THESIS OF DOCTORAL (PhD) DISSERTATION

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Population genetic, morphological, and morphometric studies of certain pikeperch (*Sander lucioperca*) populations in Hungary.

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1 BACKGROUND AND OBJECTIVES OF THE WORK

The pikeperch (Sander lucioperca Linneaus 1758) is one of the most characteristic species of the perch family in Eurasian freshwater and brackish water communities. The Hungarian pikeperch population differs significantly from other European freshwater and brackish water populations and has a unique genetic background (TSAPARIS et al., 2022). Despite this, no detailed genetic studies have been conducted in Hungary to date, not even in Lake Balaton, which is considered the most emblematic habitat for pikeperch.

The species' population in Lake Balaton has suffered significant losses in recent decades. Population genetic studies, which are becoming increasingly common and cheaper, may be suitable for detecting the presumed effects of these losses. However, until recently, morphometric methods were almost exclusively used to detect species-level separation or intraspecific differences, such as sexual dimorphism or population differences.

The study of these morphometric characteristics can be traced back to the beginnings of biological taxonomy. The classification of living organisms and the first steps towards understanding the evolution of life were both achieved through the morphological description of different forms. In morphological studies, we use countable, "meristic" variables, while in morphometric studies, we use continuous, "measurable" variables (ROHLF 1990; TAKÁCS et al., 2016). In the latter case, the selected morphometric characteristics are measured on a continuous scale and analyzed using appropriate statistical methods (CARPENTER et al., 1996; GOODALL 2010).

In my doctoral thesis, I focus on the European pikeperch, analyzing the population genetics of the Lake Balaton stock in particular, as well as presenting the morphological and morphometric differences between the Hungarian stocks of the species. Since the Lake Balaton stock is genetically unique and differs from other European stocks (TSAPARIS et al., 2022), I am conducting morphological and morphometric studies in addition to population genetic studies of the Lake Balaton population, using populations from the Danube and Tisza river basins in addition to those from the Lake Balaton river basin. I also collected tissue samples from populations originating from the Danube and Tisza river basins in order to conduct a nationwide population genetic study, but this has not yet been done, so this part is not the subject of this thesis.

Morphological and morphometric examinations are necessary in addition to genetic testing because they provide information about the extent of any differences between individual stocks. We can find out whether the extent of any differences between individual stocks can only be detected using genetic methods, or whether they also manifest themselves in the form of different body parameters or even meristic marks that can be counted.

I hope that the results of my population genetic, morphological, and morphometric analyses will provide useful information for the better utilization and long-term conservation of domestic populations of this species, which is of great importance from both an economic and ecological point of view.

1.1 Objectives:

1. Are there detectable population genetic differences between the pikeperch populations living in different areas of Lake Balaton, or do the pikeperch populations of the lake form a single population?

2. Can the mass extinctions that have occurred in recent decades and the effects of treatments carried out by the land user (e.g., stocking) be detected in the population genetics of the Balaton stock?

3. How unique is the external appearance of the pikeperch populations in Lake Balaton? Are there detectable differences in quantifiable (meristic) and measurable (morphometric) characteristics between the pikeperch populations living in Lake Balaton and those living in other areas of the country?

4. How much do the number and quality of variables included in morphometric analysis influence the results of morphometric studies? Is there a generally applicable set of variables, or must the most suitable variables be defined in each case? (I sought answers to these questions in a preliminary experiment. I then used the results obtained in the morphometric analysis of the pikeperch.)

2 MATERIALS AND METHODS

2.1 Genetic analysis of the Balaton pikeperch population

2.1.1 Sample collection

The fin tissue samples used for the study were collected between 2016 and 2017 from six different locations on Lake Balaton. The sampling points were: Keszthely, Balatonboglár, Balatonakali, Tihany, Siófok, and Balatonfűzfő. The individuals were caught exclusively by fishing, and the standard body length (SL) of each individual exceeded 35 centimeters in all cases. I cut the tissue samples from the caudal fin and stored them in 1.5 ml Eppendorf tubes in 96% alcohol at -20°C until DNA extraction.

2.1.2 DNA extraction

Total genomic DNA extraction was performed using the QIAGEN DNeasy Blood and Tissue Kit according to the manufacturer's instructions. The DNA concentration of each sample was determined using a Maestro NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware, USA) and then adjusted to a concentration of 55 ng/µl. The samples were stored at -20 °C until further use.

