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Patterns of aquatic macrophyte species composition and distribution in Bulgarian rivers

Gana GECHEVA[1,*], Lilyana YURUKOVA[2], Svetoslav CHESHMEDJIEV[3]
[1]Faculty of Biology, University of Plovdiv, Plovdiv, Bulgaria
[2]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria
[3]SI Eco Consult Ltd., Sofia, Bulgaria

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Abstract: The composition of aquatic bryophyte and vascular plant assemblages, and 4 environmental variables (water velocity, shading, mean depth, and substrate type), together with altitude, ecological status, and river type were monitored at 223 sites along 204 rivers during 2009 in the Pontic Province and Eastern Balkan Ecoregions, Bulgaria. The relationships between macrophytes themselves and the parameters studied were tested. Forty-nine bryophytes and 86 vascular plants were identified. Canonical correspondence analysis (CCA) showed that aquatic bryophytes and vascular plants are in inverse correlation. Bryophytes occurred mainly in conditions of higher altitude and ecological status; at the same time vascular plants were found in lowland river types defined by lower status and altitude. CCA also established that flow velocity was of prime importance for bryophyte distribution, while the substrate type had no significant correlation. In contrast, most vascular plants depended considerably on substrate and prefer finer types. The data also showed that shading explained the major part of vascular species variance. This study represents an important contribution to environmental monitoring and biodiversity conservation programmes. For the first time relationships between 135 macrophyte taxa and 7 environmental variables were tested. Abiotic factors were highlighted with major influence on river bryophyte and vascular plant communities.

Key words: Aquatic macrophytes, river, environmental variables, canonical correspondence analysis

1. Introduction

Aquatic macrophytes are considered photosynthetic organisms of freshwater habitats, easily seen with the naked eye, that are normally found growing in or on the surface of water, or where soils are flooded or saturated long enough. These plants have evolved some specialised adaptations to an anaerobic environment. They are represented in 7 plant divisions: Cyanobacteria, Chlorophyta, Rhodophyta, Xanthophyta, Bryophyta, Pteridophyta, and Spermatophyta (Chambers et al., 2008). Macrophytes may be floating, floating-leaved, submerged, or emergent (Sculthorpe, 1967), and may complete their life cycle in water (still and flowing) or on hydric soils (inundated and noninundated).

Aquatic communities reflect anthropogenic influence and are very useful for detecting and assessing human impacts (Solak et al., 2012). Aquatic macrophytes as primary producers and habitat providers are important component of river ecosystems. Their compositional patterns are sensitive to a number of factors such as water flow velocity and level, eutrophication, pollution, and additional pressures. As stipulated by the European Water Framework Directive (WFD) 2000/60/EC (European Union, 2000) they are an obligatory element in the monitoring of ecological river quality. Macrophytes were included in assessment methods: French Indice Biologique Macrophytique en Riviere, German Reference Index, British Mean Trophic Rank, and Dutch Macrophyte Score (Birk et al., 2006). While high ecological status is determined via dominance of reference species in type-specific vegetation density, it is very important to determine whether patterns of aquatic macrophyte composition and distribution including cases of depopulation are a result of anthropogenic pressure or natural habitat variables.

Local habitat characteristics determine river macrophyte communities, particularly light availability, current velocity, sediment patterns, and nutrient supply (Birk & Willby, 2010). Both hydrologic dynamics and human impact expressed as land-use types are found to be responsible for the variability of aquatic macrophyte assemblages along the Danube corridor in Slovakia (Otahelová et al., 2007). Light condition is another important factor determining macrophyte species composition (Hrivnák et al., 2006). Substrate type also

* Correspondence: ggecheva@mail.bg

directly affects macrophyte development. Rocks and hard, immobile substrates are associated with bryophytes (Janauer & Dokulil, 2006). Data indicate that coarse substrate and variable flow regime contributed both to the success of bryophytes and the exclusion of vascular hydrophytes (Scarlett & O'Hare, 2006). Bryophytes are a dominant component in lotic ecosystems, especially in undisturbed conditions, and their relations with environmental factors were studied (Glime & Vitt, 1987; Suren, 1996; Duncan et al., 1999; Suren & Duncan, 1999; Suren et al., 2000; Scarlett & O'Hare, 2006; etc.). The combined influence of underlying geology, water physicochemistry, current velocity, and substrate morphology on 17 bryophyte species was investigated in 3 minimally impacted high-latitude headwater streams in Scotland (Lang & Murphy, 2012). Aquatic bryophytes have also been studied and used to rank contaminated sites (Samecka-Cymerman et al., 2002; Cesa et al., 2006, 2010; Vieira et al., 2009; etc.).

A basic study of aquatic vegetation in Bulgaria was accomplished 30 years ago by Kochev and Jordanov (1981). In recent years in Bulgaria, separate studies have focused on aquatic macrophyte composition itself in single rivers or river sections (Yurukova, 2002; Yurukova & Gecheva, 2004; Papp et al., 2006; Valchev et al., 2006; Valchev & Stoeva, 2010). Studies on aquatic plants' relations with ecological variables are still scarce (Yurukova & Kochev, 1993, 1994; Yurukova, 2004; Janauer et al., 2006; Gecheva et al., 2010, 2011).

The present study aimed to enhance our knowledge of the relationships between environmental variables (water velocity, shading, mean depth, and substrate type), together with altitude, ecological status, and river type, and aquatic macrophyte species' composition and distribution in Bulgarian rivers.

