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Composition of fish communities and fish-based method for assessment of ecological status of lakes in Lithuania

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ABSTRACT

Multiyear data collected in Lithuanian lakes (Europe ecoregion 15) using standardized methods formed the basis for an analysis to determine interrelations between lake fish community composition and environmental variables. Mean and maximum depths have significant impact on fish community structure in Lithuanian lakes, therefore lakes were classified into polymictic, stratified and deep stratified. The relative abundance of stenothermic fishes such as vendace (*Coregonus albula*) and burbot (*Lota lota*) was found to correlate positively with lake depth, while tench (*Tinca tinca*), rudd (*Scardinius erythropthalmus*) and bream (*Abramis brama*) correlated negatively. Nutrient concentration in lakes positively correlated with roach (*Rutilus rutilus*) and bream abundance and negatively with abundance of perch (*Perca fluviatilis*). In different types of lakes only seven non-redundant candidate fish metrics showed a significant correlation with variables describing human pressure. Those metrics were used to develop fish-based method for the assessment of the ecological status of lakes — the Lithuanian lake fish index LEZI (*Lietuvos Ezeru Zuvu Indeksas*). In all types of lakes, LEZI values most significantly correlate with the concentration of chlorophyll a and Secchi depth.

1. Introduction

Fish are highly responsive to changes in the trophic status of lentic water bodies (Persson et al., 1991; Jeppesen et al., 2000; Mehner et al., 2005; Garcia et al., 2006) and serve as indicators of changes in the hydromorphological status of lakes (Launois et al., 2011). The European Union (EU) water policy recommends fish as a biological quality element for the assessment of the ecological status of lentic waters (European Commission, 2000). Up to now, a number of fish-based systems have been developed for the assessment of European lakes (Appelberg et al., 2000; Gassner et al., 2003; Holmgren et al., 2007; Rask et al., 2010; Launois et al., 2011; Volta et al., 2011; Kelly et al., 2012). Equally a fish-based index designed to assess the eutrophication status of lakes at the European scale was developed based on the fish data collected using a standardized protocol (Argillier et al., 2013). However, data for development of this index were mainly collected in North and Western Europe.

Many principal factors affecting fish community composition in temperate climate lakes were identified: surface area, dissolved oxygen levels, the acidity of the system (Matuszek and Beggs, 1988; Robinson and Tonn, 1989; Jackson et al., 2001; Olin et al., 2002; Lehtonen et al., 2008); turbidity, macrophyte coverage and compexity (Brazner and Beals, 1997; Eadie and Keast, 1984); nutrient load (Matuszek and Beggs, 1988; Olin et al., 2002; Lehtonen et al., 2008), commercial and amateur fishing and stocking (Lehtonen et al., 2008), piscivory and isolation (Robinson and Tonn, 1989), substrate diversity (Eadie and Keast, 1984). Among natural variables, characteristics of thermal stratification were recognized as important factors determining fish community structure and species composition in lakes of ecoregion (thereafter ER) 14 (Illies, 1978; Holmgren and Appleberg, 2000; Diekmann et al., 2005; Mehner et al., 2005, 2007). Diekmann et al. (2005) and Mehner et al. (2007) found that mean depth criterion can be used for differentiation of polymictic (thereafter POLY) lakes from stratified lakes (thereafter S), and for differentiation of S lakes dominated by different fish species. Ritterbusch et al. (2014) proposed maximum depth in addition to mean depth to divide lakes into types for fish communities' characterization and estimation of fish metrics, because combined use of both criteria makes it possible to classify lakes according to stratification peculiarities more precisely. Garcia et al. (2006) also used maximum depth to differentiate fish assemblages in shallow lakes from those in deep lakes. Selection of proper variables for differentiation of lake types and fish communities is crucial for assessment of lake status using fish metrics. Therefore, it seems

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appropriate to test the depth criterion for fish based typology of lakes in ecoregions, neighboring ER14.

The new data collected using standardized methods enabled us to analyze the dependence of the fish community composition in lakes in Lithuania (ER15) on environmental variables and human pressures in more detail. The objectives of the current study were (1) to determine interrelations between lake fish community composition and environmental variables; (2) to test the validity of lake classification into types in accordance with mean and maximum depth; (3) to determine fish metrics that are the most responsive to changes in environmental quality due to pressure from human activities; (4) to develop a fish-based method for the assessment of the ecological status of lakes.

2. Methods

The area studied covers the southern part of Water Framework Directive (thereafter WFD) ER 15. In this part of ER 15, all lakes lie below 200 m a.s.l. and, in terms of geology, almost all of them are calcareous (>1.0 meq/lg; Ca > 15 mg l^{-1}). During the 2005–2013 state monitoring of water quality and biological indicators, fish were sampled in 142 natural lakes with an area exceeding 50 ha. In some of the lakes, investigations were carried out several times every 6 year, thus data for our study were obtained from 162 fishing occasions.

All lakes studied are natural in origin. Hydromorphological characteristics are close to natural in 53 lakes and altered in 89 lakes. The most common hydromorphological changes include water level increase due to impoundment (39 lakes) and partial or complete destruction of riparian vegetation (49 lakes). Significant morphological alteration of different kinds were recorded only at 9 lakes: 25% of the shoreline was found to be embanked or eroded at 5 lakes; 25–50% of the shoreline was embanked or eroded at 2 lakes, the water level lowered due to land reclamation in 1 lake. The majority of lakes were relatively small (median – 124 ha). There were just 9 lakes with the area exceeding 1000 ha. The maximum depth in the deepest lake of Lithuania (Lake Tauragnai) is 60.5 m, but the maximum depth of the majority of lakes does not exceed 15 m (Table 1). The annual mean value of TN most often varies within 560–1200 $\mu g\,l^{-1}$ (with maximum of 3800 $\mu g\,l^{-1}$) and that of TP within 18–45 $\mu g\,l^{-1}$ (maximum 140 $\mu g\,l^{-1}$). Natural land cover most often constitutes from 29% to 59% of the lake catchment area.

Fish were captured with multimesh benthic gillnets, each of which was 40 m in length and 3 m in height. Mesh size varied every 5 meters and was 14, 18, 22, 25, 30, 40, 50, 60 mm. Fishing was carried out in the second half of summer – at the beginning of autumn with water temperature being >15° C. Depending on the lake area, at least 12 (<200 ha lakes), 16 (<500 ha), 24 (<1000 ha) or 32 (>1000 ha) benthic nets were used following the standardized method by the Ministry of Environment of Lithuania (20-10-2005 Order No.D1-501). Nets were positioned randomly to cover different parts and lake depths of each lake. In deep (>17 m maximum depth) lakes, 8 or 12 m height multimesh benthic gillnets for vendace *Coregonus albula* and smelt *Osmerus eperlanus* (14, 18, 22 and 26 mm mesh size) were also used as fish catches with standard height benthic gillnets fail to reflect the abundance of these pelagic fishes representatively (Diekmann et al., 2005). Nets remained in lakes for at least 12 hrs during the night covering sunset and sunrise periods.

