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Study of variation patterns for body shape in four endemic mullet species (Mugilidae) from Umm-Hufayn Lagoon, on the Cyrenaica coast of Libya



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Received: 26 August 2023

Accepted: 13 June 2024

Publish online: 30 June 2024

Abstract::The study investigates the morphometric characteristics of four mullet species from the Umm-Hufayn lagoon on the Cyrenaica coast to analyze their morphometric variation and possible factors contributing to it. The morphometric analysis showed significant variation in the body shape of mullet species. The results of Discriminant Function Analysis (DFA) show significant variation to separate the four mullet species without any overlap. While Canonical Variate Analysis (CVA) shows the presented four distinct phenotypes differing by mullet species of body shape, Principal Component Analysis (PCA) revealed a wider distribution of body shape variations among the data points, indicating a greater range of body shapes within the sample population. The morphometric differences may be attributed to weight, size, or genetic differences. The study highlights the importance of geometric morphometric analysis to in identifying and differentiating the selected fish species in the same habitat.

Keywords: Mullet species, Umm-Hufayn Lagoon, goemetric morphometric, body shape.

دراسة أنماط التباين في شكل الجسم لأربعة أنواع من أسماك البوري المستوطنة (Mugilidae) في بحيرة أم الحفين في ساحل برقة الليبية

المستخلص: تتناول هذه الدراسة الخصائص المورفومترية لأربعة أنواع من أسماك البوري من بحيرة أم حفين في ساحل برقة، لتحليل تباينها المورفومرتي والعوامل المحتملة التي تساهم في ذلك .حيث أظهر التحليل المورفومتري وجود تباين كبير في شكل الجسم لأنواع البوري .أظهرت نتائج تحليل الوظيفة التمييزية (DFA) تباينًا كبيرًا في فصل أنواع البوري الأربعة دون أي تداخل .بينما يُظهر تحليل التباين القانوني (CVA) الأنماط الظاهرية الأربعة المميزة التي تختلف حسب أنواع البوري في شكل الجسم .تحليل المكونات الرئيسية (PCA)، يُظهر أنه يحتوي على تباين أكثر انتشارًا بين نقاط البيانات، مما يعني أن هناك المزيد من التباين في شكل الجسم .قد تعزى الاختلافات المورفومترية إلى الوزن أو الحجم أو الاختلافات الجينية .تسلط الدراسة الضوء على أهمية التحليل المورفومتري في تحديد وتمييز أنواع الأسماك المختارة في نفس الموطن

الكلمات المفتاحية: اسماك البوري، بحيرة ام حفين، دراسات الشكل الظاهري، ساحل برقة، الاراضى الرطبة.



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INTRODUCTION

The diversity of body shapes among fish species often reflects their adaptations to specific habitats. However, the precise causes of these morphological variations remain challenging to elucidate. It is hypothesized that genetic makeup, environmental factors, and their interplay shape the morphological characteristics of fish (Rocamontes-Morales et al., 2021). Understanding body shape is essential for comprehending a species' ecology, life history, and evolutionary trajectory, as well as for comparing populations across different regions (Kitano, 2004). Additionally, patterns of morphometric variations in fishes can reveal observable differences in growth rates and developmental constraints as body form emerges from ontogeny (Azzurro et al., 2014).

Data from morphometric measurements can also be used to distinguish between fish populations. The environmental explanations of morphometric differences could contribute to the development of conservation strategies. Geometric morphometric methods have become increasingly popular in biological research, enabling the examination of diverse structures, from sperm cells to dinosaur skulls (MacLeod et al., 2013). Parallel to various other phenotypic approaches, this methodology allows for the measurement of a vast number of morphometric dimensions, facilitating the investigation of variation patterns. This enables the study of general morphometry, encompassing the overall shape of the organism or the shape of its individual features.

Geometric morphometric methods are useful for measuring the differences in phenotypes between closely related sympatric species that live in different ecological niches (Russo et al., 2008). These methods excel in exploratory studies, as a large number of landmarks can be used to capture the overall body shape rather than relying solely on traditional morphometric measurements. This approach provides detailed insights into subtle variations in specific body segments or traits and can serve as an 'early detection' method for investigating hybridization events.