2. Materials and methods

2.1. Study objects and area

For the purposes of the research, we studied bryophytes (Bryophyta) and vascular plants (Pteridophyta and Spermatophyta) and included them in 3 groups: hydrophytes, amphiphytes (species capable of growing on land or in water), and helophytes (emergent plants, rooted under water). Aquatic macrophytes were monitored in the period June–September 2009 at 223 sampling sites in Bulgaria (Figure 1), representative of various river types.

2.2. Sampling

All vascular plants and bryophytes were recorded at each site with length varying between 50 and 100 m. Structural characteristics, unusual features regarding colouration, and odour of the water were noted as well (Schaumburg et al., 2006). As far as possible, vascular plant species were determined on site according to the field guide of vascular plants of Bulgaria (Kozhuharov, 1992). Bryophyte samples were stored in collecting packets, observed under the light microscope in the laboratory, and determined according to Petrov (1975) and Smith (1980, 2004). The nomenclature described in Grolle and Long (2000) for liverworts and in Hill et al. (2006) for mosses was followed. The taxonomy of vascular plants followed *Flora Europaea* (Tutin et al., 1964–1980, 1993). The abundance of each species was estimated according to a 5-degree scale (1 = very rare, 2 = infrequent, 3 = common, 4 = frequent, 5 = abundant, predominant) according to Kohler (1978). Macrophyte relative abundances were quantified based on percent frequency of occurrence at 223 sampling sites.

Four main characteristics of each site were recorded: speed of the water flow, substrate type, shading, and mean depth. Shading was noted based on the 5-degree scale (1 = completely sunny, 2 = sunny, 3 = partly overcast, 4 = half-shaded, 5 = completely shaded) of Wörlein (1992). The other 3 parameters were determined according to Schaumburg et al. (2004, 2006) in a semiquantitative way using class scales, to enable a fast and easy field application. Velocity of flow was recorded via a 6-point scale: I = not visible, II = barely visible, III = slowly running, IV = rapidly running (current with moderate turbulences), V = rapidly running (turbulently running), VI = torrential. The substratum conditions at the sampling site are classified in 5% steps according to an 8-point scale: % mud, % clay/loam (<0.063 mm), % sand (0.063–2.0 mm), % fine/medium gravel (2.0–6.3/6.3–20 mm), % coarse gravel (20–63 mm), % stones (63–200 mm), % boulders (>200 mm), and % organic/peat. Mean depth was noted on a 3-degree scale (I = 0–30 cm, II = 30–100 cm, III >100 cm).

In situ measurements of acidity (pH) and electrical conductivity (C, $\mu S\ cm^{-1}$) of river water were made using a Multiline P3 (WTW, Germany).

Integrated ecological status (ES) is based on assessed biological, physico-chemical, and hydromorphological quality elements of the studied rivers as required by the Water Framework Directive (European Union, 2000). Assessment results, expressed as the 'Ecological Quality Ratio' (EQR), followed Cheshmedjiev et al. (2010) and were applied to express the overall anthropogenic impact. The EQR uses a numerical scale between 0 and 1, where 1 represents (type-specific) reference conditions and values close to 0 indicate bad ecological status.

2.3. Data analysis

Initially, we elaborated a floristic list of higher plants and additional list of sites, indicating the presence/absence of aquatic bryophytes and vascular plants. The dataset included the main environmental variables studied: water velocity, shading, mean depth, and substrate type, as well as altitude, ecological status, and river type. First, the dataset was analysed to test the hypothesis that basic patterns of

