Response of macrophyte communities to flow regulation in mountain streams

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Abstract
River macrophytes are widely used in fresh-water ecosystem assessment because of their sensitivity to anthropogenic pressures, even if there are only a few studies that investigated how macrophytes respond to water regime alterations. In this study, we analyzed the effects of dams on river macrophyte communities through a comparison between upstream and down-stream sides from 18 dams located in Alps and Apennines. A co-inertia analysis and a Mantel test were applied to assess if the analysis of environmental pa-rameters could be effective in predicting macrophyte community structure. We analyzed morphological and physicochemical inter-site differences and tested the influence of dams on various aspects of community structure (composition, richness, diversity, dominance, coverage) using multivariate randomized block permu-tation procedure. Plant similarity between sites was evaluated at the level of phylum, and indicator species analysis was performed to identify the taxa most sensi-tive or tolerant to water regulation. We found that the overall environmental setting overwhelms the dam im-pact and that the influence of hydrological alteration became apparent when comparing upstream and down-stream assemblages at the same dam. In particular, we found that most of taxa had a higher affinity with the downstream side and that in general, stream regulation increases plant richness and coverage, but reduces com-munity evenness. Analyses based on higher taxonomic groups (phyla) demonstrated that this community can be effectively used in bioassessment even at phylum level analysis. In particular, we found that bryophytes, strictly linked with changes in substrate stability, show particu-lar sensitivity to water regulation in mountain streams.

Introduction

An increased interest in biodiversity conservation and sustainable development prompted the study of freshwater ecosystems in the last decades. The European Water Framework Directive (WFD) 2000/60/EC identified biomonitoring of different bi-ological quality elements (fish, diatoms, macroinver-tebrates, macrophytes) as a fundamental tool for a comprehensive evaluation of the conservation status of inland water ecosystems. Macrophytes of fresh-water habitats have been studied in detail since the 1960s (Sculthorpe 1966; Haslam 1978) and their ecology has been widely investigated in several recent papers (e.g. Janauer et al. 2010; Ceschin et al. 2012; Vieira et al. 2012; Mebane et al. 2014). Macrophytes are known to be particularly sensitive to different anthropogenic alterations, such as eutrophication, pollution or hydromorphological changes (Baatt rup-Pedersen and Riis 1999; Barendregt and Bio 2003; Schaumburg et al. 2004). Thus, several authors proposed the use of these organisms as bioindicators of ecosystem quality (e.g. Haury et al. 1996; Newman et al. 1997; Schneider et al. 2000; Ferreira et al. 2005; Minciardi et al. 2009). The interest in the use of macrophytes as river bioindicators led to the development of indices for the evaluation of trophic status, but which resulted only partially suitable when used to assess other environmental impacts, in particular the hydromorphological alterations (Wiegleb 1984; Daniel et al. 2006; Poff and Zimmerman 2010; Demars et al. 2012). For this reason, it is important to develop new approaches specifically conceived for this purpose (Friberg et al. 2013).

All over the world, river ecosystems are affected by hydromorphological pressures, and hydroelectric power production (HEP) is one of the commonest and most impacting pressures (Vörösmarty et al. 2010). In particular, water abstraction and flow reg-ulation for hydropower activities have severe im-pacts on water regime (magnitude, duration, timing, rate of change and frequency of flows) and on mor-phological features of watercourse (river cross pro-file, substrate stability, grain size, etc.), which in turn affect habitat structure, connectivity and func-tionality, as well as river biodiversity and biological community structure (Arthington et al. 1992; Poff et al. 1997; Jowett and Biggs 2008; Tombolini et al. 2014). Macrophyte communities of lotic ecosystems are strongly influenced by water regime (e.g. Lowe 1979; Suren and Duncan 1999; Casanova and Brock 2000; Madsen et al. 2001), which is considered one of the main factors influencing macrophyte abun-dance and diversity in rivers (Riis and Biggs 2003; Franklin et al. 2008). These considerations suggest that macrophyte communities could be particularly useful in the evaluation of this type of impact (Poff et al. 1997; Bunn and Arthington 2002; Tharme 2003). Macrophyte responses to hydrological alter-ations, however, are still poorly investigated. Available studies indicate changes in plant coverage (Rørslett et al. 1989; Baattrup-Pedersen and Riis 1999), biomass (Fayolle et al. 1998), richness (Englund et al. 1997; Bernez et al. 2004), diversity (Bernez and Ferreira 2007; Riis et al. 2008) or presence/abundance of single species (Englund et al. 1997). However, most of these investigations presented single-case studies and produced contrast-ing results. In addition, only a very few studies refer to mountain stream stretches (Vanderpoorten and Klein 2000; Downes et al. 2003), although the hydromorphological changes often represent the most severe anthropogenic impact on river ecosys-tems in these areas.

In this paper, we analyzed river macrophyte re-sponses to hydrological alterations by comparing spe-cies assemblages collected at both upstream and down-stream sides in a set of several dams. In particular, the main topics of our research were to assess the following: (i) how macrophyte assemblage responds to water reg-ulation taking into account the effects of other environ-mental variables; (ii) if macrophyte responses show recurrent patterns in different ecological and geograph-ical contexts; and (iii) if regulated stream stretches can be characterized by particular macrophyte assemblages or taxa. The identification of macrophyte response pat-terns under water regulation could

clarify the bioindicator potential of these organisms for this type of alteration and may contribute to the future develop-ment of specific metrics for the evaluation of this an-thropogenic impact.

Materials and methods

Sampling sites

The effects of hydrological alterations on river eco-systems are extremely complex, being dependent on the type and intensity of alterations, river typology and environmental features of the target waterbody (Petts 1984). For these reasons, we focused our research on mountain or piedmont stretches, where the hydrological alteration is the main anthropogenic pressure on river ecosystems, whereas sources of water pollution are often negligible and synergistic effects of different anthropogenic pressures are avoidable. In order to analyze sampling sites pre-senting a water regulation as homogeneous as pos-sible, we considere d only st rea m se gme nts subtended by dams constructed for hydroelectric exploitation. To avoid the risk of drawing conclu-sions from single case-studies, which are subject to an increase rate type 1 errors and potential positive publication bias (Ioannidis 2005), we considered a number of dams dispersed over large geographical areas (western Alps and central Apennines) and which have therefore different ecological character-istics. Moreover, we focused on barrages that par-tially differ for size, in order to investigate how macrophytes respond to a wide range of hydrologi-cal alteration intensities. In particular, the hydraulic residence time (HRT) parameter (Poff and Hart 2002), defined as the ratio of the storage volume (m^3) of the reservoir to its flow-through rate (m^3 per year; generally range between 0 and 1), varied for an order of magnitude in our sampling sites, from about 0.01 to 0.1.

