GENDER, AGE, AND SEASONAL VARIATION IN SCALE CHARACTERISTICS OF *ALBURNUS SELLAL* HECKEL, 1843 FROM THE TIGRIS RIVER (TURKEY) A GEOMETRIC MORPHOMETRIC STUDY

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DOI: 10.2478/trser-2024-0017

KEYWORDS: Cyprinidae, landmark, morphometric, geometry, scale, shape, Turkey.

ABSTRACT

In this study, 37 female and 40 male *Alburnus sellal* individuals from the Tigris River were analyzed. Using geometric morphometrics, significant differences in scale size between sexes were found, but none in shape. Seasonal and age groups showed significant differences in both size and shape, with females and autumn individuals having larger scales. Scale size increased with age. PCA showed variation across age, season, and sex, while CVA and DFA revealed shape differences between age and seasonal groups, but not between sexes.

RESUMEN: Variación sexual, de edad y estacional en las características de escamas de *Alburnus sellal* Heckel, 1843 del río Tigris (Turquía): un estudio morfométrico geométrico.

En este estudio, se analizaron 37 individuos hembras y 40 machos de *Alburnus sellal* del río Tigris. Utilizando morfometría geométrica, se encontraron diferencias significativas en el tamaño de las escamas entre los sexos, pero no en la forma. Los grupos estacionales y de edad mostraron diferencias significativas tanto en tamaño como en forma, con las hembras y los individuos del otoño teniendo escamas más grandes. El tamaño de las escamas aumentó con la edad. El análisis PCA mostró variaciones según la edad, la estación y el género, mientras que el CVA y el DFA revelaron diferencias en la forma entre los grupos de edad y de estación, pero no entre los sexos.

REZUMAT: Sexul, vârsta și variația sezonieră a caracteristicilor dimensionale la *Alburnus sellal* Heckel, 1843 din râul Tigru (Turcia) un studiu morfometric geometric.

În acest studiu, au fost analizați 37 de indivizi femele și 40 de masculi de *Alburnus sellal* din râul Tigru. Folosind morfometrie geometrică, s-au găsit diferențe semnificative între mărimea solzilor între sexe, dar nu și în forma lor. Grupurile sezoniere și de vârstă au prezentat diferențe semnificative atât în mărime, cât și în formă, femelele și indivizii de toamnă având solzi mai mari. Mărimea solzilor a crescut odată cu vârsta. Analiza PCA a arătat variații în funcție de vârstă, sezon și sex, în timp ce analizele CVA și DFA au evidențiat diferențe de formă între grupurile de vârstă și de sezon, dar nu și între sexe.

INTRODUCTION

Image techniques have revealed numerous new features of fish scales. Fish scales differ depending on the sex, age, diet, habitat, and genetic makeup of the fish. The silhouette of the scales is important not only in species discrimination in systematic studies but also in revealing intra-specific variations and determining differentiation between populations (Roberts, 1993, Braeger et al.2017; Ibáñez and Jawad, 2018; Ibáñez et al. 2023). Consequently, fish scales are an important tool in identifying and monitoring fish populations (Trueman and Moore, 2007).

Geometric morphometric methods for analyzing fish scales have been shown to be a reliable and practical tool for distinguishing between challenging genera, species, geographic variants, and local populations. In addition, these methods are also effective for assessing the effects of habitat on scale morphology, as well as for indicating age and seasonal variation. These analyses provide important biometric data related to scale shape (Bilici, 2020; Dörtbudak et al., 2022; Rohlf and Marcus, 1993; Vignon, 2012; Zelditch et al., 2004).This method is also more economical, easier, and harmless compared to other methods, allowing for sampled fish to be released again and for the inspection and monitoring of many samples from populations (Bilici et al., 2016; Çicek et al., 2016; Ibáñez et al., 2007, 2009; Poulet et al., 2005; Staszny et al., 2013).

The Sellal bleak, *Alburnus sellal* Heckel, 1843, also known as "Gümüşbalığı" in Turkey, belongs to the Leuciscidae family and is native to the Euphrates, Tigris, Zoreh, Persis, and Hormuz River basins (Turkey, Syria, Iraq, and Iran) (Coad, 2010; Çiçek et al., 2023a, b; Jouladeh-Roudbar et al., 2020; Saad et al., 2023; Shahraki et al., 2022, 2023). This species inhabits lakes, reservoirs, and all kinds of streams and rivers from the cold Anatolian highlands down to the subtropical Shatt al Arab and Iranian Gulf rivers (Çiçek et al. 2023b; Saad et al. 2023).