Figure 1. Location of Bulgaria, river sites and Ecoregion No. 12 Pontic Province and No. 7 Eastern Balkan (above and under purple dotted line). Legend: Nishava - Kalotina village: 1. Erma - Strezimirovtsi village: 2. Erma - Tran town: 3. Timok - Bregovo town: 4. Topolovets - Vidin town: 5. Voynishka - mouth: 6. Archar - Archar village: 7. Skomlya - after Dobri Dol village: 8. Lom - before Gorni Lom village: 9. Lom - before Lom town: 10. Tsibritsa - Valchedram village: 11. Tsibritsa - Dolni Tsibar village: 12. Chiprovska Ogosta - above Chiprovtsi town: 13. Ogosta - after Montana town: 14. Ogosta - Kobilyak village: 15. Botunya - Ohrid village: 16. Scat - after Byala Slatina town: 17. Scat - after Miziya town: 18. Ogosta - mouth: 19. Barziya - after Borovtsi village: 20. Botunya - above Varshets town: 21. Botunya - Golyamo Babino village: 22. Varteshnitsa - before Krivodol town: 23. Ribine - before inflow into Ogosta River: 24. Scat - Golyamo Peshtene village: 25. Cherni Iskar - Govedartsi village: 26. Iskar - before Iskar Dam: 27. Iskar - before Kokalyane Dam: 28. Iskar - Novi Iskar town: 29. Iskar - Rebarkovo village: 30. Iskar - before Roman town: 31. Iskar - Orehovitsa village: 32. Iskar - Gigen village: 33. Malyovitsa - before tourist complex Malyovitsa: 34. Shipochnitsa - before inflow into Iskar Dam: 35. Bistrishka - before inflow into Pancharevo Dam: 36. Gradska - before inflow into the Iskar River: 37. Rakovishka - Golema Rakovitsa village: 38. Lesnovska - after Makotsevska River: 39. Lesnovska - Dolni Bogrov village: 40. Lesnovska - mouth: 41. Kakach - mouth: 42. Blato – mouth: 43. Batuliyska - before Yablanitsa village: 44. Batuliyska - mouth: 45. Iskretska - mouth: 46. Gabrovnitsa - mouth: 47. Malak Iskar - Laga village: 48. Malak Iskar - Svode village: 49. Malak Iskar - Roman town: 50. Zlatna Panega - Cherven Bryag town: 51. Gostilya - mouth: 52. Beli Vit - above Ribaritsa village: 53. Vit - after Teteven village: 54. Vit - before Sadovets village: 55. Vit - after Gulyantsi town: 56. Kostina - above Karvavoto kladenche: 57. Kamenka - Bejanovo village: 58. Tuchenitsa - Opanets village: 59. Cherni Osam - water catchment: 60. Osam - after Lovech town: 61. Osam - after Levski town: 62. Krushuna - karst springs: 63. Osam - Cherkovitsa town: 64. Shavarna – mouth: 65. Lomya - after Varana village: 66. Yantra - Yabalka district: 67. Yantra - after Gabrovo town: 68. Yantra - Debelets village: 69. Yantra - after Veliko Tarnovo town: 70. Yantra - Draganovo village: 71. Yantra - Karantsi village: 72. Stara - mouth: 73. Yantra - Nov Grad village: 74. Panicharka - Gabrovo town: 75. Belitsa - before Debelets town: 76. Dryanovska - mouth: 77. Stara - Maysko village: 78. Stara - after Kesarevo village: 79. Veselina - after Yovkovtsi Dam: 80. Golyama reka - HMS Strajitsa: 81. Djulyunska - Djulyunitsa village: 82. Veselina - before Yovkovtsi Dam: 83. Rositsa - after Stokite village: 84. Rositsa - after Sevlievo town: 85. Rositsa - mouth: 86. Vidima - open catchment: 87. Vidima - mouth: 88. Choparata - Sevlievo town: 89. Krapets - before Krapets Dam: 90. Krapets - before inflow into A. Stamboliyski Dam: 91. Magara - before inflow into Stamboliyski Dam: 92. Eliyska - mouth: 93. Studena - mouth: 94. Beli Lom - above Beli Lom Dam: 95. Beli Lom - mouth: 96. Rusenski Lom - mouth: 97. Malak Lom - above Lomtsi Dam: 98. Cherni Lom - Svetlen village: 99. Cherni Lom - Ostritsa village: 100. Baniski Lom - mouth: 101. Cherni Lom - Cherven village: 102. Seyachka - before Kavatsite Dam: 103. Kayadjik - above Boyka Dam: 104. Kayadjik - after Boyka Dam: 105. Tsaratsar - Malak Porovets village: 106. Chairlak - Cherkovna village: 107. Suha - Novo Botevo village: 108. Batova - before Batovo village: 109. Batova - mouth: 110. Provadiyska – mouth: 111. Madara - mouth: 112. Kriva - Enevo village: 113. Devnenska - mouth: 114. Ticha - above Ticha village: 115. Kamchiya - Milanovo village: 116. Kamchiya - Grozdyovo village: 117. Kamchiya - "Poda": 118. Vrana - after Targovishte town: 119. Vrana - Han Krum village: 120. Andere - mouth: 121. Brestova - mouth: 122. Medvenska - Medven village: 123. Dvoynitsa - mouth: 124. Hadjiyska - Tankovo village: 125. Aheloy - "Aheloy": 126. Rusokastrenska - mouth: 127. Sredetska - after Sredets town: 128. Fakiyska - Fakiya village: 129. Fakiyska - Varovnik village: 130. Izvorska - Izvor village: 131. Ropotamo - Veselie village: 132. Ropotamo - "Velyov vir" reserve: 133. Dyavolska - before Primorsko town: 134. Karaagach - Fazanovo village: 135. Veleka - Brashlyan village: 136. Veleka - Sinemorets village: 137. Maritsa - Raduil village: 138. Maritsa - Pazardjik town: 139. Maritsa - Plovdiv town: 140. Maritsa - Parvomay town: 141. Maritsa - Harmanli town: 142. Maritsa - Svilengrad town: 143. Chepinska - Marko Nikolov station: 144. Topolnitsa - before Topolnitsa Dam: 145. Topolnitsa - Pamidovo village: 146. Topolnitsa - mouth: 147. Mativir - Ihtiman town: 148. Luda Yana - Rosen village: 149. Stara - mouth: 150. Vacha - Devin town: 151. Vacha - Yoakim Gruevo village: 152. Kalavashtitsa - Malo Krushevo village: 153. Chepelarska - Chepelare town: 154. Chepelarska - Bachkovo village: 155. Chepelarska - mouth: 156. Stryama - Klisura town: 157. Stryama - Slatina village: 158. Stryama - Banya village: 159. Stryama - Manole village: 160. Pikla - Matenitsa village: 161. Srebra - Zlatosel village: 162. Sazliyka - Rakitnitsa village: 163. Sazliyka - Radnevo town: 164. Sazliyka - mouth: 165. Blatnitsa - Konyovo village: 166. Blatnitsa - Radnevo town: 167. Sokolitsa - Vladimirovo village: 168. Harmanliyska - Harmanli town: 169. Biserska - Dolno Botevo village: 170. Azmaka - Ivanovo village: 171. Tundja - Kalofer town: 172. Tundja - Koprinka Dam: 173. Tundja - Nikolaevo town: 174. Tundja - Gavrailovo village: 175. Tundja - Samuilovo village: 176. Tundja - Hanovo village: 177. Belenska - mouth: 178. Mochuritsa - mouth: 179. Dereorman - mouth: 180. Popovska - mouth: 181. Melnishka - mouth: 182. Manastirska - before Mramor mine: 183. Arda - Mogilitsa village: 184. Arda - Vehtino village: 185. Arda - before Kardjali Dam: 186. Arda - Madjarovo village: 187. Arda - after Ivaylovgrad Dam: 188. Cherna - Smolyan town: 189. Malka Arda - Kutela village: 190. Varbitsa - after mill tailings dam: 191. Varbitsa - before Studen kladenets Dam: 192. Byala - Meden Buk village: 193. Cherna Mesta - Cherna Mesta village: 194. Mesta - before Iztok River inflow: 195. Mesta - Bukovo village: 196. Mesta - state border: 197. Demyanitsa - before Banderitsa river inflow: 198. Glazne - after Bansko town: 199. Iztok - mouth: 200. Matnitsa - after Petrelik village: 201. Struma - before Studena Dam: 202. Struma - before Zemen town: 203. Struma - Rajdavitsa village: 204. Struma - Boboshevo town: 205. Struma - Mursalevo village: 206. Struma - before Kresna town: 207. Struma - state border: 208. Arkata - mouth: 209. Svetlya - mouth: 210. Treklyanska - mouth: 211. Dragovishtitsa - state border: 212. Eleshnitsa - mouth: 213. Dupnishka Bistritsa - Bistritsa village: 214. Razmetanitsa - mouth: 215. Blagoevgradska Bistritsa - before Blagoevgrad town: 216. Stara - before Jeleznitsa village: 217. Vlahinska - mouth: 218. Sandanska Bistritsa - before Sandanski town: 219. Lebnitsa - before Lebnitsa village: 220. Strumeshnitsa - state border: 221. Melnishka - mouth: 222. Pirinska Bistritsa - state border: 223.