On the basis of these selective criteria, a total of 18 site pairs (upstream vs downstream side) were identified in northern and central Italy. Half of them are located in the western Alps, while the others are in the central Apennines (Fig. 1). The possible lack of independence between sites within the same region was taken into account in the choice of statistical methods (see below). At each selected dam (site), we sampled macrophytes in the upstream side and in the downstream side. To reduce the environmental variability that might affect macro-phyte assemblage, we selected stream reaches with the following characteristics:

– availability of upstream and downstream sides with similar environmental features, such as river chan-nel confinement, average slope and shading
– absence of relevant water pollution sources between up/downstream sides of each site

It was almost always possible to select sampling sites with these characteristics except for sites 1, 9, 14 and 17 (Table 1) because of the lack of a suitable upstream side. In these cases, as already done by Ferreira and Moreira (1999), we used a reference upstream side in a tributary, which, besides fulfilling the selective criteria, had sim-ilar orography and sizes. In all cases, the upstream side was used as a benchmark to test the effects of the dam on the downstream side. Each side was located at a distance ranging from 0.5 to 3 km from the dam and, for the upstream sides, out of dam's influence.

Macrophyte sampling

Macrophyte sampling was performed in 2011–2012, between June and September, always under moderate/ low flow regime. All samples were collected at least 10 days after rainfall events to prevent alterations in the plant assemblage. Up/downstream sides related to the same dam were sampled in the same day. For each side, a 50-m-long stream reach was checked to pinpoint all taxa present in the wet

riverbed and to visually assess species abundance as percent coverage respect to the entire sampled area. The sampling procedure followed a standardized protocol described by Minciardi et al. (2014), even if the length of sampling reach was short-ened to reduce the sampling effort. All macrophyte groups were considered: macroalgae and cyanobacteria, bryophytes and vascular plants.

Environmental factors

Each sampling site was characterized from both mor-phological and physicochemical points of view. In par-ticular, the following parameters were measured twice in the field: riverbed width, average depth, shading (per-centage of shaded riverbed), granulometry (percent coverage of six size classes: bedrock, boulders, cobbles, gravels, sand, silt), slope of the sampled reach and turbulence (as a ratio of turbulent to laminar flow). Temperature, pH, oxygen concentration and conductivity were measured with a multiparameter probe (Hach-Lange HQ40d). Concentration of nitrates, ammonium ions, phosphates and COD was measured through spec-trophotometric analysis (Hach-Lange DR2800 spectro-photometer). Such environmental characterization allowed us to verify if there are upstream/downstream trends of variation, also as a side effect of flow regulation.

Statistical analysis

In order to analyze abiotic-biotic relationships and to detect environmental and biological differences between upstream and downstream sides, we used a set of multivariate and univariate approaches.

We applied a coinertia analysis (CoIA) to relate macrophyte abundance data with environmental data in a symmetric way. This technique performs a double inertia analysis of two datasets maximizing covariance on axes of CoIA (Dolédec and Chessel 1994). The intensity of correlation between the data tables is described by the RV coefficient that varies between 0 and 1 (with 1 as maximum correlation between them) and expresses the similarity between squared symmetric matrices (Robert and Escoufier 1976). CoIA was performed using the PCA ordination method for both matrices with 999 permutations in order to assess the significance of the costructure of the data sets. To reduce the noise due to rare taxa, those with less than five occurrences were removed from the dataset. To improve the distribution of abundance data toward the normality, evaluated by Shapiro-Wilk test, the biotic matrix was logarithmically transformed $[\ln(x + 1)]$. To perform the CoIA analysis, the Ade4 package in R (R Core Team 2014) was used.

In order to evaluate potential environmental shifts between upstream and downstream sides, we tested differences in the median value of each environmen-tal variable by pairing sides of the same site with a Wilcoxon sign test, which takes into account the non-independence of two sides belonging to the same dam. Then, in order to test the presence of a significant difference between upstream and downstream sampling site groups in terms of environmental conditions, excluding differences in hydrology, we used a multi-response permutation procedure by applying the permutation test to each pair of sites separately (a technique known as multivariate randomized block permutation procedure, MRBP; 999 permutations). MRBP is a non-parametric analysis that provides a measure of within-group agreement (A), which ranges from -1 to 1 (with values close to 0 suggesting that group membership tends toward randomness) (Mielke et al. 1976). MRBP was performed by using the vegan package in R (R Core Team 2014). Because environmental variables are expressed in different units, we applied standardized Euclidean distance in this MRBP. Similarly, we used a MRBP based on Bray-Curtis distance matrix to test for differences in macrophyte assemblages between upstream and downstream sides. To verify if the measured environmental parameters represented good predictors of macrophyte species composition, we correlated (by Mantel test) a matrix of inter-site dissimilarity in species composition (based on Bray-Curtis distances) with a matrix of environmental dissimilarity

(based on Euclidean distances between sites). Mantel tests were performed both on the entire dataset and on the two upstream and downstream sub-sets separately.

In order to describe possible assemblage changes between upstream and downstream sites, we used indices of diversity (Shannon entropy), dominance (Simpson dominance) and evenness (Buzas and Gibson's evenness) (Hayek and Buzas 2010). Differences in the median values of these community descriptors between upstream and downstream sides were tested not only by pairing sides of the same site (Wilcoxon sign test) but also comparing globally the sets of upstream and downstream sites (Mann-Whitney U test). Probability levels were calculated using Monte Carlo simulations, based on 99,999 random reassignments of values within each up/downstream pair (Wilcoxon paired test) or 10,000 reassignments to the two upstream/ downstream groups (Mann-Whitney test). We used this Monte Carlo approach to deal with the possible non-independence of sites belonging to the same river/watershed (Schank and Koehnle 2009).

