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Development of Bulgarian Fish Based Index for Ecological Classification and Monitoring of Natural Riparian Lakes (Type L5/L-EC-1)

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Abstract: The proposed new index BFBI-L5 was developed by using the data from 15 years ichthyological investigations in the Srebarna Lake (Danube floodplain). The method is based on the comparison of composition and structure of the fish community with the reference model ichthyocenosis as defined by historical data and data of specific hydromorphological features of the water body. Fish sampling shall be performed twice a year with standard bottom multi-mesh gillnets (NORDIC type) covering proportionally all main fish biotopes. Eleven indicators calculated through seven ichthyological parameters characterizing both fish community and type specific guilds are used to assess the ecological status of the lake. Each indicator is converted to score values according to its significance and to the specific metrics. The total sum of scores of all 11 indicators is used to calculate the ecological quality ratio (EQR) as the ratio of calculated sum of scores and the reference total score. The normalized EQR values equal the BFBI-L5, with equidistant class boundaries from 0 to 1. Although the index was developed for the lakes of type L5/L-EC1, the methodology for ecological assessment could be adapted also for other lowland natural lakes in Bulgaria, such as the lakes along the Black Sea coast.

Key words: Water Framework Directive, biological quality element fish, Danube floodplain, riparian lakes, reference ichthyocoenosis, EQR

Introduction

One of the key actions identified by the Water Framework Directive (Directive 2000/60/EC, WFD) is to develop ecological assessment tools and carry out a European intercalibration exercise. The aim of the intercalibration is to ensure that the values assigned by each Member State to the good ecological class boundaries are consistent with the Directive's generic description of these boundaries and comparable to the boundaries proposed by other member states (Poikane et al. 2011). The intercalibration was carried out in the 13 Lake Geographical Intercalibration Groups (LGIGs) according to the ecoregion and biological quality element (Poikane et al. 2014). The national methods of Bulgaria should be intercalibrated within the Eastern Continental Lake GIG. Currently the riparian lakes of type L-EC1 (corresponding to L5 of the Bulgarian national water typology) are considered the only common lake type which should be intercalibrated within the Eastern Continental Lake GIG with Romania and Hungary (EC Decision 20 Sep 2013 No.2013/480/EU). In Bulgaria this lake type is presented by natural shallow (maximum depth ≤ 3.0–3.5 m) meso- to hypereutrophic water bodies on the Danube floodplain usually significantly overgrown with macrophytes (Cheshmedjiev et al. 2010).

Fish are considered one of the Biological Quality Elements (BQE) according the Water Framework

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Directive (Directive 2000/60/EC, WFD), but until now neither Bulgaria nor the other two countries of the same intercalibration group have developed a fish based method for the ecological assessment of riparian lakes of type L-EC1, while the existing national methods of other European countries are also not applicable for different reasons. This makes it imperative for Bulgaria to develop a national method using the BQE Fish, which shall be applicable for future monitoring of waters, but can also be intercalibrated in the future, as soon as the other member states of the EC GIG have developed their own methods.

Within the targetlake type L-EC-1, the only lake, which has to be included in the River Basin Management Plans, is Srebarna Lake (Danube floodplain, Northeast Bulgaria), as it is the only lake with a surface area >50ha. In most of the other lakes, which can be assigned to the national lake type L5, permanent natural fish communities are largely missing at all or they are in a continuous process of recovering after degradation of the aquatic ecosystems due to disturbed flooding regime. Furthermore, in most of these lakes, the fish communities are affected by strong fishing pressure (including illegal fishing), which causes significant impact on their development. In contrast, the Srebarna Lake is a relatively less affected riparian lake on the Bulgarian Danube floodplain because of its protected status as a Biosphere Reserve.

The purpose of this work is to present a method (index) based on fish community parameters for ecological assessment and water monitoring of the natural lakes of type L5/L-EC-1on the Danube floodplain meeting the requirements of WFD.