aquatic macrophyte composition and distribution depend on ecological status, which is correlated with altitude and river type, respectively. Second, the dataset was split into sites where bryophytes were registered (n = 51); the other 172 locations were characterised by absence of bryophytes (divided into 130 sites with vascular plants and 42 sites characterised by macrophyte depopulation), and analysed separately to detect relationship with the 4 main environmental variables.

One-way analysis of variance (ANOVA) and Tukey's HSD test were applied to the main 4 variables to test differences.

Data analyses using Canoco ver. 4.5 were conducted (Ter Braak & Šmilauer, 2002). Canonical correspondence analysis (CCA) was applied to study the relationship between aquatic macrophytes and the main variables. The data were transformed (x' = log (x + 1)), automatically centred, and standardised by the Canoco analysis program. Monte Carlo permutation tests (number of permutations 499) and forward selection were used within CCA to detect significant (P = 0.05 probability threshold level) and independent environmental variables.

The underlying relations between parameters of the selected river sites were investigated using factor analysis (Schaug et al., 1990). The analysis was performed using principal component extraction, with 1 as the eigenvalue factor selection criterion, and VARIMAX rotation of the extracted factors. Variables with factor loadings higher than 0.7 were assumed to contribute significantly to a given factor.

3. Results

3.1. Taxonomic composition

The assessed river sites showed significant variability in aquatic macrophyte composition: bryophytes were registered at 51 sites, while vascular plants had patterns of distribution at 130 sites. Macrophyte depopulation was assessed at 42 sites. A total of 135 species were observed, of which 49 were bryophytes (Table 1). The 86 registered vascular plants included 28 hydrophytes and

Table 1. List of registered bryophytes and vascular plants.

Species	Number of registered sites	Relative abundance, %
Bryophytes		
Amblystegium serpens (Hedw.) Schimp. [AS]	1	0.45
Atrichum undulatum (Hedw.) P.Beauv. [AU]	2	0.90
Blindia acuta (Hedw.) Bruch & Schimp. [BA]	1	0.45
Brachytheciastrum velutinum (Hedw.) Ignatov & Huttunen [BV]	1	0.45
Brachythecium rivulare Schimp. [BRi]	11	4.93
Brachythecium rutabulum (Hedw.) Schimp. [BRu]	2	0.90
Bryum capillare Hedw. [BC]	1	0.45
Bryum dichotomum Hedw. [BD]	1	0.45
Bryum elegans Nees [BE]	1	0.45
Bryum pallens Sw. ex anon. [BPa]	1	0.45
Bryum pseudotriquetrum (Hedw.) P.Gaertn. et al. [BPs]	6	2.69
Campylium protensum (Brid.) Kindb. [CP]	1	0.45
Chiloscyphus pallescens (Ehrh. ex Hoffm.) Dumort. [ChPa]	1	0.45
Chiloscyphus polyanthus (L.) Dumort. [ChPo]	1	0.45
Cinclidotus aquaticus (Hedw.) Bruch & Schimp. [CiA]	1	0.45
Cinclidotus riparius (Host ex Brid.) Arn. [CiR]	1	0.45
Climacium dendroides (Hedw.) F.Weber & D.Mohr [ClD]	1	0.45
Cratoneuron filicinum (Hedw.) Spruce [CrF]	4	1.79
Dichodontium palustre (Dicks.) M.Stech [DPa]	1	0.45

Table 1. (Continued).

