*Genetics of Aquatic Organisms 7(3), GA603*
https://doi.org/10.4194/GA603

RESEARCH PAPER

# Gender Determination of Pearl Mullet by Geometric Morphometric Method

**Veysel Delibaş[1,*], Zafer Soygüder[1], Gamze Çakmak[1]**

[1] Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Anatomy, 65040, Van, Türkiye.

**How to Cite**

Delibaş, V., Soygüder, Z., Çakmak, G. (2023). Gender Determination of Pearl Mullet by Geometric Morphometric Method. *Genetics of Aquatic Organisms, 7(3), GA603*. https://doi.org/10.4194/GA603

**Article History**
Received 06 April 2023
Accepted 07 September 2023
First Online 13 September 2023

**Corresponding Author**
Tel.: +905413479282
E-mail: Veyseldelibas@hotmail.com

**Keywords**
Discrimination
Geometric morphometric
Landmark
Morphological difference
Pearl mullet

## Abstract

Pearl mullet (*Alburnus tarichi*, Güldenstadt 1814) is an endemic fish species of great importance worldwide. This species is an important economic and cultural resource for the region since it lives in Lake Van, one of the world's largest sodic lakes. It is often not possible to determine the sex of fish based on morphological characteristics. In this study, landmark-based geometric and morphometric method was preferred to identify the shape difference between male and female sexes of pearl mullet fishes species in Lake Van. A total of 100 pearl mullet fish (50 females and 50 males) were used as material. Significant morphological differences were found between the male and female sexes ($P<0.001$ in canonical analysis of variance). These differences were found that the male fishes were longer than the female fishes on the lateral side and the head of the male fishes were larger than the female fishes. In addition, female fishes formed a deeper abdominal line towards the ventral side in the wireframe graph. The presence of a large gill positioned cranially is another important feature that distinguishes female fishes from male fishes. Lateral and ventral body differences between the sexes were statistically determined by ANOVA test results.

## Introduction

Pearl mullet (*Alburnus tarichi*, Güldenstadt 1814) is an endemic species of carp living in Lake Van, which is Turkey's largest and most sodic lake (Kaptaner *et al*., 2021). Since its habitat is Lake Van, one of the largest sodic lakes in the world, this species is also called Van Fish (Oguz *et al*., 2022). This endemic species has managed to be a great source of income for the region for years due to the high protein content of the pearl mullet and the fact that Lake Van has a large coast and a large number of freshwater rivers flowing into its center. Although it has great economic importance with an average annual catch of 10,000 tons, it is worrying that this species is under threat of extinction (Çiftci *et al*., 2022).

Changing the shape of fish depending on sex and population has been an important problem for many years (Meng *et al*., 2018). It is often difficult to determine directly whether a fish is female or male by looking at its morphological features from the outside (Faggion *et al*., 2019). The process of determining the population and sex of fish simply by looking at their shape without an invasive procedure; plays an important role in the breeding and control of many species (Fernandino & Hattori, 2019). In this context, by distinguishing endemic fish species based on population and sex characteristics, it will be useful to breed endangered species and control their reproduction. Although there are multiple methods of quantitatively defining the shape of an object or creature, the Landmark point-based geometric morphometric

Published by Central Fisheries Research Institute (SUMAE) Trabzon, Türkiye.

method is widely used today (Rohlf & Bookstein, 1990). The fact that the geometric morphometric method is more quantitative than the traditional morphometric method has made the use of this method attractive (Dennis, 2007; Mitteroecker & Gunz, 2009).

Within the scope of some studies with morphometric methods; the presence of sex-dependent morphological shape variation in trout has been demonstrated (Cetinkaya, 1995). In addition, with the standardization of Landmark point-based measurements in *Cypriniform* fishes, the geometric morphometric method has become a more quantitative and usable method (Jonathan, 2012). Using the geometric morphometric method, it was found that there were shape differences between linesand growing conditions in species such as European sea bass fish (*Dicentrarchus labrax*), and brown trout (*Salmo trutta*) (Costa *et al*., 2010; Fragkoulis *et al*., 2017). Similarly; in a study conducted on *Cyphotilapia frontosa* fishes, it has been demonstrated that there is shape varies depending on the sex factor (Altun *et al*., 2015).

In this study, the aim was to identify shape variations depending on sex using the method of geometric morphometric in the pearl mullet, which is an endemic species.

## Material and Methods

### Animals

In this study; total 100 mullet, 50 females (Fork length: 11.83-16.45 mm, average: 12.40±0.32 mm; Weight: 23.25-68.60 g, average: 33.60±0.70 g) and 50 males (Fork length: 12.00-17.87 mm, average: 13.18±0.35 mm, weight: 21.20-35.10 g, average: 26.80±0.40 g) was used. The fishes by professional fishermen at Çatakdibi station of the freshly flowing Zilan Stream (39º 02' 20.11'' North latitude and 43º 18' 24.75'' East Longitude) (Figure 1.) with fan and scatter nets (number of meshes: 450; eye opening: 17-20 mm; Average weight: 5kg, Height: 2.20 m) was caught. The process of catching fish to pinpoint the sex difference; It was carried out in May and June when breeding migration is active. Adult fish to be used in the study were brought alive to the anatomy laboratory of the Faculty of Veterinary Medicine.

### Sampling

Kavambe *et al*. (2016) were taken as reference in photography and image analysis. Although the

