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A botanical classification of standing waters in Serbia and its application to conservation

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ABSTRACT

1. The aim of this study was to describe a botanical classification for lakes in Serbia based on vegetation assemblage, basic water quality parameters and geographic region.
2. Between 2008 and 2010 records from more than 1000 relevés were collated into one dataset. All relevés were derived from synoptic syntaxon tables (1956–2010).
3. In order to fill geographical and methodological gaps in the database, additional field research was carried out during 2008, 2009 and 2010 at 18 new locations, producing a final total of 98 sites, 748 relevés and approximately 22,500 floristic records.
4. Cluster analysis using TWINSPAN (Two-Way INdicator SPecies ANalysis) revealed two main lake vegetation types (LVT1 and LVT2). LVT1 is characterized by typically species-rich, eutrophic sites with *Ceratophyllum demersum* dominant, followed by *Hydrocharis morsus-ranae*, *Lemna minor*, *Salvinia natans* as constants, while LVT2 comprises species-poor, meso-eutrophic sites with *Myriophyllum spicatum* constant. These two main lake vegetation types are further divided into four sub-types (LVT1a, LVT1b, LVT2a, LVT2b), and seven site groups.
5. Instead of predicting the vegetation lake groups using predefined physico-chemical categories, the reverse approach has been applied. Altitude and BOD/COD values (as proxies of trophic status) verified the TWINSPAN-derived classification based on lake vegetation.
6. Brief reviews are given to describe national conservation strategy. The results from this study illustrate their value in amplifying descriptions of sites already recognized as important nationally and internationally. In addition, Groups 5 and 6 are considered a potential conservation resource, with six site-targets in particular, representing specific oases for many aquatic species whose natural habitats are endangered by human activity or natural succession.

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KEY WORDS: lake; pond; wetland; vegetation; relevés; classification; Serbia

INTRODUCTION

The Balkans is not the land of lakes. In fact, natural lakes are a rare phenomenon within extraglacial areas of Central and Eastern Europe as almost all have been completely terrestrialized during the Holocene (Pokorný and Jankovská, 2000). Indeed, standing waters in the area include a relatively limited number of natural, catchment drainage lakes, having at least one natural outflow in the form of a river or stream. Some of these contain water for 2 months in spring only, being grazed wetlands for the rest of the year – such as Slano Kopovo, an ephemeral, relict salt lake, situated in the floodplain of the River Tisa. Generally, in the Balkans peninsula natural lakes are greatly outnumbered by endorheic lakes (which lose water solely by evaporation or underground seepage or both, excavated gravel-pit lakes and angling ponds), ornamental pools and reservoirs. In other words, many lakes in Serbia are artificial, constructed for hydro-electric power generation, flood and erosion control, water retention, recreation, industrial use or domestic water supply. At the same time, over decades, some of these lakes have developed many natural characteristics, including hydrological connectivity typical for natural waters, so even a basic, historical classification of standing waters in Serbia is rather difficult.

Although rivers in the region are monitored regularly for various biological, water quality and hydromorphological

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parameters, this is not the case for lakes. According to Moss *et al.* (1996), the reason (which applies around Europe) is that standing bodies of water have been less affected by organic pollution and generally not associated with epidemic-prone centres of urban population, so have often been ignored. For Serbia, this statement does not fully hold, as many lakes and reservoirs are subject to diffuse pollution (mainly from agricultural runoff) and some even to point-source organic pollution, such as Palic, which receives direct discharges from a wastewater treatment plant. Nevertheless, the official surface water quality monitoring programme, which is carried out by RHMZ (Republic Hydro Meteorological Service of Serbia) includes only 30 lakes and reservoirs, with a low sampling frequency (1–4 samples per year), in contrast to more than 150 sites on rivers, which are sampled monthly, weekly or even daily. Results from the RHMZ lake monitoring programme and narrative annual reports are available at http://www.hidmet.gov.rs, and since 2003 the data have been fed into WATERBASE (EEA, 2011). Moreover, the status assessment is strongly biased towards chemistry, with plankton communities, and in exceptional cases bottom fauna, being the only biological quality elements monitored. In addition, according to old legislation (Official Gazette of Republic of Serbia, 1994) and the recently adopted new Water Law (Official Gazette of Republic of Serbia, 2010a), public water supply companies and municipal public health institutions are obliged to monitor a limited number of water quality variables in reservoirs intended for domestic water supply. Although Serbia is not yet a member of the EU, as a contracting party to the Convention for the Protection of the River Danube and a full member of ICPDR (International Commission for Protection of the Danube River), it is part of the Transnational Monitoring Network (TNMN) and committed to implementation of the EC Water Framework Directive (WFD) (European Commission, 2000). Unfortunately, not a single lake in Serbia is large enough to be included in a group of significant lakes that are subject to detailed assessment and monitoring programmes intended to yield data for the reports to the European Commission on the Danube River Basin.

Consequently, almost all studies on lakes in Serbia have been carried out irregularly and unsystematically, each with different aims (defining biological status, conservation status, vegetation mapping, water quality condition, etc.), using different methods, relying on modest datasets of physico-chemical measurements, and with varied interpretations. As such, and in contrast to rivers, there has been no comprehensive study of lake typology in Serbia. Paradoxically, there are more internationally available data for lakes filled with methane on Saturn's moon Titan (the only stable bodies of surface liquid known to exist anywhere other than Earth) than for lakes in the central Balkans region. Two approaches to lake typology were described by Moss *et al.* (2003). The first challenge is to establish a typology; the second to develop a system for assessing ecological quality, where the classification of vegetation is fundamental to the process of evaluating the importance of sites on a regional, national and international scale and for defining target sites in habitat restoration programmes (Kennison *et al.*, 1998). Hence, the aim of this study was to tackle at least one of those problems and to erect a lake typology for Serbia based on geographic region, vegetation assemblage and selected water quality variables. A secondary aim was to demonstrate the value of the typology in identifying and describing lakes important for nature conservation.

STUDY AREA

Serbia is situated in the central part of the Balkan Peninsula, a territory that covers 88 361 km^2. The climate is continental in the north and south-east, with semi-arid summer and cold winter periods, while in the west the climate is humid-temperate. In Midland Serbia the climate is semi-arid temperate-continental (or sub-continental), with transitional sub-Mediterranean parts (Stevanović and Stevanović, 1995; Stevanović and Šinžar, 2005).

Northern lowland Serbia consists of the south-east Pannonian plain (the Danube floodplain), the wide alluvial lowlands and surrounding loess plateaus along the Danube, Sava, Tisa, Tamiš, and Begej rivers, a mosaic of alluvial plains, river terraces, loess plateau, and hills. The great majority of lakes are located in the Danube floodplain, lowland areas (70–80 m above sea level) of northern Serbia, although the largest lake is Vlasinsko jezero (16 km^2) in the Vlasina highlands of southern Serbia.

Southern Serbia is mountainous, except the grand valleys of the Great Morava, Western Morava, Southern Morava, Nišava and Ibar rivers. Occasional standing waters are dispersed within this area; these are mainly artificial reservoirs, ponds and ornamental lakes. The mountains (15 more than 2000 m elevation) belong to four systems: Dinaric Alps, Carpathian-Balkan Mountains, the Rhodopes, and Scardo-Pindhic massifs (Stevanović and Šinžar, 2005). In the east, old igneous rocks, limestone, and siliceous bedrocks support croplands with some steppe and sand-steppe vegetation (with remnants of diverse continental psammophyte vegetation). West and central Serbia is formed of limestone, serpentine, and igneous rocks. Along the main rivers, alluvial forest of white willow, white and black poplar, ash, and pedunculate oak, as well as dispersed fragments of marshes occur. The limestone and serpentine gorges and canyons hold a rich flora of numerous relict and endemic taxa. Mountain areas above the tree line are also rich in diverse chasmophytic, scree and rocky ground communities composed of endemic and Alpine orophytes (Stevanović and Šinžar, 2005).

Although there are many fresh waters in the mountains of Montenegro, relatively little information is available for this area. Crno jezero is the largest and deepest lake in the Durmitor Mountain National Park. It lies at the concave amphitheatre corrie valley, formed at the head of a valley created by glacier erosion (the foot of Međed peak, an area of 0.515 km^2). This lake was included in this study as an outlier.

METHODS

Most of the historical data on aquatic vegetation in the database were presented according to Braun-Blanquet methodology (Braun-Blanquet, 1928, 1932, 1964). Between 2007 and 2010 data from more than 1000 relevés were collected, analysed, and collated. Some of the oldest relevés (about 300) were excluded as the data could not be verified in the field (e.g. some of the water bodies do not even exist any more or because there are no reliable relevés for a particular site after Slavnić (1956). A final selection consisting of 748 relevés from 80 lakes was eventually extracted from the database and included in this study.

