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Geometrik Morfometri Analiz Yöntemi Kullanılarak Genç ve Erişkin Gökkuşağı Alabalıklarında Cinsiyet Tayini

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ÖZET

Gökkuşağı alabalıklarında cinsiyet ayrımındaki genel yaklaşım, eşeysel olgunluğa erişmiş bireylerde vücut şekli ve renginin öznel değerlendirmesidir. Bu çalışmanın amacı, genç ve erişkin gökkuşağı alabalıkları arasındaki vücut geometrisine dayalı cinsiyet farklılıklarını geometrik morfometri ile değerlendirmektir. Çalışma 40 genç (20 dişi, 20 erkek) ve 40 erişkin (20 dişi, 20 erkek) olmak üzere toplam 80 adet gökkuşağı alabalığı *(Oncorhynchus mykiss)* üzerinde gerçekleştirildi. Tüm alabalıkların sol lateral yönlü görüntüleri üzerinde 16 adet homolog landmark kullanılmıştır. Cinsiyetler arasında ayrımın yapılabilmesi için genç ve erişkin balıklara ayrı ayrı geometrik morfometrik prosedürler uygulanmıştır. Yapılan analizler sonucunda, genç erkek alabalık örneklerinde dişilere göre, pelvik yüzgecin orijin noktasının posterioventral yönlü olduğu ve burnun uç noktasının ise anteriodorsal yönelim gösterdiği belirlendi. Erişkin erkek alabalıkların dişilerle karşılaştırıldığında, burnun uç noktasının anteriodorsal yönlü olduğu, dorsal yüzgeçin anteriodorsal köşe noktasının ise dorsal yönlü olduğu tespit edildi. PCA (Principal Component) skorları, PCA 1-2'nin cinsiyet grupları arasındaki toplam varyansın gençlerde %61.49'unu erişkinlerde %43.48'ini açıkladığını göstermektedir. Geometrik morfometri ile özellikle genç, kısmen de erişkin gökkuşağı alabalıklarında eşeysel farklılıklar kolaylıkla tespit edildi. Sonuç olarak ucuz, invaziv olmayan ve herkesin erişebileceği pratik bir yöntem olan geometrik morfometrinin cinsiyetin tanımlamasına yardımcı olarak, alabalık yetiştiriciliği yapan işletmelerin kısa sürede kolay, sürdürülebilir, ekonomik ve katma değeri yüksek balık yetiştiriciliği yapabilmelerine katkı sağlayabileceğini düşünüyoruz.

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Sex Determination in Young and Adult Rainbow Trout Using Geometric Morphometrics Analysis

ABSTRACT

Sex determination in rainbow trout generally involves the subjective evaluation of body shape and color in sexually mature individuals. The present study aimed to evaluate sex differences in body geometry in both young and adult individuals of rainbow trout using geometric morphometry. A total of 80 rainbow trout (*Oncorhynchus mykiss*) individuals were studied, including 40 young individuals (20 females and 20 males) and 40 adult individuals (20 females and 20 males). Sixteen homologous landmarks were evaluated in the left lateral images of the studied trout. Geometric morphometrics procedures were applied separately to young and adult fish to determine the sex differences. The results revealed that in young male trout individuals, the origin point of the pelvic fin was posteroventral, and the anterior tip of the nose was anterodorsal, compared to females. When adult male trout fish were compared to their female counterparts, the anterior tip of the nose was observed to be anterodorsal, and the anterodorsal corner of the dorsal fin was dorsal. In the Principal Component Analysis (PCA), PCA 1–2 explained 61.49% of the total variance between the sexes in young trout and 43.48% of the total variation in adult trout. The use of geometric

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morphometry enabled easy determination of sex differences, particularly in young rainbow trout and partly in adult rainbow trout. Therefore, geometric morphometry could serve as a cost-effective, non-invasive, and feasible approach for the sex determination of fish in trout farms to achieve simple, sustainable, economical, and high-value-added fish farming within a short duration.

