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Assessing the influence of environmental parameters on aquatic plants of ponds in the hinterland of Long Beach in Montenegro

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ABSTRACT

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The distribution and richness of aquatic plants in 19 ponds in the hinterland of Long Beach and Ada Bojana Island in the southern part of Montenegro in the period from March 2014 to October 2014 have been studied in relation to some environmental factors. The presence of 74 species was recorded, 10 of which were submerged, 4 floating-leaved and 60 emergent plants. *Phragmites australis* was the most frequent species that is present in 16 of the analysed ponds. The results of Canonical Correspondence Analysis (CCA) have shown that statistically significant physicochemical parameters (concentration of nitrates, distance from the sea and salinity) accounted for 28.3% of variance in aquatic plant distribution. Species richness was rather high, 20 species per pond, on average, and was moderately related to the physicochemical parameters according to the Generalized Additive Models (GAM).

Key words: Aquatic plants, Mediterranean ponds, species richness, CCA, GAM.

RESUMEN

Evaluación de la influencia de parámetros ambientales en las plantas acuáticas de las lagunas del interior de la Gran Playa en Montenegro

La distribución y riqueza de plantas acuáticas en las 19 lagunas situadas en el interior de la Gran Playa y la Isla de Ada Bojana en el sur de Montenegro, en el periodo comprendido entre marzo 2014 y octubre 2014, han sido estudiadas en relación a diversos factores ambientales. Se ha registrado presencia de 74 especies, de las cuales 10 eran sumergidas, 4 flotantes y 60 emergentes. La especie Phragmites australis *fue la más frecuente, se encuentra en 16 de las lagunas analizadas. Los resultados del Análisis de correspondencia canónica (CCA) han mostrado que los parámetros fisicoquímicos estadísticamente significativos (concentración de nitratos, distancia desde el mar y salinidad) representan un 28.3% de varianza en la distribución de las plantas acuáticas. La riqueza de especies fue más bien alta, 20 especies de media por laguna, y estaba moderadamente relacionada con los parámetros fisicoquímicos, de acuerdo con el Modelo aditivo generalizado (GAM).*

Palabras clave: *Plantas acuáticas, lagunas Mediterráneo, riqueza, CCA, GAM.*

INTRODUCTION

It is a well-known fact that the Mediterranean Basin represents one of the world's major biodiversity hot spots (Médail & Quézel, 1999; Myers *et al.*, 2000; Coll *et al.*, 2010). This region also features a high aquatic plant richness, especially in countries such as Spain, France, Italy and Portugal in which there is a considerable number of suitable freshwater or/and brackish habitats (Chappuis *et al.*, 2012).

Aquatic plants which are also referred to as aquatic macrophytes are regarded as large photosynthetic organisms growing permanently or temporally in aquatic environments (Bornette & Puijalon, 2011). “True” macrophytes inhabit permanently water bodies, while the others known as helophytes are more amphibious and can tolerate seasonal drying (Lacoul & Freedman, 2006). These plants have an essential role in the structure and functioning of freshwater ecosystems, given that they provide habitat and shelter, as well as breeding sites and food resources for other aquatic and terrestrial species (Carpenter & Lodge, 1986).

Distribution of aquatic plants, as well as the composition of their communities, is largely affected by climate, hydrology, sediment and water chemistry (Koch, 2001; Lougheed *et al.*, 2001; del Pozo *et al.*, 2011). Out of the water chemical parameters the most influential ones are alkalinity, hardness and conductivity, pH and limiting nutrients such as nitrogen and phosphorus (Jackson & Charles, 1988; Toivonen & Huttunen, 1995; Vestergaard & Sand-Jensen, 2000) or excessive supply of these nutrients through eutrophication (Lehmann & Lachavanne, 1999). Namely, macrophyte-environment relationships have been studied in various types of aquatic ecosystems, as well as in different geographical areas, including Mediterranean region (Grillas, 1990; Christia & Papastergiadou, 2007; del Pozo *et al.*, 2011; Chappuis *et al.*, 2014, Gallego *et al.*, 2015; Lauridsen *et al.*, 2015).

