	0.31	0.72	0.17	0.67	0.37	0.20	0.41	0.46	0.37	0.37	0.24	0.58
Limnohalacaridae	0.40	0.51	0.63	0.18	0.73	0.40	0.45	0.45	0.51	0.20	0.40	0.00	0.63
Alona nuragica	0.49	0.46	0.58	0.17	0.33	0.37	0.41	0.41	0.31	0.37	0.37	0.00	0.29
Sminthuridae	0.37	0.46	0.58	0.33	0.50	0.55	0.41	0.41	0.31	0.37	0.18	0.00	0.29
Ceriodaphnia sp.	0.40	0.51	0.32	0.18	0.18	0.20	0.22	0.45	0.34	0.00	0.20	0.00	0.32
C. dubia	0.35	0.22	0.41	0.24	0.24	0.26	0.29	0.00	0.22	0.26	0.26	0.00	0.82
Cypridopsis	0.21	0.00	0.50	0.00	0.29	0.00	0.00	0.00	0.27	0.00	0.00	0.00	1.00
Diacyclops nanus	0.64	0.53	0.63	0.58	0.58	0.47	0.53	0.35	0.67	0.47	0.63	0.41	0.25
D. bicuspidatus	0.76	0.36	0.45	0.52	0.13	0.28	0.32	0.32	0.36	0.57	0.57	0.55	0.22
Notonectidae	0.75	0.27	0.50	0.29	0.43	0.32	0.18	0.53	0.53	0.47	0.32	0.20	0.25
Chydorus sphaericus	0.85	0.40	0.63	0.58	0.43	0.47	0.53	0.18	0.53	0.79	0.63	0.41	0.25
Proales sp.	0.54	0.34	0.47	0.55	0.18	0.40	0.45	0.22	0.34	0.60	0.60	0.26	0.32
Megacyclops viridis	0.75	0.40	0.63	0.43	0.43	0.32	0.35	0.18	0.67	0.63	0.47	0.41	0.00
Pleuroxus letourneouxi	0.49	0.62	0.29	0.67	0.33	0.37	0.41	0.41	0.46	0.18	0.18	0.24	0.00
Keratella sp.	0.74	0.31	0.43	0.50	0.17	0.37	0.41	0.20	0.46	0.73	0.55	0.24	0.00
Simocephalus vetulus	0.67	0.17	0.47	0.55	0.18	0.20	0.22	0.00	0.51	0.60	0.40	0.52	0.00
Daphnia (Ctenodaphnia) chevreuxi	0.60	0.76	0.35	0.41	0.41	0.67	0.75	0.50	0.19	0.67	0.45	0.00	0.00
Daphnia (Ctenodaphnia) atkinsoni	0.60	0.57	0.18	0.61	0.20	0.45	0.50	0.50	0.38	0.45	0.67	0.29	0.00

4. Discussion

The study of 16 sites of the habitat MTP 3170* in the same geographical area (South Apulia) allowed one to establish that dolines (Ds) and waterlogged soils (WSs) share a common floristic assemblage representing the floristic type g-3 (Table 3, Figure 4). Ds, however, mostly host plant species of the floristic type g-2, and CPs are characterised by the floristic type g-1. It is easy to recognise that CPs (four sites) have the lower species richness (5–26 floristic species, and 8–19 faunal species) and that this could be due to both their small size and short hydroperiod, which impede a more thorough collection of samples. In fact, in Figure 2, it is evident that the highest number of taxa (both flora and fauna) belong to the two sites (DTO, PMA) subject to repetitive plankton collection. This notwithstanding, some species can be recognised as highly characteristic: *Lythrum hyssopifolia* and *Poa infirma*, very typical of floristic type g-1, are highly characteristic of CPs (Table 3). The floral–faunal association study individuates *Poa infirma* as highly associated with the Cladoceran *Ceriodaphnia dubia* and the Ostracoda of the genus *Cypridopsis* (Table 4). In general, CPs show a prevalence of Insecta taxa on the Crustacea ones, and this also justifies the association between *Lythrum hyssopifolia* and Culicidae (Diptera) in the same CPs. The lack of faunal species identification (mostly Insecta larvae, not identified at the level of species), however, does not encourage one to see such a result as useful for an unequivocal choice of CPs' animals as indicators of MTPs 3170*. The Ostracoda of the genus *Cypridopsis*, on the contrary, are typical and exclusive of CPs in the present study, and they can be proposed as characteristic (if not indicators, because rare) of the habitat.

