# Investigation of size and shape differences depend to sex, age and season on scales of smallmouth lotak (*Cyprinion kais)*

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**Abstract**

In this study, a total of 82 (49♀♀, 33♂♂) *Cyprinion kais* samples from the same location in the river of Tigris were collected and ages of scales taken from front and upper section of lin lateral of dorsal fins of fishes were determined and photographed by an Olympus digital camera with Canon SX7 model binocular under the same conditions and then six landmarks were taken by using tpsDig ver. 2.32 software. Afterwards, procrustes analysis was conducted. After separating shapes and sizes, ANOVA, PCA, CVA/MANOVA and DFA analyses were performed. According to the results of the analyses, there was a significant difference between the samples in terms of gender and size (Size ANOVA: F= 50.6, $p$<.0001); at the same time the difference between samples in terms of shape was at the level of (Shape ANOVA: F= 3.92, p =0.0002); there was a significant difference between samples at different ages in terms of size (Size ANOVA: F= 44.08, $p$<.0001), however, no difference was found in terms of the shape of the samples; there was a significant difference between size (Size ANOVA: F= 17.87, $p$<.0001) of the samples but no difference was found between shape of them depending on the season. For gender, age and seasons, the first two components explain 61.4%, 63.6% and 63.4% of total variance, respectively. As a result, there was a difference between sizes of the *C. kais* species depending on gender, age of the fishes and the season; however, although there was a difference between shapes of the samples depending on gender variable, no difference was found depending on age of the fish and the season.

**Keywords:** Smallmouth lotak **(***Cyprinidae, kais*), Scale, 2D Landmark, Geometric Morfometri.

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## Introduction

Cyprinidae family is quite a large family in terms of number of species and it is distributed in Asia, Europe and Africa. The majority of bony fish living in our country belong to the Cyprinidae family and this is particularly relevant to freshwater fish. Although this family is represented by 1500 species, there are 30 genus and 70 species inhabiting in Turkey. Pharynx teeth that are characteristic of this family are usually located under the operculum and on pharyngeal bones behind $4^{th}$ gill arc and their order, number and shapes can vary greatly according to different species.

*Cyprinion* species is distributed in Asi, Qweig, Dicle-Fırat basins as well as Iran-Arab Peninsula, Afghanistan and Pakistan (Banarescu and Herzing-Sraschil, 1995). According to Banarescu and Herzing-Sraschil (1995) and Geldiay and Balık (2002), the number of branched rays in dorsal fin of *C. kais* is around 13-15 and this number is 9-11 in *C. macrostomus.* It is flattened from side and its head length is less that the body height. The ratio between the standard length and body depth is ranged from 2.9 to 3.4. The mouth of fish is small and on the ventral and there is a lobe on it. There are a couple of barbels around the mouth. The last branched ray of the dorsal fin is boned and there are denticles on the ray. The form of pharyngeal tooth is different compared to *C. macrostromus. C. kais* has hooks, but *C. macrostomus* doesn't have hooks on their teeth (Krupp, 1985c). The color of back of the fish is grey, sides are whitish. The number of spines on the first gill arch is between 15 and 16. Caudal fin is forked, free edges of dorsal and anal fins are concave. Lin lateral is complete and it gets closer to ventral as approaching to the tail.

Mouth shape is apparent. It is small and it the shape of a semicircular with a width of diameter of an eye and it has large lateral lobes (the lower lip) (Kafuku, 1969). Cartilaginous sheath thickens in corners of the mouth and rounds towards the direction of back side of the fish with a significant margin. The cartilage is in the form of a tooth from lower lip to inward. Mouth of *C. macrostomus* is wider, arched and it doesn't have lateral lobes (Kafuku, 1969; Krupp, 1985c and Banarescu and Herzig-Straschil, 1995).

In the breeding season, there is a tuberculation formation around nose of adult males and they are distinguished from female fishes in this way. In Tigris River, 2 years is the first maturity age and they leave eggs on soil, stones and pebbles between May and June (Ünlü, 1999). Coad (2010) indicated that female *C. macrostomus* are heavier compared to males, their reproductive age is 2+ and reported maximum age as 7 years.

