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Taxonomic, phylogenetic and functional diversity of leeches (Hirudinea) and their suitability in biological assessment of environmental quality

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Abstract – Different components of biological diversity of leeches (Hirudinea), sampled in lowland watercourses in Poland were compared between sites differed in terms of environmental quality, measured by officially approved procedures. Indices based on taxonomic diversity, rarefied species richness, taxonomic distinctness, phylogenetic diversity and functional diversity were considered in the analysis, for sites belonging to three biocoenotic types of watercourses. In most cases values of all indices except Pielou's evenness show unimodal relationships with environmental quality and in any case monotonic increase in diversity was not shown. Median values of diversity were significantly higher and its highest values were significantly more frequent at sites with moderate than with low or high environmental quality. This pattern of relationships was observed both for indices based on presence/absence data and those based on relative abundance of species. The significance of this mode of relationships differed between biocoenotic types and between procedures using to measure environmental quality. The results obtained show low and doubtful usefulness of each component of leech diversity as an element of biological assessment in lowland watercourses.

Keywords: taxonomic diversity / phylogenetic diversity / functional diversity / Hirudinea / biological assessment

Résumé – Diversité taxonomique, phylogénétique et fonctionnelle des sangsues (Hirudinea) et leur aptitude à l'évaluation biologique de la qualité environnementale. Différentes composantes de la diversité biologique des sangsues (Hirudinea), échantillonnées dans les cours d'eau de plaine en Pologne, ont été comparées entre des sites différents en termes de qualité environnementale, mesurée par des procédures officiellement approuvées. Les indices fondés sur la diversité taxonomique, la richesse en espèces raréfiées, la diversité phylogénétique et la diversité fonctionnelle ont été pris en compte dans l'analyse, pour des sites de cours d'eau appartenant à trois types biocénotiques. Dans la plupart des cas, les valeurs de tous les indices, à l'exception de l'équitabilité de Pielou, montrent des relations unimodales avec la qualité de l'environnement et, en tout état de cause, une augmentation monotone de la diversité. Les valeurs médianes de la diversité étaient significativement plus élevées et les valeurs les plus élevées étaient significativement plus fréquentes dans les sites de qualité environnementale modérée plutôt que faible ou élevée. Ce patron de relations a été observé à la fois pour les indices fondés sur les données de présence/absence et ceux fondés sur l'abondance relative des espèces. L'importance de ce mode de relations différait entre les types de biocénoses et entre les procédures utilisées pour mesurer la qualité de l'environnement. Les résultats obtenus montrent que chaque composante de la diversité des sangsues est peu utile et douteuse en tant qu'élément de l'évaluation biologique dans les cours d'eau de plaines.

Mots-clés : diversité taxonomique / diversité phylogénétique / diversité fonctionnelle / Hirudinea / évaluation biologique

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1 Introduction

Environmental (ecological) quality (EQ) as a general term is a "set of properties and characteristics of the environment as they impinge on organisms" (European Environment Agency, 2012). When applied to aquatic ecosystems EQ is an overarching term coined by the Water Framework Directive (WFD, Directive 2000/60/EC of the European Parliament and of the Council – 2000) and is defined as a quality of structure and functioning of an ecosystem (Furse *et al.*, 2006). EQ can be also described as the inverse of the total effect of many factors constituting together the ecological impact or ecological stress observed in ecosystem. It can be quantified and used in biological assessment if such effects will be standardized and then compared with those under natural (reference) conditions (Hering *et al.*, 2003). Thus, it would appear reasonable to analyse the diversity of organisms in relation to environmental quality treated as a measure of the intensity of ecological stress.

Biological diversity of aquatic organisms is considered in WFD (Directive 2000) as being among the most important parameter characterizing structure and functioning of lotic environments and describing the ecological status and environmental quality of an ecosystem. The influence of different ecological stressors on biological diversity is one of the most important and most intensively researched the problems of modern ecology (Darling and Côté, 2008; Jackson *et al.*, 2016). Thus, the values of different measures of biological diversity can be treated both as the result of natural and anthropogenic ecological stress and as a parameter used to assess their intensity. The negative impact of such factors on biological diversity of benthic invertebrates in freshwater environments is frequently assumed in numerous studies (*e.g.* Reice, 1985; Lorenz *et al.*, 2004; Sánchez-Montoya *et al.*, 2010; Stranko *et al.*, 2012), but the lack of such direct effects was also found (*e.g.* Heino *et al.*, 2007).

On the other hand, certain well-known and widely discussed models try to describe the mechanisms of the commonly observed unimodal (hump-like, peaked) relationship observed in the gradient of different disturbing factors. The explanations of the mechanism leading to higher taxonomic richness at moderate levels of stressor intensity have been proposed by Collins and Barber (1986) and Collins *et al.* (1995) as the intermediate disturbance hypothesis, by Huston (1979) as the dynamic equilibrium model and later as other models by, *e.g.* Porter et al. (2001), Kondoh (2001), Tonkin *et al.* (2013). IDH seems to be a one of the most widely discussed modern concepts concerning biological diversity in unimodal relation to ecological impact (*e.g.* Wilkinson, 1999; Svensson *et al.*, 2012). While the critical approach to these models focuses primarily on showing weaknesses in predictive modelling of causal mechanisms describing relationships between populations and disturbing factors (*e.g.* Fox, 2013), specialists agree that the maxima of biodiversity typically occur at their intermediate intensity.

The reaction of different components of biological diversity to increase intensity of environmental stress in freshwaters can be carried out according to at least two different types of responses: monotonic decrease or unimodal. The type of response may be dependent (among the others) on the level of taxonomic identification (Wright *et al.*, 1995; Wright and Ryan, 2016), type of stressor (Gutiérrez-Cánovas *et al.*, 2013) and spatial scale of an analysis (Heino *et al.*, 2003). Determining which type of response is responsible for relationships between EQ at a sampling site and the diversity of different groups of benthic animals is important from the point of view of biological assessment. Monotonic decrease in abundance or diversity along the gradient of ecological stress is one of the most important reason why a given taxon has high indicative value (Hering *et al.*, 2006b). It seems that the unimodal relationship between environmental quality and the diversity of a taxon makes the latter unsuitable in biological assessment.

Most of the bioassessment methods based on the taxonomic composition, abundance and diversity of many groups of benthic fauna and Hirudinea are used as one of many measured metrics. The history of the use of diversity indices in freshwater monitoring is long and complex and involves numerous methods (Magurran, 2013), especially the diversity of benthic invertebrates has been expressed in biological assessment in different ways (Bailey *et al.*, 2004). Leeches are used as a group included in macrobenthos, which is the basic element of monitoring in freshwater habitats, as is required by WFD (Directive 2000/60/EC). Observations that particular taxa of Hirudinea have an indicative value in the biological assessment of environmental quality are common in the literature (Grosser *et al.*, 2001; Koperski, 2005; Kubová *et al.*, 2013; Kazanci *et al.*, 2015). Abundance, percentage and diversity of Hirudinea, treated as ones of moderately tolerant taxa are taken into account in practical bioassessment protocols (Armitage *et al.*, 1983). Leeches are often used as an element of standardized metrics (family richness, Shannon diversity index, ASPT index) clearly responding to organic pollution, changes in stream morphology, acidification and general environmental degradation (Bis and Mikulec, 2013). The level of pollution in lowland streams correlates stronger with changes in taxonomic diversity of leeches than of certain commonly occurred groups of benthic invertebrates like, *e.g.* Chironomidae and Odonata (Koperski, 2010). Due to the low species richness of European leeches, the relative easiness of their taxonomic determination and widespread occurrence in different types of watercourses, this group seems to be potentially very useful for the assessment of the ecological status of lotic environments. Phylogenetic relationships among leeches based on modern molecular methods seems to be relatively better understood than among many other groups of freshwater animals. However, erroneous taxonomic identification in Hirudinea impedes a clear understanding of their phylogeny and diversity. European leech species are highly divergent in terms of their ecological preferences: some of them are common and tolerant for water pollution (*e. g. Erpobdella octoculata*, *Glossiphonia complanata*) while other ones are more sensitive (*E. vilnensis*, *G. nebulosa*) or even treated as highly specialized (*Calliobdella mamillata*, *Trocheta bykowskii*). Indicative values of Hirudinea for purposes of biological assessment are typically determined on the level of family: in BMWP method (Wright *et al.*, 1989), in its Polish modification BMWPpl (Kownacki and Soszka, 2004) and in recently developed official Polish index MMI (Bis and Mikulec, 2013).