Material and Methods

Methodological approach

The methodological approach follows the main principles of the theory for biological integrity of the aquatic ecosystems (Karr 1981) and is expressed in deviation from the ideal (reference) state of fish community. The assessment is carried out by biotic indices only, without using abiotic parameters.

The proposed method is based on the analysis of the composition and the structure of the fish assemblages and its comparison with the reference communities. Since in Bulgaria there are no real reference conditions for lakes, the reference parameters of the fish communities are modelled based on historical data (from previous studies, information about the fish catches etc.) and based on data for type specific hydro-morphological characteristic of the relevant lake.

Data basis

The ichthyofauna in Srebarna Lake was explored systematically under different projects during the last 15 years, which together with the data from the literature (Bulgurkov 1958, Michev et al. 1998; Hiebaum et al. 2000, Pehlivanov 2000, Pehlivanov et al. 2005, 2011, Pehlivanov & Pavlova 2009, 2012, Pavlova & Pehlivanov 2012), enabled us to determine the potential referent ichthyocoenosis for the whole target lake type.

A model of reference ichthyofauna for the lake type L5 (L-EC-1) in the flood plains of Lower Danube River

The ichthyofauna is presented by a large number of species, typical for standing waters or slow-running waters in the region of Lower Danube River. They represent several trophic guilds and include species which primarily inhabit the open water areas (in the water column and bottom layer) slightly overgrown with submerged vegetation as well as species, which prefer small peripheral “pools”, heavily overgrown with submerged vegetation. Under reference conditions, the fish density and the biomass are well-balanced as is the ratio between small and big fish (in terms of species, but also size classes). The balance between species in the fish community also requires several predator species inhabiting different biotopes. Their total biomass represents a significant proportion of the total biomass of the community. Generative rheophilous Danube species are present and form a significant part of the fish assemblage. The presence of invasive alien species by itself is not an indicator of deteriorated condition, since it is only dependent on the hydraulic connection with the Danube River, which is a prerequisite for natural invasion of alien species into the lake. They may be present with low numbers. Also some sensitive species form populations in low, but stable numbers. Under reference conditions, the percentage of fish with external signs of diseases and malformations, as well as the proportion of interspecific hybrids is low or zero.

Results and Discussion

General description of the assessment method

Sampling methodology

Due to the conservation status and the existing protecting regimes and limits in the Srebarna Lake it is not possible to apply sampling of fish with electricity with boat. Therefore the assessment method is based on quantitative sampling of fish with standard multi-mesh gill nets (type “NORDIC”) according

the Standard CEN EN 14757:2006 (Water quality. Sampling of fish with multi-mesh nets). This international standard has been transposed in BSS EN 14757:2015 and is the basis for the simplified methodology (Belkinova et al. 2013). Due to the shallowness of the Srebarna Lake (and the other riparian lakes of the same type), it is appropriate to use mainly bottom multi-mesh nets with a standard height of 1.5 m. At depths more than 3 m the use of pelagic nets with standard height of 6 m is necessary. Sampling is carried out twice per year, during the period from April to November, in order to cover differences in spatial distribution and fish activity during warm and cold months.

Sampling is implemented at two main areas, covering the two dominant types of specific biotopes (>20%) in Srebarna Lake: 1) Central open water body and 2) Peripheral pools (Fig. 1).

Within each of the two specific biotope types 4-6 nets are exposed. Due to the relatively high abundance of fish in the Srebarna Lake, the nets should be exposed during the day between May and September (preferably at dawn or dusk). The exposure shall not exceed 4 hours; if necessary, the nets could be checked after two hours. At lower water temperatures (in April, October, November) the nets should be placed at dusk and stay in the water overnight (for 8-10 hours).

The sampling of fish is accompanied by measurement of water temperature, transparency, dissolved oxygen content (concentration and saturation), pH and electric conductivity. In addition, the depth is measured at each net exposition site.

