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Original Research

Environmental Conditions and Macrophytes of Karst Ponds

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Abstract

Man-made karst ponds are the only surface water bodies in the Karst region (SW Slovenia, NE Italy) and support variable biotic communities, depending on ecological parameters and human activities. The aim of our study was to establish the relationship between environmental conditions (land-use of the catchment, vegetation of the banktop zone, morphological properties of the ponds, water quality parameters, the presence of fish) and aquatic macrophyte species composition and abundance. The survey of 28 ponds was performed in the peak vegetation period in 2009. The most abundant emergent macrophytes found were *Typha latifolia*, *Alisma plantago-aquatica*, *Eleocharis palustris* agg., and *Alisma lanceolatum*, while the most common hydrophytes were *Lemna minor*, *Chara* spp., *Nymphaea alba*, and *Potamogeton natans*. About 15% of all species found in ponds are included in the "Red" list of endangered plant species of Slovenia. The presence of fish, water transparency, pH, land-use of the pond catchment and quality of shore substrate were detected as the most influential factors determining aquatic macrophyte communities.

Keywords: Karst, pond, environmental parameter, floristic composition

Introduction

Ponds are small lentic ecosystems that permanently or temporarily hold water [1]. They are shallow and their size ranges from a few square metres to several hectares. They can be natural or man-made. Their number is much higher than that of large lakes, which constitute a small percentage of the total number of lakes [2]. Despite this, studies of lentic ecosystems have concentrated mainly on moderately large lakes [2].

Ponds differ functionally from larger lakes [3], since their littoral structure and its productivity dominates the ecosystem [2]. Despite their small size they contain a significant part of aquatic biodiversity on the landscape scale [4, 5]. Humans have created millions of ponds for multiple purposes [2], but today they serve as refugia for a variety of freshwater biota [5] and are, as such, an irreplaceable type of habitat [6-8].

The biotic communities that develop in ponds depend on environmental conditions and human activity. Several studies document clear associations between the communities and a variety of environmental gradients, such as hydro-period [9, 10], surface area [11], and water properties like water transparency, nutrient availability, and pH [12-14]. Compared to rivers and lakes, ponds exhibit greater environmental and, consequently, biotic amplitudes [15].

Macrophytes are important components of aquatic ecosystems (including ponds), affecting their function through energy flow, nutrient cycling, sedimentation processes, and habitat provision [16, 17]. Their presence and abundance reflect the quality of the ecosystem as a whole. Aquatic macrophytes not only are affected by water quality, but they also affect water quality and provide food and refugia for aquatic invertebrates and fish [11, 18, 19]. Their growth is controlled by a variety of factors, namely water quality, water depth, water movement, substrate characteristics, and biotic interactions [20, 21]. Therefore, a survey of aquatic macrophytes is important from the point of

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view of plant species diversity, as well as of factors explaining the diversity of other groups (e.g. macro-invertebrates, periphyton) [22] and as indicators of water and habitat quality [e.g. 23-28]. Deterioration of the physical environment and eutrophication of water bodies result in changes in macrophyte distribution, a decline in aquatic macrophyte species richness, and a greater abundance of more resistant species [29].

Karst ponds are of human origin, presenting an outstanding natural and cultural heritage [30]. Following mass regulation of rivers and streams all over Europe, man-made systems have become a substitute habitat for numerous organisms [6]. This is the case in the karst ponds, which provide an important network of water-bodies in the Karst Region. The fact, that they are the only surface water-bodies on the Karst Plateau [31] makes them even more important.

The aims of our study were to examine the habitat characteristics and/or ecological conditions in karst ponds, as well as surveying the presence and abundance of aquatic macrophyte species. We also aimed to establish the relations between habitat characteristics and aquatic macrophyte species composition. The specific questions addressed were:

- Which environmental conditions significantly influence aquatic macrophyte species composition in karst ponds?
- How much of the variation of aquatic macrophyte species composition is explained by considered environmental conditions and how do they affect macrophytes species richness?