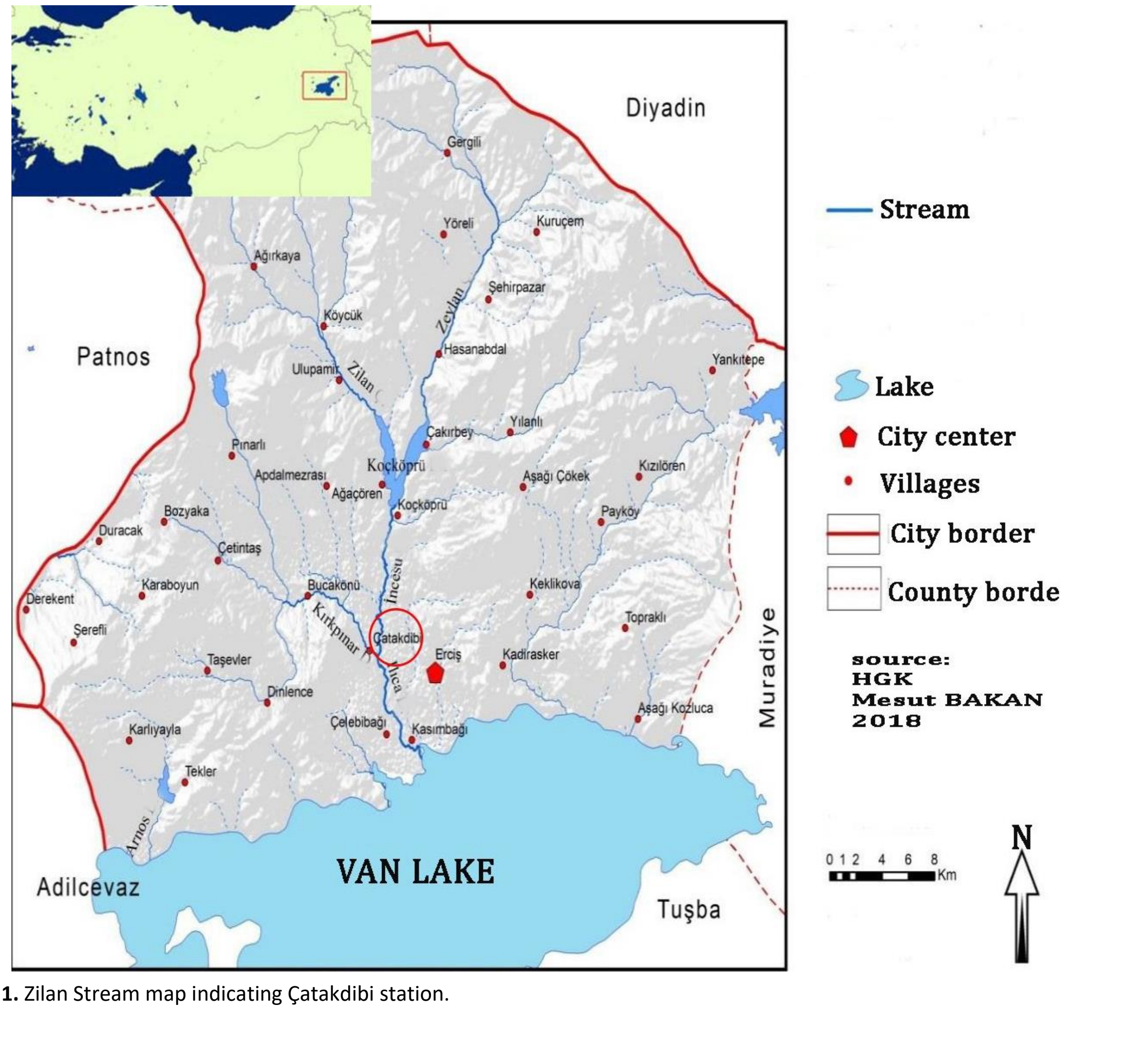


**Figure 1.** Zilan Stream map indicating Çatakdibi station.

disadvantages of the traditional manual milking method such as it takes a lot of time and cannot be applied to all fish species are known, it is aimed to compare the results we obtained at the end of our study with traditional manual milking. For this reason, all fishes were first separated into male and female using the traditional manual milking method. Fishes were anesthetized with phenoxyethanol (320µl/L) just before photographing.

Fishes were positioned on their right side for lateral photographs and then on their dorsal side for ventral photographs (Figure 2). To create the images, the 18 megapixel EOS 7D digital camera (Canon USA Inc) with 50mm 1:2:5 lens was fixed with a Manfrotto MT190XPRO3 tripod with an object distance of 30cm.

Captured photos were turned into TPS files sequentially with TpsUtility v1.78 (Rohlf, 2019). Then, using the TpsDig2 v2.31 program (Rohlf, 2017), coordinates of 21 anatomical landmarks in lateral position images (Figure 2 a) and 13 anatomical landmarks in ventral positioned images (Figure 2 b) was digitized separately as TXT files (Helland *et al*., 2009; Arbour *et al*., 2010). In order to determine whether the points in the contents of these files are safe for statistical analysis, the average slope, correlation (uncentered) and root MS error coefficients of all points were calculated separately with the Tps small v1.36 program (Rolhf, 2020). For image analysis, data files containing previously digitized XY coordinates were transferred to MORPHO.J v 1.60 program (Klingenberg, 2011). In this program, primarily the images of objects were standardized into two separate categories as male and female. Then, the general covariance matrix of the marked point coordinates was extracted with the MORPHO J v1.60 program (Klingenberg & Monteiro, 2005; Slice, 2007). In this context, by minimizing the sum of the total squares of the images, a new image was created that provides a complete Procrustes harmony by rotating, rotating and scaling all fish images relative to each other in a way that creates a reflection to the tangential space (Dryden & Mardia, 1998).