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INTRODUCTION

Rainbow trout *(Oncorhynchus mykiss* Walbaum, 1792) is a member species of the Salmonidae family. This Pacific salmonid species is carnivorous and has a characteristic adipose fin (Scott & Crossman, 1973). The species is particularly preferred in aquaculture due to its adaptability to the existing environmental conditions, short incubation period, ease of feeding, resistance to diseases, and high level of nutritional value (Bristow, 1992; Çelikkale, 1994; Baki et al., 2021; Özyılmaz et al., 2023). The males and females of this species reach sexual maturity in 2 years and 3 years, respectively. Adult rainbow trout fish are sexually dimorphic, although the evaluation of body structure and color changes in these fish for sex determination is conducted subjectively (Ekingen, 1975; Bristow, 1992). In the case of young trout, the general morphological differences that would facilitate sex determination have not been established to date.

The study of fish morphology generally involves evaluating the length, depth, and width of the body and the shape, color, and location of the other body structures of the fish (Strauss & Bond, 1990; Oliveira & Almada, 1995; Berns, 2013; Gurkan & Innal, 2021). External appearance does not always facilitate the determination of sex differences in fish as the fish may not exhibit evident sexual characteristics, which depend on the larval stage and the completion of morphological development of juvenile fish, as well as on the genetic and environmental factors of the fish (Hanson et al., 2008; Wearmouth & Sims, 2008; Tenugu & Senthilkumaran, 2022). Experts may be able to distinguish sexes in fish by examining the morphological features, although such morphological differences may not be distinctly visible or could be absent entirely. Surgical methods and ultrasonography may, therefore, be used for sex determination in fish. Surgical procedures are, however, not suitable for all species as these may cause stress to the fish. Ultrasonography, on the other hand, is an expensive procedure requiring specific equipment (Baroiller et al., 1999; Guiguen et al., 1999; Baroiller & D'Cotta, 2001; Sarıeyyüpoğlu et al., 2003).

Morphometry is the approach of conducting multiple variance analyses using quantitative variables such as length, height, width, etc. Followed by a mathematical shape analysis to reveal shape variations (Bookstein, 1991; Dryden, 2014). The greatest progress in determining shape differences in biological structures has been achieved by using the landmark-based Geometric Morphometric (GM) method (Rohlf & Marcus, 1993; Adams et al., 2004). This method enables an inexpensive and feasible determination of the relationship between anatomical points through the statistical analysis of the data obtained from reproducible and reliable anatomical points (Zelditch et al., 2012). According to Takács et al. (2016), the GM method is effective due to the similarity between the measurers and the reduced effect of the measures, which reduces or eliminates the data-related errors. GM is used widely to determine the morphological differences between individuals in various fields of science, such as biology, anatomy, forensic medicine, and anthropology. Sex or shape differences have been determined using the GM method in various fish species, such as *Cyphotilapia frontosa* (Altun et al., 2015), *Hysterocarpus traskii* (Parvis, 2016), *Barbus balcanicus* (Radojković et al., 2019), *Danio rerio* (Duff et al., 2019), *Salvelinus confluentus* (Nitychoruk et al., 2013), *Salmo trutta* (Monet et al., 2006; Závorka et al., 2020; Špelić et al., 2021; Salehi et al., 2022), *Oncorhynchus kisutch* (Hard et al., 2000), *Oncorhynchus tshawytscha* (Wessel et al., 2006*), and Oncorhynchus mykiss* (Salinas et al., 2022; Sevastei et al., 2024). However, sex differences in rainbow trout (*O. mykiss*), one of the most preferred species in aquaculture, have not been determined using morphometry to date. Therefore, the present study aimed to determine whether geometric differences at the morphometric level exist between sexes in young and adult rainbow trout. The effect of sex on body shape was also investigated using homologous landmarks. Sex determination in fish is important for both aquaculture production and maintaining fish sex ratios in the wild.

MATERIALS and METHODS

Selection and Description of Subjects

The present study was conducted with 1–2-year-old young (20 females and 20 males) and 2–4-year-old adult (20 females and 20 males) rainbow trout (*O. mykiss*) individuals sampled from a trout production facility in Konya/Sarayönü region. All procedures were conducted after receiving approval from the Ethics Committee (2015/90) of the Experimental Animal Production and the Research Center at the Faculty of Veterinary Medicine, Selcuk University. The young trout individuals were photographed on the same day they were obtained from the production facility, following which the fish were examined for their gonads through necropsy and then placed in containers filled with formaldehyde. Since two fish in the young female trout fish group were determined to be male during the autopsy, these individuals were removed from the group, and in their place, two female samples were added to the group. The adult rainbow trout individuals were photographed immediately after milking under anesthesia [25 mg/L MS-222 (tricaine methanesulfonate)] (Topic Popovic et al., 2012). All rainbow trout individuals were first compared in terms of sex at the macro-anatomical level, following which geometric morphometric analyses were performed.

