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How many indicator species are required to assess the ecological status of a river?

by

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Abstract

The presented research focused on macrophytes, which constitute a primary element in the assessment of the ecological status of surface waters following the guidelines of the Water Framework Directive. In Poland, such assessments are conducted using the Macrophyte Index for Rivers (MIR). The objective of this study was to characterize macrophyte species in rivers in terms of their information value in the assessment of the ecological status of rivers. The macrophyte survey was carried out at 100 river sites in the lowland area of Poland. Botanical data were used to verify the completeness of samples (the number of taxa). In the presented research, the information provided by each species was controlled. Entropy was used as the main part of information analysis. This analysis showed that the adoption of a standard approach in the studies of river macrophytes is likely to provide sample underestimation (with missing species). This may potentially lead to incorrect determination of MIR and thus result in a wrong environmental decision. On this basis, a sample completeness criterion was developed. Using this criterion, the average value of information for macrophyte species in medium-sized lowland rivers is sufficient to be considered representative.

Key words: entropy, indicator taxa, information vector, macrophytes, Macrophyte Index for Rivers (MIR)

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Introduction

Preferences of individual macrophyte species for specific environmental conditions make it possible to determine the degree of flowing water degradation (Szoszkiewicz et al. 2017). In every ecosystem, nutrient concentrations change under the influence of contaminants. Changes may also result from fluctuations in river water flow, seasons, weather conditions and the changing self-purification capacity of rivers (Westlake 1975; Dawson 1988). Water quality assessments based on biological characteristics, physical and chemical parameters are complementary, and reflect the state of an aquatic ecosystem. Aquatic organisms are permanently exposed to environmental pressure. If their sensitivity to a specific contaminant has been determined, then the degree of degradation of an aquatic environment resulting from a given pressure can be determined based on field studies, with macrophytes (similarly as fish) facilitating the identification of changes over a longer timeframe, as opposed to phytoplankton and zooplankton, which tend to respond promptly (Wiegleb 1979; Haslam 1982; Holmes et al. 1999; Ceschin et al. 2010; Szoszkiewicz et al. 2017).

Ecological assessment methods for rivers based on macrophytes are applied in many European countries. In the UK, the Mean Trophic Rank (MTR) is commonly used (Holmes et al. 1999). The research conducted in France resulted in the development of the Indice Biologique Macrophytique En Rivière (IBMR) method (Haury et al. 2006). The Polish macrophyte method for rivers is largely based on the British MTR method and the French IBMR method. Their Polish counterpart, using the Macrophyte Index for Rivers (MIR), was developed in 2006 (Szoszkiewicz et al. 2007; Szoszkiewicz et al. 2010) and implemented in the monitoring of flowing waters.

Regardless of the type of biological characteristic (index), in each case the primary problem relates to accurate estimation of the sample size. Spatial and temporal variability are the most important sources of uncertainty affecting the variance of indicator values and classification results for various groups of organisms (Staniszewski & Szoszkiewicz 2006; Staniszewski et al. 2006; Carvalno et al. 2013). The main sources of variability related to macrophytes are associated with the method of on-site data collection (Dudley et al. 2013; Kolada et al. 2011) and the accuracy of macrophyte identification and classification, particularly the estimation of their abundance and cover. Another important source of errors, so far neglected in the literature on the subject, is the underestimation of the sample size – meaning that not all the taxa present were found. On the other hand, new species records are sometimes discovered during on-site studies, which do not provide relevant information, because they may occur anywhere.

The development of the information theory initiated by Shannon (1948) was of great importance for the progress of the probability theory and mathematical statistics. Furthermore, it was also applied in ecological studies (Kullback 1959; Sherwin et al. 2017). The creator of information theory, Eryomin (1998), proposed new scientific directions of research related to information ecology, such as research into the value of information or identification of quantitative and qualitative criteria of information. The concept of entropy in ecology means the expected value of a discrete variable, which is the sum of products of frequencies of species at surveyed sites and their corresponding information values (or relative total percentage cover of a species over the entire study area and its corresponding information value). The amount of information obtained while finding a species at a site may be considered as one of the aspects of diversity.

The aim of this study was to characterize selected macrophyte species in rivers in terms of their indicator value, related to the information they provide in the assessment of the ecological status of rivers. In practice, this will facilitate the assessment of the site completeness in terms of reported taxa, based on the informative value of indicator species for the Macrophyte Index for Rivers (MIR). On this basis, a decision can be made as to whether studies at a given site should be repeated or whether they may be considered complete.

The paper evaluates macrophytes found in medium-sized lowland rivers in Poland in terms of their informative value reflecting the quality of an aquatic ecosystem. A criterion for the sample completeness required for the determination of MIR is proposed.

Materials and methods

Site selection

The research was conducted in Poland at 100 river sites on medium-sized lowland rivers covered by the national environmental monitoring system, which were classified as representing a single abiotic type of sandy lowland rivers. These rivers are located below 200 m a.s.l. Their catchments are less than 1000 km^2 in area (small and medium-sized rivers according to WFD; WFD Intercalibration 2011; Fig. 1).

Figure 1

Location of the survey sites (n = 100)

Data collection

Research on macrophytes, including vascular plants, algae, mosses and liverworts (of the total number of 153 indicator taxa reported in the literature), was conducted at water sampling sites in the period 2008–2013 from July to early September (once in the analyzed period) along 100 m sections of river channels. Macrophytes were identified to the species level (except for six algae taxa: *Cladophora* sp., *Enteromorpha* sp., *Oedogonium* sp./*Ulothrix*, *Rhizoclonium* sp., *Stigeoclonium* sp., *Vaucheria* sp.). Twenty sites were selected for each of the five river quality classes based on physicochemical analyses. Similarities and differences in species composition between rivers representing the five quality classes were demonstrated according to the geombinatoric approach (as problems in discrete, convex, and combinatorial geometry), using Venn diagrams (Henderson 1963; Ruskey et al. 2006; Fig. 2).