2.1.3 PCR amplification and microsatellite analysis

I used 15 microsatellite markers to genotype the individuals, which were as follows: MSL1, MSL2, MSL3, MSL5, MSL9—(KOHLMANN and KERSTEN 2008), Svi-4, Svi-6, Svi-L7, Svi-18—(WIRTH et al., 1999), Pfla-L9—(LECLERC et al., 2000), Za038, Za144, Za199, Za207, Za237—(DUBUT et al., 2010). In the PCR reaction, using primers labeled with NED, PET, VIC, and FAM, in a reaction volume of 20 µl, the markers were amplified in three multiplex and one simplex reactions. The length of the PCR products was examined on an eight-capillary ABI 3500 sequencer (POP-7 polymer, GeneScan standard 600 LIZ). The size of the fragments was calculated using GeneMapper 4.1 software.

2.1.4 Processing of genetic data

I used the MICRO-CHECKER 2.2.3 program to evaluate the presence of null alleles (number of random tests: 1000, 95% confidence interval) (VAN OOSTERHOUT et al., 2006).

The allele number (Na), effective allele number (Nef), observed heterozygosity (Ho), expected heterozygosity corrected for population size (uHe), and fixation index (F) were calculated using GenAlex 6.5 (PEAKALL and SMOUSE 2012). Allele richness (AR) and individual allele richness (ARp) were calculated using the HP RARE 1.0 program (KALINOWSKI 2005). To compare the genetic values of the individual subpopulations, I used one-way ANOVA (Tukey post hoc test for Na, AR, Ho, uHe, and F) and the Kruskal-Wallis test for Nef and ARp, taking into account Bonferroni-corrected significance values (using a significance value of 0.008). The statistical analysis was performed using the SPSS 11.5.0 software package.

I performed molecular variance analysis (AMOVA), determined the degree of genetic differentiation between populations (Fst) and calculated Nei's genetic distance values (NEI 1978) using the GenAlEx 6.5 program. The phylogenetic tree (Neighbor Joining tree NJ (SAITOU and NEI 1987)) was constructed using Nei's genetic distance values with the help of MEGA11 software (TAMURA et al., 2021). To determine the population structure, I used the Bayesian algorithm of the STUCTURE program (FALUSH et al., 2003; PRITCHARD et al., 2000). To estimate the most probable number of clusters (K), I used the highest lnP(D) and ΔK (EVANNO et al., 2005) values in the STUCTURE HARVESTER program (EARL and VONHOLDT 2012). To determine the number of clusters, we chose a setting in which allele frequencies were correlated, set the burn-in value to 10,000, and set the number of additional MCMC runs to 200,000. The calculation was repeated 10 times for each K value.

I performed principal component-based discriminant analysis (DAPC) on microsatellite loci and populations in the R environment (4.2.1) using the adegenet 2.1.1.7 software package (JOMBART 2008). The paired Mantel test (with 9999 permutations) of the pairwise Fst values and the geographical distance values between populations was performed using the GenAlEx 6.5 program. The genetic bottleneck effect predicting the potential decline of populations was assessed using the Bottleneck 1.2.02 software (CORNUET and LUIKART 1996) based on a two-phase mutation model (TPM) with a 0% stepwise mutation model (SMM) in TPM and a 36% variance in geometric distribution. The significance level was estimated using the Wilcoxon rank test with 1000 repetitions.

The effective population size (Ne) was estimated using the LD and heterozygote surplus methods implemented in the NeEstimator 2.01 software (DO et al., 2014). To calculate the migration network, I used the divMigrate-online software (SUNDQVIST et al., 2016) applying the Jost D method with a step size of 1000. A threshold value of 0.5 was used as a filter when presenting the main migration models.

2.2 Morphometric analyses

2.2.1 The effect of the number of variables analyzed (preliminary experiment)

2.2.1.1 Fish species included in the study

The aim of the preliminary study was to demonstrate how the number of variables involved in the measurement affects the morphometric results. For the preliminary studies, I used data from measurements taken from photographs of a total of 303 individuals from five populations of each of three fish species. The fish species studied were: the gudgeon species complex (Gobio gobio complex

TAKÁCS 2018), Petényi's barbel (Barbus petenyi Heckel, 1852) and the pumpkinseed (Lepomis gibbosus Linneaus, 1758).

2.2.1.2 Sample collection

For the study, 20 individuals were collected from each of five populations using an electric fishing machine (License No.: PE-KTF/659-15/2017, ANPA Agentia Nationala pentru Pescuit si Acvacultura: 08/21.03.2016). The caught individuals were placed on a polystyrene surface, and then photographs were taken of the left side of the individuals using a fixed magnification lens and a Nikon D5300 digital camera mounted on a tripod.

