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Research Article

Gender, Age and Seasonal Variation in Scale Characteristics of *Carassius Gibelio* (Bloch, 1782) from the Tigris River, Turkey: A Geometric Morphometric Study

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Abstract

In this study, individuals of *Carassius gibelio* (Bloch, 1782) comprising 85 females and 34 males were collected from the Tigris River in Şırnak, Turkey. The size and shape of the scales were analyzed separately using 2D geometric morphometric methods, with scale size treated as the center size. Procrustes ANOVA indicated significant differences between the groups in both size (center size) and shape. Scale size increased with age among the age groups, while seasonal analysis revealed that autumn samples exhibited the largest scale size, whereas summer samples had the smallest. Female specimens were generally larger than their male counterparts. In the principal component (PC) analysis based on gender, the first two principal components (PC1 and PC2) accounted for 59.8% of the total variance, contributing 42.7% and 17.1%, respectively. When analyzing by age, PC1 and PC2 explained 57.7% of the variance, with contributions of 41.2% and 16.5%, respectively. Seasonal PC analysis showed that PC1 and PC2 accounted for 59.3% of the total variance, with 42.7% and 16.6% contributions, respectively. Canonical Variate Analysis (CVA) for gender demonstrated a significant difference between the two genders. In the seasonal CVA, significant differences were observed among the autumn-summer, summer-spring, and spring-winter group comparisons. For age groups, significant differences were found between age groups 2-5, 2-6, and 2-7 as well as between 3-5 and 3-6; however, the differences among the other age groups were not statistically significant. In the shape analysis through Discriminant Function (DF) analysis, the female individuals exhibited larger dorso-ventral scale dimensions, with the difference reaching significance according to both parametric and permutation p-values for T2.

Keywords: Cyprinidae; Geometric Morphometrics; Landmark Analysis; Scale Shape; Turkey

Introduction

Cyprinidae is the largest family of freshwater fish, with a broad geographical distribution that spans North America, Africa, and Eurasia [1]. In Turkey, approximately 15% of freshwater fish species, totaling 59 species, belong to the family [2,3]. The focus of this study, *Carassius gibelio* (Bloch, 1782) (Figure 1), is found in the Tigris and Euphrates river systems [4-7]. *Carassius gibelio* is considered one of the first cyprinid species used by humans in fisheries outside its native distribution area [8,9]. This omnivorous species thrives in both lentic and lotic habitats and is recognized as a highly invasive species, often outcompeting native fish populations [10,11]. Notably, *Carassius gibelio* can survive for extended periods under extreme environmental conditions, such as high turbidity and eutrophication, even without oxygen [12,13].

Several aspects of *Carassius gibelio* have been investigated, such as its distribution, gender ratio, growth [14], reproductive biology [14,15], and feeding biology [16]. However, few studies have focused on the morphological variations of *Carassius gibelio* [17-19].

Figure 1: General appearance of *Carassius gibelio* from the Tigris River.

Fish scales serve as valuable tools for identifying fish at the genus or species level, as well as for studies on fish phylogeny, sexual dimorphism, age determination, and habitat effects on development [20-28]. While fish scales are acknowledged as important for classification, they have been found to be less effective for species-level identification. Instead, they are more suitably used as an age index [29]. The external morphology of fish and scale models has been reported as useful for establishing phylogenetic relationships [29]. Recent scanning electron microscopy (SEM) studies have provided detailed insight into the shapes of teleost fish scales, enhancing their use for taxonomic purposes [30]. Fish populations exhibit varying growth characteristics influenced by factors such as environmental conditions, seasonal changes, habitat type, and food availability, and scales reflect these phenotypic variations [31].

Geometric morphometrics (GMM) offers a powerful approach to detect subtle morphological variations often overlooked by conventional methods [32]. Geometric morphometric analyses of scales have proven to be a reliable tool for distinguishing habitat impacts on scale morphology, as well as identifying age and seasonal differences, despite the challenges in differentiating between genera, species, geographical variants, and local populations. This method allows for the examination and monitoring of samples, which can be safely released back into their habitats, enabling the collection of large sample sizes from populations. Fish scales are ideal for 2D geometric morphometric techniques, as they can vary with age, gender, and season. Additionally, scales can help identify sources of variation in fish size and shape [3,18,27,33-36].

