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Development of a River Macrophyte Index (RMI) for assessing river ecological status

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ABSTRACT

We present the process of developing a macrophyte based index (River Macrophyte Index – RMI) for assessing river ecological status, that would be applicable for rivers with moderate to high water alkalinity, flowing over low slope terrain. A reference value and boundary values were determined for five ecological classes. The relation between the developed index and two existing indices, the Reference Index (RI) and the Trophic Index of Macrophytes (TIM), and selected environmental variables was established. The RMI is based on species composition and abundance from 208 sampling sites being in reference or good hydromorphological conditions and differing in the catchment land use. The percentage of natural areas in the sub-catchment was used for classifying macrophyte taxa into 5 ecological groups. 65 plant taxa, of which 47 were identified as indicator taxa, were included in the analysis. To assess the ecological status of a river site, the presence of at least 3 indicator taxa is necessary, otherwise the assessment is considered inconclusive. RMI is expected to indicate multiple pressures on the river, including trophic level. The developed index and RI and TIM indices differed in relation to slope, distance to source and catchment size.

Introduction

Macrophytes are fundamental to the structure and functioning of river habitats (Baattrup-Pedersen and Riis, 1999), being involved in energy flow, nutrient cycling, and sedimentation processes. They affect water quality, provide food and refugia for aquatic invertebrates and fish, and are also valuable as indicators of water and sediment quality (Haslam, 1987; Carbiener et al., 1990). Their presence and diversity depend on water quality, water depth, flow velocity, and substrate characteristics (Bornette et al., 1994; Baattrup-Pedersen and Riis, 1999). Macrophyte species composition and abundance reflect the quality of an ecosystem as a whole. They are one of the biological elements required by the EU Water Framework Directive (WFD) (Council of the European Communities, 2000) for the assessment of ecological status of rivers. In Europe, several macrophyte based systems have been developed recently for assessing water quality (mostly trophic status), e.g. the British Mean Trophic Rank (MTR) (Holmes et al., 1999), the German Trophic Index of Macrophytes (TIM) (Schneider and Melzer, 2003), and the French Biological Macrophytes Index for Rivers (IBMR) (Haury et al., 2006). In all these indices the general hypothesis taken into account presumes that aquatic macrophyte distribution in lotic ecosystems responds to phosphorus (P) and/or nitrogen (N) enrichment. On the other hand, some authors (e.g. Wiegleb, 1984; Demars and Edwards, 2009) showed that it is not easy to separate the individual effect of nutrient enrichment (inorganic P, N) from other environmental variables, especially in the presence of a strong environmental gradient. Therefore, Demars and Edwards (2009) suggested that responses of macrophyte species to nutrient enrichment should be studied in homogeneous groups defined by factors such as alkalinity and slope. WFD (Council of the European Communities, 2000) requires the use of a river type-specific reference condition approach in the assessment of ecological status. In the Reference Index (RI) (Schaumburg et al., 2004; Meilinger et al., 2005) a reference condition approach is used for the assessment of the four German river types, and a river site typology is also taken into account.

The present study was aimed at developing a macrophyte based index for river ecological status assessment that would be applicable for rivers with moderate to high water alkalinity flowing over low slope terrain, including karst rivers. For a defined river type a reference value and five ecological classes boundary values were determined in accordance with the WFD ecological status classes definition. Furthermore, we aimed to establish the relation between the developed index and two existing indices, namely RI and TIM, and selected environmental variables.

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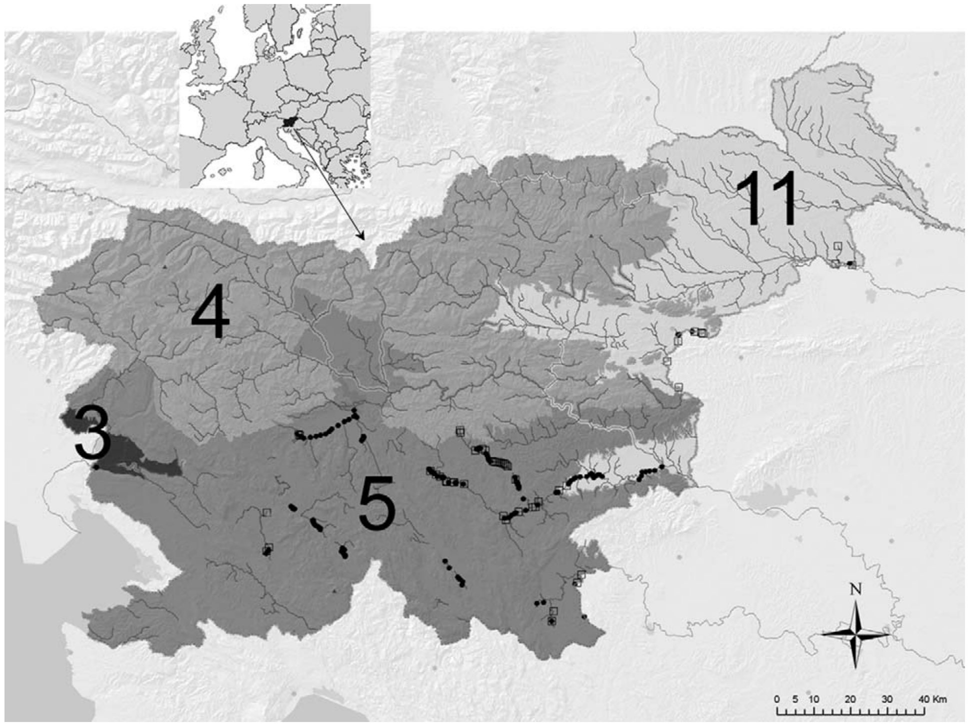

Fig. 1. Slovenian ecoregions and location of the sampling sites. ● – sampling sites with at least three indicator taxa, □ – sampling sites with less than three indicator taxa. 3 – Po lowland, 4 – Alps, 5 – Dinarids, 11 – Pannonian lowland.

Materials and methods

Study area

For the development of the index we used the data sets gained in the years 2002–2007, including plain terrain rivers occurring in 7 bioregions of three Slovenian ecoregions: "Pannonian lowland", "Dinarids" and "Po lowland" (Urbanič, 2008a,b) (Fig. 1). Altogether, 208 sampling sites were surveyed in small to large rivers with a catchment area between 10 km^2 and 15,000 km^2, with a distance to source of up to 470 km, with low slope (Table 1) and moderate to high water alkalinity (ARSO, 2008). All surveyed sites are in reference or good hydromorphological conditions, but differ in the catchment land use and some other pressures like wastewater treatment plants, industrial outflows and hydroelectric power plants.

