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Morphological Variations among the Different Populations of Estuarine Croaker (*Pseudotolithus elongatus*) in the Cross-River Estuary, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study examines the morphological variation of the estuarine croaker (*Pseudotolithus elongatus*) in the Cross River Estuary, Nigeria, to understand how environmental factors shape fish populations. A total of 815 fish specimens were collected from five stations along the estuary, representing diverse salinity gradients and levels of anthropogenic influence. Morphometric analysis focused on 11 key body traits, adjusted for size using allometric scaling. Principal

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Component Analysis (PCA) revealed that the first component (PC1) accounted for 91.58% of the total variance, capturing the majority of morphological variation. Discriminant Function Analysis (DFA) further identified three distinct morphological groups, with an overall classification accuracy of 61.44%. Monte Carlo simulations indicated that sampling variability could impact observed morphological distinctions, particularly at sites with minor environmental differences. Findings underscore the role of environmental gradients in driving morphological diversity and suggest the need for refined sampling protocols and classification models to improve species differentiation in estuarine ecosystems. The study recommends managing the croaker population as distinct stocks based on local environmental conditions to support effective conservation, thereby sustaining population diversity and resilience in the estuary.

Keywords: Pseudotolithus elongatus; commercial fisheries; ecosystems; food webs.

1. INTRODUCTION

The African croaker (*Pseudotolithus elongatus*) is a species of substantial economic, social, and ecological importance in West Africa, particularly in Nigeria's coastal and estuarine ecosystems. In Cross River State, this species sustains local fisheries, serving as a crucial source of food security, employment, and income for coastal communities (Ajah & Udoh, 2012). It also plays a vital role in commercial fisheries, contributing significantly to regional economies and export markets. Ecologically, *P. elongatus* is a key component of the estuarine food web, acting as both predator and prey, and its abundance reflects the health of these dynamic ecosystems (Nwosu, et al., 2010). As a predator, *P. elongatus* primarily feeds on smaller organisms, including crustaceans, mollusks, and small fish species. Its diet reflects its position as a mid-level predator, which allows it to regulate the populations of these prey species and maintain ecosystem stability. The species' predatory behaviour is adapted to its diverse habitats, such as muddy estuarine bottoms and sandy coastal areas, where it uses its sensory adaptations, including a well-developed lateral line system, to detect prey in turbid waters. By consuming benthic invertebrates and small fish, *P. elongatus* also facilitates energy transfer from lower trophic levels to higher ones, supporting the productivity of estuarine food webs (Uchenna, et al., 2023). Simultaneously, *P. elongatus* serves as prey for larger predators, including piscivorous fish species such as barracudas (*Sphyraena spp*.), groupers (*Epinephelus spp*.), and sharks (Asuquo, et al., 2013; Abiaobo, et al., 2024). Marine mammals and certain bird species, particularly those inhabiting coastal and estuarine regions, also feed on *P. elongatus* (Otogo, et al., 2023). Its role as prey makes it an integral link in the trophic network, providing energy and nutrients to top predators that rely on its abundance. More so, the high fecundity and adaptability of *P. elongatus* to diverse habitats contribute to its availability as a food source, ensuring its significance in supporting higher trophic levels. Its adaptability to freshwater and marine environments, including the Cross River estuary, highlights its importance in understanding population dynamics and informs broader fisheries management and conservation efforts (Paugy, et al., 2003; Asuquo & Ifon, 2019).

In the Cross River estuary, local fishers have reported significant reductions in the abundance of *P. elongatus* over the past two decades (Nwosu, et al., 2010; Asuquo & Ifon, 2019). Historical data indicate that this species once constituted a substantial portion of the total fish landings, supporting local economies and food security. However, increased fishing pressure, habitat degradation, and environmental fluctuations have contributed to the observed decline. Artisanal fishing practices, including the use of non-selective gear such as small-mesh gillnets and beach seines, have disproportionately affected juvenile populations, further exacerbating the species' vulnerability (Macário, et al., 2021). Beyond the Cross River estuary, similar trends have been documented along the West African coastline. In countries such as Ghana, Côte d'Ivoire, and Senegal, *P. elongatus* populations are under intense pressure due to overfishing, climate change, and pollution (Paugy, et al., 2003; Ssentongo et al., 1986). These challenges are particularly pronounced in estuarine ecosystems, where the species relies on specific habitats for spawning and nursery grounds. The degradation of mangroves, which provide critical shelter and feeding areas, has further disrupted the life cycle of *P. elongatus*, limiting its ability to replenish stocks (Ifon & Asuquo, 2021). The declining catch rates of *P. elongatus* have significant ecological, economic, and social implications. As

a mid-level predator and prey species, its reduced abundance disrupts trophic dynamics in the estuarine food web. Economically, it threatens the livelihoods of artisanal fishers and reduces the availability of a critical protein source for local communities. Socially, the decline has led to increased competition and conflict among fishers, as well as reduced incomes in communities dependent on fishing.

African croakers exhibit a complex life cycle in the dynamic estuarine environments, particularly in regions like the Cross River Estuary. Adults of this species are typically found in brackish waters and nearshore marine habitats, including sandy and muddy bottoms, tidal creeks, and saline zones (Ajah & Udoh, 2012; Holzlohner & Nwosu, 2014). While detailed migratory patterns are less well understood, it is believed that *P. elongatus* may exhibit site fidelity or natal homing behaviour, where individuals return to specific estuarine or nearshore areas for spawning. The early life stages of *P. elongatus* are likely influenced by the unique environmental conditions found within these estuarine ecosystems. Juveniles may spend time in the freshwater or brackish zones of the estuary before migrating to marine environments, where they grow and mature. The duration of their stay in estuarine habitats could vary depending on factors such as salinity, temperature, and habitat complexity, with different regions offering varying ecological pressures that could shape their growth and development. These environmental conditions, including fluctuating salinity, temperature, and habitat structure, may lead to local adaptations in *P. elongatus*, potentially contributing to morphological differences between populations in different parts of the estuary. For example, populations inhabiting more saline creeks or mud flats might exhibit distinct phenotypic traits compared to those found in less saline or more vegetated areas. This ecological variation provides an opportunity to study potential stock differentiation based on natal origin and habitat-driven selection pressures.