Table 1

Maximum, minimum, mean and median MIR values in five quality classes

MIR	class I	class II	class III	class IV	class V
MIR_{max}	56.11	46.67	46.90	38.00	38.70
$MIR_{min.}$	35.77	34.09	32.41	20.78	18.29
MIR_{mean}	43.32	39.29	37.94	32.02	29.01
MIR_{med}	42.98	39.31	38.06	33.13	29.06

Data analysis

The Macrophyte Index for Rivers (MIR) is a biological quality indicator for flowing waters (Szoszkiewicz et al. 2010). This index was calculated for each site based on the formula:

$$MIR = \frac{\sum (L_i \times W_i \times P_i)}{(W_i \times P_i)} \times 10 \qquad (1)$$

where:

L_i is the indicator value for the *i*th reported taxa;
W_i is the weighting factor for the *i*th taxa;
P_i is the coefficient of cover for the *i*th taxa on a 9-point scale.

The MIR value was determined based on macrophytes identified at each site, with specific L_i, W_i and P_i for indicator species. L_i refers to the mean trophic level of the habitat in which a given taxon is found (assuming a value of 1 for advanced eutrophy and 10 for oligotrophy). W_i denotes the weighting factor as a measure of the ecological tolerance of a species (assuming a value of 1 for eurytopic species and 3 for stenotopic species, i.e. highly sensitive). For each species, the P_i value was determined, i.e. the degree of cover in a 100 m section of a river on a 9-point scale (Szoszkiewicz et al. 2010). The basic statistical characteristics were determined for each water quality class, i.e. the maximum, minimum, mean and median MIR (Table 1).

To determine the information value of each species, the probability D_i was estimated as the relative frequency of the *i*th species ($i = 1,\ldots,90$), i.e. the quotient of incidence of this species (rarity) and the number of all reported incidences for all recorded species. Next, S_i was determined ($i = 1,\ldots,90$) as the relative total percentage cover of a species over the entire study area. On this basis, for vectors *D* and *S***,** the corresponding information vectors were determined for the frequency of species ln(1/*D*) and the relative percentage cover ln(1/*S*) (Mazur 1970; Cover et al. 1991; Li et al. 1997; Jones et al. 2000; MacKay 2003). Table 2 shows L_i and W_i values reported in the literature and values of the numerical parameters presented above: D_i, S_i, ln(1/D_i), ln(1/S_i) for each species (*D* and *S* denote the *n*-element vectors of the characteristics, D_i and S_i denote the *i*th coordinates of the vector).

The occurrence of the *i*th species in a given environment is reflected by its D_i (percentage occurrence) or S_i (relative percentage coverage). The information theory shows that these characteristics

Table 2

Indicator values of L_i and W_i for macrophytes and determined S_i (percentage cover) and D_i (incidence) and their informative value ($i = 1, 2, \ldots, 90$)