This study employs geometric morphometric methods to investigate the characteristic structure of *Carassius gibelio* scales. Additionally, it aims to evaluate differences in scale morphology between male and female individuals, providing insights into potential sexual dimorphism within the species.

Material and Method

A total of 119 fish specimens were collected from four different locations in the Tigris River, specifically from the Güçlükonak locality in Şırnak, Türkiye. This collection comprised 85 females and 34 males (Figure 2).

Figure 2: Map of the study area showing the sample locations: 1) Tigris River (Güçlükonak), 2) Tigris River (Güçlükonak), 3) Tigris River (Akdizgin), 4) Tigris River (Damlarca).

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The scales of *Carassius gibelio* specimens were collected from the anterior dorsal fin and the upper part of the lateral line. The age-determined scales were photographed under consistent conditions using a Canon SX7 binocular equipped with an Olympus digital camera (see Figure 3). Six landmarks were recorded using tpsUtil and tpsDig ver. 2.32 [37]. Geometric morphometric analysis (GPA) was then performed.

Following the shape and size discrimination, Procrustes ANOVA, PCA, CVA, and CFA analyses were conducted with MorphoJ 1.08.02 [38]. Box-violin plot charts of LogCS for different groups were created using R software [39] and Jamovi (2023).

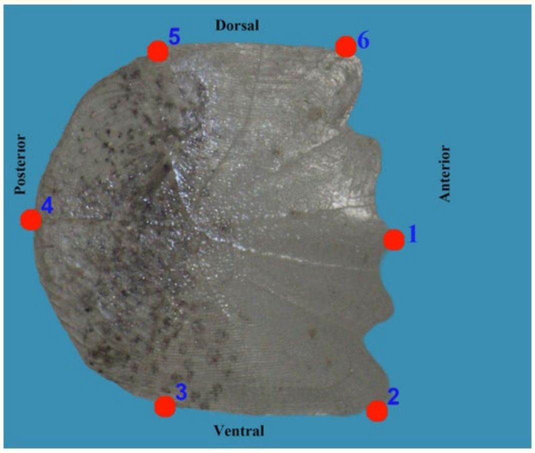

Figure 3: Definitions of landmarks used in the fish scales of *Carassius gibelio*.

Results

The Procrustes ANOVA results indicated that the groups differed significantly in both size (CS) and shape (Table 1).

Group	Size/Shape	F	p	Pillai tr.	p
Age	CS	56.02	<.0001		
	Shape	2.13	<.0001	0.43	0.11
Season	CS	4.35	0.01		
	Shape	1.94	0.0046	0.29	0.07
Gender	CS	7.35	0.01		
	Shape	1.88	0.06	0.15	0.02

Table 1: Procrustes ANOVA results (F: Goodal's F, CS: Centroid Size).

Analysis of the CS box plot reveals that scale size increases with age among the different age groups. Among the seasonal groups, autumn (Au) scales are the largest, while summer (Sm) scales are the smallest. Additionally, female specimens tend to be larger in size (Figure 4).

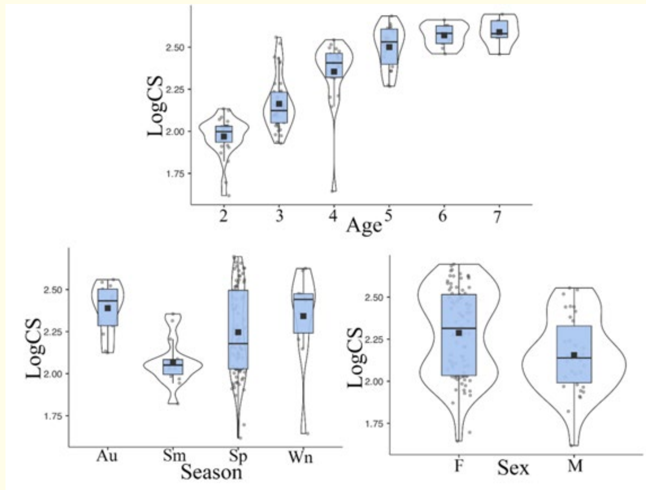

Figure 4: Box and Violin plot of CS of scales by gender, age and season).