All relevés were derived from published synoptic syntaxon tables (Slavnić, 1956; Babić, 1971; Rauš *et al*., 1980; Butorac, 1995; Ranđelović, 2002; Radulović, 2000, 2005; Radulović and Vučković, 2001; Nikolić, 2004; Panjković, 2005; Polić, 2006). Each relevé was assigned a value on the Braun-Blanquet Cover Abundance Scale, where: 5 = > 75% cover; 4 = 50–75% cover; 3 = 25–50% cover; 2 = 5–25% cover; 1 = numerous but less than 5% cover, or scattered with cover up to 5%; + = few, with sparse cover; and r = rare, solitary, with sparse cover. The use of descriptive attributes such as '+' and 'r' was avoided by converting the cover values into the numerical scale of van der Maarel (1979) as follows:

[+][r][1][2][3][4][5] – Braun-Blanquet
[1][2][3][5][7][8][9] – van der Maarel

The method is semi-quantitative in the sense that species cover is estimated instead of measured, and qualitative in the sense that it gives a complete list of species for the plot. The average number of species per relevé was approximately 30, making a total of 22 500 floristic records included for the whole study. Most (90%) of the relevés date from 1980–2010. Relevés from the earlier period (1956, 1971) were related to stable lake ecosystems (Obedska bara, Ludos and Carska bara), and they were confirmed and updated in the additional field study carried out in 2009.

Field survey

During the reconnaissance phase of the survey in 2008 (which covered all 98 lakes in the final analysis) the relationships between various vegetation types and topography (toposequences), history (chronosequences), geology, soil conditions, floods, and landslides were analysed in order to find the gaps in the database. To fill these geographical and methodological gaps, additional field survey was undertaken during 2008, 2009 and 2010 at 18 new locations, giving in total, 98 standing water sites (Figure 1).

Plot locations were selected in line with the Braun-Blanquet approach, which is based on replicate sampling in the central areas of homogeneous stands of aquatic and semi-aquatic vegetation. Frequent replicate samples were collected from other similar stands, where each sample site satisfied the homogeneity criteria (homogeneity of the vegetation cover as well as homogeneity of the soil and other habitat factors). The extension of relevés into the water varied between 10 m^2 and 20 m^2. These minimal-area guidelines have been suggested by Westhoff and van der Maarel (1978) for typical vegetation physiognomic types, although actual plot size should be larger than the minimal area and should remain constant within a vegetation type. The minimum sampling area depends on the point where the species–area curve levels off. Plots enclosed all the species characteristic for the community – indicator species, differential species, and constant companion species – which very often included terrestrial plants such as swamp meadowgrass *Poa palustris*.

Apart from cover–abundance estimates, information on sociability, vigour, and phenology was also recorded. 'Sociability' is particularly important as a measure of the degree of clustering (contagion) of individuals of a plant species. However, those results are not included in this study as they are difficult to quantify and had only minimal value for lake-type analysis.

Lakes were circumnavigated and surveyed from the lake shore or from a boat, while deeper areas were sampled by a modified, nine-spike lighter anchor (attached to a length of rope), which is more suitable for this purpose than grapnels. Depending on the size of the sampling area and the amount of vegetation cover varying numbers of random grab samples were taken. This was repeated where necessary to ensure the accuracy of submerged vegetation sampling and thus to fulfil the requirements of the Braun-Blanquet method (a combined qualitative–quantitative approach).

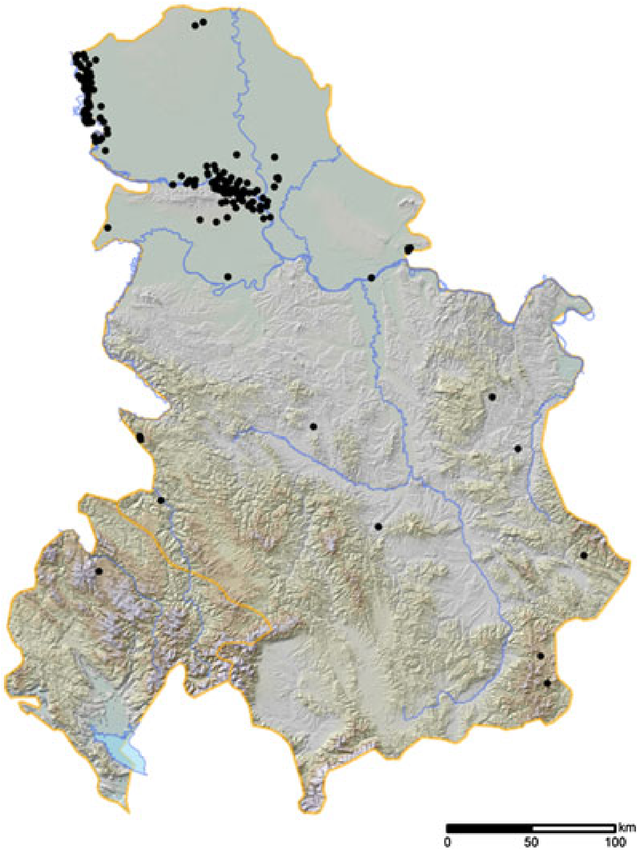

Figure 1. Distribution of lakes included in the analysis

Water quality was assessed by the following set of basic water quality parameters: temperature (T), dissolved oxygen content (DO) and oxygen saturation, pH (measured electrochemically with WTW Inco Lab 4), while biological and chemical oxygen demand (BOD, COD), total organic carbon (TOC) and total suspended solids (TSS) were analysed using a portable SECOMAM Pastel UV spectrophotometer. Alkalinity was determined according to the standard method in ASTM (1992) and expressed as ppm $CaCO_3$ (median value of two samples, two replicates each). For all chemical analyses samples were taken at 0.5 m depth at each of the lakes studied. Water quality was measured in the field for 53 lakes. A further 42 comprised groups of lakes where water quality measurements were made in the nearest connected water body. For the remaining three lakes data on water quality were obtained from the literature (see Supplementary Material).

Although the official monitoring programme includes highly relevant determinants (such as concentration of total nitrogen (N) and phosphorus (P), total ammonium, nitrates, nitrites, orthophosphates, chlorophyll *a*) measured in stratified samples taken at three sampling sites per lake, it covers only 10 of the 98 lakes included in this study. Therefore, the official data were summarized (Table 2) and used only for discussion and verification of the proposed classification. Other observations were made on substrate, water colour and clarity, adjacent land-use, wetland edges, artificial features, use of or damage to the site, inflows and outflows and obvious fauna. All geo-spatial data were derived using a Trimble Nomad GPS, integrated into a Microsoft Access LHS (Lake Habitat Survey) database (Rowan *et al*., 2006), but hydromorphological data were not included in this study.

Field work on Crno jezero in Montenegro was carried out separately and at the same time as field trials for LHS (Rowan *et al*., 2004), during the summer months of 2005 and 2006 (Radulović *et al*., 2010).

Dataset and data analysis

Relevés undoubtedly have been a valuable source of field information, as they combine lists of species per quadrat (homogeneous, typical stands of the plant community) together with estimates of their abundance, dominance and plant sociology.

Phytosociological databases are primarily designed for vegetation classification and the spatial distribution of vegetation or habitat diversity. Nevertheless, these data can be used for other purposes, such as mapping the distribution of individual species, modelling the potential distribution of species and plant communities, and calibrating indicator values for species. Even though this method produces a very large dataset on the vegetation, many authors have warned that the approach creates some fundamental problems (Podany, 2006), primarily concerning the specific manner in which data are numerically treated in the method for sampling relevés. For that reason, the attributes of relevés were transformed into a numerical scale to avoid descriptive terms such as ‘+’ and ‘r’, according to van der Maarel (1979).

Different authors, over decades, have used different taxonomic concepts for many species, subspecies or aggregate species. To allow standardization of the dataset, species or subspecies had to be transformed to species *sensu lato* in order to enable a consistent taxonomic treatment for the whole dataset.

Geographical coverage in the dataset has varied with time; in general it is fairly comprehensive but there are still some gaps. Another, more fundamental, source of gaps in the dataset is the under-representation of species-poor vegetation stands. Although these may be common, researchers often tend to avoid them, believing that they are difficult to classify (Podany *et al*., 2005). To fill this gap additional sites were surveyed at random within any area of homogeneous vegetation (including species-poor areas) and repeated where necessary.

The final list of species, with the overall abundance data (pseudospecies) for each lake, was derived from the Braun-Blanquet specific abundance–cover data and analysed using TWINSPAN (Two-Way INdicator SPecies ANalysis; Hill, 1979; Oksanen and Minchin, 1997). Overall abundance was calculated using FLORA software (Karadžić *et al*., 1998) which includes several tools for numerical classification and analysis of phytosociological data. TWINSPAN is fundamentally based on indicators; hence this method was considered the most suitable. The resulting clusters are described as LVTs (Lake Vegetation Types) Groups 1–7. Range, median, extreme and outlier values of basic water quality parameters were plotted using Statistica 8.1 Whisker Plots.

RESULTS

Lake classification based on vegetation

TWINSPAN analysis, with three cut-off levels resulted in seven site types (Figure 2, Table 1). Table 1 shows the species occurring at a constancy of more than 20% in the TWINSPAN end-groups of seven site types. Two main Lake Vegetation Types were recognized (LVT1 and LVT2) and divided further into four sub-types (LVT1a, LVT1b, LVT2a, LVT2b), and finally into seven site groups (1: LVT1a (i), 2: LVT1a (ii), 3: LVT1b (i), 4: LVT1b (ii), 5: LVT2a, 6: LVT2b (i) and LVT2b (ii), the single lake in the outlying Group 7: Crno jezero, Montenegro. Table 2 contains data on water chemistry for 10 lakes used in the classification process.