Field processing of catches

The processing of the catches is done on the shore immediately after completion of the sampling. The caught fish are removed from the nets and transferred in containers with a minimum of 50 l of water, as the catch of each net is processed and reported separately. If necessary, the water in the container shall be aerated and periodically changed. After species determination the body length of each fish is measured by the end of the caudal fin (TL) with an accuracy of 1 cm. All fish are separated in different containers by species and at the end it's the total weight per species is measured with an accuracy of 1 g.

The number of individuals with visible symptoms of diseases as well as the identified hybrids is counted.

Fig. 1. Sampling areas (specific biotopes) within the Srebarna Lake

Table 1. Rating by frequency

Frequency (pF)	
Class	%
Very rear (0)	0 – 10
Rear (I)	11 – 25
Widespread (II)	26 – 45
Frequent (III)	46 – 70
Common (IV)	71 – 100

Table 2. Rating by absolute dominance

Dominance (D)	
Class	%
Eudominant	> 16 (2^4)
Dominant	8 (2^3) – 16
Subdominant	4 (2^2) – < 8
Recedent	2 (2^1) – < 4
Subrecedent	1 (2^0) - < 2
Occasional	< 1

Table 3. Assessment of the type specific species

Species number	Score
≥ 13	10
9-12	5
< 9	0

Data analysis

The analysis is performed on an annual basis, which means that the data from the two sampling campaigns are aggregated before the assessment. To determine the ecological status the following parameters are used:

- Species composition of the ichthyofauna;
- Total and specific abundance:numbers (density) and biomass;
- Frequency (pF) and dominance (D) of the species/guilds/groups;
- Age structure of the species;
- Trophic structure;

Relative proportion (%) of fish with diseases, malformation, hybrids etc.

The quantitative parameters are calculated as Catch per Unit Effort (CPUE), where Unit Effort = 100 m^2 net for conditional exposure of 1 hour. The species are grouped in guilds, due to their ecological/biological specifics.

The number of the age-groups in the populations of each target species is determined by the distribution of the individual lengths of fish in the catches, compared with data for the ratio „length:age“ in different fish species received from previous studies.

The frequency of each species/guild/group is calculated at 5 classes (Table 1) for the whole area of the lake.

The dominance is determined by 6 classes (Table 3), according Sindrilariu et al. (2002) and Năvodaru & Năstase (2008). The absolute dominance of the species/guild/group is defined as the arithmetic average of the degrees of dominance by numbers and biomass.

Description and calculation of used indicators

Total number of native (type-specific) species

For the lakes of type L5/L-EC-1 at least 15 type-specific native species are identified: *Esox lucius, Abramis brama, Alburnus alburnus, Blicca bjoerkna, Carassius carassius, Carassius gibelio, Cyprinus carpio, Rutilus rutilus, Scardinius erythrophthalmus, Tinca tinca, Misgurnus fossilis, Silurus glanis, Gymnocephalus cernua, Perca fluviatilis, Sander lucioperca.* The calculation of the score of this indicator is done according Table 3.

Number and Dominance of predator species

The predators balance the structure of the ichthyocoenosis. They are a key element in the structure of the community, since they are the only group with clear determined trophic status. The number of the predator species is connected with the variety of biotopes and their condition and the level of dominance reflects the place of the group/guild in the structure of the community. In the target lake type the guild of native predator species includes: *Esox lucius*, *Sander lucioperca*, *Silurus glanis*, *Aspius aspius* and *Perca fluviatilis* with length L > 15 cm. The calculation of the score of this indicator is done according Table 4.

Relative biomass and frequency of predator species

The relative biomass (%) reflects the functional role of the group/guild in the ecosystem, but the frequency gives information for the density and spatial distribution of the population, respectively for suitability and efficient use of the potential biotopes. The calculation of the score of this indicator is also shown in Table 4.

