ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University

Research Article /Araştırma Makalesi
22(1), 7-12, 2025
DOI: 10.32707/ercivet.1540573

Shape Analysis of Otoliths of *Capoeta trutta* (Heckel, 1843) and *Capoeta umbla* (Heckel, 1843) Species by Geometric Morphometry

Muhammed Yaşar DÖRTBUDAK[1,a], Yasin DEMİRASLAN[2,b], İsmail DEMİRCİOĞLU[3,c], Funda AKSÜNGER KARAAVCI[4,d]

[1]Harran University, Faculty of Veterinary Medicine, Department of Fisheries and Diseases, Şanlıurfa-TÜRKIYE
[2]Dokuz Eylül University, Faculty of Veterinary Medicine, Department of Anatomy, İzmir-TÜRKIYE
[3]Harran University, Faculty of Veterinary Medicine, Department of Anatomy, Şanlıurfa-TÜRKIYE
[4]Bingöl University, Faculty of Veterinary Medicine, Department of Anatomy, Bingöl-TÜRKIYE
ORCID No: [a]ORCID: 0000-0001-7966-5678, [b]ORCID: 0000-0003-3612-6142, [c]ORCID: 0000-0002-0724-3019, [d]ORCID: 0000-0001-8311-1723

Corresponding author: İsmail DEMİRCİOĞLU, E-mail: idemircioglu@harran.edu.tr
How to cite: Dörtbudak MY, Demiraslan Y, Demircioğlu İ, Aksünger Karaavcı F. Shape analysis of otoliths of *Capoeta trutta* (Heckel, 1843) and *Capoeta umbla* (Heckel, 1843) species by geometric morphometry. Erciyes Univ Vet Fak Derg 2025; 22(1):7-12

Abstract: *Capoeta (C.) trutta* (Heckel, 1843) and *Capoeta umbla* (Heckel, 1843) are the most common species living in the Tigris and Euphrates rivers. This study aimed analyze the shape of the otoliths of *C. trutta* and *C. umbla* fish from the Capoeta genus, which inhabit the Tigris River in Turkey. The geometric morphometric method was used to identify and compare the similarities and differences between these fish species. A total of 72 right otoliths, (for each species 36), were used. Geometric morphometric analyses were performed by marking semi-landmark and landmarklar on the otoliths. When the data were examined, it was determined that the first principal component accounted for 25.15% of the total shape difference, while the first four principal components (PC1+PC2+PC3+PC4) accounted for 59.14% of the total shape difference. According to the regression analysis of shape over size, 9.2615% of shape can be predicted in terms of size by groups and it was determined as 26.0817% of shape according to PC1 (P: 0.0001), 7.1205% according to PC2 (P: 0.0233) and 10.2255 (P: 0.0051) according to PC3 can be predicted. According to the cross-validation score, 7 (19.44%) samples from *C. trutta* and 4 (11.11%) samples from *C. umbla* were involved in the opposite group, and these results showed that the two groups had a successful grouping (at least 80%) according to the shape. Consequently, it is thought that the data of the present study can be reference data for many disciplines, especially the taxonomic classification of the species in question.
Keywords: Capoeta, geometric morphometry, otolith, semilandmark, shape analysis

Capoeta trutta (Heckel, 1843) ve *Capoeta umbla* (Heckel, 1843) Türlerinin Otolitlerinin Geometrik Morfometri ile Şekil

Öz: *Capoeta (C.) trutta* (Heckel, 1843) ve *Capoeta umbla* (Heckel, 1843) Dicle ve Fırat nehirlerinde yaşayan en yaygın türlerdir. Bu çalışmada, Türkiye'de Dicle Nehri'nde yaşayan Capoeta cinsine ait *C. trutta* ve *C. umbla* balıklarının otolitlerinin geometrik morfometrik yöntem kullanılarak şekil analizlerinin yapılması ve aralarındaki benzerlik ve farklılıkların ortaya konulması amaçlanmıştır. Her biri 36 adet olmak üzere toplam 72 adet sağ otolit kullanılmıştır. Geometrik morfometrik analizler otolitlerin üzerine semilandmarklar ve landmarklar konularak gerçekleştirilmiştir. Veriler incelendiğinde, ilk temel bileşen toplam şekil farkının %25.15'ini oluştururken, ilk dört temel bileşenin (PC1+PC2+PC3+PC4) toplam şekil farkının %59.144'ünü oluşturduğu belirlenmiştir. Boyut üzerinden şeklin regresyon analizine göre, gruplar tarafından boyut açısından şeklin %9.2615'inin tahmin edilebildiği ve PC1'e göre şeklin % 26.0817'sinin (P: 0.0001), PC2'ye göre %7.1205'inin (P: 0.0233) ve PC3'e göre 10.2255'inin (P: 0.0051) tahmin edilebildiği belirlenmiştir. Çapraz doğrulama skoruna göre *C. trutta'dan* 7 (%19.44) ve *C. umbla'dan* 4 (%11.11) örnek zıt grupta yer almış ve bu sonuçlar iki grubun şekle göre başarılı bir gruplama (en az %80) yaptığını göstermiştir. Sonuç olarak, mevcut çalışmanın verilerinin söz konusu türlerin taksonomik sınıflandırması başta olmak üzere birçok disiplin için referans veri olabileceği düşünülmektedir.
Anahtar kelimeler: Capoeta, geometrik morfometri, otolit, semilandmark, şekil analizi

