Substrate specificity and fine-scale distribution of epiphytic diatoms in a shallow tarn in the Brenta Dolomites (south-eastern Alps)

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Background and aims – The host-specificity of epiphytic diatom species has long been debated. Scuba divers sampled epiphytic diatoms in the shallow Alpine Lake Valagola (average depth c. 2 m) along seven transects (length: 30–144 m) in West-East direction. The bottom of the tarn was covered by macrophytes dominated by *Chara aspera* and *Potamogeton gramineus*. Factors affecting epiphytic-diatom spatial distribution at a fine scale were tested.

Methods – Dataset was tested using Redundancy Analysis (CANOCO package) and one-way ANOVA (NCSS package).

Key results – The analysis separated sampling sites into two groups: the tarn shore dominated by *Potamogeton gramineus*, and the central area dominated by *Chara aspera*. Diatom species richness, diversity, and composition differed significantly between the two main host plants. *Potamogeton gramineus* assemblages were characterized by higher species richness and diversity, and by the large-celled, adnate diatom species *Epithemia adnata*, *Rhopalodia gibba*, *Eunotia arcus* and *E. arcubus*. *Chara aspera* was preferred by the small-celled, motile diatom species *Brachysira neoexilis* and *Encyonopsis cesatii*.

Conclusions – The spatial distribution of epiphytic diatoms in the shallow, oligo-mesotrophic Lake Valagola is influenced by host plant composition and distribution. Epiphyton size structure suggests that *Chara* represents a less appropriate substrate for long diatoms.

Key words – Epiphyton, mountain lake, host specificity, epiphyton size structure, *Chara aspera*, *Potamogeton gramineus*, diatoms.

INTRODUCTION

While lake environments are separated in habitats, zones, and gradients (Poulíčková et al. 2008, 2014), microphototrophs are typically classified as benthic and planktic. Both categories perform a range of ecosystem functions and contribute significantly to lake biodiversity. However, photoautotrophs that inhabit benthic environments have received less attention than the phytoplankton (Cantonati & Lowe 2014, Poulíčková et al. 2014). Aquatic macrophytes are key components in spatial heterogeneity (Thomaz et al. 2008). Epiphytic microalgae living in association with macroalgae and aquatic macrophytes contribute significantly to the primary production of lakes, particularly in the littoral zone (Cattaneo & Kalff 1980, Vander Zanden et al. 2006, Cano et al. 2008). Epiphyton is an important source of food for invertebrates (Cattaneo 1983), and has been proposed as a target community for the assessment of lake trophic status (Lalonde & Downing 1991, Poulíčková et al. 2004).

Epiphytic algae are challenging to be studied quantitatively because they are difficult to separate from their substrate, and because their spatial distribution is heterogeneous and not fully understood. Diatoms, cyanobacteria, and green algae are the most common benthic microalgae (Poulíčková et al. 2014), comprising the majority of the epiphyton biomass (Pomazkina et al. 2012, Neif et al. 2013). Diatoms are represented by motile species gliding on various substrates, and species attached mostly via mucilagenous structures.

Epiphyton species composition on submerged macrophytes differs between lakes (Kiss et al. 2003) and phosphorus has been shown to have a significant influence (Cattaneo & Kalff 1980, Fairchild et al. 1985). Lake trophic status can influence phytoplankton abundance (and consequently light availability at the bottom), biomass of submerged plants (substrate availability), and subsequent vertical distribution of epiphyton (Lalonde & Downing 1991, Romo et al. 2007). Substrate specificity, which in real ecosystems is combined with other possible influencing factors (physical, chemical,

biological), represents a multivariate problem. Thus previous studies were mostly observational (Cattaneo & Kalff 1980, Millie & Lowe 1983, Lalonde & Downing 1991, Potapova & Charles 2005, Cantonati et al. 2012).

This study aims to analyse the detailed spatial distribution of epiphytic diatom assemblages in the shallow Alpine Lake Valagola. We tested differences in epiphyte composition and distribution on a single sampling date: (1) between the marginal and central part of the lake, and (2) between different macrophytes.

MATERIAL AND METHODS

Study site

Lake Valagola (46°9′56,462″N 10°49′13,980″E) is located in the western part of the Brenta Dolomites (south-eastern Alps, Adamello-Brenta Nature Park, Italy), at an elevation of 1,595 m a.s.l. The lake was formed by a Daun stadial moraine (Trevisan 1939). The lake does not have direct inlets. The Valagola stream, flowing down the Nardis Valley and collecting the meltwaters of the small Agola and Prato-Fiorito glaciers, disappears into the alluvial fan formed by the stream itself slightly upstream of the tarn. Since the average discharge of the Valagola stream is about four times that of the tarn outlet, it must be assumed that its waters reach the groundwater through the fan deposits, and part of this water re-emerges from the bottom of the tarn feeding it, which would be consistent with the tectonic and carbonate context. The lake level can consequently undergo important fluctuations, almost reaching the height of the small forest-operations road bordering the eastern bank and the hiking path during very-rainy periods. The morainic rim is permeable, and the depths reached by the lake today are possible
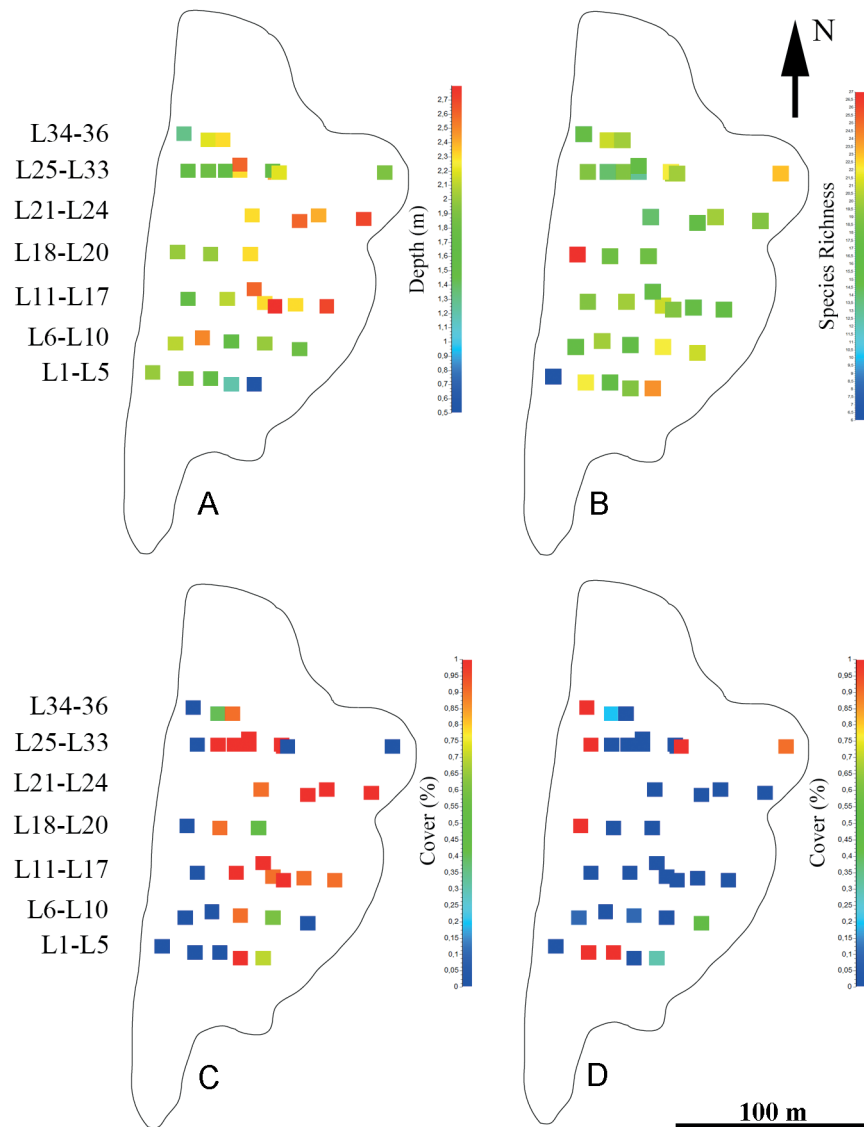