“Incoming”/transient (Danubian) species

The presence of the rheophilous Danubian species in the riparian lakes is an indicator for hydraulic connection with the river and regular flooding, which is considered as a pre-condition for allowing the lake to reach or maintain the good ecological status. Species abundance is indicative for the frequency of flooding. The most common tran-

Table 4. Assessment of the predator species

Metrics	Score
4 species, ≥ 1 eu-, dominant	15
4 species, ≥ 1subdominant	10
3 species, ≥ 1 dominant/subdom. or 4 sp., no dominant/subdominant	5
< 3 species	0
Proportion in biomass ≧ 20%, ≧ 1 species of pF ≥ II	20
Proportion in biomass 15-19%, pF I-II	15
Proportion in biomass 8-14%, pF ≤ I	5
Proportion in biomass <8%	0

Table 5. Assessment of the transient (Danubian) species

Metrics	Score
≥ 1 species, ≥ 1 sp. with pF II and/or dominant	10
≥1sp.,≥ 1sp. pF I and/or subdominant-recedent	5
absent	0

Table 6. Assessment of the sensitive species

Metrics	Score
4 species, normal age structure, ≥ 3 with pF III and/ or ≥ 2 eu-, dominants	20
4 species, normal age structure, ≥ 1 pF ≥ II and/ or eu-, dominants	15
3 species, normal age structure, pF I-II and/or sub-, dominants	8
3 sp., abnormal age structure, pF I-II, subdominant-recedent	3
< 3 species, only juveniles, sub-, recedent and/ or pF 0-I	0

Table 7. Assessment of the invasive/alien species

Metrics		score
Proportion in numbers	≤ 5%	10
	5,1-10%	5
	>10%	0

Table 8. Assessment of the total abundance

Metrics	Score
Total numbers > 15 ind./CPUE	0
Total numbers 5-15 ind./CPUE	-3
Total numbers < 5 ind./CPUE	-5
Total biomass > 4 kg/CPUE	0
Total biomass 1-4 kg/CPUE	-3
Total biomass < 1 kg/CPUE	-5

sient (Danubian) species in the Srebarna Lake is asp *Aspius aspius*, but at least 5 other species of this group were recorded in the lake during the last years. The calculation of the score of this indicator is done according Table 5.

Sensitive species

The number and frequency of the sensitive species, as well the condition of their populations, expressed by both the dominance and age structure reflect the state of typical biotopes/habitats in the Srebarna Lake as a model of riparian lake on the Danube floodplain (open water areas, slightly overgrown with submerged vegetation, heavily overgrown lateral water bodies, pelagial, benthal).

Six species are identified as sensitive to specific impacts such as water quality changes, composition and abundance of the trophic base (zoo- and phytoplankton, macrozoobenthos), prolonged fall of the water level (lack of regular flooding), change in biotopes structure, fishing pressure, etc: *Alburnus alburnus, Aspius aspius, Misgurnus fossilis, Sander lucioperca, Scardinius erythrophthalmus, Tinca tinca.* The calculation of the score of this indicator is done according Table 6.

Relative numbers of invasive/alien species (including cultivated forms of carp)

By itself, the presence of alien/invasive species is not an indicator for deteriorated condition of the lake ecosystem, since the connectivity with the Danube River, which is an important factor for reaching or maintaining the good ecological status, is also a precondition for natural penetration of alien species from the river. Therefore, they can be considered a natural element of the species diversity of the lake ichthyofauna. However, a strong increase of the relative numbers of the invasive/ alien species is an indicator for anthropogenic impacts and in consequence a disruption in the structure of the ichthyocoenosis (e.g. vacant ecological niches) or deterioration of the ecological conditions in the lake (e.g. general tendency to more tolerant invasive or alien species). Typical representatives of this group are: *Ameiurus melas, Cyprinus carpio* (cultivated forms), *Hypophthalmichthys molitrix, Aristichthys nobilis,* hybrids of *H. molitrix x A. nobilis, Pseudorasbora parva, Lepomis gibbosus, Perccottus glenii.* The calculation of the score of this indicator is done according Table 7.