Macrophyte data set

Macrophytes were surveyed once per site in the peak vegetation period. Surveys were carried out in the years 2002–2005 over the whole stream course. Streams were divided into stretches of different lengths according to changes in macrophyte species composition and abundance, or to environmental changes (Kohler, 1978; Kohler and Janauer, 1995). Additionally we collected data in years 2005 and 2007 when approximately 100 m long stretches were surveyed. All surveys were performed from the bank of the stream or from a boat, using a rake with hooks to reach plants. We record submerged, floating and emergent vascular plants, bryophytes and charophytes. Macrophyte species abundance was estimated as a relative plant biomass using a five-degree scale: 1 = very rare; 2 = rare; 3 = common; 4 = frequent; 5 = abundant, predominant (Kohler, 1978; Kohler and Janauer, 1995). Plants that were sampled in the phenological phase, that prevents identification to the species level, were recorded as a genus.

Table 1
Environmental features of sampling sites.

Variable	Mean	Median	Min	Max	Range
Slope (‰)	1.6	0.9	0.1	14.0	13.9
Distance to source (km)	27.1	12.1	0.03	468.7	468.7
Catchment size class	1.99	2	1	4	3

Catchment size class: 1 – 10–100 km^2, 2 – 100–1000 km^2, 3 – 1000–10,000 km^2, 4 – >10,000 km^2.

Reference conditions and pressures on the rivers in Slovenia

Criteria for the selection of potential reference sites in the rivers include the hydromorphological and physico-chemical condition of the site, land use, riparian vegetation and floodplain properties, saprobic index values, and the presence of certain pressures (Urbanič and Smolar-Žvanut, 2007) and were in accordance with Wallin et al. (2003). Two proposals were made on the basis of these criteria: (1) potential reference sites considering all the criteria and (2) potential reference sites without considering the criteria of biotic pressures, i.e. alien species and fishery management (Urbanič, 2007). The latter were used in our study that resulted in 23 reference sites used for determining the reference values of the developed index.

Pressures at each sampling site, established on the basis of the national data sets, were used for determining the gradient of pressures. The percentage of the natural, agricultural and urban areas, determined according to the Corine Land Cover (CLC, 2000), and the presence of power plants, wastewater treatment plants, fish farms, industrial outflows and agglomerations were taken into account. Data were recorded for upstream catchment (whole catchment upstream of the sampling site) and/or sub-catchment (upstream of the sampling site) (Table 2). Sub-catchments along approximately 5 km river segments were used, but the actual length depended on the river network characteristics (Fig. 2).

Table 2
Summary of variables used in the canonical correspondence analyses.

Variable	Code	Mean	Median	Min	Max	Range
Agricultural land use (%)_Catchment	Agri_Cat	31.8	31.1	10.5	76.3	65.7
Agricultural land use (%)_Sub-catchment	Agri_Scat	42.4	44.5	10.5	97.3	86.7
Natural land use (%)_Catchment	Natur_Cat	65.0	67.0	23.7	89.5	65.7
Natural land use (%)_Sub-catchment	Natur_Scat	51.9	53.5	0.0	89.5	89.5
Urban land use (%)_Catchment	Urban_Cat	1.5	1.3	0.0	3.6	3.6
Urban land use (%)_Sub-catchment	Urban_Scat	3.5	2.0	0.0	74.4	74.4
Number of wastewater treatment plants_Sub-catchment	WWTP_no	1.3	1.0	0.0	4	4
Number of hydroelectric power plants_Sub-catchment	HePP_no	1.3	0.0	0.0	6	6
Number of fishing ponds_Sub-catchment	Fponds_no	0.9	0.0	0.0	4	4
Number of agglomerations_Sub-catchment	Agglomer_no	26.6	20.0	0.0	77	77
Number of industrial outflows_Sub-catchment	Industr_no	7.3	9.0	0.0	21	21

Development of an index

The index was developed in the following steps:

1. The pressure variables were tested and the one that explained most of the variability of taxa distribution at the sampling sites was selected by Canonical Correspondence Analysis (CCA). As most of the variability was explained by the percentage of the natural areas in the sub-catchment (Fig. 3), this variable was chosen as the pressure gradient.
2. The values of the variable – the percentage of natural areas in the sub-catchment – were ranged into 10 classes (by steps of 0.1).
3. The frequency of the presence (n_{ji}) of the taxon *i* in a given class of the percentage of natural area *j* was determined.
4. The probability of the presence (p_{ji}) of the taxon *i* in the class of the percentage of the natural area *j* was determined according to the following equation:

$$p_{ji} = \frac{n_{ji}}{N_j}$$

where n_{ji} = frequency of the presence of the taxon *i* in the class of the natural area *j*, N_j = number of samples found in the class of the natural area *j*.

5. The valence (v_{ji}) of taxon *i* was calculated according to the following equation:

$$v_{ji} = \frac{10p_{ji}}{\Sigma p_{ji}}$$

where p_{ji} = probability of the presence of the taxon *i* in the class of the natural area *j*.

6. TWINSPAN analysis was carried out, based on the classes of natural areas and taxa present at at least 3 sampling sites. The analysis distinguished three groups of classes of natural areas: (1) classes comprising up to 30%, (2) classes comprising up to 70% and (3) classes comprising over 70% of natural areas. The latter also includes reference sites.
7. Six ecological groups were defined and taxa ranged into one of them.
8. The equation of the new index – the River Macrophyte Index (RMI) – was determined.
9. The relation between the chosen pressure variables and RMI values was tested.
10. The conditions for the use of the RMI were determined, based on the relation between RMI and the percentage of the natural areas.
11. The boundary values of RMI corresponding to the 5 classes of the ecological status were determined. A reference value (Ecological Quality Ratio (EQR) = 1) was determined as the median of the calculated values of the RMI at the reference sites. The lower

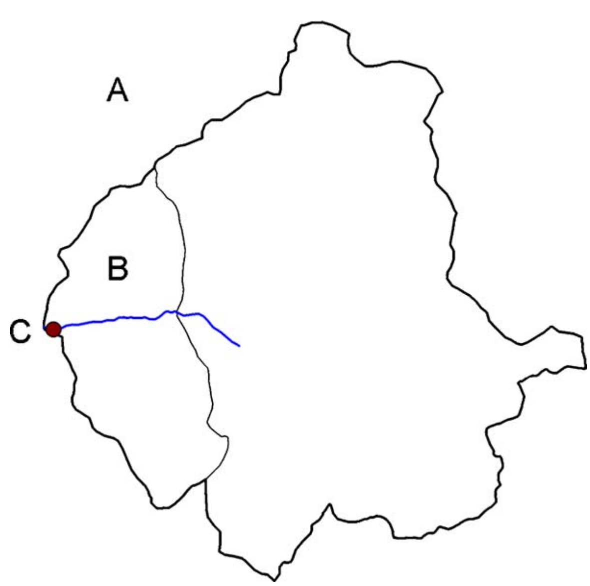

Fig. 2. Schematic presentation of A – catchment, B – sub-catchment and C – sampling site.