In the PCA based on gender, PC1 and PC2 accounted for 59.8% of the total variance, with 42.7% explained by PC1 and 17.1% by PC2. For the PCA based on age groups, PC1 and PC2 explained 57.7% of the total variance, with 41.2% attributed to PC1 and 16.5% to PC2. In the PCA based on seasonal groups, PC1 and PC2 accounted for 59.3% of the total variance, with 42.7% explained by PC1 and 16.6% by PC2. The graph indicates that there is significant overlap among all groups on both the PC1 and PC2 axes (Figure 5).

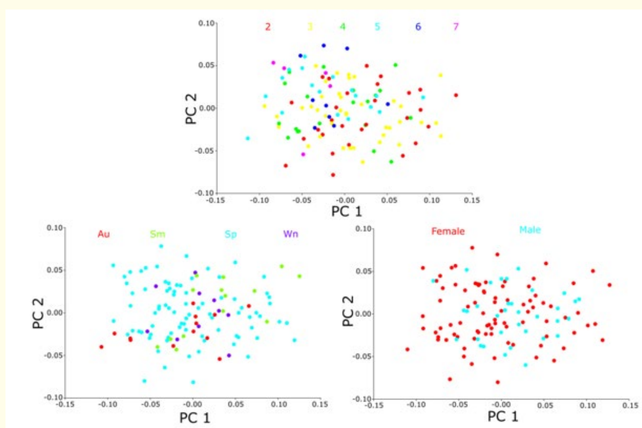

Figure 5: Scatter plot of principal component analysis (PCA) showing the distribution of scales by gender, age, and season.

The CVA conducted on gender reveals a significant difference between the genders (Table 2, Figure 6).

	Female	
	Mah. Dist/P val.	**Proc. Dist/P val.**
Male	0.9060/0.0242	0.0221/0.0999

Table 2: CVA result of scales by gender (Mah.Dist: Mahalanobis distance, Proc. Dist: Procrustes distance, P val: P value of permutation test).

In the CVA for age groups, significant differences were observed between the 2-5, 2-6, and 2-7 age groups compared to the 3-5 and 3-6 age groups. However, differences between the other age groups were not statistically significant (Table 3, Figure 6).

	Age									
	2		**3**		**4**		**5**		**6**	
	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.**	**Mah.Dist/P val.**	**Proc.Dist/P val.**
3	0.6082/0.4826	0.0149/0.6879								
4	1.0701/ 0.2451	0.0339/0.0797	1.0599/0.1892	0.0254/0.2188						
5	1.3430/ 0.0394	0.0500/0.0047	1.0215/ 0.2004	0.0386/0.0215	0.9445/0.7111	0.0247/0.4521				
6	1.2931/ 0.1177	0.0486/0.0317	1.3417/ 0.3676	0.0418/0.0581	1.1513/0.6714	0.0286/0.5253	0.9565/0.9221	0.0209/0.8239		
7	1.9920/ 0.0212	0.0804/0.0031	1.8838/0.1586	0.0711/0.0087	1.5836/0.4506	0.0520/0.1328	1.2440/0.8885	0.0384/0.3932	1.0173/0.9346	0.0367/0.4708

Table 3: CVA result of scales by age (Mah.Dist: Mahalanobis distance, Proc. Dist: Procrustes distance, P val: P value of permutation test).

The CVA conducted for seasonal groups shows significant differences between autumn and summer, summer and spring, as well as spring and winter (Table 4, Figure 6).

	Autumn		Summer		Spring	
	Mah.Dist/ P val.	**Proc.Dist/ P val.**	**Mah.Dist/ P val.**	**Proc.Dist/ P val.**	**Mah.Dist/ P val.**	**Proc.Dist/ P val.**
Summer	1.6100/0.2592	0.0605/0.012				
Spring	1.1033/0.1977	0.0318/0.1210	1.2029/0.0730	0.0398/0.0196		
Winter	0.8173/0.8353	0.0301/0.4468	1.1574/0.2903	0.0363/0.2083	1.0481/0.0229	0.0229/0.5789

Table 4. CVA result of scales by season (Mah.Dist: Mahalanobis distance, Proc. Dist: Procrustes distance, P val: P value of permutation test).