Taxa	L_i	W_i	P_i	S_i	$ln(1/S_i)$	D_i	$ln(1/D_i)$	Taxa	L_i	W_i	P_i	S_i	$ln(1/S_i)$	D_i	$ln(1/D_i)$
Acorus calamus	2	3	1	0.007	4.955	0.006	5.053	*Oedogonium* sp./*Ulothrix*	4	1	1	0.002	6.166	0.005	5.303
Alisma plantago-aquatica	4	2	1	0.012	4.465	0.019	3.953	*Oenanthe aquatica*	5	1	2	0.001	6.869	0.003	5.863
Amblystegium riparium	1	1	1	0.003	5.902	0.006	5.170	*Peucedanum palustre*	5	2	1	0.001	7.550	0.001	7.249
Berula erecta	4	2	1	0.048	3.028	0.039	3.242	*Phalaris arundinacea*	2	1	2	0.050	3.004	0.050	3.001
Butomus umbellatus	5	2	1	0.012	4.412	0.014	4.253	*Polygonum amphibium*	4	1	1	0.001	6.592	0.005	5.303
Calla palustris	6	2	1	0.000	7.955	0.001	7.249	*Polygonum hydropiper*	3	1	2	0.002	6.011	0.007	4.947
Calliergonella cuspidata	8	2	2	0.001	6.586	0.001	7.249	*Polygonum persicaria*	2	2	1	0.003	5.717	0.005	5.303
Caltha palustris	6	2	1	0.003	5.869	0.003	5.863	*Potamogeton alpinus*	7	2	1	0.000	8.748	0.001	7.249
Carex acuta	5	1	2	0.004	5.570	0.009	4.684	*Potamogeton compressus*	4	2	2	0.002	6.120	0.002	6.151
Carex acutiformis	4	1	4	0.006	5.050	0.010	4.610	*Potamogeton crispus*	4	2	1	0.017	4.095	0.013	4.359
Carex paniculata	5	1	2	0.002	6.143	0.005	5.303	*Potamogeton lucens*	4	3	6	0.005	5.391	0.003	5.863
Carex riparia	4	2	1	0.017	4.074	0.015	4.205	*Potamogeton natans*	4	1	3	0.006	5.130	0.009	4.684
Carex rostrata	6	3	5	0.003	5.658	0.001	7.249	*Potamogeton nodosus*	3	2	1	0.002	6.290	0.002	6.151
Carex vesicaria	6	2	2	0.002	6.334	0.002	6.151	*Potamogeton obtusifolius*	5	2	1	0.000	8.161	0.001	7.249
Catabrosa aquatica	5	1	2	0.003	5.875	0.004	5.640	*Potamogeton pectinatus*	1	1	3	0.020	3.897	0.016	4.114
Ceratophyllum demersum	2	3	1	0.033	3.397	0.018	4.030	*Potamogeton perfoliatus*	4	2	3	0.008	4.788	0.006	5.052
Ceratophyllum submersum	2	3	1	0.003	5.843	0.002	6.151	*Potamogeton praelongus*	6	3	3	0.005	5.284	0.004	5.457
Cicuta virosa	6	2	2	0.004	5.583	0.005	5.303	*Potamogeton pusillus*	4	2	2	0.001	6.558	0.001	6.556
Cladophora sp.	1	2	3	0.025	3.678	0.018	3.991	*Ranunculus aquatilis*	5	3	2	0.009	4.689	0.004	5.640
Conocepohalum conicum	7	1	5	0.002	6.254	0.001	7.249	*Ranunculus circinatus*	5	2	1	0.003	5.870	0.004	5.640
Eleocharis palustris	6	2	2	0.001	7.309	0.001	6.556	*Ranunculus fluitans*	7	2	2	0.009	4.659	0.005	5.303
Elodea canadensis	5	2	2	0.070	2.666	0.039	3.242	*Ranunculus lingua*	8	2	1	0.004	5.621	0.003	5.863
Enteromorpha sp.	1	2	2	0.001	7.563	0.001	7.249	*Ranunculus peltatus*	4	3	2	0.001	6.908	0.001	7.249
Equisetum fluviatile	6	2	2	0.017	4.093	0.014	4.305	*Ranunculus sceleratus*	2	1	1	0.001	6.727	0.004	5.640
Equisetum palustre	5	2	2	0.003	5.794	0.006	5.052	*Ranunculus trichophyllus*	6	2	4	0.002	6.201	0.002	6.151
Fontinalis antipyretica	6	2	1	0.007	4.923	0.006	5.170	*Rhizoclonium* sp.	1	1	1	0.013	4.363	0.011	4.477
Glyceria fluitans	5	2	2	0.012	4.436	0.011	4.477	*Rhynchostegium riparioides*	5	1	2	0.001	7.550	0.001	7.249
Glyceria maxima	3	1	2	0.036	3.324	0.041	3.189	*Rorippa amphibia*	3	1	2	0.014	4.264	0.028	3.586
Glyceria plicata	5	1	2	0.001	7.090	0.001	7.249	*Rumex hydrolapathum*	4	1	2	0.010	4.616	0.028	3.586
Hippuris vulgaris	4	1	1	0.000	9.306	0.001	7.249	*Sagittaria sagittifolia*	4	2	3	0.047	3.052	0.035	3.357
Hydrocharis morsus-ranae	6	2	1	0.013	4.329	0.011	4.477	*Scirpus lacustris*	4	2	1	0.001	7.376	0.001	6.556
Hydrocotyle vulgaris	5	1	2	0.001	7.170	0.001	7.249	*Scirpus sylvaticus*	5	2	2	0.015	4.190	0.014	4.253
Iris pseudacorus	6	2	2	0.021	3.880	0.021	3.882	*Scrophularia umbrosa*	4	1	2	0.008	4.772	0.018	4.030
Lemna gibba	1	3	7	0.021	3.879	0.009	4.764	*Sium latifolium*	7	1	1	0.010	4.592	0.019	3.953
Lemna minor	2	2	2	0.075	2.590	0.053	2.945	*Sparganium emersum*	4	2	2	0.069	2.668	0.041	3.206
Lemna trisulca	4	2	1	0.011	4.482	0.014	4.305	*Sparganium erectum*	3	1	1	0.030	3.498	0.036	3.317
Lysimachia thyrsiflora	7	3	1	0.001	7.313	0.001	7.249	*Spirodela polyrhiza*	2	2	1	0.016	4.162	0.023	3.783
Lysimachia vulgaris	4	1	1	0.001	6.805	0.004	5.640	*Stachys palustris*	2	1	1	0.008	4.771	0.021	3.882
Mentha aquatica	5	1	1	0.014	4.245	0.029	3.536	*Stigeoclonium* sp.	1	1	2	0.000	7.844	0.001	7.249
Menyanthes trifoliata	9	3	1	0.001	7.444	0.001	7.249	*Typha angustifolia*	3	2	2	0.002	6.040	0.003	5.863
Myosotis palustris	4	1	1	0.015	4.219	0.033	3.399	*Typha latifolia*	2	2	3	0.014	4.287	0.013	4.359
Myriophyllum spicatum	3	2	1	0.005	5.226	0.005	5.303	*Vaucheria* sp.	2	1	1	0.003	5.904	0.009	4.764
Nasturtium officinale	5	2	1	0.001	7.550	0.001	7.249	*Veronica anagallis-aquatica*	4	2	2	0.029	3.538	0.028	3.560
Nuphar lutea	4	2	2	0.030	3.513	0.021	3.848	*Veronica beccabunga*	4	1	1	0.005	5.277	0.013	4.359
Nymphaea alba	6	2	1	0.001	7.137	0.001	6.556	*Viola palustris*	9	1	1	0.000	8.412	0.001	7.249

are assigned the corresponding information values $\ln(1/D_i)$ and $\ln(1/S_i)$. These are used to describe a given environment.

In order to determine whether a sample is representative of a macrophyte population in rivers of a given type, entropy was defined as average information. It was compared with the maximum value of entropy for the complete pool of taxa found.