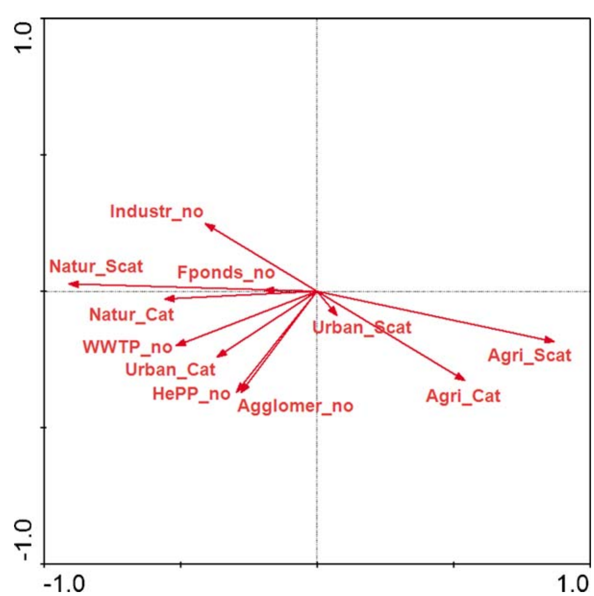

Fig. 3. CCA ordination diagram showing pressure variables (arrows). For code legend see Table 2.

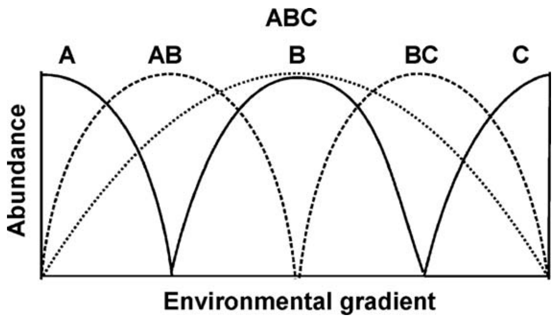

Fig. 4. Distribution of six macrophyte ecological groups along the environmental gradient used in the River Macrophyte Index.

boundary was determined as the lowest possible value of the index. To determine other boundary values, we used the procedure in three steps: (1) for each sampling site we calculated the proportion of the values of the "good" taxa (group A and AB) and the proportion of the values of the "bad" taxa (group C and BC) of RMI, (2) we established the relation between the proportions of the values of positive and negative taxa and the value of the RMI, and (3) the boundary values of the ecological status classes were determined where significant change in the proportions of the values of positive or negative taxa groups occurred.

Statistical analysis

Relations between the developed index, indices RI and TIM, and environmental variables were established using SPSS 16.0 software. Pearson correlation coefficients were calculated between indices, slope and distance to source, and Spearman correlation coefficients between indices and catchment size class.

TIM was calculated according to the following equation (Schneider and Melzer, 2003):

$$\mathrm{TIM} = \frac{\sum_{i=1}^{n} \mathrm{IV}_a W_a Q_a}{\sum_{i=1}^{n} W_a Q_a}$$

where IV_a = indicator value of species a, W_a = weighting factor of species a, Q_a = quantity of species a in the river section.

RI was calculated according to the following equation (Schaumburg et al., 2006):

$$\mathrm{RI} = \frac{\sum_{i=1}^{n_\mathrm{A}} Q_{\mathrm{A}i} - \sum_{i=1}^{n_\mathrm{C}} Q_{\mathrm{C}i}}{\sum_{i=1}^{n_\mathrm{g}} Q_{\mathrm{g}i}}$$

where $Q_{\mathrm{A}i}$ = quantity of the ith taxon of group A, $Q_{\mathrm{C}i}$ = quantity of the ith taxon of group C, Q_{gi} = quantity of the ith taxon of all groups (A, B, C), n_A = total number of taxa in group A, n_C = total number of taxa in group C, n_g = total number of taxa in all groups (A, B, C). Species group A taxa have high abundance under reference conditions and low or no abundance under non-reference conditions; species group B taxa show no preference for reference or non-reference conditions; species group C taxa are rarely found under reference conditions.

In the procedure of calculating TIM and RI, all requirements and criteria needed to obtain a reliable assessment were considered. For the calculation of RI Slovenian river types were classified into the corresponding German river types.

Results

Pressure gradient

Eleven pressure variables were tested (Table 2). Each of them significantly explained the variability of the macrophyte data. The percentage of natural land use in the sub-catchment has the highest eigenvalue, followed by percentage of agricultural land use in the sub-catchment. For each of the other variables a lower eigenvalue was observed (Table 3). Since the percentage of natural land use in the sub-catchment explained the highest variability, this variable was selected as the pressure gradient. It includes all non-natural land use (agricultural and urban land use pressure) and thus represents changes in the macrophyte assemblage due to different reasons but not to hydromorphological alterations. In order to measure deviation from the reference conditions of the macrophyte community, a selected variable better represents a pressure gradient than if only the percentage of the agricultural areas or any other variable was selected. Nevertheless, along the first two axes, most of the pressure gradient is oriented along the first axis and the percentage of natural land use in the sub-catchment has the highest correlation coefficient with the first axis (Fig. 3).

River Macrophyte Index (RMI)

65 taxa were present at at least three sampling sites and were classified into one of the six ecological groups (Table 4, Fig. 4). Taxa present only at the reference sites (percentage of natural areas > 70%) were classified into group A, taxa present only at the moderately loaded sites (percentage of natural areas 30-70%) were classified into group B, and those present only at the heavily loaded sites (percentage of natural areas < 30%) were classified into group C. Taxa present at both reference and moderately loaded sites were classified into group AB, and those present at both moderately and heavily loaded sites were classified into group BC. The taxa found at the heavily loaded sites and at the reference sites as well were classified into group ABC. The taxa in that group do not have an indicator value and are not included in the RMI calculation.