Introduction

There are *C. trutta* and *C. umbla* species belonging to the genus Capoeta in the Tigris and Euphrates river systems. These species are among the most common and dominant fish of the river system (Kuru, 1975; Ünlü, 1991). It is inevitable for species with a wide distribution to show some genetic variations due to the different ecological conditions in which they live in river systems. Morphometric variation is used to distinguish similar fish species and to identify hybrids (Carlson et al., 1985). For this reason, morphometric

Geliş Tarihi/Submission Date : 29.08.2024
Kabul Tarihi/Accepted Date : 06.12.2024

and meristic variations of *C. trutta* (Heckel, 1843) and *C. umbla* species (Heckel, 1843), which are widely distributed in the Tigris and Euphrates river basins, were attempted to be determined by discriminant analysis (Çiçek, 2009).

Diagnostic characteristics of *C. trutta* are D.III-IV/8, A.III/5, L.lat: 71-85 with an average of 76.5. Its body is high and laterally compressed. Its mouth is ventral and has 1 pair of barbels. Pharyngeal dental formula is 4.3.2-2.3.4. In terms of color, the dorsal contains black spots against an off-white backdrop, whereas the ventral is white. The longest unbranched ray on the dorsal fin is greater than the head length (Dağlı and Erdemli, 2011).

Diagnostic characteristics of *C. umbla* are D.III-IV/ (8) 9(10), A.III/5, L. Lat: 72-93 with an average of 80.8. Its body is elongate and cylindrical. Its mouth is ventral and has a pair of short barbels. Pharynx dental formula is 4.3.2-2.3.4. The lips are covered with a horny skin and the upper lip is well developed. The color is dark brown or gray on dorsal and muddy yellow on lateral and ventral. The last ossified simple ray of the dorsal fin is serrated with thin and flexible distal (Dağlı and Erdemli, 2011).

One of the preferred bony formations for the age determination of Osteichthyes is otoliths. There are three types of otoliths ("sagitta", "lapillus" and "asteriscus") in the semicircular canals on the right and left sides of the head, which act both as a balance organ and hearing ad in fish. The location, structure, size and shape of these three types of otoliths are different (Kontaş et al., 2020). They are usually ranked by size as sagitta, lapillus, and asteriscus. Sagittas are most preferred for age determination (Atilgan et al., 2010). Otoliths are widely used in fish biology. In addition, otolith morphometry is employed to obtain information about fish length, growth and ecomorphology, population identification, age estimation, determination of growth rates, the field of paleontology, and changes in the habitat conditions (Volpedo and Fuchs, 2010; Ilkyaz et al., 2011; Jawad, 2018). Otoliths are also used to estimate size and identity of the prey due to interspecies differences in shape and resistance to digestion (Battaglia et al., 2010).

The aim of the study is to reveal the similarities and differences of *C.trutta* and *C. umbla* and the changes depending on the shape according to the effect of species by conducting the shape analysis of these species' otoliths with geometric morphometry.

Material and Method

Ethical Statement Samples

The materials utilized in this study were obtained as waste products from fishermen engaged in fishing activities in the Tigris River located in Şırnak province. The acquisition of these samples was conducted in compliance with the guidelines outlined in the Regulation on the Working Procedures and Principles of Animal Experimentation Ethics Committees, as published in the Official Gazette. This regulation explicitly states that the use of deceased animals, tissues, slaughterhouse materials, and waste fetuses does not require permission from the HADYEK.