Materials and Methods

Study Area

Twenty-eight karst ponds were investigated in the Karst region in SW Slovenia. The ponds are distributed on the Karst Plateau along the border with Italy (from 13º38’08” E, 45º50’43” N to 13º59’36” E, 45º39’54” N) (Table 1). The climate is sub-Mediterranean, annual precipitation is 1,500 mm [32] (about two-fold higher than evapotranspiration). The largest amount of precipitation occurs in November, and the least in the peak of the vegetation period in July [32]. The mean annual temperature is 11ºC [33]. The altitude ranges from 195 to 420 m a.s.l.

Aquatic Macrophyte Survey

The abundance of aquatic macrophytes was evaluated using a 5-degree estimation scale [34]:

1 = very rare
2 = infrequent
3 = common
4 = frequent
5 = abundant, predominant

The whole pond was a sampling unit. Plants were identified using the following keys: Martinčič et al. [35] and Preston [36]. Plants whose reproductive organs were not present at the time of sampling are listed by genus (e.g. *Bidens*, *Epilobium*, *Sparganium*). The survey was performed in July and August in 2009. The nomenclature of ferns and flowering plants follows Martinčič et al. [35]. The definition of aquatic macrophytes is in accordance with Hutchinson [37] and Wetzel [2], therefore woody species and other non-hygrophilous species were excluded from analyses.

Plant species richness in the ponds was calculated as species number (N) per surface area and/or approximate volume of the studied water bodies.

Table 1. Locations of the studied karst ponds (y,x: Gauss-Krüger coordinates).

No.	Location	y	x
1	Brje	400900	71766
2	Dutovlje	410018	68693
3	Gorjansko	399759	74392
4	Klanec	398653	74742
5	Kobjeglava	408070	75514
6	Lukovec	408706	77326
7	Pliskovica	404645	70288
8	Ponikve	411870	72180
9	Dobravlje	413580	69726
10	Coljava	405687	73453
11	Gorjansko	400565	74089
12	Kobjeglava	407750	75870
13	Kobjeglava	407593	75500
14	Lukovec	408368	77438
15	Komen	403264	75733
16	Lipa	399416	78933
17	Škrbina	401753	78508
18	Škrbina	402083	78595
19	Komen	403383	75734
20	Coljava	405481	73703
21	Coljava	405481	73703
22	Tomaj	411472	68720
23	Dutovlje	410096	68135
24	Lipa	399319	78752
25	Matavun	421734	58156
26	Škocjan	421942	58350
27	Škrbina	401070	79535
28	Tomaj	411110	68917

Assessment of Environmental Conditions

Parameters were recorded in the field, measured and/or calculated in the laboratory (Table 2). Independent environmental variables were tested for their significance in shaping aquatic macrophyte species composition. These variables can be classified into three groups [38]: quantitative variables, semiquantitative estimates, and categorical variables. Quantitative variables include morphometric parameters of the specific pond (length, width or diameter (m), surface (m^2), depth (m), approximate volume (m^3), surface:depth ratio (S/d)), and water quality parameters (pH, temperature (T), conductivity (EC), O_2 concentration, O_2 saturation, transparency, chlorophyll-*a*). Semiquantitative estimates include habitat characteristics (land-use of the catchment, bottom substrate, shore substrate, type of vegetation in banktop zone, width of woody and/or wetland banktop zone). Categorial variables include data about the presence/absence of: fish, filamentous algae, slime, woody species in the pond.

Water samples were taken from each pond in August 2009. The majority of the water quality parameters (pH, O_2 concentration, O_2 saturation, T and EC of water) were measured *in situ* using the portable multi-meter PCD 650 (Eutech Instruments, Singapore). Water transparency and concentration of chlorophyll-*a* were analyzed spectrophotometrically in the laboratory on the same day.