Many indices of diversity allow us to compare different aquatic communities in different types of environments, but their divergence makes biological diversity difficult to quantify for the purposes of biological assessment. Treating species as being equal in spite of their functional divergence and phylogenetic distance seems to be an important weakness of traditional measures of taxonomic diversity (Schweiger *et al.*, 2008) and the use of taxonomic diversity indices based on identification to a level higher than the species is prone to different errors (Koperski, 2010, 2011; Šiling and Urbanič, 2016). Three groups of methods have been developed to solve these problems: taxonomic distinctness (*e.g.* Warwick and Clarke, 1995), phylogenetic diversity based on mutual evolutionary relationships between species (*e.g.* Faith and Baker, 2006) and functional diversity based on the analysis of the biological traits of benthic animals (*e.g.* Usseglio-Polatera *et al.*, 2000; Statzner *et al.*, 2005). These components of diversity provide certain additional value to theoretical and applied ecology (Mason *et al.*, 2005; Schweiger *et al.*, 2008). Vamosi and Vamosi (2007) and Carew *et al.* (2011) suggest that biological assessment of freshwater environments based on phylogenetic data on certain smaller taxa seems to be potentially effective and reliable.

The main aims of the study were:

- to determine the modes of relationships between diversity of leeches in lowland watercourses and environmental quality;
- to determine the suitability of leeches' diversity for the purposes of biological assessment on the basis of the modes of these relationships.

The most probable modes of distribution of the diversity along the gradient of environmental quality are: (i) monotonic – the higher EQ, the higher diversity and (ii) unimodal – the highest level of diversity observed at sites with moderate EQ. The study was also aimed into searching and comparing the differences in these relationships between

- different components of diversity, those measured by traditional diversity indices as well as taxonomic distinctness, phylogenetic diversity and functional diversity;
- different types of lowland watercourses.

2 Methods

The analysis was performed on the basis of data on benthic invertebrates collected from 268 sites at watercourses on territory of Poland (Fig. 1). Samples were collected once at each site between 2010 and 2012 by Regional (Voivodship) Inspectorates of Environmental Protection (RIEP) and by the scientific team cooperating with the National Foundation of Environmental Protection, Warsaw, Poland. Sampling was performed in accordance with the procedure recommended after minor modifications under the name RIVECO to assess the ecological status of flowing waters and conform with the requirements of the WFD (Bis and Mikulec, 2013). Leeches were separated from the samples and then identified on the basis of the key by Nesemann and Neubert (2000). All individuals were identified to species level. Only samples including at least two individuals belonging to at least two species were taken into consideration to analysis.

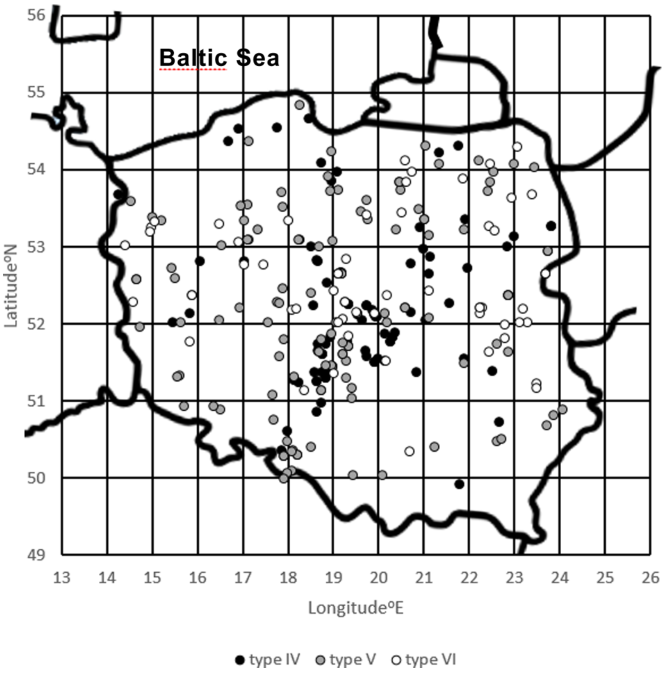

Fig. 1. Map of Poland showing 268 sampling sites, belonging to the three biocoenotic types.

The ecological status at each sampling site was taken from the data-bases available on the RIEP's websites (available at web portal http://www.gios.gov.pl/). The classification is based on the results of biological assessment expressed by the values of BMWPpl and MMI indices as they occurred between 2010 and 2012. Each sampling site was classified to adequate one of 26 abiotic types of watercourses described for Poland, and then classified into one of the three biocoenotic types of watercourses (type IV – lowland sandy streams, 94 sites; type V – estuarine rivers and lowland streams and rivers other than in types IV and VI, 114 sites; type VI – lowland streams and rivers with organic bottoms and lowland streams and rivers connecting lakes, 60 sites) chosen from six types described for Poland (Bis and Mikulec, 2013) on the basis of RIEPs' databases (Fig. 1). All analyses were performed separately for each biocoenotic type.

Data on the geographical variables (parameters) at each site: latitude, longitude, altitude and distance from the site to the source have been obtained from the Geoportal website (http://mapygeoportalgovpl/).

2.1 Biological diversity

Five components of biological diversity (1–5) were taken into account to assess its relationships with EQ. Nine indices describing leech diversity were calculated for each sample:

- Richness – to assess species richness in samples with different sizes, containing data on abundances Krebs' individual rarefaction procedure (IR) was used (PAST software) while to assess rarefied number of species in groups of samples containing presence/absence data procedure of Mao Tau sample rarefaction (SR) was applied (PAST software).

Q2 **Table 1.** Taxonomic composition of leeches sampled in the studied sites, classified to three biocoenotic types and presented as the frequency of occurrence. Basic parameters characterizing sites are added.