Samples

Fish samples were taken from the Tigris River (Şırnak/Turkey), and their sagittal otoliths were removed, cleaned, dried, and stored in labeled boxes. Otoliths were photographed using stero-microscope (Olympus SZ61TR+Olympus LC20). A total of 72 right otoliths, (for each species 36), were used.

Imaging and Digitization

The right otoliths were zoomed in and photographed. Photographing was performed at 500 µm. The JPG photos were saved to the computer. A total of 80 markings, consisting of 2 homologous landmarks (rostrum and antirostrum) and 78 semilandmarks, were made using TpsUtil (Version 1.79)[12] and TpsDig2 (Version 2.31) (Rohlf, 2019) software. Thus, the x and y cartesian coordinates of the points representing the general shape of the otolith were determined. Before statistical analysis, a confirmation test was performed for landmarks in TpsSmall software (Version 1.34) (Rohlf, 2018). As a result of this test, uncentered correlation and root mean square error values were found to be 0.999999 and 0.000075, respectively. These outcomes proved that the marked semilandmarks were accurate.

Statistical Analysis

General Procrustes Analysis was performed. Since there were differences in direction, position and size in the photographs (Slice, 2007). The new coordinates from the Procrustes analysis were subjected to principal component analysis. In addition, in areas where shape differences are concentrated, allometry and grouping features were determined by the Discriminant Function Analysis (DFA). MorphoJ (Klingenberg, 2011) software was used for all these analyses.

Results

Based on the principal component analysis, the two fish species that were part of the study had 71 components total. Table 1 displays the results from this research. The first principal component (PC1) accounted for 25.150% of the total shape variation, while the first four principal components accounted for 59.144% of the total shape variation. A significant difference was observed among principal components after PC4 and PC5.

Table 1. Results of the principal component analysis, PC: principal component

PC	Eigenvalue	% Variance	PC	Eigenvalue	% Variance	PC	Eigenvalue	% Variance
1	0.003119	20.15	**25**	8.96E-05	0.579	**49**	1.96E-05	0.127
2	0.002257	14.58	**26**	8.42E-05	0.544	**50**	1.91E-05	0.124
3	0.00204	13.178	**27**	7.97E-05	0.515	**51**	1.87E-05	0.121
4	0.001739	11.236	**28**	7.49E-05	0.484	**52**	1.8E-05	0.116
5	0.000756	4.881	**29**	7.08E-05	0.457	**53**	1.61E-05	0.104
6	0.000539	3.483	**30**	6.59E-05	0.426	**54**	1.48E-05	0.095
7	0.000489	3.161	**31**	5.99E-05	0.387	**55**	1.32E-05	0.086
8	0.000402	2.598	**32**	5.8E-05	0.374	**56**	1.2E-05	0.078
9	0.000312	2.019	**33**	5.61E-05	0.362	**57**	1.16E-05	0.075
10	0.000285	1.843	**34**	5.13E-05	0.331	**58**	1.08E-05	0.07
11	0.000239	1.545	**35**	4.95E-05	0.32	**59**	1.05E-05	0.068
12	0.000211	1.364	**36**	4.58E-05	0.296	**60**	9.65E-06	0.062
13	0.000206	1.331	**37**	4.1E-05	0.265	**61**	9.1E-06	0.059
14	0.000192	1.243	**38**	3.99E-05	0.258	**62**	7.67E-06	0.05
15	0.000161	1.039	**39**	3.81E-05	0.246	**63**	6.91E-06	0.045
16	0.000153	0.986	**40**	3.64E-05	0.235	**64**	6.79E-06	0.044
17	0.00015	0.968	**41**	3.47E-05	0.224	**65**	5.83E-06	0.038
18	0.000141	0.913	**42**	3.27E-05	0.211	**66**	4.97E-06	0.032
19	0.000131	0.848	**43**	3.11E-05	0.201	**67**	4.34E-06	0.028
20	0.000127	0.817	**44**	2.97E-05	0.192	**68**	4.27E-06	0.028
21	0.000117	0.756	**45**	2.65E-05	0.171	**69**	3.35E-06	0.022
22	0.000112	0.725	**46**	2.47E-05	0.159	**70**	3.07E-06	0.02
23	0.000104	0.671	**47**	2.32E-05	0.15	**71**	2.53E-06	0.016
24	9.69E-05	0.626	**48**	2.28E-05	0.147			

