OTKA PD 115801

Functional diversity of the Carpathian fish fauna. Preliminary studies and database development.

Final Report

In this OTKA proposal I had three major tasks:

1. **Method testing**: to test the usability of different morphometric methods potentially usable to create the functional trait database
2. **Compilation**: to compile a functional and life history trait database of the Carpathian fish fauna
3. **Comparison**: to compare the results of functional trait and „traditional" diversity computations

1. Method testing

1. a) Since as morphometric methods are widely used to record functional trait data, we compared the repeatability, reproducibility (intra- and inter-measurer similarity), separative power and subjectivity (measurer effect on results) of four morphometric methods frequently used in ichthyological research. The "traditional" caliper-based (TRA) and truss-network (TRU) distance methods and two geometric methods that compare landmark coordinates on the body (GMB) and scales (GMS) were tested usin three-three populations' morphometric data of three common Cyprinid fish. TRA measurements were made on conserved specimens using a digital caliper, while TRU, GMB and GMS measurements were undertaken on digital images of the bodies and scales. In most cases, intra-measurer repeatability was similar. While all four methods were able to differentiate the source populations, significant differences were observed in their repeatability, reproducibility and subjectivity. GMB displayed highest overall repeatability and reproducibility and was least burdened by measurer effect. While GMS showed similar repeatability to GMB when fish scales had a characteristic shape, it showed significantly lower reproducability (compared with its repeatability) for each species than the other methods. TRU showed similar repeatability as the GMS. TRA was the least applicable method as measurements were obtained from the fish itself, resulting in poor repeatability and reproducibility. Although all four methods showed some degree of subjectivity, TRA was the only method where population-level detachment was entirely overwritten by measurer effect. Based on these results, we recommended a) avoidance of aggregating different measurer's datasets when using TRA and GMS methods; and b) use of image-based methods for morphometric surveys. These results are published. For more details, see: Takács et al. 2016., PLoS ONE.

1. b) Morphometric measurements usually made on stictly one bodyside of the studied specimens. Since there is no consensus about which side is more appropriate for morphometric studies, one finds notes originating from datasets of both the right and left sides. Moreover, no information has been published about how the bodyside-specific data processing influences the comparability of population-level morphometric studies, and how this feature changes if different morphometric methods are used. Therefore in our other methodologic study the effect of bodyside specific data management was investigated. To facilitate the analyses, data of four common fish species (bleak, roach, perch, pumpkinseed sunfish) collected from three closely related sites were used. The separative powers of the datasets derived from opposite bodysides do not show systematic differences in any of the studied species. Therefore the bodyside "per se", does not affect the results of the morphometric studies. Results show that the population origin is of significantly (up to 35 times) greater importance than the bodyside if the geometric method is used. While the traditional method demonstrates a similar trend, due to the general uncertainties of this method, the bodyside origin of the data must be taken into consideration. Our findings are significant for datasets containing different aggregated or merged bodyside-originated data, or if the results of different investigations are to be compared. These results are published. For more details, see: Takács et al. 2018., FAL.

1. c) In one of our previous studies phylogenetic features of Hungarian stream dwelling gudgeons (*Gobio*, Cyprinidae) was studied. Five geneticaly distinct haplogroups (cryptic species?) indicated from the study area, but no information have been published about that the revealed genetic differences how manifest in morphometric or meristic features. In our present work we wanted to compare the usability of geometric and distance based image processing morphometric methods for the designation of these cryptic entities. Moreover we wanted to test how the number of the measured morphometric variables can be reduced. Additionaly we tried to find out if there any detectable morphometric differences among the three dominant haplogroups indicated from the NW, SW and NE area of Hungary. For this reason simultaneous genetic (608 b long mitochondrial Control Region sequenation) and morphometric studies were made on 103 individuals collected from five sampling localities across Hungary (situated to the NW, SW and NE area of Hungary, **Figure 1**).

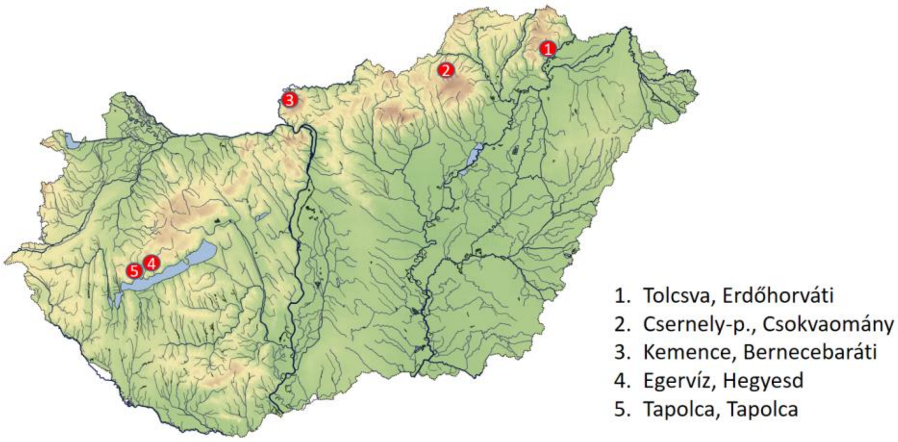


Figure1: Five sampling sites for the gudgeon morphometric studies

The results of mtCR sequenation showed that the studied individuals can be classified into nine haplotypes which grouped into four cryptic entities (**Figure 2**). Only one individual from te Tolcsva stream proved to be *G. carpathicus*. 37 and 27 individuals collected from the Danubian watershed groupped into the *G. obtusirostris*, and the „Southern" haplogroup respectively, moreover 38 individuals from the Tisza drainage identified as *G. „sp1"* (see: Mendel et al. 2008). Morphometric features of these three latter cryptic groups were investigated further.