Similarity between sites was also evaluated at the phylum identification level by using Bray-Curtis distance. The Bray-Curtis distance matrix was then used to run a non-metric multidimensional scaling (NMDS), using Past ver. 1.94b (Hammer et al. 2001) in order to plot sampling sites according to their similarity in phylum composition and abundance. Inter-site similarity was analyzed at phylum identification level in order to lower the noise due to rare taxa.

Finally, an indicator species analysis (ISA) was performed to identify taxa most sensitive or tolerant to water regulation (Dufrêne and Legendre 1997). This analysis represents an asymmetrical approach to identify indicator taxa by integrating the degree of uniqueness (specificity) and abundance/occurrence (fidelity) of a taxon within a given group into a single metric (the indicator value, IndVal, varies between 0 and 1, with 1 as perfect indication). The statistical significance of ISA, evaluated by means of a permutational test, was checked by blocking the permutations to each pair of site (999 permutations). ISA was performed with the indicspecies package of R, where an extension of the original indicator value method is implemented (De Cáceres et al. 2010).

Results

We collected 97 taxa (13 cyanobacteria, 23 algae, 5 liverworts, 26 mosses, 5 pteridophytes and 25 flowering plants) from 18 pairs of sampling sites (Online Resource 5). Most of the taxa (65 %) were found in only one or two sites, whereas nine taxa occurred in ten or more sites (Cladophora glomerata, Lemanea fluviatilis, Hydrurus foetidus, Vaucheria geminata, Oscillatoria sp., Spirogyra sp., Ulothrix sp., Platyhypnidium riparioides and Hygrohypnum luridum). The number of taxa per site ranged between 4 and 18 (mean value ± SD, 10 ± 3.5). Water surface covered by macrophytes ranged between 3 and 62 % of the total water surface (mean value ± SD, 29 ± 18).

CoIA showed a significant but relatively low correlation between macrophyte species abundance and environmental features (RV = 0.50, p < 0.001), with the first two eigenvalues accounting for 77.5 % of the total variance (Fig. 2). Some sampling sites showed a relatively strong correlation (shorter arrows) between environmental data and macrophyte species abundance (sites 5, 17, 18 for upstream sides; sites 1, 16, 17 for downstream sides), but in other cases, correlation was very weak (longer arrows: sites 6, 11, 13, 16 for up-stream sides; sites 2, 8, 10 and 13 for downstream sides). CoIA did not reveal any overall difference between macrophyte assemblages sampled at the upstream sides and those found at the respective downstream sides (Fig. 2). However, looking at the direction of the arrows of each upstream/downstream pair, in most cases (16 out of 18 pairs), the two sides of each sampling site were more similar in terms of environmental characteristics than in terms of macrophyte assemblages.

A comparison of upstream and downstream characteristics for each sampling site pair revealed no concordant trends for any environmental factor with no significant difference between the two sides (Wilcoxon paired test). The two groups did not result to be significantly different also when MRBP was applied (A = −0.014; p > 0.05), i.e. by comparing the two sides of the same site. By contrast, upstream and downstream sides appeared to be significantly different in terms of macrophyte assemblages when using the MRBP approach (A = 0.014, p < 0.01).

Mantel tests, based on macrophytes and environmental dissimilarity matrices, revealed an overall significant correlation between macrophyte species composition (inter-site dissimilarity) and environmental distances (r_M = 0.40, p < 0.001). Mantel tests conducted separately for upstream and downstream sides indicated that environmental factors were more strongly correlated with species composition for upstream macrophyte assemblages (r_M = 0.41, p < 0.001) than for downstream assemblages (r_M = 0.29, p < 0.001).

Macrophyte assemblages showed significant differences between upstream and downstream sides in terms of species richness, total abundance and evenness, both when the two sides were compared in association for the same site (Wilcoxon test) and when compared with no reference to site (Mann-Whitney U test) (Fig. 3). In particular, downstream macrophyte assemblages had more species and higher plant coverage, but lower evenness, than upstream assemblages. No difference was found for Shannon entropy and dominance.

When the 18 sampling sites were ordered according to their composition similarity based on higher taxonomical level (phylum) (NMDS in Fig. 4), 14 of them shared a common trend along the first NMDS axis: for each sampling pair, the downstream side was placed at a higher x value than the respective upstream side, with the exception of the pairs: 4, 6, 7, 9. The second NMDS axis separated sites into two groups: one (sites 1, 3, 4, 8, 9, 10, 11, 16, 17), in which the down-stream side had a y value higher than the respective upstream side; and another (sites 2, 5, 6, 7, 12, 13, 14, 15, 18) with an opposite trend. The two groups showed important differences in the abundance of particular phyla when comparing the two sides of each pair: bryophytes were more abundant on the downstream side than on the upstream side in the sites belonging to the first group, whereas chlorophytes were more abundant on the downstream side than on the upstream side in those belonging to the second group (Fig. 4).

The indicator species analysis (restricted to species which were found in at least seven sampling sites) revealed that taxa with highest IndVal resulted associated with downstream conditions (five taxa with IndVal higher of |0.30| in down-stream group against zero taxa for the upstream group). Significant differences in the IndVal scores between upstream and downstream sides were recorded for a bryophyte (Cinclidotus riparius) and two chlorophytes (Ulothrix sp. and Spirogyra sp.). Other species, such as the mosses Brachythecium rivulare and Fontinalis antipyretica, showed large but not significant differences (Table 2).

An analysis based on a higher taxonomical level, i.e. phylum, indicated that bryophytes are significantly associated with downstream conditions (Table 3). Pteridophytes and magnoliophytes appeared also associated with downstream conditions, whereas marchantiophytes and ochrophytes (represented by only V. geminata) appeared to be more associated with up-stream conditions, although in these groups, indicator values were not significant.