Dichodontium pellucidum (Hedw.) Schimp. [DPe]	1	0.45
Ditrichum pusillum (Hedw.) Hampe [DitP]	1	0.45
Fissidens crassipes Wilson ex Bruch & Schimp. [FiC]	1	0.45
Fontinalis antipyretica Hedw. [FA]	19	8.52
Fontinalis hypnoides C.Hartm. [FH]	1	0.45
Hygroamblystegium tenax (Hedw.) Jenn. [HT]	2	0.90
Hygrohypnum duriusculum (De Not.) D.W.Jamieson [HhD]	1	0.45
Hygrohypnum luridum (Hedw.) Jenn. [HhL]	1	0.45
Leptodictum riparium (Hedw.) Warnst. [LR]	14	6.28
Marchantia polymorpha L. [MP]	4	1.79
Orthotrichum diaphanum Schrad. ex Brid. [OD]	1	0.45
Oxyrrhynchium speciosum (Brid.) Warnst. [OxS]	2	0.90
Philonotis fontana (Hedw.) Brid. [PhF]	1	0.45
Philonotis seriata Mitt. [PhS]	1	0.45
Plagiochila porelloides (Torrey ex Nees) Lindenb. [PlP]	5	2.24
Plagiomnium rostratum (Schrad.) T.J.Kop. [PmR]	1	0.45
Plagiomnium undulatum (Hedw.) T.J.Kop. [PmU]	4	1.79
Platyhypnidium riparioides (Hedw.) Dixon [PR]	21	9.42
Rhizomnium pseudopunctatum (Bruch & Schimp.) T.J.Kop. [RhPs]	1	0.45
Rhizomnium punctatum (Hedw.) T.J.Kop. [RhPu]	3	1.35
Ricciocarpos natans (L.) Corda [RiN]	1	0.45
Sanionia uncinata (Hedw.) Loeske [SUnc]	1	0.45
Scapania undulata (L.) Dumort. [SUnd]	2	0.90
Schistidium agassizii Sull. & Lesq. [SchA]	1	0.45
Schistidium rivulare (Brid.) Podp. [SchR]	2	0.90
Sciuro-hypnum plumosum (Hedw.) Ignatov & Huttunen [ShP]	5	2.24
Straminergon stramineum (Dicks. ex Brid.) Hedenäs [StrS]	1	0.45
Thamnobryum alopecurum (Hedw.) Gangulee [ThA]	1	0.45
Timmia bavarica Hessl. [TB]	1	0.45
Warnstorfia exannulata (Schimp.) Loeske [WE]	1	0.45
Vascular plants		
Hydrophytes		
Azolla filiculoides Lam.	1	0.45
Callitriche brutia Petagna	1	0.45
Callitriche stagnalis Scop.	3	1.35
Ceratophyllum demersum L.	32	14.35
Ceratophyllum submersum L.	4	1.79
Elodea canadensis Michx.	2	0.90
Elodea nuttallii (Planch.) H.St.John	9	4.04

Table 1. (Continued).

Lemna gibba L.	6	2.69
Lemna minor L.	45	20.18
Lemna trisulca L.	17	7.62
Myriophyllum spicatum L.	51	22.87
Myriophyllum verticillatum L.	5	2.24
Najas marina L.	1	0.45
Najas minor All.	1	0.45
Nuphar lutea Sm.	1	0.45
Polygonum amphibium L.	4	1.79
Potamogeton berchtoldii Fieber	2	0.90
Potamogeton crispus L.	27	12.11
Potamogeton gramineus L.	1	0.45
Potamogeton natans L.	35	15.70
Potamogeton nodosus Poir.	16	7.17
Potamogeton pectinatus L.	12	5.38
Potamogeton pusillus L.	1	0.45
Ranunculus aquatilis L.	13	5.83
Ranunculus trichophyllus Chaix	1	0.45
Spirodela polyrrhiza (L.) Schleid.	4	1.79
Trapa natans L.	4	1.79
Zannichellia palustris L.	13	5.83
Helophytes & Amphiphytes		
Alisma lanceolatum With.	7	3.14
Alisma plantago-aquatica L.	14	6.28
Berula erecta (Huds.) Coville	30	13.45
Bidens cernua L.	3	1.35
Bidens tripartita L.	21	9.42
Butomus umbellatus L.	25	11.21
Calystegia sepium (L.) R.Br.	1	0.45
Carex pseudocyperus L.	1	0.45
Carex riparia Curtis	1	0.45
Cyperus fuscus L.	10	4.48
Cyperus longus L.	1	0.45
Echinochloa crus-galli (L.) P.Beauv.	14	6.28
Eleocharis palustris (L.) Roem. & Schult.	3	1.35
Epilobium hirsutum L.	5	2.24
Equisetum arvense L.	2	0.90
Equisetum palustre L.	1	0.45
Equisetum telmateia Ehrh.	1	0.45

Table 1. (Continued).

