Acta Zoologica Academiae Scientiarum Hungaricae 58 (Suppl.), pp. 19–27, 2012

SCALE-MORPHOMETRY STUDY TO DISCRIMINATE GIBEL CARP (*CARASSIUS GIBELIO*) POPULATIONS IN THE BALATON-CATCHMENT (HUNGARY)

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An examination was made to find out whether the shape of the scales is suitable to discriminate gibel carp (*Carassius gibelio* BLOCH, 1782) populations. The tools of landmark-based geometric morphometrics were used. Fish were collected from four sampling areas. Three are connected with each other (Kis-Balaton Water Protection System stage I, Kis-Balaton Water Protection System stage II, Lake Balaton western basin), while one is separated from the others (Nagyberek, located on the Balaton-catchment). Two of the sampling areas (Kis-Balaton Water Protection System stage I, Kis-Balaton Water Protection System stage II) were hypertrophic, whilst the western basin of Lake Balaton and Nagyberek was mesotrophic. *Carassius gibelio* populations could be differentiated into three distinct groups based on their scale morphology, with good reliability (97.3%). Populations of the two stages of the Kis-Balaton Water Protection System could not be separated. Based on the linkage relationships and environmental parameters, the results suggest that the environmental and the possible genetic effects on scale morphology separated with this method. Although the genetic differences were not proved, only assumed.

Key words: geometric morphometrics, landmarks, GLS, Canonical Variates Analysis, Discriminant Function Analysis

INTRODUCTION

It has always been a crucial issue how to achieve a more precise separation of fish species or population. This is not always easy to accomplish by exploring the differences amongst them due to the frequent natural hybridization of related species or populations (HUBBS 1955, ARNOLD *et al.* 1999, IBAÑEZ *et al.* 2007). In the last fifty years a variety of methods were developed to solve these problems, such as the investigation of genetic markers (BERNATCHEZ & WILSON 1998), traditional morphometrics (SPECZIÁR *et al.* 2009), outline-based and landmark-based geometric morphometrics (LOY *et al.* 2000). Morphometric studies based on the full body are highly stressful for each specimen, causing death in most cases. So a structural component variable enough to distinguish populations and easily collectable, without permanent damage was needed (GARDUÑO-PAZ *et al.* 2010). As such, researchers started to focus on finding out whether the fish scales would be suitable to differentiate genera, species or populations, like wild and bred populations of striped bass *Morone saxatilis* (WALBAUM, 1792) based on scale shape (RICHARDS & ESTEVEZ 1997*a*, *b*) or rostrum dace *Leuciscus burdigalensis* (VALENCIENNES, 1844) stocks along River Viaur (France) with Fourier analysis (POULET *et al.* 2005). In Mullets (Mugilidae), IBAÑEZ *et al.* (2007) studied whether genera, species and populations could be distinguished on the basis of their scales' shape.

In these studies, the differences are generally regarded as genetic difference alone, but in fact the morphological differences amongst the groups could be explained by genetic differences, the effect of dissimilar environmental parameters and the covariance of them (MINER 2005, MARCIL 2006). IBAÑEZ *et al.* (2012) started to find out whether the environmental effects could influence the scale shape, and their main finding was the role of compensatory growth in the development of scale shape.

The basic questions of this study were to find out whether 1) the scales of gibel carp are suitable for the separation of populations or not and 2) the results can be explained solely by the differences of the environmental parameters (e.g., turbidity, conductivity, surface area, the emersed plants in littoral zone or the coverage of submersed plants).

MATERIALS AND METHODS

Samples were collected by electrofishing (a 12 V battery powered AGK-Kronawitter IG200/2 device was used) in the summer of 2009. The sampling sites were in the shallow, littoral zone, and the sampling times were after the spawning season (in July); therefore the differences could not come from the described spawning migration of gibel carp (PASCHOS *et al.* 2004). In this study 164 Prussian carp scales (1 scale/specimen) were examined from the following 4 sampling sites (41 each): Kis-Balaton Water Protection System stage I (KBWPS I), Kis-Balaton Water Protection System stage II (KBWPS II), Lake Balaton western basin and Nagyberek, Hungary (Fig. 1). KBWPS I, KBWPS II and Balaton connected to each other, while Nagyberek has no direct connection with any of them. KBWPS I is a hypertrophic pond, with large open water areas, a surface area of 18 km^2 and a mean depth of 1.1 m. KBWPS II is also a hypertrophic pond, with a surface area of 54 km^2 mostly covered with reed vegetation and a mean depth of 1.2 m (TÁTRAI *et al.* 2000). Lake Balaton is a large (596 km^2), oligo-mesotrophic shallow lake (mean depth 3.2 m) (ISTVÁNOVICS *et al.* 2007). The sampling site was situated in the western basin, about 1.5 km north of the Zala estuary. Nagyberek is a mesotrophic wetland (6 km^2), with patches of reeds and bulrush. The water supply mostly comes