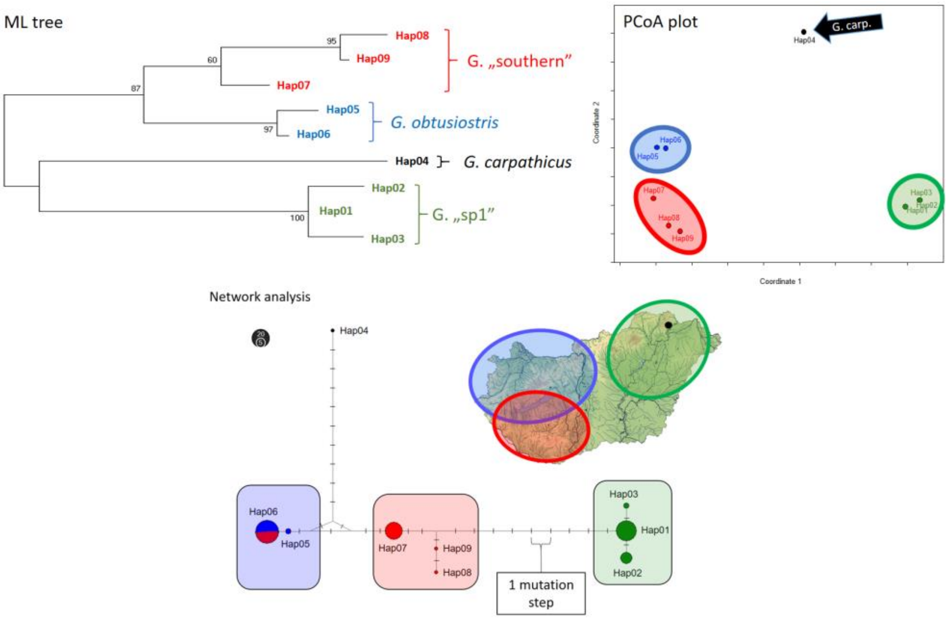
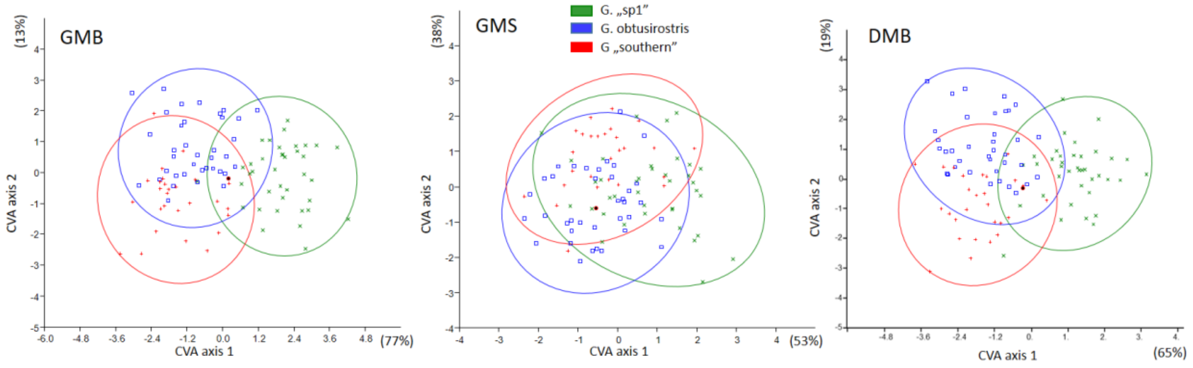


Figure 2: ML tree, PCoA plot and ther resuult of Network analysis of the 608 b long mtCR data of the investigated 103 individuals. (Morphometric features of *G. „southern"*, *G. obtusirostris*, and *G. „sp1"* were analysed further. on the inserted map the presumed distribution of the four haplotype groups is indicated, by Takács et al. 2014.)

Geometric morphometric investigations made on photos taken from the entire fish body (11 landmarks), and from scales (seven landmarks), moreover for the distance based morphometric computations 34 distance measurements were made on photos taken from the entire fish body also. All the morphometric data were standardized by appropriate methods (for more details see: Takács et al. 2016., PLoS ONE.)



Confusion matrix

	G.Sp1	G. obt	Southern	Total
G. Sp1	**97%**	0%	3%	38
G. obt	3%	**73%**	24%	37
Southern	4%	11%	**85%**	27

85%

Sign (p<0.05)	G. Sp1	G. obt	Southern
SP1	-		
G. obt	0.0000	-	
Southern	0.0000	0.0973	-

SQ MAHAL DIST	G. Sp1	G. obt	Southern
SP1	-		
G. obt	9.36	-	
Southern	12.76	3.05	-

Confusion matrix

	G. Sp1	G. obt	Southern	Total
G.Sp1	**61%**	21%	18%	38
G. obt	13%	**55%**	29%	37
Southern	13%	13%	**47%**	27

50%

Sign (p<0.05)	G. Sp1	G. obt	Southern
G. SP1	-		
G. obt	0.0067	-	
Southern	0.0282	0.1364	-

SQ MAHAL DIST	G. Sp1	G. obt	Southern
G. SP1	-		
G. obt	1.94	-	
Southern	1.94	1.58	-

Confusion matrix

	G. Sp1	G. obt	Southern	Total
G. Sp1	**89%**	5%	5%	38
G. obt	5%	**81%**	14%	37
Southern	4%	11%	**85%**	27

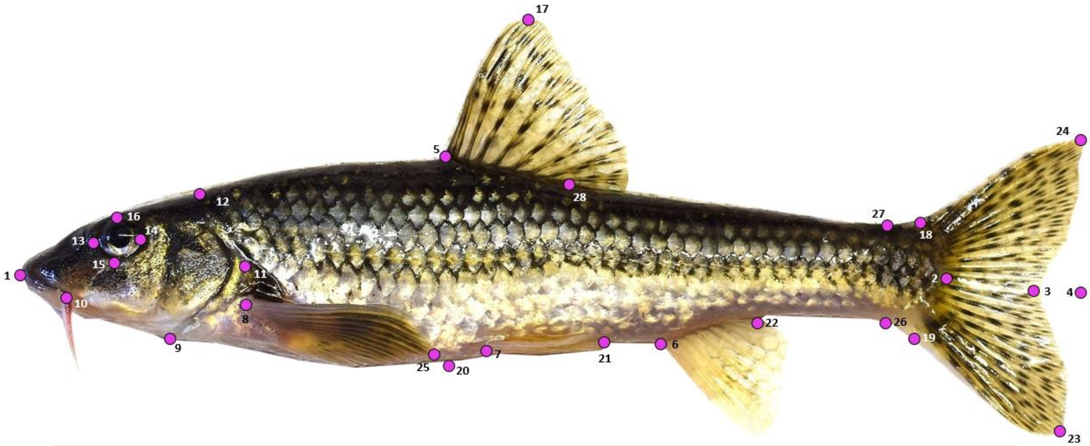
85%

Sign (p<0.05)	G. Sp1	G. obt	Southern
G. SP1	-		
G. obt	0.0000	-	
Southern	0.0002	0.6782	-

SQ MAHAL DIST	G. Sp1	G. obt	Southern
G. SP1	-		
G. obt	9.66	-	
Southern	10.95	3.72	-

Figure 3: CVA plots, confusion matrices, significances, and squared Mahalanobis distances of the compared three haplogroups. Green: *G. „sp1”*, blue: *G. obtusirostris*, red: *G. „southern”*. Used methods: Geometric morphometric measuements on body (GMB) and scales (GMS) and the distance based body measurements (DMB)

Results of CVA analyses (**Figure 3**) show that geometric morphometry and the distance based analysis of the photos taken from the entire body showed highly similar patterns with the results of genetic analyses. The G. „sp1” isolatate almost completely from the other two cryptic entities, at the same time these latter two did not differ significantly. By our results the 85% of the studied individuals were correctly classified by the GMB and DMB methods, at the same time this value is only 50% in the case of GMS (**Figure 3**). Therefore it seems that the two prevoius methods are appropriate to isolate these close relative cryptic entities, but the scales shape analysis is not usable for this purpose.