For the fish caught, species, number, total length (mm), and wet weight (±1 g) were determined. Catches from benthic gillnets for vendace and smelt were re-calculated to standard area of the section of the same mesh size and then merged with catches from standard benthic gillnets. The catch per unit of effort was standardized in relation to the benthic gillnet's area (m^2).

Four eutrophication variables were analysed for assessment of human pressure, and considerably simplified version of the Scotish lake habitat survey method (Rowan et al., 2003) was used for the assessment of hydromorphological changes in lakes. The most common hydromorphological pressures in Lithuania are change of water level (water level elevation, as water level lowering was recorded only in two lakes in the whole country), natural riparian vegetation destruction and, on a much lesser extent, shoreline stabilization or erosion. Therefore only these hydromorphological metrics were measured and the dominant bottom substrate of the littoral zone was determined in order to compute the hydromorphological index (thereafter HMI) (Table 2). Each of the metrics were scored, summed and translated into the 0–1 scale. HMI was computed according to the formula:

HMI = (sum of scores–maximal sum of scores)/(minimal sum of scores–maximal sum of scores)

Lakes with altered water level (due to impoundment of the outflow or amelioration of the catchment) and lakes water level alterations (m) were determined based on the information available in the State register of Rivers, Lakes and Ponds of the Republic of Lithuania (https://uetk.am.lt). The length of the natural riparian vegetation (forest) belt and the scope of changes in the shoreline as a result of lake shore embankment or erosion were estimated visually by analyzing high resolution (50 cm) aerial photos (www.maps.lt). The composition of substrate in the littoral zone was determined visually in the course of the study.

Data on lake area, the mean and maximum lake depths (thereafter Z mean and Z max) and on eutrophication variables — the mean annual concentrations of total phosphorus (thereafter TP), total nitrogen (thereafter TN), chlorophyll α (thereafter Chl α) and Secchi depth were obtained from state authorities (Lithuanian Environmental Protection Agency). According to the state monitoring program variables are measured every 3rd or 6th year (depending on the WFD monitoring type), at least four times per year (from April till October) in the deepest part of the lake. Depending on lake depth and stratification, several samples are collected during each measurement, but only those taken in the euphotic layer were used to calculate average annual concentrations. Variables measured in the same year as fish sampling, or measured in the previous or next year (if sampling year did not coincide) have been used for analysis.

Two datasets were used in testing the impact of environmental factors on fish community structure. The first dataset included data from all lakes, whereas the second one contained data only from reference status lakes. Lakes falling into the category of potentially reference status lakes were selected by common intercalibration criteria (Poikane, 2009). When selecting lakes, an exception was made to the criterion of natural land cover, i.e. lakes with >50% of their catchment area covered by natural vegetation were also attributed to potentially reference status lakes because in Lithuania there are just a few lakes with >90% of their catchment covered by natural vegetation, i.e.forests.

Principal component analysis (PCA) was carried out on both data sets separately, with the relative abundance of fish species selected as an active variable and maximum and mean depths of lakes, area, Secchi depth, mean annual concentrations of TN, TP and Chl a, and the hydromorphological index as suplementary variables. Mean data from lakes that were studied several times was used for analysis. Non-native fish species and typical riverine (rheophilic) fish species were not included in the analysis.

The correspondence between the fish community composition and lake types derived only on the basis of mean depth (Z mean <3, 3–9 or >9 m; national typology) or maximum depth (Ritterbusch et al., 2014) combined with mean depth criterion (Z max <11 m polymictic lakes, Z max 11–30 and Z mean >3 stratified lakes, and Z max >30 deep lakes) was tested by discriminant analysis (thereafter

Table 1
Descriptive statistics of environmental characteristics of the studied lakes.

	Mean	Median	25%	75%	Min–Max
Surface area (ha)	321	124	74	291	50–3622
Average depth (m)	5.9	5.4	3.1	7.7	0.9–18.7
Maximum depth (m)	17.3	14.0	6.9	24.0	1.5–60.5
Secci depth (m)	3.0	2.8	1.7	4.0	0.5–7.9
TN (annual average, $\mu g\,l^{-1}$)	940	780	560	1200	310–3800
TP (annual average, $\mu g\,l^{-1}$)	36	29	18	45	6–140
Chl a (annual average, $\mu g\,l^{-1}$)	14	7	4	17	2–95
Natural land cover (% catchment)	46.8	41.7	29.1	59.3	13.8–100
Hydromorphological index	0.85	0.88	0.75	0.94	0.5–1

DA) with the relative abundance of fish species individuals selected as variables. Significance of differences in the relative abundance of fish species individuals in different types of lake was additionally analyzed separately for all species using ANOVAs and Bonferroni post hoc test.

In total 56 fish metrics, representing species diversity, overall abundance and biomass, feeding, habitat, overall tolerance and spawning guilds as well as individual fish species metrics and combinations of individual metrics were calculated for analysis. Candidate fish metrics were selected based on the published information on the metrics used in methods devised in other countries and/or response of different fishes to changes in lake status. Lithuanian lakes fish species diversity and distribution of species in different ecological groups were also considered when selecting potential metrics. For assessment of deviation in species composition, number of native species (Appelberg et al., 2000; Gassner et al., 2003), and number of type specific obligatory species (full list and combinations with rejection of various species tolerant

Table 2
Variables of hydromorphological quality elements used for the calculation of the lake hydromorphological index and their description.