Figure 1 – Location of 36 sampling sites (L1–L36; for details see table 3) within seven transects in West-East direction and their characteristics as follows: A, sampling site depths; B, epiphytic diatom species richness; C, *Chara aspera* cover; D, *Potamogeton gramineus* cover.

Table 1 – Hydrochemical analysis.
Average from nine measurements (9 September, 16 October, 13 November, 16 December 2013; 19 March, 19 June, 25 July, 3 September, 14 October 2014) and minimum and maximum value.

Depth (m)	Temperature (°C)	Conductivity (µS cm⁻¹)	pH	Redox
0	8.0 (0.2–13.8)	239 (187–305)	8.2 (7.9–8.7)	264 (208–320)
0.5	10.9 (3.3–18.2)	222 (159–295)	8.2 (7.8–8.8)	247 (208–320)
1	10.9 (3.3–17.9)	222 (160–295)	8.3 (7.9–8.8)	249 (207–320)
1.5	9.7 (3.3–14.8)	232 (187–296)	8.2 (8.0–8.6)	248 (206–320)
2	10.7 (3.4–17.7)	222 (159–296)	8.3 (8.1–8.8)	249 (206–321)
2.5	10.6 (4.1–17.7)	240 (195–355)	8.3 (8.1–8.6)	250 (206–321)

Table 2 – Hydrochemical analysis.
Data measured on 11 September 2013 and 3 September 2014. *measurement only on 11 September 2013, ** measurement only on 3 September 2014.

Depth (m)	Alkalinity (mg/l)	TN (µg/l)	N-NO₃⁻ (µg/l)	N-NH₄⁺ (µg/l)	P-PO₄ (µg/l)	TP (µg/l)	SiO₂ (mg/l)	SO₄²⁻ (mg/l)	F (µg/l)	Chl-*a* (mg/l)
0.5	81–116	427–407	190*	12–39	1–1	14–12	2.2–0.5	1.4*	68*	0.002**
1.5	80–116	401–403	187*	13–38	1–1	15–14	2.2–0.5	1.5*	54*	0.001**
2.5	81–116	341–408	190*	12–70	1–1	17–16	2.3–0.5	1.3*	89*	0.001**

only thanks to an artificial rim, which was completed in the 1970s. Lake Valagola might thus have been characterized by a very long period of fluvio-lacustrine regime during its post-glacial history.

Lake Valagola is a shallow tarn with depths ranging from 1.6 to 2.7 m in the western portion and from 0.5 to 2.8 m in the deeper eastern part (fig. 1A). From the thermal point of view, Lake Valagola is a cold polymictic lake (table 1) with average surface temperature 8°C. Snow and ice cover last 5–6 months. Conductivity and slightly alkaline pH are consistent with the carbonate lithology of the area. The values of the main algal nutrients (table 2) measured during the present investigation (in particular nitrates and total phosphorus) do not differ significantly from the few hydrochemical data available in the literature (ISMA 1997). Nitrate values even appear to be lower today. A meso-oligotrophic status can thus be confirmed. This condition is favored by its shallowness, and it is mainly determined by the nearby cattle barn with pastures reaching the shores of the lake. Phytoplankton abundance is low (average Chl-*a* is 1.3 µg l⁻¹) with diatoms, Cryptophyceae, Chrysophyceae, and Dinophyceae being the numerically best represented groups (ISMA 1997). Bentic assemblages are well developed during the ice-free period and dominated by Zygnematophyceae (*Spirogyra*), diatoms and cyanobacteria (data not shown).

Sampling

Macrophytes were sampled with the assistance of Scuba divers on 10 Sep. 2013. Seven transects (length: 30–144 m) in c. West-East direction (i.e. perpendicular to the maximum dimension of the lake) were marked by strong ropes firmly

assured to the opposing shores (table 3). A boat moved along the ropes and distance from the shore (with a measuring tape ribbon), GPS position, and depth (with an echosounder) of each site (five sites per each transect, L1–L36, table 3) were measured. Samples of epiphytic algae together with their substrate were obtained by collecting the upper 10–20 cm of macrophytes (enough plant material to fill a large transparent polyethylene bag) growing on the bottom at sites mentioned above (L1–L36). Samples close to lake banks belong to the group "marginal part of the lake" (sites L1, 5, 6, 10, 11, 15, 16, 20, 21, 25, 26, 30, 31, 36). Subsamples (100 ml sampling bottles) for the study of epiphytic diatoms were taken and fixed with formaldehyde (2–4% final conc.). Samples and slides are kept in the Museo delle Scienze – MUSE, Trento diatom collection under numbers cLIM005 DIAT 2316-2351. The rest of plant material served for identification of macrophytes and a selection will be deposited in the herbarium of the Museo delle Scienze – MUSE (TR). General limnological characterization of the site was based on measurements of ecological variables in 2013–2014 and single sampling of epilithon, epipelon, and plankton taken from the southern shore (close to L1) on 10 Sep. 2013. Phytoplankton was sampled qualitatively with a 10-µm mesh net towed by a boat operated as to describe a sinusoidal trajectory.