	Var 01	Var 02	Var 03	Var 04	Var 05	Var 06	Var 07	Var 08	Var 09	Var 10	Var 11	Var 12	Var 13	Var 14	Var 15	Var 16	Var 17	Var 18	Var 19	Var 20	Var 21	Var 22	Var 23	Var 24	Var 25	Var 26	Var 27	Var 28	Var 29	Var 30	Var 31	Var 32	Var 33	Var 34
Startpoint	1	1	1	1	1	1	1	1	1	1	13	14	15	9	9	5	5	5	5	5	5	5	5	7	7	7	6	6	6	19	19	18	8	5
Endpoint	3	5	6	7	8	9	10	11	12	13	14	11	16	12	5	17	18	19	6	12	8	20	7	21	12	9	18	22	19	23	18	24	25	28

Figure 4: The satrt and endpoints of the measured 34 distances. The start- and endpoints of each variable are indicated in the embedded table

Using the available distance based datasets (34 standardized distance data -see: **Figure 4-** for the 103 genetically identified individuals) I compared each measured variables among the mentioned cryptic species. The most important three morphometric variables (which can be characterised by the highest F values) are indicated in the **Figure 5 A-C.** Several variables of the *Gobio „Sp1"*showed significatntly lower values from the G. obtusirostris and the Southern haplogroup. But the pairwise comparisons of these values in most cases show considerable overlaps. Moreover we found statisticaly significant differences in a meristic feature as well. The number of scales on the lateral line was higher in the case of *Gobio „Sp1"* than in the other three cryptic species.

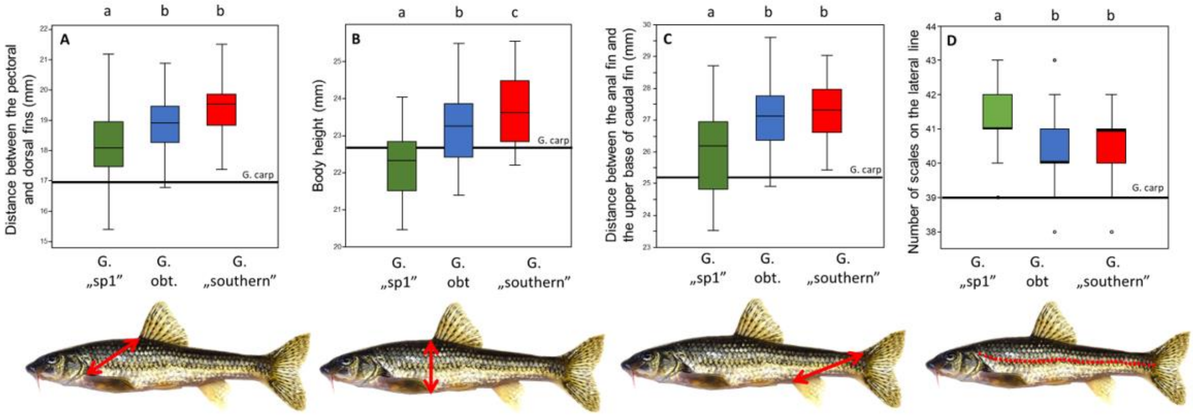
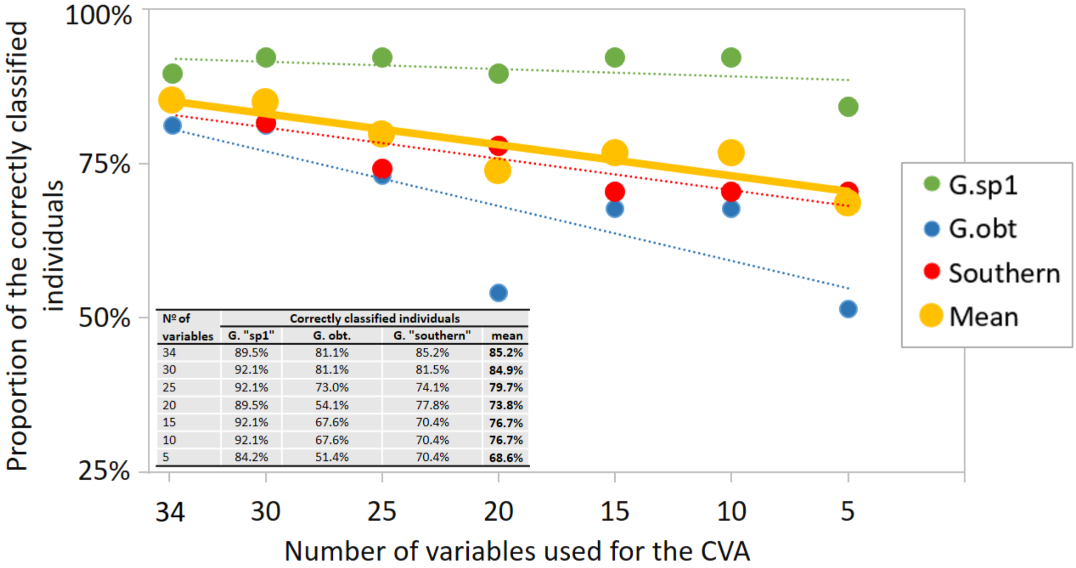


Figure 5: The three most important morphometric features discriminating the studied *Gobio* groups (A-B-C) and the differences in the lateral line scale numbers (D). Since as the *G. carpathicus* appeared with a single individual its data was indicated as a black line in each boxplots. All morphometric data were standardized to SL= 86.794mm. Boxplots with the same letters do not differ significantly based on Kruskall-Wallis tests ($p \geq 0.05$). The box represents the 25% and 75% quartiles, the band in the box is the median. The whiskers represent the highest and lowest values that are not outliers or extreme values. Outliers (values that are more than 1.5 times the interquartile range) are represented by circles beyond the whiskers

The above mentioned results suggest, that the *Gobio „sp1"* is separated from the other studied cryptic *Gobio* species, but due to their considerable overlaps the revealed meristic and morphometric variables are still not usable for the identification in the field. By my opinion the morphometric and genetic differentiation of Gobio „sp1" from its congeners is enough to describe it as a valid species in the future, but further detailed genetic investigations (nuclear genom analyses), and more detailed morphometric surveys using multiple variables are needed.