Variables	Description of lake ecological status according to parameters/indices/metrics of hydromorphological quality elements		Score
Water level and water exchange	There are no water level alterations caused by unnatural factors (water level is neither raised nor lowered, there is no water extraction, water flow is not regulated).		1
	Water level is raised, but water flow is naturalized.		2
	Water level is raised and stabilized (adjustments of water level are done to ensure safety of operation of hydro technical installation).		3
	Water level is raised and periodically alters due to operation of the electric power plant built on the lake ouflow or water level and/or water exchange are periodically regulated because of other reasons. Or water level is lowered, but alteration is less than 1 m, lake area alteration is <10%.		4
	Water level is regulated, water level alteration exceeds 1m or an alteration in lake area is >10%.		5
Shore structure	Length of natural riparian vegetation belt	Not less than 70 % of the lake shoreline is covered by the belt of natural riparian vegetation (forest)	1
		70–30 % of the lake shoreline is covered by the natural riparian vegetation (forest) belt	2
		29–5 % of the lake shoreline is covered by the natural riparian vegetation (forest) belt	3
		<5 % of the lake shoreline is covered by the natural riparian vegetation (forest) belt	5
	Shoreline allterations	Shoreline is natural (neither straightened nor embanked) or <5 % of the lake shoreline is altered	0
		5–25% of the shoreline is altered	1
		26–50% of the shoreline is altered	2
		>50% of the shoreline is altered	3
	Shore erosion	There is no shore erosion caused by unnatural factors (water level elevation/lift or water level alternation) or <5% of the shoreline is eroded	0
		5–25% of the shoreline is eroded due to unnatural factors	1
		26–50% of the shoreline is eroded due to unnatural factors	2
		>50% of the shoreline is eroded due to unnatural factors	3
Predominant substrate in the littoral zone	Clean, hard substrate (gravel and/or sand)		1
	Heterogeneous substrate: silty sand and/or gravel and/or clay, or hard substrate covered by a thin layer of silt		2
	Silt		3

to degradation) were tested. The weight and number of individuals per unit of effort (WPUE and NPUE respectively) were used for testing of response to changes in overall productivity of the lake (Appelberg et al., 2000). Abundance of piscivorous percids (Appelberg et al., 2000), piscivorous fish and biomass ratio of piscivorous fish to plankti-benthivorous cyprinids (Jeppesen et al., 2000), and abundance of omnivorous fish (Launois et al., 2011) were selected as feeding guilds. Abundance of benthic fish in different combinations of species, phytophilic (Perrow et al., 1999), lithophilic and tolerant (Launois et al., 2011), and oxygen deficit sensitive stenothermic species (coregonids, smelt and burbot *Lota lota*) were selected for testing for changes in habitat, spawning and overall tolerance guilds. Among individual metrics selected were - the mean body weight of roach *Rutilus rutilus* and bream *Abramis brama* (Holmgren et al., 2007), abundance of perch *Perca fluviatilis*, bream (Perrow et. al., 1999), ruff *Gymnocephalus cernua* and white bream *Blicca bjoerkna*, abundance of cyprinids (Persson et al., 1991; Appelberg et al., 2000), percids/cyprinids biomass ratio (Olin et al., 2002), roach/perch biomass ratio, the mean biomass of fish individuals (Holmgren et al., 2007), abundance of non-native species (Appelberg et al., 2000), abundance of translocated species and combined metrics (abundance of perch + stenothermic species, abundance of non-native + translocated species). Each of the abundance metrics were computed as number and biomass of individuals, and percentage of number and biomass of individuals. Origin of fish species (native, non-native, translocated) was defined based on Reyjol et al. (2007) and Virbickas (2000), type specific obligatory species (species that should be present in the reference status lakes of appropriate type) were defined based on available data on species composition in reference lakes and historical data on commercial catches (unpublished data).

Pearson's correlation matrix was calculated between fish metrics and variables describing nutrient conditions (TP, TN, Chl *a*), Secchi depth and hydromorphological index. For the development of fish index, we selected only those metrics, whose coefficient of correlation with at least one of the above listed variables was $\geq$0.4 (when $P < 0.05$). A correlation matrix was calculated to select non-redundant metrics. Metrics were assumed to be redundant if the correlation coefficient was >0.8. Reference values of the selected metrics were determined by estimating the 75th percentiles for metrics with a negative response to anthropogenic pressure, and the 25th percentiles for metrics with a positive response to anthropogenic pressure in potentially reference status lakes.

Data sets of metrics were normalised according to reference value. The fish index was expressed as the mean of normalized estimates of all metrics. Preliminary status class boundaries were set by analysing discontinuities in the distribution of index values. These boundaries were used for pre-classification of lakes into status class. Afterwards, status class boundaries were additionally calibrated by calculating averages between 25th percentile in the lakes of higher status class and 75th percentile in the lakes of lower status class.

The index was tested by calculating the regression between the index and variables characterising nutrient and hydromorphological conditions. ANOVAs and the Bonferroni post hoc test were used to test significance of differences in TN, TP, Chl α and Secci depth values in different types of lakes of the same status according to fish index, and among different status lakes of the same type.

- Threshold values of TN, TP, Chl α and Secci depth corresponding to high, good and moderate status classes according to the fish index were determined as follows: lakes with unaltered or only slightly altered hydromorphology (HMI > 0.8) were selected and divided into ecological status classes according to the fish index.
- 25th and 75th percentiles of the dispersion of TN, TP, Chl *a* and Secci depth values were calculated for the group of lakes of high ecological status according to the fish index.

In the group of lakes of good ecological status according to the fish index, environmental variables values lower than 75% of the corresponding values in high status group (in the case of Secci depth value-higher than 25%, thereafter value in brackets) were rejected and only then the 25th and 75th percentiles of the dispersion of variables values were estimated.

In the group of moderate status lakes according to the fish index, environmental variables values lower than 75% of the corresponding values in the group of good ecological status (higher than 25%) were rejected and only then the 25th and 75th percentiles of the dispersion of variables values were estimated.

- Threshold values of parameters/indices were selected/ as the mean of the 75% (25%) value of the metric of the higher ecological status class and the 25% (75%) value of the metric of the lower ecological status class.

Prior to analysis, species richness, abundance and biomass metrics as well as environmental variables, characterizing lake morphometry and nutrient conditions were log2 or log10 transformed to best fit normal distribution. Percentage metrics were transformed to a range within 0–1 and then arcsin transformed. *Chi*-square test was used to assess significance of deviation from normal distribution. All statistical calculations were done using STATISTICA 6.0 (StatSoft, Inc.).

3. Results

3.1. Fish assemblages

Twenty four species were recorded, three of which (common carp *Cyprinus carpio*, gibel carp *Carassius gibelio* and silver carp *Hypophthalmichthys molitrix*) are non-natives, and 1 species (pikeperch *Sander lucioperca*) has been translocated into lakes from transitional waters (Table 3). Five fish species (asp *Leuciscus aspius*, gudgeon *Gobio gobio*, dace *Leuciscus leuciscus,* ide *Leuciscus idus* and chub *Squalius cephalus*), which are are not typical lake dwellers, were found only once or twice (chub). These species, together with non-native species were not included in the analysis of the impact of environmental variables on native fish fauna in the lakes.