During 2013–2014, physical and chemical factors (temperature, conductivity, pH, redox) were measured with a multiparametric Hydrolab probe (2013: 9 September, 16 October, 13 November, 16 December; 2014: 19 March, 19 June, 25 July, 3 September, 14 October). On 19 Mar. 2014 no operations could be carried out on the water column, since the lake was covered by ice and snow (several layers). In September 2013 and 2014, complete (major ions and algal nutrients)

Table 3 – Basic characteristics and host plants of sampling points.

| Sampling point	Transect	Depth(m)	Latitude WGS84_N	Longitude WGS84_E	*Calliergon giganteum*	*Chara aspera*	*Chara virgata*	*Potamogeton gramineus*
L1	1	2	4609894	1049180	0	0	0	0
L2	1	1.9	4609891	1049196	0	0	0	1
L3	1	1.6	4609891	1049208	0	0	0	1
L4	1	1.2	4609888	1049218	0	1	0	0
L5	1	0.5	4609888	1049229	0	0.7	0	0.3
L6	2	1.8	4609906	1049251	0	0	0.5	0.5
L7	2	2	4609909	1049234	0	0.6	0.4	0
L8	2	1.6	4609910	1049218	0	0.9	0	0.1
L9	2	2.5	4609912	1049204	0	0	0	0
L10	2	2.1	4609909	1049191	0	0	0.9	0.1
L11	3	1.6	4609932	1049197	0	0	0	0
L12	3	2.1	4609932	1049216	0	1	0	0
L13	3	2.3	4609930	1049234	0	0.9	0.1	0
L14	3	2.3	4609929	1049249	0	0.9	0.1	0
L15	3	2.7	4609928	1049264	0	0.9	0.1	0
L16	4	2	4609956	1049192	0	0	0	1
L17	4	2	4609955	1049208	0	0.9	0.1	0
L18	4	2.3	4609955	1049227	0	0.5	0.5	0
L19	4	2.6	4609937	1049229	0	1	0	0
L20	4	2.8	4609928	1049239	0	1	0	0
L21	5	1.9	4609977	1049292	0.1	0	0	0.9
L22	5	2.7	4609973	1049282	0	1	0	0
L23	5	2.6	4609972	1049251	0	1	0	0
L24	5	2.3	4609975	1049228	0	0.9	0.1	0
L25	5	2.4	4609975	1049200	0	1	0	0
L26	6	1.6	4609998	1049197	0	0	0	1
L27	6	1.8	4609998	1049207	0	1	0	0
L28	6	1.6	4609998	1049215	0	1	0	0
L29	6	2.3	4609998	1049222	0	1	0	0
L30	6	2.4	4609997	1049239	0	1	0	0
L31	7	1.3	4610017	1049195	0	0	0	1
L32	7	2.2	4610014	1049207	0	0.4	0.4	0.2
L33	7	2.3	4610014	1049214	0	0.9	0.1	0
L34	7	2.6	4610001	1049222	0	1	0	0
L35	7	1.7	4609998	1049238	0	1	0	0
L36	7	2.2	4609997	1049241	0	0	0	1
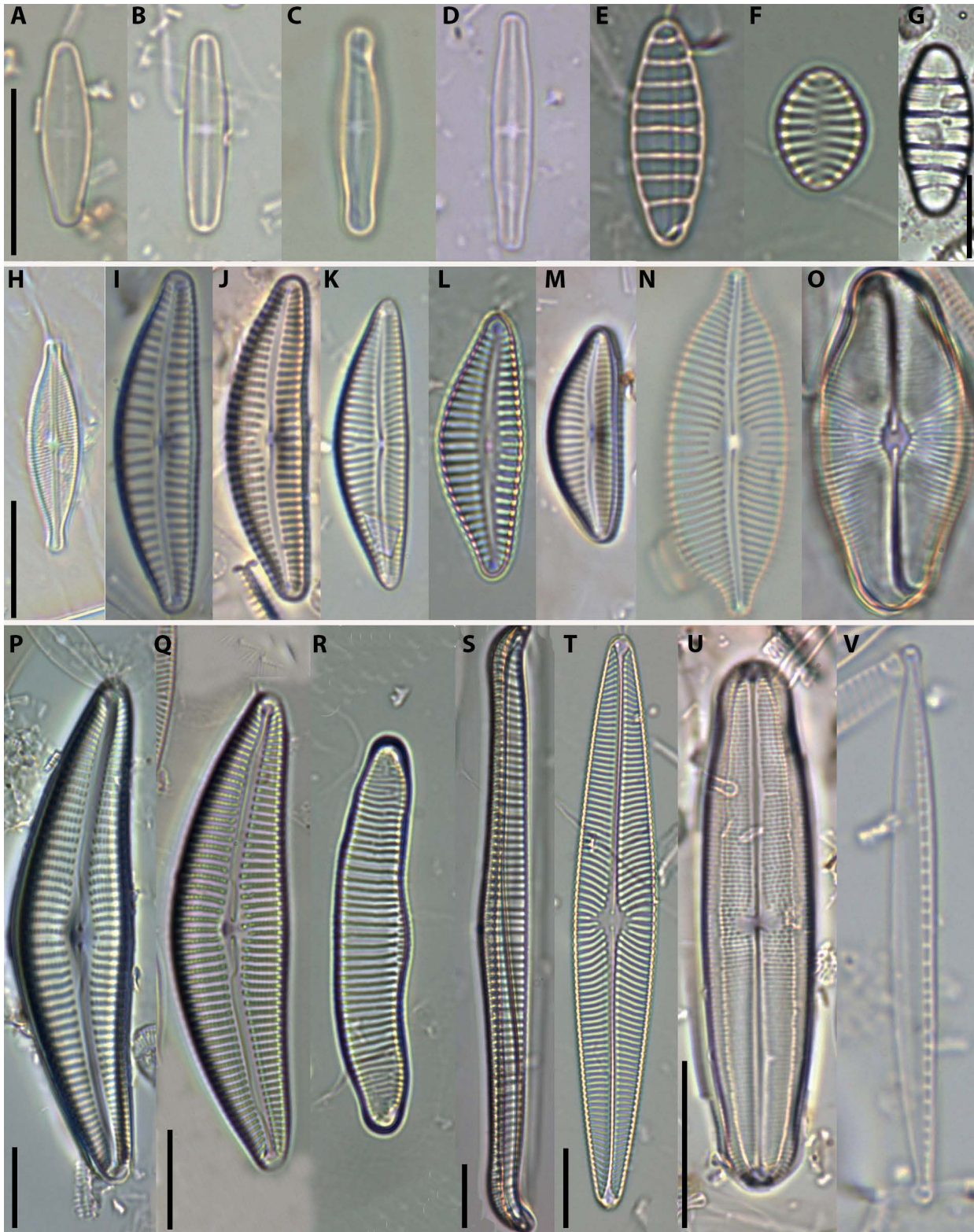
Figure 2 – Selected species: A, *Achnanthidium dolomiticum* M.Cantonati & Lange-Bert.; B, *Achnanthidium lineare* W.Sm.; C, *Achnanthidium minutissimum* var. *jackii* (Rabenh.) Lange-Bert.; D, *Achnanthidium minutissimum* var. *minutissimum*; E, *Denticula tenuis* Kütz.; F, *Staurosira venter* (Ehrenb.) Cleve & J.D.Moeller; G, *Diatoma mesodon* (Ehrenb.) Kütz.; H, *Brachysira neoexilis*; I, *Cymbella excisiformis* Krammer; J, *C. parva* (W.Sm.) Kirchn.; K, *C. levis* Nägeli; L, *Cymbella subleptoceros*; M, *Cymbella* cf. *hustedtii* var. *rhombica* Krammer; N, *Cymbopleura frequens* Krammer; O, *Eucocconeis flexella* (Kütz.) F.Meister; P, *Cymbella cymbiformis* C.Agardh; Q, *Cymbella scutariana* Krammer; R, *Eunotia soleirolii* (Kütz.) Rabenh.; S, *Rhopalodia parallela* (Grunow) O.Müll.; T, *Navicula radiosa* Kütz.; U, *Neidium affine* (Ehrenb.) Pfitzer; V, *Nitzschia oligotraphenta* (Lange-Bert.) Lange-Bert. Scale bar = 10µm.