Species	Biocoenotic type			Total
	IV	V	VI	
Alboglossiphonia heteroclita (L.)	9.6	7.9	16.7	10.4
Alboglossiphonia hyalina (O.F. Müller)	0	0.9	0	0.4
Alboglossiphonia striata (Apáthy)	0	2.6	3.3	1.9
Caspiobdella fadejevi (Epshtein)	0	3.5	0	1.5
Dina apathyi Gedroyć	0	0.9	0	0.4
Dina lineata (O.F. Müller)	1.1	2.6	3.3	2.2
Erpobdella monostriata (Lindenfeld et Pietruszyński)	1.1	0.9	1.7	1.1
Erpobdella nigricollis (Brandes)	37.2	40.4	33.3	37.7
Erpobdella octoculata (L.)	97.9	94.7	66.7	89.6
Erpobdella testacea (Savigny)	4.3	3.5	5.0	4.1
Erpobdella vilnensis Liskiewicz	21.3	13.2	41.7	22.4
Glossiphonia complanata (L.)	52.1	43.0	10.0	38.8
Glossiphonia concolor (Apáthy)	12.8	13.2	10.0	12.3
Glossiphonia nebulosa Kalbe	13.8	10.5	0	9.3
Glossiphonia paludosa (Carena)	1.1	0	1.7	0.7
Haemopis sanguisuga (L.)	22.3	10.5	11.7	14.9
Helobdella stagnalis (L.)	34.0	44.7	38.3	39.6
Hemiclepsis marginata (O.F.Müller)	7.4	8.8	10.0	8.6
Piscicola geometra (L.)	2.1	3.5	1.7	2.6
Placobdella costata (Fr. Müller)	2.1	2.6	1.7	2.2
Theromyzon tessulatum (O.F. Müller)	1.1	2.6	5.0	2.6
Species richness (range/median)	2–9/3	2–8/3	2–7/3	21
Abundance (range/mediane)	3–314/17	3–202/11	3–184/12	
Individuals	2574	2531	1562	6667
BMWPpl (range/median)	18–129/54.5	18–165/74.5	14–140/70	
MMI (range/median)	0.086–0.968/0.481	0–0.942/0.492	0.016–0.904/0.532	
Number of samples	94	114	60	268

- Species diversity – two widely known and commonly used indices of diversity were chosen: Shannon index and Pielou evenness index J′.
- Distinctness – two indices based on number of taxonomic levels between pairs of species in a sample were used: taxonomic diversity index (TDI, Clarke and Warwick, 1998, PAST software) to analyze data on species abundance while the net relatedness index (NRI) (Webb, 2000) was applied to analyze presence/absence data.
- Phylogenetic diversity – estimated genetic distances between leech species were revealed on the basis of the distance matrix constructed on the basis of previously published data. The distance between each pair of species was determined on the basis of the standardized, relative average or resultant distance between species presented by Siddall and Burreson (1998), Apakupakul *et al.* (1999), Utevsky and Trontelj (2004), Siddall *et al.* (2005), Rousset *et al.* (2007), Oceguera-Figueroa *et al.* (2011), Kaygorodova and Mandzyak (2014), and Kaygorodova *et al.* (2014). For this purpose distances were estimated only on the basis of individuals collected in Central or Northern Europe. The average values of genetic distance within closely related group of species was applied in case of the lack of published data on distance between certain, relatively rare species within genera *Alboglossiphonia* (*A. hyalina*, *A. striata*) and *Glossiphonia* (*G. nebulosa*, *G. paludosa*). Mean phylogenetic distance (MPD) between pair of species in a sample (Vellend *et al.*, 2011) was applied to analyze presence/absence data while Rao's quadratic entropy (QE) (Botta-Dukát, 2005) calculated for estimated phylogenetic distance was applied to analyse data on species abundance. To calculate this index software package FunctDiv was used (Lepš *et al.*, 2006).
- Functional diversity – value of Rao's index (RAO) for each sample based on functional traits dissimilarity was calculated using software package FunctDiv (Lepš *et al.*, 2006). For this purpose values of eight traits was determined for each species: diet, feeding mode, width of the feeding niche, parental care, amphibiotic life style, size of the body, morphological variability, habitat specialization.

To calculate values of taxonomic distinctness and phylogenetic diversity two software procedures were created in Visual Basic (Koperski and Meronka, 2017) and applied into the data base in the Excel 2013 software package, Microsoft Office. Values of all indices are presented as the first, second (median) and third quartile, percentile 0.9 and maximal value in each group of sites, independently for each biocoenotic type of watercourses. Groups contain sites in classes of 0.1 MMI or 10 points of BMWPpl (*e.g.* 0.11–0.20, 0.21–0.30 MMI and 21–30 BMWPpl). The limits separating low, moderate and

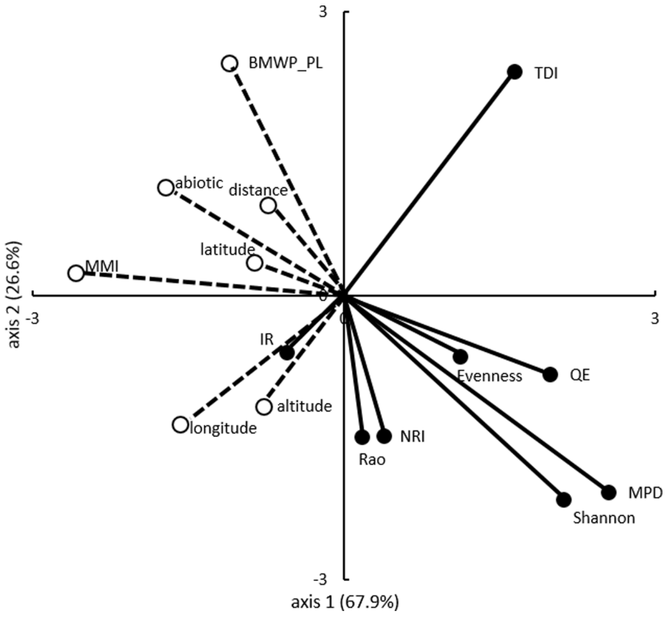

Fig. 2. Results of canonical correspondence analysis presented as an ordination map, where the components of leech diversity are shown as vectors pointed with black circles and environmental parameters and metrics shown as vectors pointed with white circles. Percentages of variance explained by each axis (canonical function) are added.

high values of BMWPpl and MMI were established for sites of each biocoenotic type on the basis of relative number of sites included into groups. In IVth type of watercourses moderate values of EQ range between 30 and 80 BMWPpl and between 0.3 and 0.7 MMI, in Vth type between 40 and 100 BMWP and between 0.3 and 0.8 MMI and in VIth type between 40 and 90 BMWPpl and between 0.4 and 0.8 MMI.

2.2 Numerical analysis

Relationships obtained between different components of leech diversity in samples and environmental parameters (indices expressing environmental quality, abiotic type and the geographical parameters of altitude, longitude, latitude and distance from the source to the sampling site) were assessed with canonical correspondence analysis (CCA, PAST software). It was performed to show the relative importance of relationships between and among variables divided into two groups: metrics characterizing leech diversity and geographical/environmental parameters describing sampling sites (Legendre and Legendre, 2012).

2.3 Statistical analysis

Values of different diversity indices were compared between sites belonging to groups with moderate values of EQ indices and those belonging to groups with extreme (over and under limits) values (PAST software). Mann–Whitney's test was used to compare medians while the contingency tables approximated with Chi^2 function were used to compare number of values belonging to percentile 0.9 between groups of moderate and extreme values of EQ.

3 Results

In 268 samples analyzed in this study 6667 individuals were found and identified to 21 species. During the study, four wide spread species were detected, among which *E. octoculata* was the most common, being collected in *ca.* 90% of samples, while three other species occurred with frequency more than 30% (*Erpobdella nigricollis*, *G. complanata*, *Helobdella stagnalis*). Six species should be treated as rare (*Dina apathyi*, *Alboglossiphonia hyalina*, *A. striata*, *Caspiobdella fadejewi*, *Erpobdella monostriata*, *Glossiphonia paludosa*) because their frequency of occurrence did not range 2% (Tab. 1).