2.2.1.3 Morphometric measurement and standardization

The 35 variables used for morphometric measurements were measured on digital photographs using ImageJ software (RASBAND 2012). The measured data were standardized using the method of Elliott et al. (1995) with standard body length (SL). To ensure the success of the standardization, I performed a Spearman rank correlation test between the standard body lengths and the standardized data.

2.2.1.4 Statistical analysis

I was able to measure the discriminating power of each variable using F statistics (POPE and WEBSTER 1972). This gave me the ratio of variances between and within groups. Subsequently, based on the F value associated with each variable, the variables were ranked according to their effect on group separation. The higher the F value, the more uniform the mark within the group and the greater the difference between the groups. Using this method, we were able to select the characteristics that distinguished the groups.

First, I performed canonical variance analysis (CVA) on each group using 34 variables, excluding standard body length. Then, starting with the variable with the lowest discriminating power, I reduced the number of variables included in the analysis one by one, followed by another CVA in each case, until only the two most significant variables remained in the analysis (CADRIN 2000).

To examine the discriminatory power of the analysis based on the CVA results, I used the square values of the Mahalanobis distances of the group centroids, the percentage of correctly classified individuals, and the percentage of pairwise group separations. In the analysis, I took into account Bonferroni-corrected significance values (Hotelling's P<0.05) (ZELDITCH et al., 2012). I plotted the square values of the Mahalanobis distances of the group centroids and the percentage of correctly classified individuals using LOESS smoothing (CLEVELAND 1979) with a 95% confidence interval. Statistical analyses were performed using the Past 2.17 program (HAMMER et al., 2001).

2.2.2 Morphological and morphometric examination of the pikeperch

2.2.2.1 Sample collection, measurement, standardization

For morphometric measurements, I used pikeperch samples from three different river basins in the country to determine how uniform the Hungarian population of this species is. The sampling points were located in the Balaton, Danube, and Tisza river basins. For morphological measurements, I counted the number of scales on the lateral line and the number of swim rays on each fin of the collected individuals, and calculated the body profile index from the standard body length and height of the individual.

A total of 78 specimens were collected for the study using an electric fishing machine (License No.: HAGF/69/2022, HAGF/79/2022) The method of taking photos of the caught fish, the measurements and standardization in the photos were the same as the methods used in the preliminary experiment. Based on the results of the preliminary experiment, it was sufficient to start the study with a lower number of variables in this case, so I used only 30 variables. After taking the photos, I cut tissue samples from the caudal fin for subsequent DNA testing.

I stored the tissue samples in 1.5 ml Eppendorf tubes in 96% alcohol at -20°C until the subsequent tests began.

2.2.2.2 *Statistical analysis*

The measured variables were ranked based on their corresponding F values. Since preliminary experiments showed that 5-10 variables were ideal for detecting significant group differences, I used 9 variables with F values greater than 5 from the variables ranked based on their F values for the CVA analysis, and the classification test was also performed using these 9 variables. In the analysis, I took into account the Bonferroni-corrected significant values (Hotelling's $P<0.05$).

I used the Kruskal-Wallis test to compare the morphological measurement values. I performed the statistical analyses using the Past 2.17 program (HAMMER et al., 2001).

3 RESULTS AND DISCUSSION

3.1 Genetic analysis of the Balaton pikeperch population

3.1.1 Genetic diversity of populations

The Microchecker test found no evidence of significant allele loss. Furthermore, the presence of null alleles could only be detected in the PfaL9 (Tihany population) and MSL-5 (Balatonfűzfő population) markers in two populations due to the general homozygote surplus.

The allele number (Na) of the Tihany population showed a significant difference compared to the other populations, with the exception of the Keszthely population. In terms of effective allele numbers (Neff), the Keszthely population showed a significantly higher value. The same can be observed in terms of allele richness (AR) and individual allele richness (ARp), with the Keszthely population showing the highest values, but only showing a significant difference in terms of individual allele richness. The Keszthely population also showed a significant difference in terms of expected heterozygosity corrected for population size (uHe) and observed heterozygosity (Ho). The fixation index or inbreeding coefficient (F) showed low negative values in all cases except for Balatonfűzfő. The deviation from Hardy-Weinberg equilibrium was significant at the Za237 locus in Balatonboglár and Balatonakali at the MSL-5, MSL-9, PfLa-L9 locus in Tihany, the Svi-4 locus in Siófok, and the Svi-6, MSL2, and MSL-5 loci in Balatonfűzfő.