By analyzing the morphometric traits of *P. elongatus* across different estuarine habitats, it could be possible to detect measurable differences in body shape, size, and other phenotypic characteristics (Opeh, et al., 2023; Chindo, et al., 2024). Such differences could reflect adaptations to local conditions during early life stages, contributing to the stock structure of this species within the estuary (Suleiman, et al., 2019; Ekpo, et al., 2021). Understanding the stock structure of *Pseudotolithus elongatus* is fundamental to developing effective fisheries management strategies in the Cross River Estuary, Nigeria. The absence of such knowledge can lead to several ecological and management challenges, including the erosion of genetic diversity, alterations in key biological traits such as reduced body size, overfishing of less productive stocks, and inaccuracies in predicting the outcomes of management interventions (Smith, et al., 1991; Ricker, 1981). Despite its ecological and economic significance, the stock structure of *P. elongatus* in the Cross River Estuary remains poorly understood. This species occupies a variety of estuarine habitats, such as mangrove-lined creeks, sandy and muddy bottoms, and saline inlets, which are characterized by dynamic environmental conditions, including fluctuating salinity, temperature, and habitat complexity (Hanif et al., 2019). These environmental factors likely influence the morphology and behavior of *P. elongatus*, potentially leading to the development of distinct populations within the estuary (Ifon & Asuquo, 2021). Identifying such stock structures is essential for ensuring the sustainability of *P. elongatus* fisheries and protecting the species from overexploitation.

Morphometric analysis, which involves the quantitative study of fish shape and form, has proven to be a valuable tool for identifying stock structure. Morphometrics is especially suitable for regions with limited access to advanced molecular techniques, as it provides cost-effective insights into population differentiation and environmental adaptations (Cadrin & Silva, 2005). For *P. elongatus*, morphometric analyses can reveal whether distinct populations exist within the estuary, driven by variations in habitat conditions or other ecological factors. In the Cross River Estuary, stock structure analysis of *P. elongatus* is particularly relevant given the species' declining catch rates (Ajah & Udoh, 2012; Asuquo & Ifon, 2019). Overfishing, habitat degradation, and the use of non-selective fishing gear are likely driving this decline (Ameh, et al., 2023). Understanding the stock structure could help identify critical spawning and nursery areas, assess habitat-specific productivity, and protect vulnerable populations. For example, if distinct stocks are linked to specific habitats, targeted conservation efforts could be developed to preserve these areas and ensure the sustainability of the fishery.

Moreover, recognizing the stock structure of *P. elongatus* is crucial for mitigating the risks of overfishing less productive populations. If populations within the estuary differ in their growth rates, reproductive potential, or response to environmental pressures, failing to account for these differences in management plans could result in the depletion of less resilient stocks (Agi-Odey, et al., 2024). Effective stock-specific management strategies would help optimize the fishery, ensuring the long-term viability of *P. elongatus* populations and the livelihoods they support. By identifying stock structures through morphometric analysis, this study aims to provide critical insights into the ecological adaptations and population dynamics of *P. elongatus*. These findings will contribute to informed decision-making for sustainable fisheries management and the conservation of this economically and ecologically significant species in the Cross River Estuary.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in the Cross River Estuary, a vital ecological zone located in southeastern Nigeria, within Cross River State. This estuarine system is one of the most extensive and biologically diverse in West Africa. It supports a wide range of aquatic and terrestrial species. Geographically, the Cross River Estuary is positioned between latitudes 4°45' and 6°15' N and longitudes 8°00' and 8°55' E of the Greenwich meridian (Fig. 1). It originates as the Manyu River in the Mamfe region of Cameroon, approximately 74 km from the Nigerian border. The Cross River flows southward into Nigeria, where it expands into a broad estuarine area before emptying into the Atlantic Ocean at the Gulf of Guinea (Zapfack, et al., 2001).

The estuary covers approximately 54,00 sq. km. serving as a navigational route to different ports (Calabar, Port Harcourt, Onne, Okrika, and Bonny) in Nigeria (Emeka, et al., 2023). The estuary's open connection to the Gulf of Guinea significantly influences its ecological dynamics. Marine and freshwater inputs create a gradient of environmental conditions along the estuary, with salinity levels ranging from nearly freshwater in the upper reaches to higher salinity near the mouth where it meets the Atlantic Ocean (Otogo et al., 2023). This variation, along with tidal fluctuations and seasonal changes, creates numerous ecological niches that shape the distribution, growth, and behavior of fish populations, including the African croaker (*P. elongatus*), the focus of this study.

Fringed by extensive mangrove forests, primarily composed of *Rhizophora* and *Avicennia* species, the estuary provides crucial habitats that support high biodiversity. These mangroves, interspersed with mudflats and sandy shores, serve as breeding, nursery, and feeding grounds for various fish and invertebrate species, each adapted to distinct habitats within the estuary. Local fishing communities depend heavily on the estuary for their livelihoods, with artisanal fishing being a predominant activity. Species like *P. elongatus* hold substantial commercial value, supporting small-scale fisheries that supply local and regional markets (Asuquo & Ifon, 2022). However, anthropogenic pressures such as overfishing, habitat degradation, and pollution threaten the ecosystem. Sustainable management of fisheries within the estuary is therefore critical to conserving biodiversity and supporting the local economy.

2.2 Data Collection

Fish specimens for this study were collected from a total of 815 individuals across multiple sites within the lower Cross River Estuary in Nigeria. Sampling was conducted systematically from March to August 2024, encompassing five sites: Itu Beach, Calabar River Mouth, Oron Beach, Great Kwa River Mouth, and the estuary mouth (Fig. 1). These sites were chosen based on varying salinity gradients, habitat types, levels of anthropogenic influence, and proximity to the Atlantic Ocean. The total length of the fish ranged from 10.2 cm at Itu Beach to 53.2 cm at the estuary mouth. The specimens from the estuary mouth exhibited the longest mean total length (28.22 ± 2.38 cm), while those from Itu Beach had the shortest mean total length (22.44 ± 1.03 cm) (Table 1).

This map provides a spatial reference for the study locations, illustrating the environmental gradients affecting fish populations across the estuary. Stations S1-S5=Estuary Mouth, Great Kwa River Mouth, Oron Beach, Calabar River Mouth, and Itu Beach respectively.

Table 1. Sampling Stations (S1 – S5) and Characteristics of Fish Specimens Collected from the Lower Cross River Estuary, Nigeria

Station	Description	Latitude	Longitude	Distance (km) to the Atlantic Ocean	No. of fish samples	Size range (cm)	Mean ±SD (cm)
S1	Estuary mouth	4°35'8"	8°24'28"	8.38	150	15.0-53.2	28.22±2.38
S2	Great Kwa River Mouth	4°46'7"	8°22'32"	28.4	200	12.5-51.2	25.12±1.73
S3	Oron Beach	4°49'6"	8°15'59"	38,5	140	11.8-50.8	24.24±0.84
S4	Calabar River Mouth	4°54'36"	8°15'44"	47.7	190	11.4-51.0	23.82±0.76
S5	Itu beach	5°12'54"	7°59'50"	98.9	135	10.2-52.4	22.44±1.03