Habitat characteristics were assessed as semiquantitative estimates in a similar way as in many studies of aquatic ecosystems [39-41]. The estimated gradients were as follows:

(1) land-use of the pond catchment, according to decreasing human influence (1 = settlement or arable land/vineyard or road, 2 = mixed arable land and grassland, 3 = grassland, 4 = mixed grassland and forest, 5 = forest)
(2) the type and/or structure of the shore substrate, according to decreasing coarseness of the substrate (1 = concrete, 2 = stone-wall, 3 = stones and stone-wall, 4 = stones, 5 = loam and clay)
(3) of the bottom (1 = concrete, 2 = stones and concrete, 3 = stones, 4 = stones and loam, 5 = loam and clay)
(4) width of the littoral zone (1 = <1 m, 2 = 1-5 m, 3 = >5 m)
(5) vegetation of the banktop zone, according to decreasing human influence (1 = absent, 2 = ruderals, 3 = perennial herbs and grasses, 4 = pioneer woody species (*Salix* sp., *Populus* sp.), 5 = climax species of trees and shrubs).

Statistical Analyses

Detrended correspondence analysis (DCA) was used for exploratory data analysis. The eigenvalue for the first DCA axis was greater than 0.4 and indicated strong unimodality [42], therefore canonical correspondence analysis (CCA) was chosen. Relationships between site conditions and vegetation were analyzed using direct gradient analysis (CCA). Separate CCAs for each environmental variable were performed to test the significance of its influence and the explanation of species composition variation. The analysis was run with scaling for inter-sample distances, and bi-

Table 2. Ecological parameters (morphometric and water quality parameters) of the studied karst ponds. 1-7: species-poor "walled" ponds with prevailing hydrophytes, 8-22: species-rich ponds with prevailing emergent plants, 23-28: ponds without macrophytes.

Pond No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
surface (m^2)	32	177	65	130	79	127	213	28	603	85	745	9	52	35	547	391	255	72	269	64	50	131	98	79	228	51	7	87
depth (m)	0.7	1.5	0.3	1.2	3.3	0.4	0.8	0.2	1.3	0.6	3.0	0.7	0.5	0.4	0.5	0.6	1.0	1.1	0.3	0.2	0.3	0.9	2.6	2.2	1.0	0.9	0.3	1.3
volume (appr.) (m^3)	22	265	18	156	255	51	170	5	783	51	2235	6	26	13	274	234	247	79	67	11	14	118	255	173	228	47	2	113
surface/depth ratio (S/d)	45	118	232	108	24	318	266	156	464	141	248	13	103	92	1095	651	262	65	1075	353	186	146	38	36	228	55	24	67
pH	7.2	6.3	6.5	6.9	6.4	6.5	6.1	6.0	6.0	6.4	7.0	7.2	8.2	6.9	6.5	7.6	6.8	7.2	6.3	6.7	6.2	6.3	6.5	7.2	8.3	7.9	6.7	6.5
T_{water} (ºC)	21.7	22.9	18.5	23.8	24.6	17.9	29.0	19.9	27.1	26.5	23.7	23.1	22.2	19.9	20.5	27.9	25.7	25.1	20.7	22.5	22.1	19.3	24.4	22.4	28.5	26.8	23.3	19.5
conductivity (µs/cm)	144	195	367	167	290	305	66	202	300	209	132	212	124	138	188	213	226	106	279	430	134	765	189	275	152	128	225	222
O_2 (mg/l)	11.3	1.6	0.2	12.6	2.0	1.0	14.7	2.8	10.3	11.0	7.3	8.9	14.7	8.1	5.5	21.9	7.0	8.2	1.7	3.5	8.4	0.2	6.5	7.3	19.2	11.3	9.8	3.1
O_2 saturation (%)	127	20	5	150	25	11	187	36	126	140	86	104	170	85	71	274	87	101	18	42	96	10	79	88	243	140	120	35
transparency	0.09	0.01	0.60	0.01	0.02	0.07	0.07	0.00	0.03	0.01	0.13	0.01	0.35	0.28	0.01	0.03	0.05	0.02	0.078	0.03	0.03	0.09	0.01	0.01	0.122	0.06	0.00	0.01
Chl *a* (µg/l)	0.86	27.11	9.24	36.33	58.38	35.03	116.76	6.81	157.60	22.87	16.97	0.95	708.07	4.87	311.36	0.57	1.52	36.49	65.19	49.26	0.01	13.34	9.34	27.80	98.27	7.78	0.28	2.24

Table 3. Floristic composition of the studied ponds. 1-7 species-poor “walled” ponds with prevailing hydrophytes, 8-22: species-rich ponds with prevailing emergent plants.