The RMI was calculated using the following equation:

$$\mathrm{RMI} = \frac{\sum_{i=1}^{n_\mathrm{A}} Q_{\mathrm{A}i} + \frac{1}{2}\sum_{i=1}^{n_\mathrm{AB}} Q_{\mathrm{AB}i} - \frac{1}{2}\sum_{i=1}^{n_\mathrm{BC}} Q_{\mathrm{BC}i} - \sum_{i=1}^{n_\mathrm{C}} Q_{\mathrm{C}i}}{\sum_{i=1}^{n_\mathrm{S}} Q_{\mathrm{S}i}}$$

where $Q_{\mathrm{A}i}$ = abundance of the taxa i from the group A, $Q_{\mathrm{AB}i}$ = abundance of the taxa i from the group AB, $Q_{\mathrm{BC}i}$ = abundance of the taxa i from the group BC, $Q_{\mathrm{C}i}$ = abundance of the taxa i from the group C, $Q_{\mathrm{S}i}$ = abundance of taxa i from all groups (group A, AB, B, BC, C; taxa from the group ABC are not considered), n_A = total number of taxa in group A, n_AB = total number of taxa in group AB, n_BC = total number of taxa in group BC, n_C = total number of taxa in group C, n_S = total number of taxa in all groups (group A, AB, B, BC, C; taxa from the group ABC are not considered).

The relation between the pressure variable and RMI explained 58% of the variability (Fig. 5a). At some sampling sites only one indicator taxon was present. We therefore tested the influence of the number of indicator taxa on the value of the explained variability. More than 70% of the variability was explained when at least 3 indicator taxa were present (Fig. 5b). Thereafter the presence of at least 3 indicator taxa was taken as the criterion for the calculation of RMI.

Reference conditions and boundary values for 5 classes of ecological status

The reference value was determined as the median value of the RMI at the reference sites. This value is 0.72 (Table 5). Boundary

Table 3
Eigenvalue of each variable before and after forward selection (FS), using canonical correspondence analysis.

Variable	Before FS	p	After FS	p	Selection order
Natural land use (%)_Sub-catchment	0.43	0.001	0.43	0.001	1
Agricultural land use (%)_Sub-catchment	0.41	0.001	0.11	0.006	9
Agricultural land use (%)_Catchment	0.29	0.001	0.22	0.001	3
Natural land use (%)_Catchment	0.29	0.001	0.25	0.001	2
Number of wastewater treatment plants_Sub-catchment	0.24	0.001	0.18	0.001	6
Number of agglomerations_Sub-catchment	0.23	0.001	0.14	0.001	7
Number of industrial outflows_Sub-catchment	0.23	0.001	0.12	0.001	8
Number of hydroelectric power plants_Sub-catchment	0.22	0.001	0.08	0.016	11
Number of fishing ponds_Sub-catchment	0.22	0.001	0.19	0.001	4
Urban land use (%)_Catchment	0.21	0.001	0.18	0.001	5
Urban land use (%)_Sub-catchment	0.09	0.001	0.14	0.002	10
All together	2.04	0.001	2.04	0.001	

values for the five classes of the ecological status were determined on the basis of the changing of the proportion of frequency of so-called "good" and "bad" RMI taxa. Proportions were calculated on the basis of the frequency of the taxa. Taxa from groups A and AB were taken as "good" and taxa from groups C and BC as "bad". The boundary value between high and good ecological status was determined to be where "bad" taxa started to appear. The boundary value between good and moderate status was determined where there was an intersection of curves of the proportions of "good" and "bad" taxa. The boundary value between moderate and poor ecological status was determined where the proportion of frequency of "good" taxa dropped below 10%, and the boundary value between poor and bad status where "good" taxa no longer appeared (Fig. 6). Boundary values for the 5 classes of ecological status are listed in Table 5. To adjust boundary values to the boundary values for multimetric indices, already developed in Slovenia, we transformed them using following equations:

RMI_EQR	RMI_EQR_transformed
>0.79	RMI_EQR
0.79–0.58	$0.6 + 0.2 \times (\text{RMI\_EQR} - 0.58)/(0.22)$
0.57–0.38	$0.4 + 0.2 \times (\text{RMI\_EQR} - 0.38)/(0.20)$
0.37–0.19	$0.2 + 0.2 \times (\text{RMI\_EQR} - 0.19)/(0.19)$
<0.19	$0.2 \times (\text{RMI\_EQR})/(0.19)$

Indices and environmental variables

The data on calculated indices (RMI, TIM and RI) are given in Table 6. RMI, TIM and RI were not significantly correlated with slope ($p > 0.05$). RMI and TIM were significantly correlated with distance to source ($r = -0.756$ and $r = 0.484$, respectively, $p < 0.01$). The only significant correlation with catchment size class was obtained for

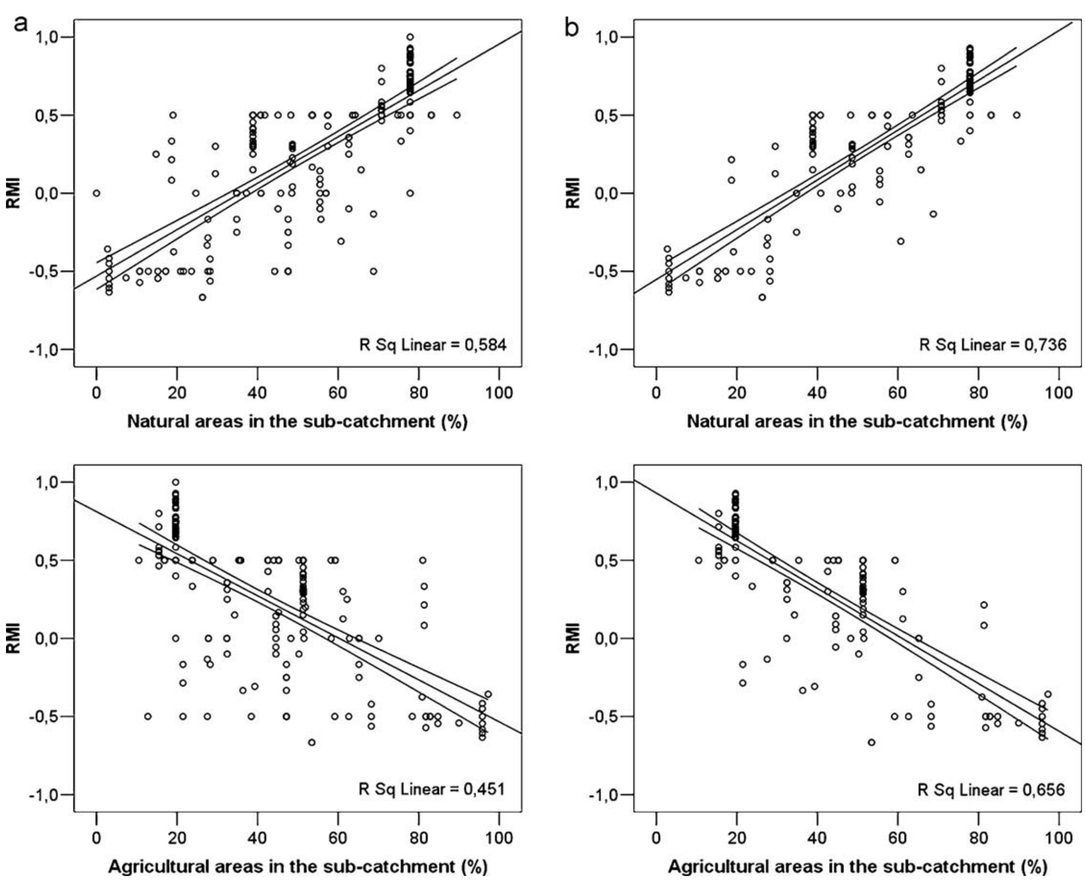

Fig. 5. Relation between the percentage of natural areas in the sub-catchment area and the River Macrophyte Index (RMI), and between the percentage of agricultural areas in the sub-catchment area and the River Macrophyte Index (RMI). (a) All sampling sites. (b) Sampling sites with at least three indicator taxa.