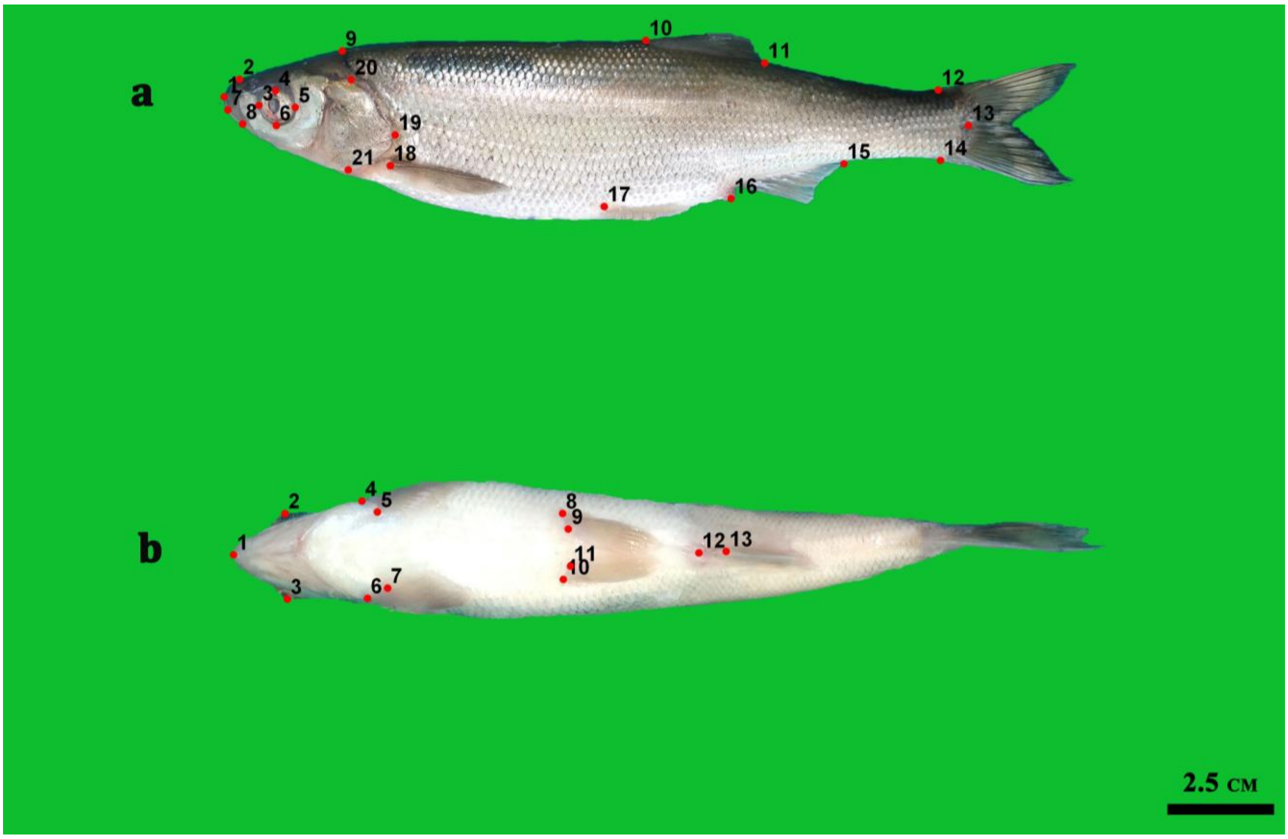


**Figure 2.** Sets of landmarks used for geometric morphometric analysis and calculation of interlandmark distances. Landmarks indicated by red dots; (a) The 21 landmark points used for the lateral side geometric morphometric analysis: (1) tip of snout, (2) posterior margin of posterior nare, (3) most dorsa point of orbit (4) posterior point of orbit, (5) ventral point of orbit, (6) anterior point of orbit, (7) opening of mouth, (8) posterior end of jaw, (9) posteriomedial tip of supraoccipital, (10,11) origin and insertion of dorsal fin, (12) dorsal origin of caudal fin, (13) end of vertebral column, (14) ventral origin of caudal fin, (15) insertion of anal fin, (16) vent (centered on opening), (17) origin of pelvic fin, (18) origin of pectoral fin, (19) posterior point of operculum, (20) most dorsal point of operculum, (21) ventral point of operculum; (b) The 13 landmark points used for the ventral side geometric morphometric analysis: (1) tip of snout, (2,3) left and right posterior edge of lip, (4,5) origin and insertion of left pectoral fin, (6,7) origin and insertion of right pectoral fin, (8,9) origin and insertion of left pelvic fin, (10,11) origin and insertion of right pelvic fin, (12) vent (centered on opening), (13) origin of anal fin.

**Statistical analysis**

Finally, Procrustes, Canonical variance, Discrimination analysis and ANOVA test were applied to the newly acquired image. Test results: Correctly reclassified all but two fish (1 male and 1 female) in the discrimination analysis test, the correct reclassification rate was 98%. The Procrustes distance and Mahalanobis distance between the male and female sexes were found to be too far. (the previous distance was 0.0351 and the next distance was 7.3094). Significant gender differences were detected by canonical analysis of variance (P<0.0001 in all cases out of 10,000 permutations). ANOVA detected significant differences in the shapes of the male and female sexes (F: 22.29; P<0.001)

## Results

The mean slope, correlation (uncentered) and root MS error coefficients of 21 different landmark points determined in the lateral position were determined with the Tps small v1.36 program. These were found to be 0.999785, 1.000000 and 0.000001, respectively. These demonstrated that the points are statistically compatible. The group-centered scores of the dependent variable Procrustes analysis of these points showed the following (Figure 3 a).

The examined pearl mullet fish are expressed with 38 different shape variations (eigenvalues) in total (Figure 3 b). The first three of these shape variations (first eigenvalues 31.759%, second eigenvalues 12.487% and third eigenvalues 11.732%) represent

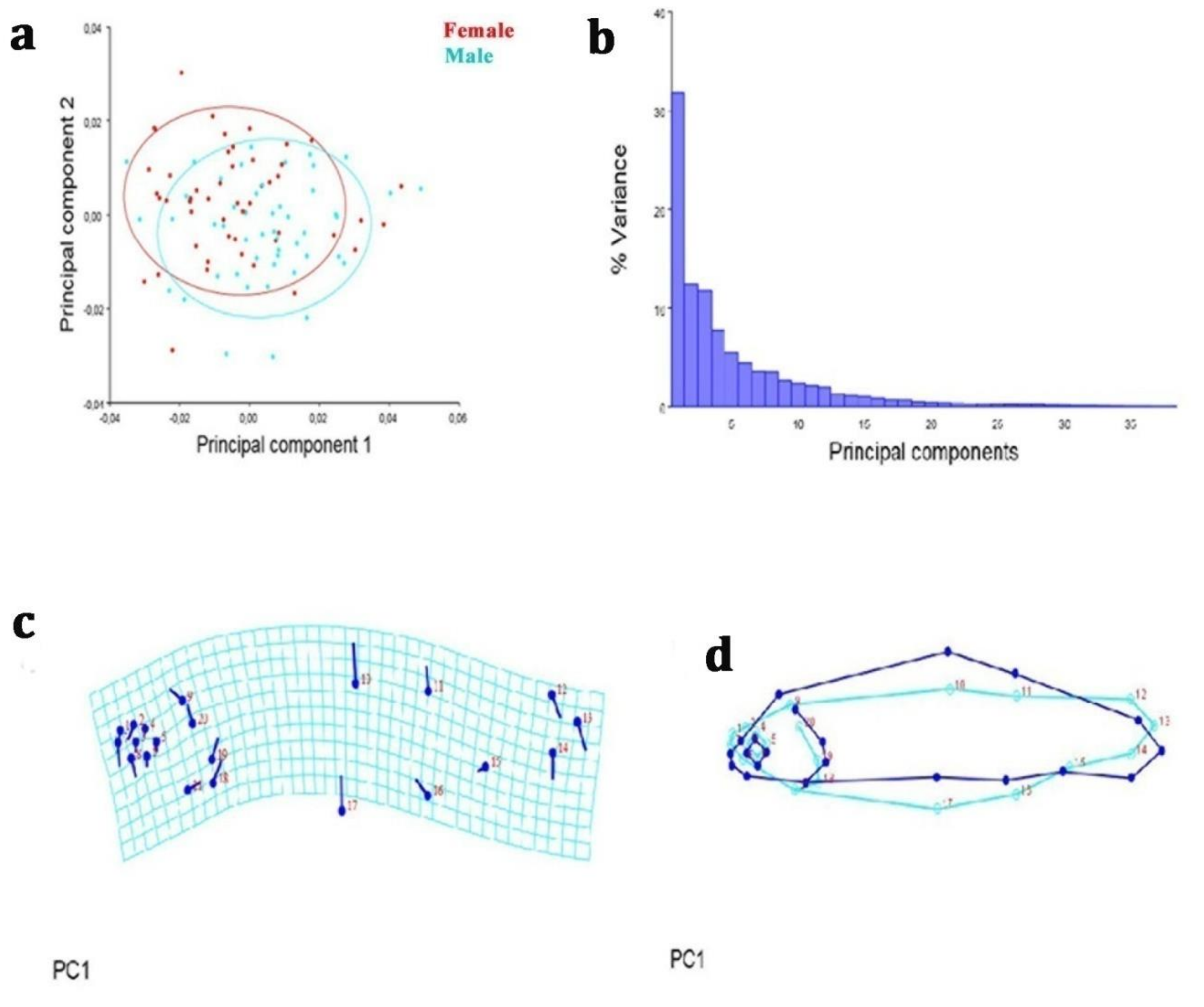


**Figure 3.** The result plots of the lateral side geometric morphometric analysis 1: (a) Scatterplot of principal components 1 and 2 from principal component analysis of Cartesian coordinates of 21 landmarks for all individuals (n = 100) among the two morphotypes (female and male pearl mullet genders). (b) Bar chart of percent variance explained by 38 principal components of geometric morphometric analysis, the first five PCs together account for 69.29% of the total variation. (c) Principal component 1 Transformation grid graph of the analysis result of pearl mullet fishes (scale factor = 4). (d) Wireframe graph of the Principal component 1 Analysis result of pearl mullet fish (scale factor = 4, bluish green dots and lines indicate the average of landmarks of all individuals).