Pond No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
No. of plant taxa	2	4	4	3	2	3	5	9	16	5	12	7	7	4	17	14	9	15	17	12	9	11
Submerged hydrophytes																						
Ceratophyllum demersum	.	4	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.
Potamogeton crispus	5	2	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Potamogeton trichoides	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.
Chara spp.	.	.	.	3	.	.	.	.	1	3	.	1	.	.	2	.	.	1	.	1	.	.
Floating hydrophytes																						
Potamogeton nodosus	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Potamogeton natans	.	3	.	.	.	.	2	.	3	.	.	.	.	.	.	2	.	.	.	.	.	.
Lemna minor	.	2	2	3	.	5	4	1	.	.	.	.	.	.	2	1	.	.	.	.	.	5
Nymphaea alba	.	.	.	.	3	.	.	.	1	.	1	.	.	.	.	.	.	1	1	1	.	.
Emergent macrophytes																						
Typha latifolia	.	.	.	.	.	.	.	.	4	1	.	1	1	2	4	2	.	1	4	1	.	5
Alisma lanceolatum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	2	2	1	4
Eleocharis palustris	.	.	.	.	.	.	.	2	1	.	.	.	.	.	2	.	1	1	1	3	1	.
Typha angustifolia	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	1	.	1	.	.	.
Bolboschoenus maritimus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	2	.	.	.
Schoenoplectus lacustris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	2	.	.	.
Schoenoplectus tabernaemontani			.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
Phragmites australis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.
Alisma plantago-aquatica	.	.	1	.	.	4	.	1	2	.	1	.	.	.	.	.	1	2	1	.	.	.
Marsh taxa																						
Juncus inflexus	.	.	.	.	.	.	.	.	2	2	.	1	3	.	1	.	1	2	1	.	2	2
Juncus effusus	.	.	.	.	.	.	.	.	2	.	1	.	2	.	1	.	.	1	2	.	.	.
Juncus articulatus	.	.	.	.	.	.	.	.	2	.	.	.	.	2	2	.	.	1	1	.	.	.
Polygonum lapathifolium	.	.	.	.	.	.	.	.	.	.	1	.	2	.	.	.	.	2	.	2	.	.
Lycopus europaeus	.	.	.	.	.	3	1	.	.	.	.	1	1	.	.	1	1	.	.	.	.	2
Sparganium sp.	1	.	.	.	.	.	.	.	1	.	1	.	.	.	.	.	.	.	.	1	.	.
Juncus compresus	.	.	.	.	.	.	.	.	2	.	1	.	.	.	.	.	.	.	.	.	.	.
Carex otrubae	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	1	.
Galium palustre	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Iris pseudacorus	.	.	1	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	1	.	.	.
Phalaris arundinacea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.
Rorripa palustris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.
Stachys palustris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.
Flood-meadow taxa																						
Agrostis stolonifera agg.	.	.	.	.	.	.	.	1	2	.	1	.	.	.	2	2	2	.	2	2	.	.
Bidens sp.	.	.	.	.	.	.	.	.	1	.	1	1	.	.	1	.	.	1	1	.	1	.
Carex hirta	.	.	.	.	.	.	.	.	.	.	2	.	.	.	1	.	.	1	.	2	2	2
Echinochloa crus-galli	.	.	.	.	.	.	2	1	.	.	.	1	.	.	.	.	2	2	.	1	.	.

Table 3. Continued.