PCA analysis with all lake data produced two axes with eigenvalues higher than 1.5. The first axis was positively correlated with Secchi depth ($R = 0.61$), lake area ($R = 0.49$) and maximum depth ($R = 0.52$) as well as the relative abundance of perch ($R = 0.55$), vendace ($R = 0.52$), burbot ($R = 0.65$) and ruffe ($R = 0.62$), and negatively with the relative abundance of roach ($R = -0.59$). The second axis was negatively correlated with concentrations of TP ($R = -0.35$), TN ($R = -0.36$) and Chl a ($R = -0.37$) as well as with the relative abundance of silver bream ($R = -0.66$) and pikeperch ($R = -0.53$). Both axes represented only 23.4% of the total variance (Fig. 1a).

PCA analysis with reference lake data produced three axes with eigenvalues higher than 1.5, but only the first axis was significantly positively correlated with environmental variables: the mean ($R = 0.62$) and maximum ($R = 0.63$) depth, Secchi depth and lake area (both $R = 0.55$) (Fig. 1b). The relative abundance of vendace, burbot and ruffe correlated with the first axis positively (accordingly, $R = 0.78$, 0.73 and 0.57), and that of tench *Tinca tinca*, rudd *Scardinius erythropthalmus* and bream negatively (accordingly, $R = -0.45$, −0.40, and −0.43).

DA results showed that when reference status lakes were classified into types by the mean and maximum depth, the observed

Table 3
Frequency of occurence of fish species in the studied lakes and relative abundance of species in reference lakes of different types (POLY — polymictic, S — stratified, DS — deep stratified). Relative abundances of fish species significantly differing between lake types (ANOVA, Bonferroni test, $P < 0.01$) are indicated in bold.

Common name	Species	Frequency of occurence	Relative abundance in reference lakes		
		all lakes (*n*-142)	POLY(*n*-13)	S(*n*-20)	DS(*n*-12)
White bream	*Blicca bjoerkna*	83.1	10.69	10.71	6.85
Bream	*Abramis brama*	92.3	6.53 ^{SDS}	2.07	1.47
Bleak	*Alburnus alburnus*	73.9	2.32	3.10	2.11
Asp	*Leuciscus aspius*	0.7	0.00	0.00	0.00
Crucian carp	*Carassius carassius*	26.8	0.14	0.22	0.00
Gibel carp	*Carassius gibelio*	19.7	0.00	0.00	0.00
Vendace	*Coregonus albula*	23.2	0.00	0.69	**6.10** ^{POLYS}
Common whitefish	*Coregonus lavaretus*	2.8	0.00	0.00	**3.07** ^{POLYS}
Common carp	*Cyprinus carpio*	3.5	0.00	0.00	0.00
Northern pike	*Esox lucius*	89.4	0.72	0.98	0.88
Gudgeon	*Gobio gobio*	0.7	0.00	0.00	0.00
Ruffe	*Gymnocephalus cernua*	78.9	1.14	2.50	**7.93** ^{POLY}
Silver carp	*Hypophthalmichthys molitrix*	0.7	0.00	0.00	0.00
Dace	*Lauciscus leuciscus*	0.7	0.00	0.00	0.00
Ide	*Leuciscus idus*	0.7	0.00	0.00	0.00
Burbot	*Lota lota*	5.6	0.00	0.00	**0.36** ^{POLYS}
Smelt	*Osmerus eperlanus*	4.2	0.00	0.00	**2.99** ^{POLYS}
Perch	*Perca fluviatilis*	100	**23.55** ^{SDS}	31.46	34.17
Roach	*Rutilus rutilus*	100	**47.3** ^{DS}	42.06	33.14
Pikeperch	*Sander lucioperca*	12	0.00	0.00	0.00
Rudd	*Scardinius erythrophthalmus*	79.6	6.15	2.47	2.79
Wels catfish	*Silurus glanis*	6.3	0.01	0.01	0.07
Chub	*Squalius cephalus*	1.4	0.00	0.00	0.00
Tench	*Tinca tinca*	70.4	1.42	1.11	0.76

relative abundances of fish species corresponded to the predicted ones in 86% of the lakes investigated. Deep stratified (thereafter DS) lakes differ from the rest of lakes in significantly higher relative abundances of vendace, whitefish *Coregonus lavaretus*, burbot and smelt, as bream was significantly more abundant and perch less abundant in POLY lakes (Table 3). DS and POLY lakes also differed in relative abundances of roach and ruffe. When lakes were classified into types by mean depth, DA revealed that the coincidence rate of observed and predicted relative abundances decreased to 75%. At the individual species level, only differences between relative abundances of bream and perch in POLY lakes and those in S lakes remained significant. Likewise, the relative abundance of vendace in DS lakes remained significantly different from that in other lakes (ANOVA, Bonferroni test, $P < 0.01$)

3.2. Fish metrics and assesment system

As the use of both mean and maximum depth criteria in fish community discrimination proved more efficient than the use of mean depth alone, the classification of lakes into POLY, S and DS ones carried out for the purpose of fish metrics selection was based on both criteria (mean and maximum depth).

In different types of lakes, only 11 candidate fish metrics showed a significant correlation with human pressure. Two of them, representing the relative abundance of cyprinidae species and roach/perch ratio in POLY lakes were rejected as highly redundant ($R > 0.80$) with the metric of the relative abundance of perch. Another two metrics, representing biomass and proportion of biomass of stenothermic fish in DS lakes were rejected as redundant with the metric representing the combined relative biomass of perch and stenothermic species. Finally, only 7 metrics were selected (Table 4).

Selected metrics significantly correlate with either Chl *a* concentration or Secchi depth (or both) in all types of lakes, except the relative biomass of non-native and translocated species (thereafter Non-nat_W%) in S and DS lakes. The relative biomass of benthivorous fish (thereafter Benthiv_Sp_W%) and the mean weight of roach individuals (thereafter Roach_Q_av) in S lakes as well as the number of obligatory species (thereafter Nb_Oblig_Sp) in DS lakes were found to significantly correlate with the concentration of TP. The Non-nat_W% in POLY lakes and the relative biomass of perch and stenothermic species (thereafter Perch_Steno_W%) in DS lakes significantly correlate with the concentration of TN. Benthiv_Sp_W% and Nb_Oblig_Sp significantly correlate with the variables describing environmental degradation in all types of the lakes studied. Both these metrics significantly correlated with the HMI in DS lakes. The relative abundance of perch (thereafter Perch_N%) and the relative biomass of silver bream (thereafter S_bream_W%) correlated only with Chl *a* concentration and Secchi depth.