The entropies $H_{(D)}$ and $H_{(S)}$ were determined as the mean amount of information required to characterize a site in terms of the number of observed species (Bremer et al. 2004), including the entropy $H_{(D)}$ for all species found in the entire study area (2) and the entropy $H_{(S)}$ for relative percentage cover for all species in the entire study area (3):

$$H_{(D)} = D' \ln(1/D) \tag{2}$$

$$H_{(S)} = S' \ln(1/S) \tag{3}$$

Based on the matrix of six characteristics for the indicator species recorded in the study (Table 2), the synthetic Perkal index was constructed (Smith 1972; Parysek et al. 1979; Chojnicki et al. 1991; Sobala-Gwóźdź 2004). This is a sum of standardized partial values in two versions: ($Pe_{L,W}$) for L and W known from the literature and information vectors ($Pe_{ln(1/D),ln(1/S)}$) for vectors S and D presented in this study (Table 3). The standardized value, also called the normal deviate, is the distance of one data point from the mean, divided by the standard deviation of the distribution.

For the newly determined characteristics from Table 2, Pearson's correlation coefficient r was used to determine the linear correlation between the variables $\ln(1/D)$ and $\ln(1/S)$.

To verify the hypothesis that there is no significant difference between the investigated entropies $H_{(D)}$ and $H_{(S)}$, the structural comparison test proposed by Hutcheson (1970) was applied. In case of high correlation between $\ln(1/D)$ and $\ln(1/S)$ and no significant differences between $H_{(D)}$ and $H_{(S)}$, one of the parameters S or D should be taken into account.

These values were used to determine the information threshold required for a site to be

Table 3

Species profiles for macrophytes and the Perkal indices $Pe_{Li,Wi}$ and $Pe_{ln(1/Si),ln(1/Di)}$

Taxa	Perkal indices		Taxa	Perkal indices		Taxa	Perkal indices	
	$Pe_{Li,Wi}$	$Pe_{ln(1/Si),ln(1/Di)}$		$Pe_{Li,Wi}$	$Pe_{ln(1/Si),ln(1/Di)}$		$Pe_{Li,Wi}$	$Pe_{ln(1/Si),ln(1/Di)}$
Amblystegium riparium	−2.94	0.23	*Catabrosa aquatica*	−0.77	0.57	*Equisetum palustre*	0.75	0.08
Potamogeton pectinatus	−2.94	−1.85	*Glyceria plicata*	−0.77	2.55	*Glyceria fluitans*	0.75	−1.23
Rhizoclonium sp.	−2.94	−1.28	*Hydrocotyle vulgaris*	−0.77	2.60	*Nasturtium officinale*	0.75	2.85
Stigeoclonium sp.	−2.94	3.04	*Mentha aquatica*	−0.77	−2.05	*Peucedanum palustre*	0.75	2.85
Phalaris arundinacea	−2.39	−3.26	*Oenanthe aquatica*	−0.77	1.38	*Potamogeton obtusifolius*	0.75	3.25
Ranunculus sceleratus	−2.39	1.12	*Rhynchostegium riparioides*	−0.77	2.85	*Ranunculus circinatus*	0.75	0.56
Stachys palustris	−2.39	−1.46	*Myriophyllum spicatum*	−0.33	−0.11	*Scirpus sylvaticus*	0.75	−1.56
Vaucheria sp.	−2.39	−0.06	*Potamogeton nodosus*	−0.33	1.21	*Calla palustris*	1.29	3.11
Glyceria maxima	−1.85	−2.91	*Typha angustifolia*	−0.33	0.84	*Caltha palustris*	1.29	0.73
Polygonum hydropiper	−1.85	0.14	*Lemna gibba*	0.10	−1.38	*Carex vesicaria*	1.29	1.24
Rorippa amphibia	-1.85	-2.00	*Alisma plantago-aquatica*	0.21	-1.60	*Cicuta virosa*	1.29	0.13
Sparganium erectum	−1.85	−2.70	*Berula erecta*	0.21	−3.06	*Eleocharis palustris*	1.29	2.18
Cladophora sp.	−1.42	−2.09	*Carex riparia*	0.21	−1.67	*Equisetum fluviatile*	1.29	−1.58
Enteromorpha sp.	−1.42	2.86	*Lemna trisulca*	0.21	−1.33	*Fontinalis antipyretica*	1.29	−0.40
Carex acutiformis	−1.31	−0.73	*Nuphar lutea*	0.21	−2.30	*Hydrocharis morsus-ranae*	1.29	−1.30
Hippuris vulgaris	−1.31	3.99	*Potamogeton compressus*	0.21	1.10	*Iris pseudacorus*	1.29	−2.04
Lysimachia vulgaris	−1.31	1.17	*Potamogeton crispus*	0.21	−1.54	*Nymphaea alba*	1.29	2.07
Myosotis palustris	−1.31	−2.17	*Potamogeton perfoliatus*	0.21	−0.58	*Ranunculus trichophyllus*	1.29	1.16
Oedogonium sp./*Ulothrix*	−1.31	0.51	*Potamogeton pusillus*	0.21	1.69	*Viola palustris*	1.40	3.41
Polygonum amphibium	−1.31	0.78	*Sagittaria sagittifolia*	0.21	−2.96	*Potamogeton lucens*	1.73	0.42
Potamogeton natans	−1.31	−0.63	*Scirpus lacustris*	0.21	2.22	*Ranunculus peltatus*	1.73	2.43
Rumex hydrolapathum	−1.31	−1.78	*Sparganium emersum*	0.21	−3.33	*Potamogeton alpinus*	1.83	3.63
Scrophularia umbrosa	−1.31	−1.35	*Veronica anagallis-aquatica*	0.21	−2.50	*Ranunculus fluitans*	1.83	−0.48
Veronica beccabunga	−1.31	−0.77	*Conocepohalum conicum*	0.32	2.00	*Ranunculus aquatilis*	2.27	−0.21
Lemna minor	−0.88	−3.57	*Sium latifolium*	0.32	−1.52	*Calliergonella cuspidata*	2.38	2.22
Polygonum persicaria	−0.88	0.21	*Acorus calamus*	0.64	−0.47	*Ranunculus lingua*	2.38	0.56
Spirodela polyrhiza	−0.88	−1.92	*Ceratophyllum demersum*	0.64	−2.24	*Carex rostrata*	2.81	1.62
Typha latifolia	−0.88	−1.42	*Ceratophyllum submersum*	0.64	0.92	*Potamogeton praelongus*	2.81	0.04
Carex acuta	−0.77	−0.34	*Butomus umbellatus*	0.75	−1.41	*Lysimachia thyrsiflora*	3.35	2.69
Carex paniculata	−0.77	0.49	*Elodea canadensis*	0.75	−3.30	*Menyanthes trifoliata*	4.44	2.78