Total abundance expressed as Catch per Unit Effort (CPUE) includes:

Total numbers (Ntot)/CPUE

Total biomass (Btot)/CPUE

The high abundance of the fish assemblage, expressed by these two indicators, is not implicitly associated with good water quality and/or good status of the environment. On the other hand, as a rule, the significant reduction of the abundance is usually a sign of deteriorated conditions and/or strong anthro-

Table 9. Assessment of the mean individual biomass

Values	Score
≥ 0.2	5
0.1 – 0.19	3
□ 0.1	0

Table 10. Assessment of the tolerance of dominant species

Metrics	Score
> 1 sensitive species in eu-, dominants, pF II	10
1 sensitive sp. in eudominants/dominants	5
Only tolerant/eurytopic spp. eu-, dominants, pF ≥II	0

Table 11. Assessment of the health status

% fish with diseases	Score
□ 10%	0
10-20%	-5
□20%	-10

pogenic pressure. Therefore the high values of these indicators are ignored, while only the reduction of the biomass numbers is considered as indicative. The calculation of the score of these two indicators is done according Table 8.

Mean individual biomass

The mean individual size of the hydrozoocoenoses is an indicator of the water ecosystems stability (Alimov 2000). The decrease of the mean individual biomass within the fish community(calculated as a ratio B_{total}/N_{total})can be interpreted as a responseof increasing system instability, which usually is a result of growing eutrophication. The calculation of the score of this indicator is done according Table 9.

Tolerance of dominant species

The character of the dominant speciesis a very good integral indicator for the general status of the aquatic ecosystem. The ranking by dominance is adopted from Romanian researchers in contemporary studies on the ichthyofauna in lakes in the Danube River Delta (Sindrilariu et al. 2002, Năvodaru & Năstase 2008, Pehlivanov & Pavlova 2012). The absolute dominance of the species is defined as the arithmetic mean of the dominance values calculated by numbers and by biomass. The calculation of the score of this indicator is done according Table 10.

Health status

The health status is assessed as a percentage of individuals with external signs of diseases/ malformations/hybridization at catch. By itself the presence of fish with signs of carp erythrodermatitis, infestation, interspecific hybridization or certain proportion of malformations are not unusual in meso- and eutrophic water bodies such as the lakes of type L5/EC-1. It is considered that under normal conditions the natural selection leads to the removal of the individuals with significant deviations and they do not affect the health status of the community as a whole. Therefore, the reference value of this indicator is zero. The increase in the number of infected individuals, however, is an indicator for deterioration of the ecological status (primarily as a result of water contamination). The increase in the number of hybrids is a sign of poor condition of the populations and violated conditions for reproduction. The calculation of score of this indicator is done according Table 11.

Reference value and the calculation of the ecological quality ratio (EQR)

There are numerous different anthropogenic pressures, which affect the lake ecosystem in general and specifically the fish community (Table 12). Therefore an increase of the metric values are assessed by increasing scores in some cases (e.g. Table 10), while they decrease in others (e.g. Table 11). The assessment is carried out by summing up all scores.

The ecological status (ES) is expressed through Ecological Quality Ratio (EQR) and calculated by the formula:

$$EQR = \frac{Total\ score\ of\ the\ assigned\ values}{Maximum\ (Reference)\ total\ score}$$

The sum of scores can reach a maximum of 100, which also represent the highest EQR value possible (1.0). As some scores are negative, the minimum sum possible is –20 (EQR -0.2).

Class boundaries are defined by dividing the total range into 5 classes of equal width. Finally, in order to achieve EQR values between 0 and 1 with equidistant class widths (which gives a High/Good boundary at 0.8, Good/Moderate at 0.6 etc.), the EQR values are transformed by the following formula:

$$nEQR = \frac{EQR - minimum\ EQR}{Maximum\ EQR - Minimum\ EQR}$$

which equals:

$$nEQR = \frac{Total\ score\ of\ the\ assigned\ values - Minimum\ total\ score}{Maximum\ total\ score - Minimum\ total\ score}$$

This nEQR is defined as the Bulgarian Fish Based Index for Lakes of type L5 (BFBI-L5).

Assessment of the Ecological status according Bulgarian Fish Based Index-L5 is presented in the Table 13.