Results of CCA shows that the model based on the first and second canonical functions explains big part (90.5%) of total variance, moreover both functions were highly significant when tested with permutation test ($p < 0.03$ and $p < 0.001$, respectively, PAST software). Both measures of EQ: BMWPpl and MMI as well as the longitude and abiotic typology of the watercourses were the most important environmental variables included into the model, while Shannon index, mean phylogenetic diversity and distinctness (TDI) were the most important metrics describing diversity (Fig. 2). Variability of longitude and altitude were independent on other environmental variables while variability of rarefied species richness (IR) and TDI were independent on other components of diversity.

To show the differences in estimated species richness between groups of sites (Fig. 3) the curves describing best fitted logarithmic estimation were designed, each one with fit accuracy (R^2) higher than 0.964. Species richness estimated with SR for biocoenotic types IV and V were significantly higher at sites with moderate values of BMWPpl and MMI (Kolmogorov–Smirnov's test, PAST software: $p < 1 \times 10^{-14}$ type IV, BMWPpl; $p < 1 \times 10^{-5}$ type IV, MMI; $p < 0.0112$ type V, BMWPpl; $p < 0.025$ type V, MMI) while for type VI differences between groups with extreme and moderate values of EQ were non-significant.

In most cases median, third quartile and maxima of diversity indices show unimodal relationships with EQ: Shannon diversity (Fig. 4), taxonomic distinctness (Fig. 5), phylogenetic diversity (Fig. 6) and functional diversity (Fig. 7). Low values of diversity components were not dependent on the values of EQ, thus the distributions of the values of the diversity indices were, in most cases typically bell-shaped. In any case median and maximal values of any index of diversity did not increased monotonically along with the values of EQ. In most cases median values of the diversity indices and were significantly higher (65 of 84 comparisons) and their very high values (higher than percentile 0.9) were significantly more frequent (74 of 84 comparisons) at sites with moderate level of EQ (Tab. 2). The level of difference in particular components of diversity and the level of statistical significance between moderate and extreme values of EQ were visibly dependent on typological classification of the sites. Evenness was the only index which did not depend clearly on EQ in any biocoenotic type of watercourses (Fig. 4). All other indices used to measure diversity differed significantly between moderate and extreme values of environmental quality measured by BMWPpl and MMI in biocoenotic type V and those measured by BMWPpl in type IV (Tab. 2). Only indices of phylogenetic and functional diversity differed

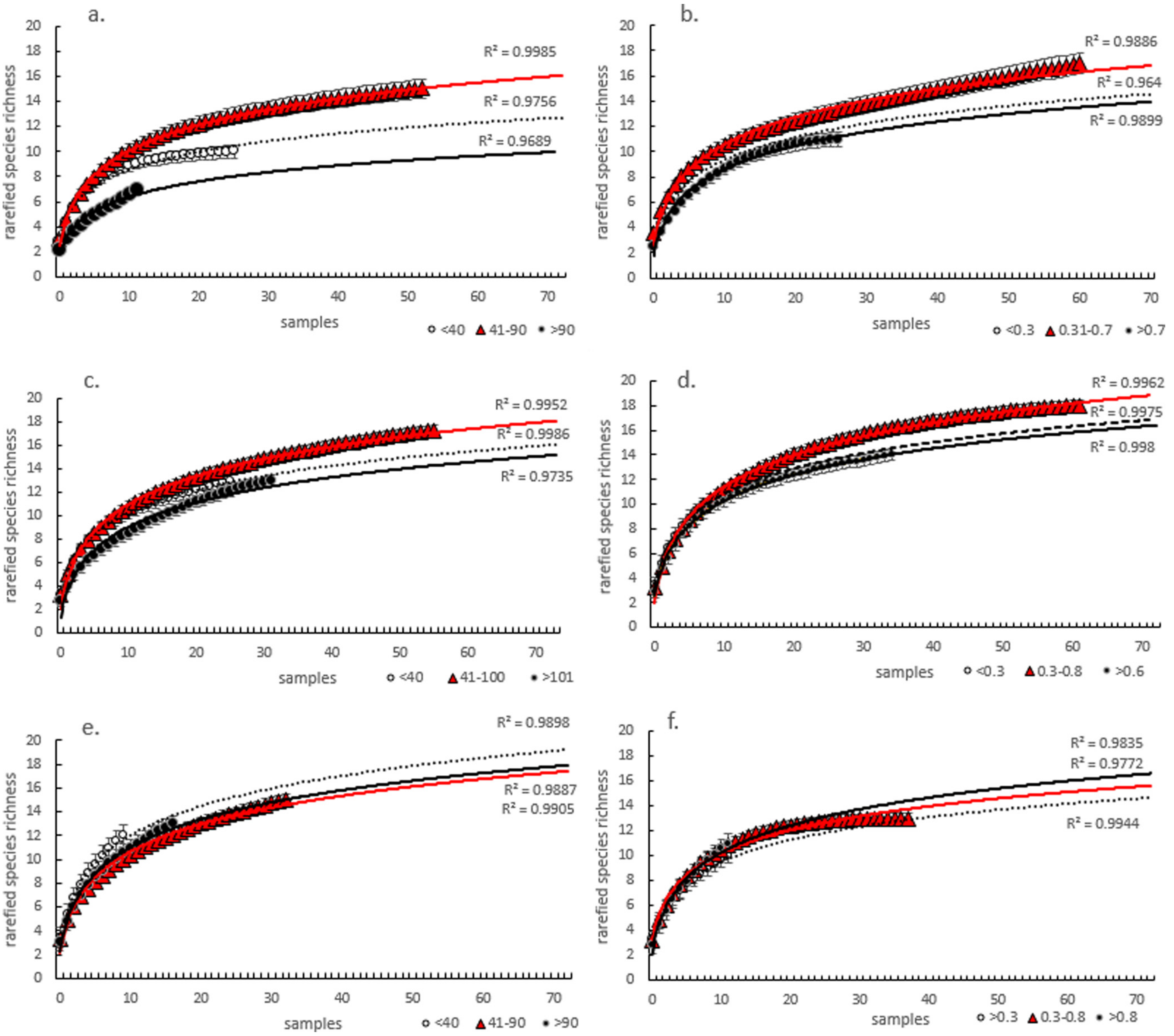

Fig. 3. Values of rarefied species richness with standard deviation (sample rarefaction with Mao Tau estimation) for groups of sites differed in terms of biocoenotic type (IV – a, b; V – c, d; VI – e, f), method of quality assessment (BMWPpl and MMI) and values of environmental quality (white circles – low, triangles – moderate, black circles – high). Values of fit accuracy of logarithmic regression curves are presented as R^2. Differences in richness between sites with moderate and extreme values presented on plots (a)–(d) are statistically significant (Kolmogorov–Smirnov's test).

significantly between moderate and extreme values of MMI in type IV (Tab. 2). Median values of Shannon diversity and QE differed significantly between sites with moderate and extreme values in biocoenotic type VI but only when EQ was measured by BMWPpl – in case of MMI those differences in any components of diversity was not significant (Tab. 2). Levels of statistical significance were higher for phylogenetic diversity than for other components in type IV, but higher for distinctness than for other components in type V (Tab. 2). When EQ was assessed with values of MMI at sites classified to type VI median values of any indices of diversity did not distributed unimodally (Figs. 4–7f) and their considerable variability seems to indicate other environmental factors than EQ as a determinants of diversity. Numbers of the values higher than percentile 0.9 of almost all indices (except evenness) were significantly higher at sites with moderate than extreme values of EQ in all biocoenotic types.