Geometric Morphometrics

Young and adult trout individuals were placed on a flat surface and then photographed from a distance of 35 cm in the left lateral direction using a tripod (Sony Alpha DSLR-A330 digital camera). All obtained images of trout fish in JPEG format were then transferred to the TpsUtil v.1.69 software program on a personal computer. A total of 16 homologous landmarks were determined in all images of trout fish using the TpsDig2 (v.2.26) software (Figure 1) (Rohlf, 2016).

The identified homologous landmarks were subjected to a validation analysis using the TpsSmall (v.1.33) software (Rohlf, 2015). TpsRelw (v.1.62) and Past (v.3 .12) were employed to perform translation, rotation, and scaling for all samples (Hammer et al., 2016; Rohlf, 2016). The distance values of the landmark points to each other were stabilized using the GPA (Generalized Procrustes Analysis)-superimposition method, and an average consensus image was generated. This enables the elimination of the differences in shape caused by any reason other than the original image. TpsRelw and Past software were then employed to determine the displacement, direction, slope, and deformation of the homologous landmark points identified in the trout images, and the groups were accordingly compared in terms of sex. A Principal Component Analysis (PCA-Principal Component & RWA-Relative Warp) was performed for each image, in which evaluations were presented on the X–Y coordinate axis, and PCA percentages of the components were determined. The GM procedures were applied separately to young and adult trout individuals.

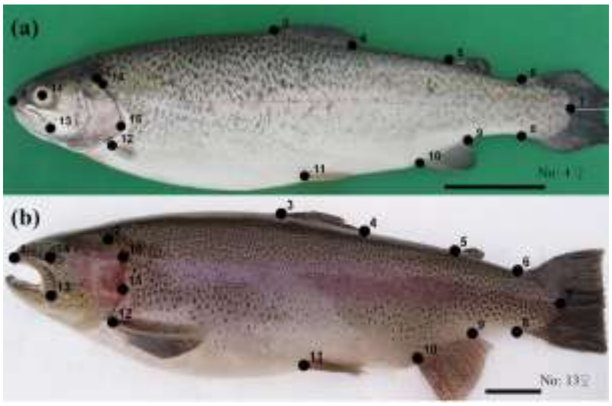

Figure 1. A total of 16 homolog landmarks. (a) Young female rainbow trout. (b) Adult female rainbow trout. 1, anterior tip of nose; 2, most dorsal point of the operculum; 3, anterior and 4, posterior of base of the dorsal fin; 5, anterior of base of adipose fin; 6, anteriodorsal point of the caudal fin; 7, posterior end point of the lateral line; 8, anterioventral point of the caudal fin; 9, posterior and 10, anterior of base of anal fin; 11, origin of the pelvic fin; 12, origin of the pectoral fin; 13, the posterior point of maxilla; 14, midpoint of the eye; 15, most posterior point of the operculum; 16, anterior endpoint of the lateral line. Scale bar=50mm.

Şekil 1. Toplam 16 homolog landmark. (a) Genç dişi gökkuşağı alabalığı. (b) Erişkin dişi gökkuşağı alabalığı. 1, burnun anterior uç noktası; 2, operkulum'un dorsal orijin noktası; 3. dorsal (sırt) yüzgecinin anterio-dorsal köşe noktası; 4, dorsal yüzgecinin posterio-dorsal köşe noktası; 5, yağ yüzgecinin anterio-dorsal köşe noktası; 6. caudal (kuyruk) yüzgecinin anterio-dorsal köşe noktası; 7, lateral (yanal) çizginin caudal sonlanma noktası; 8, caudal yüzgecinin anterio-ventral köşe noktası; 9, anal yüzgecin posterio-ventral köşe noktası; 10, anal yüzgecin anterio-ventral köşe noktası; 11, pelvic yüzgecin orijin noktası; 12, pektoral yüzgecin orijin noktası; 13, maksilla'nın posterior köşe noktası; 14, gözün orta noktası; 15, operkulum'un posterior köşesi; 16, lateral çizginin cranial orijin noktası.