This fis species, frequently reported as *Albumus mossulensis* in several studies (Coad, 1996; Kaya et al. 2016; Kuru, 1978), was finnally determined to be a synonym for *Alburnus sellal* mainly due to significant overlaps in lateral line, gill rake, anal fin ray, and also vertebra number intervals (Bogutskaya, 1997). Subsequent morphological and molecular studies further supported Bogutskaya's interpretation, leading to its acceptance as a synonym of the *Alburnus sellal* species (Bektas et al., 2020; Mangit and Yerli, 2018; Mohammadian-Kalat et al., 2017).

Alburnus sellal has been the subject of extensive and multidisciplinary research, with studies delving into various aspects (Banaee et al., 2023; Banaee et al., 2014; Bostanci et al., 2015; Dane and Şişman, 2020; Esmaeili et al., 2018; Mangit and Yerli, 2018; Mousavi-Sabet et al., 2013). Additionally, numerous investigations into its biology and ecology have been carried out in Iraq (Jawad, 2004; Mohamed et al., 2016), Iran (Esmaeili and Ebrahimi, 2006; Ergene, 1993; Mousavi-Sabet et al., 2013; Parsa et al., 2011), and Turkey (Basusta and Cicek, 2006; Ozdemir et al., 1993; Parmaksız et al. 2018; Türkmen and Akyurt, 2000; Uçkun and Gökçe, 2015; Yıldırım et al., 2003; Yıldırım et al., 2007).

This research aims to evaluate the effectiveness of using a landmark-based, geometric morphometric approach to describe fish scale morphology in *Alburnus sellal* and to distinguish differences between seasons, age groups, and male and female individuals.

MATERIAL AND METHODS

In this research, we collected 77 specimens (37 female and 40 male) of *Alburnus sellal* from the Tigris River (Fig. 1).

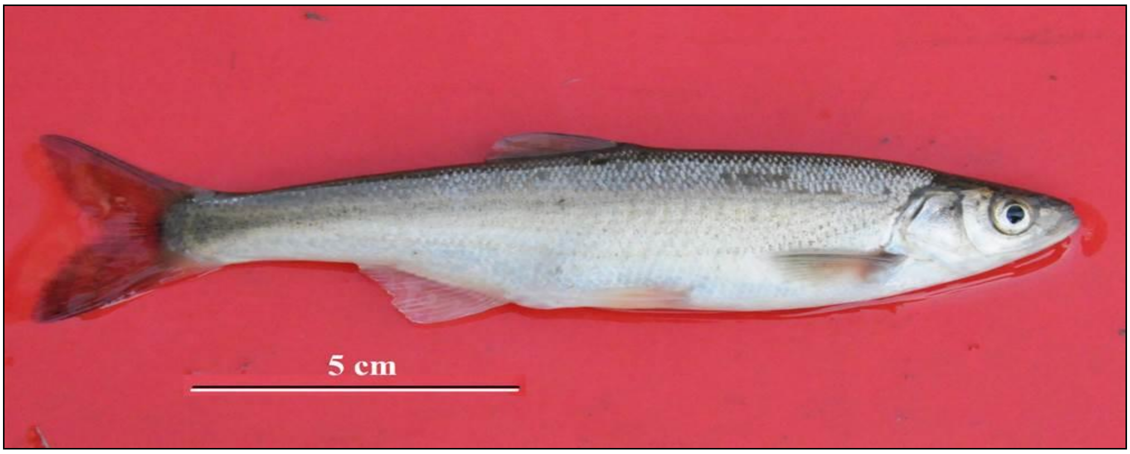

Figure 1: The overall body appearance of *Alburnus sellal* (Tigris bream), Tigris River (photo E. Ünlü).

Samples localities are shown in figure 2, and the seasonal, sexual, and age distributions of the samples, as well as some water parameters of the locality where they were collected, are given in table 1.