4 Discussion

Species richness observed in studied watercourses – most common 3–5 species per sample and 17–20 species per each biocoenotic type seem to be typical for aquatic environments in Central Europe (Nesemann and Neubert, 1999; Koperski, 2006). In Polish freshwater environments 47 species of Hirudinea (Euhirudinea) were found (Bielecki *et al.*, 2011), but

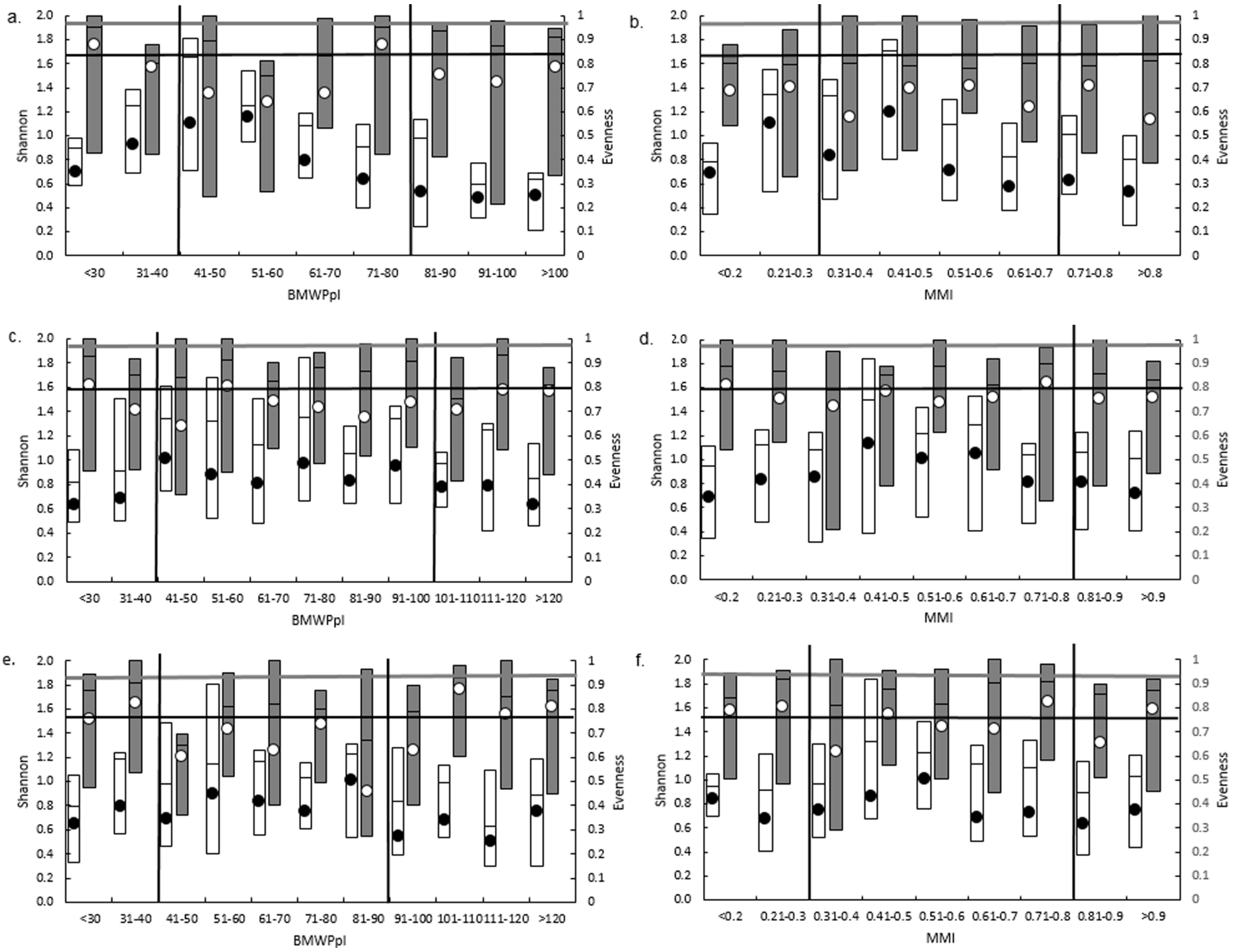

Fig. 4. The relationships between environmental quality (BMWPpl and MMI) and taxonomic diversity of leeches, expressed by the values of Shannon diversity index (white bars) and Pielou's evenness index (grey bars) for groups of sites differed in terms of biocoenotic type (IV – a, b; V – c, d; VI – e, f). First quartile, third quartile and maximal value are shown on the bar and median values are presented as the circles. Vertical lines separate moderate and extreme values of EQ, while horizontal lines mark percentile 0.9 of both indices. Statistical significance between sites with moderate and extreme values of EQ is presented in Table 2.

the taxonomic status of certain morphological distinctive forms, mainly those belonging to family Piscicolidae are debatable (Bielecki, 1997). Nevertheless 21 species of leeches sampled in analyzing watercourses does not constitute all what one might have expected even if we ignore few species with strong preferences for standing waters. It must be emphasized that the method of sampling used in this study was not adequate for determining the abundance and diversity within the fish-leeches, family Piscicolidae – a group of obligatory fish parasites that should be analyzed on the basis of fish sampling in springtime. This may be the explanation of the relative rarity and low taxonomic richness of this group observed in sampled material. In general, the ecological preferences of particular species of piscicolid leeches remain poorly understood (Jueg *et al.*, 2004).

Detailed data on the main geographic parameters have been taken into account as the fundamental factors for the classification of polish watercourses into biocenotic and abiotic types (Błachuta *et al.*, 2010), consistently with the methodology recommended in other countries of the European Union (Bis and Usseglio-Polatera, 2004; Furse *et al.*, 2006) and analogously to the hierarchical classification made for biological assessment (*e.g.* Frissell *et al.*, 1986). A significant effect of abiotic typology and longitude on leech assemblages, found in this study reflects abiotic diversity within the same biocoenotic type. Weaker relationships between diversity and EQ observed in the watercourses of type VI may lead to a conclusion that this biocoenotic type is too divergent to be treated as a homogenous category. In fact, type VI contain highly disparate abiotic types of watercourses, independent on classification into ecoregions: either small streams with organic substrate, streams and rivers impacted by peatlands or rivers connecting lakes. The effect of longitude on diversity, more important than other geographic parameters probably reflects serious climatic differences between the western part of Poland (prevalence of the maritime type of climate) and an eastern one (prevalence of the continental type of climate).