The distribution of the samples according to PC1 and PC2 is shown in the graph in Figure 1. Accordingly, *C. trutta* samples were more distinctly separated from *C. umbla* samples (C. trutta: 55%, C. umbla: 27%)

As a result of the regression analysis of the shape (PCs) on the centroid size, it was determined that 9.2615% of the shape could be predicted in terms of size (P: 0.0001 at confidence interval of 95%). 26.0817% (P: 0.0001) of the shape according to PC1, 7.1205% (P: 0.0233) according to PC2, and 10.2255% (P: 0.0051) according to PC3 were predictable in terms of size. Figure 2 shows the graphs showing at which landmark level the shape differences were concentrated according to PC1 and PC2 . Therefore, the variations in shape, as indicated by PC1 and PC2 (Figure 2), were clearly observed in the dorsal edge and antirostrum.

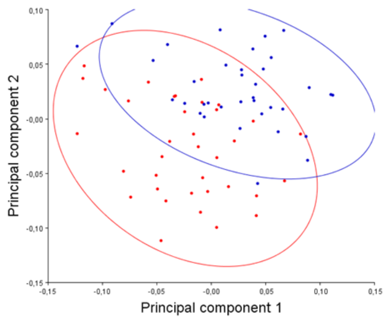

Figure 1. Graphical distribution of the samples according to PC1 and PC2

As a result of discriminant function analysis, Mahalanobis and Procrustes distances were determined as 5.8335 and 0.07999546 (P: 0.0001), respectively. Table 2 shows the grouping characteristics obtained as a result of this analysis. According to the cross-validation score, 7 (19.44%) samples from *C. trutta* and 4 (11.11%) samples from *C. umbla* were included in the opposite group. These results showed that the two groups had a successful grouping (at least 80%) according to the shape.

Figure 3 show that the variations in shape based on the groups found in the otolith wire-frame warp graph. Accordingly, it was observed that *C. umbla* was wider than *C. trutta* at the level of its dorsal and ventral edges. The rostrum of *C. trutta* was larger and sharp pointed. Additionally, the *C. trutta* had a more indented area at the dorsoventral intersection point (symmetrical of the rostrum).

Erciyes Üniv Vet Fak Derg 2025; 22(1): 7-12

Table 2. Grouping characteristics of the samples according to discriminant function analysis

Discriminant Function			
Group	***C. trutta***	***C. umbla***	**Total**
C. trutta	36	0	36
C. umbla	0	36	36
Cross-Validation			
Group	***C. trutta***	***C. umbla***	**Total**
C. trutta	29	7	36
C. umbla	4	32	36

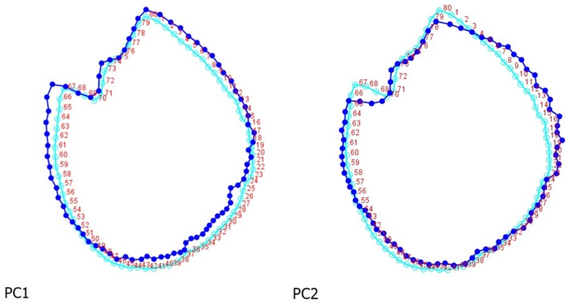

Figure 2. Wireframe graphical representation of shape differences according to PC1 and PC2. Blue represents the average shape relative to the principal component.

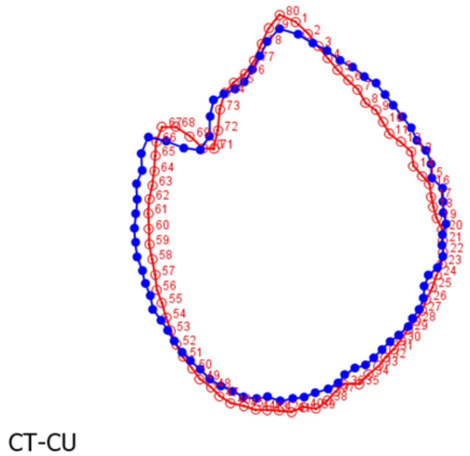

Figure 3. Wire-frame warp graph of otolith shape according to discriminant function analysis. Red: *C. trutta*, Blue: *C. umbla. (CT: C. trutta, CU: C. umbla)*

Discussion and Conclusion

The morphology of otoliths is important because it provides a plethora of information that may be used to infer the systematics and evolution of fish, as well as information about the age and general biology of fish (Samsun and Samsun, 2006; Avşar et al., 2007). Traditional morphometric method enable us to make measurements such as length, depth, and width obtained by distance, angles and ratios between points, but it cannot reveal complete data in terms of the shapes of the objects examined. The morphometric geometry method enables the shapes of objects to be revealed fully through analysis (Zelditch et al., 2012; Aytek; 2017). In addition, it has been reported that the morphometric geometry method is effective in revealing the similarities and differences of taxonomically close species (Ibanez et al., 2012; Teimori; 2016). In the study, the similarities and differences of the *C. umbla* and *C. trutta* were revealed by determining the shape analyses of the otoliths of the two species living in the same zones, using the morphometric geometry method.