The genetic diversity of the Balaton pikeperch population (He=0.55-0.74; AR=4.17-5.42) is comparable to the available literature data. These values are also well known in Finland's coastal populations (He=0.30-0.37; AR=3.3-3.9) and lake populations (He=0.36-0.46; AR=4.0-5.5) (SÄISÄ et al., 2010). In addition, other publications are available for lake populations (He=0.51; AR=3.6-4.6) (SALMINEN et al., 2012); in all cases, the two indicators are lower. In the Rhône delta estuary (He=0.64-0.74; AR=4.0-6.0) (POULET et al., 2009) and the lower Volga (He=0.79-0.82; AR=9.57-10.64) (KUSISHCHIN et al., 2018).

In the Danube river system, the German pikeperch population shows lower genetic diversity (He=0.62; AR=4.5) (ESCHBACH et al., 2014) than the Balaton population. A study covering several populations in Europe (TSAPARIS et al., 2022) shows that, despite the large overlap in the markers used, the genetic diversity of the Lake Balaton population is high not only in the natural population (He=0.40-0.69; AR=2.7-4.9) but also in the farmed populations (He=0.35-0.72; AR=2.6-5.5) that originated from Lake Balaton.

3.1.2 Population dynamics of the pikeperch stock in Lake Balaton

AMOVA revealed low genetic separation between the groups studied (Fst=0.028, P<0.001). Ninety-two percent of genetic variance was within individuals, while 5% was between individuals (P<0.001). However, pairwise comparisons revealed greater genetic separation between pairs of populations. Based on the Fst values, the Keszthely population differed significantly from all other populations. Furthermore, when comparing Tihany, Siófok, and Balatonfűzfő, the Fst values exceeded the 0.05 level.

The separation of the Keszthely population is also supported by the phylogenetic tree (NJ) based on Nei's genetic distance values. Based on the lnP(D) values of the STUCTURE program, the Bayesian algorithm determined the most probable number of clusters (K=2). However, using the method of EVANNO et al. (2005), a different value (K=5) was determined based on the change in the ΔK value. With low Fst values between populations, the STRUCTURE program was unable to identify a distinct cluster in either the 2 or 5 clusters.

Based on the results of the DAPC analysis, the Keszthely population stands out. Almost every individual in the Keszthely population showed clear separation in the classification probability test, which is not observed in the other populations.

3.1.3 Gene flow, population size, and bottlenecks

The Mantel test showed a strong correlation between genetic distance (Fst) and geographical distance. Based on the results, a geographical distance of 42 km is required to achieve an Fst value of 0,05, which can be considered a significant difference.

The results of population genetic studies showed only weak separation between the individual populations. The pikeperch population tended to show a metapopulation structure. This is also indicated by the significant but relatively low Fst value per pair compared to the Hungarian and Baltic populations (BJÖRKLUND et al., 2007; KÁNAINÉ SIPOS et al., 2019; SÄISÄ et al., 2010). Spawing migration rarely exceeds 35 km (LAPPALAINEN et al., 2003), whereas Lake Balaton is 79 km long. Therefore, the shape and size of the lake already create the possibility for distance-based isolation. The Fst value of 0.05 observed above 50 km already borders on the minimum threshold for genetic isolation.

The Balatonboglár population had the smallest estimated effective population size, with a shifted allele frequency distribution, indicating a recent bottleneck effect. Significantly fewer heterozygotes were observed in the Tihany population, while significantly more were observed in the Keszthely population, but the allele frequency distribution of the populations showed a normal L-shaped distribution in both cases.

Although the bottleneck effect (indicating a recent significant decline in population size) cannot be demonstrated in most populations, the results are not entirely clear. In the case of the Tihany population, several markers showed deviations from Hardy-Weinberg equilibrium and a significant heterozygote deficiency was observed, while allele frequency showed an L-shaped normal distribution. Based on these findings, it can be assumed that, at least in this population, there has been a recent change in population size.

The relative migration matrix showed the lowest migration value between Keszthely and Siófok (0.063) and the highest between Siófok and Tihany (1.000). With the threshold set at 0.5, a partial separation of the Keszthely population can be observed, as well as the fact that the Tihany population shows strong two-way migration with all populations except Keszthely. Bootstrapping (LARGET and SIMON 1999) revealed statistically significant asymmetric migration, which pointed towards Keszthely in the case of all populations.

Thus, a unidirectional east-west trend can be observed in the migration of pikeperch. This migratory movement is fully consistent with the results obtained in fish tagging studies (SPECZIÁR and TURCSÁNYI 2017), according to which pikeperch stocked in mesotrophic areas travel shorter distances and are less scattered than individuals stocked in oligotrophic areas (SPECZIÁR and TURCSÁNYI 2017). It therefore appears that the movement and spread of pikeperch is strongly related to the trophic gradient observed in Lake Balaton (ISTVÁNOVICS et al., 2007).