Table 1. The measured and estimated environmental parameters of the sampling sites.

Sampling site	Emerse plants in littoral zone	Coverage of submerse plants	Sub-strate	Turbidity (NTU)	Conductiv-ity (μS/cm)
KBWPS I	reed-mace >51%	coverage <5%	mud	152	646
KBWPS II	reed >51%	coverage 5–50%	mud	220.5	640.5
Balaton	reed and reed-mace ca. 50–50%	coverage 5–50%	sand	5	804
Nagyberek	reed and reed-mace ca. 50–50%	coverage <5%	sand	45	717

Table 2. Scoring system for environmental characterization of sampling sites.

Score	1	2	3
Emerse plants in littoral zone	reed >51%	reed and reed-mace ca. 50–50%	reed-mace >51%
Coverage of submerse plants	<5%	5–50%	>50%
Area	canal	<1 km^2	>1 km^2
Substrate	sand	mud	gravel

from precipitation, and the water level is controlled with a floodgate. Turbidity and conductivity were measured on sampling; the emersed plants in littoral zone, the coverage of submersed plants and the substrate were estimated (Table 1) and encoded in Table 2.

The sex ratio of the specimens which were involved to the investigation was 1:1; all of the populations could be regarded gonochoristic (FERINCZ *et al.* 2010).

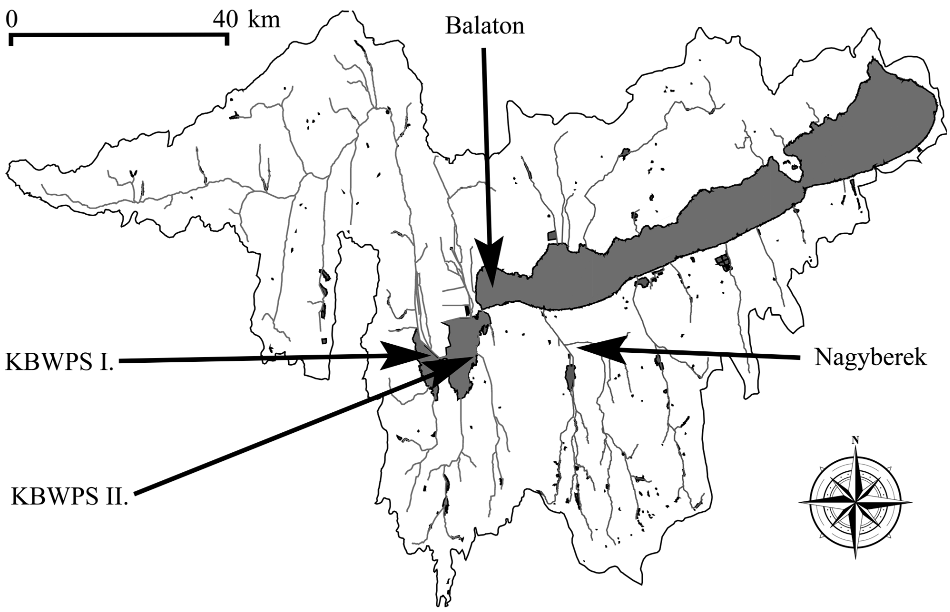

Fig. 1. Overlooking map of sampling areas

Scales were collected from the flank, anterior to the dorsal fin (GARDUÑO-PAZ *et al.* 2010). Scales were fixed between slides and digitalized with an upper lighting (XPA) scanner using 2400 dpi resolution. Shape was analysed using landmark-based geometric morphometric methods (ZELDITCH *et al.* 2004). The digital images were first compiled using the software TpsUtil (ROHLF 2010*a*). Seven landmarks were defined and located on one scale from each fish (Fig. 2) with tpsDig2 (ROHLF 2010*b*).