Pond No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
No. of plant taxa	2	4	4	3	2	3	5	9	16	5	12	7	7	4	17	14	9	15	17	12	9	11
Epilobium hirstum	.	.	.	.	.	.	.	.	.	1	1	.	.	2	1	.	.	.	.	.	.	.
Epilobium parviflorum	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.	.	1	.	.	.
Juncus tenuis	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.
Lysimachia nummularia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.
Mentha x verticillata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.
Polygonum mite	.	.	.	.	.	.	2	1	.	1	.	.	.	.	.	1	.	.	.	.	.	2
Polygonum persicaria	.	.	.	.	.	.	.	.	1	.	.	1	.	1	.	.	2	.	.	.	.	.
Potentilla reptans	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.
Rorripa silvestris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1	.	.	.	.
Rumex conglomeratus	.	.	.	.	.	.	.	.	.	.	.	.	2	.	1	1	.	.	1	.	1	2
Rumex crispus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
Solanum dulcamara	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	2	.	2	.	.	1	3
Epilobium sp.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
Other taxa																						
Polygonum sp.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.
Potamogeton sp.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.
Rumex sp.	.	.	.	.	.	.	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.

plot scaling to relate gradients in floristic composition to the explanatory variables. Forward selection of explanatory variables was used to provide a ranking of the relative importance of the specific variables and to avoid co-linearity [43]. We used the unrestricted Monte Carlo test with 999 permutations to test the statistical significance of the variables and canonical axes. Another CCA was done with a subset of selected variables only ($p<0.1$), and the proportions of variance explained by these variables were calculated. Some of the variables were not normally distributed so the data were log transformed. Ordination of the ponds according to the most important environmental parameters was made using CCA. The whole set of analyses was performed using CANOCO 4.5 [44].

Differences in environmental parameters between ponds with and without macrophytes were tested for significance using t-test. Species richness was correlated with environmental parameters using Spearman coefficient in SPSS version 17.

Results

Aquatic Macrophyte Survey

Different growth forms of plants were found: submerged hydrophytes, floating hydrophytes, helophytes, marsh species, and flood-meadow species (Table 3). The most abundant aquatic macrophyte species found in karst ponds were: *Typha latifolia*, *Lemna minor*, *Alisma plantago-aquatica*, *Eleocharis palustris*, *Chara* spp., *Alisma lanceolatum*, and *Potamogeton natans*. Species from the Red List of Slovenia [45] are relatively numerous, constituting 15% of the total of 50 plant species recorded in ponds.

Floristically Defined Types of Karst Ponds

Leaving aside the smaller group of ponds that contained no aquatic macrophytes, the ordination plot based on results of preliminary DCA showed clustering of the ponds according to floristic composition. Two groups of ponds that were ecologically sound were delineated – a group of seven less similar species-poorer, mostly walled ponds, and the larger group of 15 species-richer ponds with loamy shores.

The group of walled ponds was species-poorer and characterized by the presence of different pond-weeds (*Potamogeton crispus*, *P. nodosus*, *P. natans*, *P. trichoides*) and a higher proportion of other submerged and/or floating hydrophytes, which constitute half of the species. The group of relatively shallow ponds was characterized by a significantly higher total number of plant taxa, with *Typha latifolia*, *Alisma lanceolata*, *Eleocharis palustris* agg., *Juncus inflexus*, *J. effusus,* and other emergent macrophytes and marsh species. Emergent species constitute a quarter of all species, hydrophytes only 12%, and marsh species were most common, constituting 36% of the plant taxa.

Environmental Conditions and Gradients

A preliminary DCA revealed a first gradient length of 6.49 SD, which indicates strong unimodality, so CCA was chosen. Separate CCAs for each environmental variable revealed that some of the variables influence floristic composition significantly: fish presence, water transparency, pH, land-use of the catchment, and shore substrate. Analysis with forward selection provided a ranking of the relative importance of the specific significant variables (Table 4).

The presence of fish, water transparency, pH, land-use of the catchment area of the pond, and shore substrate were revealed to be the most influential factors determining aquatic macrophyte communities, explaining species variation significantly. These variables explain 32% of the total variation of the data set.