Morphological characters of fish scales are useful tools to separate populations in the same collection basin (Poulet *et al*., 2005; Jawad and Al Jufaili, 2007). Ibanez *et al*., (2009) stated that the fish scales are associated with swimming of the species. Ibanez *et al*. (2007) stated the shape of scales is a useful tool for separating species in the

Mugilidae by the geometric morphometric method. In the population growth characters, habitat, location and seasonal changes may affect the morphology of scales. Therefore, the morphological characters containing metric and meristic information provide information for differences reflecting the season and location where populations are growing (Parsons and Hodder, 1971; Ihssen *et al*., 1981; Ibanez *et al*., 2007). Changes in the limnology and hydro-geomorphology of waters, large fish stocks and productivity of these stocks explain the differences between morphology of scales and also explain why shape of scale is used for separation of stocks (De Pontual and Prouzet, 1987; Margraf and Riley, 1993; Watkinson and Gillis, 2005; Ibanez *et al*., 2007).

In this study, it was aimed to determine whether variations of scales depending on gender, age and seasons and changes are monitored since it is much easier and cheaper to transport, protect and examine fish scales compared to a whole fish.

## Materials and Methods

In this study, a total of 82 (49♀♀, 33♂♂) *Cyprinion kais* samples from the same location in the river of Tigris were collected and ages of scales taken from front and upper section of lin lateral of dorsal fins of fishes were determined and photographed by an Olympus digital camera with Canon SX7 model binocular under the same conditions. Then, six landmarks were collected by tpsDig ver. 2.32 (Rohlf, 2016) software (Fig. 1) and procrustes analysis was performed. After separating shape and size of the samples, ANOVA, PCA, CVA/MANOVA and DFA analyses were performed by using MorphoJ 1.06d (Klingenberg, 2011) and PAST 3.11 (Hammer *et al*., 2001) programs.

## Results

As a result of the analyses, significant difference was found between the sizes of scales depending on gender and age of the fishes and the seasons. In addition, significant difference was found between shapes of the scales (ANOVA) depending on gender of the fishes; however, no difference was found depending on age and seasons. There was no significant difference between shapes of scales according to the results of MANOVA analysis (Table 1).

Considering the boxplot graph of CS, female fishes are larger than males (Fig. 2A), size of fishes increase as they are aged (Fig. 2B), and size of scales are largest in fall and smallest in spring (Fig. 2C).

In the principal component analysis (PCA), the first component explains 61.4% of the variation between the genders, 63.6% of the variation between the ages and 63.4% of the variation between the seasons (Figs. 3A, B and C).

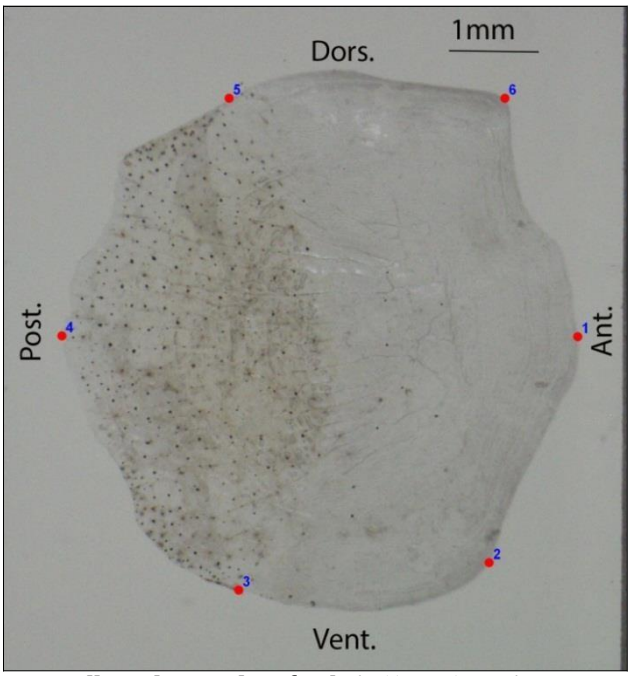


**Figure 1: Landmarks were collected on scales of *C.kais* (Ant: Anteriör, Post: Posteriör, Dors: Dorsal, Vent: Ventral).**