This study was focused on relationships among aquatic plans and local environmental characteristics of ponds along the southernmost part of Montenegrin coast. According to the results of the latest investigations, flora of Montenegro includes 272 species of aquatic plants (Blaženčić, 2007). Previous floristic surveys of aquatic plants were focused on the Skadar Lake (Černjavski *et al.*, 1949; Adams, 1981), glacial lakes on the mountains Durmitor, Bjelasica and Volujak (Birkis & Walters, 1972/1973; Blaženčić & Blaženčić, 1989, 1994, 2005), reservoir in the town of Nikšić (Blaženčić, 2004). Furthermore, these studies also included the coastal part of Montenegro with regard to the saline wetlands in the area of Budva (Adam *et al.*, 1971/1972), Tivat (Janković & Stevanović, 1983) and Ulcinj (Biberdžić & Blaženčić, 2013).

However, previous studies in Montenegro did not comprise any analysis of the relationship between aquatic plants and environmental parameters of their habitats. Furthermore, aquatic plants of Montenegro were not included in broader ecological research of these plants at the European level (Chappuis *et al.*, 2012). The main objective of this study was to analyze the data on the distribution and richness of these plants in ponds along the southernmost part of Montenegrin coast, as well as their relationships with several physicochemical variables in order to provide elements for the management and protection of these valuable habitats.

MATERIAL AND METHODS

Study area

Long Beach and Ada Bojana Island are located in the southernmost part of Montenegro. Long Beach, with the length of about 13 kilometres and an average width of about 1.5 km, is the largest sandy beach on the Montenegrin coast. It extends from Port Milena channel to the Bojana River, which separates it from the Ada Bojana, the island situated at the mouth of the river Bojana. In the hinterland of the Long Beach, there are numerous ponds which are remnants of the former lake Zoganjsko that dried up and transformed into great Ulcinj saltern after the construction of the Port Milena channel. There are still many brackish and freshwater springs in the area whose waters fill these ponds, along with rain water.

This study included 19 ponds of different size in the hinterland of the Long Beach (Table 1). The size of the analysed ponds varies considerably during the year. They have the largest area in the early spring due to the inflow of atmospheric precipitation and groundwater. In addition, their size is also affected by the higher inflow of sand sediments, caused by strong winds in this area. During the spring and early summer temporary ponds are filled with water, while during the dry season (July-September) the water level significantly decreases. They start filling up with water again at the beginning of the new rainy season in October. The area of analysed ponds, as well as their distance from the sea, was determined in March 2014, at the beginning of the growing season. The calculation was done by means of the ArcGIS 10.0 software (ESRI, 2011).

Floristic data

Field research was carried out twice a month during the growing seasons, from March till October 2014. Aquatic plants were surveyed by observing the shore, as well as the water surfaces of 19 analysed ponds along transects perpendicular to the shoreline and collected during the peak of the vegetation season in August 2014. Transects were made every 5 meters and their number per pond depended on the pond size (from 15 in small, to 50 transects in larger ponds). The collected plant material was partially stored in a herbarium or preserved in 60% alcohol and deposited in the botanical collection of the Natural History Museum of Montenegro. Species diversity in each pond was expressed as species richness, which represents the total number of different species of aquatic plants.

Water sampling

The water in the littoral part of the investigated ponds was sampled in March and August 2014 at the depth of about 70-80 cm. However, in August, temporary ponds were completely dried, therefore the physicochemical parameters were determined only in permanent ones. The mean values of parameters that were measured in March and August were used for further statistical analyses. Water temperature, pH, conductivity and salinity were measured in the field using PCSTestr 35 (Eutech Instruments Multi-Parameter PCSTestr 35), while other parameters (total hardness, the hardness of $CaCO_3$, the hard-

Table 1. Main characteristics of the study ponds and species richness of aquatic plants. *Principales características de las lagunas estudiadas y riqueza de especies de plantas acuáticas.*