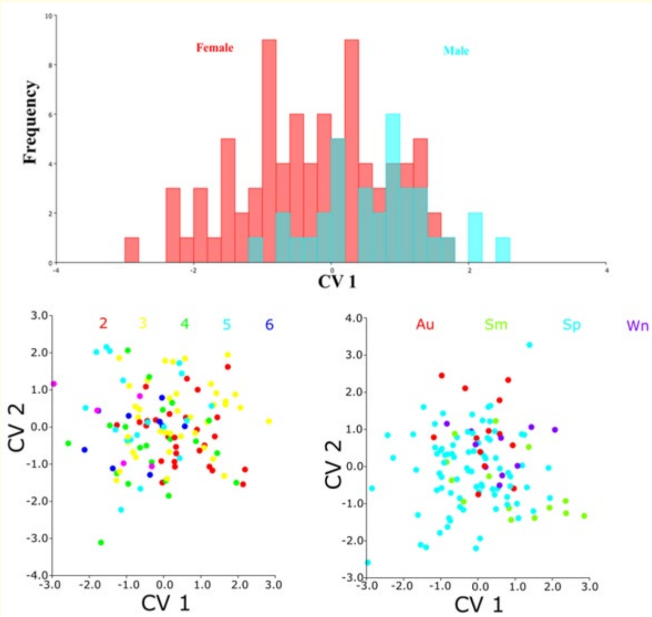

Figure 6: CVA plot of scales by gender, age and season.

The shape difference analysis in DF revealed that female scales were larger dorso-ventrally. This difference was significant based on both the parametric p-value and the permutation p-value for T2 (Table 5, Figure 7).

		Female
Male	T^2	19.9365
	Param. p	0.0230
	Perm. P (Proc./T^2)	0.0910/0.0220

Table 5: DFA results f of scales by gender (T2: T-square, Param. p: Parametric P values, Perm. P: Permutation P value, Bolded: significant)

Among the seasonal groups, the summer group exhibited larger dorsal and antero-ventral scales compared to the autumn group, with significant differences based on the permutation p-value for Procrustes. Additionally, the summer group had smaller dorso-ventral scales and was longer anteriorly compared to the spring group, with these differences also being significant according to the permutation p-value for Procrustes (Table 6 and Figure 7).

		Autumn	Summer	Spring
Summer	T^2	15.8752		
	Param. P	0.2509		
	Perm. P (Proc./T^2)	0.0130/0.2640		
Spring	T^2	12.4051	16.2079	
	Param. P	0.1941	0.0741	
	Perm. P (Proc./T^2)	0.1280/0.1940	0.0230/0.0760	
Winter	T^2	5.9650	15.7411	9.2987
	Param. P	0.8389	0.2968	0.3907
	Perm. P (Proc./T^2)	0.4390/0.8220	0.2130/0.3010	0.5940/0.4010

Table 6: DFA results f of scales by season (T2: T-square, Param. p: Parametric P values, Perm. P: Permutation P value, Bolded: significant).

Among the age groups, the 2-year-old group had longer dorso-ventral scales and shorter anterior-posterior scales compared to the 5, 6, and 7-year-old groups. Significant differences were found for both parametric and permutation p-values between the 2- and 5-year-old groups. For the 2- and 6-year-old groups, differences were significant based on the permutation p-value for Procrustes. Similarly, both parametric and permutation p-values indicated significant differences between the 2- and 7-year-old groups. The 3-year-old group also had longer dorso-ventral scales than the 5 and 7-year-old groups, with these differences being significant according to the permutation p-value for Procrustes (Table 7, Figure 8).

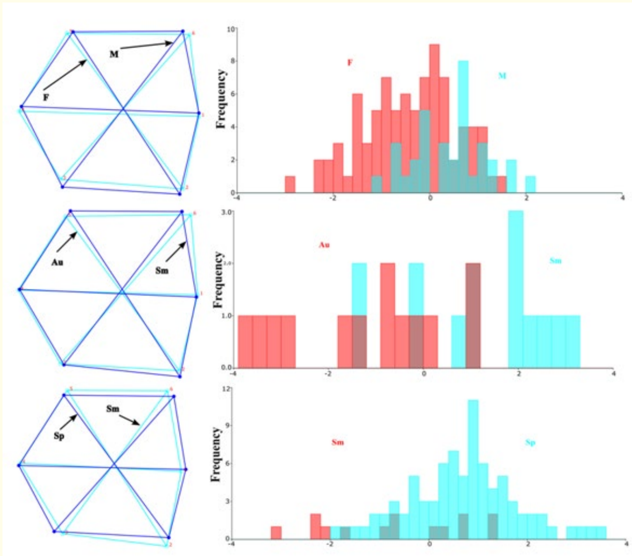

Figure 7: Shape differences and DFA histograms of scales by gender, season (F: female, M: male: Au: autumn, Sm: summer, Sp: spring).