**Table 1: Size(CS) and Shape Procrustes ANOVA and MANOVA values on scales of *C.kais* that depend on gender, age and season (F: Goodal's F, Pillai tr: Pillai trace, P(param): Parametric p value $p<0.05$, CS: centroid size, written in bold: significant difference).**

| | **Gender** | | **Age** | | **Season** | |
|---|---|---|---|---|---|---|
| | **F/Pillai tr.** | **P(param)** | **F/ Pillai tr.** | **P(param)** | **F/ Pillai tr.** | **P(param)** |
| Size(CS)-ANOVA | 50.60 | **<.0001** | 44.08 | **<.0001** | 17.87 | **<.0001** |
| Shape-ANOVA | 3.92 | **0.0002** | 1.15 | 0.2423 | 1.22 | 0.2482 |
| Shape-MANOVA | 0.17 | 0.0793 | 0.61 | 0.1301 | 0.24 | 0.2476 |

In the canonical variance analysis (CVA), the Mahalanobis distance between the gender groups is 0.9098, Procrustes distance is 0.0398 and p values are (10000 permutation rounds) 0.0301 and 0.0058 and the difference between these values is significant. Although the permutation p values of Mahalanobis distance of 1-2, 1-3, 1-4 and 1-5 age groups show that the difference is significant, p values of Procrustes distance show that the difference is not significant (Table 2). In the spring and autumn groups, although p values of Mahalanobis distance show that the difference is significant, p values of Procrustes distance show that the difference is not significant (Table 3).

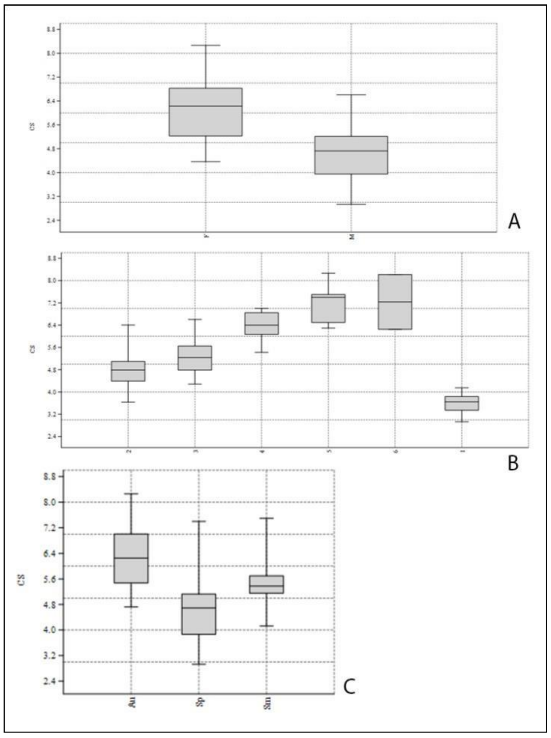


**Figure 2: CS boxplot graphics (CS: Centroid size, F: female, M: male, 1-6: age, Au: Autumn, Sp: Spring, Sm: Summer) of scale variations depending on Gender (A), Age (B) and Seasons (C).**