Additionaly the distance based dataset of the above mentioned project was used to reveal how the deduction of analysed variable numbers may affect the classification power of Canonical Variable Analysis. The morphometric variables were ranked by their F values (the proportion of the among and within groups sum of square values). Than I compared the resulst of CV analyeses using decreasing (34, 30, 25, 20, 15, 10, 5) number of variables, Where in each run the last five variables characterised by the lowest F values were omitted. The group Gobio „Sp1" showed significant differentiation from the *G. obtusirostris* and *G. „southern"* groups, at the same time these latter two groups did not differed, and this feature was independent from the number of variables used for the analyses. It means in our case only the five morphometric variables characterized by the highest F values can separate the three groups as if all the 34 variables would be used for the analyses. At the same time we have to note, that the proportion of correctly classified cases decreases with the increasing number of omitted variables (**Figure 6**). Althoght for this trend mostly the datasets of geneticly close related *G. obtusirostris* and *G. „southern"* groups are the responsible.

Our results have already been presented party in national and international conferences, and our preliminary results inspired one publication in Hungarian language (see: Takács, 2018, Pisces Hungarici) manuscript under preparation. I plan to submit it to the journal Zoologica Scripta (https://onlinelibrary.wiley.com/journal/14636409) in the summer of 2019.



Nº of variables	Correctly classified individuals			
	G. "sp1"	G. obt.	G. "southern"	mean
34	89.5%	81.1%	85.2%	**85.2%**
30	92.1%	81.1%	81.5%	**84.9%**
25	92.1%	73.0%	74.1%	**79.7%**
20	89.5%	54.1%	77.8%	**73.8%**
15	92.1%	67.6%	70.4%	**76.7%**
10	92.1%	67.6%	70.4%	**76.7%**
5	84.2%	51.4%	70.4%	**68.6%**

Figure 6: The proportion of the correctly classified individuals in relation with the number of variables used for the Canonical Variate Analyses in the case of the three haplogroups. lines: linear trendlines. For the indicated values see the inserted table.

2. **Compilation**

Using the results of our methodical researches and literature notes I recorded morphometric data of the Carpathian fish species which from the functional trait database was created. Several field collections were executed in Hungary and in the surrounding countries to collect information, moreover several rare fish species' data were obtained from conserved specimens can be found in the institutional (MTA CER BLI and DRI) and museum (e.g. Hungarian Natural History Museum, Budapest) collections. For the functional trait analyses altogether 42 morphometric data of five-five inidividuals for each species recorded and averaged. From the morphometric variables functional traits were created using the formulas published by Villéger et al. 2010. (see: Appendix A). Parallely life history trait database of the Carpathian fish fauna were broadened by literature data.

As the result of the collections and data mining from literature notes, the database contains recently 15 functional and 10 life history traits of 78 Carpathian fish species. Of course we would like to widen this database, to fill the gaps with the missing information, moreover in the future several other species data will be uploaded to the database. The excel file containing the functional and life history database is available from the following link: (https://www.dropbox.com/s/so7gmm2jpa94d7n/FunctionalTrait_Table.xlsx?dl=0)

Our countrywide morphometric data collection had two non planned results. Namely A) during the morphometric data collections, some features of the investigated pike specimens showed remarkable differences from the litterature data published for the Northern Pike - *Esox lucius*. Their striped body pattern and fewer scales (<115 vs. 120-144) counted on the lateral line made them similar to the smaller bodied *Esox* species which have been described from Italian and French freshwaters recently. Additionaly these 'atypic' pike phenotypes occurred from several, distant sample sites as well during our countrywide field investigations. Therefore the taxonomic position of the most frequent predatory fish species in Hungary became questionable (for more datails see: Takács et al., 2018, Pisces Hungarici). Additionaly B) during our fish faunistic researches executed on the litoral zone of the Lake Balaton the recent distribution pattern, and the still ongoing invasion of a non native Prosobranch snail was recorded (for more details see: Takács et al. 2019, Bioinvasions Records).

To reveal the usability of the functional trait database I compared the results of traditional diversity and the functional trait diversity computations using the same faunistic datasets. For this reason from the available faunistic data recorded by the Fish and Benthos working group of the MTA CER BLI a country wide database was compiled. The faunistic surveys were executed in the last some years on 767 sites using electric fishing gear, following the recommendations habitat type specific sampling protocols (See: **Figure 7**). On **Table 1** the total catch is indicated by habitat types.