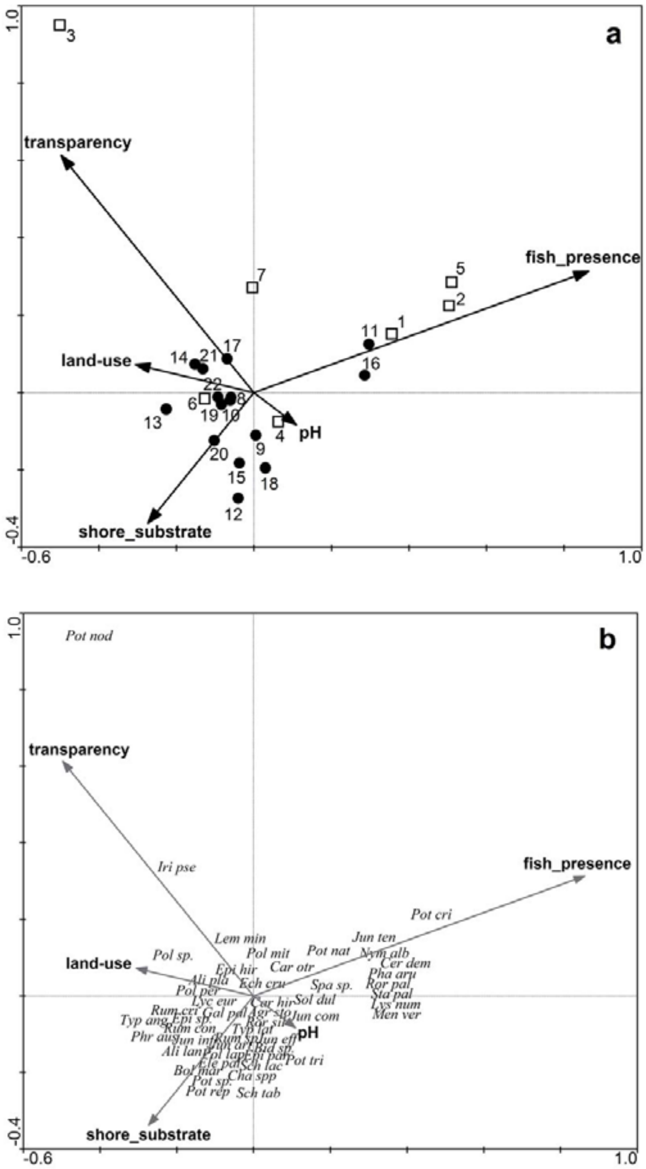

Fig. 1. Ordination diagrams of karst ponds based on Canonical Correspondence analysis (CCA), with most important environmental variables. Eigenvalues of the first two axes were 0.5 and 0.45, respectively. Fig. 1a: □ = species-poor "walled" ponds with prevailing hydrophytes, ● = species-rich ponds with prevailing emergent plants. Fig. 1b: Abbreviations of taxon names: *Agr sto = Agrostis stolonifera agg., Ali lan = Alisma lanceolatum, Ali pla = Alisma plantago-aquatica, Bid sp. = Bidens sp., Bol mar = Bolboschoenus maritimus, Car hir = Carex hirta, Car otr = Carex otrubae, Cer dem = Ceratophyllum demersum, Cha sp. = Chara spp., Ech cru = Echinochloa crus-gali, Ele pal = Eleocharis palustris agg., Epi hir = Epilobium hirstum, Epi par = Epilobium parviflorum, Epil sp. = Epilobium sp., Gal pal = Galium palustre, Iri pse = Iris pseudacorus, Jun art = Juncus articulatus, Jun com = Juncus compresus, Jun eff = Juncus effusus, Jun inf = Juncus inflexus, Jun ten = Juncus tenuis, Lem min = Lemna minor, Lyc eur = Lycopus europaeus, Lys num = Lysimachia nummularia, Men ver = Mentha x verticillata, Nym alb = Nymphaea alba, Pha aru = Phalaris arundinacea, Phr aus = Phragmites australis, Pol lap = Polygonum lapathifolium, Pol mit = Polygonum mite, Pol per = Polygonum persicaria , Pol sp. = Polygonum sp. , Pot cri = Potamogeton crispus, Pot nat = Potamogeton natans, Pot nod = Potamogeton nodosus, Pot tri = Potamogeton trichoides, Pot sp. = Potamogeton sp., Pot rep = Potentilla reptans, Ror pal = Rorripa palustris, Ror sil = Rorripa silvestris, Rum con = Rumex conglomeratus, Rum cri = Rumex crispus, Rum sp. = Rumex sp., Sch lac = Schoenoplectus lacustris, Sch tab = Schoenoplectus tabernaemontani, Sol dul = Solanum dulcamara, Spa sp. = Sparganium sp., Sta pal = Stachys palustris, Typ lat = Typha latifolia, Typ ang = Typha angustifolia.*