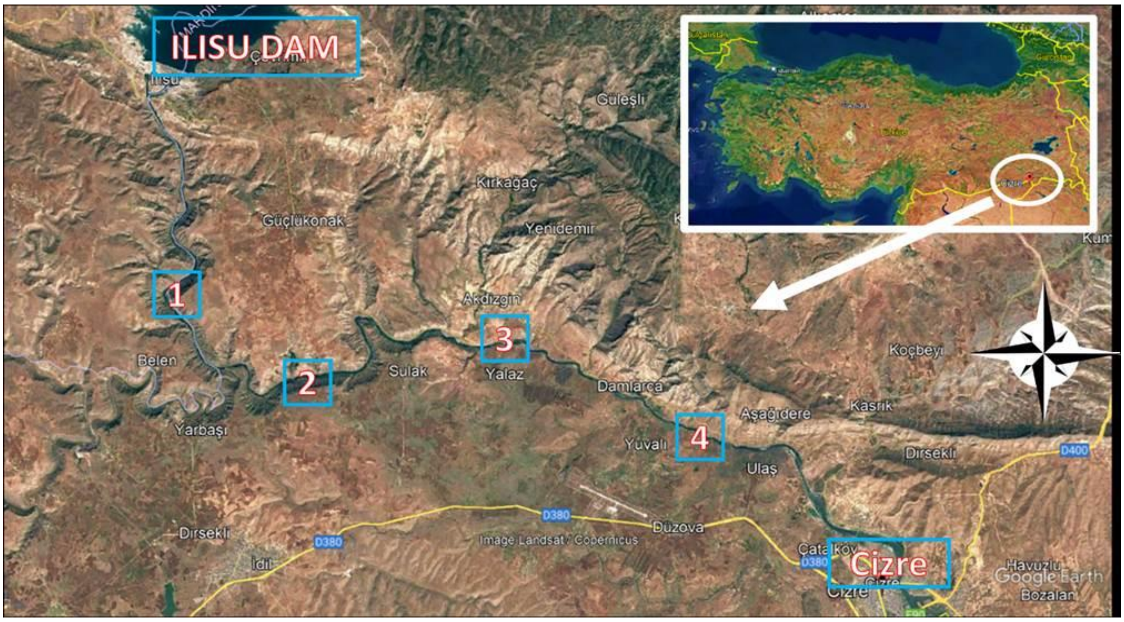

Figure 2: Map of the study area were the smples were obtained.
Sample localities (1-Tigris River (Güçlükonak), 2-Tigris River (Güçlükonak), 3-Tigris River (Akdizgin), 4-Tigris River (Damlarca).

Table 1: Samples distribution and water parameters of the study.

Season	Female	Male	Total
Autumn (November)	2	–	2
Winter	1	–	1
Spring (April)	22	25	47
Summer (July)	13	14	27

	Age				
	II	III	IV	V	VI
Sample number	12	45	17	2	1

Date	Water temperature (°C)	pH	Dissolved oxygen (O_2)	$\%O_2$	Electrical conductivity (EC) µS/cm
26.04.2021	17.7	8.3	9.11	101	306
01.07.2021	24.6	7.86	7.67	97.2	474
04.11.2021	13.5	8.2	8.62	96.8	365

The sex of each fish was determined by observing their gonads. Scales from the front and upper sections of the lateral lines of the dorsal fins were taken to determine their age and morphology. The fish scales tissue was cleaned with 5% NaOH for two hours, then washed with distilled water, and immersed in 96% ethanol for several minutes to remove any remaining water. Following this the scales were placed between two slides and photographed by an stereo microscope (Olympus SZX7, Tokyo, Japan) and a digital camera (OLYMPUS Camedia C-5060 5.1 MP w/4x Optical Zoom, Tokyo, Japan) under 20× and 40× magnifications. Images were analyzed by geometric morphometric procedure (Zelditch et al., 2004; Rohlf and Marcus, 1993; Bookstein, 1991). Subsequently, six landmarks (Fig. 3) were digitized using tpsDig ver. 2.32 (Rohlf, 2015) software, and Procrustes analysis was conducted. Following the separation of shape and size (centroid size = CS)of the samples, Procrustes ANOVA, PCA, CVA/MANOVA, and DFA analyses were performed using Morpho J1.06d (Klingenberg, 2011). R Core Team (2019) and Jamovi Ver. 2.4 (2023) programs.