approximately half of the total variance (55.978%). Similarly, 90% of the total variance is the sum of the first 12 eigenvalues. This showed that there was a clear variation in shape in fish in terms of sex (Figure 3 c, d). In addition, the applied canonical variable analysis shows that there is a clear distinction between male and female genders (Figure 4 a). In this analysis, the rate of shape variation was determined as 100% (Figure 4 a).

Although the 9th landmark point, which is considered as the reference limit of the head region in male individuals, did not change, the lip region (1st, 7th and 8th landmark points) was located more in front of the orbit (Figure 4 b). This was also supported by the wireframe graphics and transformation grids shown. These results showed that the head part of the males was larger than that of the females. Likewise, the gill part, which is indicated by the 18th, 19th and 20th landmark points, is shorter in male fishes and is located behind the orbit, which shows a great difference compared to female fishes (Figure 4 b). On the other hand, although the 15th and 21st landmarks did not change in females, the 16th and 17th landmarks were located more ventrally in the orbit, indicating that female fish had a deeper and more voluminous abdomen (Figure 4 b). Similarly, the 12th, 13th and 14th landmark points representing the tail region are located more dorsally compared to male individuals, which is an important difference for female individuals (Figure 4 b). The 15th landmark point representing the end point of the anal fin did not change, but the 16th landmark point representing the anal papilla was found further than the orbit in male fish (Figure 4 c). This result indicates that the tail stem (distance between anal papilla and fork length of the tail) is longer in males. As a result, it was determined that male fish were longer than female fish in terms of fork length.

The mean slope, correlation (uncentered) and root MS error coefficients of 13 different landmark points determined in the ventral position were determined with the Tps small v1.36 program. These were found to

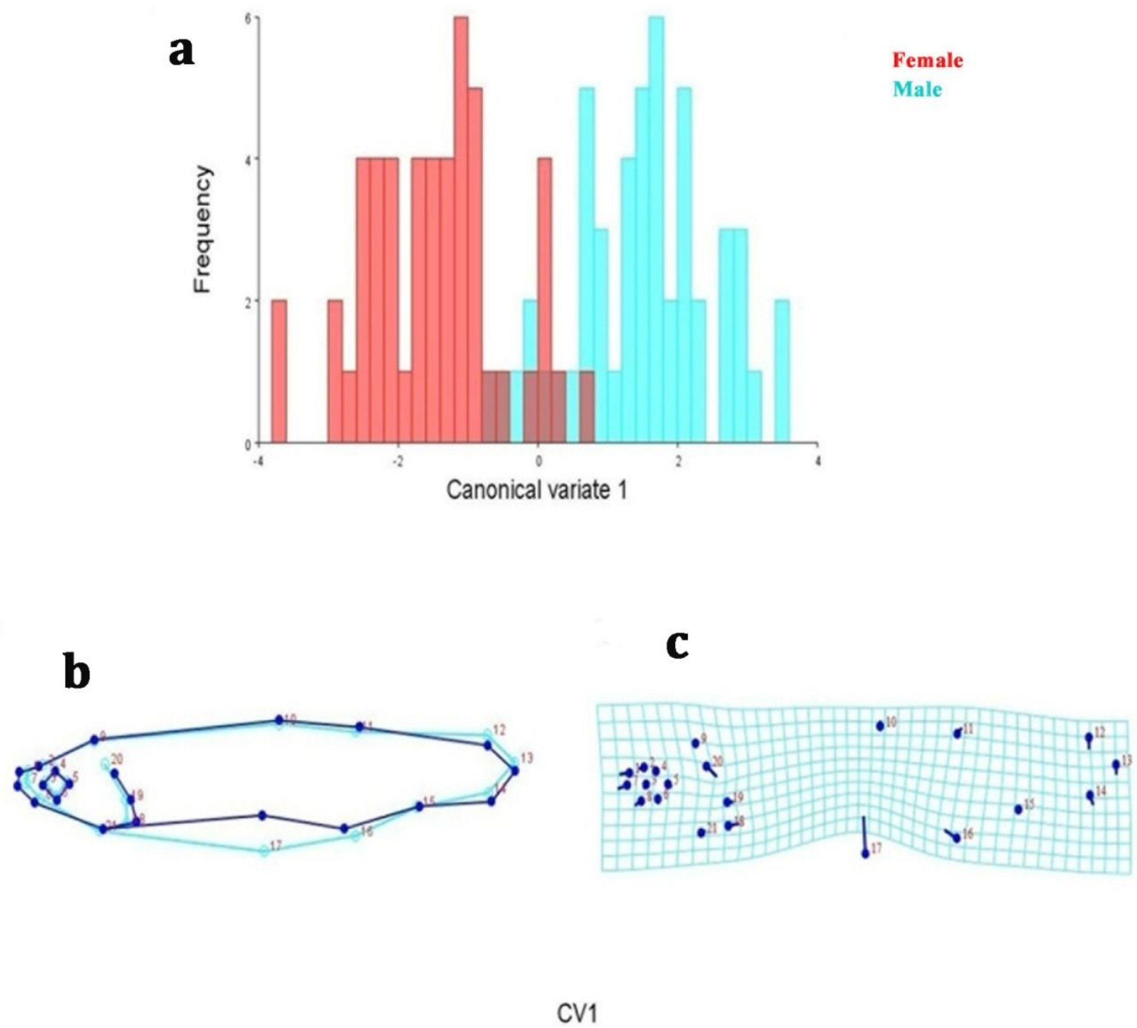


**Figure 4.** The result plots of the lateral side geometric morphometric analysis 2: Body changes in the morphotype of male and female sexes in pearl mullet. (a) Variable analysis for shape variation of the morphotype of female and male pearl mullet. The first standard variable (CV1) represents all the observed variance (100%) between the two genders. (b) Wireframe graph showing the difference in shape along the axis of the male sex (CV1) in pearl mullet fish (scala factor = 4). (c) Transformation grid plot (scala factor= 4) that can show the shape difference along the axis of the male sex (CV1) in pearl mullet fish (scala factor = 4).

be 0.999687, 1.000000 and 0.000003, respectively. These demonstrated that the points are statistically compatible. The group-centered scores of the dependent variable Procrustes analysis of these points showed the following (Figure 5 a).