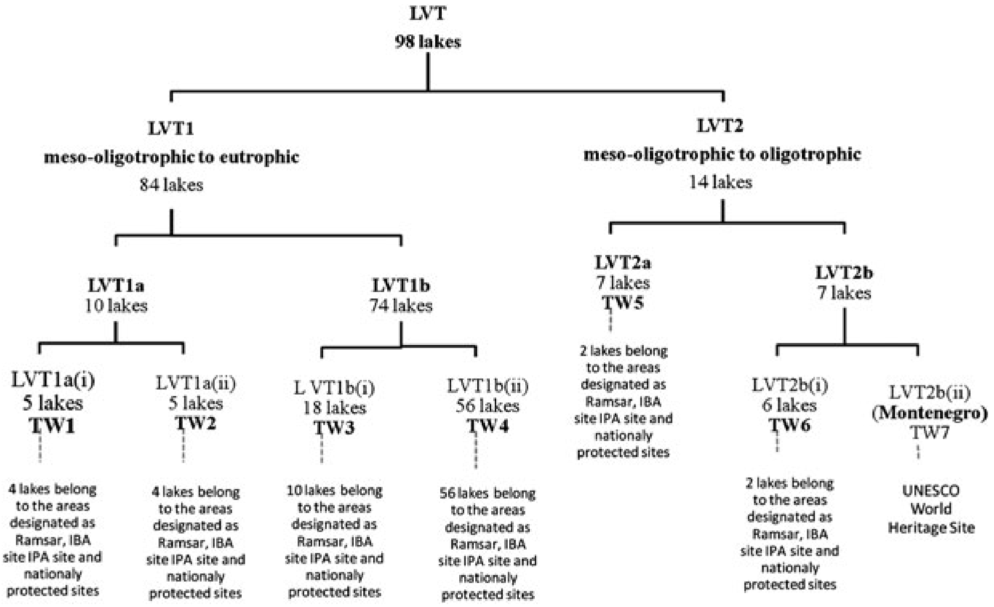

Figure 2. TWINSPAN dendrogram for the Lake Vegetation Types showing divisions and sample sizes

Table 1. Constancy table for TWINSPAN lake groups (constancy classes over 20% only)

No.	Species	1	2	3	4	5	6	7
1	*Rorippa amphibia* (L.) Besser	V	V	III	II	II		
2	*Lemna trisulca* L.	IV			IV		II	
3	*Lemna minor* L.	IV		III	V		III	
4	*Nymphoides peltata* (S. G. Gmelin) O. Kuntze	III		II	IV	IV		
5	*Spirodela polyrhiza* (L.) Schleiden	III			V		II	
6	*Salvinia natans* (L.) All.	III			IV		II	
7	*Oenanthe aquatica* (L.) Poiret	II		II				
8	*Hydrocharis morsus-ranae* L.	II			IV		II	
9	*Polygonum amphibium* L.	II	III	III	II	IV	II	
10	*Carex vesicaria* L.	II						
11	*Carex riparia* Curtis	II	II	II		II		
12	*Iris pseudoacorus* L.	II	III	IV	II	II		
13	*Rumex hydrolapathum* Hudson	II	IV	III	II			
14	*Carex elata* All. subsp. *elata*		IV	II				
15	*Lysimachia vulgaris* L.		IV	IV				
16	*Senecio paludosus* L.		IV	II				
17	*Phragmites australis* (Cav.) Trin. ex Steudel		III	IV	II		III	
18	*Scirpus lacustris* L.		III	III	II			
19	*Lythrum salicaria* L.		III	IV		II	II	
20	*Poa palustris* L.		III					
21	*Polygonum hydropiper* L.		III	II		V		
22	*Sium latifolium* L.		III	IV				
23	*Acorus calamus* L.		II					
24	*Myosotis scorpioides* L.		II	III		III		
25	*Teucrium scordium* L. subsp. *scordium*		II		IV	III		
26	*Alisma plantago-aquatica* L.		II	II		III	II	
27	*Galium palustre* L.		II	IV				
28	*Lysimachia nummularia* L.		II	II	III	V	II	
29	*Mentha aquatica* L.		II	IV		III	III	
30	*Rubus caesius* L.		II	III				
31	*Sagittaria sagittifolia* L.		II					
32	*Stachys palustris* L.		II	IV		II		
33	*Myriophyllum spicatum* L.			V	IV	II	V	V
34	*Najas marina* L.			V	II	II		
35	*Robinia pseudacacia* L.			IV			III	
36	*Glyceria maxima* (Hartman) Holmberg			IV				
37	*Calystegia sepium* (L.) R. Br. subsp. *sepium*			IV				
38	*Lycopus europaeus* L.			IV				
39	*Typha latifolia* L.			III			III	
40	*Salix alba* L. subsp. *alba*			III			III	
41	*Typha angustifolia* L.			III			II	
42	*Eupatorium cannabinum* L. subsp. *cannabinum*			III			II	
43	*Potamogeton nodosus* Poiret			III			III	
44	*Sparganium erectum* L. subsp. *erectum*			III				
45	*Amorpha fruticosa* L.			III				
46	*Butomus umbellatus* L.			II		II	II	
47	*Solanum dulcamara* L.			II				
48	*Euphorbia palustris* L.			II				
49	*Carex vulpina* L.			II				
50	*Euphorbia lucida* Waldst. & Kit.			II				
51	*Solidago gigantea* (L.) Vill. subsp. *serotina* (O. Kuntze)			II				
52	*Echinochloa crus-galli* (L.) Beauv.			II	IV	III		
53	*Najas minor* All.			II			IV	
54	*Potamogeton crispus* L.			II	III		V	V
55	*Eleocharis palustris* (L.) Roemer & Schultes subsp. *palustris*			II		IV		V
56	*Cirsium vulgare* (Savi) Ten.			II				
57	*Potentilla reptans* L.			II				
58	*Ranunculus repens* L.			II				
59	*Rorippa sylvestris* (L.) Besser			II	IV	V		
60	*Symphytum officinale* L. subsp. *officinale*			II				
61	*Thalictrum flavum* L.			II				
62	*Bidens tripartita* L.			II		IV		
63	*Scutellaria galericulata* L.			II				
64	*Cyperus michelianus* (L.) Link subsp. *michelianus*				V	III		
65	*Polygonum mite* Schrank				V	III		
66	*Agrostis stolonifera* L.				V	IV		
67	*Riccia crystallina*				V	IV		
68	*Eleocharis acicularis* (L.) Roemer & Schultes				V	IV	IV	
69	*Crypsis alopecuroides* (Piller & Mitterp.) Schrader				V	II		
70	*Polygonum lapathifolium* L.				V	V		

(*Continues*)

Table 1. (Continued)

No.	Species	1	2	3	4	5	6	7
71	*Cyperus fuscus* L.				IV	III		
72	*Gnaphalium uliginosum* L.				IV	III		
73	*Limosella aquatica* L.				IV			
74	*Ceratophyllum demersum* L. subsp. *demersum*				IV		IV	
75	*Polygonum minus* Hudson				III			
76	*Chenopodium rubrum* L.				III			
77	*Inula britannica* L.				III	II		
78	*Plantago major* L. subsp. *intermedia* (DC.) Arcangeli				III			
79	*Rumex dentatus* L.				III			
80	*Trapa natans* L.				III		II	
81	*Nymphaea alba* L.				III		II	
82	*Potamogeton lucens* L.				II			
83	*Nuphar lutea* (L.) Sm.				II			
84	*Azolla filiculoides* Lam.				II			
85	*Ranunculus circinatus* Sibth.				II			
86	*Lemna gibba* L.				II			
87	*Utricularia vulgaris* L.				II		II	
88	*Marsilea quadrifolia* L.					III		
89	*Juncus effusus* L.					II	II	
90	*Xanthium strumarium* L. subsp. *strumarium*					II		
91	*Equisetum palustre* L.					II	III	
92	*Juncus articulatus* L.					III		
93	*Juncus conglomeratus* L.					II		
94	*Veronica anagallis-aquatica* L.					II		
95	*Alisma gramineum* Lej.					II		
96	*Dichostylis michelianus* (L.) Nees					II		
97	*Gratiola officinalis* L.					II		
98	*Equisetum fluviatile* L.							V
99	*Potamogeton pectinatus* L.						IV	
100	*Potamogeton perfoliatus* L.						III	
101	*Potamogeton natans* L.						II	V
102	*Mentha longifolia* (L.) Hudson						III	V
103	*Hypericum humifusum* L.						II	
104	*Chara aspera* Deth. ex Willd							V
105	*Chara contraria* A.Br. ex Kutzing							V
106	*Chara delicatula* Agardh							V
107	*Chara globularis* Thuill							V
108	*Chara rudis* (A.Braun) Leonh							V
109	*Deschampsia cespitosa* (L.) Beauv. subsp. *cespitosa*							V
110	*Nitella flexilis* (L.) C.Agardh							V
111	*Nitella opaca* (C.Agardh ex Bruzelius)							V
112	*Potamogeton pusillus* L.							V
113	*Ranunculus trichophyllus* Chaix subsp. *trichophyllus*							V
Species richness per TWINSPAN group		27	48	127	143	116	118	16
Species richness with constancy >20% per TWINSPAN group		13	24	54	43	40	33	16
Mean species richness per site for TWINSPAN group		10	18.2	23.2	19.4	27.4	32.6	16
Number of lakes per TWINSPAN group		5	5	18	56	7	6	1

LVT1

LVT1 contains typically species-rich water lily (*Nymphoides peltata*, *Nymphaea alba*, *Nuphar lutea*) sites with *Ceratophyllum demersum* dominant, followed by *Hydrocharis morsus-ranae*, *Lemna minor* and *Salvinia natans* as constants, while water bodies in LVT2 are, on average, more species-rich, but only because of the heterogeneity of the group), and characterized by the presence of *Myriophyllum spicatum*, which frequently occurs together with *Ceratophyllum demersum*. In such situations *C. demersum* is the stronger competitor, being more tolerant of nutrient-enriched habitats. *Nymphaea alba* occurs with a high cover value (Radulović *et al*., 1998, 2004; Radulović, 2005) which is not obvious from the TWINSPAN constancy table. Although such assemblages are distinct communities, playing different roles in the successional colonization of open waters (Rodwell *et al*., 1995), their presence and spatial overlap is essential for understanding lake typology.