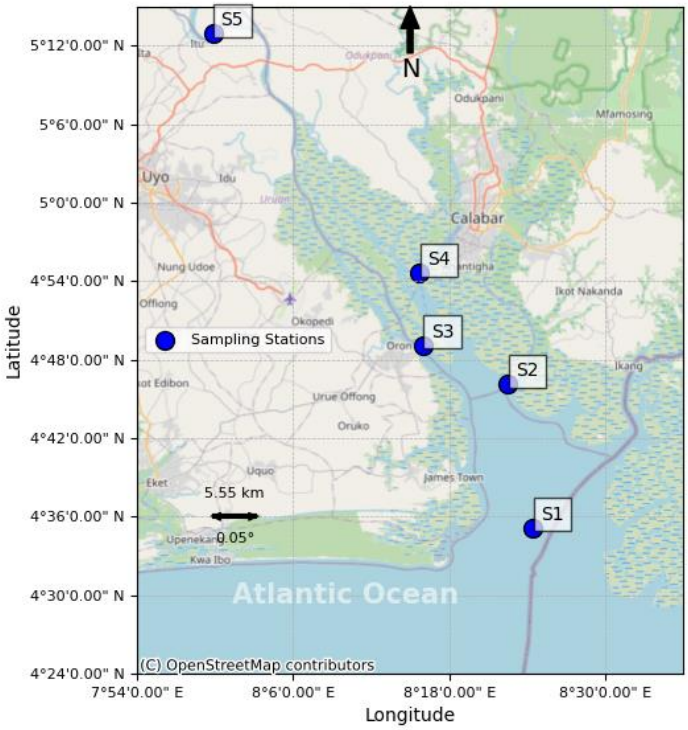


Fig. 1. Map of the Cross River Estuary with sampling stations (S1–S5) marked

2.3 Morphometric measurements

Morphometric measurements focused on 11 key body features commonly used to distinguish fish populations (Turan, 2004). These features included: Orbital Diameter (OD), Relative Eye Diameter (RED), Mouth Width (MW), Mouth Depth (MD), Trunk Thickness (TT), Length of the Pectoral Fin (LPF), Pectoral Fin Width (PFW), Length of the Caudal Peduncle (LCP), Width of the Caudal Peduncle (WCP), Depth of the Caudal Peduncle (DCP), and Length of the Caudal Fin (LCF). These traits reflect both ecological adaptations and population structure (Bookstein, 1991). To ensure accuracy and minimize human error, measurements were taken using digital calipers, recorded to the nearest 0.01 cm (Zelditch, et al., 2012). Each morphometric trait was measured three times per individual, and the average value was used in subsequent analyses to enhance reliability. A detailed methodological description of the croaker from the lower Cross River Estuary can be found in Asuquo and Ifon (2021).

2.4 Size Adjustment

In morphometric studies, variations in size can confound the results by masking true shape differences, as both the shape and relative proportions of measured characters often scale with size (Reist, 1985). To address this issue, we applied an allometry index to adjust for size-related variability among individuals. This adjustment was based on the allometric scaling equation with a standard size measure (total length) to normalize the remaining morphometric traits (Jolliffe, 2002). Specifically, the following formula was applied to each character:

$$M' = M\left(\frac{L_{mean}}{L}\right)^{b} \quad (1)$$

where M′ is the adjusted measurement, M is the raw measurement, L is the individual's total length, L_{mean} is the mean length of all sampled individuals, and b is the allometric growth coefficient for the trait (Jolliffe, 2002). This formula allows for size-independent comparison of morphometric traits, facilitating shape-based assessments across different fish populations (Elliott, et al., 1995).

2.5 Data Standardization

Following size adjustment, the data were standardized to prepare for multivariate analysis. Standardization involved normalizing the

morphometric data by subtracting the mean and dividing by the standard deviation for each trait. This step is essential in multivariate analysis, as it ensures that all variables contribute equally to the analysis, preventing traits with larger ranges from disproportionately influencing the results (Tabachnick & Fidell, 2007). Standardization also helps manage any skewed distributions within the data, ensuring that outliers do not unduly influence the results (Zar, 2010). By scaling and centering the data, we enable Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) to identify true shape variation that may otherwise be masked by differences in measurement units or scales.

The selection of morphometric traits for this study was guided by their ecological relevance and prior research demonstrating their efficacy in differentiating fish populations (Rohlf & Marcus, 1993). For instance, traits like Orbital Diameter and Relative Eye Diameter provide insights into sensory adaptations, while body depth and caudal peduncle length are indicative of swimming efficiency and habitat use, respectively (Wimberger, 1992). The preprocessing steps—size adjustment and data standardization—were carefully chosen to control for potential biases and ensure that the analyses accurately reflect shape differences influenced by environmental factors, rather than by size discrepancies or measurement variability.

3. STATISTICAL ANALYSIS

3.1 Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a widely used technique in morphometric studies for dimensionality reduction, effectively summarizing the shape-related variance of morphometric traits (Jolliffe, 2002). In this study, PCA is applied to reveal patterns in morphometric data by reducing redundant dimensions while retaining critical variance associated with shape, enabling effective separation of fish populations based on morphological characteristics (Asuquo & Asangusung, 2019).

3.2 Standardization of Data

Before performing PCA, the dataset was standardized to ensure that each variable contributed equally to the analysis. Standardization was achieved using the formula:

$$Z_{ij} = \frac{X_{ij} - \mu_j}{\sigma_j} \quad (2)$$

where Z_{ij} is the standardized value of the *i-th* observation of the *j-th* variable, X_{ij} is the original value, μ_j is the mean of the *j-th* variable, and σj is the standard deviation of the *j-th* variable. This step transformed each variable to have a mean of 0 and a standard deviation of 1.

1. **Covariance Matrix Computation:** The first step in PCA was to compute the covariance matrix Σ, which provided a measure of how each variable relates to the others. The covariance matrix is calculated as:

$$\Sigma = \frac{1}{n-1} X^T X \quad (3)$$

where n represent number of data points on all traits, X^T represents the transpose of X.

2. **Eigenvalues and Eigenvectors:** To identify the principal components, we solved the characteristic equation of the covariance matrix:

$$\Sigma e_i = \lambda_i e_i \quad (4)$$

where e_i is the i-th eigenvector (or principal component direction) and λ_i is the corresponding eigenvalue. Each eigenvalue λ_i represents the amount of variance captured by the principal component e_i , ordered such that $\lambda_1 \geq \lambda_2 \geq \cdots \geq \lambda_p$.

3. **Projection and Principal Components:** The original data X was projected onto the eigenvectors to obtain the principal components:

$$PC_i = X e_i \quad (5)$$

where PC_i denotes the projection of X onto the i-th eigenvector, yielding the principal component scores. By selecting the first k components that explain most of the total variance (determined by a cumulative variance threshold of 90%), dimensionality is reduced while retaining essential shape-related information.

4. **Variance Explained:** The percentage of total variance explained by each principal component was computed as:

$$\frac{\lambda i}{\sum_{j=1}^{p} \lambda_j} \times 100 \quad (6)$$

This measure aids in understanding the contribution of each principal component to the total variability, informing the selection of components for further analysis.