When compiling the Lithuanian Lake Fish Index (LEZI), based on expert judgement, the relative abundance of non-native species was included in the ecological status assessment of S and DS lakes as the representativeness of this metric in the above mentioned types of lakes is limited due to insufficient data amount. Non-native species were present in seven S lakes (amounting to 3–16.3% in WPUE) and only three DS lakes (1–5%). By pooling S and DS lakes into a single group (10 lakes), the correlation of the above mentioned index/metric with the concentration of TP becomes statistically significant ($R = 0.72$, $P < 0.05$).

The LEZI index is calculated from the mean of S_bream_W%, Benth_Sp_W%, Roach_Q_av, Perch_N%, Perch_Steno_W% metrics normalized in relation to the reference value, the normalized and weighted Nb_Oblig_Sp metric, and adjusted Non-nat_W% metric (for index description see Appendix A).

Regression analysis demonstrated that in all types of lakes the index value varied depending on the nutrient and hydromorphological conditions. LEZI values most significantly correlate with the concentration of Chl *a* and Secchi depth, which in different types of lakes accounted for 43–48% and 27–53% of the total variance respectively (Table 5). Changes in TP concentration explained 23–37% of the total variance. Variables of hydromorphological conditions and the concentration of TN explained the least part of the total variance. Regression analysis of all lake types showed that the hydromorphological index alone explains only 19% of the total LEZI variance.

The threshold values of LEZI from the analysis of index values discontinuities and representing boundaries between lake status classes were as follows: 0.86 between high/good, 0.61 between

Table 4
Fish metrics selected for status assessment of different type lakes and metrics correlations (Pearson's R) with environmental variables (correlations significant when $P < 0.05$ are indicated in bold).

Type	Variable	1 S_bream_W%	2 Benth_Sp_W%	3 Roach_Q_av	4 Perch_N%	5 Perch_Steno_W%	6 Nb_Oblig_Sp	7 Non-nat_W%
POLY	(n)	(52)	(65)		(65)		(65)	(17)
	TP	0.21	0.13		−0.23		**−0.33**	0.35
	TN	0.17	**0.28**		−0.12		**−0.34**	**0.48**
	Chl *a*	**0.45**	**0.43**		**−0.40**		**−0.43**	**0.56**
	Secchi depth	**0.31**	**−0.33**		**0.32**		**0.27**	**−0.47**
	HMI	−0.01	−0.04		0.04		**0.27**	−0.09
S	(n)		(70)	(70)		(70)	(70)	(7)
	TP		**0.57**	**−0.43**		**−0.29**	**−0.32**	0.68
	TN		0.20	−0.20		−0.15	**−0.25**	0.63
	Chl *a*		**0.44**	**−0.68**		−0.40	**−0.41**	0.16
	Secchi depth		**−0.63**	**0.66**		**0.45**	**0.36**	0.07
	HMI		**−0.26**	**0.35**		**0.40**	**0.29**	0.09
DS	(n)		(27)	(27)		(27)	(27)	(3)
	TP		0.29	−0.13		0.02	**−0.48**	–
	TN		**0.47**	−0.34		**−0.47**	−0.33	–
	Chl *a*		**0.59**	**−0.44**		−0.26	**−0.57**	–
	Secchi depth		**−0.45**	**0.61**		**0.37**	**0.46**	–
	HMI		**−0.48**	−0.11		−0.01	**0.40**	–

1—Relative biomass of silver bream;
2—Relative biomass of silver bream, bream, and ruff;
3—Mean weigth of roach individuals;
4—Relative abundance of perch;
5—Relative biomass of perch, burbot, smelt, vendace and whitefish;
6—Number of obligatory species except for the species included in Benth_Sp_W% metric. POLY lakes - bleak, rudd, pike, tench, perch, roach; S lakes — vendace, bleak, rudd, pike, perch, roach; DS lakes — vendace, smelt, burbot, bleak, rudd, pike, perch, roach;
7—Relative biomass of non-native and translocated species (common carp, gibel carp, silver carp, pikeperch).

Table 5
Results of regression analysis of LEZI and environmental variables.

	Polymictic				Stratified				Deep stratified			
	R	R^2 adj.	F(1, 65)	P	R	R^2 adj.	F(1, 68)	P	R	R^2 adj.	F(1, 25)	P
TP	−0.48	0.23	18.7	<0.001	−0.58	0.33	33.9	<0.001	−0.61	0.37	14.5	<0.001
TN	−0.37	0.12	10.0	<0.001	−0.40	0.14	12.6	<0.001	−0.63	0.40	16.2	<0.001
Chl a	−0.70	0.48	61.9	<0.001	−0.69	0.46	59.2	<0.001	−0.66	0.43	19.2	<0.001
Secchi depth	0.52	0.27	23.3	<0.001	0.70	0.50	67.0	<0.001	0.74	0.53	30.5	<0.001
HMI	0.26	0.05	4.6	<0.05	0.52	0.26	25.2	<0.001	0.39	0.15	4.6	<0.05

Table 6
Fish index-based values of variables characterizing nutrient conditions for high (H), good (G) and moderate (M) ecological status classes of lakes (POLY-polymictic lakes; S-stratified lakes; DS-deep stratified lakes). Status classes and lake types, significantly differing in values of variables, are indicated in superscript (ANOVA, Bonferroni test, $P < 0.05$).

Status class	Lake type	Secchi depth (m)	TN ($\mu g\, l^{-1}$)	TP ($\mu g\, l^{-1}$)	Chl a ($\mu g\, l^{-1}$)
H	DS (*n*-11)	5.6 ± 1.3	536 ± 128	14 ± 10	3.1 ± 0.6
	S (*n*-10)	5.4 ± 1.1	670 ± 193	17 ± 13	2.9 ± 1.0
	POLY (*n*-7)	2.3 ± 0.5	835 ± 406	31 ± 5^{SDS}	5.5 ± 1.6^{SDS}
G	DS (*n*-14)	4.2 ± 0.9^{H}	795 ± 403	26 ± 16	4.3 ± 1.3^{H}
	S (*n*-31)	3.9 ± 1.1^{H}	677 ± 183	22 ± 12	6.4 ± 3.2^{H}
	POLY (*n*-20)	2.2 ± 0.9	939 ± 334	31 ± 12	12.9 ± 12.5^{SDS}
M	S (*n*-24)	2.7 ± 1.1^{HG}	1262 ± 727^{HG}	43 ± 24^{HG}	10.7 ± 6.5^{HG}
	POLY (*n*-28)	1.6 ± 0.8^{HG}	1129 ± 463	50 ± 28^{SDS}	29.5 ± 8.3^{SHG}

good/moderate, 0.37 between moderate/poor, and 0.18 between poor/bad ecological status classes. After grouping of lakes into fish based status classes, the number of high and good status lakes of all types and the number of moderate status POLY and S lakes were sufficient to compare eutrophication variables between different types of same status lake, and between different status of same type lakes. The number of moderate status DS lakes, and poor or bad status S and DS lakes were too small for comparison.