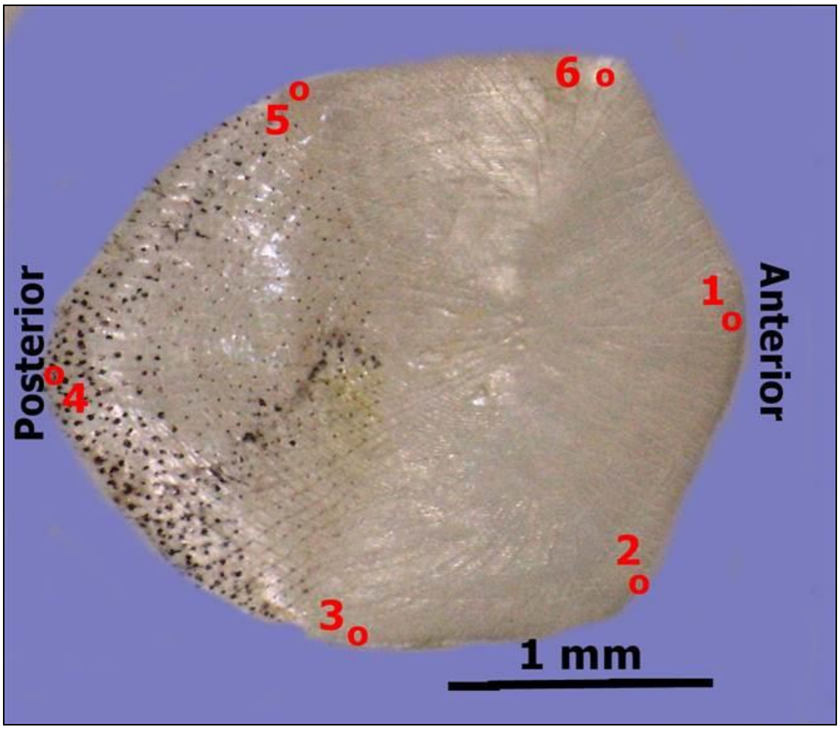

Figure 3: Landmark definitions used in the fish scales.

RESULTS

When the results of Procrustes ANOVA are examined, no significant difference in size (LogCS) (p = 0.1960) and shape (p = 0.9350) between sexes is found. Groups based on season and age are significant in size (p < 0001), but not for shape (Tab. 2).

Table 2: Procrustes ANOVA results of scales for amos groups (df: Degree of freedom, F: Goodal's F, CS: Centroid Size, P: p value).

Procrustes ANOVA						
		df	F	P (param.)	Pillai tr.	P (param.)
Sex	CS	1	1.70	0.1960		
	Shape	8	0.37	0.9350	0.06	0.8330
Season	CS	3	9.78	<.0001		
	Shape	24	1.26	0.1822	0.31	0.5086
Age	CS	4	13.89	<.0001		
	Shape	32	0.43	0.9978	0.16	0.9996

Scale size is larger in females than in males, and the summer and winter groups' scales are larger than other groups. Scale size increases with age groups (Fig. 4).

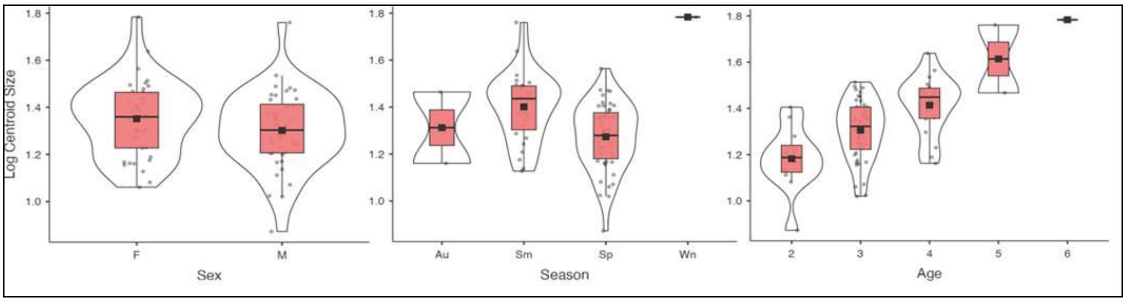

Figure 4: Box-violin chart of scale's size of amos groups.

In PCA analysis, when examined by age, first two PC explain 49.4%, by season, first two PC explain 49.7% and by sex, first two PC explain 50.2% of the total variation (Tab. 3).