PCA was applied to the morphometric data to examine the variation in morphometric traits across fish populations from different sampling sites. The components retained based on the variance threshold were analyzed to assess site-specific morphological patterns. This approach enables the identification of shape-related differences between populations, with each principal component representing a linear combination of the original morphometric traits (Asuquo & Asangusung, 2019). By focusing on components with high variance, PCA allows for an efficient yet comprehensive comparison of morphometric variation between groups, providing insights into how environmental factors may drive phenotypic divergence.

3.3 Discriminant Function Analysis (DFA)

Discriminant Function Analysis (DFA) is employed as a classification tool to differentiate between groups based on morphological traits, which are often shaped by ecological and environmental factors (Asuquo & Ifon, 2021). In the context of this study, DFA was used to classify individual fish into pre-defined groups based on morphometric characteristics, facilitating the detection of distinct population structures within the estuarine environment. By creating a discriminant function that maximizes the separation between these groups, DFA helps to quantify the morphological divergence between populations sampled from different locations, shedding light on habitat-induced phenotypic variation (Segherloo, et al., 2018; Asuquo, et al., 2024).

Validation of the DFA model is essential to ensure the accuracy and reliability of the classification results. Cross-validation is conducted to assess the model's robustness, where the dataset is divided, and the discriminant function is applied to classify samples that were not included in model training. The model's performance was quantified through classification success rates, indicating the percentage of correctly classified samples in each group. Two key criteria guide the assessment of model reliability:

1. **Proportional Chance Criterion:** This criterion tests whether the model performs better than chance by comparing the observed classification success rate to a random allocation (Asuquo & Ifon, 2021). A statistically significant result indicates that DFA accurately classifies the samples beyond what could be expected from chance alone.
2. **Maximum Chance Criterion:** This criterion evaluates the likelihood that the model's classification rate exceeds that of the largest group in the dataset. This ensures that the DFA model is not biased toward over-represented groups, offering a balanced evaluation across all population groups (Cronin-Fine, et al., 2013).

DFA operates by creating linear combinations of predictor variables (morphometric traits) to form discriminant functions, each of which maximizes separation between predefined groups. Given k groups and p predictors, DFA identifies up to $min(k-1, p)$ discriminant functions, each contributing to group differentiation (Tabachnick & Fidell, 2007). The core DFA equation is:

$$D = w_1X_1 + w_2X_2 + \cdots + w_pX_p \quad (7)$$

where D is the discriminant score, X_i are the predictor variables, and w_i are the weights (coefficients) assigned to each predictor to maximize the between-group variance relative to within-group variance. The discriminant functions are calculated as follows:

1. **Within-Group and Between-Group Variance:** The total variance in the dataset was divided into between-group and within-group components. The DFA maximizes the ratio of between-group to within-group variance by finding coefficients w_i that best separate the groups. This ratio, termed Wilks' Lambda (Λ), assesses the discriminatory power of the function, with values closer to zero indicating greater separation.
2. **Canonical Correlation:** Canonical correlation measures the relationship between the discriminant scores and the grouping variable, with higher values indicating stronger discriminatory ability. Canonical discriminant functions are derived from eigenvalues of the matrix:

$$S_b{S_w}^{-1} \quad (8)$$

where S_b and S_w are the between-group and within-group scatter matrices, respectively. The

eigenvectors corresponding to the largest eigenvalues form the discriminant functions, while the eigenvalues represent the discriminatory power of each function.

3. **Classification:** Once the discriminant functions are computed, they are used to calculate discriminant scores for each sample, which are then compared to group centroids. A sample is classified into the group with the nearest centroid, thereby minimizing the Mahalanobis distance between the sample and group means. To enhance the model's reliability, we applied a cross-validation method, specifically the leave-one-out classification method (Lachenbruch, 2014), to estimate the potential error rates in the grouping process. This method involves sequentially omitting each sample, classifying it based on the remaining data, and assessing the accuracy of the classification.

Additionally, we tested the precision of the classification model by randomly selecting 75% of the data as a training set to develop the model, while the remaining 25% served as a test set to independently evaluate the model's efficacy. This approach allowed us to verify the model's ability to correctly classify new observations, as advised by the maximum and proportional chance criteria (Cronin-Fine, et al., 2013), which provide benchmarks for classification success rates beyond random chance.

4. SIMULATIONS AND GRAPHICAL REPRESENTATIONS

4.1 Monte Carlo Simulations

Monte Carlo simulations were utilized in this study to assess the robustness and reliability of the multivariate models, particularly Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA). In ecological and morphometric studies, Monte Carlo simulations provide a statistical method for estimating the performance of models under varied, randomized conditions, which helps ensure that results are not artifacts of sampling bias or specific parameter choices (Pokropek, et al., 2019). By generating numerous synthetic datasets, these simulations allowed us to test the stability of PCA and DFA results across potential sampling variations, reinforcing the models' validity in identifying true morphometric structures within estuarine fish populations.

The simulation process begins with the calculation of the original data parameters from the measured morphometric traits (Rauf, et al., 2024). Specifically, the means and covariance matrices are derived from the morphometric data of the 815 specimens collected across the sampling sites. The mean vector μ for each morphometric trait is calculated as follows:

$$\mu = \frac{1}{n}\sum_{i=1}^{n} x_i \quad (9)$$

where n is the total number of specimens, and x_i represents the individual measurements for each trait.

The covariance matrix Σ was computed to quantify the relationships between the traits, using the formula:

$$\Sigma = \frac{1}{n-1}\sum_{i=1}^{n}(xi - \mu)(x_i - \mu)^T \quad (10)$$

where $(x_i - \mu)$ is the deviation of each observation from the mean, and T denotes the transpose operation. This matrix captures how much the morphometric traits vary together.

Using the mean vector μ and covariance matrix Σ, we generated synthetic datasets by drawing random samples from a multivariate normal distribution. This step, essential to the Monte Carlo process, requires a random number generator (RNG) to ensure variability in the generated samples. This RNG facilitates drawing values from the specified distribution, which mimics real-world sampling conditions and enables us to evaluate how variability might impact PCA and DFA results. Each generated synthetic dataset shared the same means and covariances as the original data, mathematically expressed as:

$$x_{sim} \sim N(\mu, \Sigma) \quad (11)$$

where μ is the mean vector of the original data, Σ is the covariance matrix, and N denotes a multivariate normal distribution. In this study, 1,000 synthetic datasets were generated, each representing plausible variations in sampling conditions.

These synthetic datasets were then subjected to PCA and DFA to evaluate the consistency of trait differentiation under hypothetical sampling

conditions. For PCA, the covariance matrix was calculated as follows:

$$C = \frac{1}{n-1}\sum_{i=1}^{n}(x_i - \mu)\,(x_i - \mu)^T \quad 12$$

where n is the sample size, and x_i represents individual sample vectors. We calculated the eigenvalues and eigenvectors of this covariance matrix to identify principal components, which capture the maximum variance within the data.