Table 1 Dams and site characterization

Geographical location	Dam name	Up/downstream code	Watercourse	Watershed	Altitude (m a.s.l.)	Source distance (km)
CA	Campotosto	1U	Leomogna	Vomano	590	4
		1D	Rio Fucino	Vomano	830	8
	San Giacomo	2U	Vomano	Vomano	422	28
		2D	Vomano	Vomano	320	32
	Provvidenza	3U	Chiarino	Vomano	1099	9
		3D	Vomano	Vomano	878	14
	Talvacchia	4U	Castellano	Tronto	566	20
		4D	Castellano	Tronto	446	26
	Presa gronda orientale	5U	Tordino	Tordino	452	20
		5D	Tordino	Tordino	436	21
	San Cosimato	6U	Aniene	Aniene	301	58
		6D	Aniene	Aniene	281	60
	Monterano dam	7U	Mignone	Mignone	187	22
		7D	Mignone	Mignone	165	24
	Posta	8U	Velino	Nera	732	20
		8D	Velino	Nera	718	21
	Scandarello	9U	Neja	Tronto	802	10
		9D	Scandarello	Tronto	837	13
WA	Malciaussia	10U	Stura Viù	Stura di Lanzo	1819	7
		10D	Stura Viù	Stura di Lanzo	1376	13
	Usseglio	11U	Stura Viù	Stura di Lanzo	1298	14
		11D	Stura Viù	Stura di Lanzo	1236	18
	Pourrieres	12U	Chisone	Chisone	1480	19
		12D	Chisone	Chisone	1402	23
	Gurzia	13U	Chiusella	Chiusella	517	21
		13D	Chiusella	Chiusella	350	27
	Agnel	14U	Rio del Carro	Orco	1904	5
		14D	Rio d'Agnel	Orco	1891	7
	Ceresole	15U	Orco	Orco	1682	11
		15D	Orco	Orco	1515	18
	Rasiga-Variola	16U	Rasiga	Toce	1618	5
		16D	Rasiga	Toce	1604	6
	Rovina	17U	Gesso della Barra	Gesso	984	11
		17D	Gesso di Entracque	Gesso	989	12

| Brusson | 18U | Evancon | Dora Baltea | 1402 | 24 |
| | 18D | Evancon | Dora Baltea | 1278 | 26 |

Table 2 Indicator species analysis. Indicator values (IndVal) and values are reported for taxa with at least seven occurrences. Significant IndVal (p < 0.05) are in bold

	Taxa	Phylum	Occurrence number	IndVal Upstream	IndVal Downstream	Δ
Increasing affinity toward upstream sides (unregulated condition)	*Cladophora glomerata*	Chlorophyta	17	0.51	0.45	0.06
	Hydrurus foetidus	Ochrophyta	10	0.39	0.33	0.06
	Vaucheria geminata	Heterokontophyta	11	0.42	0.36	0.05
	Fissidens rufulus	Bryophyta	7	0.32	0.30	0.02
Increasing affinity toward downstream sides (regulated condition)	*Platyhypnidium riparioides*	Bryophyta	21	0.52	0.56	−0.04
	Nostoc sp.	Cyanobacteria	8	0.30	0.35	−0.05
	Microspora sp.	Chlorophyta	8	0.28	0.38	−0.09
	Oscillatoria sp.	Cyanobacteria	25	0.51	0.61	−0.10
	Lemanea fluviatilis	Rhodophyta	13	0.35	0.49	−0.14
	Hygrohypnum luridum	Bryophyta	11	0.30	0.45	−0.15
	Palustriella commutata	Bryophyta	7	0.20	0.41	−0.20
	Cinclidotus riparius	**Bryophyta**	**7**	**0.14**	**0.48**	**−0.34**
	Fontinalis antipyretica	Bryophyta	9	0.13	0.51	−0.38
	Brachythecium rivulare	Bryophyta	7	0.08	0.51	−0.43
	***Ulothrix* sp.**	**Chlorophyta**	**17**	**0.23**	**0.70**	**−0.47**
	***Spirogyra* sp.**	**Chlorophyta**	**14**	**0.14**	**0.68**	**−0.54**

Table 3 Indicator species analysis at phylum level. Indicator values (IndVal) and for all phyla are reported. Phyla with significant IndVal (p < 0.05) are in bold