$Pe_{Li.Wi}$ – Perkal indices: sum of standardized values $Li+Wi$; $Pe_{ln(1/Si). ln(1/Di)}$ – sum of standardized values of information $\ln(1/Si)+\ln(1/Di)$

considered sufficiently surveyed, regardless of its quality class. Furthermore, based on literature data, it is assumed that a thorough study requires on average at least eight or nine indicator species (Szoszkiewicz 2013; Budka 2018).

As a consequence, a criterion was proposed to indicate whether a given site was sufficiently surveyed. The total arithmetic mean of the informative value for the recorded species at a given site may not be lower than the arithmetic mean of the informative value determined separately based on all sites belonging to each of the river quality classes.

Statistical analyses were performed using the R computational platform. The available packages, i.e. “VennDiagram” v.1.6.20 , “ggplot2” v.1.0.0, “gplots” v. 2.14.1, “graphics” v.3.1.1, were used.

Results and discussion

A total of 90 indicator species with specific indicator values L_i and weighting factors W_i were identified at 100 sites representing five water quality classes (Fig. 2).

It is estimated that the total aquatic flora in the analyzed types of watercourses (lowland, with sandy bottom substrate) comprises approx. 115 vascular plant species (Bernatowicz et al. 1969; Rutkowski 2008; Jusik 2012).

It should be noted that this study identified 19 strongly stenotopic species that were reported in only one quality class. Six species were reported in quality class I: *Conocepohalum conicum*, *Hippuris vulgaris*, *Hydrocotyle vulgaris*, *Lysimachia thyrsiflora*, *Viola palustris*, and *Menyanthes trifoliata*. *Carex rostrata*, *Peucedanum palustre* and *Ranunculus lingua* were recorded in class II. Among the reported taxa, *Calla palustris*, *Calliergonella cuspidate*, *Enteromorpha* sp., *Nasturtium officinale*, *Potamogeton alpinus*, *Potamogeton obtusifolius*, *Ranunculus peltatus* and *Rhynchostegium riparioides* were observed only in quality class III, *Glyceria plicata* – only in class IV and *Stigeoclonium* sp. – only in class V. Furthermore, a set of 37 taxa was characterized by a very high tolerance (eurytopicity) and comprised a pool of species common to all quality classes. Of the total number of 90 indicator species recorded, 66 species belonged to class I, 68 species – to class II, 66 species – class III, 53 species – class IV, and 50 species – class V.

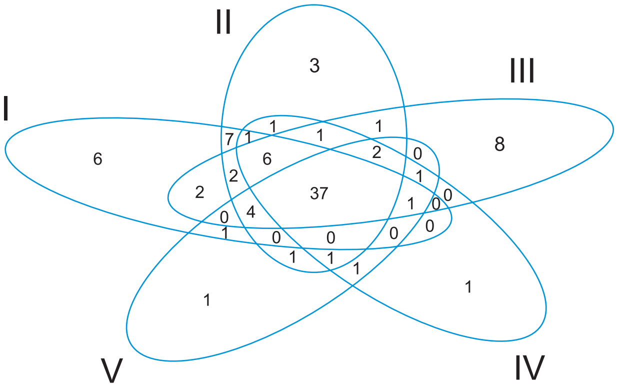

Figure 2

Venn diagram analysis of taxonomic differentiation of the five trophic classes (I–V). Numbers show the distribution of shared species.

The basic characteristics of the MIR index (maximum, minimum, mean and median values) in five quality classes were determined for the obtained database (Table 1) in order to present a general description of the environment.

The largest range (20.3), i.e. the MIR values were the most diverse at sites with waters of the highest eutrophic levels. Furthermore, the range for quality class IV was 17.2, followed by 14.4 for class III and 12.6 for class II. It can be observed that the highest disproportions occurred among the MIR values for the sites in class I. This may be due to the fact that at some sites mainly rare and scarce species of high indicator value were identified, while other sites were dominated by species typical of all water quality classes. Furthermore, it should be noted that the decreasing trend of the MIR mean value in the trophic gradient was maintained. Values of the above-mentioned measures are typical of medium-sized lowland rivers. It can be assumed that the data characterize well ecoregions established by the EU WFD (2000/60/EC): Central Plains (Ecoregion no. 14) and Eastern Plains (Ecoregion no. 16). As a good representation of lowland rivers in Europe, they constitute an adequate group of bioindicators for Europe (Klijn 1989; 1994; Groen et al. 1993; Kondracki 1995).

The L_i and W_i values for indicator species (i = 1,…,90) and the obtained characteristics D_i, S_i, ln(1/D_i), ln(1/S_i) are presented in Table 2.

The highest recorded incidence of the ith species (D_i) was 0.0526, which shows that *Lemna minor* was a species commonly found at sites belonging to all quality classes: at 8 sites in class I, at 13 sites in class II, at 15 sites in class III, and at 19 sites in classes IV and V of the total number of 20 sites. For this reason, based on the relative frequency of occurrence, *Lemna minor* provided the least information (was the least informative) on the water quality status (ln(1/D_i) = 2.945). Similarly, this species was found to have the highest value of relative percentage cover throughout the study area (S_i), amounting to 0.0750, which confirms its lowest informative value related to its abundance ($\ln(1/S_i) =$ 2.590).