In addition to the different sizes and depths of the individual basins, the unique shape of Lake Balaton may also have influenced the separation of the Keszthely population. In addition to environmental factors, it should not be forgotten that the individual basins have different fish fauna compositions (BÍRÓ 1997). All these factors together may have led to local adaptation within the population, which may be important information for stock replacement operations.

3.1.4 Effects of stock utilization

Based on data from Balatoni Halgazdálkodási Nonprofit Zrt., we can track annual catches of pikeperch from Lake Balaton from 1901 to 2020. During this period, the total catch of zander was 10,022 tons, with an average annual catch of 83.51±50.18 tons. Since 2014, pikeperch can only be legally caught from Lake Balaton using fishing rods. Fish tagging studies (SPECZIÁR 2010; SPECZIÁR and TURCSÁNYI 2017) estimate that the quantity of pikeperch caught by anglers (over 35 cm SL) accounts for a significant proportion (49-56%) of the total stock each year. The fact that traces of the bottle neck effect could only be found in one of the populations studied suggests that, in addition to breeding and stocking practices, fish mortality in the past century (BÍRÓ 1997; VIRÁG 1997) have not had a negative impact on the genetic composition of the pikeperch population in Lake Balaton. This is probably due to the fact that the initial size of the pikeperch population in Lake Balaton was sufficiently large and the populations in the individual areas were able to mix freely in the absence of physical barriers between the basins, and can still do so today.

3.2 Morphometric analyses

3.2.1 The effect of the number of variables (preliminary experiment)

3.2.1.1 The most informative morphometric variables

The standardized variables did not show a significant correlation (Spearman D, $p<0.05$) with standard body length (SL), thus eliminating the size effect, so that all morphometric variables can be used for further analysis. The standardized variables were ranked in descending order based on their F-values for all three species studied. In the case of the two species belonging to the order Cypriniformes, almost half of the 15 most important variables (DPC, DVPL, EH, HL, Hh, Hmax, PPEC) were common. In addition, four variables (EH, HL, Hh, PPEC) were among the 15 most significant variables for all three fish species.

3.2.1.2 Effects of reducing variable numbers

The Mahalanobis distance square values ranged from 0.05 to 29.65 for the Gudgeon species complex, from 0.11 to 57.09 for Barbus petenyi, and from 0.03 to 41.04 for Pumpkinseed. The percentage of correctly classified individuals ranged from 26 to 100%, 25 to 100%, and 20 to 100% in the order of the fish species mentioned above. The percentage of significant ($P>0.05$) pair-wise group differences ranged from 0-90% for Gudgeon and the Barbus petenyi, while for the Pumpkinseed this percentage ranged from 0-60%.

Based on the analysis performed on three different fish species, it can be concluded that reducing the number of variables included in the study significantly affects the results of morphometric measurements. The Mahalanobis distance squared values of the group centroids showed a continuous decrease as the number of variables decreased. The percentage of correctly classified individuals also decreased with the reduction in the number of variables, but only a slight decrease was observed in the initial stage for these values. By reducing the number of variables from 34 to 17, the percentage of correctly classified individuals decreased from 100% to only 92% in the case of Barbus petenyi, and from 92% to 83% in the case of the Gudgeon species complex and the Pumpkinseed. Subsequently, however, the rate of decline accelerated for all three fish species.

No significant group separation was detected in Gudgeon species complex 25 in Barbus petenyi 29 and in the Pumpkinseed 28 in terms of the percentage of pair-wise group separations above the number of variables included in the analysis. In the case of the two species belonging to the order Cypriniformes, the proportion of significant group differences was quite significant, reaching 90% during the reduction of the number of variables. However, with further reduction of the variables included in the study, this proportion also deteriorated. In the case of the Pumpkinseed, after an initial increase, the curve flattened out at 50% significant group differences, with only one case showing an outlier value of 60% when using 10 variables.

The three characteristics examined showed similar trends for all three fish species. Only the Pumpkinseed showed a lower rate of significant group differences compared to the other two fish species. This difference may be due, on the one hand, to the smaller spatial scale of the sampling area. This is because the

Pumpkinseed samples came exclusively from the artificial shoreline of Lake Balaton, while the sample sites for the other two fish species were located in more distant water bodies. Furthermore, the non-native Pumpkinseed only appeared in Lake Balaton in the last century (VUTSKITS 1912). Its invasion may have reduced its genetic variability (GRAPPUTO et al., 2006), which may have had an impact on the morphology of the population (HAUSER et al., 1995).