Table 3: PCA resulsts of sclaes for amos groups.

	PC	Eigenvalue	% variance
Sex	1	0.0019	30.8
	2	0.0012	19.4
	3	0.0010	16.5
Season	1	0.0017	29.5
	2	0.0012	20.2
	3	0.0010	16.5
Age	1	0.0019	30.4
	2	0.0012	19.0
	3	0.0010	16.7

When PCA plots are examined, there is no clear separation between the groups along the PC1 and PC2 axes (Fig. 5).

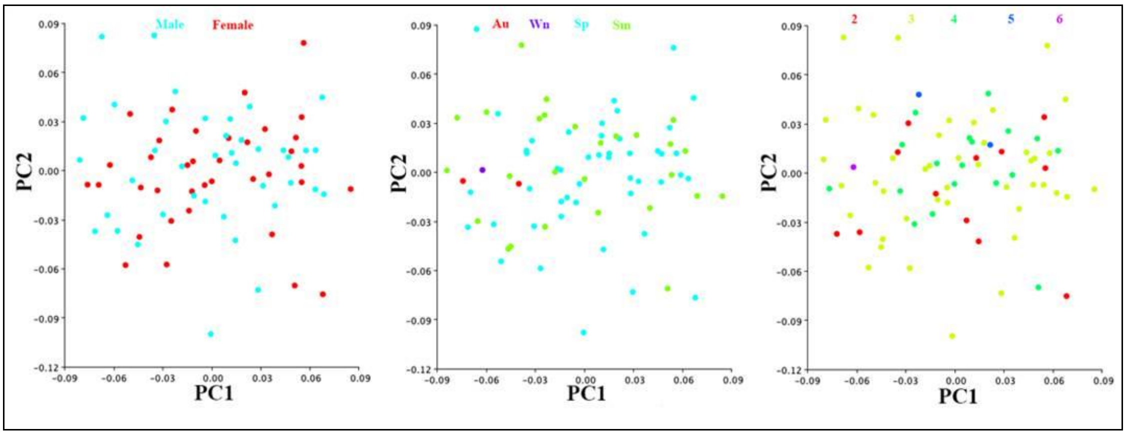

Figure 5: Scatter plot of principal component analysis (PCA)
showing the distribution of scale shapes by sex, age, and season.

When looking at the CVA results, there is not clear separation and significant difference between any groups (Tab. 4, Fig. 6).

Table 4: CVA results of scales for amos groups (Mah. Dist.: Mahalanobis distance, Proc. Dist.: Procrustes distance, p val: Permutation p-value).

Age								
	2		3		4		5	
	Mah.Dist/ p val.	Proc.Dist/ p val.	Mah.Dist/ p val.	Proc.Dist/ p val.	Mah.Dist/ p val.	Proc.Dist/ p val.	Mah.Dist/ p val.	Proc.Dist/ p val.
3	0.6658/ 0.8921	0.0150/ 0.9398	–	–	–	–	–	–
4	0.6631/ 0.8821	0.0168/ 0.9119	0.3960/ 0.9815	0.0100/ 0.9823	–	–	–	–
5	1.4983/ 0.9156	0.0477/ 0.7395	1.1786/ 0.9602	0.0363/ 0.9007	1.9158/ 0.9501	0.0356/ 0.8516	–	–
6	1.8235/ 0.8713	0.0702/ 0.6626	1.8328/ 0.9497	0.0662/ 0.6868	1.1345/ 0.8262	0.0716/0.3921	2.2266/ 1.0000	0.0752/ 0.6629
Season								
	Au		Sm		Sp			
Sm	2.5778/0.1333	0.0792/ 0.0923	–	–	–	–	–	–
Sp	2.4285/ 0.2009	0.0781/0.0763	0.7899/ 0.2211	0.0196 / 0.3625	–	–	–	–
Wn	2.5473/ 0.6631	0.0614/0.6631	1.6775/ 0.9826	0.0631/ 0.7042	2.1015/ 0.8228	0.0734/ 0.4500	–	–
Sex								
	Female		–		–		–	
Male	0.4914/ 0.8114	0.0109/ 0.9034	–	–	–	–	–	–

CVA graphs show that the groups are very similar in terms of scales and there is a lot of overlap (Fig. 6).