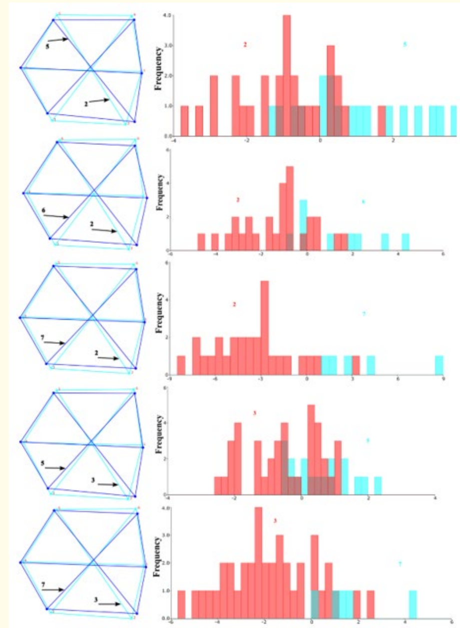

Figure 8: Shape differences and DFA histograms of scales by age, numbers represent ages.

2		Age				
		3	4	5	6	
3	T^2	8.4627				
	Param. P	0.4882				
	Perm. P (Proc./T^2)	0.6940/0.4950				
4	T^2	12.7682	13.4079			
	Param. P	0.2473	0.1920			
	Perm. P (Proc./T^2)	0.0830/0.2550	0.2340/0.1970			
5	T^2	21.8731	13.2473	6.7229		
	Param. P	0.0413	0.2026	0.7077		
	Perm. P (Proc./T^2)	0.0070/0.0430	0.0270/0.1930	0.4480/0.7160		
6	T^2	17.9728	10.5078	7.74010	4.2005	
	Param. P	0.1200	0.3755	0.6748	0.9186	
	Perm. P (Proc./T^2)	0.0360/0.1240	0.0680/0.3560	0.5250/0.6940	0.8370/0.9150	
7	T^2	30.5530	15.5623	11.9073	5.2067	5.8829
	Param. P	0.0188	0.1543	0.4504	0.8814	0.9330
	Perm. P (Proc./$T^{2)}$	0.0050/0.0110	0.0120/0.1660	0.1270/0.4460	0.4130/0.8930	0.4670/0.9380

Table 7: DFA results f of scales by age (T2: T-square, Param. p: Parametric P values, Perm. P: Permutation P value, Bolded: significant).

Discussion

Fish scales contain small growth rings that help determine the age of the fish. These rings, typically composed of $CaCO_3$, are arranged around a central point [40,41]. Variations in these rings occur due to periods of accelerated scale growth during times of abundant feeding, usually in spring and summer, and reduced or halted growth during periods of scarce feeding, particularly in winter [42]. The structure of annual growth rings in fish scales can be influenced by environmental conditions, making this differentiation important for understanding the physicochemical parameters of the environment and feeding patterns [18]. Changes in the shape of fish scales can thus help differentiate between populations [26,34]. Moreover, interspecific morphological variability can indicate genetic differences or reflect environmental conditions within the context of phenotypic plasticity [43,44].

Geometric morphometrics is particularly valuable in fish scale studies because it allows for a quantitative analysis of shape and size variation that traditional morphometrics cannot achieve [44,45]. This method provides a detailed understanding of shape and size variations and can address questions related to taxonomy and ecology. It enables the visualization and analysis of complex patterns of shape change, making it an essential tool for researchers studying fish scales [46,47].

[48] successfully applied geometric morphometric methods to *Capoeta trutta* and *Capoeta umbla*. Similarly, this study achieved success with *Carassius gibelio*. Analysis based on gender revealed that female specimens were larger than males, demonstrating the effectiveness of geometric morphometric analysis in distinguishing fish species.

Previous studies have also successfully utilized this type of analysis. For instance, research on fish scale and otolith morphometry and geometry has provided significant insights [18,28,33,34,48-53]. Additionally, studies examining the relationship between fish size and otolith morphometry have proven effective for species identification [18,50].

In this study, Procrustes ANOVA results revealed significant differences in size (CS) and shape among age and gender groups, with seasonal groups showing significant differences only in size. Specifically, scale size increased with age, with autumn (Au) scales being the largest and summer (Sm) scales the smallest. Female specimens were larger overall (Table 1, Figure 4).