The results of the preliminary study show that the most significant group distinctions can be obtained by analyzing 5-10 variables. At the same time, the number of correctly classified individuals can be maximized by including as many variables as possible in the analysis.

Based on a literature review (BÁNÓ and TAKÁCS 2022), authors of morphometric studies use an average of 15 variables for their analyses. According to the results of our preliminary studies, this number of variables is too high for the examination of significant group differences, but too low for the examination of the proportion of correctly classified individuals and the Mahalanobis distance of group centroids. It is therefore worthwhile to select the number of morphometric variables to be used according to the purpose of the study and, if necessary, to select them based on the F statistic. In our case, since the purpose of our studies was to demonstrate the differences between populations, we significantly reduced the number of variables included in the analysis.

3.2.2 Morphological and morphometric examination of the pikeperch

3.2.2.1 Morphological examination

The morphological data of the populations characteristic of each catchment area did not show any significant differences from each other and corresponded in all respects to the data published in the literature (FERINCZ et al., 2023). There was a significant difference in body profile index values, but this value is greatly influenced by the amount of food consumed by the individual immediately before sampling. The significant difference observed here can also be explained by this.

3.2.2.2 Morphometric variables according to their significance

After standardization, the individual variables did not show a significant correlation (Spearman D, $p<0.05$) with standard test length (SL), so the size effect no longer needed to be taken into account and the variables could be used for the individual measurements. I ranked the individual variables using their F-values.

Since the results of the preliminary study showed that the degree of significant group differences could be maximized using 5-10 variables, I used only the 9 most significant variables in further analyses.

Of the nine most significant variables, five are located on the head of the examined individuals, while three further variables characterize the elongation of the body and caudal pendulance. In contrast, there is only one variable that connects the points of origin of the caudal fin. In this case, too, it can be said that the most informative variables are mostly located on the front part of the body. Eight of the nine most important variables characterize horizontal elongation, which clearly plays an important role in the streamlined body shape of fish. The magnitude of the variable characterizing the caudal fin's points of origin—which is the only vertical determinant variable—refers to the robustness of the tail fin, which is the most decisive factor in terms of the animal's movement.

3.2.2.3 Separation of the three groups examined

Based on the results of canonical variance analysis (CVA), it can be stated that the Balaton population is significantly more distinct from the two river populations than they are from each other.

The Bonferroni-corrected significance values of the pairwise group differences were significant ($P<0.05$) in all comparisons. However, the significance values are lower in the comparison between the Lake Balaton and river populations than in the comparison between the two river populations.

In the case of the Danube catchment population, the defining marks are located on the front part of the head, while in the case of the Tisza catchment population, the defining marks are located on the caudal side of the body. The population characteristic of Lake Balaton is mainly located on the opposite side of the vectors of the variables on the head. In my opinion, this may indicate that the front half of the body of the Lake Balaton population, including the head, is smaller than that of the other two groups analyzed.

The classification test correctly classified 27 of the 33 individuals (81.8%) from the Balaton catchment area, 19 of the 30 individuals (63.3%) from the Tisza catchment area, and 10 of the 15 individuals (66.7%) from the Danube catchment area. On average, the test classified 56 of the 78 individuals, or 71.79% of the total population, into the correct location. According to the results, the populations of the three areas can be relatively well distinguished by morphometric analysis.

According to the results of the current study, the population characteristic of the Tisza river basin has an elongated caudal peduncle (DAUC) and a more powerful caudal fin (UPLC) compared to the other populations studied. At the same time, no significant differences can be observed in the front, head part of the body in this population.

4 CONCLUSIONS AND RECOMMENDATIONS

4.1 Population genetic analysis of pikeperch stocks in Lake Balaton

The genetic composition of the pikeperch population living in Lake Balaton differs from that of Western and Northern European populations (TSAPARIS et al., 2022). In our study, with the exception of one locus, we were unable to detect any signs of inbreeding, so it can be said that the major fish kills that occurred several times in the second half of the last century, as well as the subsequent intensive restocking, did not cause a bottleneck effect in the population genetics of the species. The main reason for this may be that the lake originally had a large and presumably genetically diverse pikeperch population, which was able to survive these negative events without significant impoverishment. In addition, the results of the studies also suggest that the pikeperch stocked in Lake Balaton probably originated from Lake Balaton in all cases. The eggs from the artificial nests placed in Lake Balaton are returned to Lake Balaton after hatching by the staff of Balatoni Halgazdálkodási Nonprofit Zrt. In the case of a sufficiently large natural population, such as the pikeperch population in Lake Balaton, this stocking practice does not have an adverse genetic effect on the stock.