Pond	Lat/Long	Pond type	Species richness
L1	41°54′38.25″N 19°14′8.19″E	Permanent	5
L2	41°54′42.33″N 19°14′25.41″E	Permanent	11
L3	41°54′9.11″N 19°16′25.16″E	Permanent	14
L4	41°54′4.21″N 19°16′37.46″E	Permanent	23
L5	41°53′55.48″N 19°17′20.55″E	Permanent	19
L6	41°53′45.02″N 19°17′44.37″E	Permanent	28
L7	41°53′40.14″N 19°17′51.69″E	Temporary	29
L8	41°53′41.81″N 19°18′15.12″E	Permanent	25
L9	41°53′42.98″N 19°18′50.89″E	Permanent	24
L10	41°53′29.75″N 19°18′38.37″E	Permanent	18
L11	41°52′16.39″N 19°20′17.87″E	Permanent	26
L12	41°51′53.82″N 19°21′35.13″E	Temporary	23
L13	41°52′37.34″N 19°21′26.76″E	Permanent	36
L14	41°52′32.92″N 19°21′36.55″E	Permanent	19
L15	41°52′32.62″N 19°22′9.78″E	Permanent	25
L16	41°52′32.93″N 19°21′47.76″E	Permanent	11
L17	41°52′29.10″N 19°22′7.15″E	Temporary	14
L18	41°53′0.43″N 19°20′15.33″E	Permanent	17
L19	41°53′7.06″N 19°19′55.01″E	Permanent	18

ness of $CaCO_3$ Ca^{2+}, and Mg^{2+}, NO_3^- and SO_4^{2-}) were determined immediately after sampling in laboratory according to standard methods (APHA, 1975).

Statistical analysis of the data

In order to reveal the overall variation of analyzed physicochemical parameters, a principal component analysis (PCA) was performed. Data used in PCA were log transformed and standardized. Physicochemical parameters were also checked for the collinearity using the variance inflation factor. Variables that showed significant correlations with other parameters (VIF > 10) were excluded from further analyses (McCune & Grace, 2002). Relationships between the presence of aquatic plants and environmental parameters were estimated by means of Canonical Correspondence Analysis (CCA). Environmental variables were chosen by forward selection and only the significant ones ($p < 0.05$) were included in the model. The statistical significance of physicochemical parameters was tested by Monte Carlo permutation test with 999 runs. Finally, Generalized Additive Models (GAM) were used to determine the relationships between individual physicochemical parameters and species richness (Hastie & Tibshirani, 1990). A Poisson probability distribution with a log link function was used (Crawley, 1993). An initial model included the predictor (environmental parameters) smoothed with four degrees of freedom (Heikkinen *et al*., 2007). The best GAM model was selected on the base of Akaike information criterion-AIC, a measure of goodness of fit of the model for a given set of parameters (Akaike, 1974). Statistical analyses were performed by means of CANOCO 5 (Ter Braak & Šmilauer, 2002).

RESULTS

Physicochemical characteristics of ponds

In the hinterland of Long Beach and Ada Bojana Island, it was noted that sixteen of 19 analysed ponds were permanent, while three of them were temporary ones because they dried up during the summer due to smaller amounts of atmospheric precipitation, high air temperature, evaporation and reduction of the river Bojana water level, which usually floods the surrounding area in the spring.

Table 2. Factor-variable correlations, eigenvalue and percentages of variance for the first three principal components (factor loadings > 0.70 are indicated in bold). *Correlaciones de variables factoriales, de valores propios y los porcentajes de varianza para los tres primeros componentes principales (las cargas de los factores > 0.70 se indican en negrita).*

Parameter	Abbreviation	PC1	PC2	PC3
Area (m^2)	Area	–0.011	0.1406	**–0.9519**
Distance (m)	Dist	–0.3463	**0.8269**	0.0296
Water temperature (°C)	Twat	0.1047	**0.9174**	–0.0101
pH	pH	0.121	**0.7298**	0.0189
Salinity (‰)	Sal	**0.9652**	–0.2021	0.0607
Conductivity (µS/cm)	EC	**0.9912**	0.0224	0.0642
Total hardness (mg/l)	dH	**0.9941**	0.0361	0.0688
Carbonate hardness (mg/l)	dCa	**0.9936**	0.0415	0.0716
Ca^{2+} (mg/l)	Ca	**0.9762**	0.0177	0.0663
Mg^{2+} (mg/l)	Mg	**0.9545**	–0.0063	0.0653
NO_3^- (mg/l)	NO3	0.6511	0.6061	–0.1514
SO_4^{2-} (mg/l)	SO4	–0.5474	0.3775	0.5329
Eigenvalue		6.62	2.63	1.24
% of Total Variance		55.2	21.9	10.3