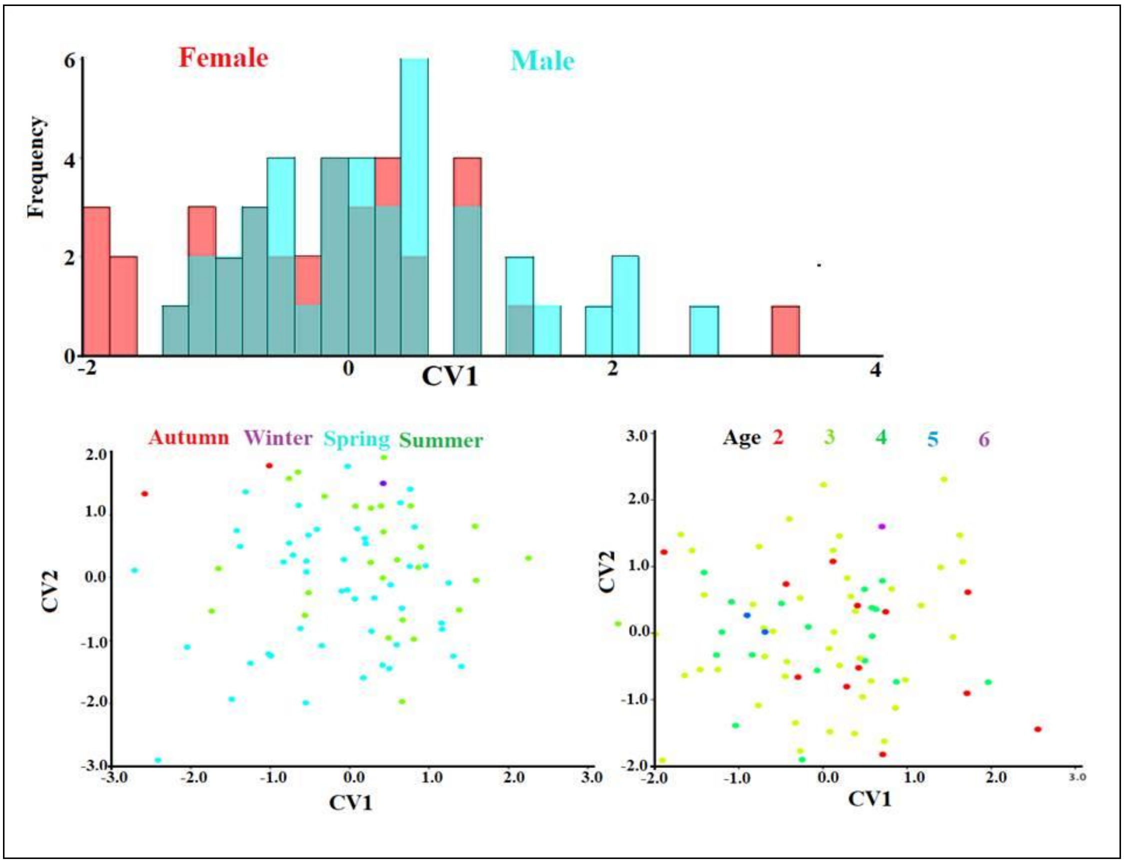

Figure 6: CVA plots of scales for amos groups.

Upon reviewing the DFA results, there are not significant differences between any studied groups (Tab. 5, Fig. 7). Looking at the warp line graphs (Fig. 7) produced by the DF analysis, it shows that there are no significant differences in scale shape between the groups.

Table 5: DFA results of scales for amos groups (Mah. D.: Mahalanobis distance, Proc. D.: Procrustes distance, T^2: T-square, Par. p: Parametric p value, Perm. p: Permutation p-value).