Nº	**Scientific name**	**code**	**MS**	**HS**	**HR**	**LS**	**LR**	**D**
1.	*Abramis brama* (Linnaeus, 1758)	Abrbra		+	+	+	+	+
2.	*Alburnoides bipunctatus* (Bloch, 1782)	Albalb	+	+	+	+	+	+
3.	*Alburnus alburnus* (Linnaeus, 1758)	Albbip	+	+	+	+	+	+
4.	*Ameiurus melas* (Rafinesque, 1820) #	Amemel		+	+	+	+	
5.	*Anguilla anguilla* (Linnaeus, 1758)	Angang				+		
6.	*Babka gymnotrachelus* (Kessler, 1857) #	Babgym					+	+
7.	*Ballerus ballerus* (Linnaeus, 1758)	Balbal		+		+	+	
8.	*Ballerus sapa* (Pallas, 1814)	Balsap			+	+	+	+
9.	*Barbatula barbatula* (Linnaeus, 1758)	Ortbar	+	+	+	+	+	
10.	*Barbus barbus* (Linnaeus, 1758)	Barbar	+	+	+	+	+	+
11.	*Barbus carpathicus* (Kotlík, Tsigenopoulos, Ráb and Berrebi, 2002)	Barcar	+	+	+	+	+	
12.	*Blicca bjoerkna* (Linnaeus, 1758)	Blibjo	+	+	+	+	+	+
13.	*Carassius carassius* (Linnaeus, 1758)	Carcar	+	+		+	+	
14.	*Carassius gibelio* (Bloch, 1782) #	Cargib	+	+	+	+	+	+
15.	*Chondrostoma nasus* (Linnaeus, 1758)	Chonas	+	+	+	+	+	+
16.	*Cobitis elongatoides* Băcescu and Maier, 1969	Cobelo	+	+	+	+	+	+
17.	*Ctenopharyngodon idella* (Valenciennes, 1844) #	Ctenid		+		+	+	
18.	*Cyprinus carpio* (Linnaeus, 1758)	Cypcar	+	+	+	+	+	+
19.	*Esox lucius* (Linnaeus, 1758)	Esoluc	+	+	+	+	+	+
20.	*Gambusia holbrooki* (Girard, 1859) #	Gamhol		+		+		
21.	*Gasterosteus aculeatus* (Linnaeus, 1758) #	Gasacu		+		+		+
22.	*Gobio gobio* (Linnaeus, 1758) sensu lato *	Gobgob	+	+	+	+	+	
23.	*Gymnocephalus baloni* Holčík and Hensel, 1974	Gymbal				+	+	+
24.	*Gymnocephalus cernua* (Linnaeus, 1758)	Gymcer	+	+		+	+	+
25.	*Gymnocephalus schraetser* (Linnaeus, 1758)	Gymsch			+		+	+
26.	*Hypophthalmichthys nobilis X molitrix (hybride)*** #	HypHYB				+	+	
27.	*Knipowitschia caucasica* (Berg, 1916) #	Knikau					+	
28.	*Lepomis gibbosus* (Linnaeus, 1758) #	Lepgib	+	+	+	+	+	+
29.	*Leuciscus aspius* (Linnaeus, 1758)	Leuasp		+	+	+	+	+
30.	*Leucaspius delineatus* (Heckel, 1843)	Leudel	+	+	+	+	+	
31.	*Leuciscus idus* (Linnaeus, 1758)	Leuidu	+	+	+	+	+	+
32.	*Leuciscus leuciscus* (Linnaeus, 1758)	Leuleu	+	+	+	+	+	+
33.	*Lota lota* (Linnaeus, 1758)	Lotlot	+	+	+	+	+	+
34.	*Misgurnus fossilis* (Linnaeus, 1758)	Misfos		+		+	+	
35.	*Neogobius fluviatilis* (Pallas, 1814) #	Neoflu	+	+	+	+	+	+
36.	*Neogobius melanostomus* (Pallas, 1814) #	Neomel		+	+	+	+	+
37.	*Oncorhynchus mykiss* (Walbaum, 1792) #	Onkmyk	+	+				
38.	*Pelecus cultratus* (Linnaeus, 1758)	Pelcul					+	
39.	*Perca fluviatilis* (Linnaeus, 1758)	Perflu	+	+	+	+	+	+
40.	*Perccottus glenii* (Dybowski, 1877) #	Pergle		+		+	+	
41.	*Phoxinus phoxinus* (Linnaeus, 1758)	Phopho	+	+	+	+		
42.	*Ponticola kessleri* (Günther, 1861)	Ponkes					+	+
43.	*Proterorhinus semilunaris* (Pallas, 1814) #	Prosem		+	+	+	+	+
44.	*Pseudorasbora parva* (Temminck and Schlegel, 1842) #	Psepar	+	+	+	+	+	
45.	*Rhodeus sericeus* (Pallas, 1776)	Rhoser	+	+	+	+	+	+
46.	*Romanogobio kesslerii* (Dybowski, 1862)	Romkes		+	+		+	
47.	*Romanogobio vladykovi* (Fang, 1943)	Romvlad	+	+	+	+	+	+
48.	*Rutilus pigus virgo* (Heckel, 1852)	Rutpig		+		+	+	+
49.	*Rutilus rutilus* (Linnaeus, 1758)	Rutrut	+	+	+	+	+	+
50.	*Sabanejewia aurata* (Filippi, 1865)	Sabaur		+	+		+	+
51.	*Salmo trutta morpha fario* (Linnaeus, 1758)	Saltru	+	+	+	+		
52.	*Sander lucioperca* (Linnaeus, 1758)	Sanluc		+	+	+	+	+
53.	*Sander volgensis* (Gmelin, 1788)	Sanvol				+		+
54.	*Scardinius erythrophthalmus* (Linnaeus, 1758)	Scaery		+	+	+	+	+
55.	*Silurus glanis* (Linnaeus, 1758)	Silgla			+	+	+	+
56.	*Squalius cephalus* (Linnaeus, 1758)	Squacep	+	+	+	+	+	+
57.	*Tinca tinca* (Linnaeus, 1758)	Tintin		+	+	+	+	
58.	*Umbra krameri* Walbaum, 1792	Umkra				+		
59.	*Vimba vimba* (Linnaeus, 1758)	Vimvim		+	+	+	+	+
60.	*Zingel streber* (Siebold, 1863)	Zinstr		+	+		+	
61.	*Zingel zingel* (Linnaeus, 1758)	Zinzin			+		+	+
		species number	**29**	**48**	**42**	**51**	**53**	**38**
		number of individuals	**7886**	**49775**	**15524**	**75971**	**43207**	**6358**

Table 1: List of species and their occurrences in the six habitat types. Species code: scientific name abbreviation, MS: Mountain Stream, HS: Hilly Stream, HR: Hilly River, LS: Lowland stream, LR: Lowland River, D: Danube. Non-native species are highlighted by # and red colour. * The taxonomic position of stream dwelling gudgeons are still not cleared in detail (see. Takács 2018), ** for more details see: Vital et al. 2017.

Althought beside the climate change and habitat degradation the expansion of non native species is considered to be the third most acute problem which threatens native biota; there is a scarce information available about how their occurrence modify the fish assemblage structure of fluvial systems. We compiled this database primarily to reveal how the different (traditional and functional trait) diversity indices may change by the occurrence of non native species.