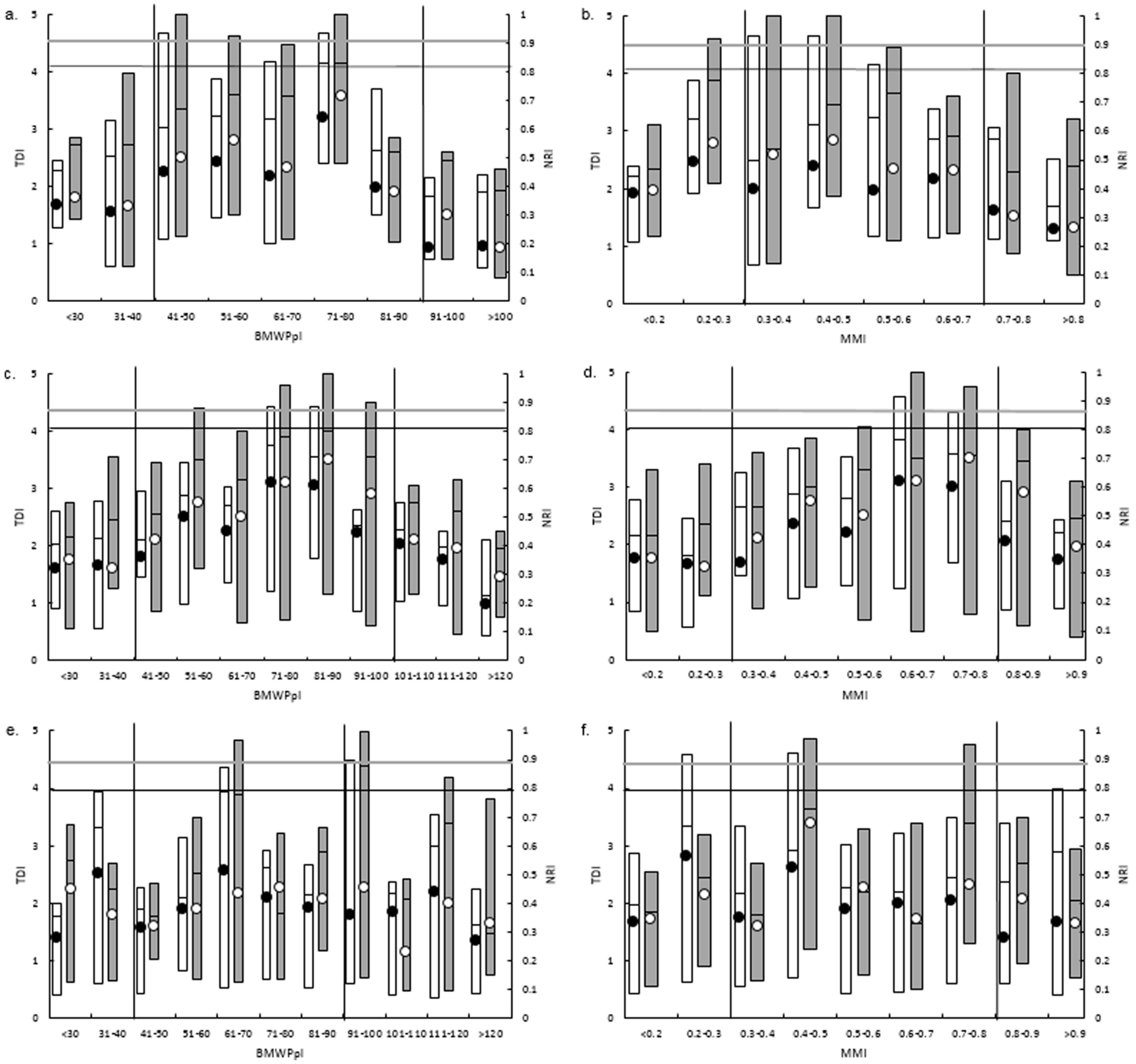

Fig. 5. The relationships between environmental quality (BMWPpl and MMI) and taxonomic distinctness of leeches, expressed by the values of taxonomic diversity index (TDI – white bars) and net relatedness index (NRI – grey bars) for groups of sites differed in terms of biocoenotic type (IV – a, b; V – c, d; VI – e, f). First quartile, third quartile and maximal value are shown on the bar and median values are presented as the circles. Vertical lines separate moderate and extreme values of EQ, while horizontal lines mark percentile 0.9 of both indices. Statistical significance between sites with moderate and extreme values of EQ is presented in Table 2.

Habitat stability and predictability as the combined effects of natural disturbance in freshwater environments were shown by Death and Winterbourn (1994, 1995) as important factors shaping the diversity of benthos in streams (Townsend *et al.*, 1997). The negative relationship of invertebrate diversity with the level of degradation was observed in numerous studies, but it concerns mainly higher than species taxonomic levels of identification (Cortelezzi *et al.*, 2013; Gutiérrez-Cánovas et al., 2013; Johnson and Angeler, 2014), *e.g.* family richness, calculated for benthic invertebrates strongly correlates positively with environmental quality in different watercourses (*e.g.* Barbour *et al.*, 1996; Koperski and Meronka, 2017) being one of the most important metric used to assess their ecological status (*e.g.* Armitage *et al.*, 1983; Bis and Mikulec, 2013). The relationships observed in present study involving identification to the species level were completely different but it would not surprise. Level of family in case of Hirudinea seems to be too coarse to bring information suitable in terms of biological assessment. Substantial differences in ecological preferences of leech species representing the same families and even the