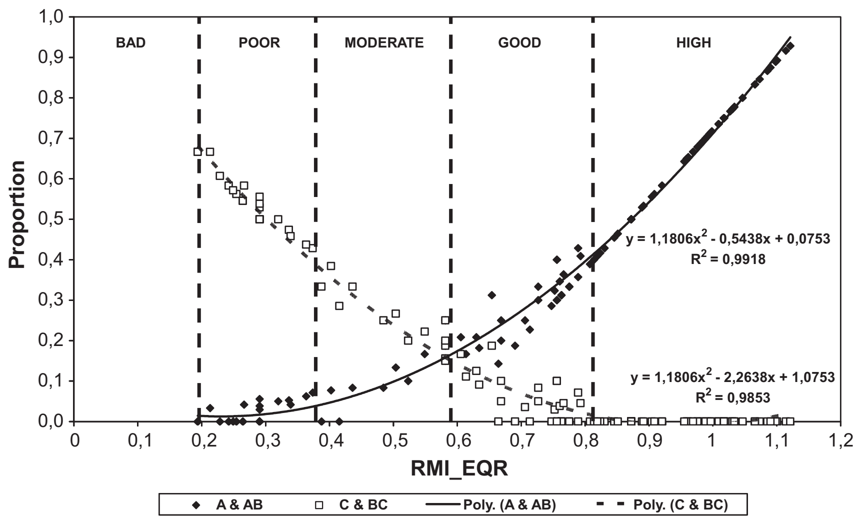

Fig. 6. Proportions of the sum of "high" status taxa (A and AB) and of the sum of "bad" status taxa (C and BC) in relation to the Ecological Quality Ratio of the RMI (RMI_EQR) and boundary values for the 5 classes of the ecological status.

RMI ($r = -0.587$, $p < 0.01$), but the values for single size class were very scattered (Table 7, Fig. 7). RMI exhibited a significant negative correlation with TIM ($r = -0.587$, $p < 0.01$), while was not significantly correlated with RI ($r = 0.022$, $p > 0.05$). A negative correlation was obtained between TIM and RI ($r = -0.247$, $p < 0.05$).

Discussion

Several macrophyte based systems have been developed recently for rivers assessment. In the British MTR (Holmes et al., 1999), the German TIM (Schneider and Melzer, 2003) and the French IBMR (Haury et al., 2006), macrophytes are assigned scores according to their tolerance to eutrophication, so these methods provide information about the trophic status of waters. The German RI (Schaumburg et al., 2004) reflects river degradation, estimating the deviation of observed macrophyte communities from stream type specific reference communities. All methods are based on species composition and abundance, the latter being specified as relative macrophyte biomass (Kohler and Janauer, 1995) in German indices or as plant coverage in MTR and IBMR. Although most

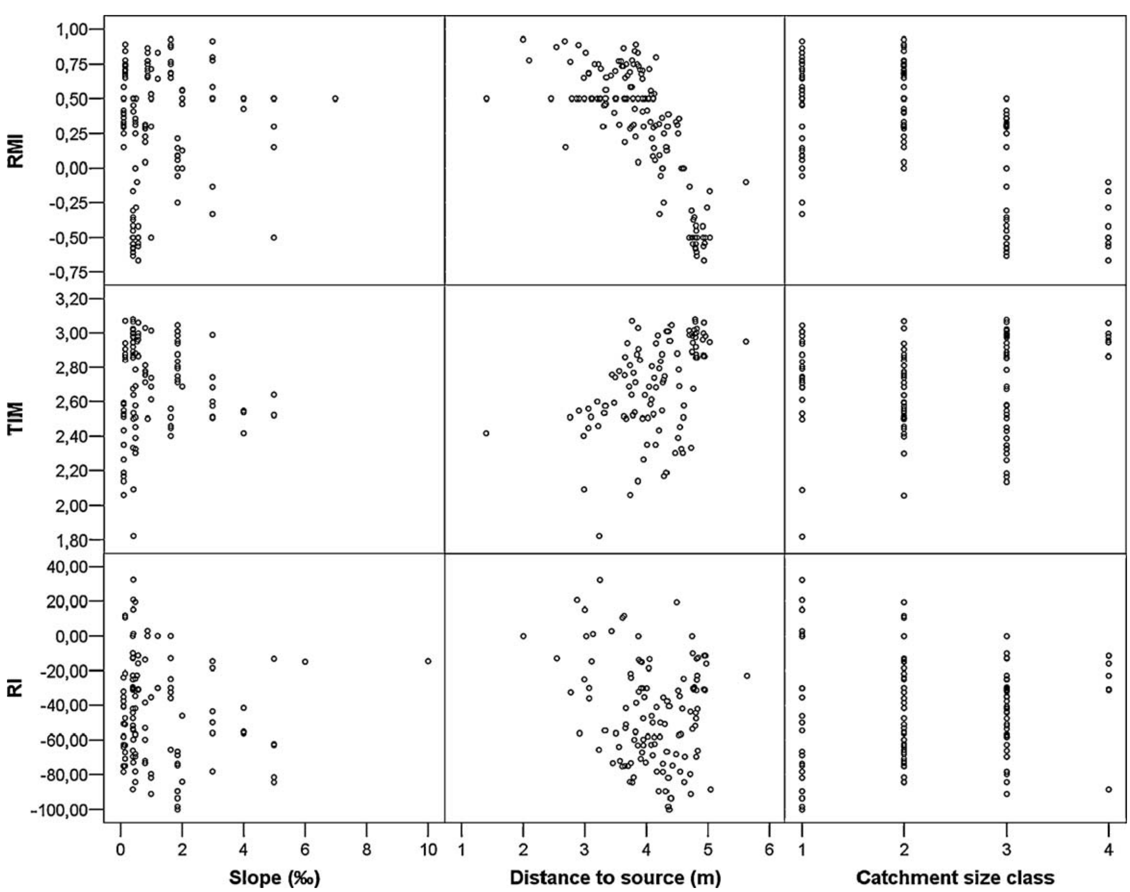

Fig. 7. Relation between RMI, TIM and RI indices and certain environmental variables. Distance to source values are log-transformed. For catchment size class see Table 1.