| Phyla	Occurrence	IndVal		Delta
		Upstream	Downstream	
Marchantiophyta	5	0.32	0.21	−0.11
Ochrophyta	10	0.39	0.33	−0.06
Heterokontophyta	13	0.37	0.48	0.10
Chlorophyta	35	0.63	0.75	0.12
Rhodophyta	20	0.46	0.59	0.14
Cyanobacteria	28	0.55	0.69	0.14
Pteridophyta	7	0.18	0.45	0.27
Magnoliophyta	12	0.26	0.54	0.27
Bryophyta	36	0.53	0.85	0.32
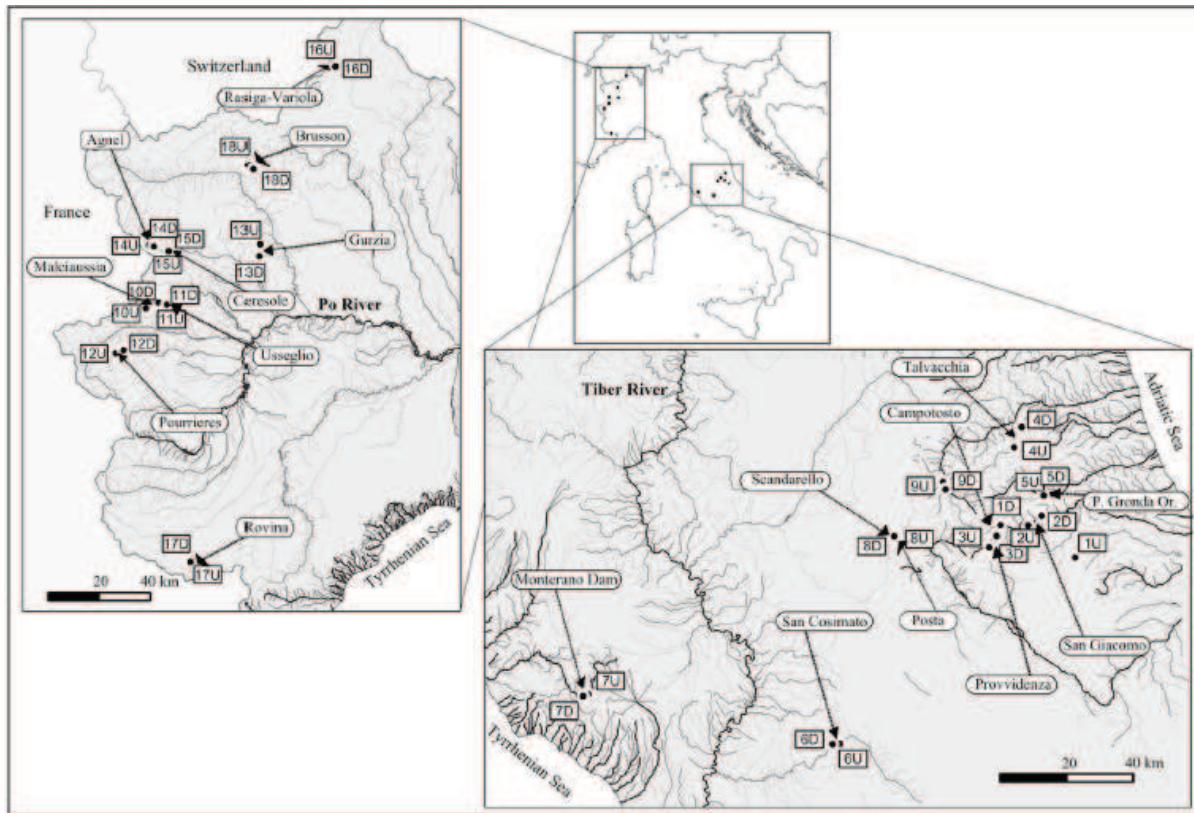
Fig. 1 Study areas and location of sampling sites. Dam names are reported. For site coding, see Table 1. According to the European typization system (European Union 2013), the Alpine and Apennine river stretches belong to the R-A1 (Alpine, high altitude, calcareous, boulders/cobbles dominance, high alkalinity) and R-M4 (Mediterranean mountain streams, with seasonal flow regime) typologies, respectively
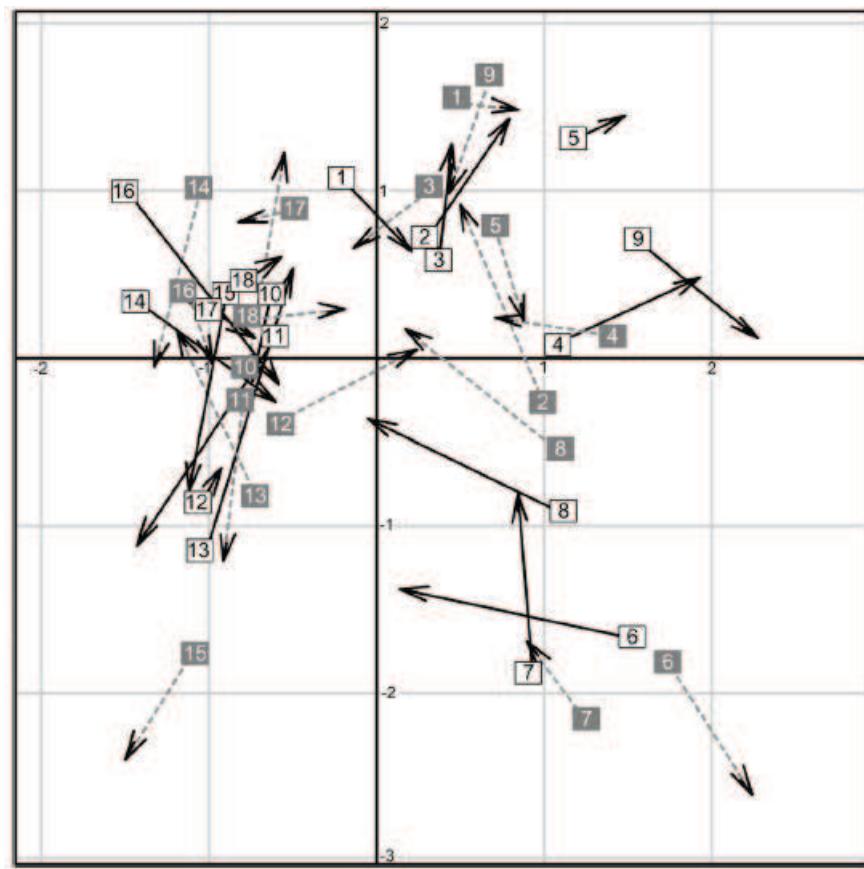
Fig. 2 Co-inertia analysis (CoIA) of environmental and macrophyte matrices. Beginning of arrows indicates site position described by the environmental dataset; end of arrows indicates site position described by macrophyte species abundances. White squares indicate upstream sides; grey squares downstream sides. For site codes, see Table 1
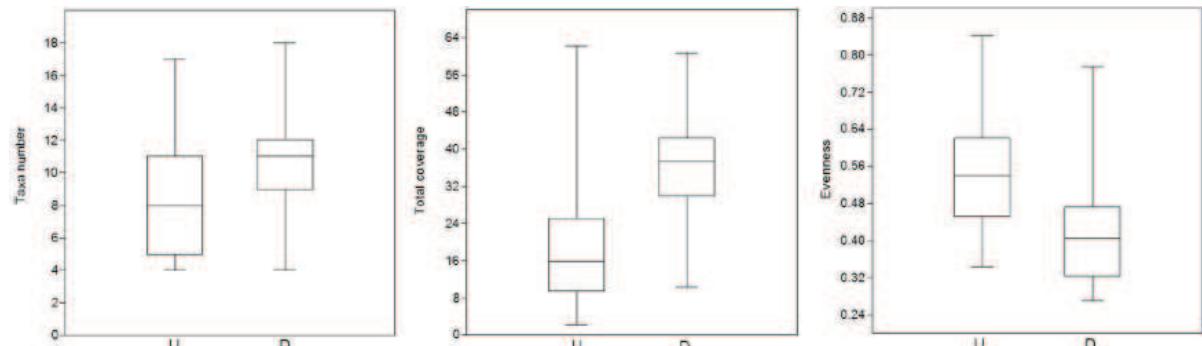
Fig. 3 Variations in species richness, total coverage and evenness in macrophyte assemblages between the upstream (*U*) and downstream (*D*) sides of river dams