For further analysis, the MorphoJ software package was used (KLINGENBERG 2011). Generalized least-squares Procrustes superimposition (GLS) was applied to the co-ordinates of raw landmarks to scale, to translate and rotate them and get new shape variables, independent of the scale size (ROHLF 1990). A regression analysis was performed between the logarithm of centroid sizes and Procrustes coordinates to find out whether the scale size influences the shape, and thus whether the growth of scales is allometric or not. Canonical Variate Analysis (CVA) and Discriminant Function Analysis (DFA) were performed to examine group separation. Permutation testing was performed (10 000 iterations) to test the reliability of the results. A redundancy analysis (RDA) was performed, based on the results of CVA, and the environmental parameters, to find out the relations of the sampling sites and the environmental parameters. Redundancy analysis was performed with SYN-TAX2000 software (PODANI 2000).

RESULTS

Regression of scale body shape (Procrustes coordinates) on centroid size indicates notable allometry (i.e. non-independence of shape and size). The percentage of variation for which allometry accounts is given in the percentage predicted, the pooled percentage was 8.01% ($p < 0.0001$).

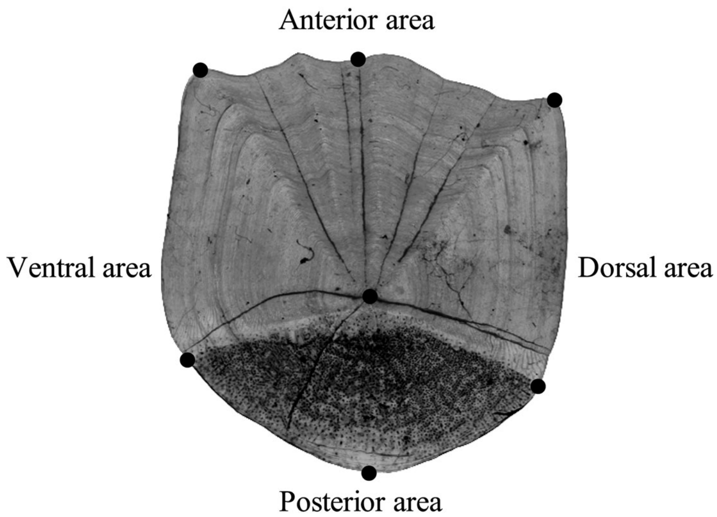

Fig. 2. Landmarks used to define the shape of the scales (Prussian carp). The areas of the scales are described with respect to the fish position

Table 3. Mahalanobis distances and T-square statistic of *C. gibelio* populations discrimination from KBWPS I, KBWPS II, Balaton, Nagyberek, Hungary with landmark-based geometric morphometrics based on scale shape.

		T-square statistic			
		Balaton	KBVR I	KBVR II	Nagyberek
Mahalanobis-distances (D)	Balaton	–	943.618	531.2176	679.343
	KBVR I.	5.937	–	15.056	307.582
	KBVR II.	5.546	0.736	–	179.407
	Nagyberek	5.501	3.441	3.276	–

The specimens could be divided into 3 groups based on their scale shape, from the 4 pre-defined groups (4 sampling area) (Fig. 3). The populations of KBWPS I and KBWPS II could not be separated correctly. The Mahalanobis distances and T-square statistics are shown in Table 3. Based on the results of permutation tests, all results were highly significant ($p < 0.0001$) except KBWPS I vs.