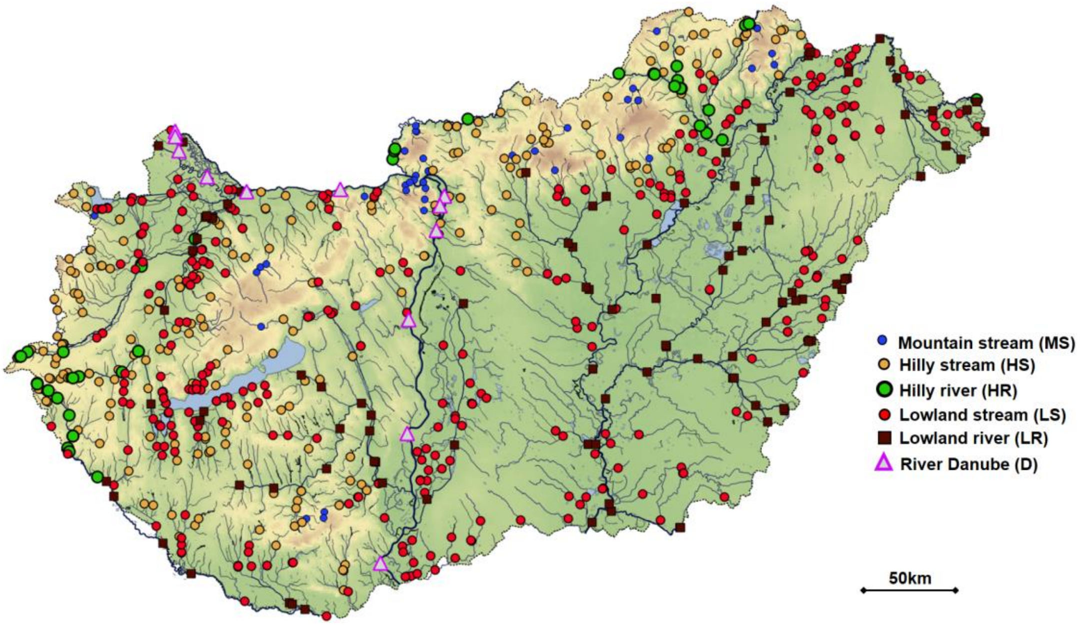


Figure 7. Sampling sites, classified into six habitat types used for the functional trait database testing

Additionaly this database can be used to reveal the actual distribution pattarens of the non native fish species in the Hungarian fluvial system as well. Moreover till 2017 there was no comprehensive information published about the appearance and distribution of non indigenous fish in Hungary. For this reason at first we collected the available historic and recent data of the non native species occurrences, moreover our faunistic database was used to present the countrywide distribution of the eight most frequent non indigenous species, and their distribution in the six habitat types also evaulated. For more details, see: Takács et al. 2017., Hydrobiologia. These latter results were used in the evaulation and interpretation of the functional trait computations' results as well.

Moreover this countrywide fish faunistic dataset was used for other purposes as well. E.g. to present the recent distribution pattern of the most frequent protected fish species in Hungary. Fot more details see: Takács et al. 2018, Hidrológiai Közlöny

3. Comparison

The compiled fish faunistic database was used to reveal how the functional diversity of an assemblage may change by the occurrence of non native species. For this reason we evaulated how the non indigenous fish species modify the results of „traditional" and functional diversity computations. At first only the species lists of the studied sites were investigated. To reveal the place of the collected 61 species in the functional trait space a Principal Component Analyses were made using of their 15 functional trait data (**Figure 8**.).

The species' position on the plot corresponds with their role in the assemblages. The closer positioned species on the plot supposedly have more similar function in the fish assemblages. The funcional diversity of the certain habitat types' fish fauna are in correspondance with the area covered by the species indicated from them. To reveal the importance of non native species the polygon areas covering the native and entire species pools are compared. In this case the convex hull areas were calculated using the xy coordinates of species by GIS software.

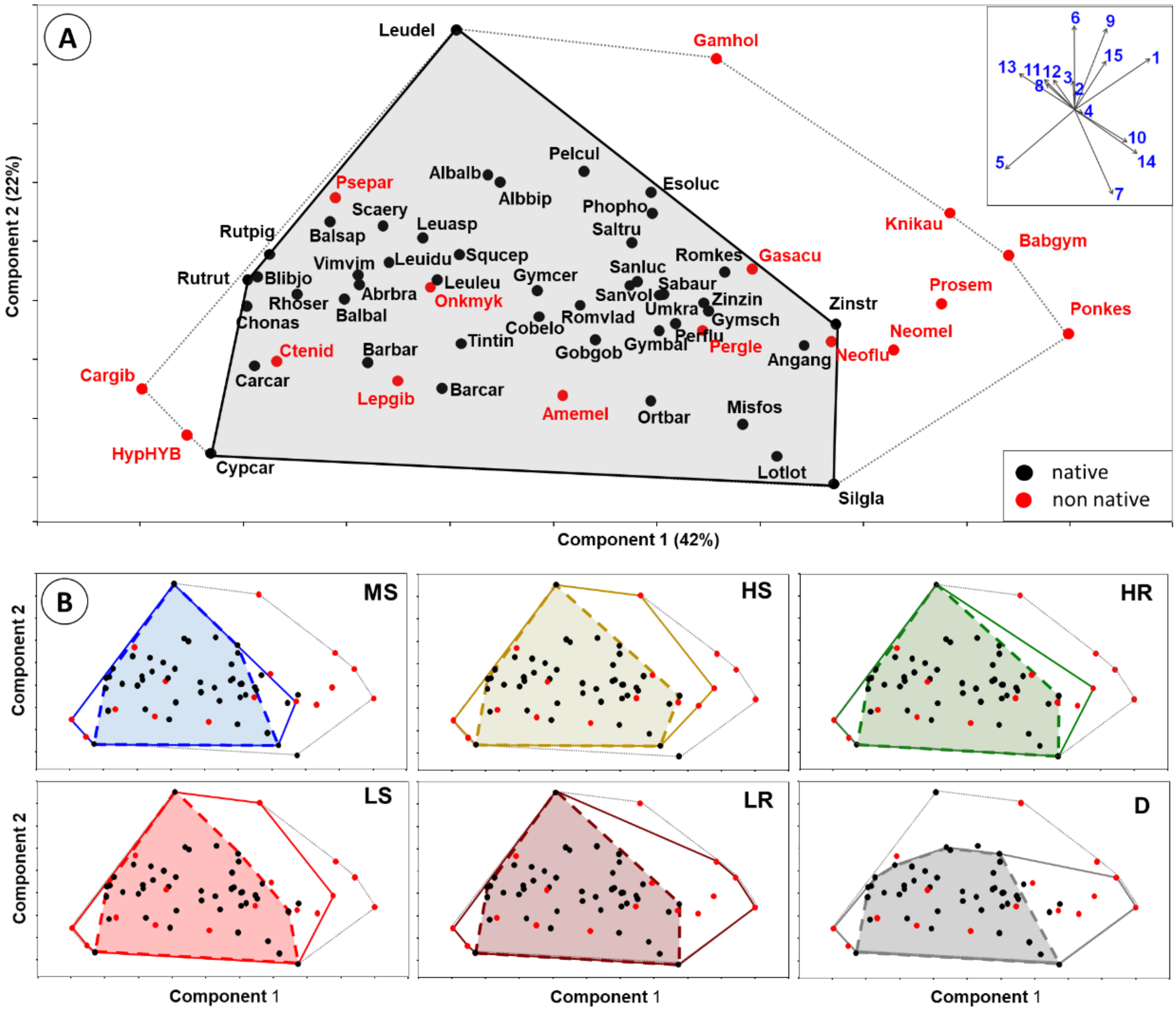


Figure 8: Principal Component Analysis plots of the 61 Middle Danubian fish species based on their 15 functional trait attributes, where the convex hulls of the native species is shaded by grey colour (A); the differences of the entire (straight line framed polygons) and the native species pool (dashed line framed polygons) for each of the the six habitat types (B). Blue numbered insert in the the top right corner shows the correlations of the 15 functional traits. For codes see database. Species code indicated in the Table 1 and trait database. Non-indigenous species are highlighted by red. Abbreviations and colour codes of the certain habitat types corresponds with the Figure 6. The overall species pool is farmed by grey dotted polygon in each plot.