The examined pearl mullet fish are expressed with 22 different shape variations (eigenvalues) in total (Figure 5 b). The sum of the first 9 eigenvalues constitutes 90% of the total variance. This showed that there was shape variation in the ventral position, although not as much as the lateral position in terms of sex (Figure 5 c). In the applied canonical variable analysis, differences are observed in male and female individuals (Figure 6 a).

These support lateral findings. In the male fishes the 9th and 11th landmark points belonging to the line between the 8th and 9th points and to the line between the 10th and 11th points, which represent the exit points of the anal fins, were fixed. This shows that the abdominal fins of male fish are closer to each other (Figure 6 b). Likewise, it was seen that the pectoral fins are close to each other in male fish, so males have a narrower and smaller abdomen's volume as seen in the lateral images (Figure 6 b, c). In addition, the 1st landmark point, which indicates the lip region, and the 2nd and 3rd landmark points, which represent the boundaries of the head region, is located further along the trajectory, reiterating that the head region is narrower and longer in male fish than in females (Figure 6 c).

Finally, the discriminant analysis and ANOVA test results applied separately to the lateral and ventral image data showed that there is a gender-related shape variation in pearl mullet with quantitative values. Discriminate analysis and ANOVA test applied separately to the lateral and ventral positions resulted in statistical values of P-value<0.0001 at 1000 permutation runs.

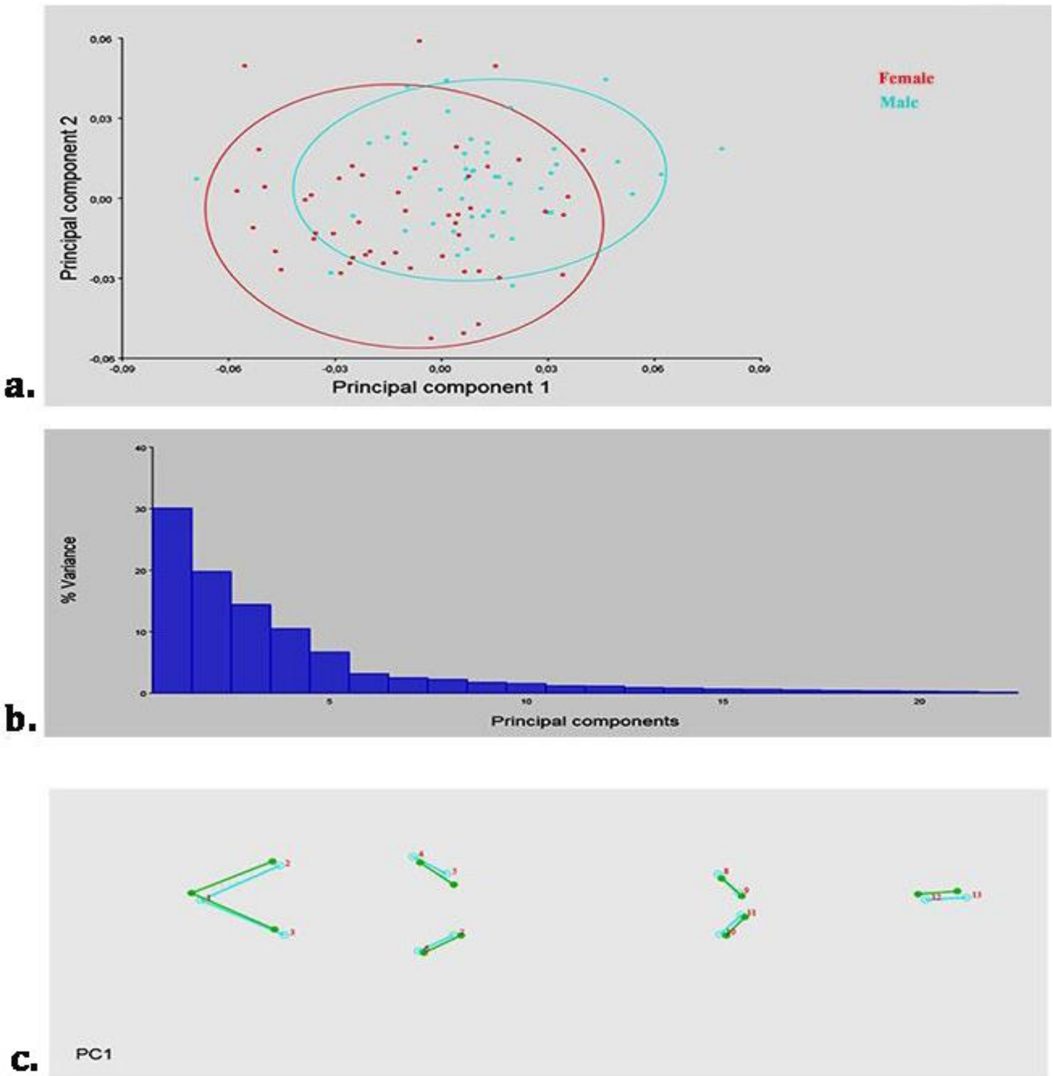


**Figure 5.** The result plots of the ventral side geometric morphometric analysis 1: Body changes in the morphotype of male and female sexes in pearl mullet. (a) Scatterplot of principal components 1 and 2 from principal component analysis of Cartesian coordinates of 13 landmarks for all individuals (n = 100) among the two morphotypes (female and male pearl mullet genders). (b) Bar chart of percent variance explained by 22 principal components of geometric morphometric analysis, the first five PCs together account for 81.50% of the total variation. (c) Wireframe graph of the Principal component 1 Analysis result of pearl mullet fish (scale factor = 4, bluish green dots and lines indicate the average of landmarks of all individuals).

## Discussion and Conclusion

In our study, we noticed that there are morphological differences in pearl mullets in terms of sex. Since fish are evolutionarily the first vertebrates, they are likely to differ morphologically. When studying this difference between the sexes, one should take into account the seasonal and climatic conditions in which the species exhibits sexual activity. The pearl mullet, found in the soda Lake Van, breeds in the freshwater streams around Lake Van in May and June. However, for some fishes species, this period may be from December to February or from July to August (Silva *et al*., 2020). Our studies have shown that the growing conditions of both sexes and different populations of the same species; showed that this may be the cause of morphological differences (Wimberger, 1992).

Arslan *et al.* (2010) stated in their study on adult rainbow fishes that the female fish had a swollen and wide abdomen during sex separation during the reproductive period, and that the anal region was reddish in color. This situation was similar in female pearl mullet fish.

The high accuracy of the geometric morphometric method, due to its technological and software capabilities, has led to its application in many fields of science, replacing the classical morphometric method (Rohlf & Slice, 2004; Delibas *et al*., 2022). The main ones include anthropology, archeology, forensic medicine, animal science and anatomy. With this method, gender and species differences can be detected in hard structures, such as bone tissue, that belong to anthropologically different societies, and in the same way, gender and species differences can be detected in living beings that contain soft tissues (such as muscles) (Yahyaoglu, 2015).