Principal Component Analysis (PCA) was used in order to determine the structure of variability of the analysed environmental data set (Table S1, supplementary material available at www.limnetica.com). PCA rendered three principal components with eigenvalues greater than 1.00, accounting for 87.5% of the total variability of analysed data set (Table 2). The first component (PC1), explaining 55.8% of the total variance, was highly correlated with the variables related to the salinity and water hardness (Fig. 1). The second component (PC2), which explained 21.9% of the total variance, was mostly correlated with water temperature, defining a gradient from temporary ponds which are warmer and have a higher concentration of sulphates and nitrates, to permanent, less warm ponds, with lower concentrations of nutrients. Given that the second component was also correlated with the distance from the sea, permanent ponds that are located farther, were plotted on the positive side of the second components, as well. Finally, the third component (PC3) was explained by differences in the surface area of the analysed ponds. It accounted on 10.3% of the total variance.

Relationship between plants and environmental variables

A total of 74 taxa were found in 19 analysed ponds in the hinterland of Long Beach and Ada Bojana Island from which 10 (13.5%) were sub-

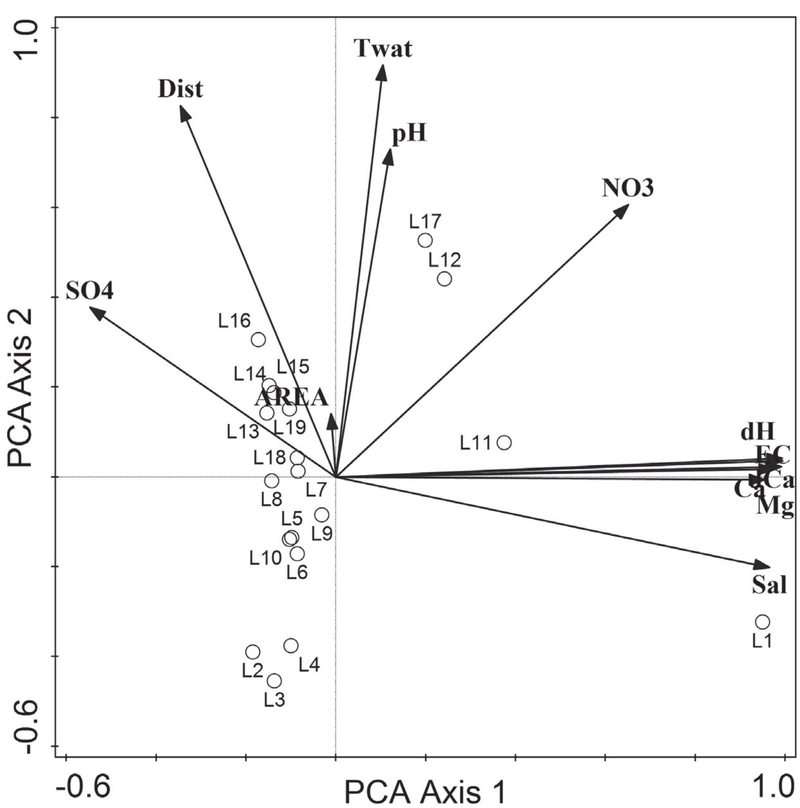

Figure 1. Ordination plot of PCA of physicochemical variables; Area-pond area, Dist-distance from the sea, Twat-water temperature, pH-pH value, Sal-salinity, EC-conductivity, dH-total hardness, dCa-carbonate hardness, Ca-concentration of calcium, Mg-concentration of magnesium, NO3-concentration of nitrates, SO4-concentration of sulphates. *Diagrama de ordenación PCA con las variables ambientales. Area-área de la laguna, Dist-distancia del mar, Twat-temperatura del agua, pH-valor del pH, Sal-salinidad, EC-conductividad, dH-dureza total, dCa-dureza debida a carbonatos, Ca-concentración de calcio, Mg-concentración de magnesio, NO3-concentración de nitratos, SO4-concentración de sulfatos.*

merged, 4 (5.4%) floating-leaved and 60 (81.1%) helophytes. Out of these 74 taxa, 14 occurred in more than 50% of the studied ponds, while 15 occurred at only one site (Table S2, supplementary material available at www.limnetica.com). *Phragmites australis* was the most frequent species that was recorded in 16 ponds, while *Utricularia vulgaris* (recorded in 9 ponds) and *Potamogeton nodosus* (recorded in 13 ponds) were the most frequent submerged and floating ones, respectively. Besides *Phragmites australis*, plants that occurred in more than 50% of the ponds were mainly helophytes, including *Carex vulpina*, *Lythrum salicaria*, *Juncus acutus* and *J. articulatus*.