Taxa found in only one quality class (19 species) had the lowest D_i values, reaching 0.0007 or 0.0028, and provided the greatest informative value: $\ln(1/D_i)$ = 7.2492 or 5.8629. Similarly, these species had the lowest S_i values, ranging from 0.0001 to 0.0036, and were the most informative ones, with $\ln(1/S_i)$ ranging from 5.621 to 9.306.

Such results are consistent with the scale of species occurrence and dynamic trends (Rutkowski 2008). The abundance score denotes the abundance of species on a nationwide scale of five classes: 1 – very rare (1–10 sites), rare (10–100 sites), quite frequent (more than 100 sites), frequent in many regions, common throughout (or nearly throughout) the territory of Poland. Macrophytes that are highly ecologically specialized, such as *Nasturtium officinale*, *Potamogeton alpinus*, *Potamogeton obtusifolius*, *Hippuris vulgaris*, are listed as rare in the Polish Flora (10–100 sites in Poland, abundance score 2/5) or frequent only in some areas (abundance score 3/5). They bring the highest information values $\ln(1/D_i)$ and $\ln(1/S_i)$ in our research. Even if they are found as single macrophytes, they guarantee sufficient knowledge about a site. On the other hand, taxa that are often frequent in the Polish flora in many regions (abundance score 4/5) and the most common taxa (abundance score 5/5) (Rutkowski 2008), such as *Lemna minor*, *Carex acuta*, *Carex acutiformis*, *Glyceria fluitans*, *Rhizocolonium* sp., occur in each of the studied water quality classes and contribute little to the general knowledge about a site, which is also confirmed by their information value $\ln(1/Di)$ and $\ln(1/Si)$. In the case of species which, according to the frequency scale, can be regarded as frequent in many regions (abundance score 4/5) and can be found in every environment, the information vector remains a valuable indicator of the information at that site.

Entropy for all macrophytes found in all examined river sections was $H_{(D)} = D' \ln(1/D) = 3.948$, while entropy for the percentage cover over the entire study area was $H_{(S)} = S' \ln(1/S) = 3.775$. Values of the indicators S and D show a similar reaction to the detection of another species at a given site. The maximum value of entropy for a total of 90 identified indicator macrophyte species was 4.5. Furthermore, based on the Hutcheson test comparing the structural indicators, no statistically significant differences were found between the entropies $H_{(\mathbf{S})}$ for the relative percentage cover over the entire study area (2) and $H_{(D)}$ for all species found over the entire study area (3) ($t = 0.03112$ and $p = 0.5$).

The correlation coefficient between the information vectors $\ln(1/D)$ and $\ln(1/S)$ was statistically significant and amounted to 0.917. When such high values of correlation between the information vectors were recorded, without compromising the generalization value of the study, the discussion was limited to the analysis of one of these indices. Since in practice the determination of the percentage cover requires additional skills and, according to the literature, is a major source of error, the value $\ln(1/D_\mathrm{i})$ was selected. This confirms the correctness of the statement that further analyses are possible based on one of the selected parameters D.

The next step consisted in the standardization of data from Table 2, which resulted in the so-called species profiles for individual taxa. Standardization involves converting measurements expressed in different units into a scale expressed in the same measurement unit (the variable obtains an average equal to 0 and standard deviation equal to 1). The transformed data are presented in Table 3.

Two synthetic Perkal indices were constructed based on the species profiles (Table 3). The maximum value of the Perkal index $\mathrm{Pe}_{Li,Wi}$ was 4.438, while the minimum and the median were −2.936 and 0.208, respectively. For the information value, $\mathrm{Pe}_{\ln(1/Si),\ln(1/Di)}$ reached a maximum of 3.991, a minimum of −3.569 and a median of −0.009. The Perkal index $\mathrm{Pe}_{Li,Wi}$ assumes 22 different values for 90 recorded macrophytes. The lowest values are ascribed mainly to species found in all five quality classes. Taxa representing one group for the Perkal index based on L_i and W_i ($\mathrm{Pe}_{Li,Wi}$), with the lowest value of −2.936, include *Potamogeton pectinatus*, *Rhizoclonium* sp., *Amblystegium riparium* and the alga *Stigeoclonium* sp. The $\mathrm{Pe}_{\ln(1/Si),\ln(1/Di)}$ for those macrophytes was −1.853, −1.281, 0.235 and 3.039, respectively. The latter, clearly different value characterizes *Stigeoclonium* sp., which was not found in all quality classes like the other species – i.e. it was identified only in quality class V. This may indicate the necessity to verify L_i and W_i values or to use additional informative values. Group IV was composed of the algae *Cladophora* sp. and *Enteromorpha* sp. with the identical value of $\mathrm{Pe}_{Li,Wi} =$ −1.418, but with markedly different $\mathrm{Pe}_{\ln(1/Si),\ln(1/Di)}$ values (−2.086 and 2.856, respectively). Similarly, this may confirm the reliability of values for the characteristics L and W or the inclusion of additional information on a given taxa. Group V comprises ten species: *Myosotis palustris*, *Rumex hydrolapathum*, *Scrophularia umbrosa*, *Veronica beccabunga*, *Carex acutiformis*, *Potamogeton natans*, *Oedogonium* sp./*Ulothrix*, *Polygonum amphibium*, *Lysimachia vulgaris* and *Hippuris vulgaris*, with $\mathrm{Pe}_{Li,Wi}$ equal to −1.309. The latter species has a much greater $Pe_{\ln(1/Si),\ln(1/Di)}$ value of 3.991, reflecting its affiliation with quality class I only.