The nine D sites showed a high species richness (9–53 floristic taxa, and 6–34 faunal taxa, per site), but this category comprises a couple of sites (DTO and PMA) repeatedly visited and sampled; thus, probably the result has been conditioned by the overall methodological approach for aquatic micro-invertebrates. *Polypogon subspathaceus*, *Juncus pygmaeus*, *Lythrum borysthenicum,* and *Isolepis cernua* were the most characteristic plant species of the floristic type g-2 and, consequently, also of D environments. For all these four species, the association with faunistic taxa had the maximum values with *Daphnia* (*Ctenodaphnia*) *chevreuxi,* and *Daphnia* (*Ctenodaphnia*) *atkinsoni.*

The three sites of WS were the less represented morphological type of MTP 3170*, and they share plant species also with D environments. In any case, *Ranunculus sardous*, *Paspalum distichum*, *Lotus angustissimus,* and *Cynodon dactylon* can be considered characteristic of the WS environment of the habitat 3170*. *P. distichum* and *L. angustissimus* (g-3 floristic group), in detail, are highly correlated with *Chydorus sphaericus* (Cladocera) and *Proales* sp. (Rotifera).

Both flora and fauna showed species characteristic of single morphological types of the habitat MTP 3170*, and their association encourages one to work for a description of the whole biological assemblage that could be defined as typical of MTPs, with the aim to propose indicator species of the habitat, although the low number of sites analysed (see WS environments) suggests caution in this very preliminary proposal. On the contrary, some taxa (such as Cyclopoida *Diacyclops bicuspidatus* and *Megacyclops viridis*) are generally common in the habitat, and it could be good, in future, to clarify if they can be assumed as indicators of the habitat (without floristic association preferences) or simply common in any kind of temporary pond.

The conservation status of the habitat MTP 3170* was assessed as "favourable" only in 7 out of 16 sites. This is not a surprise in a territory hosting human activities for thousands of years and deprived of pristine and undisturbed sites (i.e., inaccessible to human activities). Perturbations may result in an impoverishment of the characteristic fauna of the habitat [83,84], but our analysis is not able to detect this phenomenon, since the three floristic types (g-1, g-2 and g-3) are not differently considered in different conservation statuses.

A series of studies [6,24–32] demonstrated that the hydroperiod of temporary ponds has a role in the composition of faunal assemblages. In the present research, however, we tried to focus our attention on the possibility of proposing animal species as indicators of the habitat MTP 3170*, distinguishing ponds on the basis of floristic assemblages. The analysis showed a high degree of linkage of some animal species with the floristic assemblages considered (passing the threshold of 0.4 degree of belonging). This result confirms an already known characteristic of the habitat MTP 3170*, namely that plant species appear more linked to local conditions than crustaceans, and floristic assemblages appeared to be better differentiated among sites, probably in dependence on different responses deriving from the turn-over rate and/or by dispersal capacity of each taxon [85].

Habitat MTP 3170* studies have revealed relevant knowledge gaps among different taxonomic groups. Vascular plants are the most studied organisms and the only ones to be used as habitat MTP 3170* indicators, despite this habitat being a biodiversity hotspot for other taxonomic groups, such as macroinvertebrates [86]. For the strictly temporary (ST) ponds of Alta Murgia, the only animal species identified as possible habitat indicators are Crustacea (the calanoid *Diaptomus cyaneus*, the anostracan *Chirocephalus diaphanus*, the cyclopoid *Diacyclops lubbocki*, and the Cladoceran *Macrothrix hirsuticornis*, [6]). The new analysis, conducted on a larger territory, taking into account the heterogeneity of the habitat MTP 3170* in terms of morphological types (CP, D, WS) and their related floristic types (g-1, g-2, g-3), allowed us to reveal a higher number of taxa. Of the 103 animal taxa here considered, 20 showed a degree of belonging higher of 0.4 to one or more floristic assemblages (Figure 6), thus justifying their proposal for the role of indicators for the habitat MTP 3170* or for some specific subtype. More than an independent study of faunal assemblages to find MTP indicators, what the present study encourages is the extension of this comparative approach to more sites, to confirm or to refine the search and the identification of animal species useful as indicators of the habitat MTP 3170*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d16090559/s1, Table S1: Environmental variables of water; Table S2: Vegetation survey results; Table S3: Faunal survey results.

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