For DFA, discriminant functions were derived using the means and covariances of each group to enable classification based on morphological traits. The discriminant function for group k was defined as:

$$D_k(x) = b_T^k x + c_k \quad 13$$

where b_k is the vector of coefficients derived from the means of the groups and c_k is a constant term for group k (Daikwo, et al., 2016). The classification accuracy is then evaluated by applying the discriminant functions to the synthetic datasets.

The outcomes were interpreted by examining the stability of principal components and discriminant functions across simulations. Consistency in these elements suggests that the morphometric patterns identified were likely genuine reflections of population structures, rather than statistical artifacts, thus supporting the reliability of the results (Fisher & Tipton, 2015).

4.2 Graphical Representations

To visually convey morphometric variation across different sampling sites, PCA and DFA plots were generated. These plots serve as graphical representations of the high-dimensional morphometric data reduced to two or three dimensions, providing intuitive insight into population structure and spatial morphometric differentiation (Jolliffe & Cadima, 2016). In PCA, each data point represents an individual fish, plotted based on its scores on the first few principal components, which capture the majority of morphometric variance. The spatial arrangement of points in PCA plots indicates the degree of morphological similarity or dissimilarity among individuals, with tighter clusters suggesting more homogenous populations, while dispersed points may imply diverse morphometric traits due to geographic or environmental influences.

For DFA, scatter plots of the first two discriminant functions were employed to illustrate group separation. These plots provide visual evidence of DFA's capacity to classify fish populations by morphometric traits linked to specific sites, with clearer boundaries between clusters suggesting higher classification accuracy and better-defined group distinctions.

The PCA plot highlights the primary axes of morphometric variation across populations, with component loadings indicating which traits most strongly differentiate groups. For example, elongation and body depth traits may load heavily on the first component if they contribute significantly to shape variation due to habitat adaptation. The DFA plot, on the other hand, serves as a visual confirmation of the model's classification power, displaying the spatial separation of groups as a function of discriminant scores. Each cluster represents a sampling site or population, with minimal overlap indicating effective classification based on morphometric traits (Asuquo & Ifon, 2022).

4.3 Statistical analysis

The statistical analyses and visualizations were conducted using Python (ver. 3.12.6) for windows.

5. RESULTS

5.1 Within-Group Morphometric Variation

Principal Component Analysis (PCA) revealed that the first principal component (PC1) accounted for the majority of the variance, with an eigenvalue of 0.9158, representing 91.58% of the total variation (Fig. 2). This suggests that PC1 captures the primary differences between the fish samples. The second principal component (PC2) accounted for an additional 3.66%, bringing the cumulative explained variance to 95.24%. As we move to the third through fifth components, the explained variance progressively decreased, reaching 98.94% by PC5. By PC11, 100% of the variance was explained, with the majority of variation concentrated in the first few components. These results indicate that focusing on PC1 and PC2 can effectively summarize the variation in the dataset, allowing for a simplified analysis without significant loss of information.

5.2 Between-Group Variation

Discriminant Function Analysis (DFA) showed clear morphological differentiation among the sampling stations. A 2-Dimensional DFA plot (Fig. 3) revealed three distinct morphometric groups, with Station 1 (near the estuary mouth) clearly separating from the other stations (2–5), which are located farther inland. This suggests that the proximity to the Atlantic Ocean plays a significant role in shaping the fish's morphology.

This plot shows the contribution of each principal component to the total variance, highlighting the main components that capture most of the morphological variation in the fish populations.

This visualization indicates clear separation between groups, supporting the hypothesis of morphological differentiation due to environmental influences. Stations 1-5=Estuary Mouth, Great Kwa River Mouth, Oron Beach, Calabar River Mouth, and Itu Beach respectively

The contour plot (Fig. 4) further demonstrated these patterns, with dense clusters of discriminant function scores at Stations 2–5, away from the oceanic influence. The color gradients in the plot (yellow for high-density areas and blue for low-density) highlighted the clustering of morphometric traits at each station, with Station 1 showing distinct traits from the other stations. The scatter plots (Fig. 5) of pairwise comparisons of key morphological traits confirmed these results, with Stations 2–5 showing more overlap, indicating a similar morphological group.

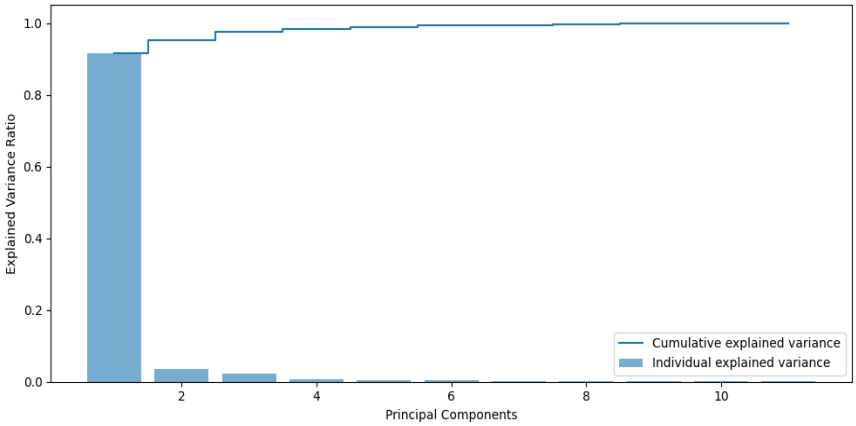


Fig. 2. Explained variance by principal components in the morphometric data set

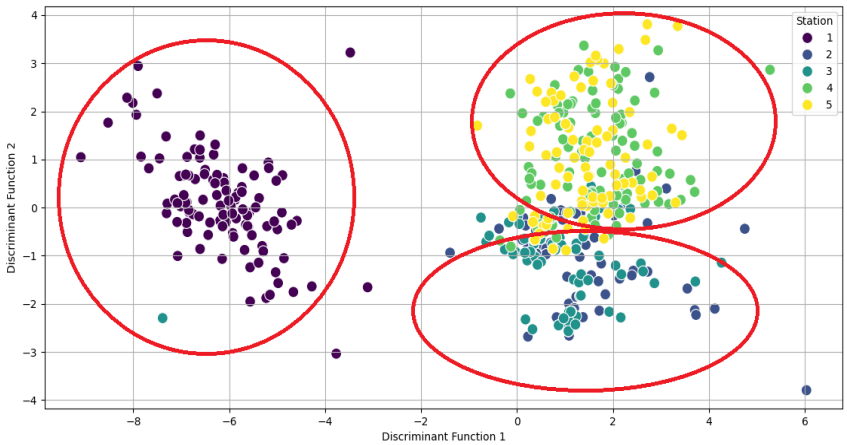


Fig. 3. Discriminant Function Analysis (DFA) plot displaying distinct morphological groupings among sampling stations