Eupatorium cannabinum L.	1	0.45
Glyceria maxima (Hartm.) Holmb.	1	0.45
Gratiola officinalis L.	1	0.45
Hypericum tetrapterum Fr.	1	0.45
Iris pseudacorus L.	4	1.79
Juncus articulatus L.	17	7.62
Juncus effusus L.	4	1.79
Juncus tenageia L.f.	6	2.69
Lythrum salicaria L.	40	17.94
Lycopus europaeus L.	14	6.28
Lycopus exaltatus L.f.	2	0.90
Mentha aquatica L.	26	11.66
Mentha longifolia (L.) Huds.	1	0.45
Mentha pulegium L.	21	9.42
Mentha spicata L.	51	22.87
Myosoton aquaticum (L.) Moench	3	1.35
Nasturtium officinale R.Br.	2	0.90
Paspalum paspalodes (Michx.) Scribn.	24	10.76
Petasites hybridus (L.) P.Gaertn. B.Mey. & Scherb.	8	3.59
Phalaris arundinacea L.	21	9.42
Phragmites australis (Cav.) Trin. ex Steud.	22	9.87
Polygonum hydropiper L.	52	23.32
Polygonum mite Schrank	1	0.45
Portulaca oleracea L.	1	0.45
Ranunculus repens L.	3	1.35
Ranunculus sardous Crantz	5	2.24
Rorippa palustris (L.) Besser	1	0.45
Ruppia maritima L.	4	1.79
Sagittaria latifolia Willd.	1	0.45
Scirpus holoschoenus L.	1	0.45
Scirpus lacustris L.	29	13.00
Scirpus maritimus L. subsp. *maritimus*	3	1.35
Scirpus triqueter L.	1	0.45
Scrophularia umbrosa Dumort.	1	0.45
Scutellaria galericulata L.	2	0.90
Sparganium erectum L.	80	35.87
Stachys palustris L.	4	1.79
Typha angustifolia L.	38	17.04
Typha latifolia L.	39	17.49
Typha laxmannii Lepech.	2	0.90
Veronica beccabunga L.	26	11.66

58 helophytes and amphiphytes. The most frequently distributed hydrophyte was *Myriophyllum spicatum* L. (at 51 sites, relative abundance near 23%), followed by *Lemna minor* L. (45 sites), *Potamogeton natans* L. and *Ceratophyllum demersum* L. (35 and 32 sites, respectively), and *Potamogeton crispus* L. (27 sites). The most common species from the group of helophytes and amphiphytes was *Sparganium erectum* L. (registered at 80 sites; relative abundance above 35%), followed by *Polygonum hydropiper* L. and *Mentha spicata* L. (52 and 51 sites, respectively).

3.2. Relationship with environmental variables

Acidity of the river water at both mountain (n = 114) and lowland (n = 109) sites was in the range of 7.1–9.3, with a median of 8.3. Electrical conductivity had a median of 364 µS cm^{-1} at mountain (31–1320) and 629 µS cm^{-1} at lowland (47.2–2820) sites.

The relationships between presence of aquatic bryophytes and vascular plants, and effects of altitude, river type, and ecological status (ES) represented by ecological quality ratio (EQR) were assessed by CCA (Figure 2). The first and second axes together explained 100% of the species variation. The sum of all canonical eigenvalues was 0.185. EQR was the main variable responsible for the aquatic macrophyte variance. All 3 main parameters tested correlated with the first axis: altitude and EQR (respectively ES) with its positive and river type with its negative part. The ordination plot represented a gradient between sites located at higher altitude and characterised by predominantly good or high ecological status (upper part of the plot) to localities belonging to river types established in lowlands and normally subject to anthropogenic pressure. CCA showed that aquatic bryophyte species occur mainly in conditions of higher altitude and ecological status (the right of the diagram), while vascular plants prefer lowland river types defined by lower status and altitude.

One-way ANOVA and Tukey's HSD test indicated that all variables were significantly different between sampling sites ($P < 0.001$).

The relationship between distribution of bryophyte species with assessed abundance at 51 sites and environmental characteristics was also identified using CCA (Figures 3, 4). Four environmental variables were tested. Velocity, mean depth, and shading were significantly correlated with bryophyte distribution, while the influence of the predominant substrate did not contribute significantly to the model ($P > 0.1$) (Table 2). Velocity was shown to be the parameter exerting greatest influence on bryophyte communities, explaining over one third of the variance. The first axis had an eigenvalue of 0.558 and explained 5.9% of species variation. It represents a gradient from deeper habitats with dynamic flow, mostly shaded (upper part of the ordination plot), to shallow sites with lower flow dynamic, predominantly sunny (Figure 3). The most distributed bryophyte species, *Fontinalis*

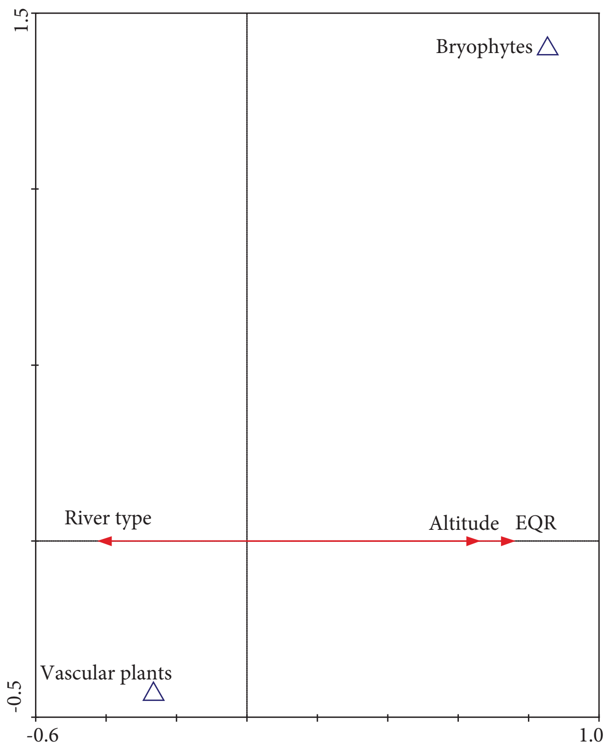

Figure 2. CCA ordination diagram of distribution of aquatic bryophytes and vascular plants and 3 environmental variables (altitude, EQR, and river type).