Values of nutrient condition variables in S and DS lakes of the same ecological status according to LEZI did not differ (Table 6). Concentration of Chl *a* in POLY lakes were significantly higher than in S and DS ones of the respective status. The concentration of total phosphorus in POLY lakes was significantly higher than in S and DS lakes only in the group of high ecological status lakes, while significant differences were not found in total nitrogen concentration among POLY, S and DS lakes of the same ecological status according to LEZI.

The concentration of Chl *a* and Secchi depth differed significantly between different status classes of DS and S lakes. Concentration of TN and TP in moderate status S lakes also was significantly higher than in the high and good status lakes. Only Secchi depth and Chl *a* concentration significantly differed in medium status POLY lakes compared to those of high or good status according to fish index.

Calculation of thresholds of eutrophication variables between high/good (H/G) and good/moderate (G/M) status classes resulted in the following values in different types of lakes: in DS lakes the H/G and G/M status threshold values of Secchi depth are, respec-

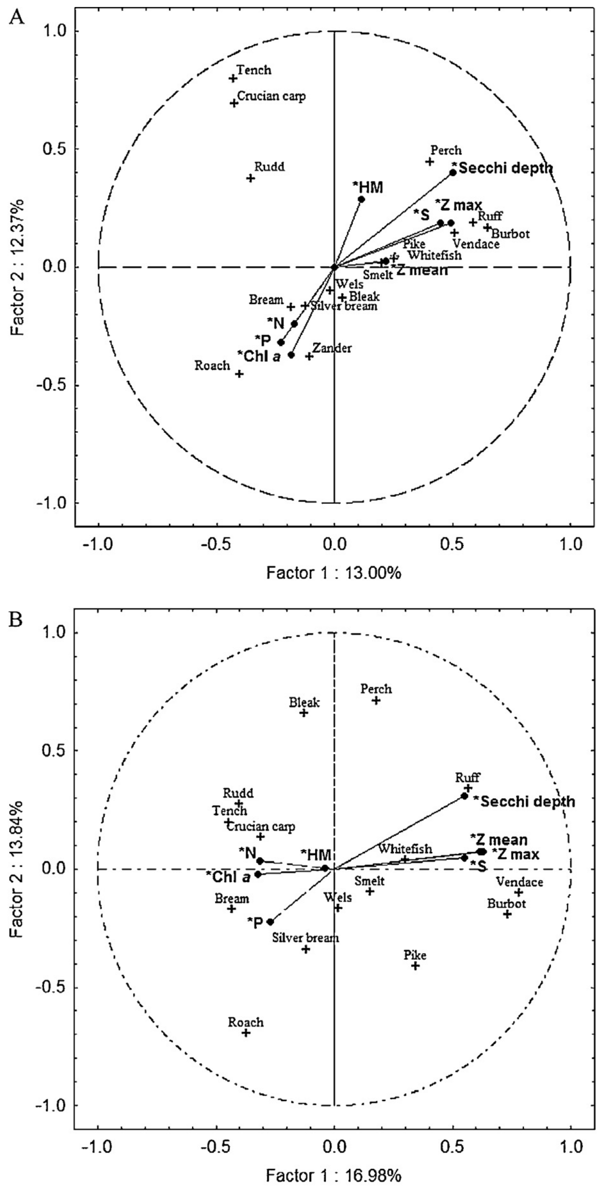

Fig. 1. Plot of the principal component analysis results with 16 fish species in all 142 lakes (a) and 15 species in 45 reference lakes (b) and 8 environmental variables, shown as vectors (Z mean = mean depth, Z max = maximum depth, *S* = lake area, *N* = total nitrogen, *P* = total phosphorus, Chl *a* = Chl a, HM = hydromorphological index).

tively, 4.1 and 2.7 m, TN–730 and 1360 $\mu g\, l^{-1}$, TP–28 and 47 $\mu g\, l^{-1}$, Chl *a* – 4.3 and 8.2 $\mu g\, l^{-1}$; in S lakes Secchi depth –4.4 and 2.5 m, TN–730 and 1280 $\mu g\, l^{-1}$, TP–20 and 43 $\mu g\, l^{-1}$, Chl *a* –4.1 and 8.8 $\mu g\, l^{-1}$; in POLY lakes Secchi depth –2.3 and 1.2 m, TN–690 and 1400 $\mu g\, l^{-1}$, TP–36 and 58 $\mu g\, l^{-1}$, Chl *a* – 7.4 and 13.0 $\mu g\, l^{-1}$.

4. Discussion

Principal component analysis showed that mean and maximum depths are important factors determining fish community structure in the lakes of Lithuania. The significance of variables characterizing nutrient conditions in a lake for fish community differentiation became more significant when reference lakes and human impacted lakes combined were included in the PCA. The relative abundance of stenothermic fishes such as vendace and burbot was found to correlate positively with lake depth, while tench, rudd and bream corelated negatively. The abundance of all stenothermic fishes (vendace, burbot, smelt and whitefish) in DS lakes is significantly higher than in other lakes. Mehner et al. (2005) showed that fish community composition in North-East German lowland lakes was mainly determined by the maximum and mean depths, Chl *a* content and lake volume. Similarly, productivity, lake area and the maximum depth were found to be most important in structuring fish communities of Swedish lakes (Holmgren and Appelberg, 2000). The mean depth correlated positively with the abundance of vendace and burbot and negatively with that of pike *Esox lucius* and rudd in ER 14 lakes. The 'Vendace type' consisting of deep lakes of low trophic state was identified as having a distinct fish assemblage both in Germany and in the entire ER 14 (Mehner et al., 2007). This type of lake is characterized by the presence of vendace, perch and pike in significant numbers. According to all above-mentioned regularities in interrelations between the structure of fish communities and environmental variables, Lithuanian lakes representing the southern part of European ER 15 'Baltic Plains' (Illies, 1978) are very similar to those of ER 14.