In the subfigure „A" the entire dataset (containing all the 61 species) was analyzed, while the lower part (B) the functional diversities in the six habitat types are shown if the whole species list and if only the native faunaelemnets were taken into account. The differences between the functional diversity of the whole species pool and native faunaelemets were calculated as the differences of polygon areas.

Results show that native species' polygon size represent only the 65% of the entire species pool's area (see: **Figure 8A**) therefore the non native species contribute considerably for the functional diversity of the Hungarian flowing waters. Several non native species' position on the plot (e.g. Ponto-Caspic gobies, or silver-, and gibel carps) indicates that they have special role in the invaded assemblages. Maybe this feature can be responsible for their invasion success.

The above mentioned computation was executed for each of the six habitat types as well, using their whole and native species pools. In these cases the area of convex hulls covering their whole species pool and the native species pool were expressed in the proportion of the area covered by entire species pool. Results (**Figure 8B** and **Table 2**) show that non native species considerably (above 20%) enlarged the polygon areas of HS, LS, LR and D while the MS and HR are less impacted by their effect. These results are in correspondance of the species list differences, namely the MS and HR habitat types are less impacted by non native species in Hungary (See: Takács et al. 2017, Hydrobiologia Table 2.)

habitat type	whole species pool	non-natives excluded	difference	number of species	
				Tot	**NN**
Entire	100%	65%	35%	61	16
MS	62%	54%	12%	30	5
HS	84%	61%	23%	50	12
HR	78%	65%	13%	43	7
LS	89%	62%	27%	58	15
LR	94%	65%	29%	53	13
D	73%	49%	24%	38	8

Table 2. The poligon sizes of the certain habitat types expressed in the percentage of the polygon's area containing the entire species pool, in case if the whole species lists is taken into consideration, and if the non-natives are excluded. Difference: differences of the polygons' areas derived from the whole species lists and from the native faunaelements data for the entire species poo and for each habitat types. The total number of species (Tot) and number of non native species (NN) indicated fromthe six studied habitat types

The effect of non native species are further investigated on sample site level by non abundance weighted and abundance weighted metrics, both for „traditional" and functional trait diversity computations. For this reason the non abundance weighted metrics as species number and the functional richness (see: Villéger et al. 2008) - moreover the „traditional" Shannon diversity and the Rao's quadratic entropy (see: Laliberté and Legendre 2010) values - as abundance weighted metrics- were computed. All these computations were executed on the entire dataset and solely on the native species' data for each site. All values were presented on boxplots for each habitat types on the **Figure 9.**

The non abundance weighted metrics show similar increasing trend for both species number and functional richness from the smaller to the larger habitat types. But while there species numbers of the entire and the native datasets show significant differences in the case HS, LS, and LR (these are the most infected habitat types) the functional richness did not show any differences. Indeed in some cases (e.g. HR, LS, LR) the medians of the functional richness values were higher for the native species pool than for the entire species list. So it seems the functional richness computation is less sensitive to the effect of non native species than if only the species number of the studied assemblages are taken into consideration.

In case the abundance weighted computations some consiredable differences were found. Higher Shannon diversity values were computed from the entire datasets at all habitat types, althought solely the LS showed significant difference between the entire datasets and the native species pool. Moreover on average the most diverse assembleages were found in the HR and D. Generally the Rao's quadratic enropy values were higher if only the native species pools' data were analysed (althought in this case only in the LS data showed significant differences). Therefore our preliminary results show that the non indigenous species may decrease the functional diversity in almost all habitat types. Contrary the datasets of Danube showed similar trend as it was indicated by the Shannon Diversity computations. That in this case both metrics showed higher values if the entire assemblages' data were analysed. This difference can be explained by the fact that the fish assemblage of the investigated Danubian shoreline habitats are dominated by the non indigenous Gobies. And due to their invasions the assemblage structure of these stocks are completely changed in the last decades. Contrary in the other six habitat types the non indigenous species are present as well, but their natural assemblage stucture is fundamentally unchanged.