A crucial objective of morphometrics in various biological contexts is to identify shape features that can aid in distinguishing between different groups or species. However, beyond distinguishing between populations or species, we may also seek to gain a comprehensive understanding of the structure of intraspecific variation among individuals in study areas. (Adams et al., 2004; Bookstein, 1991). In essence, morphometrics enables us to delve into the intricate patterns of shape variation within a species, providing valuable insights into population dynamics, evolutionary processes, and the influence of environmental factors on morphological traits. By analyzing shape variations, we can unravel the subtle distinctions that characterize individuals from different populations or geographic regions, shedding light on the intricate tapestry of biological diversity (Zelditch et al., 2012).

Mullet species exhibit uniform morphological characteristics that hinder identification, making it challenging to distinguish between species, as body shape undergoes modifications throughout their lifespan. However, knowledge regarding the genetic and phylogeographic population differentiation of Mullet species in Libyan waters remains somewhat limited.

Several studies have explored the morphological variations and geographic distribution of mullet species in the Mediterranean basin. One study by (El-Zaeem, 2011) examined the morphometric and meristic characteristics of *Mugil cephalus* and *Mugil ramada* from Tunisian lagoons and found significant differences between the two species in terms of body shape, fin ray counts, and meristic characters. Another study by (Cossu et al., 2021) investigated the genetic diversity of mullet species in the Black Sea and revealed distinct genetic groupings among the different species. Despite these advancements, a comprehensive understanding of the genetic and phylogeographic structure of mullet populations in Libyan waters remains elusive. Further research is needed to elucidate the ge-

netic relationships between mullet species in the region and identify potential population boundaries. This information would be crucial for informing conservation and management strategies for these important fisheries resources.

The current study employed geometric morphometrics to investigate the body shape variations of four Mullet species collected along the Cyrenaica coast of Libya.

MATERIALS AND METHODS

Umm Hufayan Lagoon, Libya (32° 33' 13" N, 23° 05' 57" E) is a brackish lagoon located on the eastern coast of Libya (Fig.1). Samples of *Chelon aurata, C. labrosus, C. ramada*, and *M. cephalus* have been gathered from Umm-Hufayn Lagoon, collection sites in the Cyrenaica -Libya (Fig.1). Approximately 50 specimens of each mullet species were collected from the study area, resulting in a total sample size of 200 individuals. All fish specimens were transported in ice-filled plastic boxes to the laboratories of the College of Natural Resources and Environmental Sciences in Libya.

Geometric Morphometrics

To facilitate geometric morphometric analysis, 180 fish specimens were photographed from their left lateral side against a white backdrop using a digital camera mounted on a tripod at a fixed height and zoom level. Each specimen was placed on a ruler for scale reference. The fish were photographed individually and labeled according to their respective species. *Chelon aurata* (red), *C. ramada* (light blue), *M. cephalus* (dark blue) and *C. labrosu* (green).

The photographs underwent image analysis using the software tpsDIG 2.17 (Rohlf & Evolution, 2009). During this process, 34 landmarks were identified and digitized to represent homologous points on the fish's external shape. These landmarks were scaled to ensure that the variation in shape among the specimens was not too large for the application of statistical methods. The software tpsSMALL (Rohlf & Evolution, 2008; Rohlf, 2003) was employed to verify that the shape variation within the dataset was within the acceptable range for these statistical methods.



Figure (1). Map of Umm-Hufayn Lagoon sampling location

Principal Component Analysis (PCA) was employed to explore the patterns of variation in multiple variables within the sample. The statistical significance of shape differences between groups was evaluated using Discriminant Function Analysis (DFA) applied to the matrix of partial warps, with MorphoJ software serving as the computational tool. To pinpoint the shape features that most accurately differentiate between the groups being studied, we employed Canonical Variate Analysis (CVA) within the MorphoJ software. To ensure the reliability of these classifications, we conducted a leave-one-out cross-validation procedure, involving 1000 permutations.

RESULTS AND DISCUSSION

The Umm-Hufayn lagoon is home to a wide variety of plant and animal life, including many rare and threatened species, for example, *Anguilla anguilla*. It is also an important breeding and nursery ground for fish mullet species and other marine life. It provides a habitat for various birds, including water birds, migratory birds, and shorebirds.