In the present study, the anatomical structures of the fish were named according to The Laboratory Fish (Ostrander et al., 2000) and Nomina Anatomica Veterinaria (NAV, 2017). GM analyses were performed using the SB Morphometrics software programs (https:// https://www.sbmorphometrics.org). The results of the morphometric analysis were visualized using Adobe Photoshop CC 2015.5 (Version: 25.5.0, Adobe system, San Jose, CA, USA).

RESULTS and DISCUSSION

The general morphological examination of rainbow trout fish individuals revealed certain non-specific color and structure differences among the trout samples. In particular, certain young male trout individuals exhibited a slightly brighter stripe along the lateral line than that observed in their female counterparts. The lower jaw was elongated in certain, although not all, adult male rainbow trout individuals, and the tip of the lower jaw in these fish was curved backward and shaped like a hook.

In living organisms, sex-related differences in the form of different colors have been reported in several species of birds, fish, mammals, etc. (Oliveira & Almada, 1995; Berns, 2013). Willson (1997) reported that the male members of migratory salmon exhibit brighter colors. Arslan et al. (2010) reported that male trout fish exhibit color darkening close to the breeding season. In the present study, six male and ten female adult trout individuals were darker in color compared to the other trout fish, as reported by Arslan et al. (2010).

In the male members of salmon, the anterior part of the nose is hook-shaped, and a similar condition is observed in certain female counterparts. In *Salmo* and most members of *Salvelinus* and *Oncorhynchus*, the development of a hooked lower jaw in breeding individuals is reported as a sex differentiator. The development of this hook structure is greater in stream-dwelling and anadromous forms compared to the lake-spawning or freshwater forms. While this structure is further developed in males, it has also been observed in the females of certain species (Willson, 1997). In adult trout (Brook trout) individuals sampled from the tributaries in the Savage River basin in western Maryland, males (n = 36) reportedly had a blunt snout and a rounded head, while females (n = 29) had an angular head, a pointed snout, and a well-developed lower jaw (Holloway, 2012). The body of the adult female rainbow trout is swollen, while the body of the adult male fish is flat with a hook-shaped lower jaw (Ekingen, 1975; Bristow, 1992; Aydın, 2009; Altun et al., 2015). Ouillet et al. (2004) reported that the secondary sex characteristics of adult rainbow trout may remain hidden in the case of intermediate sexuality. The authors examined the gonads of young (6–13-month-old, n = 288) and adult (2–4-year-old, n = 203) rainbow trout fish, identifying 50 inter-sex gonads in the young trout and 23 inter-sex gonads in the adult fish in both gonads. In the present study, similar to the studies reported in the literature (Ekingen, 1975; Bristow, 1992; Aydın, 2009; Altun et al., 2015), no differences were observed in the head, nose, and body structure of adult fish at the macro-anatomical level, although certain adult male individuals had an extended-forward and hook-shaped lower jaw as reported in the literature (Willson, 1997; Holloway, 2012).

In certain kinds of Pacific salmon (*Oncorhynchus spp.*), males have a larger body structure than females, while in certain kinds of coho salmon (*O. kisutch*), females have a larger body structure than males (Tamate, 2004). In 34 species of the fish genus Sebastes, males are shorter than the females (Lenarz & Echeverria, 1991). Holloway (2012) reported that Pacific salmon males exhibit larger values of the length and height of adipose fin than females. In pink salmon males, the head size and dorsal hump are larger than those in the females (Cadrin, 2000). Sockeye salmon (*O. nerka*) males have larger dorsal humps, and longer jaws compared to females (Quinn & Foote, 1994). In the morphological evaluations conducted in the present study, hump structures were observed neither in adult trout nor in young trout. Moreover, no differences in the body and fin structure were observed in adult trout, while young male fish had larger body structures than their female counterparts, as reported in previous studies for Pacific salmon (Tamate, 2004). It is generally accepted that male rainbow trout reach sexual maturity earlier than their female counterparts. Dimensional differences have also been reported as the fish approach sexual maturity. These findings might explain the differences in the size between the sexes in these fish (Ekingen, 1975; Bristow, 1992).