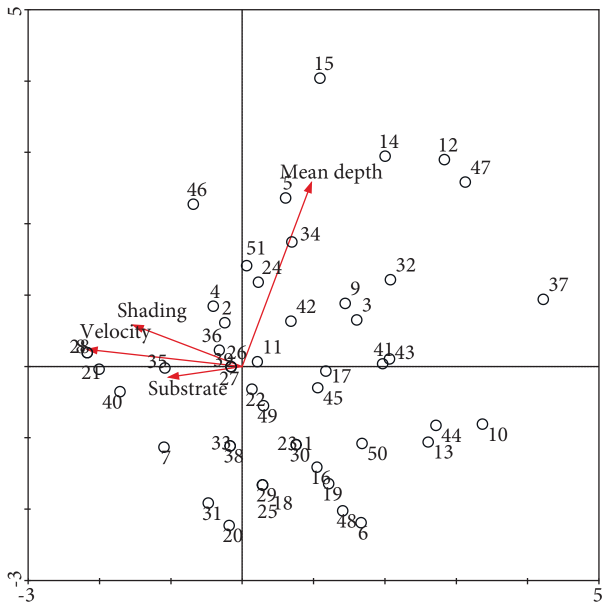

Figure 3. CCA ordination diagram with data for 51 sites with registered bryophyte species abundance and 4 environmental characteristics: mean depth, predominant substrate, velocity, and shading. Circles - relevant sampling site.

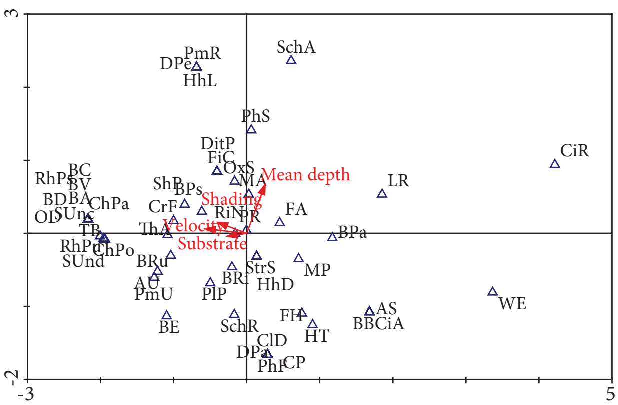

Figure 4. CCA ordination diagram with data for 51 sites with registered bryophyte species abundance and 4 environmental characteristics: mean depth, predominant substrate, velocity, and shading. Triangles - relevant species (abbreviations according to Table 1).

antipyretica (FA) and *Leptodictyum riparium* (LR), were associated with higher depth, while *Brachythecium rivulare* (BRi) was related to more shallow habitats with coarser bottoms (Figure 4). *Platyhypnidium riparioides* (PR) preferred sites with average values of the environmental parameters tested. Species whose distribution is the most restricted to shaded and dynamic flow lay in the top-left quadrant of the diagram.

The relationship between the above 4 environmental variables and aquatic macrophytes was additionally tested for the rest 172 sampling sites, characterised by absence of bryophytes. CCA revealed the following model (Figure 5). The first and second axes together explained 79.2% of species variation. Shading significantly correlated with the first axis and explained the major part of the species variance. Predominant substrate correlated with the second axis. Sites with a high extent of shading were located at the right of the ordination plot. Localities with dominance of stones and rocks were separated at the upper part of the diagram from those characterised by finer substrate (silt, clay, etc.). Sites associated with fast water flow are concentrated at the upper left corner and those with the highest depth at the bottom left corner.

Registered sites with absence of aquatic macrophytes (total number 42) belonged to various river types and ecological statuses. The majority (30 sites) were characterised by high flow speed and stones and/or rocks as substrate (upper part of the plot).

Results from factor analyses of the variables of 42 river sites with macrophyte depopulation are shown in Table 3. Factor analysis showed 2 distinct components. Factor 1 had high loadings for flow velocity, substrate type, and altitude and was obviously associated with habitats characterised by high flow speed and coarse substrates at an altitude median of 338 m a.s.l. Factor 2 had high loadings for mean depth and shading, apparently due to inappropriateness of deep and shaded habitats.

4. Discussion

Forty-nine aquatic bryophyte and 86 vascular species were identified at 223 sites along 204 rivers in Bulgaria. Based on the results it could be suggested that aquatic bryophytes and vascular plants are in inverse correlation in the rivers studied. Similar domination of bryophytes in mountain types in southern Europe was reported and the highest proportion of bryophytes in unpolluted rivers (Szoszkiewicz et al., 2006).

Table 2. Conditional effects of observed environmental variables. Lambda A - additional variance explained; P - significance of variable; F - variance ratio.

Variable	Lambda A	P	F-ratio
Ecological quality ratio (EQR)	0.13	0.002	53.71
Altitude	0.04	0.002	19.92
River type	0.01	0.03	5.28
Velocity	0.47	0.002	2.59
Shading	0.33	0.008	1.82
Mean depth	0.30	0.022	1.69
Predominant substrate	0.20	0.250	1.13
Shading	0.02	0.002	95.72
Predominant substrate	0.01	0.002	123
Mean depth	0.01	0.002	157
Velocity	0.00	0.002	381