Table 4. Canonical Correspondence analysis statistics of 22 karstic ponds. Parameters listed explained 32% of the variation in aquatic macrophyte communities composition (%TVE = percentage of the total variance explained).

Variable	%TVE	*p*	F
Fish presence	7.3	0.002	1.72
Transparency	6.6	0.046	1.45
pH	5.9	0.062	1.36
Land-use of the catchment	5.9	0.068	1.34
Bank substrate	5.7	0.079	1.35

CCA with these five variables (Table 4) was performed, allowing the distribution of the ponds according to floristic composition along the most important environmental gradients to be shown in an ordination diagram (Fig. 1). Ponds with prevailing hydrophytes are distributed on the upper-right side of the diagram in the direction of fish presence (and transparency), which indicates oxic conditions throughout the year in the permanent water phase. Hypoxic conditions normally occur in ponds with a strong input of allochthonous leaf litter in autumn. Species-rich ponds with abundant emergent and marsh species are distributed in the lower-left part of the diagram in the direction of finer shore substrate, enabling the growth of the mentioned species. Arrows representing water transparency and land-use indicate relative correlation that was expected, since the higher proportion of forest or permanent grassland in the catchment area of a pond would result in clearer water.

Species number was significantly correlated with surface/depth ratio, shore substrate, surface area of the pond and width of littoral zone (Table 5). The ponds with loamy shores and/or wider littoral zone were not only richer in total species number, but also harboured more Red list species, which were also more abundant.

Environmental Factors in Different Pond Types

The main differences in environmental conditions between ponds without macrophytes and those with more or less abundant macrophytes, are the type of shore-substrate ($p = 0.014$) and of bottom-substrate ($p = 0.099$), as well as the pH of the water ($p = 0.078$). The mean recorded pH value in ponds without macrophytes was 7.2, while in ponds with plants it was 6.7. The differences were most significant in the case of shore substrate ($p < 0.05$), which was concrete, or a stone wall in the first case and mostly loamy, or a mixture of stones and loam, in the latter.

Discussion

Floristic Gradients and Environmental Parameters

The variation of floristic composition is best explained by the following environmental variables: fish presence/absence, water transparency, pH, land-use of the catchment of the pond, and substrate of the shore. Mentioned parameters explained 32% of the variance in plant species composition that was similar to the results obtained for aquatic macrophytes in karstic watercourses in western Slovenia [46], where a similar set of the studied variables explain 28% of the variation. Capers et al. [13] obtained a similar result (27%), while the results by McElarney et al. [47] revealed higher value (56%). The unexplained variation may also be explained by sampling errors, other non-measured parameters, and by the fact that the species-specific recruitment from the soil seed bank and its persistence has a significant impact on the structure and maintenance of the plant-species diversity in wet habitats [48].

Floristic composition was most strongly correlated by fish-presence (7.9% of total variance was explained by this parameter) which, in contrast, had no influence on species richness or the presence of Red List species. A strong role of fish was also found in the study of peri-urban eutrophic ponds by De Backer et al. [14]. Water transparency explained 6.6% of species variance, in accordance with studies showing the importance of submerged macrophytes in maintaining the clear-water state [14, 49, 50]. The amount of phytoplankton, measured as the concentration of chlorophyll-*a*, was very variable and did not significantly affect plant species composition or abundance, as was shown by Bakker et al. [51].

The number of plant taxa found in 28 karst ponds was 50, which was high compared to the 53 plant taxa found in 9 streams flowing through the agricultural landscape of Eastern Slovenia, and were considered to support rich aquatic macrophyte communities [52]. The percentage of Red List species was relatively high (15%) and similar to results published by Chappuis et al. [53].