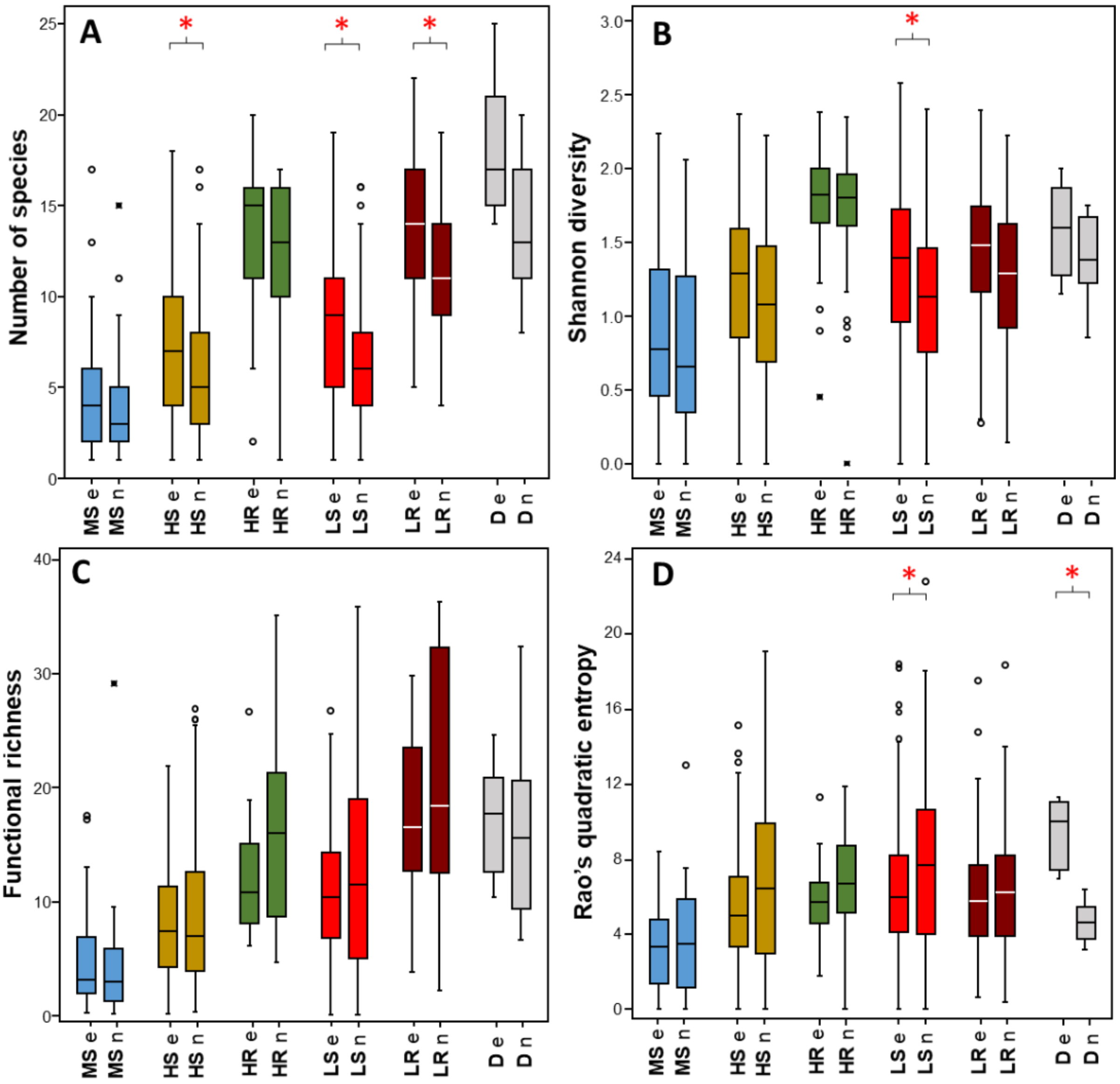


Figure 9: Box plots of non abundance weighted (A, C) and abundance weighted (B, D) metrics data of the studied 7 sites classified into the six habitat types. For habitat type codes see Figure 6. Each box represents the 25 % and 75 % quartiles of the dataset, the band in the box is the median. The whiskers are drawn from the top of the box up to the largest data point less than 1.5 times the box height from the box (the"upper inner fence"), and similarly below the box. Values outside the inner fences are shown as circles values further than three times the box height from the box (the "outer fences") are shown as stars. The same coloured boxplots show the sites' data classified into the same habitat type if the entire species pool analysed (e) and if the non-natives excluded (n). Red asterisk show significant ($p<0.05$) differences of within habitat type differences according to the Kruskal-Wallis tests. (For the better visibility only the significatnt within habitat type differences were indicated on the plots. For more details see „Sign" worksheet in the database).

In conclusion the functional diversity computations are seem to be a promising alternative methodology for the deeper understanding of non native species' effects on the natural assemblage structure. Some addioitional analyses are still going on these datasets. A manuscript dealing with the above presented results will be published hopefully in a Q1 journal (Hydrobiologia) supposedly in the summer of 2019.

Our mentioned results were published in eight scientific articles (four SCI) and eleven presentations were made in Hungarian and international conferences.

Articles:

Takács P, Vitál Z, Ferincz Á, Staszny Á (2016) Repeatability, Reproducibility, Separative Power and Subjectivity of Different Fish Morphometric Analysis Methods. PLoS ONE 11:(6) Paper e0157890. 16 p. **IF: 3.014**

Takács P, Czeglédi I, Ferincz Á, Sály P, Specziár A, Vitál Z, Weiperth A, Erős T (2017) Non-native fish species in Hungarian waters: historical overview, potential sources and recent trends in their distribution. HYDROBIOLOGIA 795:1-22. **IF: ~2.022**

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Takács P (2018) Megjegyzések a Magyarországon előforduló, Gobio genusba tartozó küllők taxonómiai helyzetével és névhasználatával kapcsolatban = Notes on the taxonomic position and naming problems of the Hungarian stream dwelling gudgeons (*Gobio*). PISCES HUNGARICI 12: 63-66.

Takács P, Bánó B, Czeglédi I, Ferincz Á, Kern B, Preiszner B, Staszny Á, Vitál Z, Weiperth A, Erős T (2018) Hány csukafaj él a Kárpát-medencében? = How many Pike (*Esox*) species live in the Carpathian Basin? PISCES HUNGARICI 12: 67-70.

Takács P, Czeglédi I, Ferincz Á, Sály P, Specziár A, Vitál Z, Weipert A, Erős T (2018) Halállományok fajgazdagsága és a védett halfajok elterjedés-mintázata magyarországi vízfolyásokban = The species richness of fish stocks and the distribution of protected fish species in the Hungarian river system. HIDROLÓGIAI KÖZLÖNY 98: 86-90.

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Presentations:

Takács P, Vitál Z, Ferincz Á, Staszny Á: Repeatability and subjectivity of different morphometric methods. - XV. European Congress of Ichthyology, Porto, Portugália, 2015. szeptember 5-11., (poster)

Takács P, Vitál Z, Ferincz Á, Staszny Á: Morfometriai módszerek ismételhetőségének, reprodukálhatóságának és szubjektivitásának vizsgálata - 17. Kolozsvári Biológus Napok, Kolozsvár, Románia, 2016. április 8-9., (oral presentation)

Takács P, Ferincz Á, Staszny Á, Vitál Z: Does the side matter? Potential effect of side-specific data processing on the results of fish morphometric studies. - X. European Symposium for Freshwater Sciences (SEFS10), Olmüc, Csehország, 2017. július 02-07., (poster)

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Takács P, Czeglédi I, Ferincz Á, Sály P, Specziár A, Vitál Z, Weiperth A, Erős T: Halállományok fajgazdagsága és a védett fajok elterjedés mintázata magyarországi folyóvizekben. - LIX. Hidrobiológus napok, Tihany, 2017 október 4-6., (oral presentation)

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Takács P, Bánó B, Czeglédi I, Ferincz Á, Kern B, Preiszner B, Staszny Á, Vitál Z, Weipeth A, Erős T: Hány csukafaj él a Kárpát-medencében? - XIV. Magyar Haltani Konferencia, Tiszafüred 2018. március 22-23., (oral presentation)