Fig. 3 Variations in species richness, total coverage and evenness in macrophyte assemblages between the upstream (U) and downstream (D) sides of river dams
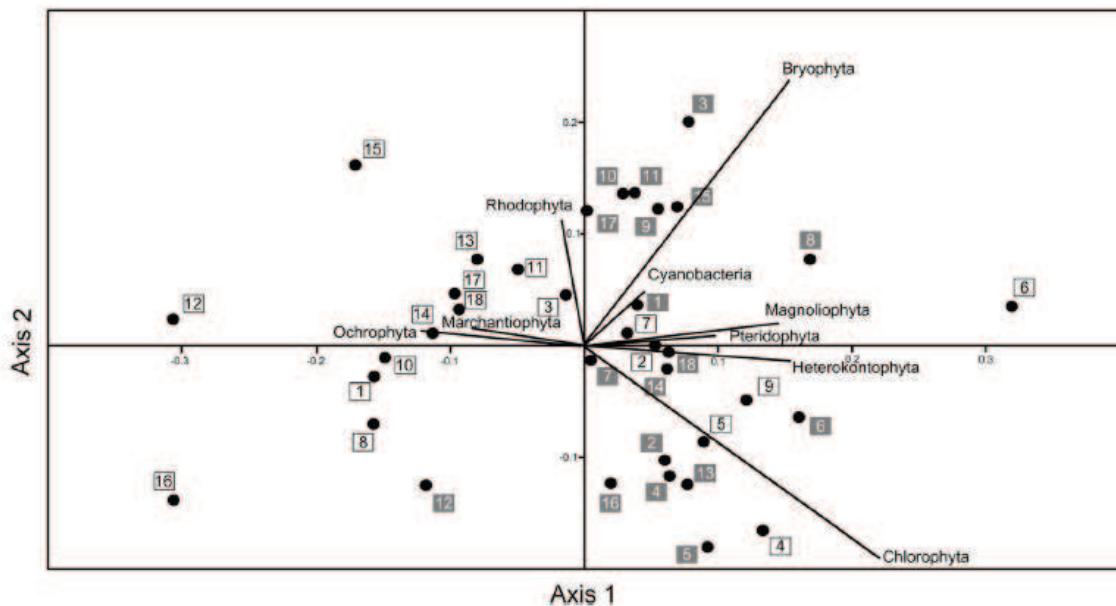
Fig. 4 NMDS biplot of macrophyte phyla matrix. Stress value = 0.19. Numbers with empty squares indicate upstream sides; numbers with grey squares indicate downstream sides.
Phyla are plotted based on Tau-Kendall correlation with the first two axes. For site codes, see Table 1

Discussion

Our analysis revealed the lack of clear trends in terms of environmental conditions between upstream and down-stream sides, which always resulted quite similar. Moreover, the correlation between environmental features and macrophyte assemblages was higher for the upstream group than for the downstream one, in which, consequently, the selected environmental features were weaker predictors of biological assemblages. These re-sults demonstrate, albeit indirectly, that the differences between upstream and downstream macrophyte assemblages are closely associated with different subtended hydrological regimes. On the other hand, our results indicate that the effects of river regulation on macrophyte assemblages are highly variable from site to site, without a clear overall trend. It is known that biotic responses to river regulation may vary considerably according to river typologies and the characteristics of the dams (Petts 1984; Rehn 2009; Benítez-Mora and Camargo 2014), and this study demonstrates that the effects on macrophytes also vary among dam sites.

Moreover, we generally found that dam effects were overwhelmed by the overall characteristics of sampling sites. In general, the presence of a dam does not disrupt macrophyte assemblages, and variations related to site characteristics prevail on the possible effects of the dam.

This, however, does not mean that dams do not have effects on the aquatic vegetation, but that these effects can be noticed more easily when comparing upstream and downstream macrophyte assemblages from the same site. In fact, we found significant differences in macrophyte species composition and abundance between the two sides of most of the studied dams.

In particular, we found that downstream sides presented a higher number of species and higher macrophyte coverage, but a lower evenness, than the respective upstream sides. An increase in coverage determined by stream regulation has been already observed in other
contexts (Rørslett et al. 1989), whereas a positive effect on species richness contrasts with the results reported by Baattrup-Pedersen and Riis (1999). However, these authors focused their study on lowland rivers, while we considered mountain streams.Actually, the increase in macrophyte coverage and richness at the downstream sides observed in this study may be a consequence of a reduction in the water force, which represents a strong natural disturb for macrophyte colonization in unregulated mountain streams (Biggs 1996). On the other hand, a reduction in the evenness of macrophyte assemblages on the downstream sides indicates that stream regulation does not favour all species equally, but may have deep and different impacts on their relative abundance.

Most taxa showed a higher affinity with the down-stream side, suggesting that the general reduction in water force favours macrophyte colonization. The indicator value analysis highlighted a small number of taxa for which differences were statistically significant and which can be considered, for the analyzed river typology, as bioindicators of community alterations due to stream regulation: Ulothrix sp., which are typical of standing or slow flowing habitats, Spirogyra sp., which are free-floating algae, and C. riparius, which is typical of flood zone of lowland rivers (Wehr and Sheath 2003).

The use of a higher taxonomical level highlighted the role of bryophytes as indicators of community alterations in regulated streams. Indeed, bryophyte colonization is strongly dependent on substrate stability (Slack and Glime 1985; Suren and Duncan 1999). Stream regulation causes a reduction in the

frequency and/or magnitude of floods and, consequently, important changes in sediment mobilization on the riverbed (Brookes 1994), which may favour bryophyte colonization. These results seem to be in contrast with previous studies that highlighted a reduction in bryophyte diversity along regulated stretches (Slack and Glime 1985; Englund et al. 1997); however, these studies included periodically submerged habitats, which were not considered in our study.

Conclusions

The effects of river regulation on aquatic macrophytes had been investigated so far in Europe mostly in single-case studies, which were mainly focused on lowland rivers. Our study represents a first comparative analysis that deals with a large number of mountain dams. We found that macrophyte responses to river regulation varied from dam to dam as a consequence of the fact that macrophyte assemblages are more deeply shaped by overall (site) environmental conditions than by the presence of a dam. Actually, a first emerging important result of our study is the evidence that using a single-case study to draw general conclusions about the effect of stream regulation on macrophytes may be strongly misleading.