analyses were carried out at the environmental chemistry lab of the E. Mach Foundation following standard methods (APHA 2000).

Diatom preparation

Samples were treated with 30% H_2O_2 for 24 hours. The Erlenmayer flasks with treated samples were subsequently heated up to a boiling point and kept boiling approximately for 60 minutes. Small amounts of $K_2Cr_2O_7$ and c. 500 µl, 37% HCl were added into the hot samples. Samples were centrifuged and cleaned with distilled water until reaching neutrality. Cleaned diatom frustules were mounted in Naphrax. At least 400 valves were counted and percentage of relative abundance for every of 36 slides were evaluated together with species level identification using the following literature: Krammer (2000, 2002, 2003), Lange-Bertalot (2001), Levkov (2009), Lange-Bertalot et al. (2011), and Hofmann et al. (2013). Nomenclature was harmonized using AlgaeBase (Guiry & Guiry 2015). Slides were observed using light microscope Zeiss Axioskop 2 (Zeiss, Jena, Germany) equipped with phase-contrast and with an Axiocam digital camera. Two permanent slides representing samples of epiphyton exclusively from *Chara/Potamogeton* (N. DIAT 2327, DIAT 2317) were used for diatom cell size measurements (200 cells were measured for each substrate). SEM images were taken from herbarized (dried) material from the stations 4.4 and 7.1, corresponding to epiphytic-diatom samples cLIM005 DIAT 2334 (for *Chara aspera* Willd. 100%) and cLIM005 DIAT 2346 (for *Potamogeton gramineus* L. 100%) respectively, using a Scanning Electron Microscope Zeiss EVO 40 XVP Zeiss after gold coating.

Statistical analysis

The statistical package Canoco for Windows 4.5 (ter Braak & Šmilauer 2002) was used to test relationship among diatom assemblages and host plants. Species data were transformed using the Hellinger transformation before carrying out multivariate analyses. Detrended Correspondence Analysis (DCA) based on detrending by segments showed short gradients on first (2.509) and second axis (1.137). Redundancy Analysis (RDA) was carried out as follows: *Chara aspera* and *Potamogeton gramineus* as the most frequent host plants in the Lago di Valagola were set as environmental data, depth as covariable, and position in the lake as supplementary variable. Scaling method was focused on inter-species correlation. Both automatic and manual forward selection of environmental variables (Monte Carlo Permutation test, 499 unrestricted permutations) was used to test statistical significance of species-environmental variables relationship. Both *Chara aspera* and *Potamogeton gramineus* showed low inflation factor (VIF_{Chaspe} = 2.602, $VIF_{Potgram}$ = 2.674). Visualisation, T-values biplot statistics and Shannon diversity index calculation were processed by CanoDraw for Windows 4.0. Differences between averages of diatom length, width, length/width ratio and Shannon diversity index between *Chara aspera* and *Potamogeton gramineus* were tested statistically using One Way ANOVA (NCSS, Hintze 2006).

Table 4 – Results of Redundancy Analysis.
Lambda1, variable explanation for Conditional Effects (%); LambdaA, variable explanation for Marginal Effects (%); P, significance of F statistics; F, result of F statistics; Chara, *Chara aspera*; Potgram, *Potamogeton gramineus*.

Marginal Effects			
Variable	**Lambda1**	**p**	**F**
Chara	0.17	0.002	7.13
Potgram	0.11	0.006	4.35
Conditional Effects			
Variable	**LambdaA**	**p**	**F**
Chara	0.17	0.002	7.13
Potgram	0.06	0.008	3.05

RESULTS

The majority of the tarn bottom was overgrown with the stonewort *Chara aspera*, with scattered populations of *C. virgata* Kütz. The lake shore in the western portion was covered by a narrow belt of *Potamogeton gramineus*. A small population of the bryophyte *Calliergon giganteum* (Schimp.) Kindb. grew only in one site (L21) located on the western shore. Host (substrate) plant composition of each sample is given in table 3 and co-dominating host plant's relative representation is shown in fig. 1C & D.

A total of 78 epiphytic-diatom species (some of them are documented in fig. 2) were identified (electronic appendix). Species richness of the individual epiphyton samples ranged from 11 to 38 taxa. In comparison, single samples of epilithon and epipelon (close to site L1) included 28 and 30 species respectively. The vast majority of species found on the macrophytes were benthic pennate, while the percentage of centric diatoms was < 36% (*Cyclotella* sp.). The most frequent epiphytic diatoms were *Pseudostaurosira polonica* (M.Witak & Lange-Bert.) E.Morales & Edlund, with relative representation ranging from 1 to 74%, *Staurosirella pinnata* (Ehrenb.) D.M.Williams & Round, with proportions up to 27%, and *Encyonopsis subminuta* Krammer & E.Reichardt, with relative abundances up to 25%. In comparison, epilithon and epipelon (site L1), were dominated by *Pseudostaurosira polonica* and *Achnanthidium straubianum* (Lange-Bert.) Lange-Bert. Phytoplankton was represented by *Cyclotella* cf. *radiosa* (Grunow) Lemmerm.