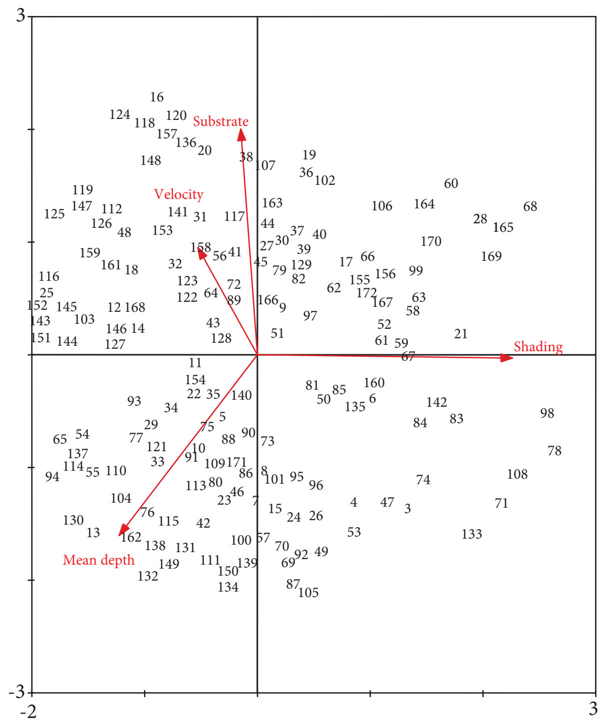

Figure 5. CCA ordination diagram with data for 4 environmental variables at 172 sampling sites, characterised by absence of bryophytes. Numbers - relevant sampling site.

Table 3. Factor analysis - dataset of 7 parameters in 42 macrophyte depopulated river sites. Factor loadings (Varimax normalised); extraction: Principal components; marked loadings are >0.700).

	Factor 1	Factor 2
Mean depth	0.06681	**0.816978**
Velocity	**–0.73322**	0.228829
Shading	0.022709	**–0.83195**
Predominant substrate	**–0.77597**	–0.13563
Altitude	**–0.77376**	–0.14234
EQR	–0.53584	–0.32961
River type	0.429074	0.520747
Explained variance	2.214651	1.830438
Principal total	0.316379	0.261491

In the upper reach, where rivers are often close to their reference conditions (i.e. high ecological status), 2 main parameters control the growth of macrophytes: fast water flow velocity, often combined with irregular run-off and hard substrates. They both prevent vascular plants' development in mountain and semimountain river types. Results from Slovak streams corroborated the importance of sediment type and bryophytes' preference for coarser sediments (Hrivnák et al., 2010). Similarly, gradients of substrate morphology and water chemistry were detected as primary drivers at 3 high-latitude upland streams for the community composition of 17 registered bryophyte species (Lang & Murphy, 2012).

Water velocity, the parameter with the greatest influence on bryophyte communities, could be related to element uptake. Additionally, velocity, as a major variable in habitats dominated by bryophytes, provokes 3 different strategies towards decreasing its strength and thus leading to increasing substrate stability (Suren et al., 2000).

A number of studies focused on substrate stability, due to flow velocity influence on bryophytes (Suren, 1996; Duncan et al., 1999; Suren & Duncan, 1999). Our results concerning the prime importance of flow velocity for bryophyte distribution confirmed the connection found between bryophyte distribution and substrate stability. Moreover, Suren and Duncan (1999) reported the importance of substrate prepossession on movement for aquatic bryophyte presence in streams.

The most abundantly distributed mosses were obligate aquatic species *Fontinalis atipyretica* and *Platyhypnidium riparioides*. Both were reported as "core" and common partner species in high-latitude streams in Scotland (Lang & Murphy, 2012).

Despite bryophyte's proven high tolerance to contaminants (Gecheva et al., 2011), most species prefer lower values of nutrients, represented by higher ecological status. Notwithstanding tolerant species like *Leptodictyum riparium* inhabiting sites with different extents of nutrient loads, the limited nutrient load in mountain and semimountain river habitats supports bryophytes and prevents the development of vascular plants.

Habitats at the lower reaches of lowland rivers characterised by slow water velocity and finer substrates create favourable conditions for abundant macrophytes. The above sites are usually subject to anthropogenic pressure and thus have predominantly lower ecological status and are inhabited by macrophyte species tolerant to eutrophic conditions such as *Potamogeton crispus*, *P. pectinatus*, *Lemna minor*, and *Zannichellia palustris*. This finding confirms the previously reported tendency of *Potamogeton pectinatus* and *Lemna minor* to dominate in highly enriched or engineered sites (Birk & Willby, 2010).

Water depth and shading are 2 additional important variables defining patterns of aquatic macrophyte composition and distribution, particularly in lowland river types. Riverside vegetation along lowland rivers can prevent macrophyte growth. With decreasing shading gradient, an increase in vascular plants occurs. Shading by shrubs and trees on the banks was a parameter affecting macrophyte composition also in Slovak streams, preceded by sediment type and followed by water depth (Hrivnák et al., 2010).

In conclusion, velocity was shown to be the parameter exerting greatest influence on bryophyte communities, since for vascular plants the most important predictor was shading. Substrate did not significantly influence aquatic bryophyte distribution, but appeared to be the prime environmental variable for vascular plants. Our findings concerning the significant role of abiotic habitats' characteristics in river plant communities' development reflect those of relevant studies (e.g., Suren, 1996; Lang & Murphy, 2012). However, we also found that hard substrates and high water velocity can lead to macrophyte depopulation.

This study represents an important contribution to the environmental monitoring and biodiversity conservation programmes. It highlights abiotic factors with major influence on bryophyte and vascular plant communities in rivers. According to the EU Water Framework Directive (European Commission, 2000), European surface waters must achieve good ecological quality by 2015, and responsibility for the quality assessment lies with the individual member states. As obligatory elements in the monitoring of river quality, aquatic macrophytes and description of environmental parameters influencing their patterns of composition and distribution are of crucial importance.

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