Evaluating the pressure – response relationships

One of the most significant environmental factors,

Table 12. Response to the anthropogenic pressure and reference scores of the used indicators. EQR = (calculated total score)/(reference total score)

Indicator	Response to anthropogenic pressure	Maximum (Reference) score
Total number of native (type specific) species	↘	10
Number and dominance of predators	↘	15
Relative biomass and frequency of predators	↘	20
"Incoming" (Danubian) transient species	↘	10
Sensitive species	↘	20
Relative numbers of invasive/alien species	↗	10
Total numbers/CPUE	↘	0
Total biomass/CPUE	↘	0
Average individual biomass	↘	5
Tolerance of dominant species	↗	10
Health status (a proportion of individuals with signs of diseases/ malformations/infestation and hybrids)	↗	0
Sum of scores = reference value		100
maximum EQR		1.00

Table 13. Assessment of the Ecological status by BFBI-L5/EQR values

Ecological status	sum of scores	EQR	nEQR = BFBI-L5
High	≥76	≥ 0.76	≥ 0.80
Good	52 – 75	0.52 – 0.75	0.60 – 0.79
Moderate	28 – 51	0.28 – 0.51	0.40 – 0.59
Poor	4 – 27	0.04 – 0.27	0.20 – 0.39
Bad	< 4	< 0.04	< 0.20

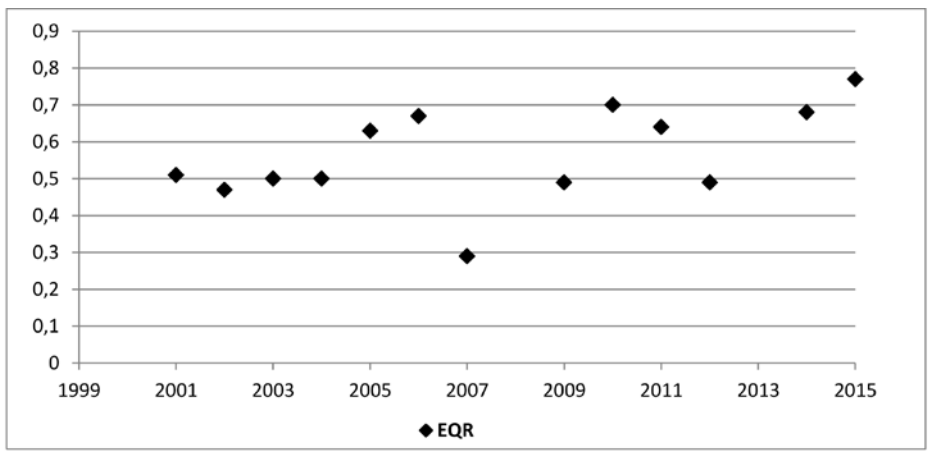

Fig. 2. Changes of the mean annual values of water depth

which affect the fish communities in floodplain lakes is the hydro-morphology and especially habitat connectivity (Amoros & Roux 1988, Bolland et al. 2012, Glińska-Lewczuk et al. 2016). As a consequence, changes of the flooding regime and the hydraulic connection with the Danube are considered as a main anthropogenic pressure.

This hypothesis was tested by using the new index BFBI-L5 and applying it in a dataset from the Srebarna Lake, in order to track and assess the changes of its ecological status against the environmental changes. The data stem from long-term ecological studies (Pehlivanov et al. 2005, 2006, 2011, 2012; Pehlivanov & Pavlova 2008, 2012, 2009; Varadinova et al. 2009, 2012, Pavlova & Pehlivanov 2012) shows that the variability of the BFBI-L5for the period from 2001 to 2013 (Fig. 2) follows the same temporal pattern as the long-term