The number of studies on the use of the geometric morphometric method is increasing, especially for fish species where species and sex discrimination are difficult (Baran *et al.,* 2011). In addition, in some studies,

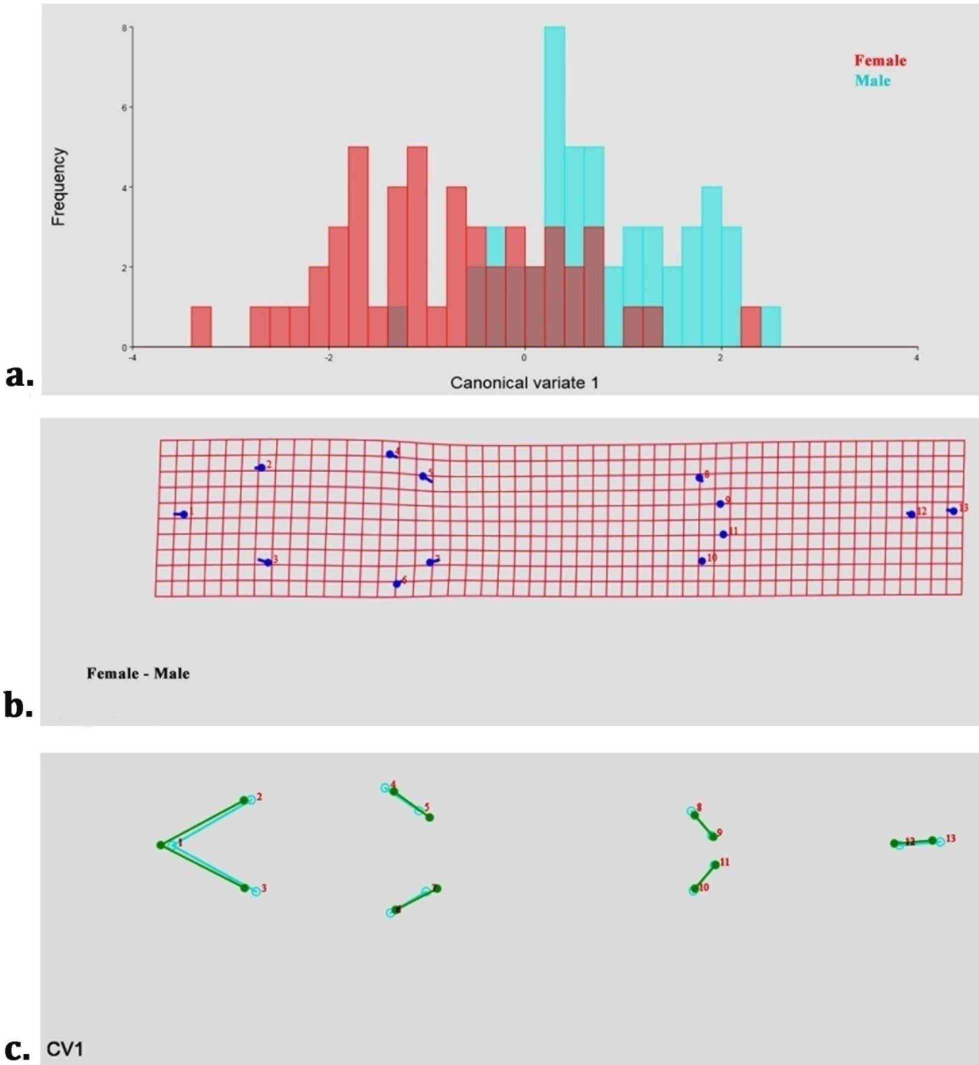


**Figure 6.** The result plots of the ventral side geometric morphometric analysis 2: Body changes in the morphotype of male and female sexes in pearl mullet. (a) Variable analysis for shape variation of the morphotype of female and male pearl mullet. The first standard variable (CV1) represents all the observed variance (100%) between the two genders. (b) Transformation grid plot (scala factor = 4) showing the difference in shape along the axis of the male sex (CV1) in pearl mullet fish. (c) Wireframe graph showing the difference in shape along the axis of the male sex (CV1) in pearl mullet (scala factor = 4).

it has been reported that morphological analysis can be made with the geometric morphometry method at different stages and sizes of many living things, especially fish (Ilić *et al.,* 2019; Lorena Martinez *et al.,* 2023). Although adult pearl mullet fish were used in the study, it is clear that the geometric morphometry method can be applied to all sizes and stages of pearl mullet. Studies such as determining the shape difference of different populations of *Oreochromis* sp. and *Trachulus picturatus* species due to environmental conditions or identification of different races of *Bentuk lutjanus* spp. can be given as examples of examining the shape difference in terms of species (Montoya-López *et al*., 2019; Ikhwani Saputra *et al.,* 2020). Likewise, studies on fish species such as *Caquetaia kraussi*, *Gambusia holbrooki* and *Cyphotilapia frontosa* can be shown as an example of examining the shape difference in terms of gender (Kodric-Brown & Nicoletto, 2001; Altun *et al*., 2015; Hernandez *et al*., 2022). The method we used in our study to determine the gender-related shape differences in pearl mullets is similar to the method used in the studies mentioned.

In our study, when pearl mullet fish were examined from both the lateral and ventral directions, the most basic shape differences defined by the geometric morphometric method were the head shape and body depth of the fish. In pearl mullet, the 1st Landmark representing the tip of the nose and the 7th 8th Landmark representing the mouth region were found to be more advanced in males than females. In addition, the 16th Landmark point representing the anal hole and the 17th Landmark point representing the beginning of the pelvic fin were found to be more ventral in females than in males. This situation was similar to the studies in which male and female gender distinctions were made (Beacham, 1984; Reyes-Gavilan *et al*., 1997; Firmat, 2012; Lorenz *et al*. 2014).

Altun *et al*. (2015) found that male individuals have a longer lip structure than females, the tail stem is longer, and the forehead part of male individuals is more prominent in their study on aquarium fish, which are *Cyphotilapia frontosa* species. This situation was found to be compatible with the results of our study on pearl mullet fish. In their study on Parvis (2016) *Hysterocarpus traski* and Turgut (2016) *Oncorhynchus mykiss*, they found that the caudal fins of the male fish were more prominent than the females in the ventral directional shape analysis. This situation was found to be compatible with pearl mullet fish. On the contrary, when the depth of the abdominal and coudal fins was examined in the studies, it was observed that while females were narrower than males, this situation was wider in pearl mullet than in males. Dorado *et al.* (2012) studied the determination of gender-related shape differences in *Barabus binotatus* using geometric morphometry method. This situation differed in pearl mullet, and it was concluded that males had a weaker and longer body than females. Elp & Çetinkaya (2000) found in their study that with the classical morphometric measurement method, the males of the pearl mullet have a fork length of 13.16 cm and the females 12.45 cm at the breeding age. The results of this study showed one-to-one compatibility with the longer trajectory graphs of the male fish in our study. This situation has led us to think that the geometric morphometric method can be an alternative method that can be used instead of the classical morphometric method (Bookstein, 1991).