The GM analyses conducted in the present study revealed sexual differences, especially in young rainbow trout and partly in adult rainbow trout (*O. mykiss*). The TpsSmall analysis, which was the first of the geometric morphometric analyses conducted in the present study, revealed the slope and correlation values of 0.999728 and 1.000000, respectively, for the landmark points determined for the young trout individuals. The slope and correlation values determined for the adult trout individuals were 0.999868 and 1.000000, respectively. These values confirmed that the landmark points used were correctly placed.

In the GPA and TPS (Thin-Plate Spline) analyses, the young rainbow trout fish that differed from the consensus image were distinctly visible in the graphics and vector figures. The young male rainbow trout fish, unlike their female counterparts, had anterodorsal landmarks 1, 5, 6, and 7 and anteroventral landmark 12. In addition, landmark points 9, 10, and 11 were posteroventral, while landmark points 3 and 4 were posterodorsal in male fish compared to female fish. Partial morphometric differences were observed in the remaining landmark points. These results were consistent with the TPS link graph (Figures 2 and 3). Morphometric differences between the sexes were also distinctly observed in the RWA-PCA analysis graphs of young trout fish. Polarization between the sexes was distinctly visible in the Cartesian coordinate system. While the male trout fish clustered on the upper left side of the graph, female trout fish clustered on the lower right side of the graph. According to the PCA scores, the first two PCA components (PCA-1 = 45. 98% and PCA-2 = 15. 51%) together explained 61.49% of the total variance between the groups. In addition, PCA-3 (7. 91%) and PCA-4 (6. 09%) together explained 14% of the total variance between the groups (Figure 3).

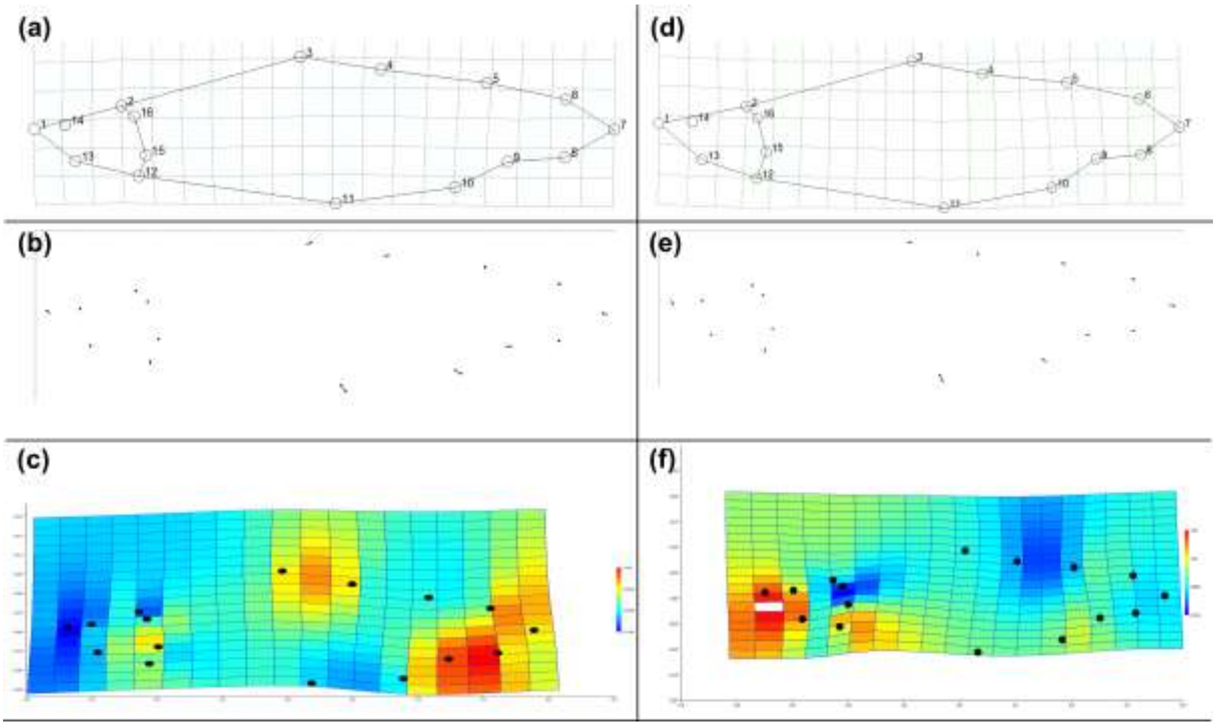

Figure 2. Thin-Plate Spline (TPS) and vectorial deformation analysis graphics. (a, b, c) Young female rainbow trout. (d, e, f) Young male rainbow trout.