Table 5. Statistically significant correlations between environmental factors and plant species richness, calculated using Spearman correlation coefficients.

	Surface	Depth	Surface/depth	Shore substrate	Width of littoral
N	0.468*	n.s.	0.608**	0.562**	0.427*
N /log Surface	n.s.	n.s.	n.s.	0.541**	n.s.
N / log Volume	n.s.	-0.536*	n.s.	0.623**	n.s.

**p < 0.01, * = p < 0.05, n.s. = not significant

Some studies relate the ratio of submerged to emerged macrophyte species with the level of eutrophication [51]. In our study the abundance of helophytes prevailed over that of submerged species. This was not necessarily the consequence of nutrient availability since, in many case, this parameter does not function as a primary factor shaping the aquatic macrophyte community; other factors affecting ecological conditions, such as water depth and fluctuation, could be of greater importance [54]. However, if we take into account the aquatic macrophyte indicator values for ponds proposed by Sager and Lachavanne [27], the surveyed ponds were shown to host species indicating a mesotrophic as well as a mesotrophic to eutrophic state.

Water pH was expected to have a significant influence on aquatic macrophyte assemblages, as indicated by a study of alpine ponds [12].

Land-use practices may affect pond characteristics [5, 13, 15, 55, 56], and these changes of the pond environment (e.g. water/sediment quality) may affect aquatic organisms, their species richness, and abundance. Dodson [56] also concluded that land-use was a good predictor of pond biodiversity. In our study, land-use of the catchment of the pond was not significantly correlated with species-richness, but with species composition.

Differences in Environmental Factors between Pond Types

As expected, the type of shore substrate provided the most significant difference between ponds with and without macrophytes. The semi-natural types of shore, with finely textured substrate (clay, silt and/or loam), were suitable sites (microhabitats) for emergent macrophytes, while in the case of concrete or stony shores, rooted aquatic macrophytes were scarce.

The difference in pH between ponds with macrophytes and "empty" ponds also was evident ($p = 0.078$). The higher pH of the water in non-colonizd ponds could be due to the contribution of Ca-rich concrete and/or stony substrate.

Species number did not correlate with depth, but was significantly correlated with size/depth ratio and surface. In the case of alpine lakes [12] depth did not affect biodiversity, while a significant influence of pond size on the aquatic macrophyte community was shown by Bagella et al. [57], who studied Mediterranean temporary ponds. They also established the important role of disturbances. The main disturbances in the ponds studied here are water level fluctuations, since the conditions of ponds in the Karst region depends directly on precipitation [30], which is unequally distributed over the year [32]. In many cases disturbances also could be the consequence of the land-use in the surroundings of the ponds, i.e. trampling, erosion, and invasive species [50]. In our survey invasive alien plant species were not detected in the ponds and were not abundant in the littoral zone. The most abundant species was the invasive tree species *Robinia pseudacacia*, which is widespread in the Karst region and is important from the point of nitrogen enrichment of ponds [58].

The ponds with more suitable measures (e.g. loamy shores, wider littoral zone) were not only richer in total species number, but also harbored more Red list species, which also were more abundant. To mitigate adverse effects on plant communities, Akasaka et al. [50] suggests that management should focus on the creation of buffer zones.

Conclusions

Understanding the environmental conditions that maintain high aquatic macrophyte species richness in karst ponds, together with the main disturbances threatening them, is crucial for the conservation of this important heritage. Land-use in the catchment area and the presence of fish-influenced plant species composition. Concrete shores and bottoms, as well as stony walls, prevented the development of complex aquatic macrophyte communities and the increase of species richness. We conclude that reinforcement of the shores with concrete is the main reason for species impoverishment in karst ponds. On the other hand, some species (*Potamogeton crispus*, *P. nodosus*) were found only in walled ponds and that contributes to gamma diversity. As was established for alpine lakes [12], karst ponds need a special approach due to the particular water regime in the Karst area. The present findings should be considered in restoration projects, in order to preserve diversity at all levels.

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