LVT1 splits further into two sub-types LTV1a and LTV1b, where LTV1b has the characteristics of lowland, eutrophic lakes (70% of lakes in Serbia fall into this category). The other cluster (LVT1a) comprises rather similar sites. These differ mainly by the higher constancy of amphibious (*Rorippa amphibia*) and riparian (*Rumex hydrolapathum*) flora, not typical for aquatic systems *sensu stricto*, but representative of woodlands and hedgerows (Harper and Chancellor, 1959), and forming a riparian zone of eutrophic, artificial or heavily modified natural lakes usually surrounded by arable land. Sites in LVT1b are divided further into LVT1b (i) and LVT1b (ii).

LVT2

Type LVT2 has a narrower range of species than LVT1, although the mean number of species per site is greater (Table 1). LVT2 sites typically have no water lilies (*Nypmhaea alba, Nuphar lutea*), and are characterized by a dominance of

Table 2. Water quality characteristics of 10 lakes used in the TWINSPAN exercise for classifying lakes according to their vegetation types

TWINSPAN Groups	1	2	3					5	7		National EQSII class	ICPDR TVII class
	Zavojsko L.	Borsko L.	Grliste	Potpec	Palic	Ludas	Glavno jezero	Gruza	Celije	Vlasina		
Total P (mg L^{-1})	0.005-0.03	0.013-0.0635	0.013-0.126	0.019-0.206	0.31-2.85	0.27-1.57	0.013-0.057	0.013-0.1	0.014-0.11	0.005-0.06		0.2
Total N (mg L^{-1})	0.23-1.3	0.4-2.85	0.425-2.7	0.5-1.733	1.5-5.4	2.4-7.1	8.5*	0.73-2	0.64-2.1	0.24-1.24		4
Chlorophyll a(µg L^{-1})	0.025-2.3	0.21-4.77	0.74-6.8	2.34-17	25.7-813.5	71-1932	0.025-16.6	2.4-21	0.76-15.21	0.72-2.56		50
BOD (mg L^{-1})	1.02-3.4	1.125-3.365	1.098-3.15	1.99-3.35	7-34.2	8.1-58.9	1.5-4.3	1.83-4.5	1.15-2.17	0.97-2.32	4	5
COD (Mn) (mg L^{-1})	1.54-5.07	2.53-5.015	1.65-5.02	1.7-5.04	19.2-72.4	27-72.4	3.4-9.1	2.84-9.72	1.92-6.51	1.22-3.61	12	10
Secchi depth (m)	1.6-4	1.2-2.4	1.2-2.5	0.2-2.4	0.1-3	0.05-1.7	0.25-4	0.9-2.85	0.3-2.5	0.8-4.5		
Saturation O_2 (%)	63-117	57.5-111.38	46.8-131.5	44-108	26-308	133-362	101-137	42-120	56.5-164	43.8-104	75-90	
Dissolved O(mg L^{-1})	5.67-9.8	6.13-10.76	4.76-14.21	4.52-9.8	2.3-22.6	12.4-27.9	7.8-12.2	3.64-11.3	4.67-13.61	3.93-9.54	6	6
TOC (mg L^{-1})	1.275-2.244	0.77-2.75	1.46-2.78	0.78-2.22	12.3-42.7	14-73.3	2.2-4.4	2.6-6	1.54-4.37	1.45-2.3		
EC (µS cm^{-1})	2.66-20.16	313-323	2.16-11.16	275-294	602-728	643-749	350-400	1.16-14.16	293-322	80-86		
Nitrates (mg L^{-1})	0.035-0.5	0.1-0.56	0.1-0.85	0.17-1.2	0.015-0.84	0.06-0.53	1.22-10.56	0.08-1.78	0.074-0.97	0.02-0.52	10	3
Nitrites (mg L^{-1})	0.001-0.085	0.005-0.297	0.002-0.11	0.0015-0.023	0.0015-0.236	0.0015-0.018	0.043-0.066	0.005-0.12	0.0032-0.088	0.0015-0.032		0.03
Total ammonium (mg L^{-1})	0.005-0.15	0.0064-0.2042	0.005-0.304	0.005-0.22	0.02-1.23	0.03-0.45	0.005-0.28	0.005-0.255	0.017-0.32	0.01-0.254	0.3	
Orthophosphates (mg L^{-1})	0.0068-0.024	0.0078-0.0319	0.008-0.05	0.011-0.044	0.016-0.56	0.014-0.398	0.0025-0.029	0.008-0.05	0.006-0.064	0.008-0.035		0.1
Silicate (mg L^{-1})	1.8-3.2	0.5-5.5	1-8.66	4.33-5	14-33	2-6	3-11	3-7	2.17-20.17	3-5.56		
pH	7.4-7.8	7.13-7.67	7.2-7.4	7.1-7.43	8.3-9.8	9.2-10	8-8.2	7-8	7.53-7.73	7-7.53	6.8-8.5	6.8-8.5
Maximum depth (m)	40	24	22	17	shallow	shallow	shallow	28	40	40		

Data were retrieved from WATERBASE – LAKES; values are the maxima and minima recorded in stratified samples taken annually as part of the official monitoring programme of Serbia (2003–2008).

Myriophyllum spicatum. The two sub-types LTV2a and LTV2b are differentiated by the presence or absence of riparian vegetation near the water-line. The first cluster (sub-type LTV2a, Group 5) is defined by the presence of *Lysimachia nummularia, Agrostis stolonifera, Polygonum hydropiper, Polygonum lapathifolium* and *Rorippa sylvestris*, indicating the absence of a natural shore zone, typical for new, artificial, oligo-mesotrophic lakes, including some ornamental lakes. Sub-clusters LTV2a and LTV2b separate sites that are typical and those where *Gnaphalium uliginosum* (*Filaginella uliginosa*) is dominant. The second cluster (LTV2b) contains typical, meso-oligotrophic lakes. Sub-cluster LTV2b (ii) confirms the oligotrophic nature of the group; here the main charophyte species occur.

The seven lake groups

Group 1 (five lakes): Small lowland pools and ponds, eroded shore zone, fens

The components of Group 1 (27 taxa per TWINSPAN group, and 13 taxa with constancy >20% per TWINSPAN group) are generally small, low-diversity fluvial lakes, occurring at 70 m above sea level with a more or less eroded shore zone (Figure 3). These sites mainly lie in the north of Serbia (province Vojvodina, ponds of Koviljski rit and Apatinski rit wetland area), except one lake in Midland Serbia (Zavojsko jezero, water chemistry Table 2). The group is characterized by *Lemna minor* (constancy IV), *Lemna trisulca* (IV), *Nymphoides peltata* (III), *Spirodela polyrhiza* (III), and *Rorippa amphibia* (V) around the shore zone (Table 1).

Group 2 (five lakes): Lowland shallow or sluggish and sheltered waters in succession

Group 2 is relatively species-rich (48 taxa per TWINSPAN group, 28 with constancy >20% per TWINSPAN group), with a high constancy of *Rorippa amphibia* (V), and the constant presence (IV) of *Rumex hydrolapathum*, *Carex elata*, *Lysimachia vulgaris* and *Senecio paludosus*, followed by significant presence (III) of *Polygonum amphibium*, *Iris pseudoacorus*, *Phragmites australis*, *Scirpus lacustris*, *Sium latifolium, Lythrum salicaria*, and *Poa palustris* (Table 1). This group contains five lakes, two of them in the hydrosere stage, beginning with swamp to fen that allows vegetation such as sedge to grow. At this stage the pH is still not acid and the soils are not too deficient in mineral elements. All lakes are situated at 70–80 m above sea level apart from one (Borsko jezero, 500 m above sea level (Figure 4)), (water chemistry, Table 2).

Group 3 (18 lakes): Lowland eutrophic lakes of the Danube floodplain, swamps and fens

Group 3 (18 lakes) is the most diverse and species-rich, with 54 taxa occurring with constancy >20% and 127 taxa for the group as a whole (Table 1). All lakes are situated at 70–80 m above sea level (Palic, Ludos, Saransko jezero, Vracevgajsko jezero, Glavno jezero (water chemistry, Table 2), Backi Monostor, Bezdan, Dubovac, Hrljak, Jamine, Karapandza, Kozjak, Provala (Figure 5), Dobrodol, Grliste, Potpec and the ephemeral lake Jarkovac). Two lakes do not fit into this geographical distribution (Grliste and Potpec: water chemistry, Table 2), and lie in Midland Serbia.