Age					
		2	3	4	5
	T^2	4.0500	–	–	–
3	Param. p	0.8904	–	–	–
	Perm. p (Proc./T^2)	0.9450/0.8780	–	–	–
4	T^2	2.7109	2.2702	–	–
	Param. p	0.9740	0.9785	–	–
	Perm. p (Proc./T^2)	0.9010/0.9690	0.9820/0.9800	–	–
5	T^2	16.6296	2.2724	4.5819	–
	Param. p	0.5931	0.9807	0.9265	–
	Perm. p (Proc./T^2)	0.7130/0.5820	0.9100/0.9840	0.8550/0.9530	–
6	T^2	79.0948	2.5351	8.6745	0.0582
	Param. p	0.1159	0.9732	0.7320	0.8494
	Perm. p (Proc./T^2)	0.6590/0.2080	0.6740/0.9210	0.3960/0.7110	0.6790/0.3330
Season					
		Au	Sm	Sp	
	T^2	16.9733	–	–	–
	Param. p	0.1958	–	–	–
	Perm. p (Proc./$T^{2)}$	0.0870/0.1880	–	–	–
	T^2	14.0309	10.7168	–	–
	Param. p	0.1906	0.3077	–	–
	Perm. p (Proc./$T^{2)}$	0.0790/0.2240	0.3840/0.3240	–	–
	Sex	–	–	–	–
	Female	–	–	–	–
	T^2	0.0373	3.0994	4.5932	–
	Param. p	0.8785	0.9635	0.8581	–
	Perm. p (Proc./$T^{2)}$	0.6420/0.3220	0.6770/0.9960	0.4540/0.8150	–
Sex					
		Female	–	–	–
	T^2	4.6405	–	–	–
	Param. p	0.8330	–	–	–
	Perm. p (Proc./$T^{2)}$	0.9020/0.8490	–	–	–