The highest values of the $Pe_{Li,Wi}$ index (approx. 20% of the values) identify the species serving as the best indicators, because they occur in only one or a maximum of two classes. The decreasing values are closely related to the fact that a given species was reported in an increasing number of sites and had an increasingly large range as regards the river quality classes. The Perkal indices constructed on the information vectors for $Pe_{\ln(1/Si),\ln(1/Di)}$ additionally distinguish species in terms of their information values $\ln(1/D_i)$ and $\ln(1/S_i)$. The control of the information values is therefore an additional crucial element in characterizing the investigated species and, in the context of the above analyses, it appears necessary to include, in addition to the standard characteristics of species, the information provided by the new indicator values. Such an approach may result in a reliable identification of biological indicators and thus produce accurate decisions related to environmental permits.

The proposed approach allowed for more precise ecological characteristics of macrophyte species. Species with the same values of L_i and W_i, may be indistinguishable when the MIR is determined, whereas in reality they may differ from one another in their ecological specialization. For example, the taxa *Amblystegium riparium*, *Potamogeton pectinatus*, *Rhizoclonium* sp. and *Stigeoclonium* sp. are of exactly the same importance for the Polish national macrophyte score of the ecological status (MIR) due to the same and lowest values of L_i and W_i. The use of additional information enables us to observe the fact that the first two species occur in five water quality classes, the third one occurs from class II to class V and the fourth one – only in class V. Similarly, macrophytes with higher values of the indicators L_i and W_i, such as *Calla palustris*, *Caltha palustris*, *Carex vesicaria*, *Cicuta virosa*, *Eleocharis palustris*, *Equisetum fluviatile*, *Fontinalis antipyretica*, *Hydrocharis morsus-ranae*, *Iris pseudacorus*, *Nymphaea alba*, *Ranunculus trichophyllus*, do not differ in terms of their indicators L_i and W_i, but they actually differ depending on their occurrence in water quality classes. *Calla palustris* was found in rivers of quite high ecological quality (class III), *Caltha palustris* occurred in classes I and II, *Carex vesicaria* was identified in the first three water quality classes, *Eleocharis palustris* was found in the first two classes. The species *Fontinalis antipyretica*, *Hydrocharis morsus-ranae*, *Iris pseudacorus* occurred in all five water quality classes. It therefore appears that this seemingly hidden variability should be assumed to determine the ecological status based on the MIR. In the current situation, it seems that these species should not be treated equally.

Table 4 presents basic descriptive statistics for information values of all macrophyte species found in the respective quality classes.

For the selected representative characteristic D_i presented in the table, the mean information value was determined for each of the river quality classes. It amounted to 4.192, 4.149, 3.883, 3.763 and 3.799, respectively, for the river quality classes ranked from the cleanest to the most polluted (Table 5), which on average gives an information value of approx. 4.0 for medium-sized lowland rivers. This value is a criterion used to determine the information threshold required for a given site to be considered sufficiently surveyed. It was assumed that irrespective of the quality class, if the total information value provided by all species found at a given site exceeds the value of 4.0, the site could be considered sufficiently inventoried.

Table 4

Descriptive statistics: maximum, minimum, mean and median values for the information value $\ln(1/D_i)$ of plants found in five quality classes

$\ln(1/Di)$	class I	class II	class III	class IV	class V
$\ln(1/D_i)_{max}$	4.80	4.40	4.70	4.10	4.20
$\ln(1/D_i)_{min.}$	3.60	3.40	3.40	3.50	3.50
$\ln(1/D_i)_{mean}$	4.19	4.03	3.94	3.78	3.80
$\ln(1/D_i)_{med}$	4.20	4.00	3.90	3.70	3.80

It is assumed that a reliable macrophyte study requires the incidence of at least nine indicator species, but the number of bioindicators may be lower if they are the most sensitive ones, i.e. mostly stenobiotic species (Szoszkiewicz 2013; Budka 2018). The analysis of the number of taxa at the investigated lowland river sites and the mean value of information introduced by those taxa showed considerable variability in individual water quality classes (Fig. 3).

Figure 3 shows the relationship between the number of species and the mean information value at a given site.

Based on the conducted study, the mean information value at thirteen sites in river quality class I exceeded 4.0, with 6 to 28 species recorded at a site. It should be noted that at some sites, despite the large number of species found (e.g. 22 species at site 686), the information value was insufficient. On the other hand, there are sites where 6, 8 or 9 species were reported, but this was sufficient in terms of information to conclude that a given site was complete. Twelve sites in quality class II exceeded the threshold for the mean information required for

Table 5

Information mean values for ln(1/D) at the study sites in five quality classes

class I			class II			class III			class IV			class V		
ST	SP	MI	ST	SP	MI	ST	SP	MI	ST	SP	MI	ST	SP	MI
32	8	4.6	30	12	4.0	3	28	4.3	4	14	3.6	79	17	3.8
36	19	4.4	38	14	3.9	14	12	3.7	8	14	3.7	82	18	3.6
37	17	4.1	43	10	3.8	58	23	3.9	63	12	3.8	84	10	3.9
39	10	3.6	49	14	4.0	66	17	3.7	200	14	3.9	95	10	4.2
224	19	4.4	51	14	3.9	109	13	4.1	238	14	4.1	170	13	3.5
237	9	4.0	137	9	3.4	141	6	3.4	259	12	3.7	171	21	4.1
529	6	4.3	140	13	3.8	210	12	4.7	295	12	3.8	176	14	3.9
591	11	3.9	143	14	3.8	226	14	4.0	427	11	4.0	190	16	4.0
598	10	4.8	151	14	4.4	236	19	3.9	429	8	3.7	243	18	3.8
599	15	3.8	153	9	4.1	435	12	3.7	432	12	3.6	260	17	4.0
601	10	4.7	201	14	3.9	439	17	3.8	444	11	3.7	262	13	3.8
616	21	4.5	256	14	3.6	451	8	4.4	474	7	3.5	277	11	3.5
639	12	3.7	423	14	4.4	462	17	3.9	475	10	3.6	426	18	3.9
640	28	4.5	452	7	4.4	469	10	3.7	530	14	4.0	430	6	3.6
645	24	4.3	600	11	4.3	531	16	3.6	562	11	3.5	437	6	3.8
647	18	4.6	602	13	4.0	552	11	4.3	573	8	4.0	438	7	3.6
686	22	3.9	625	14	4.4	634	21	4.0	628	10	3.7	443	9	3.6
693	21	4.0	630	11	4.1	694	14	3.7	670	25	4.0	549	9	3.7
703	10	3.9	641	14	4.2	697	20	3.9	671	14	3.9	650	13	4.1
723	14	3.8	699	14	4.1	707	14	4.0	713	10	3.7	667	14	3.6