PCA results showed that PC1 and PC2 explained 59.8% of the total variance for gender groups, with PC1 contributing 42.7% and PC2 17.1%. For age groups, PC1 and PC2 explained 57.7% of the total variance, with PC1 at 41.2% and PC2 at 16.5%. In the seasonal groups PCA, PC1 and PC2 accounted for 59.3% of the variance, with PC1 explaining 42.7% and PC2 16.6% (Figure 5).

CVA results indicated sufficient differences between genders (Table 2, Figure 6). For seasonal groups, significant differences were found between autumn-summer, summer-spring, and spring-winter pairs (Table 4, Figure 6). In age groups, differences were significant between the 2-5, 2-6, 2-7, 3-5, and 3-7 age groups, but not between other age groups (Table 3, Figure 6).

DF analysis showed sufficient shape differences between genders (Table 5, Figure 7), age groups 2-5, 2-6, 2-7, 3-5, and 3-7 (Table 6, Figure 8), and seasonal groups autumn-summer and spring-summer (Table 6, Figure 7). Specifically, female scales were larger dorso-ventrally, with differences being significant according to both parametric and permutation p-values for T2.

Conclusion

Fish scales are an important structure that contains important information about the biology and ecology of the fish and is important in revealing the systematics of the taxon to which it belongs. Analyzing this structure with methods such as geometric morphometrics allows us to reach many new and accurate information. As in this study, fish scales were analyzed by geometric morphometric method and provided us with important information about the scale shape and size of *Carassius gibelio* in sex, age and season groups. Thus we have the chance to obtain new information about the species with no or minimal damage to that species.

Conflict of Interests

No conflicting interests and no funding in connection with this paper are applicable.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Bibliography

1. Nelson JS. "Fishes of the World. 4th edition. Hoboken, NY, USA: John Wiley and Sons, Inc. (2006).
2. Kuru M., *et al*. "Fish Biodiversity in Inland Waters of Turkey". *Journal of Academic Documents for Fisheries and Aquaculture* 1.3 (2014): 93-120.
3. Çiçek T., *et al*. "Size and shape analysis of two close Cyprinidae species (*Garra variabilis- Garra rufa*) by geometric morphometric methods". *Survey in Fish Research* 2.2 (2016): 35-44.
4. Beckman WC. "The Freshwater Fishes of Syria and their General Biology and Management, First Edition". FAO Fish. Bio. Tec., Roma, Italy (1962).
5. Coad BW. "Zoogeography of the fishes of the Tigris-Euphrates basin". *Zoology in the Middle East* 13 (1996): 71-83.
6. Karaman M. "Süβwasserfische der Türkei. 8.Teil. Revision der Barben Europas, Vorderasiens und Nordafrikas". *Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut*e 67 (1971): 175-254.
7. Kuru M. "The fresh water fish of South-Eastern Turkey-2 (Euphrates-Tigris Sisteme)". *Hac Bull Nat Sci Eng* 7-8 (1979): 105-114.
8. Balon EK. "Origin and domestication of the wild carp, *Cyprinus carpio*-from Roman gourmets to the swimming flowers". *Aquaculture* 129 (1995): 3-48.
9. Tarkan AS., *et al*. "Are introduced gibel carp *Carassius gibelio* in Turkey more invasive in artificial than in natural waters?" *Fisheries Management and Ecology* 19 (2012): 178-187.
10. Liasko R., *et al*. "Influence of environmental parameters on growth pattern and population structure of *Carassius auratus gibelio* in eastern Ukraine". *Hydrobiologia* 658 (2011): 317-328.
11. Ruppert JLW., *et al*. "Native freshwater species get out of the way: Prussian carp (*Carassius gibelio*) impacts both fish and benthic invertebrate communities in North America". *Royal Society Open Science* 4 (2017): 170400.
12. Fagernes CE., *et al*. "Extreme anoxia tolerance in crucian carp and goldfish through neofunctionalization of duplicated genes creating a new ethanol-producing pyruvate decarboxylase pathway". *Scientific Reports* 7 (2017): 7884.
13. Froese R and Pauly D. "FishBase". World wide web electronic publication. (2021).
14. Vetemaa M., *et al*. "Distribution, gender ratio and growth of *Carassius gibelio* (Bloch) in coastal and inland waters of Estonia (North-Eastern Baltic Sea)". *Journal of Applied Ichthyology* 21 (2005): 287-291.
15. Tichopád T., *et al*. "Spermatozoa morphology and reproductive potential in F1 hybrids of common carp (*Cyprinus carpio*) and gibel carp (*Carassius gibelio*)". *Aquaculture* 521 (2020): 735092.
16. Yalçın Özdilek Ş., *et al*. "An invasive species, *Carassius gibelio*, alters the native fish community through trophic niche competition". *Aquatic Sciences* 81 (2019): 29.
17. Sakai H., *et al*. "Morphological and mtDNA sequence studies on three crucian carps (Carassius: *Cyprinidae*) including a new stock from the Ob River system, Kazakhstan". *Journal of Fish Biology* 74 (2009): 1756-1773.
18. Staszny A., *et al*. "Scale morphometry study to discriminate Gibel Carp (*Carassius gibelio*) populations in the balaton-catchment (Hungary)". *Acta Zoologica Academiae Scientiarum Hungaricae* 58 (2012): 19-27.
19. Dürrani Ö., *et al*. "Morphological variations of an invasive cyprinid fish (*Carassius gibelio*) in lentic and lotic environments inferred from the body, otolith, and scale shapes". *Acta Zoologica* 104.3 (2023): 458-472.
20. Miranda R and Escala M. "Morphological and biometric comparison of the scales of the barbels (*Barbus Cuvier*) of Spain". *Journal of Morphology* 245.3 (2000): 196-205.
21. Poulet N., *et al*. "Does fish scale morphology allow the identification of populations at a local scale? A case study for rostrum dace Leuciscus leuciscus burdigalensis in River Viaur (SW France)". *Aquatic Sciences* 67.1 (2005): 122-127.
22. Jawad LA. "Comparative morphology of scales of four teleost fishes from Sudan and Yemen". *Journal of Natural History* 39.28 (2005): 2643-2660.
23. Esmaeili HR., *et al*. "Scale structure of a cyprinid fish, *Capoeta damascina* (Valenciennes in Cuvier and Valenciennes, 1842) using scanning electron microscope (SEM)". *Iranian Journal of Science and Technology, Transaction A, Science* 31 (2007): 255-262.