Frequently occurring species (constancy V) include *Myriophyllum spicatum* and *Najas marina*, followed by (IV) the emergent plants *Iris pseudacorus*, *Phragmites australis, Lysimachia vulgaris, Sium latifolium, Lythrum salicaria, Galium palustre, Mentha aquatica, Stachys palustris, Robinia pseudoacacia, Glyceria maxima, Calystegia sepium*, and *Lycopus europaeus*.

Group 3 lakes are optimal habitats for ass *Naiadeto-Potametum* Slavnić 1956, ass *Scirpo-Phragmitetum* W.Koch 1926 subass: typhaetosum *(angustifoliae-latifoliae)* Soó *Sparganio-Glycerietum fluitantis* Br-Bl. 1925, ass *Glycerietum maximae* Hueck 1931, dominant in this type of site. However, reed habitats and riparian zones are degraded, even in protected sites

Figure 3. Zavojsko jezero TWINSPAN Group 1.

Figure 4. Borsko jezero TWINSPAN Group 2.

Figure 5. Provala TWINSPAN Group 3.

(fragments of the Koviljski rit wetland area) owing to the invasion by *Amorpha fruticosa* (III), an invasive species non-native to the catchment.

Group 4 (56 lakes): Typical lowland, eutrophic lakes of the Danube floodplain

Group 4 (56 lakes – pools, ponds, and lakes in Koviljski rit and Apatinski rit wetland areas, Carska bara, Obedska bara, Perleska bara, Monostorski rit, Labudovo okno, Figure 6) is a species-rich group, in total supporting 143 taxa, with 43 taxa occurring at a constancy >20%. These sites support species typical of eutrophic waters where sediments have accumulated, with a high constancy (V and IV) of *Lemna minor*, *Spirodela polyrhiza*, *Polygonum mite, Agrostis stolonifera, Riccia crystallina, Eleocharis acicularis, Polygonum lapathifolium, Cyperus michelianus*, *Lemna trisulca*, *Nymphoides peltata*, *Salvinia natans*, *Hydrocharis morsus-ranae, Ceratophyllum demersum, Myriophyllum spicatum, Ceratophyllum demersum*, and *Limosella aquatica* (Table 1). This group comprises all four general aquatic community types: surface and subsurface duckweed, free-floating or rooted submerged pondweed, rooted

Figure 6. Djindja TWINSPAN Group 4.

water-lily vegetation with floating leaves, and emergent reed vegetation.

Group 5 (seven lakes): Diverse group of low and high altitude meso-oligotrophic lakes

Group 5 (Gruza, Zaovine (Figure 7), Usic bara, two pools in the Koviljski rit wetland area, Lisinsko jezero) is a relatively species-rich group with 40 taxa occurring with a constancy >20% and 116 taxa for the group as a whole (Table 1). In contrast to Group 4, this group shows a dominance of swamp and riparian vegetation, apart from the aquatic species *Nymphoides peltata* (constancy IV) the most frequently occurring (V) species include exclusively semi-aquatic plants: *Lysimachia nummularia, Rorippa sylvestris*, and *Polygonum hydropiper*. The number and constancy (IV) of the wider (riparian) zone species (*Polygonum amphibium, Eleocharis palustris*, *Bidens tripartite*, and *Agrostis stolonifera*) is rather high, indicating a riparian vegetation pressure towards the water line. This group is notable for the presence of the liverwort *Riccia crystalline* (IV) and the fern *Marsilea quadrifolia* (III), which are rather rare in the catchment.

Lisinsko jezero which splits off at the next division of TWINSPAN as a separate group, but included in Group 5, is a site poor in open-water species with the commonly occurring species around the riparian zone *Filaginella uliginosa* (L.) Opiz

Figure 7. Zaovine TWINSPAN Group 5.

subsp. uliginosa syn. *Gnaphalium uliginosum*, the diagnostic indicator of the association *Dichostylidi–Gnaphalietum uliginosi* (H-ić 1931) (Soo and Timar, 1947). This dwarf-plant community on mud may play a significant role in determining the water quality class, by decreasing the nutrient content of the sediment (water chemistry, Table 2).

Group 6 (seven lakes): Artificial meso-oligotrophic lakes

This group comprises lakes for hydro-electric power generation, recreation, industrial use or domestic water supply, ornamental lakes, primarily Vlasinsko jezero and Celije (water chemistry, Table 2), (Spajic, Sljunkara, Borkovac, Kazuski Dunavac) mainly dispersed throughout Midland Serbia (Figure 8). Thirty-three taxa occur with a constancy >20% and 118 taxa for the group as a whole (Table 1). The list of the commonest taxa contains elements of the other groups *(Lemna minor, Lemna trisulca, Phragmites australis, Hydrocharis morsus-ranae*, but at a lower constancy of II–III, with a dominance of *Myriophyllum spicatum* (V), *Potamogeton crispus* (V), *Najas minor* (IV) *Eleocharis acicularis, Potamogeton pectinatus*, and *Ceratophylum demersum*. This combination is characteristic of mesotrophic/oligotrophic highland lakes.

Group 7 (one lake): High mountains, glacial, oligotrophic lake in Montenegro (outlying group)

Group 7 (Figure 9), Crno jezero, a glacial lake, 1400 m altitude, UNESCO World Heritage Site, supports only 16 taxa dominated by *Chara aspera*, *Chara contraria, Chara delicatula, Chara globularis, Chara rudis, Nitella flexilis, Nitella opaca, Potamogeton pusillus*, and *Ranunculus trichophyllus*. Despite the general floristic impoverishment with vascular plants in the area studied, the charophytes (stoneworts) are relatively diverse, related to the ability of these organisms to use an array of niches in a nutrient-poor environment where competition by major plant groups is low (Radulović *et al*., 2010).

Physico-chemical parameters underpinning the TWINSPAN classification

The median distribution of altitude and basic water quality parameters, the latter classified against the national regulations where applicable (Official Gazette of FRY, 1978a, b), Irish Guidelines (Free *et al*., 2006) and ICPDR target values for the Danube River Basin (ICPDR, 2004) was used in an attempt to underpin the classification based on lake vegetation.

The Irish Guidelines/classification system (Free *et al*., 2006) distinguishes three types of lakes based on alkalinity: low (<20 μeq L^{-1} $CaCO_3$), medium (20–100 μeq L^{-1} $CaCO_3$) and highly alkaline lakes (>100 μeq L^{-1} $CaCO_3$). Figure 10 shows that none of the lakes studied belongs to the low alkalinity category; the majority are highly alkaline lakes (Groups 1–4), while the high scatter in Groups 5 and 6 makes it difficult to classify them using this variable.

There is a clear distinction, based on altitude, between LVT1 (Groups 1–4) and LVT2 (Groups 5 and 6) (Figure 11). Apart from a few exceptional cases all the lakes classified in Groups 1–4 fall into the category of lowland lakes (median value of 80 m). In contrast, the only lake classified in Group 7 clearly belongs to the category of high altitude lakes. Groups 5 and 6 are heterogeneous with regard to altitude – both groups are composed of a mixture of mountainous and lowland lakes, but the scatter can also be attributed to the low number of lakes in each of the groups (only seven and six lakes per group, respectively).

BOD and COD (as proxies for organic load content) were used as a rough estimate of trophic status. The aim was to check whether TWINSPAN-derived groups (based on lake vegetation) really reflect the trophic status of the lakes, or the other way round – whether division between oligotrophic, mesotrophic and eutrophic lakes based on the TWINSPAN classification could be verified by chemical water quality parameters.

Figure 8. Celije jezero TWINSPAN Group 6.

Figure 9. Crno jezero Montenegro TWINSPAN Group 7.

According to BOD and COD values (Figures 12 and 13), Groups 1–5 consist mainly of eutrophic lakes, whereas Groups 6 and 7 form a separate cluster of relatively oligotrophic lakes. This supports the first TWINSPAN division into LVT1 and 2. Group 5 is an exception; according to the vegetation assessment it falls clearly into LVT2 but the BOD and COD values exceeding the national EQS place it in LVT1. The equivocal position of Group 5 may be explained partly by the fact that only seven rather diverse bodies of standing water had been clustered together. The official chemical data cannot help significantly, as only one lake belonging to this group – Gruza (which is an outlier *per se* as the only medium altitude lake in a group) – is being regularly monitored (Table 2). Yet even in Gruza, the highest recorded values of BOD are clearly above the national environmental quality standards, while COD values, although still meeting the national EQS, are considerably higher than those recorded in the lake belonging to Group 7 (unquestionably oligotrophic) and also higher than the values in Groups 1 and 2 – classified as eutrophic. In addition, the maximum recorded concentrations of chlorophyll *a* in Gruza are high compared with most other lakes, which may partly explain the drift of Group 5 towards eutrophic lakes with regard to water chemistry.

The high organic load recorded in lakes belonging to the groups clustered in LVT1 (Groups 1–4) is expected, as most of the water bodies are shallow lowland lakes, subject to significant human pressure, characterized by intensive primary production. This is shown by the high values of total organic carbon and by extremely high chlorophyll *a* concentrations, which in Palic and Ludos, for instance, surpass the ICPDR target value by orders of magnitude. The highest values of BOD and COD were recorded in Labudnjaca, an abandoned fish pond in Monostorski rit wetland area (Group 4), which is in the final stage of succession from lake to swamp.