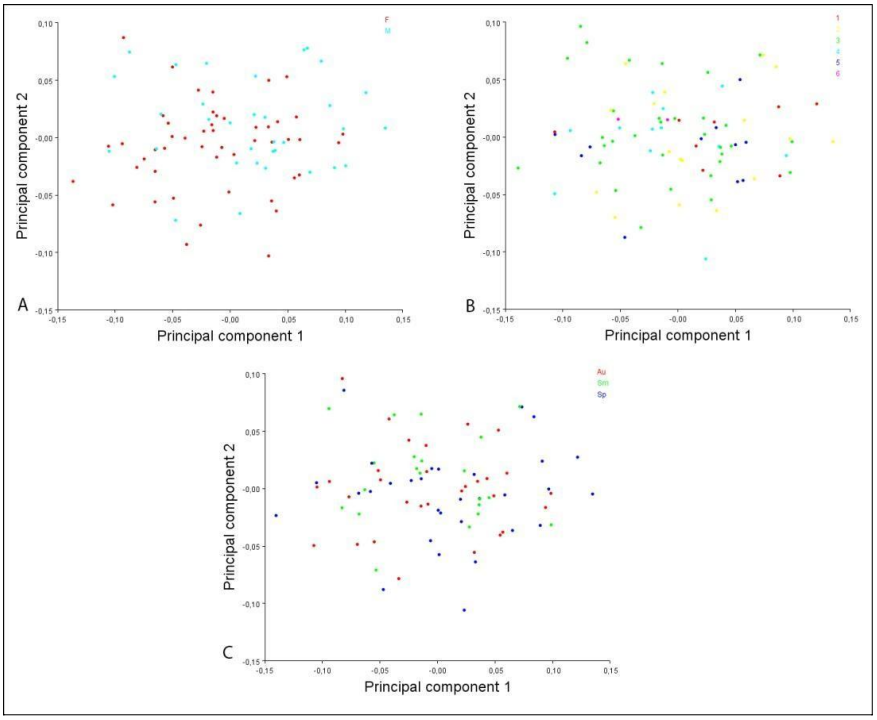


**Figure 3: PCA graphics of scale variations depending on Gender (A), Age (B) and Seasons (C) (F: female, M: male, 1-6: age, Au: Autumn, Sp: Spring, Sm: Summer).**

**Table 2: Mahalanobis distance between age groups and permutations of value (1-6: age, Mahalanobis distance/p value, written in bold: significant difference).**

| Group | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 2 | 1.7837/**0.0373** | | | | |
| 3 | 1.7140/**0.0162** | 1.0242/0.1839 | | | |
| 4 | 1.9502/**0.0242** | 1,2054/0.2568 | 0.7139/0.8186 | | |
| 5 | 2.0481/**0.0217** | 0.8054/0.8317 | 1.2757/0.0947 | 1.5160/0.0834 | |
| 6 | 2.7320/0.2099 | 2.6055/0.1484 | 1.9290/0.5473 | 1.7460/0.6831 | 2.8567/0.0786 |

**Table 3: Mahalanobis distance and permutation p-values between season groups (Au: Autumn, Sp: Spring, Sm: Summer, Mahalanobis distance/p value, written in bold: significant difference).**

| Group | Au | Sm |
|---|---|---|
| Sm | 0.3347/0.9946 | |
| Sp | 1.0772/**0.0198** | 1.1128/0.0524 |

Considering the CVA graphics between groups, 1st, 2nd, 3rd and 6th years of ages (Fig. 4B) and male and female seem to be separated (Fig. 4A); and spring and autumn seem to be partially separated in the season groups (Fig. 4C).

In the discriminant function analysis (DFA), permutation p value of Procrustes distance between genders show that the difference is significant, however parmetric p value is not at adequate level; permutation p value of Mahalanobis distance between 1-3 age groups show that the difference is significant, but parametric p-value is not at adequate level; both permutation p value of Mahalanobis distance between 2-6 age groups and the parametric p-value show that the difference is significant (Table 4).

In addition, in the re-classification made in DFA analysis, 70% of females, 61% of males, 75% of 1 years old fishes, 79% of 3 years old and 100% of 2 and 6 years old fishes are found in their original classes. Considering the shape changes in DFA analysis, scale of female fishes is longer (LM 1) in the anterior and shorter in the posterior (LM 3 and 5) and it is shorter in height. (Fig. 5A), it was observe that the scale is higher and shorter in the 1 age group among 1-3 aged fishes (Fig. 5B), and the height of the scales is decreased and length is increased among those within 2-6 years of age similar to those within 1-3 years of age (Fig. 5C ).