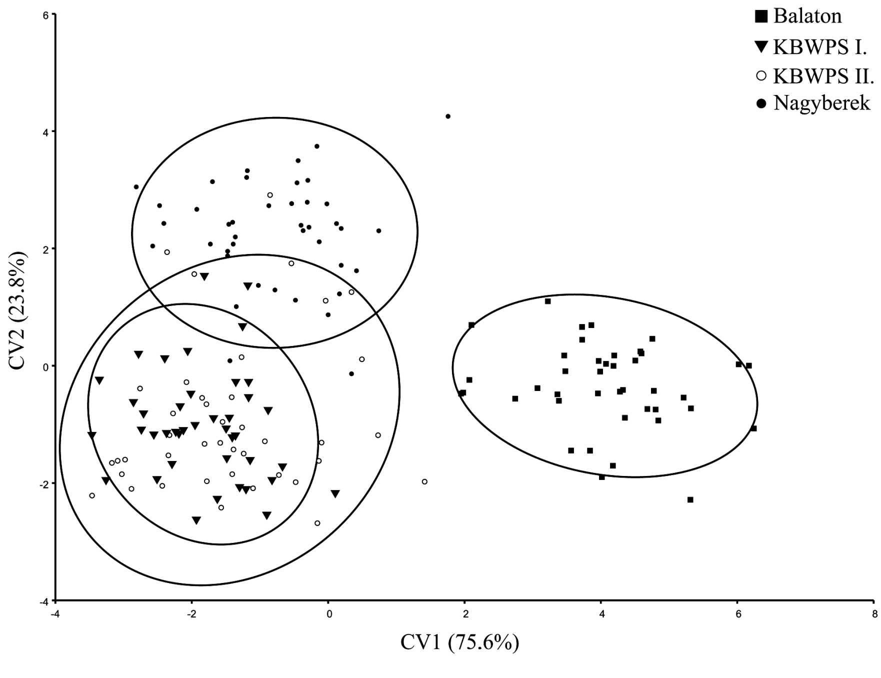

Fig. 3. Canonical Variate Analysis of *Carassius gibelio* from KBWPS I, KBWPS II, Balaton, Nagyberek, Hungary with landmark-based geometric geometric morphometrics based on scale shape. Ellipses presents the 90% confidence intervals

Table 4. Classification rates and significance of the group comparisons from DFA. Specimens are the number of specimens per group. Classifications are the average classification rate of validation and cross-validation in percentages. Significances are the results of the Chi^2 tests for the validation and cross-validation, significance levels are in the parentheses.

Groups	Speci-mens	Classifi-cation	Significance	Classifica-tion (cross-validation)	Significance (cross-validation)
KBWPS I. vs. KBWPS II	41	65.860	8.42 (0.004)	52.44	0.198 (0.656)
KBWPS I. vs. Balaton	41	100	82 (<0.001)	100	82 (<0.001)
KBWPS I. vs. Nagyberek	41	95.120	66.940 (<0.001)	93.9	63.257 (<0.001)
KBWPS II. vs. Balaton	41	100	82 (<0.001)	95.12	66.780 (<0.001)
KBWPS II. vs. Nagyberek	41	91.460	56.694 (<0.001)	86.59	43.929 (<0.001)
Balaton vs. Nagyberek	41	100	82 (<0.001)	100	82 (<0.001)

KBWPS II (from permutation test for Mahalanobis distance: $p = 0.289$; for T-square statistic: $p = 0.223$). Validation and cross-validation results were obtained from DFA (Table 4); in the least separated two groups (KBWPS I and KBWPS II) the specimens were assigned at an average of 65.9% to their real groups. For those groups which were considered distinct by the average, the value of assignment was 97.3%.

The results of redundancy analysis (Fig. 4) showed no correlation between the environmental variables and the CV 2, and strong correlation with CV 1. There was negative correlation with turbidity, emersed vegetation and the substrate, and positive correlation with conductivity, submersed vegetation and the area of the water body. The three sampling areas which are in connection with each other are located along the CV 1, which shows that the separation of Balaton, KBWPS I and KBWPS II are based on the differences in the environmental parameters. Nagyberek, which has no connection with the other water bodies, is separated based on the CV 2. This finding is consistent with the CVA plot (Fig. 3).

DISCUSSION

The results suggest that the landmark-based geometric morphometrics on fish scales is suitable to separate populations with high reliability, not just in Salmonidae (GARDUÑO-PAZ *et al.* 2010) and Mugilidae (IBAÑEZ *et al.* 2007), but probably in common cyprinid species, too (in gibel carp it was proven), which is important because this is the second biggest fish family in the world, with numerous similar threatened and endangered species (HELFMAN *et al.* 2009). The separation of the populations of *Carassius gibelio* shows reliable results which were obtained by the permutation tests, giving significant values. The groups of KBWPS I and KBWPS II are assigned to one group, probably in line with our pre-expectations, according to the geographical distance and connectivity of the two stages of KBWPS. They are only separated by a floodgate, which provides almost free migration when opened, and environmental parameters are very similar; that's why the two groups are considered functionally one population based on scale shape. However, the genetic similarity or dissimilarity of the two stocks is unknown. At the same time, Lake Balaton has close connection with KBWPS, but the environmental characteristics are significantly different (KOVÁCS *et al.* 2010, ISTVÁNOVICS *et al.* 2007). Thus the separation of these groups are strongly correlated with CV 1. The interchange of specimens between Nagyberek and KBWPS can be ex-