On the other hand, we found some recurrent trends, demonstrating the possible role of macrophytes as bioindicators of habitat alterations caused by the presence of dams. First, we found that river regulation has serious impacts on macrophyte assemblages and that the most favoured species are those that are more strictly associated with relatively slowly flowing waters. Although we detected a significant preference for the downstream conditions only for a few species, we found some others showing a strong preference and we assume that the lack of statistical significance in our analyses is a possible reflection of the low number of sites where these species were found. Therefore, our study shows that, for the considered stream typology, some macrophyte taxa can be considered as good bioindicators while others are good candidates that deserve further attention. Moreover, we found important signals of the effects of river regulation also at phylum identification level, with bryophytes showing a distinct overall preference for downstream conditions. This result is particularly important because bryophytes are the commonest macrophyte group in mountain streams (Slack and Glime 1985; Ceschin et al. 2015). Thus, bryophytes appear to be a good candidate group for the evaluation of water regime alteration, providing valuable information at lower taxonomic resolution with consequent less identification effort, but in particular through the development of metrics based on species-specific responses.

References

Arthington, A. H., King, J. M., O'Keeffe, J. H., Bunn, S. E., Day, J. A., Pusey, et al. (1992). Development of an holistic approach for assessing environmental flow requirements of riverine ecosystems. In J. J. Pilgram & B. P. Hooper (Eds.), Water allocation for the environment (pp. 69–76). Australia: The Centre for Water Policy Research, University of New England.

Baattrup-Pedersen, A., & Riis, T. (1999). Macrophyte diversity and composition in relation to substratum characteristics in regulated and unregulated Danish streams. Freshwater Biology, 42(2), 375–385.

Barendregt, A., & Bio, A. M. F. (2003). Relevant variables to predict macrophyte communities in running waters. Ecological Modelling, 160, 205–217.

Benítez-Mora, A., & Camargo, J. A. (2014). Ecological responses of aquatic macrophytes and benthic macroinvertebrates to dams in the Henares River Basin (Central Spain). Hydrobiologia, 728, 167–178.

Bernez, I., & Ferreira, T. (2007). River macrophytes in regulated mediterranean-type rivers of southern Portugal. Belgian Journal of Botany, 140(1), 136–139.

Bernez, I., Daniel, H., Haury, J., & Ferreira, M. T. (2004). Combined effects of environmental factors and regulation on macrophyte vegetation along three rivers in western France. River Research and Applications, 20, 43–59.

Biggs, B. J. F. (1996). Hydraulic habitat of plants in streams. Regulated Rivers: Research & Management, 12, 131–144.

Brookes, A. (1994). River channel change. In P. Calow & G. E. Petts (Eds.), The rivers handbook vol 2 (pp. 55–75). London: Blackwell Scientific Publications.

Bunn, S. E., & Arthington, A. H. (2002). Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environmental Management, 30, 492–507.

Casanova, M. T., & Brock, M. A. (2000). How do depth, duration and frequency of flooding influence the establishment of wetland plant communities? Plant Ecology, 147, 237–250.

Ceschin, S., Aleffi, M., Bisceglie, S., Savo, V., & Zuccarello, V. (2012). Aquatic bryophytes as ecological indicators of the water quality status in the Tiber river basin (Italy). Ecological Indicators, 14, 74–81.

Ceschin, S., Minciardi, M. R., Spada, C. D., & Abati, S. (2015). Bryophytes of Alpine and Apennine mountain streams: floristic features and ecological notes. Cryptogamie Bryologie, 36(3), 1–17.

Daniel, H., Bernez, I., & Haury, J. (2006). Relationships between macrophytic vegetation and physical features of river habitats: the need for a morphological approach. Hydrobiologia, 270, 11–17.

De Cáceres, M., Legendre, P., & Moretti, M. (2010). Improving indicator species analysis by combining groups of sites. Oikos, 119(10), 1674–1684.

Demars, B. O. L., Potts, J. M., Trémolières, M., Thiébaut, G., Gougelin, N., & Nordmann, V. (2012). River macrophyte indices: not the Holy Grail. Freshwater Biology, 57, 1745–1759.

Dolédec, S., & Chessel, D. (1994). Co-inertia analysis: an alternative method to study species-environment relationships. Freshwater Biology, 31, 277–294.

Downes, B. J., Entwisle, T. J., & Reich, P. (2003). Effects of flow regulation on disturbance frequencies and in-channel bryophytes and macroalgae in some upland streams. River Research and Applications, 19, 27–42.

Dufrêne, M., & Legendre, P. (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs, 67(3), 345–366.

Englund, G., Jonsson, B. G., & Malmqvist, B. (1997). Effects of flow regulation on bryophytes in north Swedish rivers. Biological Conservation, 79(1), 79–86.

European Union (2013). Commission Decision 2013/480/EU establishing, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the values of the Member State monitoring system classifications as a result of the intercalibration exercise and repealing Decision 2008/915/ EC.

Fayolle, S., Cazaubon, A., Comte, K., & Franquet, E. (1998). The intermediate disturbance hypothesis: application of this concept to the response of epilithon in a regulated Mediterranean river (Lower-Durance, southeastern France). Archiv Fur Hydrobiologie, 143, 57–77.

Ferreira, M. T., & Moreira, I. S. (1999). River plants from an Iberian basin and environmental factors influencing their distribution. Hydrobiologia, 415, 101–107.

Ferreira, M. T., Rodríguez-González, P., Aguiar, F. C., & Albuquerque, A. (2005). Assessing biotic integrity in Iberian rivers: development of a multimetric plant index. Ecological Indicators, 5, 137–149.

Franklin, P., Dunbar, M. J., & Whitehead, P. (2008). Flow controls on lowland river macrophytes: a review. The Science of the Total Environment, 400, 369–378.

Friberg, N., O'Hare, M. T., & Poulsen, A. M. (Eds.) (2013). Impacts of hydromorphological degradation and disturbed sediment dynamics on ecological status. REstoring rivers FOR effective catchment Management Project. Deliverable D3.1.

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica, 4(1), 1–9.

Haslam, S. M. (1978). River plants: the macrophytic vegetation of watercourses. Cambridge: Cambridge University Press.

Haury, J., Peltre, M. C., Muller, S., Tremolieres, M., Barbe, J., Dutartre, A., et al. (1996). Des indices macrophytiques pour estimer la qualitè des cours d'eau francais: premieres propositions. Ecologie, 27, 233–244.

Hayek, L. C., & Buzas, M. A. (2010). Surveying natural populations. New York: Columbia U Press.