A combination of mean and maximum depth in lake classification was found to be more efficient in differentiating fish communities than mean depth alone. This fact proves that maximum depth is an important factor determining differences in the structure of fish communities (Garcia et al., 2006; Ritterbusch et al., 2014) and describes better lake mixing characteristics. Other morphological characteristics of the lakes can also determine the structure of fish communities. It is known that species composition and abundance of different fish species depends on lake area (Holmgren and Appelberg, 2000), volume (Diekmann et al., 2005), diversity of the nearshore littoral habitats (Mehner et al., 2007; Jennings et al., 1999), mixing regimes (Garcia et al., 2006), depth gradient (Ritterbusch et al., 2014). However, due to absence of data none of these variables (except lake area) have been tested for discrimination of fish communities in the current study.

Nutrient concentration in lakes is known to have strong impact on fish assemblages (Persson et al., 1991; Jeppesen et al., 2000; Mehner et al., 2005). Positive correlations of roach, bream and ruffe abundance and negative correlation with abundance of perch with nutrient concentration were previously reported in a large number of studies on European lake fish assemblages (Persson et al., 1991; Jeppesen et al., 2000; Olin et al., 2002; Tammi et al., 2003; Mehner et al., 2005; Søndergaard et al., 2005; Garcia et al., 2006). Lithuanian lakes follow similar general trends; however, some differences have been detected. Ruff abundance does not correlate with eutrophication pressure metrics in Lithuanian lakes. Moreover, based on PCA analysis results, abundance of ruff tends to change in the opposite direction to concentrations of TN, TP and Chl α. Such a tendency can be due to significantly greater abundance of ruff in DS lakes compared to polymictic ones (see Table 4); while the range of eutrophication pressure in POLY lakes with naturally low ruff abundance is much wider than in S and DS lakes. Jeppesen et al. (2000) found a significant unimodal relationship between ruff and TP and explained it by differences in ruff competition for foraging resources at different lake trophicity. Ruff is a superior competitor to young perch at initial stages of increase in trophicity and decrease of water transparency; however, at high TP ruff abundance decreases due increase in the abundance of bream, i.e. with increasing TP ruff becomes an inferior competitor to bream and occupies an intermediate position in the shift of dominance from perch to plankto-benthivorous cypdrinids. Therefore, the combined metric of W% of silver bream, bream and ruff better reflected the change in lake trophicity, compared to the abundance of all benthic species, some of which are specialized spawners and may change in the opposite direction to non-specialized ones.

The same metrics do not represent changes in the ecological status of POLY and S lakes equally. The relative biomass of perch correlates significantly negatively with the concentration of Chl α in

POLY lakes, but in S lakes its representativeness is low. However by pooling S and DS lakes into one group the total relative biomass of perch and stenothermic fishes reflected changes in lake trophic status much better than the relative biomass of perch alone and a little bit better than the relative biomass of stenothermic fishes alone. A sufficient amount of well oxygenated cool hypolimnetic water ensures survival of stenothermic fishes, i.e. whitefish, vendace and smelt, in stratified lakes during the period of summer stagnation (Mehner et al., 2005; Garcia et al., 2006). In lakes of different bottom relief these volumes in relation to the water volume of the whole lake differ, which may determine unequal relative abundance of stenothermic fishes in the community. Perch and smelt abundance correlation in DS lakes of Lithuania revealed that the alteration in the biomass of perch and smelt in a lake is inversely proportional, i.e. a decrease in perch biomass is accompanied by a marked increase of smelt and vice versa (Virbickas unpublished data). Biomasses of these two species seem to be interrelated. For the reasons listed above the total relative biomass of stenothermic fishes and perch was selected as a metric for the lake ecological status assessment, hovewer biomass of stenothermic fishes also correlated significantly with nutrient variables.

Contrary to expectations, indices of roach and bream abundances did not correlate with nutrient variables or the HM index. The mean weight of roach specimens turned out to be a more representative index than its abundance or biomass. A decrease in the mean body weight of roach and bream with increasing TP has already been reported for Danish lakes (Jeppesen et al., 2000). The total relative biomass of silver bream, bream and ruff in all types of lakes also correlated better with nutrient metrics than individual species metrics, except for the relative biomass of silver bream in POLY lakes. The variation of relative biomass of tench, one more benthivorous species widespread in lakes of the country, did not follow patterns of other benthivorous species. The abundance of tench, which is dependent on submerged macrophytes cover, may become inversely proportional to bream abundance (Perrow et al., 1999). Based on PCA results, pikeperch is among the species correlating with an increase in nutrient concentrations. This resident of turbid eutrophicated waters (Lehtonen et al., 1996) was translocated into Lithuanian lakes from transitional waters (Virbickas, 2000). Together with other non-native eutrophication-tolerant species naturally breeding in Lithuanian waters, gibel carp in particular reflects changes in trophic status of POLY lakes. The majority of non-natives are highly tolerant to various perturbations and show high plasticity in adaptation (Copp et al., 2005). The presence of pikeperch in inland waters distorts the response of piscivorous species metrics to lake ecological status deterioration. The relative abundance of piscivorous fishes was reported as a metric negatively correlating with an increase of phosphorus (Jeppesen et al., 2000). However, the relative biomass of the obligatory predator pikeperch follows the reverse trend compared to that of perch and pike, which are native predators in lakes of the southern part of ER 15.

As it has been summed up by Launois et al. (2011), most Indexes of biotic integrity developed on lentic systems include some measure of the relative dominance of tolerant and/or intolerant species for lake status assessment. However, this metric poorly reflected the lake status change in the southern part of ER 15 as tolerant species are naturally dominant in lakes. The mean relative abundance of tolerant species in reference status POLY and S lakes accounts for 91–93%, and biomass for 84–87%. Metrics characterizing a tolerant (or intolerant) ecological guild vary within a very narrow range. Only in DS lakes the relative abundance and biomass of tolerant species are lower, 75% and 71% respectively. But, as has been mentioned already, the relative abundance of vendace and whitefish, the only intolerant fishes dwelling in Lithuanian lakes, depends on the proportion of the water volume with physiologically optimum conditions to the total water volume. Consequently, their relative biomass depends on lake morphometry and does not necessarily reflect changes in lake trophic status.

The index developed on the basis of selected metrics significantly correlates with the variables characterizing nutrient conditions (TN, TP, Chl a and Secchi depth) and the HM index in all types of lakes, but, depending on the lake type environmental variables, account for just 40–50% of variation in fish index values. Probably, not all potential metrics were selected in the pressure-response analysis or the selected indices may be insufficiently representative in certain conditions, as the available database contains only few DS lakes where the hydromorphology or water quality changed considerably. Almost all metrics finally selected for ecological status assessment, refer to relative values, thus do not account for all possible variability of fish community response to degradation. Suprisingly after classification of lakes on the basis of the LEZI index into ecological status groups and having calculated threshold values of environmental variables, it turned out that the threshold value of Chl α for good/moderate status groups in S lakes ($4.4\,\mu g\,l^{-1}$) corresponds to Chl α threshold of $4.5\,\mu g\,l^{-1}$ which was proposed by Persson et al. (1991) for distinguishing between fish communities in low to moderate productivity lakes on the one hand, and medium to high productivity lakes, on the other.