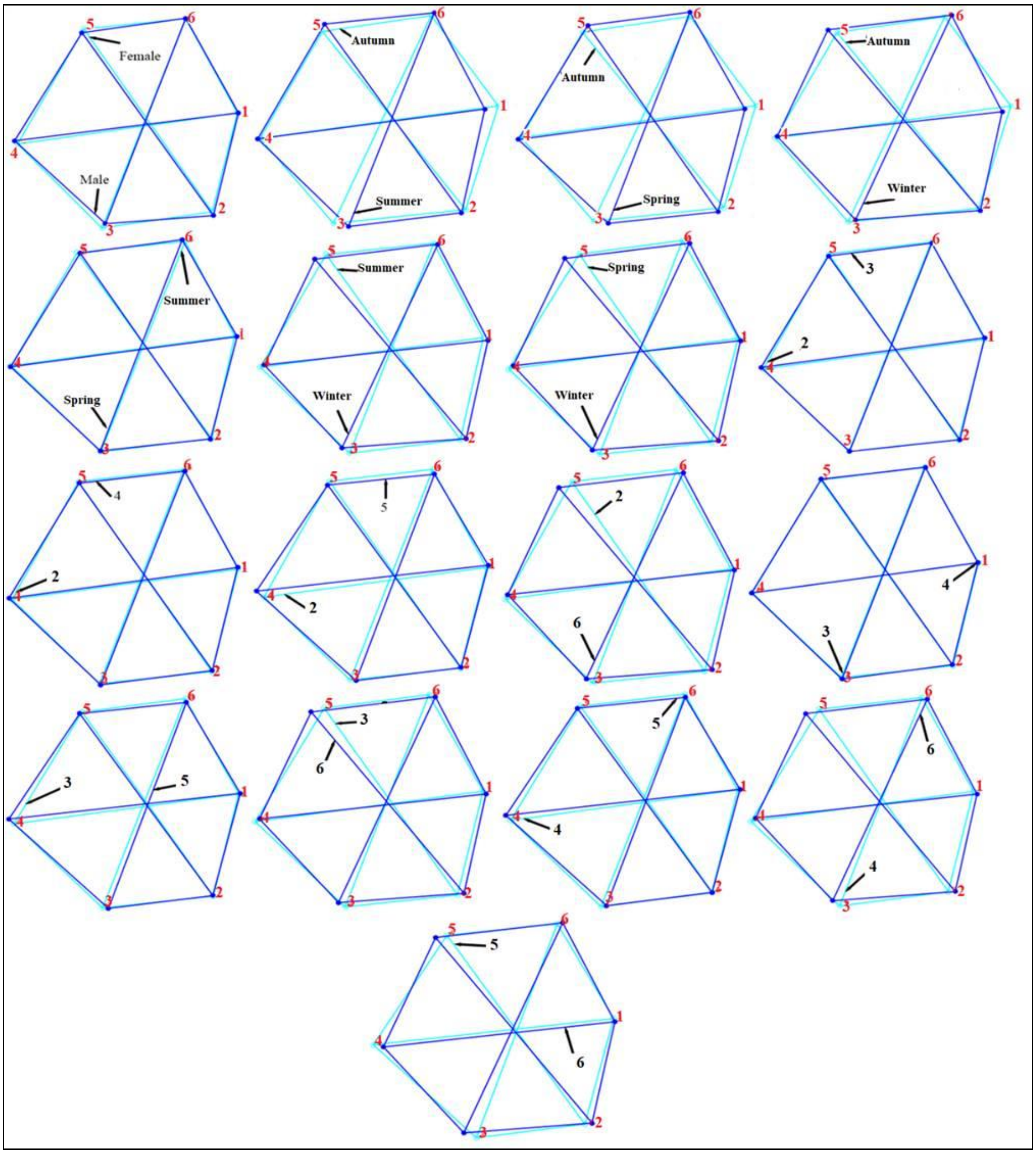

Figure 7: Warp line of scale shape difference between groups of amos.

DISCUSSION

Fish scales contain small growth rings that allow us to determine the age of the fish. These growth rings are typically arranged around a center and are composed of $CaCO_3$ compounds (Carbonara and Follesa, 2019; Chen et al., 2022). Variations in these rings occur because fish scales generally grow excessively when feeding is abundant, typically during spring and summer, and slow down or stop altogether when feeding is inadequate, especially during winter (Gümüş et al., 2002). As the structure of annual growth rings in fish scales is influenced by environmental conditions, this type of differentiation can be significant based on the physicochemical parameters of the environment and feeding (Staszny et al., 2012). In this sense, changes in the shape of fish scales can allow for differentiation in populations (Ibáñez et al., 2007; Ibáñez et al., 2009). Additionally, inter/intraspecific morphological variability may indicate genetic differences among samples or can respond to environmental conditions within the framework of phenotypic plasticity (Carro et al., 2018; Staszny et al., 2013).

Geometric morphometrics is very important in fish scales studies because it allows for the aqurate quantitative analysis of shape and size variation in a way that traditional morphometrics cannot achieve (Carro et al., 2018; Moreira et al., 2020). This method provides a detailed and comprehensive understanding of the shape and size changes in fish scales, which can be used to address questions related to taxonomy, evolution, and ecology. Additionally, geometric morphometrics allows for the visualization and analysis of complex patterns of shape variation, making it a valuable tool for researchers studying fish scales (Çiçek et al., 2017; Ibáñez and Jawad, 2018;Ibáñez et al., 2023) applied geometric morphometric methods successfully on *Capoeta trutta* and *Capoeta umbla* species. In the present study, it was achieved on *Acanthobrama marmid* species at the same success. In the size analysis performed according to sex, it was seen that female samples were larger than males. These results show that fish species can be successfully distinguished by morphometric geometric analysis.

This type of analysis has been used successfully in previous studies. For example, studies on fish scale and otolith morphometry and geometry (Bilici, 2020; Çiçek et al., 2017; Dörtbudak and Özcan, 2018; Ibáñez et al., 2019; Richards and Esteves, 1997; Staszny et al., 2012; Teimori, 2016; Wichard et al., 2005) have yielded important results in this field. In addition, studies examining the relationship between fish size and otolith morphometry (Staszny et al., 2012, 2013) were also effective in determining the species.

When the ANOVA results of the study were analysed, no significant difference was found between sex in terms of size and shape. Seasonal and age-based groups were significant in terms of size ($p < 0001$), but not in terms of shape.

Scale size is larger in females than in males, and the summer and winter groups have larger scales than all other groups. Scale size increases with age groups.

In PCA analysis, the first two PCs explained 49.4% of the total variation when analysed by age, the first two PCs explained 49.7% when analysed by season and the first two PCs explained 50.2% when analysed by sex.

When CVA results are analysed, there is no clear distinction and no significant difference between any of the groups. CVA graphs show that the groups are very similar in terms of scales and there is a lot of overlap.

When the CFA results are analysed, it is seen that there are no significant differences between any groups. Looking at the warp line graphs produced by the DF analysis, it is seen that there are no major differences between the groups in terms of scale shape.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the anonymous reviewers and the editors of the Transylvanian Review of Systematical and Ecological Research for their valuable, constructive, and insightful feedback on the manuscript.

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