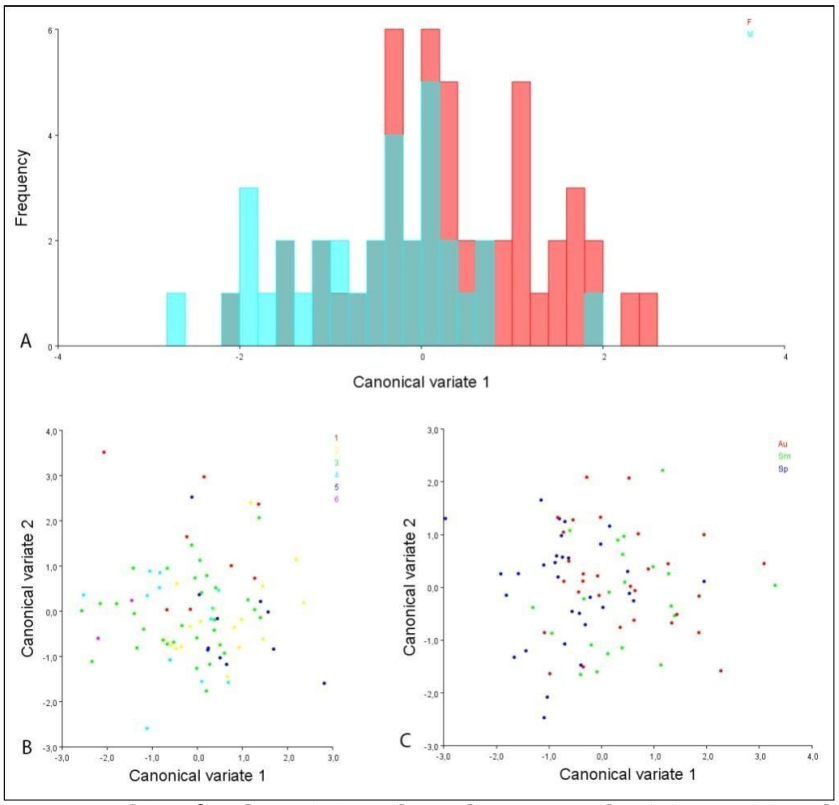


**Figure 4: CVA graphics of scale variations depending on Gender (A), Age (B) and Seasons (C) (F: female, M: male, 1-6: age, Au: Autumn, Sp: Spring, Sm: Summer).**

**Table 4: Mahalanobis distance and permutation and parametric p-values according to the DFA analysis (F: female, M: male, 1,2,3,6: age, written in bold: significant difference).**

| Gorup | p-value (permutation) | p-value(parametric) |
|---|---|---|
| F-M(Cinsiyet) | **0.0060** | 0.0793 |
| 1-3(Yaş) | **0.0470** | 0.0531 |
| 2-6(Yaş) | **0.0200** | **0.0150** |

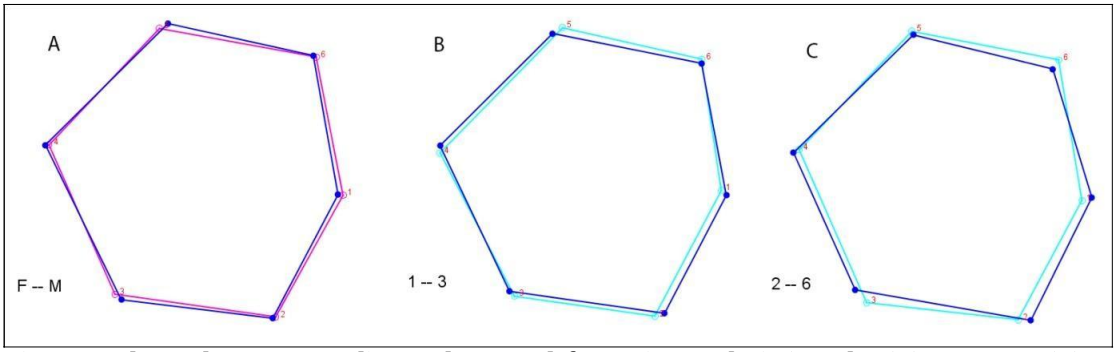


**Figure 5: Shape changes according to the DFA deformation analysis (Gender (A), 1-3 years (B) and 2-6 years (C) (F: female, M: male, 1,2,3,6: age).**

**Discussion**

According to the results of the study, there is a significant difference between sizes and shapes (Fig. 5 and Table 4) of the scale obtained for male and female fishes (Table 1) and in the CS boxplot graph, female fishes seem larger (Fig. 2A). This result is consistent with result of Coad (2010), who states that female *C. macrostostomus* fishes are heavier compared to males. Ünlü (1999) reported tuberculation in male fishes in the breeding season as a sexualdimorphic difference between male and female fishes.

The significant size difference between age groups (Table 1) and significant permutation p value for Mahalanobis distance among 1-2,3,4 age 5 groups in the CVA analysis performed for shape differences (Table 2) and the significant shape differences between 1-3 and 2-6 age groups in especially DFA analysis (Fig. 5B and C) (Table 4) is consistent with results of Coad (2010) who states that the first reproductive age is (2+).

Significant size differences between season groups (Table 1), and significant permutation p-value for Mahalanobis distance between spring and autumn season groups in terms of shape differences (Table 3) appear to be consistent with results of 1971); Ihssen *et al*., (1981) and Ibanez *et al*., (2007) who state that seasonal changes affect the morphology of scales.

As a result, scales are highly suitable materials to be used for 2D geometric morphometric methods and variations depending on age, gender and seasons can be observed on scales. Furthermore, scales can be used to determine the source of differences, and changes in size and/or shape of the fishes.

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