In the study, it was determined that PC1 accounted for 25.15% of the total shape variations and 59.144% of the first four principal components (PC1+PC2+PC3+PC4) in analysis of 71 principal components. A significant difference was observed among the principal components after PC4 and PC5.

Dörtbudak et al. (2022) in their study on *C. kais* and *C. macrostomus* otoliths, reported that PC1 accounted for 26.871% of the total shape variation, while the first three principal components (PC1+PC2+PC3) accounted for 52.235%, and a significant difference was observed among the principal components after PC5 and PC6.

In their shape analysis of 6 different fish species from the family Sebastes, Afanasyev et al. (2017), reported that the PC1 of the samples accounted for the total shape variation as 48%.

Çiçek et al. (2017), reported that the sum of the first two principal components (PC1+PC2) was 52.2% in their morphometric geometry study on fish scales of *C. umbla* and *C. trutta*. These results revealed that the two fish species analyzed in terms of otolith shape in the present study are distinguished from each other at rates similar to the literature.

Dörtbudak et al. (2022), as a result of the regression analysis of the shape (PCs) over the centroid size, reported that 2.1020% of the shape can be predicted in terms of size, according to the groups, and thus in the comparison of the otolith morphology of the individuals in terms of groups, the shape variations were not dependent on the size, therefore, there was no allometric component. In the study, regression analysis revealed that 9.2615% of the shape could be predicted in terms of size (centroid size) according to the groups. The predictability of the shape in terms of size was 26.0817% according to PC1, 7.1205% according to PC2, and 10.2255% according to PC3. These results showed that the predictability of the otolith shapes belonging to which breed was higher in the study.

In the study, it was determined that the shape differences of the otoliths in terms of PC1 and PC2 became evident in the dorsal edge and anterostrum. In the shape analysis of the otoliths of the *C. kais* and *C. macrostomus* (Dörtbudak et al., 2022) species using the morphometric geometry method, it was reported that the shape differences in terms of PC1 became evident at the level of the entire dorsal edge, rostrum, antirostrum and the middle of the ventral edge. These differences are thought to be important in species taxonomy and identification.

There are different anatomical studies determining the similarities and differences of *C. umbla* and *C. trutta* species (Çiçek, 2009; Dağlı and Erdemli, 2011). Dörtbudak et al. (2022) reported that the two groups had a successful grouping (at least 75%) according to the shape, and it was determined in their study that they showed a successful grouping of 80% on the two species in question. Findings of the present study showed that *C. umbla* and *C. trutta* species were highly similar to each other. Although they inhabit the same zone, the dams built on the rivers they live in divide these regions into sub-living areas, so even if they are in the same genus, differences may occur after a while.

Consequently, the similarities and differences between *C. umbla* and *C. trutta* were revealed by making the shape analysis of the otoliths of the two species living in different zones of the same habitat. It is thought that as a result of the determination of shape-related variations according to the effect of breed, the present study presents data that would contribute to the taxonomic classification of these two species and the fauna of the Euphrates and Tigris Rivers, which are an important source of biodiversity for the Mesopotamian region.

References

Afanasyev PK, Orlov AM, Rolsky AY. Otolith shape analysis as a tool for species identification and studying the population structure of different fish species. Biol Bull 2017; 44(8): 952-9.

Atilgan E, Erbay M, Aydin İ. Doğu Karadeniz'deki ekonomik bazı otolit özellikleri. Aquac Stud 2010; 1: 12-5.

Avşar D, Çiçek E, Yeldan H, Manaşirli M, Mavruk S. İskenderun ve Mersin körfezlerindeki Centracentidae familyasına ait (kemikli balık) bazı türlerin otolit morfolojileri. Türk Sucul Yaşam Dergisi 2007; 5: 116 - 23.

Aytek Aİ. Geometrik morfometri. MASROP E-Dergi 2017; 11(17): 1-7.