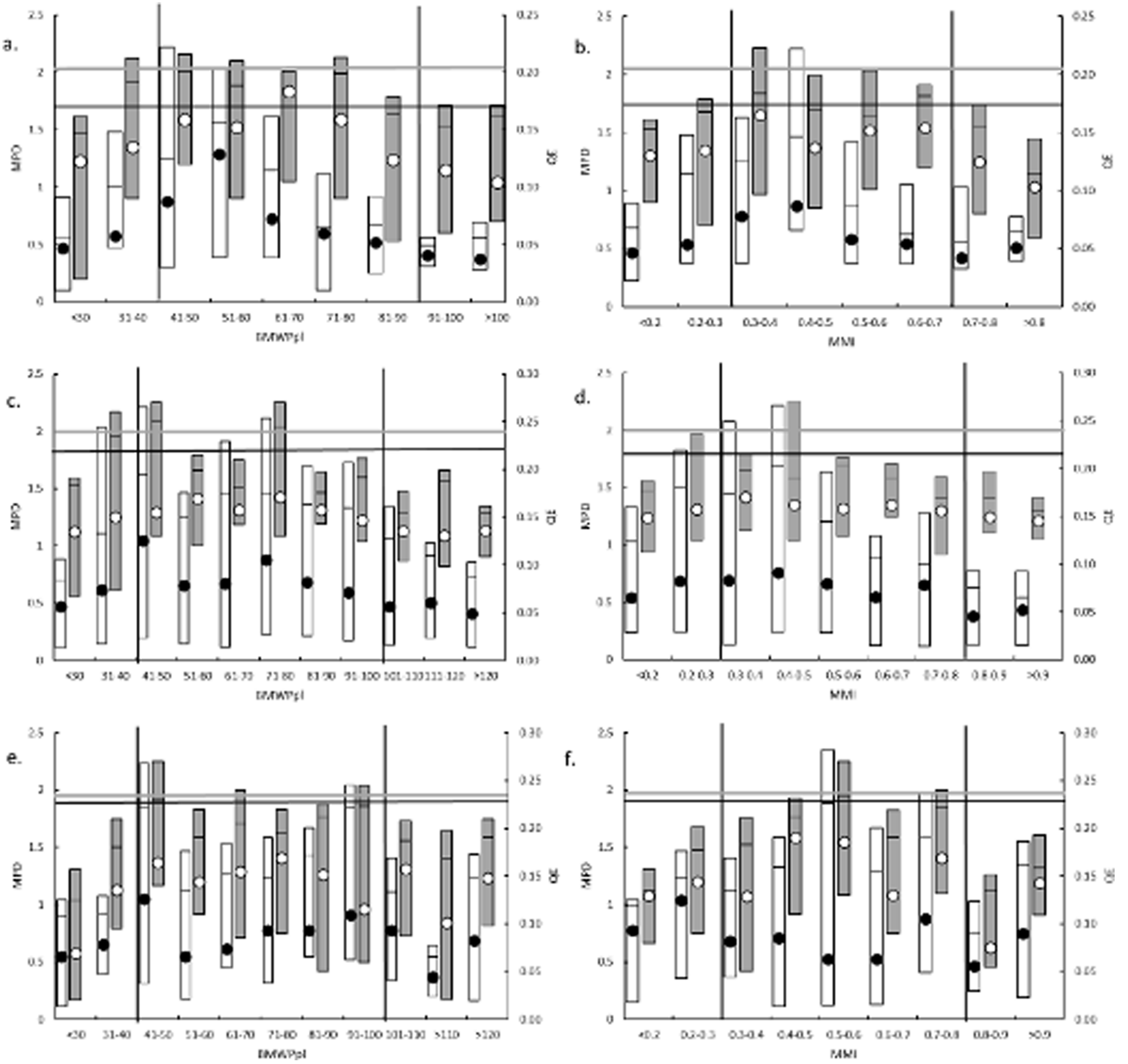

Fig. 6. The relationships between environmental quality (BMWPpl and MMI) and phylogenetic diversity of leeches, expressed by the values of mean phylogenetic distance (MPD – white bars) and quadratic entropy (QE – grey bars) for groups of sites differed in terms of biocoenotic type (IV – a, b; V – c, d; VI – e, f). First quartile, third quartile and maximal value are shown on the bar and median values are presented as the circles. Vertical lines separate moderate and extreme values of EQ, while horizontal lines mark percentile 0.9 of both indices. Statistical significance between sites with moderate and extreme values of EQ is presented in Table 2.

genera are well known and well described (*e.g.* Koperski, 2006; Kubová *et al.*, 2013). *E. nigricollis*, *T. bykowskii* and *Dina lineata* all belonging to the same family *Erpobdellidae* but inhabiting completely different habitats as well as the case of two species of *Glossiphonia*: reophilic *G. nebulosa* and pond-preferred *G. paludosa* are also illustrative examples. The observations, that informative value of sampling for the purposes of bioassessment decreasing along with the decrease in the level of identification have been commonly presented (*e.g.* Verdonschot, 2006). Contrary to that, suggestions that using of higher taxa in accordance with the procedure of “taxonomic surrogacy” provide information valuable enough are even more numerous (Bournaud *et al.*, 1996; Marshall *et al.*, 2006). An extensive discussion of the pros and cons of both concepts presents Jones (2008) and Koperski (2011).

The mode of distribution along with the EQ gradient in the present study was unambiguously bell-shaped for almost all metrics of diversity. It should be interpreted as typically unimodal relationships deformed by the results of numerous samples collected at sites differed in terms of environmental

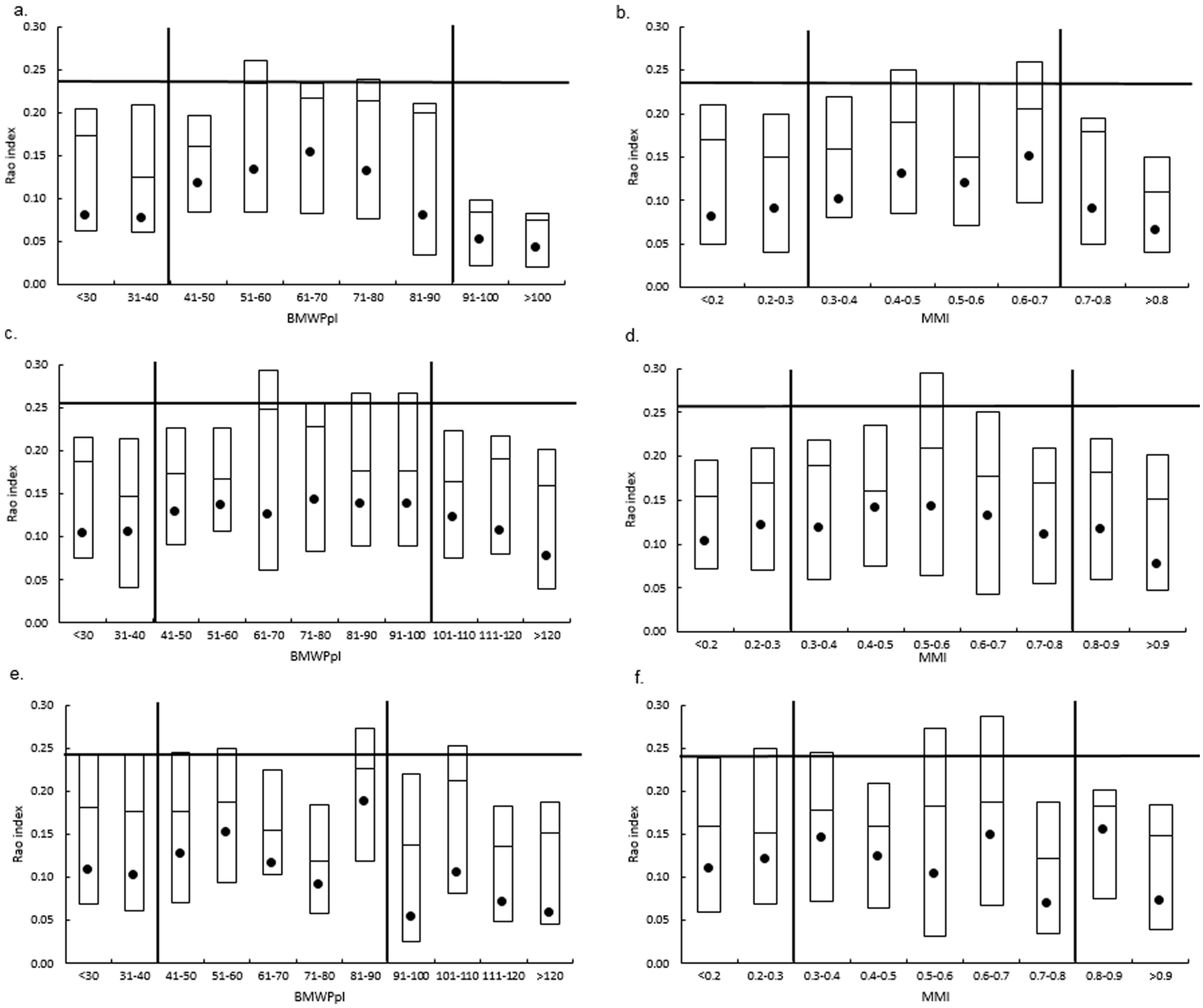

Fig. 7. The relationships between environmental quality (BMWPpl and MMI) and functional diversity of leeches, expressed by the values of Rao's index for groups of sites differed in terms of biocoenotic type (IV – a, b; V – c, d; VI – e, f). First quartile, third quartile and maximal value are shown on the bar and median values are presented as the circles. Vertical lines separate moderate and extreme values of EQ, while horizontal lines mark percentile 0.9 of both indices. Statistical significance between sites with moderate and extreme values of EQ is presented in Table 2.

quality, but with a very small number of individuals and low species richness. It should be added that this kind of bias is difficult to eliminate being a result of the sample-processing method. Sampled animals were selected randomly for further analysis using fixed-count method, in accordance with the recommended procedure (procedure RIVECO – Bis and Mikulec, 2013), required at least 350 animals randomly chosen using subsampling. Thus, as a result, leeches were found in small number in those samples which were relatively high abundances of other taxonomic groups like midge larvae or mayfly larvae.