As a result, it has been determined that there is a clear morphological variation in pearl mullet fish depending on gender. In this case, it has been revealed that the geometric morphometry method can be used to distinguish species and sex in fish as well as in domestic animals. With this study we have done, our opinion is that the geometric morphometry method is a method that can be used in all stages and sizes of pearl mullet. This study will support future research on the morphology of the pearl mullet, which is an endemic species.

## Ethical Statement

All study processes and experimental protocols were approved by Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee (No; 2021/11-01).


## Funding Information

This work is not supported by any funding.


## Author Contribution

First Author: Conceptualization, Methodology, Writing-review and editing; Second Author: Methodology, Visualization, Data Curation, Formal Analysis, Investigation, Supervision; Third Author: Resources, Writing -review and editing, Supervision.

## Conflict of Interest

The authors report no conflicts of interest.


## Acknowledgements

Contributing to the preparation of this study, Van Yuzuncu Yil Animal Experiments Local Ethics Committee Chairman, Van Yuzuncu Yil University Faculty of Fisheries Faculty Members. We thank to Prof. Dr. Muhammed ARABACI, and Hanife ÇELEBİ.


## References


Adams, D. C., Rohlf, F. J., & Slice, D. E. (2004). Geometric morphometrics: ten years of progress following the 'revolution'. *Italian journal of zoology*, *71*(1), 5-16. http://doi.org/10.1080/11250000409356545

Altun, A., Cek, S. & Cembertaş, E. (2015). Determination of gender-related shape differences in *Cyphotilapia* *frontosa* by geometric morphometry method. *Yunus Research Bulletin*, (3), 13-20. https://doi.org/10.17693/yunus.63511

Arbour, J. H., Hardie, D. C. & Hutchings, J. A. (2010). Morphometric and genetic analyses of two sympatric morphs of Arctic char *(Salvelinu salpinus)* in the Canadian High Arctic. *The Canadian Journal of Zoology*, 89, 19-30. https://doi.org/10.1139/Z10-100

Arslan, T., Guven, E. & Baltacı, M. (2010). Monosex using the hormonal sex conversion methodrainbow trout *(Oncorhynchus mykiss)* production. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, 16, 361-68. https://doi.org/10.9775/KVFD.2010.2678

Baran, Ş., Altun, A., Ayyildiz, N., & Kence, A. (2011). Morphometric analysis of oppiid mites (Acari, Oribatida) collected from Turkey. *Experimental and Applied Acarology*, 54, 411-420. https://doi.org/10.1007/s10493-011-9448-2

Beacham, T. D. (1984). Age and morphology of Chum Salmon in Southern British Columbia. *Transactions of the American Fisheries Society*, 113, 727-736. https://doi.org/10.1577/1548-8659(1984)113%3C727:AAMOCS%3E2.0.CO;2

Bookstein, F. L. (1991). *Morphometric Tools for Landmark Data: Geometry and Biology.* Cambridge University Press.

Cetinkaya, O. (1995). *Fish Production Lecture Notes. (2nd ed).* Yuzuncu Yil University Hakkari Vocational School Publications.

Çiftci, Y., Eroğlu, O., Firidin, Ş., Savaş, H., & Bektaş, Y. (2022). Genetic structure and demographic history of endangered *Alburnus tarichi* (Güldenstädt, 1814) populations from Lake Van basin in Turkey inferred from mtDNA analyses. *Mitochondrial DNA Part A*, 1-16.

Costa, C., Vandeputte, M., Antonucci, F., Boglione, C., Menesatti, P., Cenadelli, S., Parati, K., Chavanne, H., & Chatain, B. (2010). Genetic and environmental influences on shape variation in the European sea bass *(Dicentrarchus labrax)*. *Biological Journal of the Linnean Society,* 101(2), 427-436. https://doi.org/10.1111/j.1095-8312.2010.01512.x

Delibas, V., Cakmak, G. & Soyguder, Z. (2022). Landmark point based shape analysis (geometric morphometry) in veterinary anatomy. In M. Dalkılıc &, A. O. Uğur (Eds.), *INSAC New Trends in Health Sciences* (pp. 507-520). Gece Publishing.

Dennis, E. (2007). Geometric morphometry. *Annual Review of Anthropology*, 36(1), 261-281. https://doi.org/10.1146/annurev.anthro.34.081804.12061

Dorado, E. L., Torres, M. A. J., & Demayo, C. G. (2012). Sexual dimorphism in body shapes of the spotted barb fish, Puntius binotatus of Lake Buluan in Mindanao, Philippines. *Aquaculture Aquarium Conservation & Legislation*, 5(5), 321-329.

Dryden, I. L., & Mardia, K. V. (2016). *Statistical shape analysis: with applications in R* (Vol. 995). John Wiley & Sons.

Elp, M. & Cetinkaya, O. (2000). A research on reproductive biology of pearl mullet (*Cahlcalburnus tarricci*, pallas 1811). In: Proceedings of the Eastern Anatolia Region 4th Fisheries Symposium. Erzurum, Turkey.

Faggion, S., Vandeputte, M., Chatain, B., Gagnaire, P. A., & Allal, F. (2019). Population-specific variations of the genetic architecture of sex determination in wild European sea bass *Dicentrarchus labrax L*. *Heredity*, 122(5), 612-621. https://doi.org/10.1038/s41437-018-0157-z

Fernandino, J. I., & Hattori, R. S. (2019). Sex determination in Neotropical fish: Implications ranging from aquaculture technology to ecological assessment. *General and Comparative Endocrinology*, 273, 172-183. https://doi.org/ 10.1016/j.ygcen.2018.07.002

Firmat, C., Schliewen, U. K., Losseau, M., & Alibert, P. (2012). Body shape differentiation at global and local geographic scales in the invasive cichlid *Oreochromis mossambicus.* *Biological Journal of the Linnean Society*, *105*(2), 369-381. https://doi.org/10.1111/j.1095-8312.2011.01802.x

Fragkoulis, S., Christou, M., Karo, R., Ritas, C., Tzokas, C., Batargias, C., & Koumoundouros, G. (2017). Scaling of body-shape quality in reared gilthead seabream *Sparus aurata L*. Consumer preference assessment, wild standard and variability in reared phenotype. *Aquaculture Research*, *48*(5), 2402-2410. https://doi.org/10.1111/are.13076

Helland, I. P., Vøllestad, L. A., Freyhof, J., & Mehner, T. (2009). Morphological differences between two ecologically similar sympatric fishes. *Journal of Fish Biology*, *75*(10), 2756-2767. http://doi.org/10.1111/j.1095-8649.2009.02476.x