Mapping the reproductive communities of local populations and conducting a more in-depth analysis of population structure is a difficult task, even when using genetic markers. A good example of this is Lake Erie in North America, where studies have been conducted on several species of perch for this purpose. In the case of the closely related Perca flavescens, although the individual breeding groups were well separated, no distance-based genetic isolation was detected. In addition, certain groups had a greater genetic impact on the entire population than others (SEPULVEDA-VILLET and STEPIEN 2011). Numerous studies on this topic have also been conducted on the North American relative of the pikeperch, Sander vitreus. STEPIEN et al. (2012) used microsatellite markers to identify distinct breeding groups that differed in their genetic composition despite their close relationship (STEPIEN et al., 2018). This means that the individual breeding groups collectively contribute to the genetic diversity of the Lake Erie population. According to the results of my research, the pikeperch population of Lake Balaton is characterized by a metapopulation structure. Perhaps the westernmost population in Keszthely is an exception to this, as it shows partial separation from the other populations analyzed. This pattern can be explained by two factors. The first is distance-based isolation, as the spawning migration of the species does not exceed 35 kilometers according to literature data (LAPPALAINEN et al., 2003), while Lake Balaton is 79 kilometers long. The other is the east-west unidirectional gene flow detected in our studies. This suggests that there is significant migration from the eastern part of the lake to the Keszthely area, which can probably be explained by the higher trophic values at the western end of the lake. The population genetic pattern caused by the natural characteristics of the lake is a factor that should be given special attention during stock replacement planting.

The unidirectional migration pattern observed in the east-west direction is fully consistent with the results of fish tagging studies (SPECZIÁR and TURCSÁNYI 2017), which found that pikeperch stocked in mesotrophic areas travel shorter distances and are less scattered than individuals stocked in oligotrophic areas. It therefore appears that the migration direction of pikeperch is strongly influenced by the trophic gradient observed in the lake.

Since previous genetic studies have not dealt in detail with the entire population of pikeperch living in Lake Balaton, it is not possible to draw more detailed conclusions at this stage. In the future, however, it would be worthwhile to refine the methodology of the study and even compare the individual populations using SNP markers.

4.2 Morphometric analyses

4.2.1 The effect of the number of variables (preliminary experiment)

The results of the morphometric analyses of the three fish species examined confirmed the assumption that there is no universally applicable set of variables. However, the most informative variables for all species examined are located in the front part of the body.

When separating individual populations, it is worth considering the variables used for closely related species. It is advisable to start with a high number of variables, so that later in the study we have the opportunity to select from among our individual variables. This may be necessary if the aim of our study is to separate the individual populations as accurately as possible.

It is therefore advisable to apply the number of variables used in accordance with the specific objectives of the morphometric study, as different objectives require the use of different numbers of variables. Based on the data in the literature, the average number of variables used (15) is too small for classifying individuals into their populations of origin, while it proves to be too large for the maximum separation of the population into individual populations. Therefore, it is advisable to initially record a relatively large number of variables for each individual, which can later be reduced through selection if our objectives so require.

4.2.2 Morphological and morphometric examination of the pikeperch

The populations of the studied catchment areas did not show any differences in meristic (countable) morphological characteristics, either from each other or from the data published in the literature. Since the stock characteristic of Lake Balaton is genetically unique and represents a distinct type (TSAPARIS et al., 2022), we can conclude that these genetic differences have not yet manifested themselves in the form of morphological characteristics.

In my morphometric analysis, the standing water population of Lake Balaton showed significant separation from the other two riverine populations. Since eight of the nine most discriminating variables characterize horizontal elongation, it can be concluded that the individuals in the studied populations have adapted to different flow conditions. The only vertically oriented determinant variable (UPLC) is also related to a powerful caudal fin and, consequently, powerful swimming and flow conditions.

The variables with the greatest separating power have different effects on the separation of river populations. In individuals originating from the Tisza river basin, these variables are located in the rear, caudal part of the body (DAUC, UPLC), while in populations characteristic of the Danube river basin, the vast majority of these variables are located in the head region (HL, PPEC, PRE, EH). The predominance of horizontal variables among the most significant variables, which characterise the streamlined body, may therefore indicate the presence of different flow conditions, but within this, the populations characteristic of each water body show differences in different body regions.