Species richness ranged from 5 to 36, with the average value of 20 and median equal to 19 (Table 1). The lowest number of species was recorded in the pond that is closest to the sea and had the highest salinity (L1). The only five species recorded were *Cyperus longus*, *Juncus acutus*, *Phragmites australis* and *Potamogeton perfoliatus*. The highest number of species (36) was recorded in pond L13.

Canonical Correspondence Analysis (CCA) was applied in order to identify physicochemical variables that were associated with the occurrence of aquatic plants (Fig. 2). The results of CCA showed that distance from the sea, nitrate concentration and salinity were three non-

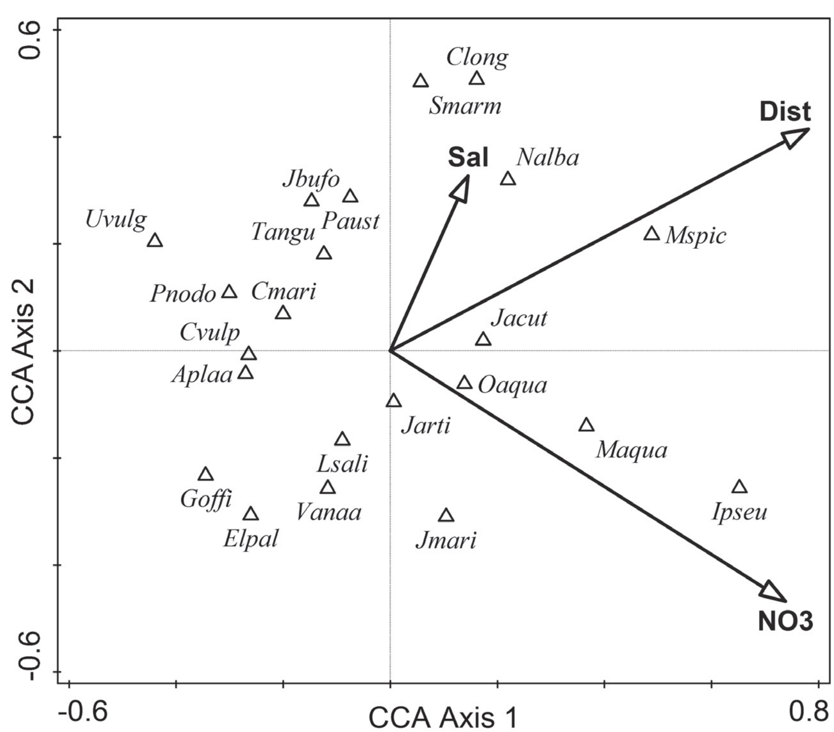

Figure 2. Aquatic plant species and environmental variables ordination obtained by CCA. Abbreviations of significant variables ($p < 0.05$): Dist-distance from the sea, NO3-concentration of nitrates, Sal-salinity. Aquatic plant species abbreviations (occurred in > 40% of ponds sampled): Aplaa-*Alisma plantago-aquatica*, Cvulp-*Carex vulpina*, Cmari-*Cladium mariscus*, Clong-*Cyperus longus*, Elpal-*Eleocharis palustris*, Goffi-*Gratiola officinalis*, Ipseu-*Iris pseudacorus*, Jacut-*Juncus acutus*, Jarti-*Juncus articulates*, Jbufo-*Juncus bufonius*, Jmari-*Juncus maritimus*, Lsali-*Lythrum salicaria*, Maqua-*Mentha aquatica*, Mspic-*Myriophyllum spicatum*, Nalba-*Nymphaea alba*, Oaqua-*Oenanthe aquatica*, Paust-*Phragmites australis*, Pnodo-*Potamogeton nodosus*, Smarm-*Scirpus maritimus* ssp. *maritimus*, Tangu-*Typha angustifolia*, Uvulg-*Utricularia vulgaris*, Vanaa-*Veronica anagalis-aquatica*). *Ordenación de las diferentes especies de plantas acuáticas y de las variables ambientales obtenidas mediante un CCA. Abreviaturas de las variables significativas* (p < 0,05)*: Dist-distancia del mar, NO3-concentración de nitratos, Sal-salinidad. Abreviaturas de las plantas acuáticas (presentes en > 40% de lagunas muestreadas).*