Şekil 2. TPS ve vektörel deformasyon analiz grafiği. (a, b, c) Genç dişi gökkuşağı alabalığı. (d, e, f) Genç erkek gökkuşağı alabalığı.

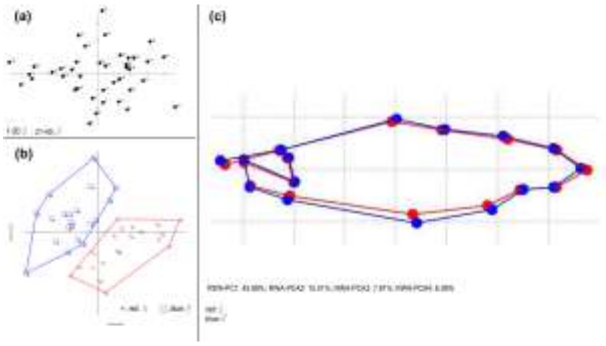

Figure 3. Analyzes in young rainbow trout. (a) Relative Warp Analysis (RWA). (b) Principal Component Analysis (PCA). (c) Thin-Plate Spline (TPS) link graph.

Şekil 3. Genç gökkuşağı alabalığında analizler. (a) Relative Warp Analysis (RWA). (b) Principal Component Analysis (PCA). (c) Thin-Plate Spline (TPS) link grafiği.

Further, according to the results of the GPA and TPS (Thin-Plate Spline) analyses, the adult rainbow trout fish individuals that differed from the consensus image were partially observed in the graphics and vector figures. The adult male fish, unlike their female counterparts, had anterodorsal landmark point 1, dorsal landmark point 3, posterior landmark point 15, posteroventral landmark point 12, and posterior landmark point 16. No morphometric deformations in any of the remaining landmark points were observed. These results obtained were corroborated in the TPS link graph (Figures 4 and 5). Sex differences were partially observed in adult trout individuals when RWA and PCA analyses were conducted. The adult female fish were clustered to the upper left side of the Cartesian coordinate axis, while the adult males were clustered on the lower right side. According to the PCA scores, the first two PCA components (PCA-1 = 29.79% and PCA-2 = 13.69%) together explained 43.48% of the total variance between the groups. In addition, PCA-3 (12.28%) and PCA-4 (9.74%) together explained 22.02% of the total variance between the groups (Figure 5).

Morphometric differences between males and females based on the results of GM analyses have been reported in different fish species. According to Parvis (2016), the most significant difference between the sexes in Tule perch (*H. traski*) was that the anterior endpoint of the anal fin was more posterior in females than in males. In another study, it was indicated that the caudal peduncle is shorter anteriorly in the females of *C. frontosa* than their male counterparts and that the main difference is in the head region (Altun et al., 2015). In *B. balcanicus*, the dorsal fin was reported to be positioned further back, and the caudal and pectoral fins were observed to be positioned slightly more anteriorly than those in the consensus image

(Radojković et al., 2019). In zebrafish (Duff et al., 2019), the eyes are reportedly positioned slightly more ventrally in males than in females. In the present study, the anterior endpoint of the anal fin in young trout fish was observed to be more anterodorsal in females than in males, and as reported by Parvis (2016), it was slightly more posterior in adult females than in adult males. No differences were observed between the sexes of adult fish in terms of the anterodorsal and anteroventral points of the caudal fin, while in young female rainbow trout, these points were more posterior than in males, which is different from the findings reported in the literature (Altun et al., 2015; Duff et al., 2019; Radojković et al., 2019).