Employing discriminant analysis, we compared the body shapes of the four mullet species. The analysis revealed that the discriminant function was highly effective in distinguishing between the four mullet species. When tested with 10,000 permutations, it successfully separated the species into distinct groups without any overlap. This clear separation is visually depicted in Figure 2. The discriminant scores were calculated in such a way that the threshold for classification into either group was set at a value of zero.

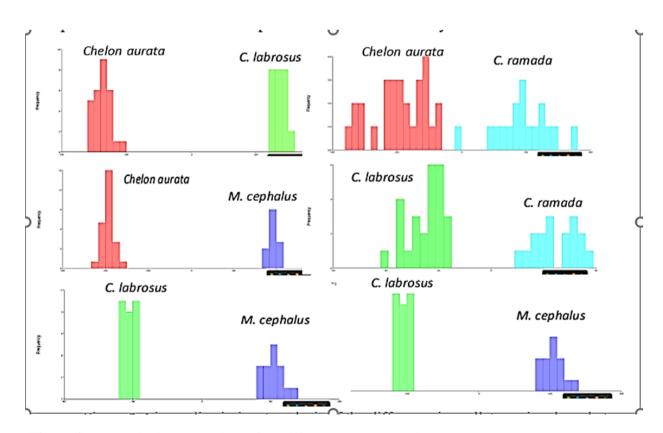


Figure (2). Linear discriminant analysis of the difference in mullet species shape between *Chelon aurata* (Red), *C. ramada* (Light-Blue), *M. cephalus* (Dark-Blue) and *C. labrosus* (Green) combining the study areas.

The Canonical Variate Analysis of *Chelon aurata* (red), *C. ramada* (light-blue), *M. cephalus* (dark-blue) and *C. labrosu* (green) from Umm-Hufayn Lagoon presented four distinct phenotypes or

forms differing by their body shape. This demonstrates a high level of morphological differentiation between the four Mullet species forms (Fig.3).

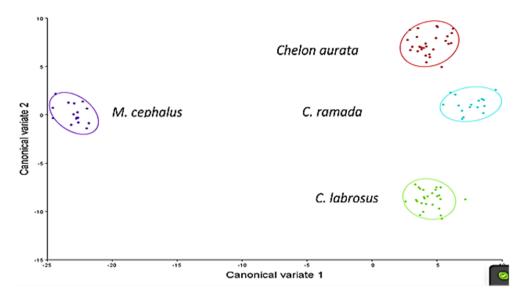


Figure (3). Scatter plot of the first two canonical variates for *Chelon aurata* (Red), *C. ramada* (Light-Blue), *M. cephalus* (Dark-Blue) and *C. labrosus* (Green).

In the mullet species, we used thin-plate splines to create deformation grids that showed how the body shape of the four mullets varied between different species. The deformation grids showed that the different species had different proportions for body shape, with some populations having longer bodies and others having wider bodies (Fig.4). Thin-plate splines are a powerful tool for visualizing variation in body shape. They can be used to visualize the variations in body shape of any organism, and they can be used to identify the factors that contribute to this variation.

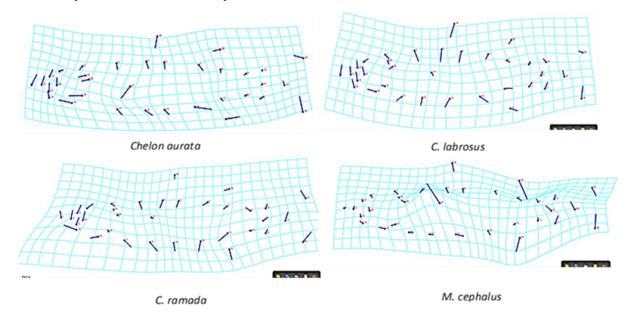


Figure (4). Grids of relative warps with the average shape of *Chelon aurata*, *C. ramada*, *M. cephalus and C. labrosus* specimens obtained from 34 landmarks from study area.