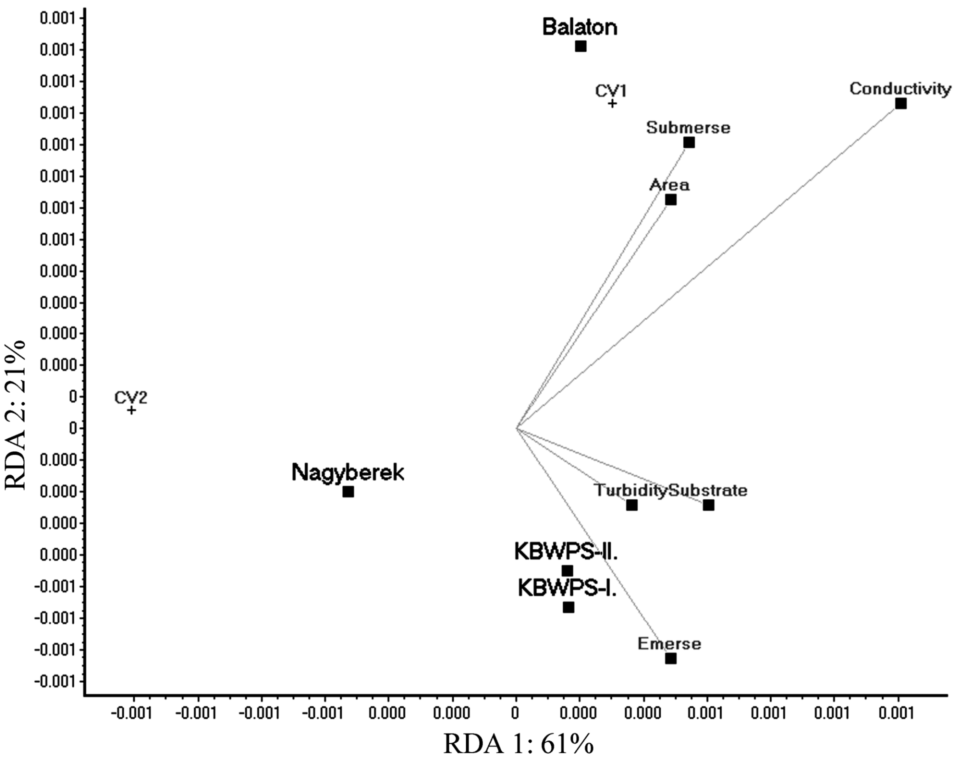

Fig. 4. Relationship between Canonical Variates and environmental variables based on sampling sites separation

cluded, but the environmental parameters were close to each other, and the groups are separated by the CV 2. The separation of the groups from Lake Balaton and Nagyberek is based on both CV 1 and CV 2, and the interchange of the specimens can be excluded, while the environmental parameters are different, too.

This study supports the results of IBAÑEZ *et al.* (2012), which showed that compensatory growth, i.e. the composition and quantity of food, can influence scale shape, as these parameters also can be defined as a special type of environmental effect. This study suggests that shape differences which were attributed to environment have a bigger role in the development of scale shape, as CV 1 has 76% of shape variability, while the CV 2 has only 24%.

It seems there is a theoretical chance to divide the scale shape differences into possible genetic and environmental influences. Further research is needed to validate this geometric morphometric method with genetic tests. It is essential to carry out a laboratory experiment in which the hypothesis can be proved under controlled conditions with known genetic and environmental effects.

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Acknowledgements – This work was financially supported by the research funding programme "KMOP-1.1.1-09/1-2009-0048". The work was supported by the National Office for Research and Technology of Hungary. We are grateful to the Balaton Uplands National Park Directorate for granting licenses. We thank to P. SÁLY for providing the map of sampling areas, and the work of reviewers which helped us to improve the quality of the interpretation of the study.

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Revised version received July 1, 2012, accepted July 23, 2012, published December 28, 2012