collinear, statistically significant ($p < 0.05$) physicochemical parameters jointly explaining 26.9% of the variation in the plant presence (9.9% distance from the sea, 9.2% nitrate concentration and 7.8% salinity). The first CCA axes, accounting on the 42.4% of the explained variance, was positively correlated to the distance from the sea ($r = 0.75$) and nitrate concentration ($r = 0.71$), while the second one accounting on 33.7% of the explained variance, was weakly positively correlated to the distance from the sea ($r = 0.37$) and. negatively correlated to nitrate concentration ($r = -0.42$). Finally, salinity was strongly correlated to the third CCA axes ($r = 0.88$), accounting on the 23.8% of the explained variance. Furthermore, Monte Carlo permutation test for the first, as well as for all three canonical axes showed that there is a significant relationship between pond to pond variation in plant presence and the environmental variables (F-ratio = 1.9, *p*-value = 0.015 and F-ratio = 1.8, *p*-value = 0.001, respectively).

Higher concentrations of nitrate were positively related to the presence of *Iris pseudacorus* and *Mentha aquatica*, while species such as *Utricularia vulgaris* and *Potamogeton nodosus* were found in ponds with the lowest nitrate concentration. Moreover, typical plants of salt marshes such as *Scirpus maritimus* ssp. *maritimus*, *Juncus acutus, Juncus maritimus* showed a positive correlation with the salinity and are found on sites with the highest values of the third axes. On the contrary, species that cannot tolerate a rather high salt concentration are found on sites with the lowest value of this axis, like *Nymphaea alba*.

GAM performed on single physicochemical predictor showed mostly moderate relationships between richness and environmental parameters. Nitrate concentration, conductivity and distance from the sea were the variables with the highest deviance reduction (54.19%, 49.14% and 42.22% of explained deviance, respectively), whereas water temperature had the lowest value (24.58% of explained deviance) (Table 3).

Table 3. Generalized Additive Models (GAM) of species richness of aquatic plants in analyzed ponds predicted by environmental variables. *Modelo aditivo generalizado (GAM) de la riqueza de especies de plantas acuáticas en lagunas analizadas predicha por las variables ambientales.*

	AIC	% of explained deviance
Null model	146.02	
NO_3^- (mg/l)	130.08	54.19
Conductivity (μS/cm)	132.25	49.14
Distance from the sea (m)	136.57	42.22
Ca^{2+} (mg/l)	134.22	32.36
Carbonate hardness(mg/l)	133.74	31.03
Total hardness (mg/l)	133.82	30.35
Salinity (‰)	132.92	29.89
Mg^{2+} (mg/l)	133.45	28.49
Area (m^2)	143.98	28.04
SO_4^{2-} (mg/l)	145.47	25.45
Water temperature (°C)	141.68	24.58

DISCUSSION

The results of the PCA revealed that the analysed ponds showed a clear pattern of variation concerning physicochemical parameters of the water. These variations were controlled by three groups of factors. The first group comprised chemical factors of the natural origin related to the salinity, conductivity, total and carbonate hardness, as well as calcium and magnesium concentrations. Distinctiveness of these factors has already been demonstrated for natural waters (Alberto *et al*., 2001; Simeonov *et al*, 2001; de Aguiar Netto, 2013.). The second one referred to the physical parameters, primarily water temperature, while the third group was related to the pond area.

The number of species that were recorded in this study represents more than a third of the total number of aquatic plants previously reported for Montenegro (Blaženčić, 2007). The average number of aquatic plants, i.e. 20 species per pond, with the range from 5 to 36, was similar to the data stated by Bagella *et al*. (2010) for Mediterranean temporary ponds in Sardinia (average of 23 species per pond, with the range from 12 to 35), and somewhat higher than those reported by Della Bella *et al*. (2008) in their study of macrophyte diversity of Tyrrhenian coastal ponds in central Italy (average of 9 species per pond, with the range from 1 to 26).