Redundancy Analysis (table 4) showed statistically significant relationships between species composition and environmental variables (F = 7.024, p = 0.004). The first ordination axis explains 18.0% and the second 6.9% of species data variation. The analysis separated sampling sites into two basic groups: (i) sites dominated by *Chara aspera* (central part of the lake) and (ii) sites dominated by *Potamogeton gramineus* (marginal parts of the lake), host plants at L1, L9 and L11 were not identified (fig. 3). Monte Carlo permutation test showed a statistically significant effect of *Chara aspera* on distribution of epiphytic diatoms (conditional effect: F = 7.13, p = 0.002; Marginal effect: F = 7.13, p = 0.002).

The influence of *Potamogeton gramineus* on diatom distribution was also significant (conditional effects: F = 3.05, p = 0.008, marginal effects F = 4.35, p = 0.006).

Diatom assemblages of both main groups differed in species richness and diversity. A significant difference (F = 9.01, p = 0.0053) in diatom distribution (expressed as Shannon index; fig. 4) was found between *C. aspera* (2.33±0.21) and *P. gramineus* (2.57±0.19). Diatom taxa positively correlated with *C. aspera* (fig. 5) included: *Brachysira neoexilis* Lange-Bert., *Cyclotella* sp., *Encyonopsis cesatii* (Rabenh.) Krammer (zone 1 in fig. 5). Diatoms positively correlated with *P. gramineus* included *Epithemia adnata* (Kütz.) Bréb., *Eunotia arcubus* Nörpel & Lange-Bert., *Eunotia arcus* Ehrenb., *Rhopalodia gibba* (Ehrenb.) O.Mül., *Cymbella subleptoceros* Krammer (zone 3 in fig. 5). Zone 2 in fig. 5 contains species present on both plants, e.g. the euryvalent species complex *Achnanthidium minutissimum* (Kütz.) Czarn. Observations

on herbarized material showed, that *Potamogeton* was colonized by higher number of diatom cells than *Chara*, moreover attached diatoms usually comprised large colonies on *Potamogeton* (fig. 6E–G). Single diatom cells were able to colonize sporadically *Chara* thalli (fig. 6A–D). Diatom size measurements exclusively from *Potamogeton/Chara* samples showed that significantly longer diatoms are present on the surface of *Potamogeton* than on *Chara* (length F = 11.49, p = 0.0008, length/width ratio F = 12.39, p= 0.0005, fig. 7). These results are in congruence with species composition typical for *Chara/Potamogeton* (fig. 5).

DISCUSSION

Freshwater benthic communities are influenced by a wide spectrum of biotic and abiotic factors (Round 1971). Benthic diatom distribution in lakes is driven by microhabitat
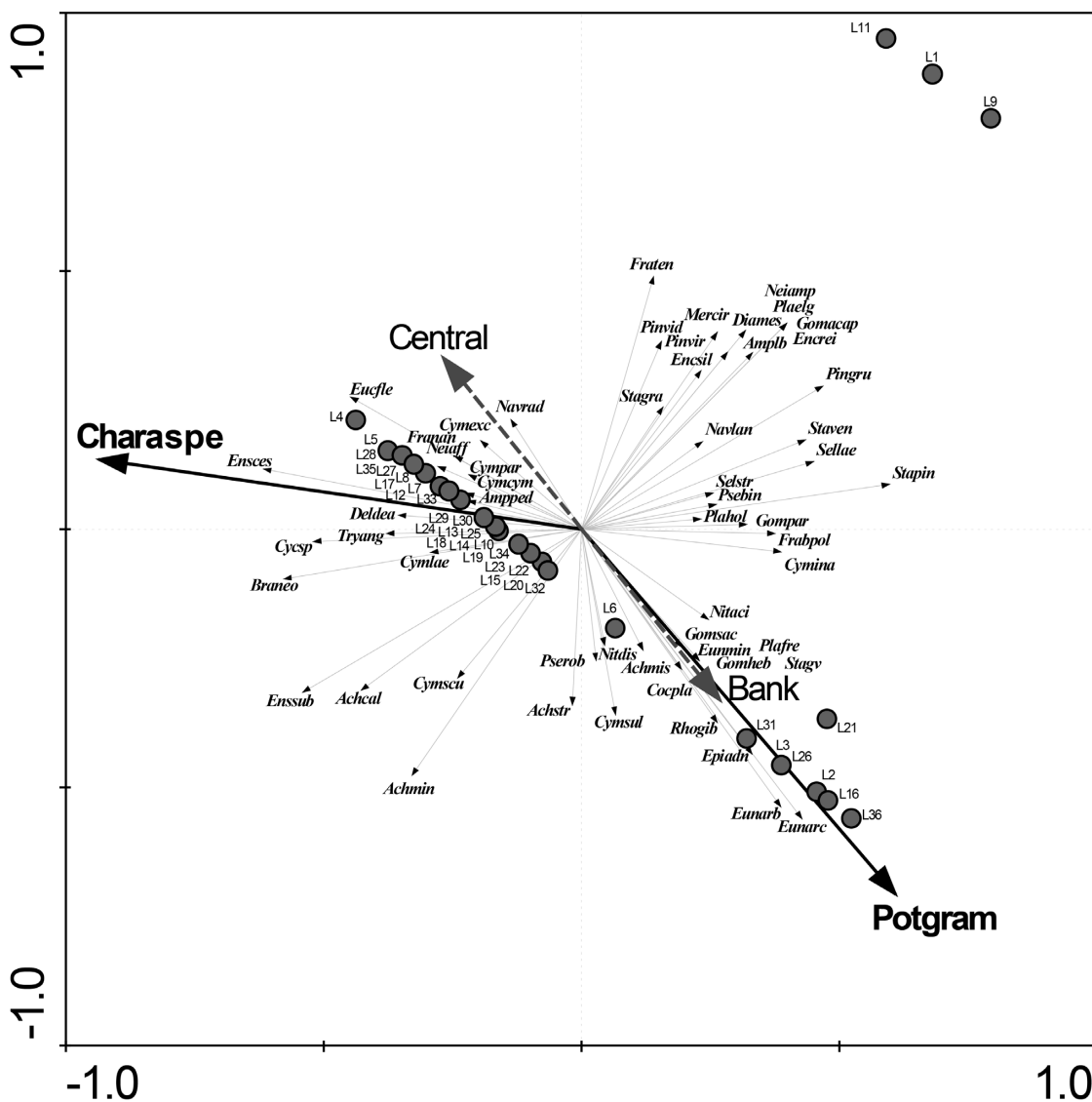