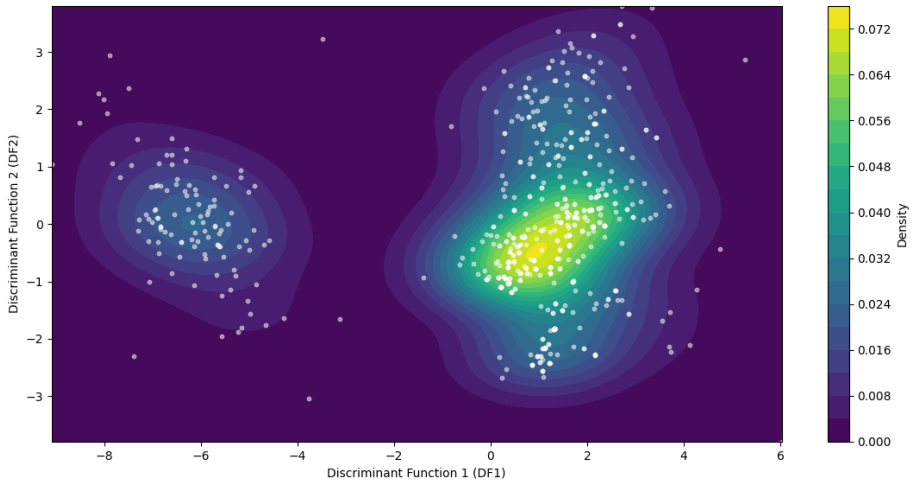


Fig. 4. Contour plot of discriminant function scores, indicating regions of low to high density across morphological groups

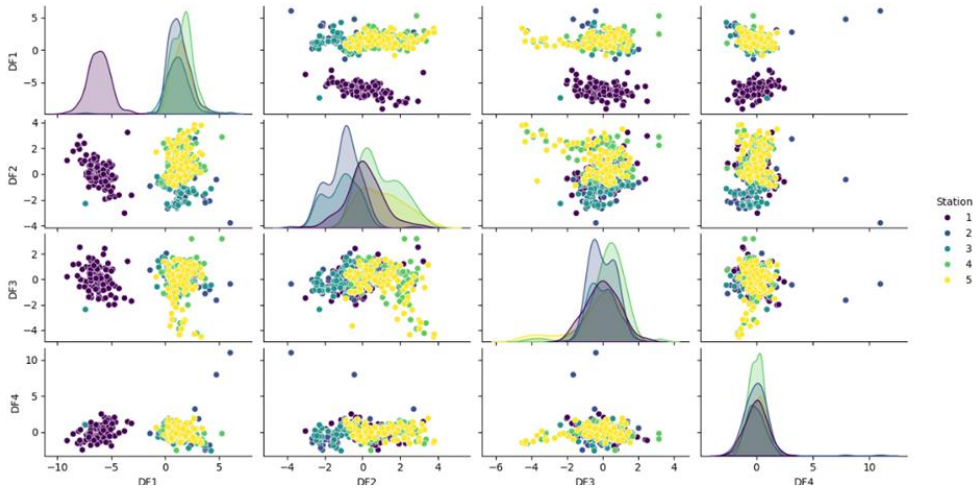


Fig. 5. Pairwise scatter plots of morphological traits across stations, illustrating relationships and clustering patterns among key traits

High-density regions, shown in yellow, represent areas where morphological traits are most similar within groups, aiding in the identification of morphological clustering patterns related to environmental conditions.

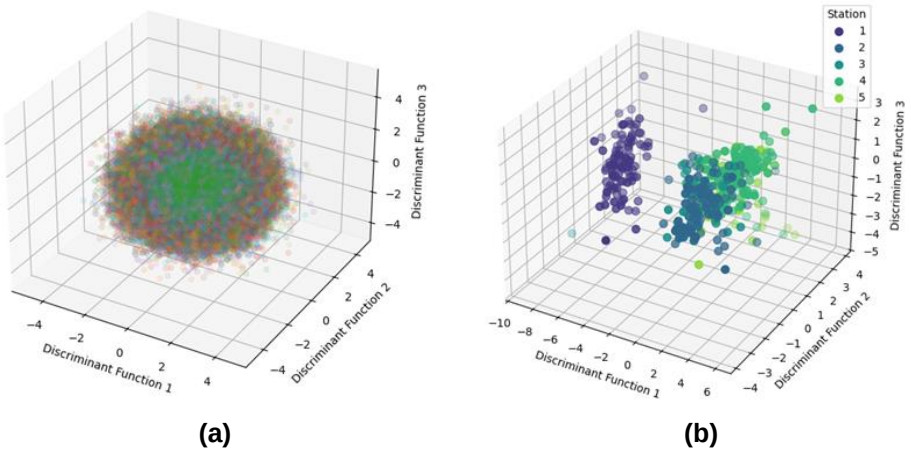
These plots provide insight into how traits vary across groups and reveal overlapping and distinct areas, supporting morphological differentiation among sampling stations. Stations 1-5=Estuary Mouth, Great Kwa River Mouth, Oron Beach, Calabar River Mouth, and Itu Beach respectively

5.3 Classification Accuracy Assessment

The DFA model's classification accuracy was evaluated using a 25% holdout set. The classification results, summarized in a confusion matrix (Table 2), indicated an overall accuracy of 61.44%. This value reflects the proportion of correctly classified fish specimens, with Group 1 and Group 4 showing higher classification accuracy. The individual group classification percentages highlighted variability, suggesting that some groups may have more distinguishing features than others. The Monte Carlo simulations (Fig. 6a) further illustrated that, under varying sampling conditions, the distinction between groups, particularly between Stations 2–5, could become less pronounced. However, the results confirmed that the DFA model was able to reliably differentiate between the groups beyond what would be expected by random chance, as shown by the p-value of <0.001.

Table 2. Confusion Matrix for Classification Accuracy of the Validation Set

		Membership						
S/N	Station	1	2	3	4	5	Sum	Correct (%)
1	Estuary Mouth	35	0	0	0	0	35	100
2	Great Kwa River Mouth	0	25	6	4	4	39	64.10
3	Oron Beach	0	17	1	1	1	20	5.00
4	Calabar River Mouth	0	2	0	24	5	31	77.42
5	Itu Beach	0	4	0	15	9	28	32.14
Total		35	48	7	44	19	153	100



(a) **(b)**

Fig. 6. Function plots comparing simulated data (a) and original data (b) to assess the robustness of DFA results

5.4 Monte Carlo simulations

The Monte Carlo simulations (Fig. 6a) evaluated the impact of sampling variability on the observed morphological distinctions. The simulations highlighted that, under certain sampling conditions, the differences between groups (Fig. 6b), especially among stations with similar environmental conditions, might be less pronounced. Nevertheless, these simulations also reaffirmed the robustness of the DFA results, suggesting that while sampling variability may influence the extent of morphological differentiation, the overall patterns remain significant.