Table 4
Classification of the taxa into the ecological groups. Group A – taxa present only at the reference sites, group AB – taxa present at both reference and moderately loaded sites, group B – taxa present only at the moderately loaded sites, group BC – taxa present at both moderately and heavily loaded sites, group C – taxa present only at the heavily loaded sites, group ABC – taxa present at the heavily loaded sites and at the reference sites as well.

Name of the taxa	Ecological group					
	A	AB	B	BC	C	ABC
Alisma spp.						+
Amblystegium riparium (Hedw.) Schimp.				+		
Berula erecta (Huds.) Coville		+				
Bryophyta						+
Callitriche spp.		+				
Caltha palustris L.	+					
Ceratophyllum demersum L.				+		
Chara spp.			+			
Cinclidotus aquaticus (Hedw.) B. & S.	+					
Cinclidotus fontinaloides (Hedw.) P. Beauv.		+				
Eleocharis palustris (L.) Roem. et. Schult.	+					
Elodea canadensis L. C. Rich.						+
Equisetum palustre L.		+				
Fontinalis antipyretica Hedw.						+
Galium palustre L.	+					
Glyceria fluitans (L.) R. Br.	+					
Hippuris vulgaris L.		+				
Iris pseudacorus L.						+
Juncus articulatus L.	+					
Juncus effusus L.		+				
Lemna minor L.						+
Lemna trisulca L.			+			
Lysimachia nummularia L.				+		
Mentha aquatica L.						+
Myosotis scorpioides L.		+				
Myriophyllum spicatum L.						+
Myriophyllum verticillatum L.			+			
Najas marina L.					+	
Nasturtium officinale R. Br. in Aiton		+				
Nitella spp.	+					
Nuphar luteum (L.) Sibth. & Sm.		+				
Oenanthe fistulosa L.	+					
Phalaris arundinacea L.						+
Phragmites australis (Cav.) Trin. ex Steud.		+				
Plantago altissima L.		+				
Polygonum amphibium L.	+					
Polygonum mite Schrank						+
Potamogeton crispus L.		+				
Potamogeton filiformis Pers.				+		
Potamogeton lucens L.		+				
Potamogeton natans L.						+
Potamogeton nodosus Poir				+		
Potamogeton pectinatus L.			+			
Potamogeton perfoliatus L.						+
Potamogeton x salicifolius Wolfg.	+					
Ranunculus circinatus Sibth.			+			
Ranunculus fluitans Lam.			+			
Ranunculus lingua L.	+					
Ranunculus trichophyllus Chaix						+
Rhynchostegium riparioides (Hedw.) Card.						+
Rorippa amphibia (L.) Besser	+					
Rumex hydrolapathum Hudson			+			
Sagittaria sagittifolia L.		+				
Schoenoplectus lacustris (L.) Palla						+
Scrophularia umbrosa Dumort.			+			
Senecio paludosus L.	+					
Sium latifolium L.	+					
Sparganium emersum Rehmann				+		
Sparganium erectum L.						+
Sparganium spp.						+
Spirodela polyrhiza (L.) Schleid.					+	
Teucrium scordium L.	+					
Typha latifolia L.			+			
Veronica anagallis-aquatica L.						+
Veronica beccabunga L.			+			
Number of taxa	15	14	10	6	2	18

Table 5
Boundary values, normalised (EQR) and transformed boundary values (EQR_transformed) of the index RMI for the 5 classes of the ecological status.

Boundary	RMI	RMI_EQR	RMI_EQR_transformed
Reference value	0.72	1	1
Boundary high/good status	0.38	0.8	0.8
Boundary good/moderate status	0	0.58	0.6
Boundary moderate/poor status	−0.33	0.38	0.4
Boundary poor/bad status	−0.66	0.19	0.2
Lower anchor	−1	0	0

Table 6
RMI, TIM and RI index values.

Index	Mean	Median	Min	Max	Range
RMI	0.28	0.42	−0.67	0.93	1.60
TIM	2.70	2.74	1.82	3.08	1.26
RI	−44.11	−44.29	−100.00	32.43	132.43

Table 7
RMI index values for different catchment size classes. For catchment size class see Table 1.

Catchment size class	Min	Max	Range
1	−0.33	0.92	1.25
2	0	0.93	0.93
3	−0.63	0.50	1.13
4	−0.67	−0.10	0.57

existing macrophyte systems are useful for assessing trophic status, none of them can be applied on a pan-European scale without modification (Szoszkiewicz et al., 2006). As shown for MTR, inclusion of additional species and re-scoring the indicator values of existing species can improve their accuracy and usefulness (Szoszkiewicz et al., 2002, 2006). However, the problem remains of the notably different classification results arising from different methods, for example RI showing better ecological status than MTR or IBMR (Birk et al., 2006). Nevertheless, these indices might address the impact of different stressors and therefore different classification results can be expected.

Slovenia is an area where four European ecoregions meet (Urbanič, 2008a). However, we developed an assessment system for rivers, defined by moderate to high water alkalinity and low slope, which can be found in three of the four ecoregions in Slovenia. Moreover, in the rivers with steep slopes only a few, if any, macrophytes can be found and were thus not applicable for ecological status assessment in Slovenia. The percentage of natural areas in the sub-catchment area was identified as the parameter that explained most of the variability in species distribution. Several studies have shown that changes in catchment land cover are associated with changes in numbers of instream parameters such as nutrient concentration, substratum quality and flow regime, and are good predictors of community structure, not only of macrophytes but also of fish, macroinvertebrates and benthic diatoms (Harding et al., 1998; Strayer et al., 2003; Johnson et al., 2007). The percentage of natural areas in the sub-catchment was used to classify macrophyte taxa into ecological groups. 65 plant taxa were included in the analysis, out of which 47 were identified as indicator taxa. Almost a third of them belonged to ecological group A species, indicative of reference conditions defined as stream sites with more than 70% natural areas in the sub-catchment. This group comprises taxa that were present almost exclusively in streams characterised by intermittence and extreme water level fluctuation that prevents intensive human activity. In spite of that in the intermittent watercourses eutrophic conditions occur temporarily due to a decrease of water level and plant decay. Such a water regime is not suitable for the growth of submerged plants and leads to the dominance of amphibious species (Jacobsen and Terneus, 2001; Mackay et al., 2003), as is evident also from our investigation. Intermittent streams were dominated by amphibious species known for morphological, physiological and reproduction features that enable survival in a gradient from water to terrestrial environment (Germ and Gaberščik, 2003; Warwick and Brock, 2003; Šraj Kržič and Gaberščik, 2005). Therefore it is not surprising that amphibious species *Rorippa amphibia* is included in A group, despite its being considered as a species characteristic of eutrophic habitats (Haslam, 1987; Szoszkiewicz et al., 2006). It occurred in streams in the rural NE part of Slovenia, but was much more frequent in intermittent streams flowing through landscapes with a low percentage of agricultural areas. Some species characteristic of eutrophic habitats, i.e. *Nuphar luteum*, *Potamogeton crispus*, *Potamogeton lucens* and *Sagittaria sagittifolia* (Haslam, 1987; Szoszkiewicz et al., 2006), were found to be indicative for the reference and the moderately loaded sites. Only two species, the eutrophic *Najas marina* and the mesoeutrophic *Spirodela polyrhiza* (Haslam et al., 1982), were found to be indicative for heavily loaded sites (percentage of natural areas < 30%). Both were frequent just in the lower part of one river (Germ et al., 2008) and occurred rarely in some other streams. According to Shelford's law of tolerance, the presence and success of an organism depend on a complex of conditions. Organisms in nature rarely live in their optimum range of a given environmental factor, but some other factor might have greater importance (Odum, 1971).