A deeper look into the subclasses of LVT1 (LVT1a and LVT1b), reveals that LVT1a consists of meso-eutrophic lakes, while typical eutrophic lakes are found in LVT1b. This division is underpinned by BOD/COD values obtained during the study and strongly supported by other determinants relevant to trophic status (such as total P, N, chlorophyll *a* content, Secchi depth, etc.) (Table 2). However, the division of LVT2 into LVT2a and b, let alone the final step of distinguishing between Groups 6 and 7, cannot be confirmed by chemical data, mainly because of the small number of water bodies that have been clustered in LVT2 (14) compared with 84 lakes in LVT1.

Dissolved oxygen content as well as oxygen saturation provide two reliable determinants of overall ecological quality, but can also be indicators of an ongoing eutrophication process. There was only one lake (Crno jezero, Group 7) where dissolved oxygen content met the national requirement set for class I waters throughout the study (Figure 14), which is to be expected as this is a clear, highland, oligotrophic lake. The lakes belonging to all the other groups in LVT1, as well as Groups 5 and 6 from LVT2, suffer from occasional oxygen depletion in their lower layers and frequent hypersaturation (Figure 15) in the surface layers of the deeper, stratified lakes. Shallow lowland lakes (such as Ludas) are characterized by constant hypersaturation throughout the whole water column (Table 2). Therefore, the TWINSPAN-derived classification cannot be fully supported by these two determinants.

The pH values recorded in all samples fluctuate within the tolerable range, except in highly eutrophic lowland lakes of Group 3 (such as Palic, Ludos, and Glavno jezero) and Group 4, where pH values constantly exceed 8.5, sometimes reaching almost 10 (Figure 16), owing to intense overall lake metabolism during summer periods. These coincided with the period of sampling undertaken in this study, as well as in the official lake monitoring programme. It is practically impossible to justify the TWINSPAN classification using this water quality parameter. Similarly, the concentration of suspended solids (Figure 17) was of little value in verifying or justifying the TWINSPAN-derived lake groups, as there was no clear pattern in median distribution within or among the groups.

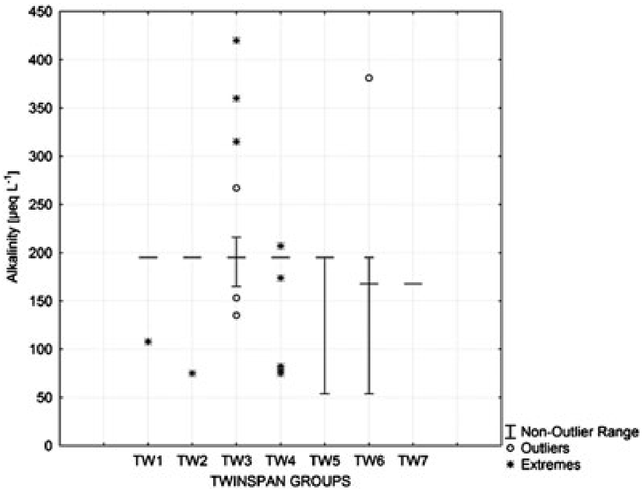

Figure 10. Whisker box plot of alkalinity (median distribution, expressed as µeq L^{-1} $CaCO_3$) in lakes classified in TWINSPAN Groups 1–7.

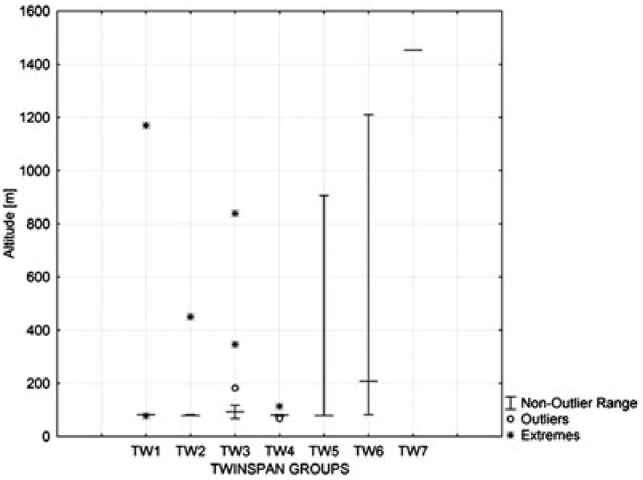

Figure 11. Whisker plot of altitude ((m) median distribution) in lakes classified in TWINSPAN Groups 1–7.

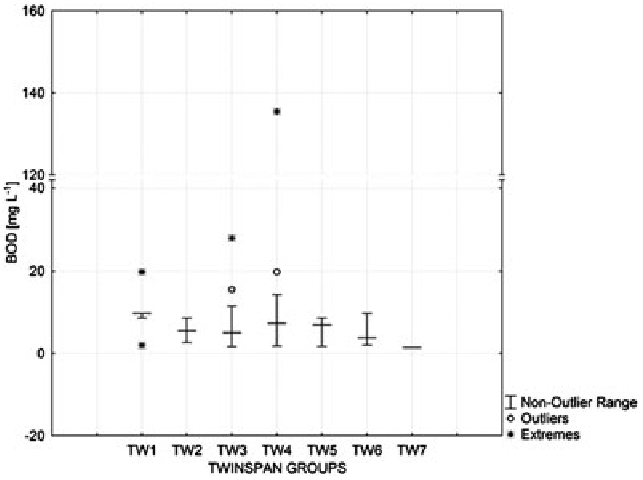

Figure 12. Whisker box plot of biochemical oxygen (BOD) demand (median distribution, expressed as mg L^{-1}) in lakes classified in TWINSPAN Groups 1–7.

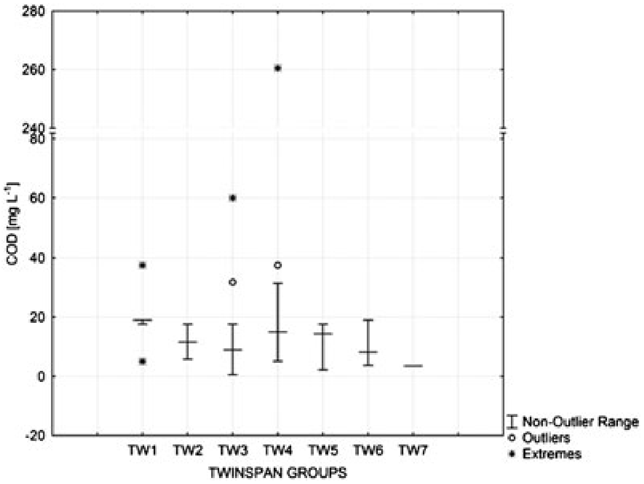

Figure 13. Whisker box plot of chemical oxygen demand (COD) (median distribution, expressed as mg L^{-1}) in lakes classified in TWINSPAN Groups 1–7.

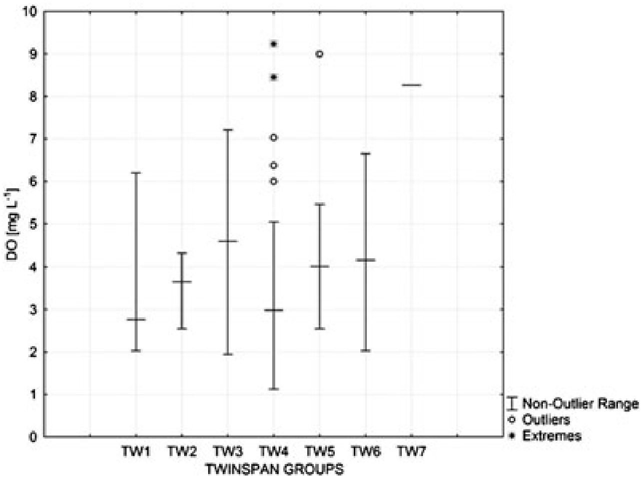

Figure 14. Whisker box plot of dissolved oxygen (DO) concentrations (median distribution, expressed as mg L^{-1}) in lakes classified in TWINSPAN Groups 1–7.

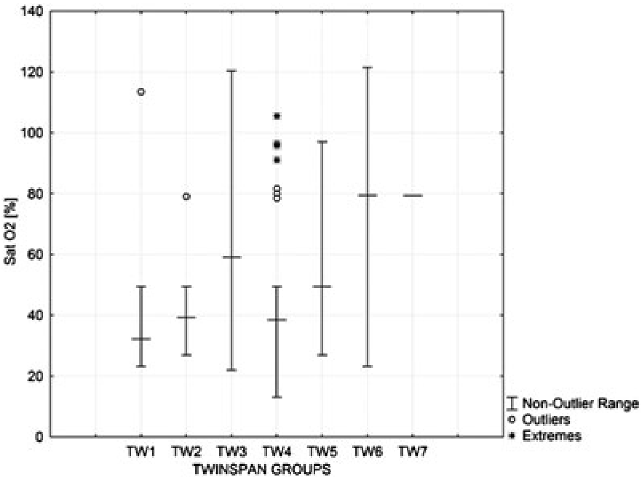

Figure 15. Whisker box plot of oxygen saturation (median distribution, expressed as %) in lakes classified in TWINSPAN Groups 1–7.