SPANGLER et al. (1977) found that even within a given body of water, groups of pikeperch with different physical characteristics can form, with spawning restricted to different locations. In addition, it has been observed that in rivers with different flow rates, the location of pikeperch changes, adapting to local conditions, which also affects their individual body parameters (KUSISHCHIN et al., 2018).

The morphometric analysis shows that the individual populations have different body parameters. The separation between the standing water and river populations is marked, while in the case of the two river populations, the most significant variables showed differences in different body regions.

5 NEW SCIENTIFIC RESULTS

1. Using microsatellite markers, I was able to demonstrate that the pikeperch population living in Lake Balaton is genetically diverse, and that the large-scale fish kills that occurred in the past and the subsequent intensive fish stocking did not cause a bottleneck effect in the genetic pattern of the species living here.
2. The pikeperch population of Lake Balaton can be characterized by a metapopulation structure, with only the Keszthely population showing partial separation.
3. From a genetic point of view, I was able to confirm the results of fish tagging studies, which showed that east-west migration can be observed in Lake Balaton, which is probably closely related to the trophic gradient characteristic of the lake.
4. After examining several fish species, I was the first to demonstrate that the number and type of variables included in distance-based morphometric analyses significantly influence the results of the measurements. I concluded that there is no universally applicable set of variables, but the most informative variables are located on the front part of the body.
5. I have demonstrated that in the case of distance-based morphometric measurements, the number of significant group distinctions can be maximized using only 5-10 variables, while the percentage of correctly classified individuals and the Mahalanobis distance between group centroids yield better values the more variables are used in the analysis.
6. Based on the most significant morphometric variables, it can be concluded that adaptation to flow conditions plays a decisive role in the separation of the populations studied. The Balaton population is statistically relatively well separated from the Danube and Tisza populations in terms of body shape.

The heads of the Balaton populations are generally smaller than those of the two river populations, but we found no differences in the countable morphological characteristics.

6 PUBLICATIONS RELATED TO THE TOPIC OF THE DISSERTATION

Bánó, B., Benedek, I., Zsolnai, A., Specziár, A., Takács, P., & Molnár, T. (2024). Population genetic structure of intensively exploited pikeperch (Sander lucioperca) in Lake Balaton (Hungary). *Hydrobiologia*, 851(3), 559-571. **(Q1)**

Bánó, B., & Takacs, P. (2022). Effects of the analysed variable set composition on the results of distance-based morphometric surveys. *Hydrobiologia*, 849(10), 2267-2280. **(Q1)**

Bánó B., Takács P. (2020) A mért változók számának hatása a morfometriai vizsgálatok eredményeire = Effect of measured variable numbers on the results of morphometric surveys. *PISCES HUNGARICI* 14 115-122.

7 PUBLICATIONS OUTSIDE THE SCOPE OF THE DISSERTATION

Bánó, B., Bolotovskiy, A., Levin, B., Mattox, G. M., Cetra, M., Czeglédi, I., & Takács, P. (2024). Scale morphology is a promising, additional tool for exploring the taxonomy and ecology of freshwater fishes. *Fish and Fisheries*, 25(4), 569-588. **(D1)**

Bánó, B., Bolotovskiy, A., Levin, B., Mattox, G. M., Cetra, M., Czeglédi, I., & Takács, P. (2024). Freshwater fish scale database. *Data in Brief*, 54, 110525. **(Q3)**

Czeglédi, I., Specziár, A., Preiszner, B., Boros, G., **Bánó, B.**, Mozsár, A., ... & Erős, T. (2024). Stable isotope analysis reveals diet niche partitioning between native species and the invasive black bullhead (Ameiurus melas Rafinesque, 1820). *NeoBiota*, 94, 57-77. **(D1)**

Takács, P., Maasz, G., Zrínyi, Z., Boross, N., Vitál, Z., Sipos, D. I. K., **Bánó, B**., ... & Kovacs, B. (2022). Infirm effect of phylogeny on morphometric features in a cryptic Gobio species complex. *Contributions to Zoology*, 91(2), 79-96. **(Q1)**

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Takács P., **Bánó B.,** Czeglédi I., Erős T., Ferincz, Á., Gál B …&; Csoma E. (2022) Kárpát-medencei csukaállományok filogenetikai és morfológiai viszonyai Phylogenetic and morphologic features of Pike populations inhabiting the Carpathian Basin. *PISCES HUNGARICI* 16. 11-20.

Takács, P., Abonyi, A., **Bánó, B.**, & Erős, T. (2021). Effect of non-native species on taxonomic and functional diversity of fish communities in different river types. *Biodiversity and Conservation*, 30(8), 2511-2528. **(Q1)**

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