Hernandez, J., Villalobos-Leiva, A., Bermúdez, A., Ahumada-Cabarcas, D., Suazo, M. J., & Benítez, H. A. (2022). An overview of interlocation sexual shape dimorphism in *Caquetaia kraussi* (perciformes: cichlidae): a geometric morphometric approach. *Fishes*, 7, 146. https://doi.org/10.3390/fishes7040146

Ikhwani Saputra, M., Ariawan, I. & Sahara, R. (2020). Analisis Morfometrik dan Klasifikasi *Bentuk Lutjanus spp*. Berdasarkan Gambar Digital. *Jurnal Ilmiah FIFO*., 12, 194-203. http://doi.org/10.22441/fifo.2020.v12i2.008

Ilić, M., Jojić, V., Stamenković, G., Marković, V., Simić, V., Paunović, M., & Crnobrnja-Isailović, J. (2019). Geometric vs. traditional morphometric methods for exploring morphological variation of tadpoles at early developmental stages. *Amphibia-Reptilia*, 40(4), 499-509. https://doi.org/10.1163/15685381-00001193

Jonathan, W. A. (2012). Standardized measurements, landmarks, and meristic counts for cypriniform fishes. *Zootaxa*, 3586, 8–16. http://doi.org/10.11646/zootaxa.3586.1.3

Kaptaner, B., Ünal, G., Doğan, E., & Aykut, H. (2021). Histology of corpuscles of Stannius in Lake Van fish (*Alburnus tarichi,* Güldenstädt 1814)(Cyprinidae). *Anatomia Histologia Embryologia*, 50, 404-410. https://doi.org/10.1111/ahe.12645

Kavembe, G. D., Kautt, A. F., Machado-Schiaffino, G., & Meyer, A. (2016). Eco-morphological differentiation in Lake *Magadi tilapia*, an extremophile cichlid fish living in hot, alkaline and hypersaline lakes in East Africa. *Molecular ecology*, *25*(7), 1610-1625. https://doi.org/10.1111/mec.13461

Klingenberg, C. P. (2011). MorphoJ: an integrated software package for geometric morphometrics. *Molecular ecology resources*, *11*(2), 353-357. https://doi.org/10.1111/j.1755-0998.2010.02924.x

Klingenberg, C. P., & Monteiro, L. R. (2005). Distances and directions in multidimensional shape spaces: implications for morphometric applications. *Systematic Biology*, *54*(4), 678-688. https://doi.org/10.1080/10635150590947258

Kodric-Brown, A., & Nicoletto, P. F. (2001). Female choice in the guppy (*Poecilia reticulata*): the interaction between male color and display. *Behavioral Ecology and Sociobiology*, *50*, 346-351. https://doi.org/10.1007/s002650100374

Lorenz, O., Smith, P., & Coghill, L. (2014). Condition and morphometric changes in tilapia (*Oreochromis sp*.) after an eradication attempt in Southern Louisiana. *NeoBiota*, *20*, 49-59. https://doi.org/10.3897/neobiota.20.5062

Martinez-Leiva, L., Landeira, J. M., Fatira, E., Díaz-Pérez, J., Hernández-León, S., Roo, J., & Tuset, V. M. (2023). Energetic Implications of Morphological Changes between Fish Larval and Juvenile Stages Using Geometric Morphometrics of Body Shape. *Animals*, *13*(3), 370. https://doi.org/10.3390/ani13030370

Meng, Y., Wang, G., Xiong, D., Liu, H., Liu, X., Wang, L., & Zhang, J. (2018). Geometric morphometric analysis of the morphological variation among three lenoks of genus Brachymystax in China. *Pakistan Journal of Zoology*, *50*(3), 885-895. http://doi.org/10.17582/journal.pjz/2018.50.3.885.895

Mitteroecker, P. & Gunz, P. (2009). Geometric morphometric developments. *Evolutionary Biology,* 36, 235-247. https://doi.org/10.1007/s11692-009-9055-x

Montoya-López, A., Moreno-Arias, C., Tarazona-Morales, A., Olivera-Angel, M., & Betancur, J. (2019). Body shape variation between farms of tilapia *(Oreochromis sp.)* in Colombian Andes using landmark-based geometric morphometrics. *Latin american journal of aquatic research*, 47(1), 194-200. http://doi.org/10.3856/vol47-issue1-fulltext-23

Oğuz, E. K., Ergöz, B., & Oğuz, A. R. (2022). Histopathological alterations in Van fish (*Alburnus tarichi,* Güldenstädt 1814) exposed to tebuconazole. *Chemistry and Ecology*, *38*(1), 17-26. http://doi.org/10.1080/02757540.2021.2017902

Parvis, E. S. (2016). Sexual dimorphism and size-related changes in body shape in tule perch, a native California live-bearing fish. Master Theses. Sacramento: California State University.

Reyes-Gavilán, F. G., Ojanguren, A. F., & Braña, F. (1997). The ontogenetic development of body segments and sexual dimorphism in brown trout (*Salmo trutta L*.). *Canadian Journal of Zoology*, *75*(4), 651-655. https://doi.org/10.1139/z97-083

Rohlf, F. J. & Bookstein, F. L. (1990). *Minutes of The Michigan Morphometry Workshop*. *(2nd ed).* University of Michigan Museum of Zoology.

Rohlf, F. J. (2017). TpsDig2, Version 2.31. Department of Ecology and Evolution and Antrophology, State University of New York at Stony Brook, Stony Brook.

Rohlf, F. J. (2019). TpsUtil32, Version 1.78. Department of Ecology and Evolution and Antrophology, State University of New York at Stony Brook, Stony Brook.

Rohlf, F. J. (2020). TpsSmall32, Version 1.36. Department of Ecology and Evolution and Antrophology, State University of New York at Stony Brook, Stony Brook.

Silva Barbato, A. C., Zubizarreta, L., & Quintana, L. (2020). A teleost fish model to understand hormonal mechanisms of non-breeding territorial behavior. *Frontiers in Endocrinology, 11, 468*. https://doi.org/10.3389/fendo.2020.00468

Slice, D. E. (2007). Geometric morphometrics. *Annu. Rev. Anthropol*, *36,* 261-281. https://doi.org/10.1146/annurev.anthro.34.081804.120613

Turgut, N. (2016). Sex Determination of Young and Adult *Rainbow Trout* by Geometric Morphometric Method. Master Thesis. Konya: Selcuk University.

Wimberger, P. H. (1992). Plasticity of fish body shape. The effects of diet, development, family and age in two species of *Geophagus* (Pisces: Cichlidae). *Biological Journal of the linnean society*, *45*(3), 197-218. https://doi.org/10.1111/j.1095-8312.1992.tb00640.x

Yahyaoglu, Ö. (2015). Applications of Geometric Morphometric Methods in Forensic Sciences. Master Thesis. Ankara: Hacettepe University.