24. Jawad L Al and Jufaili SM. "Scale morphology of greater lizardfish *Saurida tumbil* (Bloch, 1795) (Pisces: Synodontidae)". *Journal of Fish Biology* 70.4 (2007): 1185-1212.

25. Esmaeili HR and Gholami Z. "Scanning Electron Microscopy of the scale morphology in Cyprinid fish, *Rutilus frisii kutum* Kamenskii, 1901 (Actinopterygii: Cyprinidae)". *Iranian Journal of Fish Res* 10.1 (2011): 155-166.

26. Ibanez AL., *et al*. "Geometric morphometric analysis of fish scales for identifying genera, species, and local populations within the *Mugilidae"*. *Canadian Journal of Fisheries and Aquatic Sciences* 64 (2007): 1091-1100.

27. Ibáñez AL., *et al*. "Morphometric variation of fish scales among some species of the family *Lutjanidae* from Iranian waters". *Cahiers de Biologie Marine* 57 (2016): 289-295.

28. Bilici S. "A Distinction of some cyprinid species from Tigris River basin according to scales by geometric morphometric methods". Harran Üniversitesi Veteriner Fakültesi Dergisi 9.2 (2020): 148-153.

29. Oosten J. "The skin and scales". *The Physiology of Fishes* (1957): 207-244.

30. De Lamater ED and Courtanay WR. "Studies on scale structure of flatfishes. I. The genus Trinectes, with notes on related forms. Proceedings of the 27th Annual conference of the Southeast Association". *Game and Fish Communication* (1973): 592-608.

31. Şerban C and Grigoraş G. "Structural and morphometric study of scales in Petzea rudd (*Scardinuz racovitzai* MÜLLER 1958)". *Applied Ecology and Environmental Research* 16.5 (2018): 6063-6076.

32. Zelditsch ML., *et al*. "Geometric morphometrics for biologists: A primer. San-Diego etc.: Elsevier Academic (2004): 443.

33. Wichard T., *et al*. "Survey of the Chemical Defence Potential of Diatoms: Screening of Fifty Species for α, β, γ, δ- unsaturated aldehydes". *Journal of Chemical Ecology* 31 (2005): 949-958.