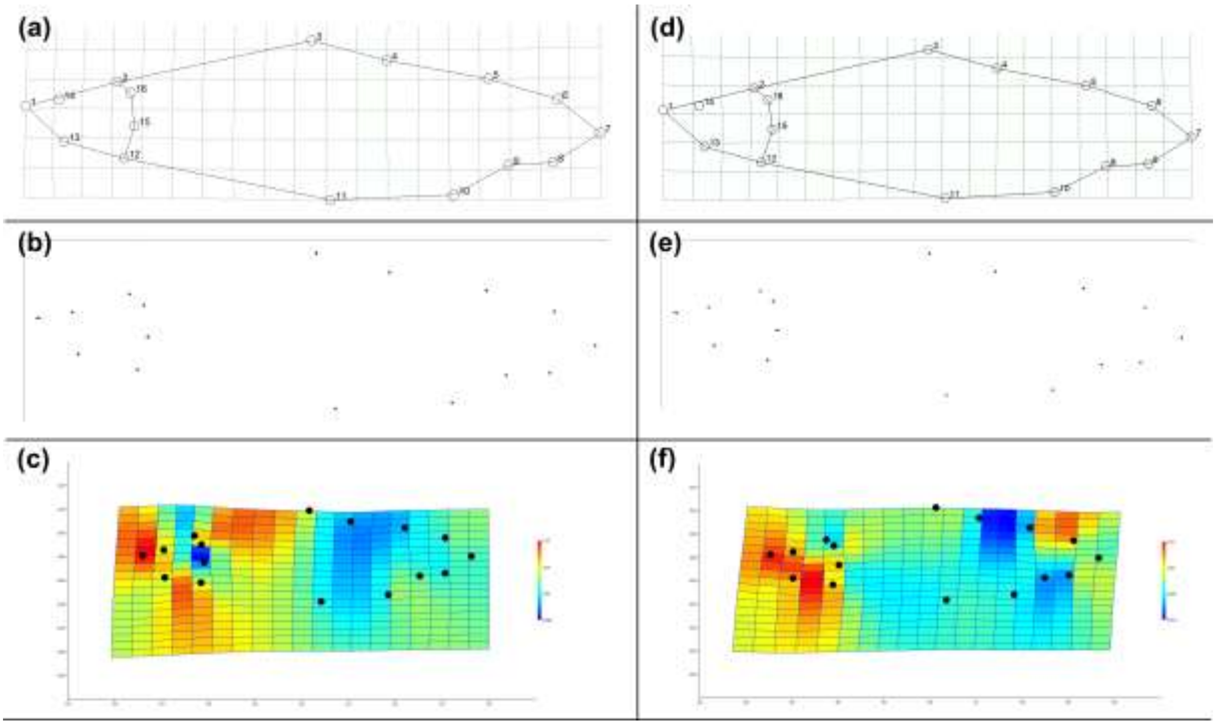

Figure 4. Thin-Plate Spline (TPS) and vectorial deformation analysis graphics. (a, b, c) Adult female rainbow trout. (d, e, f) Adult male rainbow trout.

Şekil 4. TPS ve vektörel deformasyon analiz grafiği. (a, b, c) Erişkin dişi gökkuşağı alabalığı. (d, e, f) Erişkin erkek gökkuşağı alabalığı.

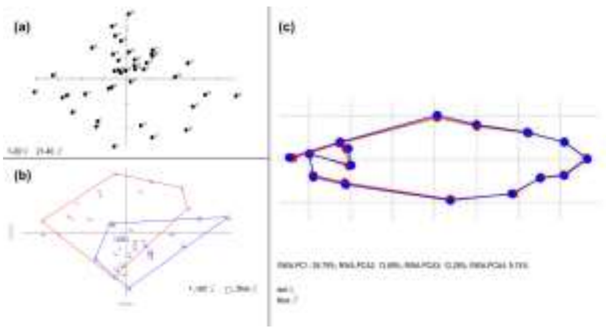

Figure 5. Analyzes in adult rainbow trout. (a) Relative Warp Analysis (RWA). (b) Principal Component Analysis (PCA). (c) Thin-Plate Spline (TPS) link graph.

Şekil 5. Erişkin gökkuşağı alabalığında analizler. (a) Relative Warp Analysis (RWA). (b) Principal Component Analysis (PCA). (c) Thin-Plate Spline (TPS) link grafiği.