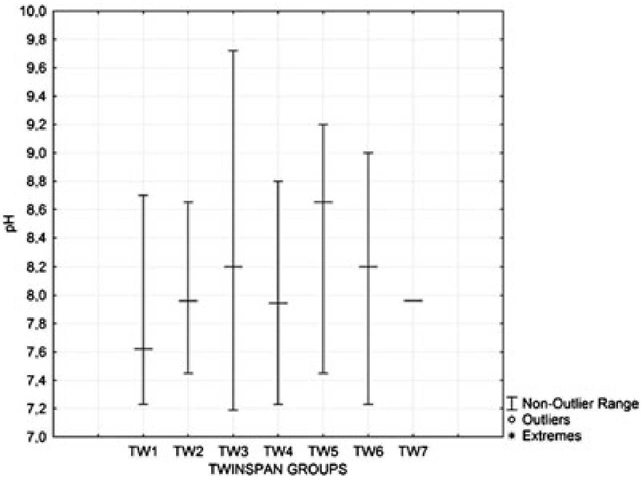

Figure 16. Whisker box plot of pH (median distribution) in lakes classified in TWINSPAN Groups 1–7.

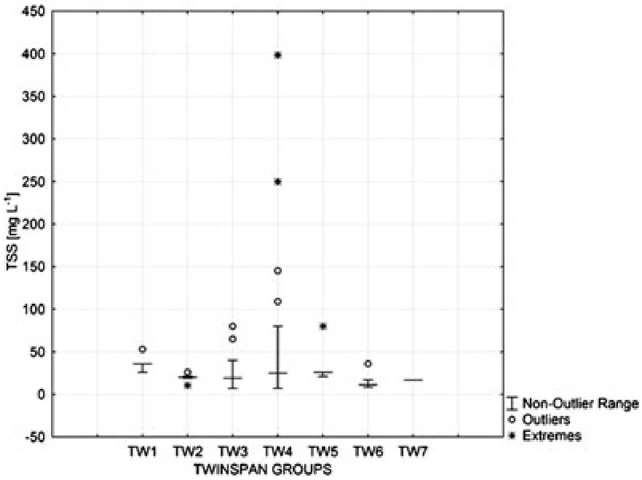

Figure 17. Whisker box plot of the concentration of suspended solids (median distribution, expressed as mg L^{-1}) in lakes classified in TWINSPAN Groups 1–7.

DISCUSSION

In some other relevant studies (Palmer *et al.*, 1992; Palmer, 2001; Duigan *et al.*, 2007), helophytes (and species for which it was unclear whether they represented the helophyte or aquatic form) were excluded from the analyses. Although their response to eutrophication is generally considered to be obscured by soil trophic characteristics, exposure, shoreline management, and their ability to emerge from the water phase (Penning *et al.*, 2008), these species were included in this study because they could not be omitted from the phytocoenological database and a large number of relevés, accumulated over decades. Excluding some species from original relevés would have constituted data manipulation, so the authors did not have any other choice but to follow the habitat concept. If one author, with complete justification, considers as lake vegetation only hydrophytes *sensu stricto*, and another one follows the habitat rather than floristic concept including amphibious species and helophytes, or includes an even wider riparian zone, the databases will contain biased samples of vegetation diversity in certain areas. In a large database containing relevés made by many authors who had different preferences for site selection, this may result in mere noise. The database included in this study was therefore collated using relevés that were compatible with the given habitat concept.

TWINSPAN-derived classification – an overview

Apart from the pragmatic reason for including the whole taxon list derived from relevés, this approach, no matter how complicated it was, turned out to be an advantage, as the authors had the chance to recognize some wider habitat characteristics. One of these was the notable lack of the buffer (reed) zone (Group 1) with the dominant vegetation type represented by ass. *Hydrochari-Nyphoidetum peltatae* Slavnić 56 and *Lemno-Spirodeletum polyrhizae* W.Koch 1926 (Radulović, 2004). The natural shore zone has been slightly degraded, so typical reed vegetation is missing. Instead, fen plants occasionally occur – *Carex riparia*, *Carex vesicaria* and *Iris pseudoacorus*. Only one lake from the same group (Zavojsko jezero, 750 m) does not belong to the lowland category. It is a reservoir in eastern Serbia, near the Bulgarian border and was constructed in 1963 after a major landslide had dammed the River Visočica; the natural dam was later replaced by a hydroelectric dam 'Zavoj'. The eroded shore zone and the presence of fen species appear to be the reason for the position of this lake in Group 1. Common reed is usually a dominant species in the buffer zone between terrestrial and aquatic ecosystems; therefore its role in maintaining stability in river and lake margins is especially important in terms of ecology and classification. The absence of this zone was the main feature distinguishing lakes in Group 1.

The other wider habitat characteristic was the recognition of the succession stage within some isolated groups (namely Group 2), with the indicators of association *Caricetum elatae* Koch 1926. All lakes in Group 2 are situated at 70–80 m above sea level (Paljevina, Jamina, Rogoznjaca and Mali Adler, apart from one – Borsko jezero (500 m altitude) – which has diametrically different hydrological characteristics. Borsko jezero probably should not have been included in the study at all, as it is an atypical highland reservoir; but indeed it falls into Group 1 in one peculiar and unforeseen manner. Most likely, the absence of macrophytes (due to succession at four sites) caused the statistical oversight or 'collateral damage' of the statistical procedure.

In an attempt to reduce this kind of error, various statistical approaches and methods were applied (the results have not been shown here), such as Fuzzy Clustering, Optimal Clustering and TWINSPAN with further divisions. Fuzzy Clustering resulted in eight rather arbitrary groups – two lakes rather similar to each other (Vlasinsko jezero and Gruza) were placed in two very distant groups, while Crno jezero (clearly a Dinaric glacial oligotrophic lake) was classified in a group dominated by standing waters in the Danube floodplain.

Optimal clustering yielded 14 groups, of which eight were Danube floodplain lakes, very similar to each other, as confirmed by TWINSPAN, which placed them all in Group 4. After running TWINSPAN in three iterations, an attempt was made to divide the largest Group 4 further. However, it was clear that the final iteration was based on seasonal–hydrological, rather than other habitat attributes, and that Group 4 is actually the most stable TWINSPAN group, covering two rather similar Danube floodplain areas: Koviljski rit and Apatinsko-monostorski rit wetland areas (Gornje Podunavlje and Donje Podunavlje).

Comparison with other studies

Compared with other classification systems (Palmer *et al.*, 1992; Rodwell*et al.*, 1995; Lukács *et al.*, 2009) the most typical lowland lakes comprise all four general types of aquatic community types: surface and subsurface duckweed, free-floating or rooted submerged pondweed, rooted water-lily vegetation with floating leaves, and emerged reed vegetation (ass. *Ceratophylletum demersi* Soó 1934, *Lemno-Spirodeletum polyrhizae* W.Koch 1926, *Salvinio-Spirodeletum polyrhizae* Slavnić 1956, *Myriophyllo-Potametum* Soo 1934, *Hydrochari-Nyphoidetum peltatae* Slavnić 1956, *Nymphaeetum albo-luteae* Nowinski, 1928, *Trapetum natantis* Muller et Gors 1960, and *Scirpo-Phragmitetum* W. Koch 1926, subass: phragmitetosum Schmalle 1939 and subass: typhaetosum angustifoliae-latifoliae Slavnić 1956, (Butorac, 1995; Radulović *et al.*, 2004; Radulović, 2005; Vukov *et al.*, 2008). Although *Nymphaea alba* does not show high constancy (III and II, Table 1), the stands where this species occurs (deeper waters) are usually monodominant, poor in associated species (Radulović *et al.*, 1998), often producing crowded masses of densely shading foliage.

Two groups showed rather specific characteristics – Group 5 dominated by the *Dichostylidi-Gnaphalietum uliginosi* community, which may be considered a continental equivalent of the sub-Atlantic association *Cyperio-Limoselletum* Oberd 1957; Korneck 1960 (Bagi, 1991) – and Group 4 (typical stands of Group 4 are *Lemno-Spirodeletum polyrhizae* W.Koch 1926, *Salvinio-Spirodeletum polyrhizae* Slavnić 1956, and *Myriophyllo-Potametum* Soo 1934 (Radulović *et al.*, 2004)). This group comprises stands of liverwort *Riccia crystallina*, fern *Marsilea quadrifolia* and bladderworts and the only carnivorous plant from the list – *Utricularia vulgaris* (Panjković *et al.*, 2004; Radulović, 2005). Group 6 contains artificial water bodies, including ornamental pools, which, especially in the early stage, have clear waters and abundant and diverse macrophytes (Sayer *et al.*, 2008).

The pioneering work in the UK on vegetation classification of British lakes by Palmer *et al.* (1992) provided the impetus for this study. The classification of standing waters in Britain was based on a large dataset: 1124 lakes in England, Wales and Scotland (Palmer *et al.*, 1992) and 3447 lakes in England, Wales and Scotland in a revised version (Duigan *et al.*, 2006). Moreover, it satisfied the prime ecological principle – island isolation and, consequently, database homogeneity, which is not the case for similar studies around the continent, including this one, based exclusively on national or administrative boundaries.