Figure 3 – Redundancy Analysis: L1–L36 sampling sites (see table 3; abbreviations of species see electronic appendix), Charaspe – *Chara aspera*, Potgram – *Potamogeton gramineus*, Central - central part of the lake), Bank - first sample of each transect situated at the lake bank: L1, L5, L6, L10, L11, L15, L16, L20, L21, L25, L26, L30, L31, L36). F = 7.024, p = 0.004.
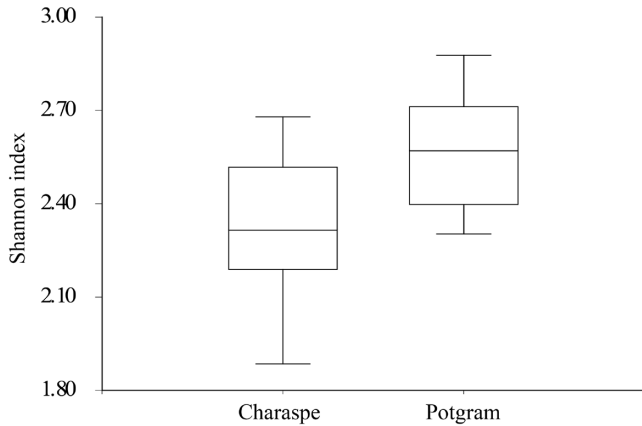
Figure 4 – One way ANOVA: epiphytic diatom diversity (Shannon index) on *Chara aspera* (Charaspe) and *Potamogeton gramineus* (Potgram). F = 9.01, p = 0.0053.

distribution, and depends upon hydrological situation (Neif et al. 2013), lake bathymetry, light (Cano et al. 2012), nutrient and substrate availability (Cantonati et al. 2009, Cano et al. 2012) and grazing (Meerhoff et al. 2007). Some results show that epiphytic diatom communities respond mainly to physical/chemical variables, and only secondarily to lake depth, size, and location (Blanco et al. 2014). Plankton in the limnetic zone have primary access to solar light, whereas benthic associations in the littoral zone to nutrients released by mineralization processes in the sediment (Wetzel 1996). The decrease of light intensity with water depth is certainly influenced by dispersed particles in the water column, and high phytoplankton abundance dramatically decreases the depth distribution of benthic algae by shading. Although competition for light between plankton and microphytobenthos has been discussed in some Alpine lakes (Poulíčková et al. 2004, Cantonati et al. 2009), phytoplankton seems to be a poor competitor in Lake Valagola (table 2 cf. chlorophyll *a* concentration).
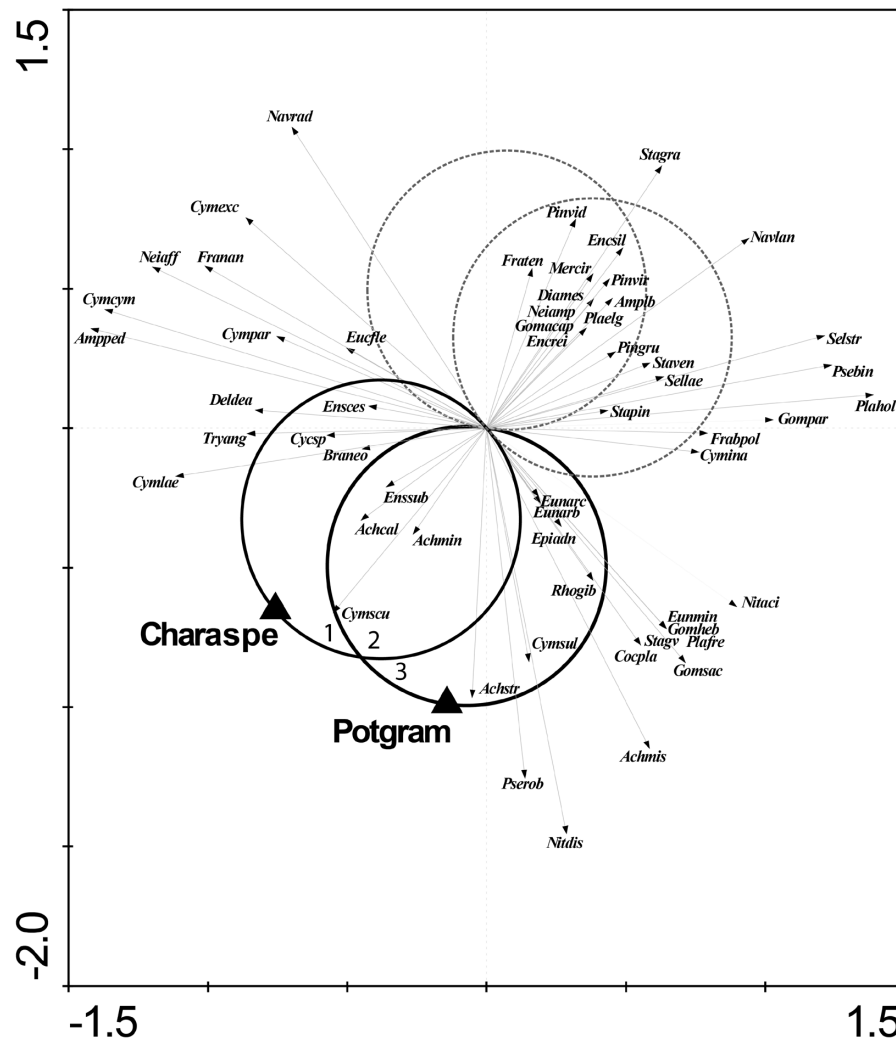
Figure 5 – T-value biplot diagram showing species sorted into Van Dobben circles showing positive or negative influence of substrate (Charaspe - *Chara aspera*, Potgram - *Potamogeton gramineus*). See electronic appendix for species names and abbreviations. Species inside circles responded significantly to the substrate. Black circles indicate positive responses, and gray circles indicate negative responses. Van Dobben circles in the case of *Chara and Potamogeton* partially cover each other, which means that in zone 2 are species colonizing both substates. Zone 1 represents species colonizing *Chara aspera*, zone 3 represents species colonizing *Potamogeton gramineus*.