The results obtained by the CCA and GAM revealed that nitrate concentration, distance from the sea, salinity (in the case of CCA) and conductivity (in the case of GAM) are the main variables which determine the distribution of the aquatic plants and species richness in the studied area. Nutrient gradients, including nitrate concentration, are proved to be significant for the distribution and species richness of plants in aquatic environment (Vestergaard & Sand-Jensen, 2000; James *et al*., 2005), as in the case of ponds in the hinterland of Long Beach in Montenegro. Namely, high concentration of nitrates has a negative impact on some species of aquatic plants that are sensitive to eutrophication, causing a difference in the distribution of species as well as species richness (Lougheed *et al*., 2001).

The key role of salinity for the distribution of macrophytes in the coastal habitats of the Mediterranean has been well documented (Grillas, 1990; Christia & Papastergiadou, 2007; Chappuis *et al*. 2014). Namely, most of the macrophytes typical for freshwaters cannot tolerate the increase in salinity (Warwick & Bailey, 1997; James et al., 2003; Nielsen et al., 2003a,b; 2007; Brock et al., 2005). Moreover, salinity, together with ionic content, is the most important factor determining the distribution of aquatic plants all over the world: in other regions of the Europe (Heegaard *et al*., 2001; McElarney & Rippey, 2009), in North (Capers *et al*., 2010), and South America (Bini *et al*., 1999). It has been shown that conductivity, as a summary variable directly related to the ionic content (Heegaard et al., 2001), represents an important predictor of species richness (Murphy *et al*., 2003; Mäkelä *et al*., 2004), as reported in this study. Thus, among the analysed ponds, the highest number of species is recorded in the one that was characterized by lowest conductivity. In addition to the distance from the sea, calcium content, carbonate and total hardness, parameters used as indirect measure of alkalinity in this study, have also shown a moderate importance as predictors of the species richness. Namely, alkalinity represents another important parameter for macrophyte distribution and richness, both in the northern, and southern Europe (Vestergaard & Sand-Jensen, 2000; Lauridsen *et al*., 2015). Other analysed parameters are proved to be weaker predictors of species richness in the analysed ponds.

However, it should be noted that even though the other tested physicochemical variables were not proven to be statistically significant in this study, they certainly affect the distribution of macrophytes (Grillas, 1990; Mäkelä *et al*., 2004, Christia & Papastergiadou, 2007; Manolaki & Papastergiadou, 2013; Chappuis *et al*., 2014). In relation to the pond size there were no significant differences concerning the number of species they host, confirming the significant contribution of small water bodies to local and regional biodiversity (Heino & Toivonen, 2008; Chappuis *et al*., 2014). Moreover, there were no significant differences in plant distribution, nor in the species richness between temporary and permanent ponds, although they were observed in similar ponds of the Mediterranean region (Della Bella *et al*., 2008; Chappuis *et al*., 2014). Furthermore, the spatial proximity of investigated ponds causes their significant ecological similarity concerning climate and geology, that ale also proven to be important factors that determine the distribution of aquatic plants (Alahuhta, 2015).

In conclusion, even though spatially closely related, the analysed ponds in the hinterland of Long Beach and Ada Bojana Island in the southern part of Montenegro have shown differences in the physicochemical characteristics, especially in regard to salinity, calcium content and water hardness. They demonstrated a high level of environmental variability that is referred to be an inherent characteristic of the aquatic ecosystems in the Mediterranean region (Ballón *et al*., 2016). Furthermore, the analysed ponds are characterized by a substantial richness of aquatic plant species, whether they are permanent or temporary ones. With a significant number of species that has been recorded therein, these ponds are extremely valuable and important habitats whose protection should be given special attention. Beside species diversity, they are particulary important because of plant communities that are characteristic of this part of the Montenegrin coast.The investigated area presents also significant wintering shelters for numerous birds, as well as shelters for large number of reptiles and amphibians. Unfortunately, the ecological status of the analyzed ponds in the hinterland of Long Beach in the southern part of Montenegro is rapidly deteriorating in the recent years. Given that they are situated directly in hinterland of the largest sandy beach on the Montenegrin coast the greatest danger they face is the rapid development of tourism and urbanization of this area.

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