The spread of data points for *Chelon labrosus* (green dots) is more extensive, indicating greater variation in body shape. *Chelon aurata* (red), *Liza ramada* (light blue), and *Mugil cephalus* (dark blue) have a more compact distribution of data points compared to *Chelon labrosus*, implying less shape variation. The Principal Component Analysis (PCA) revealed a slight overlap in morphology between *Chelon aurata*, *Liza ramada*, *and Mugil cephalus* (Fig. 5). The findings of this study align with previous research conducted by (Chien et al., 2018), which demonstrated strong correlations between whole-body growth and various morphometric and meristic characteristics in mullet species from the Karachi coast of Pakistan. This study reinforces the notion that body shape can serve as a proxy for growth and development in mullets.

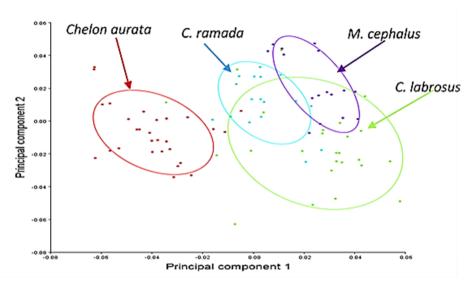


Figure (5). Principal component analyses of morphometric 34 landmarks of *Chelon aurata* (Red), *C. ramada* (Light-Blue), *M. cephalus* (Dark-Blue) and *C. labrosus* (Green).

The effects of habitat on body shape diversification in mullets are complex and vary depending on the specific species and the environment. While (Friedman et al., 2022) examined the effects of habitat on body shape diversification in marine teleost fishes and found that benthic living increases the rate of body shape evolution, leading to extreme body shapes such as wide bodies and elongate forms. Furthermore, the effects of habitat on body shape diversification in mullets are still being studied, but the research that has been done so far suggests that habitat can have a significant impact on the morphology of these fish.

Wetlands can affect the body shape of many fish species, and in Umm-Hufayn lagoon, mullet fish are no exception. The specific effects of wetlands on fish body shape will vary depending on the species of fish and the characteristics of the wetland. The study of how wetlands affect fish body shape is a growing field of research. This research is important for understanding how wetlands function and how they can be managed to protect fish populations.

(Ibañez et al., 2007) discovered that variations in fish scale morphology can effectively distinguish between the congeneric species *Mugil cephalus* and *Mugil curema*, which are distinct from other members of the Mugilidae family. The variation of fish scale morphology to between populations is influenced by their geographic proximity and habitat similarity; it is least effective for populations from nearby areas, improves for populations that are more geographically dispersed, and is most effective for distinguishing between species and genera. Where the level of similarity of habitats and geographical proximity influences morphology divergence among populations and the genetic diversity within populations.

(Asmamaw et al., 2021) reported that the biotic and abiotic factors and genetic structure interactions are believed to be the main cause of the observed morphometric variations, but we suggest that the genetic structure is the main cause of the observed morphometric variations in body shape of mullet species in Umm-Hufayn lagoon because the samples were collected from the same environment, so the biotic and abiotic factors demonstrated a negligible effect on the morphometric variables of mullet species in Umm-Hufayn lagoon.

CONCLUSION

The requirement for correctly estimating population trends, productivity, and fish stocks is to accurately identify fish species, which in turn helps define management strategies for fisheries. In the absence of fisheries data on the Libyan coast, this could will be the primary database; hence, additional studies would be welcome to make more morphological distinctions to estimate population trends for stock fisheries on the Libyan coast.

The current findings provide new tools for studying phenotypes, but the fish of the Libyan coast are still poorly described from a taxonomic standpoint. Future research on the biology and ecology of Umm-Hufayn Lagoon is required. This has additional implications for marine biodiversity conservation in wetlands.

ACKNOWLEDGEMENT

The authors are expressing their gratitude to Hamdi Al Mozini for providing them with the specimen that they studied. This specimen was essential to their research, and they are grateful for Hamdi Al Mozini's willingness to share it with them

ETHICS

Author contributions Abdulghani as an author to the methodology section , performed the analytic calculation , Data curation and writing of original draft .Fatma and Sara writing —Review &Editing and Supervision . Fatma Supervised the study.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions: Contribution is equal between authors.

Funding: The study did not receive funding from institutional, private, or corporate sources.

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