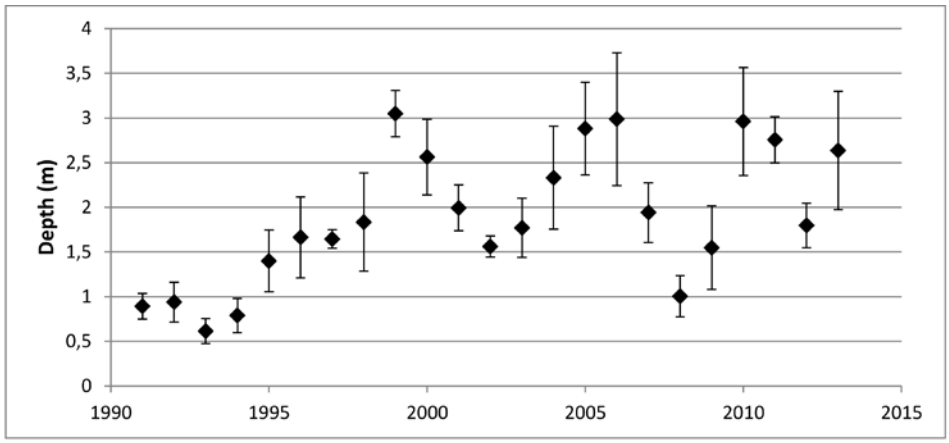

Fig. 3. Changes of the ecological status (EQR) between 2001 and 2015

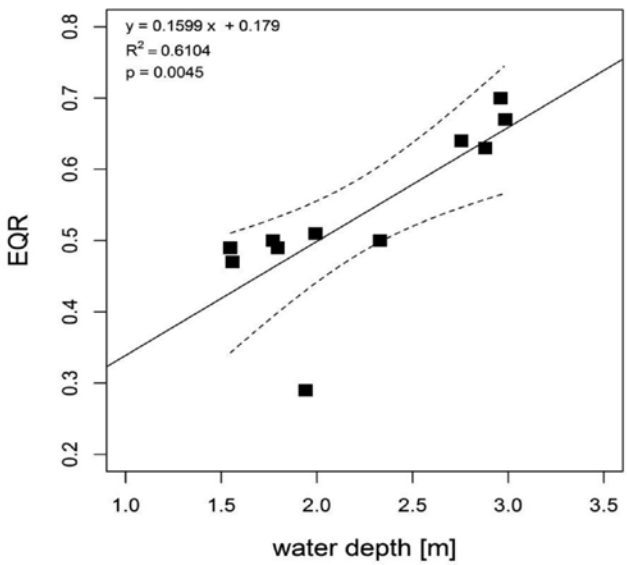

Fig. 4. Coefficient of determination between water level and the correspondent BFBI-L5 for available data concerning Srebarna lake, collected from 2001 to 2012 (incomplete data from 2008 and 2013-2015)

variations of mean annual water depth during the same period (Fig. 3) as an expression of the flooding by the Danube.

The regression model shown in Fig. 4 reveals a significant positive correlation (p=0.005). It can be concluded that the flooding regime is the key driver for the ecological integrity of the lake ecosystem.

Conclusion

The idea of this study was to develop a practical method, which is not too labour-intensive and time-consuming. The Srebarna Lake is practically the only representative of the type L5 in Bulgaria, which can become a problem during the intercalibration with methods from other member states. However, the proposed methodology allows at least tracking and assessing the temporal changes of its ecological status. It is thus recommended as a method for the national monitoring of this lake type.

A general problem of assessment methods using fish, both in Bulgaria and in the rest of Europe, is the fact that fish communities are very often affected by a specific anthropogenic pressure: fishing and/or fish stocking (cf. Poikane et al. 2015, Zick et al. 2006). Therefore final assessment must take into account the fishing pressure in order to avoid misclassifications.

Although the scope of the present fish based method/index is limited to the defined lake type, the same approach could be easily adapted also to other national lake types in Bulgaria, such as Black Sea coastal lakes of national types L7, L8, L9 and L10 (Cheshmedjiev et al. 2010). Further data and analyses are necessary to evaluate whether the parameter selection and scoring system can also become a template for assessing the ecological status/potential of lake types in higher altitudes. In all cases, however, it is first necessary to determine the reference fish communities and the reference values of the selected metrics, and to identify the representatives of the key ecological groups in the composition of the ichthyocoenosis.

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