Battaglia P, Malara D, Romeo T, Andaloro F. Relationships between otolith size and fish size in some mesopelagic and bathypelagic species from the Mediterranean Sea (Strait of Messina, Italy). Sci Mar 2010; 74: 605-12.

Carlson DM, Pflieger WL, Trial L, Haverland PS. Distribution, biology and hybridization of *Scaphirhynchus albus* and *S. platorynchus* in the Missouri and Mississippi rivers. Environ Biol Fishes 1985; 14(1): 51-9.

Çiçek T. Dicle ve Fırat Su Sistemlerinde Yaşayan Cyprinidae Familyasına Ait Bazı Türlerde Görülen Morfometrik ve Meristrik Varyasyonların Incelenmesi. Yüksek Lisans Tezi, Dicle Üniv Fen Bil Ens, Diyarbakır 2009.

Çiçek T, Kaya A, Bilici S, Dörtbudak MY. Discrimination of *Capoeta trutta* (Heckel, 1843) and *Capoeta umbla* (Heckel, 1843) from scales by geometric morphometric methods. J Surv Fish Sci 2017; 4(1): 8-17.

Dağlı M, Erdemli AÜ. *Capoeta umbla* (Heckel, 1843) ve *Capoeta trutta* (Heckel, 1843)'nın bazı meristik ve morfometrik özelliklerinin karşılaştırılması. Karadeniz Fen Bilimleri Dergisi 2011; 2(3): 46-56.

Dörtbudak MY, Demiraslan Y, Demircioğlu İ. Geometric analysis of otoliths in *Cyprinion kais* and *Cyprinion macrostomus*. Anat Histol Embryol 2022; 51 (6): 1-7.

Ibanez AL, Pacheco-Almanzar E, Cowx IG. Does compensatory growth modify fish scale shape? Environ Biol Fishes 2012; 94(2): 477-82.

İlkyaz AT, Metin G, Kinacigil HT. The use of otolith length and weight measurements in age estimations of three Gobiidae species (*Deltentosteus quadrimaculatus, Gobius niger,* and *Lesueurigobius frie-*

sii). Turk J Zool 2011; 35(6): 819-27.

Jawad LA. A comparative morphological investigation of otoliths of six parrotfish species (Scaridae) from the Solomon Islands. J Fish Biol 2018; 93: 1046–58.

Klingenberg CP. MorphoJ: an integrated software package for geometric morphometrics. Mol Ecol Resour 2011; 11(2): 353-7.

Kontaş S, Yedier S, Bostancı D. Otolith and scale morphology of endemic fish *Cyprinion macrostomum* in Tigris–Euphrates Basin. Mol Ecol Resour 2020; 60(4): 562-9.

Kuru M. Dicle-Fırat, Kura-Aras, Van Gölü ve Karadeniz Havzası Tatlı Sularında Yaşayan Balıkların (Pisces) Sistematik ve Zoocoğrafik Yönden İncelenmesi. Doktora Tezi, Atatürk Üniv Fen Bil Ens, Erzurum 1975.

Rohlf FJ. TpsDig Version 2.31. Ecology & Evolution, SUNY at Stone Brook, USA, 2018. http://life.bio.sunysb.edu/morph/index.html

Rohlf FJ. TpsUtil program Version 1.79. Ecology & Evolution, SUNY at Stone Brook, USA, 2019. http://life.bio.sunysb.edu/morph/index.html

Samsun N, Samsun S. Kalkan (*Scophthalmus maeoticus* (Pallas, 1811)) balığının otolit yapısı, yaş ve balık uzunluğu-otolit uzunluğu ilişkilerinin belirlenmesi. FÜMBD 2006; 18(2): 181-7.

Slice DE. Geometric morphometrics. Annu Rev Anthropol 2007; 36: 261-81.

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. Curr Sci 2016; 111: 1214-9.

Ünlü E. Dicle Nehrinde yaşayan *Capoeta trutta* (Heckel, 1843)'nın biyolojik özellikleri üzerinde çalışmalar. Doğa-Turk J Zool 1991; 15: 22-38.

Volpedo AV, Fuchs DV. Ecomorphological patterns of the lapilli of *Paranoplatense Siluriforms* (South America). Fish Res 2010; 102: 160-5.

Zelditch ML, Swiderski DL, Sheets HD. Geometric Morphometrics for Biologists: A Primer. Sandiego, USA: Academic Press- Elsiver, 2012.