RMI is based on species composition and abundance, both being sensitive to environmental change. It is known that eutrophication and deterioration of the physical stream environment can lead to changes in macrophyte species distribution, decline in species richness, and greater abundance of more resistant species (Preston, 1995; Sand-Jensen et al., 2000; Riis and Sand-Jensen, 2001; Germ et al., 2003; Egertson et al., 2004). For the assessment of a river site, the occurrence of at least three indicator taxa is necessary, otherwise the assessment is considered inconclusive. Further research is needed to validate the classification of macrophyte taxa into ecological groups and to test the usefulness of the RMI for all river types.

We examined the relation between the developed index, existing indices RI and TIM, and the environmental variables slope, distance to source and catchment size. None of the indices correlated significantly with slope, which was expected because the slope of examined stretches was rather uniform, ranging from 0.1 to 14‰. RMI and TIM were significantly correlated with the distance to source. This was a consequence of the fact that the ratio of natural to agricultural areas was higher in upper parts of the streams than in their lower parts. Studies on macrophytes in different regions of Slovenia have shown that the natural characteristics of the sub-catchment, that defined the extent of anthropogenic impact, resulted in characteristic macrophyte assemblages not primarily related to nutrient availability (Šraj-Kržič et al., 2007; Kuhar et al., 2007). A significant correlation of RMI was obtained with catchment size, but the RMI values exhibited rather high variability in all catchment size classes, except the 4th one representing large rivers.

RMI exhibited a significant negative correlation with TIM. This was probably a consequence of the strong connection between trophic status of the river and amount of agricultural areas in the catchment. RMI exhibited no significant correlation with RI. Previous studies have revealed the correlation between TIM and RI (Fabris et al., 2009), but in our case, the correlation was rather weak. The reason was that RI responded to different kinds of ecological stress, caused by river degradation.

Conclusions

Most of the existing macrophyte indices for assessing the ecological status of rivers cannot be used satisfactorily on the regional scale without modification. The properties of Slovenian watercourses led us to develop an index applicable for moderate to high alkalinity rivers with low slope. RMI is expected to indicate multiple pressures on a river, including trophic level. At a given location at least 3 indicator taxa are necessary, otherwise the assessment is considered inconclusive. 65 taxa were present at at least three sampling sites and were classified into one of the six ecological groups. Surprisingly, some species usually thriving in eutrophic habitats were found to be indicative for the reference and the moderately loaded sites. The possible reason was that water regime disturbances were more influential than trophic status, even though in the intermittent watercourses eutrophic conditions might occur temporarily due to the decrease of water level and plant decay.

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References

ARSO, 2008. Monitoring kakovosti površinskih vodotokov v Sloveniji v letu 2006. ARSO, Ljubljana.

Baattrup-Pedersen, A., Riis, T., 1999. Macrophyte diversity and composition in relation to substratum characteristics in regulated and unregulated Danish streams. Freshw. Biol. 42, 375–385.

Birk, S., Korte, T., Hering, D., 2006. Intercalibration of assessment methods for macrophytes in lowland streams: direct comparison and analysis of common metrics. Hydrobiologia 566, 417–430.

Bornette, G., Amoros, C., Chessel, D., 1994. Effect of allogenic processes on successional rates in former river channels. J. Veg. Sci. 5, 237–246.

Carbiener, R., Trémolières, M., Mercier, J.L., Ortscheit, A., 1990. Aquatic macrophyte communities as bioindicators of eutrophication in calcareous oligosaprobe stream waters (Upper Rhine plain, Alsace). Vegetatio 86, 71–88.

Corine Land Cover, 2000. Mapping a Decade of Change. European Environment Agency, Copenhagen.

Council of the European Communities, 2000. Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy. Off. J. Eur. Commun. L327, 1–73.

Demars, B.O.L., Edwards, A.C., 2009. Distribution of aquatic macrophytes in contrasting river systems: a critique of compositional-based assessment of water quality. Sci. Total Environ. 407, 975–990.

Egertson, C.J., Kopaska, J.A., Downing, J.A., 2004. A century of change in macrophyte abundance and composition in response to agricultural eutrophication. Hydrobiologia 524, 145–156.

Fabris, M., Schneider, S., Melzer, A., 2009. Macrophyte-based bioindication in rivers – a comparative evaluation of the reference index (RI) and the trophic index of macrophytes (TIM). Limnologica 39, 40–55.

Germ, M., Dolinšek, M., Gaberščik, A., 2003. Macrophytes of the River Ižica – comparison of species composition and abundance in the years 1996 and 2000. Arch. Hydrobiol. 147 (Suppl.), 181–193.

Germ, M., Gaberščik, A., 2003. Comparison of aerial and submerged leaves in two amphibious species, *Myosotis scorpioides* and *Ranunculus trichophyllus*. Photosynthetica 41, 91–96.

Germ, M., Urbanc-Berčič, O., Janauer, G.A., Filzmoser, P., Exler, N., Gaberščik, A., 2008. Macrophyte distribution pattern in the Krka River – the role of habitat quality. Fundam. Appl. Limnol./Arch. Hydrobiol. 162 (Suppl.), 145–155.

Harding, J.S., Benfield, E.F., Bolstad, P.V., Helfman, G.S., Jones III, E.B.D., 1998. Stream biodiversity: the ghost of land use past. Proc. Natl. Acad. Sci. USA 95, 14843–14847.

Haslam, S.M., 1987. River Plants of Western Europe. The Macrophytic Vegetation of Watercourses of the European Economic Community. Cambridge University Press, Cambridge.