This figure shows how sampling variability could impact morphological group distinctions, with the original data indicating stronger group separation than the simulated data. Stations 1-5=Estuary Mouth, Great Kwa River Mouth, Oron Beach, Calabar River Mouth, and Itu Beach respectively

6. DISCUSSION

6.1 Size Adjusted Traits

In this study, we applied an allometric scaling equation to adjust for size-related variability in morphometric data. Variations in size can confound the results in morphometric studies, as both shape and relative proportions of measured characters often scale with size (Reist, 1985). The allometric scaling approach allows for size-independent comparisons of morphometric traits, facilitating more accurate shape-based assessments across different fish populations (Elliott, et al., 1995). By using this method, we were able to effectively normalize size differences, ensuring that shape differences were not masked by variations in body size. Size adjustment is critical in morphometric studies to avoid the influence of body size on the shape of fish. Previous studies have employed various methods for this purpose. For instance, Asuquo and Ifon (2019) used Schaefer's (1992) approach and a modified version of Elliott et al.'s (1995) formula for size standardization in their study of *P. elongatus*. Asuquo and Ifon (2019) found that their method of size adjustment using the communal within-sample gradients effectively reduced size-related variance in their data. In contrast, our study opted for an allometric scaling equation based on total length as the standard size measure. The allometric scaling method used in the present study is consistent with other

shape-based morphometric studies and allows for comparisons across populations with varying body sizes without introducing redundancy or over-adjustment.

The choice of size adjustment method is important for ensuring the accuracy of subsequent analyses. While both approaches effectively address size-related variability, our decision to use the allometric scaling equation was informed by the need for consistency with widely applied methods in the field of fish morphometrics. In this regard, our findings align with studies that utilize allometric scaling for size normalization, such as those by Elliott et al. (1995), who demonstrated its effectiveness in characterizing shape variations independent of size. Our study found that after size adjustment, significant differences in morphometric traits were observed between different populations of *P. elongatus*. These differences were consistent with findings by Asuquo and Ifon (2019), who reported significant variations in the body shapes of *P. elongatus* across different locations within the Cross River Estuary. However, unlike Asuquo and Ifon (2019), who used a pooled dataset, we performed a more rigorous comparison of individual measurements across samples. This enabled a finer level of analysis, which revealed subtle shape differences that might have been overlooked in studies with broader sample pooling. The use of size-adjusted traits in our study supports the idea that shape variations in *P. elongatus* are primarily influenced by environmental factors and not simply by size. As highlighted by Elliott et al. (1995), such size-independent comparisons allow researchers to better understand the ecological and genetic factors influencing population structure and distribution. In our case, size adjustments revealed that environmental factors such as salinity gradients and tidal influences likely play a significant role in shaping the morphology of *P. elongatus* populations in the Cross River Estuary, consistent with the observations of Asuquo and Ifon (2019).

6.2 Within-Group Morphometric Variation

The results of the Principal Component Analysis (PCA) showed that the first principal component (PC1) explained 91.58% of the total variance, with the second principal component (PC2) accounting for an additional 3.66%, collectively explaining 95.24% of the variance. This high proportion of variance captured by PC1 and PC2 aligns with findings from other studies investigating morphometric variations in fish populations. For example, Turan (2004) observed that PC1 accounted for approximately 80% of variance in *Sardina pilchardus*, while Khan et al. (2012) reported that the first three components in *Channa punctatus* explained 63.41% of total variation. These findings suggest a common pattern where the primary morphological differences in fish populations are concentrated in the first few components. A related study by Asuquo and Ifon (2019) on *Pseudotolithus elongatus* in the Cross River region found that PC1 and PC2 explained 85.6% and 11.3% of variance, respectively, indicating that most morphological distinctions were captured by the initial components. This is consistent with the present study, though our PC1 explains a slightly higher percentage, likely due to species-specific differences or methodological factors, such as sample size or measurement precision.

Asuquo and Ifon (2021) also highlighted that Yakubu and Okunsebor (2011) preferred using the most discriminative character in PC2 to identify stock structure, as PC2 is less affected by fish size. However, the inclusion of variables from both PCs is supported in the current study due to the application of an allometric transformation (Elliott, et al., 1995) to minimize size-related variability. By addressing size effects through allometric scaling, the observed within-group morphological variation is attributed to shape rather than ontogenetic growth patterns (Schaefer, 1992). This approach is also consistent with morphometric research that recommends size-standardization to avoid inflated variances arising from size overlaps among samples (Reist, 1985; Asuquo & Ifon, 2019). Overall, the significant role of environmental factors in shaping morphological diversity is evident in this study, reinforcing findings from earlier research.

6.3 Between-group Morphometric Variation

The Discriminant Function Analysis (DFA) results revealed distinct morphological differentiation among the sampling stations, highlighting three morphologically distinct groups shaped by environmental gradients. Specifically, Station 1, located near the estuary mouth, exhibited clear separation from Stations 2–5, which are further inland and less affected by the direct oceanographic forces of the Atlantic. This pattern suggests that the morphological traits of

estuarine croaker populations are influenced by varying environmental conditions at different stations, consistent with studies on fish population structure and morphometric variation.

For example, Telles et al. (2014) found similar differentiation in the Amazon Basin, where *Pseudoplatystoma punctifer* populations exhibited morphological and genetic divergence due to spatial constraints on gene flow. Although Telles et al. (2014) primarily examined genetic diversity, their findings highlighted how spatially structured dispersal and isolation-by-distance patterns affected genetic similarity, with populations up to 80 km apart showing more similarity than those further away. This localized structure suggests that environmental gradients, along with geographic isolation, can influence population differentiation. Analogous to our study, where distinct morphometric groups emerged along an estuarine gradient, Telles et al.'s work indicates that environmental factors such as salinity, temperature, and substrate type may drive morphological divergence in spatially structured populations. In contrast, Asuquo and Asangusung (2019) found minimal morphometric differentiation among *Chrysichthys nigrodigitatus* populations across closely situated rivers on Nigeria's southern coast. Their findings, which align with the isolation-by-distance model (Smith & Weissman, 2023), indicated limited morphometric variation due to stable environmental conditions. This differs from our study's marked morphometric differentiation along an estuarine gradient, where sharp environmental variations likely drive distinct morphological traits, particularly between the estuary mouth (Station 1) and more inland stations.

Asuquo and Ifon (2021) similarly highlighted morphometric disparities in *P. elongatus* populations at island and estuary mouth locations, suggesting that geographic separation and environmental variation within estuaries contribute to distinct morphometric traits. This aligns with the morphometric divergence observed in our study, where environmental gradients such as salinity and substrate type create distinct groups. Further molecular analyses, as suggested by Asuquo and Ifon, could confirm whether these morphological differences indicate potential genetic divergence as well. The clustering patterns in contour plots support these findings, with stations displaying similar environmental conditions showing overlapping morphometric traits. The clustering reinforces the role of environmental gradients in shaping morphological differentiation, consistent with the ecological impact of estuarine variation on fish morphology (Wang & Bradburd, 2014).