This mode of distribution seems to confirm accuracy of concepts by Collins and Barber (1986) or Tonkin (2013). Models underlying these theories can explain the mechanisms of settlement by the species with different ecological requirements and preferences of the fragments of watercourses with different intensities of disturbance and productivity and their extinction therein. In any case monotonically negative distribution has not been shown in the present study, which means that values of diversity observed at sites with moderate quality were higher that at sites with very high quality. This monotonic relation seems to be expected in certain procedures of biological assessment based on macrobenthos identified mainly to the level of genera (*e.g.* Hering *et al.*, 2006a). Bini *et al.* (2014) found a unimodal distribution mode for the three indices of diversity against nutrient enrichment when stream invertebrates were identified to the genus level. It is not clear which mode of relation between other components of diversity and EQ, unimodal or monotonic is typical for samples identified to the genus level – it is obviously requires further research.

Table 2. Results of statistical analysis of the differences in leech diversity, expressed by seven indices between groups of sites differed in terms of environmental quality, expressed by the values of BMWPpl and MMI. Analysis is presented as the results of Mann–Whitney test (differences in median values between groups, values of function U and p level are shown) and contingency tables (differences in number of values higher than percentile 0.9 between groups, Chi^2 estimation and p level are shown). Results are presented independently for each biocoenotic type.

Comparison	Type IV BMWPpl 41–90, $N=62$ *vs* BMWPpl <40, >90, $N=32$				Type V BMWPpl 41–100, $N=61$ *vs* BMWPpl <40, >100, $N=53$				Type VI BMWPpl 41–90, $N=30$ *vs* BMWPpl <41, >90, $N=30$			
Result diversity index	U	$p<$	Chi^2	$p<$	U	$p<$	Chi^2	$p<$	U	$p<$	Chi^2	$p<$
Shannon	995	0.049	5.71	0.017	1153	0.029	8.120	0.004	292	0.0462	4.140	0.042
Evenness	808	ns	1.39	ns	1314	ns	0.307	ns	360	ns	0.387	ns
TDI	710	0.012	5.71	0.017	1138	0.022	4.248	0.039	287	ns	5.554	0.025
NRI	722	0.016	5.71	0.017	1112	0.017	4.930	0.026	289	ns	4.140	0.042
MPD	477	10^{-5}	8.29	0.004	1157	0.033	4.960	0.028	301	ns	4.140	0.042
Qent	638	7×10^{-5}	8.29	0.004	1181	0.041	4.248	0.039	299	0.0499	4.140	0.042
RAO	784	0.021	4.84	0.019	1175	0.042	4.248	0.039	306	ns	0.387	ns

Comparison	Type IV MMI 0.3–0.7, $N=66$ *vs* MMI <0.3, >0.7, $N=28$				Type V MMI 0.3–0.8, $N=82$ *vs* MMI <0.3, >0.8, $N=32$				Type VI MMI 0.3–0.8, $N=42$ *vs* MMI <0.3, >0.8, $N=18$			
Result diversity index	U	$p<$	Chi^2	$p<$	U	$p<$	Chi^2	$p<$	U	$p<$	Chi^2	$p<$
Shannon	809	ns	3.22	0.042	1221	0.033	10.11	0.001	390	ns	4.071	0.032
Evenness	829	ns	1.01	ns	1499	ns	1.499	ns	407	ns	0.532	ns
TDI	856	ns	5.71	0.017	1182	0.041	4.977	0.026	291	ns	1.554	ns
NRI	888	ns	1.13	ns	1186	0.042	4.248	0.039	310	ns	4.140	0.042
MPD	500	10^{-5}	5.78	0.016	1342	0.017	3.891	0.047	409	ns	4.140	0.042
Qent	699	0.021	3.53	0.038	1419	0.032	4.977	0.026	251	ns	4.140	0.042
RAO	780	0.039	3.53	0.038	1189	0.048	3.896	0.048	294	ns	1.304	ns

Degrading factors present in studied streams were, without any doubts, very different as well as the mechanisms leading to decrease in their environmental quality. Unimodal distributions of diversity indices were repeatedly observed along with differences of divergent types of environmental degradation: catchment urbanization (Walsh *et al.*, 2007) and nutrient loading (productivity – Rosenzweig, 1995; Svensson *et al.*, 2007). These effects can be considered by analogy to IDH and disturbance heterogeneity (Porter *et al.*, 2001) models. Nevertheless, Mackey and Currie (2001) concluded that unimodal relationships have the greatest odds of being observed when sampled area are small, when disturbances were classified as natural rather than anthropogenic in origin, and when few disturbance levels were examined.

Intensive discussion on the relative importance and suitability of particular components of macrobenthos diversity in assessment of different environmental stressors is probably still far from complete. The basic measure of biological diversity – rarefied species richness have been described as unimodally related with different types of environmental disturbance (Fore *et al.*, 1996; Townsend *et al.*, 1997; Gallardo *et al.*, 2011); in the present study their values were also higher at moderate values of EQ. The same unimodal mode of relationship was observed also in each of the applied measures of diversity except of evenness. This index turned out to be weaker predictor of environmental quality and it had been previously stated by Townsend *et al.* (1997), Mackey and Currie (2001), and Johnson and Angeler (2014).

Indices based on the analysis of the biological traits of benthic animals (Functional Diversity) and those based on mutual phylogenetic relationships between species (Warwick and Clarke, 1995; Faith and Baker, 2006) are considered the most promising in prediction of environmental alterations. Charvet *et al.* (2000), Usseglio-Polatera *et al.* (2000), and Gayraud *et al.* (2003) indicate the enhanced usefulness of functional diversity in the biological assessment of different European watercourses when compared with other measures of diversity of benthic animals. Roque *et al.* (2014) found noticeable differences between traditional diversity measures and distinctness in prediction abilities to different environmental variables. This lack of congruence may be related to the fact that distinctness expresses long-term evolutionary adaptation to ecosystem conditions, while the other traditional biodiversity metrics respond to short-term environmental changes. Taxonomic distinctness, as suggested by Gallardo *et al.* (2011) provide especially valuable information characterizing ecosystem quality, while Shannon diversity and taxonomic richness generate important information on ecosystem conditions. Faith *et al.* (2009) conclude that phylogenetic diversity indices appear to be much better predictors of EQ than traditional taxonomic indices, however the use of more advanced and precise methods to assess the phylogenetic relationships among benthic animals, *e.g.* taking into account the relative abundance of taxa would further increase the benefits of their application (Allen *et al.*, 2009). Mouchet *et al.* (2010) conclude that functional diversity indices have the potential to reveal the processes that structure biological communities but when combined with phylogenetic and taxonomic diversity it will help improve our understanding of how biodiversity interacts with ecosystem processes and environmental constraints.

In some cases, the analysis of the usefulness of diversity indices to assess the degradation seems questionable because of the extremely discontinuous distribution of environmental quality even if Authors confirmed their high usefulness in the assessment of anthropogenic degradation of watercourses (Göthe *et al.*, 2015; Saito *et al.*, 2015). When relationships between diversity and environmental quality is unimodal or U-shaped and EQ can be classified only to 2 classes accurate statistical comparison seems to be doubtful.

My results show that almost all components of biological diversity present mainly unimodal mode in response to differences in EQ. It includes traditional taxonomic indices, distinctness, functional and phylogenetic diversity and both those reflecting the relative abundance of leech species and those based on presence/absence data. Those responses were better visible in the watercourses of IV and V biocoenotic types. This conclusion seems to be interesting and can have a practical application in bioassessment but it certainly needs further research. The results suggest that all components of leeches diversity have no or at most low suitability in biological assessment of environmental quality in lowland watercourses. The use of their taxonomic compositions for these purposes also appears to be unsuitable due to strong numerical dominance of eurytopic and tolerant species in leech assemblages.

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