34. Ibáñez AL., *et al*. "Variation in elasmoid fish scale patterns is informative with regard to taxon and swimming mode". *Zoological Journal of the Linnean Society* 155.4 (2009): 834-844.

35. Ibáñez AL., *et al*. "Does compensatory growth modify fish scale shape?" *Environmental Biology of Fishes* 94.2 (2012): 477-482.

36. Avigliano E., *et al*. "Otolith elemental fingerprint and scale and otolith morphometry in Prochilodus lineatus provide identification of natal nurseries". *Fisheries Research* 186.1 (2017): 1-10.

37. Rohlf FJ. "The tps series of software". *Hystrix, the Italian Journal of Mammalogy* 26.1 (2015): 9-12.

38. Klingenberg CP. "MorphoJ: an integrated software pack- age for geometric morphometrics". *Molecular Ecology Resources* 11.2 (2011): 353-357.

39. R Core. "R: A language and environment for statistical computing". Vienna, Austria: R Foundation for Statistical Computing (2019): 20.

40. Carbonara P and Follesa MC. "Handbook on fish age determination: a Mediterranean experience. General Fisheries Commission for the Mediterranean". *Studies and Reviews* (2019): 98.

41. Chen X., *et al*. "In Age and Growth of Fish. Biology of Fishery Resources". Springer, Singapore (2022): 71-111.

42. Gümüş A., *et al*. "Relative importance of food items in feeding of *Chondrostoma regium* Heckel, 1843, and its relation with the time of annulus formation". *Turkish Journal of Zoology* 26.3 (2002): 271-278.

43. Staszny Á., *et al*. "Impact of environmental and genetic factors on the scale shape of zebrafish, *Danio rerio* (Hamilton 1822): a geometric morphometric study". *Acta Biologica Hungarica* 64 (2013): 462-475.

44. Carro SCS., *et al*. "Shape does matter: A geometric morphometric approach to shape variation in Indo-Pacific fish vertebrae for habitat identification". *Journal of Archaeological Science* 99 (2018): 124-134.

45. Moreira C., *et al*. "Landmark-based geometric morphometrics analysis of body shape variation among populations of the blue jack mackerel, Trachurus picturatus, from the North-East". *Journal of Sea Research* 163 (2020): 101926.

46. Ibáñez AL and Jawad LA. "Morphometric variation of fish scales among some species of rattail fish from New Zealand waters". *Journal of the Marine Biological Association of the United Kingdom* 98.8 (2018): 1991-1998.

47. Ibáñez AL., *et al.* The morphometry of fish scales collected from New Zealand and Turkey". *New Zealand Journal of Zoology* 50.2 (2023): 318-328.

48. Çiçek T., *et al*. “Discrimination of *Capoeta trutta* (Heckel, 1843) and *Capoeta umbla* (Heckel, 1843) from scales by Geometric Morphometric Methods”. *Journal of Survey in Fisheries Sciences* 4.1 (2017): 8-17.

49. Richards RA and Esteves C. “Use of scale morphology for discriminating wild stocks of Atlantic striped bass”. *Transactions of the American Fisheries Society* 126.6 (1997): 919-925.

50. Teimori A. “Scanning electron microscopy of scale and body morphology as taxonomic characteristics of two closely related cyprinid species of genus *Capoeta valenciennes*, 1842 in southern Iran”. *Current Science* 111.7 (2016): 1214-1219.

51. Dörtbudak MY and Özcan G. “Relationship of Otolith Size to Standard Length of the Tigris Bream (*Acanthobrama marmid* (Heckel. 1843)) in Tigris River. Sırnak. Proceedings of International Marine and Freshwater Sciences Symposium; 2018 Oct 18-21; Kemer-Antalya, Turkey (2018): 139-143.

52. Dörtbudak MY., *et al*. “Geometric analysis of otoliths in *Cyprinion kais* and *Cyprinion macrostomus”*. *Anatomia, Histologia, Embryologia* 51.6 (2022): 696-702.

53. Bilici S., *et al*. “Variation in the shape and size of the scale of the Tigris bream (*Acanthobrama marmid*, Heckel, 1843) from the Tigris River, Türkiye attributed to Seasonality, Age and Sex: A geometric morphometric study”. *Revista Cientifica de la Facultade de Veterinaria* (2024): rcfcv-e34366.