ST – site number, SP – number of species, MI – mean ln(1/D)

a complete inventory (including two sites with only 7 and 9 species). The other sites did not have a sufficient number of species or a satisfactory information value. Eight sites in class III reached a sufficient information level, including one site with only 8 species. At the other sites, a sufficient information level was obtained following a considerable effort to complete the study (as many as 23 species recorded at a site). Only five sites in quality class IV were completely inventoried (including 8 species at one site), while the information

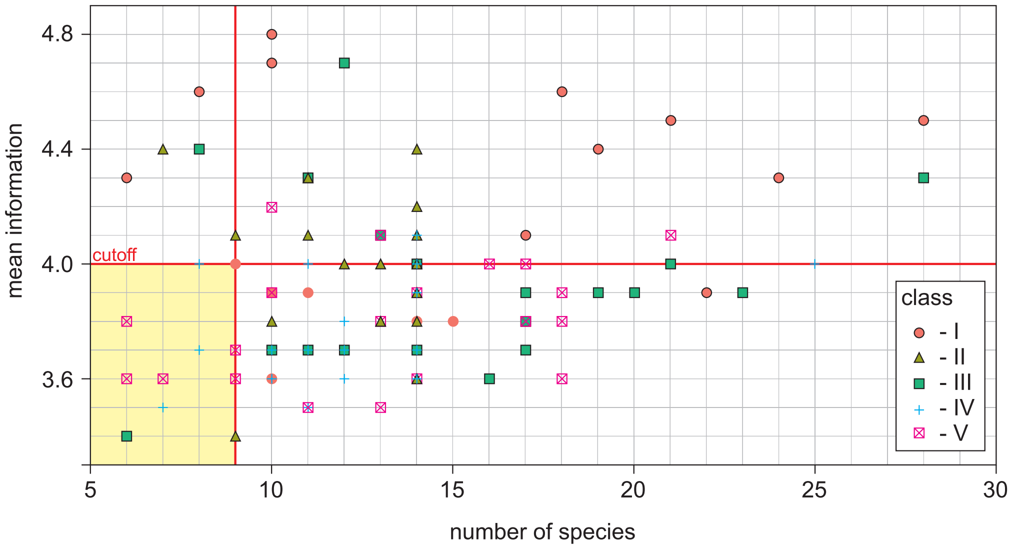

Figure 3

Division of sites in terms of the sample completeness criterion in individual water quality classes (vertical line – average number of species; horizontal line – average information necessary to determine the MIR; highlighted area contains sites that do not meet both criteria) threshold at the other sites was too low to consider the sites as completely inventoried. Five sites in quality class V achieved a high information value (from 4.2 to 4), while in the other cases the species identification must be considered incomplete. Consequently, these sites are not reliable for further analysis (Table 4).

The slow natural response of biological indicators to changing environmental conditions and the relatively small possibility of increasing the sampling frequency for biological parameters are an issue for all European water monitoring systems. Therefore, an attempt to solve some of the methodological dilemmas related to sampling, necessary to obtain reliable assessment results is very relevant and may be the first step toward solving numerous ecological issues, such as the likelihood of misclassification of biological elements in surface waters (Loga & Wierzchołowska-Dziedzic 2017). In specific cases where the indicator value is close to the threshold value between good and moderate status classes, these analyses are of particular importance as their results are crucial for water management and water protection decisions. It should be noted that statistical uncertainty in the evaluated ecological status of homogenous waters based on biological parameters may be much higher than the uncertainty based on frequently measured physicochemical indicators. The requirements of the Water Framework Directive (WFD) regarding the determination of the status of watercourses based on biological indices (in particular MIR) may contribute to an increasing risk of errors in decision making related to water management based on the WFD. This study addresses an unresolved bioethical problem by attempting to answer the question "how many species are required to obtain a reliable assessment result with a minimum level of uncertainty". Monitoring data used for this analysis represent a relatively large database of lowland rivers in Poland. Considering the results obtained while determining the criterion for lowland rivers, the proposed method could be easily extended to rivers of other types in Poland and Europe. The analyses could be performed for other indicators, such as the phytoplankton index (IFPL, Błachuta et al. 2012; Mischke et al. 2011), the diatom index (IO, Błachuta et al. 2010; Rimet et al. 2012) and the multimetric benthic invertebrate index (MMI, Bis et al. 2013; Lewin et al. 2013). With regard to these elements, there is no problem of insufficient data collection, as the relevant data have been collected by all EU countries for several decades.

Determining the completeness of a sample is consistent with the necessity to integrate many monitoring actions in order to systematize and make rational use of environmental monitoring data. The general concept of a hierarchical perspective was proposed by Loga (2012) and assumed that elementary measurement errors would be considered via a hierarchical structure of procedures applied to define the water status in compliance with WFD.

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