The HMI index accounted for just 19% of LEZI variation. Thus, LEZI potential as a tool for reflecting hydromorhological alterations is not strong: it can reflect only partly these changes. Water level elevation which represents the most common hydrological alteration in lakes of Lithuania is normally accompanied by the additional input of organic matter resulting from the decomposition of submerged vegetation and soil. Hence, the impact of water elevation should manifest itself in the increased amount of nutrients in water rather than as a direct effect of hydrological alterations. Mehner et al. (2005), who investigated the anthropogenic impact on fish communities in lowland lakes of Germany, determined that connectivity, natural shore structure, human development of the shore and human-use intensity contribute little to the determination of lake-specific fish communities and only cultural eutrophication alters fish composition in lakes substantially. Similarly, the diversity of littoral habitat features has been shown to have a relatively insignificant effect on lake fish communities (Mehner et al., 2007).

The fish-based index was developed on the basis of data collected by fishing with benthic gillnets. Special benthic gillnets, up to 12 m in height were used only in the stratified lakes inhabited by vendace and smelt, which are pelagic stenotermic fish. Diekmann et al. (2005), who compared catches from different lake habitats determined that species richness and Shannon's index of diversity are higher in littoral and benthic habitats, and the coeficient of variation of CPUE was substantially lower in the benthic habitat in comparison with the pelagic one. Therefore we assume that benthic gillnets catch method represented the structure of lake fish community reasonably well. Benthic fish communities are most often selected for analysis of the relationship between species composition or diversity and abiotic descriptors (Jeppesen et al., 2000; Radke and Eckmann, 2001; Olin et al., 2002). However, electrofishing in the littoral zone of a lake could provide additional information on species of small-sized fish which are normally not captured with benthic gillnets. Such data could allow to select more and/or more representative fish metrics. Another drawback is the use of multi-mesh benthic gillnets which do not fully comply with European Standard 14757 (2005). The use of the standardized method of lake fish community studies with multi-mesh bottom gill nets was started in Lithuania as far back as 1993. In order to ensure comparability of results, the method has not been changed beyond publication of EN standard. However, using EN 14757 multi-mesh benthic gillnets would make results of the current study much more comparable with the results of investigations conducted in the same geographical area. To sum it up, the method was not validated by an external data set, as recommended by Borja and Dauer (2008). That is the drawback of the majority of national methods (Argillier et al., 2013).

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Appendix A. Description of Lithuanian lake fish index LEZI.

Description of Lithuanian lake fish index LEZI.

Typology of lakes for calculation of LEZI	
POLY	polymictic lakes (≤3 m mean depth, or >3 m mean depth and ≤11 m maximum depth)
S	stratified lakes (>3 m mean depth and 11–30 m maximum depth)
DS	deep stratified lakes (> 30 m maximum depth)

Metrics

Type	Metric	Reference	High	Good	Moderate	Poor
POLY	S_bream_W%[1]	1.5	<4	<11	<19	<26
	Benth_Sp_W%[2]	10	<20 (>0)	<35	<47	<61 (0)
	Perch_N%[3]	30	>25	>17	>9	>4
	Nb_Oblig_Sp[4]	6	6	5	4	<4
	Non-nat_W%[5] (only when Nb of ind. >1)	0	0	0	<1	<6
S	Roach_Q_av[6]	60	>50	>34	>23	>14
	S_bream_W%	1	<2.5	<9	<17	<26
	Benth_Sp_W%	7	<16 (>0)	<29	<45	<61 (0)
	Perch_Steno_W% [7]	35	>30	>17	>9	>4
	Nb_Oblig_Sp	6	6	5	4	<4
	Non-nat_W% (only when Nb of ind. >1)	0	0	0	<1	<6
DS	Roach_Q_av	60	>50	>34	>23	>14
	Benth_Sp_W%	4	<12 (>0)	<27	<41	<56 (0)
	Perch_Steno_W%	40	>35	>24	>14	>4
	Nb_Oblig_Sp	8	8–7	6–5	4	<4
	Non-nat_W% (only when Nb of ind. >1)	0	0	0	<1	<6

1—Relative biomass of silver bream;

2—Relative biomass of silver bream, bream, and ruff;

3—Relative abundance of perch;

4—Number of obligatory species. POLY lakes—bleak, rudd, pike, tench, perch, roach; S lakes—vendace, bleak, rudd, pike, perch, roach; DS lakes—vendace, smelt, burbot, bleak, rudd, pike, perch, roach;

5—Relative biomass of non-native and translocated species (common carp, gibel carp, silver carp, pikeperch)

6—Mean weigth of roach individuals;

7—Relative biomass of perch, burbot, smelt, vendace and whitefish;

Scoring

i) for metrics S_bream_W% and Benth_Sp_W% the values are transformed to EQR with formula:

$EQR = (X - X_{MAX})/(X_{RC} - X_{MAX})$, where X—measured value, X_{RC} —reference value, X_{MAX}—theoretical maximum value.

S_bream_W% metric $X_{MAX} = 30$;

Benth_Sp_W% metric $X_{MAX} = 70$ in POLY and S lakes, and $X_{MAX} = 65$ in DS lakes

If calculated EQR values are < 0 or > 1, they are clipped at 0 and 1. If $X = 0$ than EQR $= 0$.

ii) for metrics Perch_N%, Perch_Steno_W% and Roach_Q_av the values are transformed to EQR with formula:

$EQR = X/X_{RC}$; if calculated EQR values are > 1, they are clipped at 1.

iii) for metric Nb_Oblig_Sp, the values are transformed to EQR with the formula

$EQR = X/X_{RC}$

Before transformation, $X = 4$ values are multiplied by 0.3, and $X < 4$ values are multiplied by 0.15

iv) for metric Non-nat_W%, the EQR values are adjusted as follows:

- if Non-nat_W% >0 <1%, EQR = 0.5;
- if Non-nat_W% = 1–5%, EQR = 0.2;
- if Non-nat_W%>5%, EQR = 0

Index calculation

Total EQR for the lake is the mean of the metric EQR values. If non-native and translocated species are not present in the lake, or only one individual has been recorded (occasional occurrence), metric Non-nat_W% is not used for calculation of total EQR.

Status classes:

>0.86 high

≥0.61 good

≥0.37 moderate

≥0.18 poor

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