Dramatic changes in epiphyton biomass and structure observed along transects across deep lakes from the littoral to the limnetic zone (Yang et al. 2009) were not expected in our shallow, clear lake. However, the differences between central and marginal parts are still detectable, even though a true limnetic zone is missing (fig. 3). Indeed, similar differences between the central and peripheral zone were found in other European lakes/ponds and have been explained by macrophyte density, animal and wind disturbances, wave action or solar radiation (Cano et al. 2012, Kitner et al. 2005, Poulíčková et al. 2006) irrespective to their depth.

In contrast to substrate specificity, depth influence was not found to be significant in this study (analysis not illustrated). However, some differences found in this study can be explained by the biology and ecology of both dominant macrophytes. Growth and development of *Potamogeton* follows an annual cycle (with regrowth in spring that starts from tubers or from buds on relic stems; e.g. Wiegleb & Kadono 1989) whilst *Chara* is a perennial with apical growth (e.g. Krause 1997).

The western shore of Lake Valagola is dominated by *Potamogeton* (depth 0.5 to 2.2 m) whereas *Chara aspera* grows in the rest of the lake. Their spatial distribution should be explained by competition for light and free CO_2, as previously reported for *C. aspera* and *Potamogeton pectinatus* L. (van den Berg et al. 1998). However, *Stuckenia pectinata* (L.) Börner (previously *Potamogeton pectinatus*) and *P. gramineus* differ significantly and van den Berg's model consider much more turbid conditions. The depth distribution of macrophytes in Lake Valagola suggests that *C. aspera* is tolerant to shading. This is in agreement with records highlighting that charophytes colonize deeper parts than angio-

sperms (Blindow 1992). Although depth distribution cannot explain why *Potamogeton* colonizes only the western shore of the lake, light could again be the possible factor, because the eastern shore of the tarn is shaded by wood, while the western shore is more open.

Both dominant macrophytes (*Chara aspera* and *Potamogeton gramineus*) host significantly different diatom species assemblages. Diatom species richness, diversity, and composition differed significantly between these two main host plants. Moreover, the diversity hot spot (L21; *Calliergon giganteum*; fig. 1B) might be influenced by groundwater inflow (Cantonati et al. 2012). *Potamogeton gramineus* assemblages were characterized by higher species richness and diversity, and by the large-celled, adnate diatom species *Epithemia adnata*, *Rhopalodia gibba*, *Eunotia arcus*, and *E. arcubus*. *Chara aspera* was preferred by the small-celled, motile diatom species *Brachysira neoexilis*, *Encyonopsis cesatii*. As we documented by measurements and host plant surface SEM images, *Chara* thalli seems to be a more difficult surface for diatom collonization, particularly for longer species (fig. 6).

Because macrophyte distribution is spatially structured in this lake, we can hardly separate differences induced by substrate/host from other possible influences. Substrate preferences caused either by morphology of plant species (mechanical cause; Laugaste & Reunanen 2005, Pomazkina et al. 2012) or nutrient uptake from host plants (chemical cause) are accompanied by seasonal aspects of epiphyte distribution.

Seasonal changes have been found to be significant in shallow lowland ponds (Kitner et al. 2005). Final cover and rate of colonization was higher in summer than in spring.
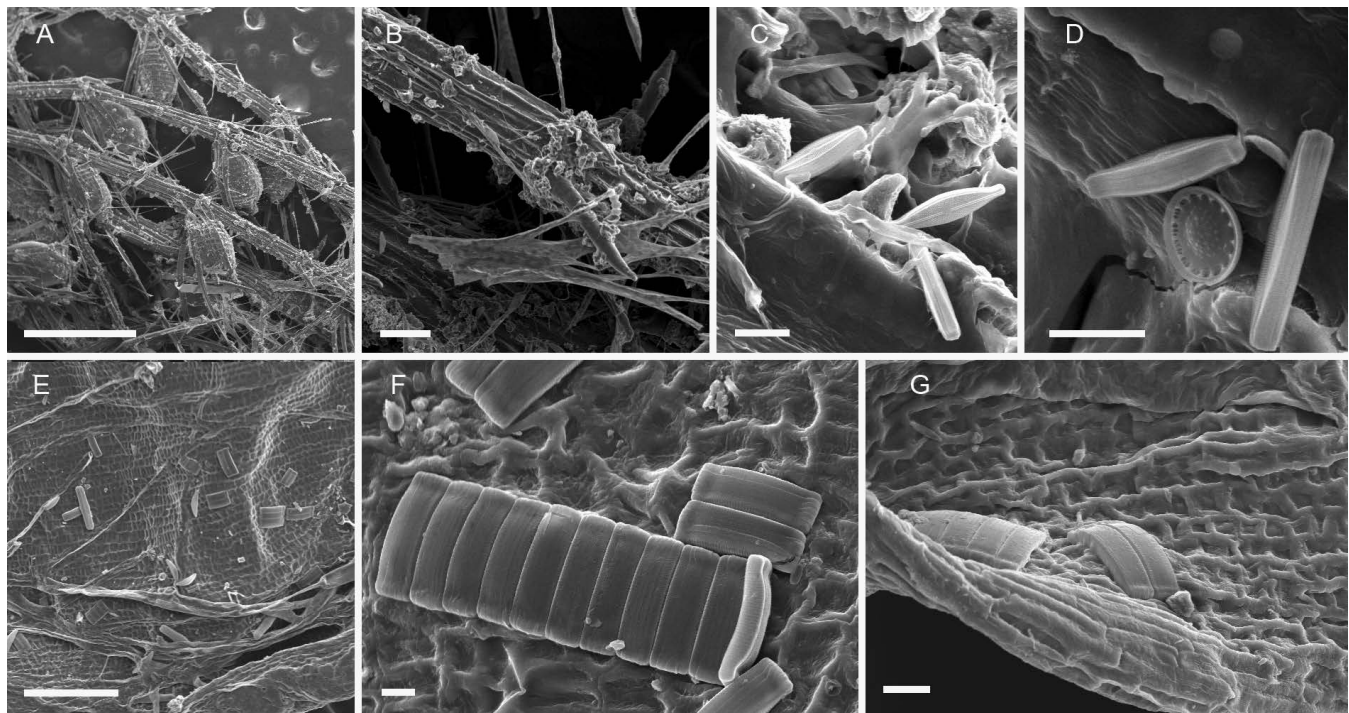

Figure 6 – SEM images of herbarium material of *Chara aspera* (A–D) and *Potamogeton gramineus* (E–G) showing diatom epiphytes on the surface. Scale bars: A, 1 mm; B, 100 µm; C, D & F, 10 µm; E, 200 µm; G, 20 µm.