Hard et al. (2000) reported that the pectoral and pelvic fins of adult farm-raised coho salmon males were more posterior than those of their female counterparts. The morphometric analyses conducted with brown trout by Monet et al. (2006) revealed that the dorsal end point of the head, the tip of the nose, and the anterior and posterior end points of the dorsal fin were in an anterior position in males compared to females. In addition, the localization of the eyes differed between the sexes. Špelić et al. (2021) reported that in brown trout of Danubian, Atlantic, and Hybrid lineages collected from ten different streams, the Danubian and Hybrid individuals had more streamlined bodies and longer heads with larger eyes compared to the Atlantic lineage. In addition, the individuals of Danubian lineages had longer heads than those of the hybrid individuals. Another study conducted with the same fish species examined the body shape of five different populations of this species in the Caspian Sea, Namak, and Urmia basins and reported that the Jajrud River population had a greater body depth, a shallower head, and a lower anterior pectoral fin origin compared to the other populations (Salehi et al., 2022). A study investigating the effect of competition on head shape in allopatric (brown trout only) and a sympatric (brown and brook trout co-occurring) trout revealed that sympatric trout had smaller eyes, shorter lower jaws, and a more terminal mouth than their allopatric counterparts (Závorka et al., 2020). In the rainbow trout populations of Valcheta (n = 140) and Guillelmo (n = 128) streams in Patagonia, body shape GM data could be distinguished among different populations as well as among Parr, smolt, and adult individuals (Sevastei et al. 2024). In a study investigating the effects of GM on body shape in the neomales and males of *O. mykiss* parr reared at the temperatures of 8 ºC and 16 ºC, dimorphism was observed in the dorsal and caudal fin regions of males for which the traditional morphometrics data could not be determined (Salinas et al., 2022). In the present study, no significant sex differences were noted in the pelvic and pectoral fins in adult fish; the origin point of the pelvic fin was posteroventral in young male fish compared to females, as also reported for coho salmon (Hard et al., 2000) and brown trout (Jajrud River population) in previous studies (Salehi et al., 2022). The origin points of the pectoral fin, on the other hand, was anteroventral. In addition, the tip of the nose in fish was observed to be anterodorsal in young male rainbow trout and anterior in adult male rainbow trout compared to their respective female, counterparts, as also reported for brown trout in a previous study (Monet et al., 2006). In the present study, the dorsal fin was revealed to exhibit dimorphism between young and adult fish, while the caudal fin exhibited dimorphism among the young fish, as reported for the males of *O. mykiss* parr (Salinas et al., 2022). Further, different from the findings reported for brown trout (Monet et al., 2006), the anterior and posterior points of the dorsal fin were posterior in the young males and dorsal in the adult males of rainbow trout compared to their female counterparts. Finally, while no sex difference was noted in the localization of the midpoint of the eye in adult trout fish, this point was slightly more dorsal in young male fish compared to young female fish.

CONCLUSION

Sex determination is important when fish populations must be compared, and the behavior and ecological life of a species must be studied (Mank et al., 2006). Appropriate sex ratios must be maintained during individual selection and broodstock management. In aquaculture, populations comprising entirely male or female fish individuals are generally preferred. In tilapia, the stocks comprising only male individuals are preferred, while in trout, stocks comprising only female individuals are preferred (Altunok et al., 2008). Further, with the increasing demand for food worldwide, the preference for healthy and natural animal products is also increasing. The limited existing resources, therefore, must be sustainable.

In the above context, the use of a limited number of animals from a limited region and the lack of geometric morphometrics data of related species may be considered limitations of the present study. Nonetheless, the study pioneers in providing detailed data on the sex differences, particularly in young rainbow trout and partly in adult rainbow trout. These data contribute to the morphological information on rainbow trout and would be beneficial for the aquaculture sector. In addition, these data may be used in various future studies and compared or evaluated using different methods for further validation.

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Author's Contributions

Nimet Turgut, Saadettin Tıpırdamaz, and Hakan Yalçın designed the study and collected by Nimet Turgut the data. Nimet Turgut and Hakan Yalçın executed the analysis executed the analysis. Nimet TURGUT wrote the article and critically reviewed it by Saadettin Tıpırdamaz and Hakan Yalçın.

Conflicts of Interest

The authors have declared no conflict of interest

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