6.4 Classification Accuracy Assessment

The discriminant function analysis (DFA) in the present study achieved a classification accuracy of 61.44%, indicating the proportion of African croaker specimens accurately classified into morphological groups based on environmental variations across sampling stations. This moderate accuracy aligns with previous studies using DFA to classify fish specimens by morphological characteristics, where environmental factors often influence classification precision. For instance, Kumari et al. (2020) reported a classification accuracy of 66.8% in a study of congeneric sciaenid species, attributing variations in accuracy to the number of discriminating traits and the environmental homogeneity of the sampling areas. The higher accuracy observed in Kumari et al. (2020) suggests that homogeneous environments contribute to clearer group differentiation, while in heterogeneous environments like the estuarine setting of the current study, ecological complexity can result in overlapping morphological traits and lower classification accuracy.

Similarly, Mendoza-Barrera et al. (2018). achieved a 66.25% classification accuracy for *the* red snapper (*Lutjanus campechanus*) populations in the Southern Gulf of Mexico, likely due to stronger environmental gradients that more distinctly separated coastal groups. In contrast, the more subtle morphological differences among African croaker populations in this study may have contributed to the lower classification accuracy, highlighting the nuanced influence of environmental variability on morphological differentiation. The present study's accuracy of 61.44% falls within the range commonly observed in DFA applications, underscoring the variability in morphological traits due to environmental factors and the challenges inherent in distinguishing groups under similar ecological conditions. This accuracy suggests that refining discriminating variables or adopting improved sampling strategies could potentially enhance classification precision in future studies.

Asuquo and Ifon (2021) achieved a slightly higher classification accuracy of 66.0% for African croaker, noting that estuary mouth

samples were correctly classified without misclassification into island samples. They attributed classification accuracy differences between studies to potential errors due to parallax in traditional measurements, although they minimized instrument error by maintaining consistency in measurement procedures. Additionally, Asuquo and Ifon (2019) previously reported a 77.5% classification accuracy for bobo croaker in the Cross River Estuary, which Zhang et al. (2020) suggests could reflect the influence of different sampling sizes. Individual group classification accuracy varied within the present study, with Groups 1 and 4 showing higher classification rates than other groups. This pattern resembles Yakubu and Okunsebor (2011), who found that groups with more distinct morphological traits (e.g., body size or fin morphology) demonstrated higher classification accuracy. In this study, such variation emphasizes that groups with subtle morphological differences are more challenging to classify accurately, reaffirming the importance of selecting robust discriminating traits to improve DFA model performance.

6.5 Monte Carlo Simulations

The Monte Carlo simulations conducted in this study provided valuable insights into the robustness of the Discriminant Function Analysis (DFA) results and the impact of sampling variability on observed morphological distinctions. These simulations revealed that under different sampling conditions, particularly at stations with similar environmental contexts (such as Stations 2–5), the groupings observed in the DFA could become less pronounced, highlighting the influence of sampling variability on the clarity of morphological differentiation. Mode and Gallop (2007) emphasize the importance of transparency and reproducibility in Monte Carlo simulations, noting that clear documentation of underlying mathematics and simulation parameters allows other researchers to replicate results accurately. Following their recommendations, we ensured that the random number generator and the sampling parameters used in this study were clearly specified, enhancing the transparency and reproducibility of our approach. Mode and Gallop (2007) also point out that rigorously selected random number generators can ensure the independence of generated samples, a factor that is critical in morphometric studies where sampling variability can easily introduce unintended biases.

Our approach aligns with the insights of Cadrin and Friedland (1999), who describe the application of advanced morphometric techniques, such as image analysis systems and landmark-based morphometry, to improve stock identification by capturing and analyzing shape variations with increased accuracy. They observed that traditional morphometrics, even when effective, benefited significantly from enhancements in image processing and geometric morphometrics, allowing for a more comprehensive and precise representation of morphological differences. This aligns with our findings, where DFA robustness was impacted by sampling variability but still retained detectable structure under realistic environmental conditions. Our simulations extend these morphometric principles by ensuring clarity and precision in the observed groupings even under fluctuating environmental contexts, providing a similar advantage in accurately distinguishing morphological traits.

Furthermore, Mode and Gallop (2007) highlight the relevance of simulating complex systems with variable conditions, a concept applicable here as morphometric group clarity varies with environmental context. Our simulations incorporate these principles, reinforcing our DFA's robustness under realistic conditions of environmental variability. This approach is consistent with Basson (2002), who discusses the use of simulation approaches, specifically Management Strategy Evaluation (MSE), for evaluating robustness in complex systems under uncertainty. While MSE typically applies to fisheries management, Basson’s focus on simulating different scenarios to test decision frameworks under varying conditions is conceptually similar to our simulations, where we evaluate the robustness of DFA results across varying environmental and sampling conditions.

Bresnahan and Jamison (2007) further demonstrate that Monte Carlo simulations can reveal biases across sample sizes, noting that smaller sample sizes often lead to increased variability and bias. Applying these insights to our study, smaller sample sizes and increased sampling variability may have impacted the clarity of morphological distinctions at certain stations, as observed in our simulations. Their findings emphasize the importance of using sufficiently large sample sizes and robust estimation methods to minimize variability and improve classification accuracy in morphometric studies. The robustness of our DFA model, as

confirmed by these simulations, is consistent with Zhang et al. (2020), who found that despite smaller sample sizes or highly variable environmental gradients, the main structure of morphological groups generally remained identifiable. This trend reinforces that while sample variability can blur group distinctions, the overall group structure in DFA remains detectable, lending further credibility to our results. Insights of Mode and Gallop (2007) on maintaining a robust simulation framework underlie our approach, further validating our Monte Carlo-based findings in assessing the robustness of morphological classifications under variable sampling scenarios (Ferrito, et al., 2007).

7. CONCLUSION

This study highlights the significant impact of environmental gradients on morphological variation within estuarine croaker populations in the Cross River Estuary. Multivariate analysis revealed distinct morphological groupings, especially between the estuary mouth, influenced by Atlantic oceanographic forces, and more inland stations. While classification accuracy in the original model was moderate, simulations underscored how sampling variability affects results, particularly in areas with minor environmental contrasts. These findings suggest the need for management strategies that consider local environmental differences, providing essential guidance for effective fisheries management and conservation in diverse estuarine ecosystems. Further meristic and genetic studies in combination with morphometrics are needed to fully discriminate stocks at scales that are not detected by the 11 morphometric variables used in the present study.

ETHICAL APPROVAL

This study adhered to the ethical standards for the welfare and care of experimental animals as outlined by the National Veterinary Research Institute (NVRI), Nigeria. The research protocol was reviewed and approved by the Animal Use and Care Committee (AUCC).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The authors hereby confirm that no generative artificial intelligence tools, such as Large Language Models (e.g., ChatGPT, Copilot) or text-to-image generation technologies, were employed in the preparation, writing, or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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