Differences have been explained by temperature and light fluctuations (Hoagland et al. 1982, Kitner et al. 2005). Moreover, seasonal changes influence the growth of macrophytes in terms of surface for algal colonization (Pizarro 1999). Seasonal differences caused by hydrological dynamics have been verified in shallow floodplain lakes (Neif et al. 2013).
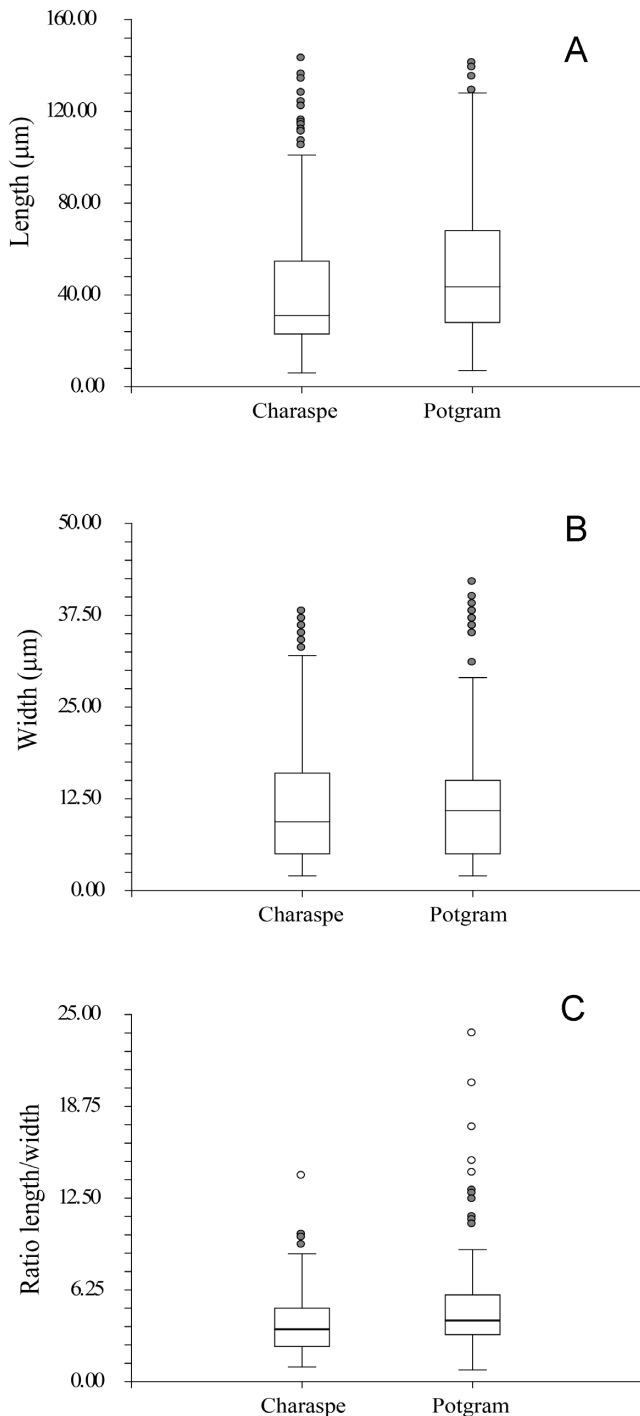
The vegetative season in Lake Valagola is shorter (about 6 months) because of the elevation, and the lake was sampled at the end of the summer when colonization of substrata and assemblage development are maximal in temperate mountain lakes (Catalan & Donato Rondón 2016). The architecture of the host plant is undoubtedly of significance, particularly, in lakes with low nutrient levels. According to Hinojosa-Garro et al. (2010) macrophyte architectural complexity leads to an increase of the epiphytic species richness and diversity. In highly-eutrophic lakes, substrate specificity can be less pronounced (Eminson & Moss 1980, Kairesalo 1984, Kitner & Poulíčková 2003, Laugaste & Reunanen 2005). No qualitative or quantitative specificity for substrata was observed in eutrophic ponds and streams in Czech Republic (Kollár et al. 2015) or in a study on Lake Erie (Millie & Lowe 1983).

However, Cejudo-Figueiras et al. (2010) rejected Blindow's (1987) neutral substrate hypothesis, and observed significant differences in the composition of diatom assemblages among host macrophytes. In contrast, diatom-based indices for trophic level assessment did not differ significantly. Thus, they hypothesize that epiphytic diatoms can be used as indicators for shallow lakes irrespective of the dominant macrophyte (Cejudo-Figueiras et al. 2010).

As we expected, we found a significantly higher diversity on *Potamogeton*, more likely due to appropriate plant architecture and surface, which is in agreement with the opinion of other authors (Pomazkina et al. 2012). The development of epiphyton can be affected also by allelopathic interactions (Gross 2003). Whereas cyanobacteria are strongly inhibited by compounds produced by *Chara aspera*, surprisingly no inhibition was noted in eukaryotic target strains including one diatom strain (Berger & Schagerl 2003, 2004).

In contrast to some studies mentioned above, our results support the existence of substrate specificity for diatom assemblages. Although, such distinct model cases as *Lemna* spp. vs. *Lemnicola hungarica* (Grunow) Round & Basson seem to be rare (Buczkó 2007). *Chara aspera* and other Charophyceae of the Balkan Peninsula were inhabited by other diatom species (Hafner & Jasprica 2013), because the localities were brackish. Variation in species composition of epiphyton growing on *Potamogeton* and other macrophytes (*Myriophyllum* sp., *Elodea* sp.) were rarely studied (Pomazkina et al. 2012). The most common epiphytic diatom seems to be *Cocconeis placentula* Ehrenberg (Birkett & Gardiner 2005, Potapova & Charles 2005, Lebreton et al. 2009). We found it to be more common on *Potamogeton* rather than on *Chara*. *Cocconeis* preferring shores exposed to wind with active wave mixing (Kozhov 1962, Pomazkina et al. 2012) was accompanied in Lake Valagola by diverse species of *Epithemia* and *Rhopalodia*.

In conclusion, our work pointed out general features of diatom assemblages colonizing two macrophytes with contrasting depth-preferences and architectures, particularly in terms of size structure of epiphytic diatoms.

SUPPLEMENTARY DATA

Supplementary data are available in pdf at *Plant Ecology and Evolution*, supplementary data site (http://www.ingenta-

Figure 7 – One way ANOVA: variability of epiphytic diatom size structure found on *Chara aspera* (Charaspe) and *Potamogeton gramineus* (Potgram). A, diatom cell length (F = 11.49, p = 0.0008); B, diatom cell width (F = 1.64, p = 0.2010); C, length/width ratio (F = 12.39, p = 0.0005).

connect.com/content/botbel/plecevo/supp-data) and consist of a list of species with abbreviations and host plants.

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