Ioannidis, J. P. A. (2005). Why most published research findings are false. PLoS Medicine, 2, e124.

Janauer, G. A., Schmidt-Mumm, U., & Schmidt, B. (2010). Aquatic macrophytes and water current velocity in the Danube River. Ecological Engineering, 36, 1138–1145.

Jowett, I. G., & Biggs, B. J. F. (2008). Application of the 'natural flow paradigm' in a New Zealand context. River Research and Applications, 25, 1126–1135.

Lowe, R. L. (1979). Phytobenthic ecology and regulated streams. In J. V. Ward & J. A. Standford (Eds.), The ecology of regulated rivers (pp. 25–34). New York: Plenum Press.

Madsen, J. D., Chambers, P. A., James, W. F., Koch, E. W., & Westlake, D. F. (2001). The interaction between water movement, sediment dynamics and submersed macrophytes. Hydrobiologia, 444, 71–84.

Mebane, C. A., Simon, N. S., & Maret, T. R. (2014). Linking nutrient enrichment and streamflow to macrophytes in agricultural streams. Hydrobiologia, 722, 143–158.

Mielke, P. W., Berry, K. J., & Johnson, E. S. (1976). Multi-response permutation procedures for a priori classifications. Communications in Statistics – Theory and Methods, 5, 1409–1424.

Minciardi, M. R., Spada, C. D., Rossi, G. L., Angius, R., Orrù, G., Mancini, L., et al. (2009). Metodo per la valutazione e la classificazione dei corsi d'acqua utilizzando la comunità delle Macrofite acquatiche. Rapporto Tecnico ENEA RT/ 2009/23/ENEA, Roma.

Minciardi, M. R., Spada C. D., Abati S., Ciadamidaro, S., & Fiorenza, A. (2014). Protocollo di campionamento e analisi delle macrofite dei corsi d'acqua guadabili, in: ISPRA. Metodi biologici per le acque superficiali interne. Manuali e linee guida, 111/2014.

Newman, J. R., Dawson, F. H., Holmes, N. T. H., Chadd, S., Rouen, K. J., & Sharp, L. (1997). Mean trophic rank: a user's manual. Bristol: Environment Agency of England & Wales. Petts, G. E. (1984). Impounded rivers: perspectives for ecological management. Chichester: Wiley.

Poff, N. L., & Hart, D. D. (2002). How dams vary and why it matters for the emerging science of dam removal. BioScience, 52, 659–668.

Poff, N. L., & Zimmerman, J. K. H. (2010). Ecological responses to altered flow regimes: a literature review to inform environmental flows science and management. Freshwater Biology, 55(1), 194–205.

Poff, N. L., Allan, J. D., Bain, M. B., Karr, J. R., Prestegaard, K. L., Richter, et al. (1997). The natural flow regime. BioScience, 47, 769–784.

R Core Team. (2014). R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Rehn, A. C. (2009). Benthic macroinvertebrates as indicators of biological condition below hydropower dams on west slope Sierra Nevada streams, California, USA. River Research and Applications, 25, 208–228.

Riis, T., & Biggs, B. J. F. (2003). Hydrologic and hydraulic control of macrophyte establishment and performance in streams. Limnology and Oceanography, 48, 1488–1497.

Riis, T., Suren, A. M., Clausen, B., & Sand-Jensen, K. (2008). Vegetation and flow regime in lowland streams. Freshwater Biology, 53, 1531–1543.

Robert, P., & Escoufier, Y. (1976). A unifying tool for linear multivariate statistical methods: the RV-coefficient. Applied Statistics, 25, 257–265.

Rørslett, B., Mjelde, M., & Johansen, S. W. (1989). Effects of hydro-power development on aquatic macrophytes in Norwegian rivers: present state of knowledge and some case studies. Regulated Rivers: Research and Management, 3, 19–28.

Schank, J. C., & Koehnle, T. J. (2009). Pseudoreplication is a pseudoproblem. Journal of Comparative Psychology, 123, 421–433.

Schaumburg, J., Schranz, C., Foerster, J., Gutowski, A., Hofmann, G., Meilinger, et al. (2004). Ecological classification of macrophytes and phytobenthos for rivers in Germany according to the Water Framework Directive. Limnologica, 34, 283–301.

Schneider, S., Krumpholz, T., & Melzer, A. (2000). Trophieindikation in Fließgewässern mit Hilfe des TIM (Trophie-Index Makrophyten) – Erprobung eines neu entwickelten Index im Inninger Bach. Acta Hydrochimica et Hydrobiologica, 28, 241–249.

Sculthorpe, C. D. (1966). The biology of aquatic vascular plants. London: Edward Arnold Ltd.

Slack, N. G., & Glime, J. M. (1985). Niche relationships of mountain stream bryophytes. The Bryologist, 88, 7–18.

Suren, A. M., & Duncan, M. J. (1999). Rolling stones and mosses: effect of substrate stability on bryophyte communities in streams. Journal of the North American Benthological Society, 18, 457–467.

Tharme, R. E. (2003). A global perspective on environmental flow assessment: emerging trends in the development and application of environmental flow methodologies for rivers. River Research and Applications, 19, 397–441.

Tombolini, I., Caneva, G., Cancellieri, L., Abati, S., & Ceschin, S. (2014). Damming effects on riparian and aquatic vegetation: the Nazzano case study (Tiber River, central Italy). Knowledge and Management of Aquatic Ecosystems. doi:10.1051/kmae/2013085.

Vanderpoorten, A., & Klein, P. J. (2000). Aquatic bryophyte assemblages along a gradient of regulation in the river Rhine. Hydrobiologia, 410, 11–16.

Vieira, C., Séneca, A., Sérgio, C., & Ferreira, M. T. (2012). Bryophyte taxonomic and functional groups as indicators of fine scale ecological gradients in mountain streams. Ecological Indicators, 18, 98–107.

Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., et al. (2010). Global threats to human water security and river biodiversity. Nature, 467, 555–561.

Wehr, J. D., & Sheath, R. G. (Eds.). (2003). Freshwater algae of north america: ecology and classification. Boston: Academic.

Wiegleb, G. (1984). A study of habitat conditions of the macrophytic vegetation in selected river systems in western Lower Saxony (Federal Republic of Germany). Aquatic Botany, 18, 313–352.