According to Palmer *et al.* (1992), charophytes and bryophytes should be included at species level (where possible), and a number of other macroalgae, such as *Enteromorpha* at generic level. This was followed where possible, but in the final analysis higher resolution data had to be omitted because of the low taxonomic resolution of the older data. Only in the case of a typical high mountain Dinaric lake (Group 7), charophyte data were presented at species level. Comparing the species list from this study with the results of the REBECCA project (Penning *et al.*, 2008) confirmed that the most sensitive species include *Chara delicatula* and *Chara rudis*, while the lists of tolerant species were very similar. Species such as *Nuphar lutea* or other more sensitive species were not expected to be present in the table with constancy classes over 20%. Although some of the species classified as tolerant in REBECCA cannot survive hypertrophic conditions, the data collected within REBECCA showed broad trends in response to eutrophication pressure. However, variability in the response of macrophytes to the pressure gradient throughout Europe, and throughout lake types, was rather high, which is confirmed by this study.

Relationship with water chemistry

The general relationships between plant species and the environmental characteristics of fresh waters, especially water chemistry, are well established (Palmer *et al.*, 1992; Jeppesen *et al.*, 2000; Murphy, 2002; Duigan *et al.*, 2007; McElarney and Rippey, 2009). Instead of predicting the vegetation groups using predefined physico-chemical categories, these studies found that it is more effective to study the biota first and then investigate its relationship with physico-chemical variables (McElarney and Rippey, 2009), the concept which has been also applied in the present study. Although it was possible to link altitude and BOD/COD values (as proxies for organic load and general trophic status) to the TWINSPAN-derived classification based on lake vegetation, the attempt failed with other water quality parameters because the set of relevant physico-chemical data available was very limited. As suggested by Moss *et al.* (2003) cost-effective determinants were used, as the number of lakes included in the study was relatively high, but relevant data from the official monitoring programme were available for only 10 out of 98 lakes included in this study. However, Lukács *et al.* (2009) used a far larger set of highly relevant determinants but found a close correlation between a TWINSPAN-derived botanical classification and only five chemical parameters: COD, nitrates, Mg^{2+}, Cl^{-} and Ca^{2+}. Recent research suggests that caution is needed when interpreting plant associations with water chemistry. Pither and Aarssen (2005) showed that environmental or habitat specialization among plants (diatoms, but the same ecological concept might be applied to other species) may be less frequent than previously thought and that highly significant correlations between vegetation composition and environmental attributes may reflect a balance between a strong signal generated by the minority specialists, and noise produced by the majority generalists. Compared with the British lake series (Palmer *et al.*, 1992; Duigan *et al.*, 2007), which includes brackish and ultra-oligotrophic lakes (the latter common in upland areas), the trophic variation of lakes in Serbia is a relatively narrow spectrum.

Application to conservation

Contemporary water management in Serbia is directed solely at flood control, power generation, and water supply, ignoring the fact that ecosystem services depend on ecosystem functioning. The first protected area in what is now Serbia was Obedska bara (Group 4) designated in 1874 (Janković, 1974). Protected areas in Serbia cover 518 003 ha, or 5.86% of the country. However, the Spatial Plan of the Republic of Serbia (Official Gazette of Republic of Serbia, 2010b) predicts that about 10% of the total area of Serbia will be protected by 2015 (listed within Supplementary Material).

One way in which the results of the present study contribute to conservation is by expanding and amplifying information already available on sites recognized as important nationally or internationally. The National Institute for Nature Conservation has so far recognized nine Ramsar sites, 61 IPAs (important plant areas) and 42 IBAs (important bird areas). In addition, 61 areas have been nominated for the 'Emerald' European Ecological Network; these are areas of special interest for European wild flora and fauna and their natural habitats, based on the Bern Convention, and form part of the Ecological Network of Serbia (Regulation on Ecological Network, Official Gazette of Republic of Serbia, 2010c).

From a nature conservation viewpoint Group 4 stands out because it consists, almost exclusively, of protected areas (56 lakes around the Apatinski and Monostorski rit area (Gornje Podunavlje, 22 480 ha, Ramsar site no. 1737), Carska bara and Perleska bara (1767 ha, Ramsar site no. 819), Obedska bara (17 501 ha, Ramsar site no. 136), and Labudovo okno (3733 ha, Ramsar site no. 1655)). Together, these make up to 90% (*ca* 45 000 ha) of internationally designated Ramsar wetland habitats in Serbia, while the area of the Koviljski rit wetland area (*ca* 15 000 ha) is on the Ramsar waiting list. All these sites are also designated as National Nature Reserves, IPAs, and IBAs. One of the criteria against which these sites were assessed was Ramsar criterion 2a – the presence of an assemblage of rare, vulnerable and/or endangered species such as *Eranthis hyemalis*, *Hottonia palustris*, and *Hippuris vulgaris* (Panjković, 2005). Ramsar site no. 1737 is also an important spawning site and a migratory route of fishes, a nesting place of the white-tailed eagle *Haliaeetus albicilla* and the black stork *Ciconia nigra*, as well as the habitat of the largest population of the red deer *Cervus elaphus* in Serbia. Ramsar site no. 819, a mixture of fish ponds, swamp, marsh, forest, meadow, and steppe intersected by rivers, canals, and embankments, consists of salt-tolerant communities, a rich aquatic flowering plant community, and steppe vegetation (Butorac, 1995; Radulović, 2005). Of the 250 recorded bird species, 140 species (including all eight European heron species) nest at the site and 100 pass through on migration.

One of the lakes in Group 4 (Carska bara) is also notable for the recently discovered stands of marsh fern *Thelypteris palustris* (Radulović, 2005) found in association with occasionally flooded relict forest *Thelyptero-Phragmitetum cinerei* M Jank (Janković, 1974). Lakes in Group 4 also contain the critically endangered *Hippuris vulgaris* L. (Vučković and Panjković, 1999), a species that is invisible in the constancy table.

The remaining two Ramsar sites included in this study do not fall into Group 4: Ludos and Vlasinsko jezero. The shallow lake Ludos is fringed by extensive reedbeds and surrounded by marshland. The area is important for many species of breeding water birds, and falls into Group 3, the most diverse lowland eutrophic lakes of the Danube floodplain. Kennison *et al.* (1998) recognized *Najas marina-Hippuris vulgaris* sites in the Norfolk Broads (eastern England) as targets for nature conservation in freshwater and brackish lakes. This study confirms the fragility of this community, which was recorded in protected areas of Groups 3 and 4. Vlasinsko jezero (Ramsar site no. 1738) is within a landscape where the peat island and peat bogs represent one of the most important refuges of boreal flora in southern Europe. More than 125 bird species are recorded, among them the endangered corncrake (*Crex crex*) which nests every year within the site and on sloping meadows of the surrounding mountains, as well as a colony of sand martins (*Riparia riparia*) with around 300 active nests, unique in this biogeographical region. Vlasinsko jezero belongs to Group 6 which comprises lakes used for hydro-electric power generation, recreation, industrial or domestic water supply and ornamental pools scattered throughout Midland Serbia. In fact, the whole LVT2 division (Group 5, Group 6 and the outlying Group 7) has been recognized as a potential conservation resource. Six sites deserve special attention: Sljunkara, Gruza, Lisinsko jezero, Borkovac, Celije and Spajic, as they are meso-oligotrophic and may represent specific oases for aquatic species whose natural habitats are endangered by human activity or natural succession. These six site targets were surveyed as a part of the additional field work (during 2008, 2009, and 2010), owing to the previous premise that there were some gaps in geographical coverage.

As Duigan *et al.* (1996) emphasized, there is a common dilemma in lake conservation: should natural succession be permitted, or changes in land-use activity which might affect these natural processes be allowed? In other words, should new lakes be created for the future or old ones dredged with the loss of valuable sediment records? The most pragmatic approach could be to retard succession as much as possible (LVT1) at natural sites, and create and maintain new open water habitats (LTV2).

According to Sayer *et al.* (2008), ornamental lakes and pools may be undervalued for nature conservation simply because of their origin. However, many ornamental lakes can be considered to be of high conservation value, owing to their diverse plant assemblages. This study generally supports the view that ornamental lakes (such as lake Sljunkara) are worthy of attention from conservation organizations. Yet, *Myriophyllum spicatum* and *Potamogeton crispus*, indicators of mesotrophic/oligotrophic conditions in highland lakes in Serbia, could also be taken as an early warning sign of impending eutrophication.

Unfortunately, natural ecosystems are still regarded as competitive users of limited water resources. Although much attention in water management in Serbia is paid to 'conservation', in practice this means the protection and conservation of water resources (both quality and quantity) intended for water supply. Evidence for this comes from the standing waters monitoring programme which covers all major reservoirs but only a few natural lakes. One option for encouraging a nature conservation assessment of lakes in Serbia would be to apply conservation criteria such as naturalness, representativeness, rarity and diversity to the data in this study. This could be achieved by using a similar procedure to LACON (Lake Assessment for Conservation) (Palmer, 2008). This is a semi-quantitative method of assessing the nature conservation interest and value of standing waters in Great Britain. It is modelled on SERCON (System for Evaluating Rivers for Conservation) (Boon *et al.*, 1998), developed by the UK statutory nature conservation agencies, in conjunction with a number of other bodies, for the assessment of river conservation value.

Meanwhile, it is hoped that the botanical classification published here will provide the means and the incentive for raising the profile of lake conservation in Serbia.

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