Haslam, S.M., Sinker, C.A., Wolseley, P.A., 1982. British water plants. Field Stud. 4, 243–351.

Haury, J., Peltre, M.-C., Trémolières, M., Barbe, J., Thiébaut, G., Bernez, I., Daniel, H., Chatenet, P., Haan-Archipof, G., Muller, S., Dutartre, A., Laplace-Treyture, C., Cazaubon, A., Lambert-Servien, E., 2006. A new method to assess water tro-

phy and organic pollution – the Macrophyte Biological Index for Rivers (IBMR): its application to different types of river and pollution. Hydrobiologia 570, 153–158.
Holmes, N.T.H., Newman, J.R., Chadd, S., Rouen, K.J., Saint, L., Dawson, F.H., 1999. Mean Trophic Rank: A Users Manual. R&D Technical Report E38. Environment Agency of England & Wales, Bristol.
Jacobsen, D., Terneus, E., 2001. Aquatic macrophytes in cool aseasonal and seasonal streams: a comparison between Ecuadorian highland and Danish lowland streams. Aquat. Bot. 71, 281–295.
Johnson, R.K., Furse, M.T., Hering, D., Sandin, L., 2007. Ecological relationships between stream communities and spatial scale: implications for designing catchment-level monitoring programmes. Freshw. Biol. 52, 939–958.
Kohler, A., 1978. Methoden der Kartierung von Flora und Vegetation von Süβwasserbiotopen. Landschaft+Stadt 10, 73–85.
Kohler, A., Janauer, G.A., 1995. Zur Methodik der Untersuchung von aquatischen Makrophyten in Flieβgewässern. In: Steinberg, C., Bernhardt, H., Klapper, H. (Eds.), Handbuch Angewandte Limnologie. Ecomed Verlag, Landsberg/Lech, pp. 3–22.
Kuhar, U., Gregorc, T., Renčelj, M., Šraj-Kržič, N., Gaberščik, A., 2007. Distribution of macrophytes and condition of the physical environment of streams flowing through agricultural landscape in north-eastern Slovenia. Limnologica 37, 146–154.
Mackay, S.J., Arthington, A.H., Kennard, M.J., Pusey, B.J., 2003. Spatial variation in the distribution and abundance of submersed macrophytes in an Australian subtropical river. Aquat. Bot. 77, 169–186.
Meilinger, P., Schneider, S., Melzer, A., 2005. The reference index method for the macrophyte-based assessment of rivers – a contribution to the implementation of the European Water Framework Directive in Germany. Int. Rev. Hydrobiol. 90, 322–342.
Odum, E.P., 1971. Fundamentals of Ecology. W.B. Saunders Company, Philadelphia.
Preston, C.D., 1995. Pondweeds of Great Britain and Ireland. Botanical Society of the British Isles, London.
Riis, T., Sand-Jensen, K., 2001. Historical changes in species composition and richness accompanying perturbation and eutrophication of Danish lowland streams over 100 years. Freshw. Biol. 46, 269–280.
Sand-Jensen, K., Riis, T., Vestergaard, O., Larsen, S.E., 2000. Macrophyte decline in Danish lakes and streams over the past 100 years. J. Ecol. 88, 1030–1040.
Schaumburg, J., Schranz, C., Foerster, J., Gutowski, A., Hofmann, G., Meilinger, P., Schneider, S., Schmedtje, U., 2004. Ecological classification of macrophytes and phytobenthos for rivers in Germany according to the Water Framework Directive. Limnologica 34, 283–301.
Schaumburg, J., Schranz, C., Stelzer, D., Hofmann, G., Gutowski, A., Foerster, J., 2006. Instruction Protocol for the Ecological Assessment of Running Waters for Implementation of the EC Water Framework Directive: Macrophytes and Phytobenthos. Bavarian Environment Agency.
Schneider, S., Melzer, A., 2003. The Trophic Index of Macrophytes (TIM) – a new tool for indicating the trophic state of running waters. Int. Rev. Hydrobiol. 88, 49–67.
Strayer, D.L., Beighley, R.E., Thompson, L.C., Brooks, S., Nilsson, C., Pinay, G., Naiman, R.J., 2003. Effects of land cover on stream ecosystems: roles of empirical models and scaling issues. Ecosystems 6, 407–423.
Szoszkiewicz, K., Ferreira, T., Korte, T., Baattrup-Pedersen, A., Davy-Bowker, J., O'Hare, M., 2006. European river plant communities: the importance of organic pollution and the usefulness of existing macrophyte metrics. Hydrobiologia 566, 211–234.
Szoszkiewicz, K., Karolewicz, K., Ławniczak, A., Dawson, F.H., 2002. An assessment of the MTR aquatic plant bioindication system for determining the trophic status of polish rivers. Pol. J. Environ. Stud. 11, 421–427.
Šraj Kržič, N., Gaberščik, A., 2005. Photochemical efficiency of amphibious plants in an intermittent lake. Aquat. Bot. 83, 281–288.
Šraj-Kržič, N., Germ, M., Urbanc-Berčič, O., Kuhar, U., Janauer, G.A., Gaberščik, A., 2007. The quality of the aquatic environment and macrophytes of karstic watercourses. Plant Ecol. 192, 107–118.
Urbanič, G., 2007. Potencialni referenčni odseki celinskih vod v Sloveniji. In: Urbanič, G. (Ed.), Dopolnitev tipologije. Poročilo o delu v letu 2007. Inštitut za vode Republike Slovenije, Ljubljana, pp. 26–30.
Urbanič, G., 2008a. Redelineation of European inland water ecoregions in Slovenia. Rev. Hydrobiol. 1, 17–25.
Urbanič, G., 2008b. Inland water subecoregions and bioregions of Slovenia. Nat. Slov. 10, 5–19.
Urbanič, G., Smolar-Žvanut, N., 2007. Criteria for selecting river reference sites in Slovenia. In: Jepsen, N., Pont, D. (Eds.), Intercalibration of Fish-based Methods to Evaluate River Ecological Quality. European Commission Joint Research Centre (JRC), Ispra, pp. 81–83.
Wallin, M., Wiederholm, T., Johnson, K.R., 2003. Guidance on establishing reference conditions and ecological status class boundaries for inland surface waters, version 7.0. CIS Working Group 2.3 – REFCOND.
Warwick, N.W.M., Brock, M.A., 2003. Plant reproduction in temporary wetlands: the effects of seasonal timing, depth, and duration of flooding. Aquat. Bot. 77, 153–167.
Wiegleb, G., 1984. A study of habitat conditions of the macrophytic vegetation in selected river systems in western Lower Saxony (Federal Republic of Germany). Aquat. Bot. 18, 313–352.