MORPHOMETRIC, MERISTIC AND TRUSS NETWORKING DISTANCES AMONG THREE SPECIES OF CROAKER (*Johnius borneensis*, *Johnius argentatus* and *Johnius belangerii*) FROM THE BAY OF BENGAL, BANGLADESH

Sonjoy Kumar Modak

Roll no: 0122/06

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Fish Biology and Biotechnology

Department of Fish Biology and Biotechnology

Faculty of Fisheries

Chattogram Veterinary and Animal Sciences University

Khulshi, Chattogram-4225

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects and that all revisions required by the thesis examination committee have been made.

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Dr Helena Khatoon	**Md.Main Uddin Mamun**
Supervisor	**Co-supervisor**

Dr. Md. Mahiuddin Zahangir

Chairman of the Examination Committee

Department of Fish Biology and Biotechnology

Faculty of Fisheries

Chattogram Veterinary and Animal Sciences University

Khulshi, Chattogram-4225, Bangladesh

June 2023

Dedicated

To

My Respectable

Teachers

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The Author

Sonjoy Kumar Modak

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Abstract

Morphometric features are efficiently used for the improved differentiation and long-term management of fish populations. Truss analysis is a frequently used taxonomic method to dissimilitude the morphologically similar species as well as a stock identification tool. It's a geometric morphometric method that uses landmarks in a fish body to acquire information about the shapes of fish. Using landmark-based truss networking techniques following 18 morphometric lengths, 10 meristic counts, and 23 truss network distance, the morphological variation of three croaker species (*Johnius borneensis*, *Johnius argentatus*, and *Johnius belangerii*) were analyzed from the northern Bay of Bengal. Data were analyzed and visualized through univariate ANOVA, canonical discriminate function analysis (DFA), and principal component analysis (PCA). Results found that the first DFA was responsible for 80.6% and 81.1%, whereas the second DFA was responsible for 19.4% and 18.9% variations among three species for the morphometric and truss distances respectively. In addition, 14 out of 18 morphometric measurements and 12 out of 23 truss-network measurements were significantly different ($p < 0.05$) among means of three different species. 1st PCA found 70.76% and 64.49% explained variations and 2nd PCA displayed 12.8% and 11.5% explained variations respectively for morphometric and truss distances among the three species. The high degree of morphological variations among the three species of croaker demonstrates *Johnius borneensis* and *Johnius belangerii* are morphologically highly similar and *Johnius argentatus* is completely distinct which may be due to their physiological and genetic variations among the studied species. The findings of the present study may be helpful in identifying species differences and for the management action in the Bay of Bengal. Further studies regarding the molecular characterization of the cytochrome c oxidase subunit I (COI) gene could finally determine the genetic variations among these three species from other croakers in the Bay of Bengal.

Keywords: morphometric and meristic characters, truss networking, croakers, principal component analysis, discriminant function analysis.

Chapter-1
Introduction

1.1 Background of the study

Stock identification is a key concept in fisheries science, which is concerned with the study of self-sustaining components within natural populations (Cadrin et al., 2005). Different stocks in a given ecosystem are constantly evolving as a result of ongoing evolution within an existing species or population and their interdependent reactions to the environment. Because individuals from geographically distant populations routinely interbreed, there are frequent variations in body shape among stocks of the same species (Anvari et al., 2011; Sajina et al., 2011). Diverse rates of growth, development, and maturation in response to environmental factors help organisms eliminate or add different ontogenetic traits that give rise to novel traits or physiology (Cadrin, 2000). Because each stock exhibits different traits than others, all emerging stocks must be identified and managed individually for improved yields and conservation. Inadequate knowledge about existing stocks can lead to overexploitation, poor management, and reproductive and conservation failure (Devaraj and Vivekanandan, 1999). Therefore, proper identification of existing stocks should be developed for better fisheries stock management.

Body shapes vary across species due to differences in growth, development, and maturation (Cadrin, 2000). Because body form is a product of ontogeny, patterns of morphometric variation in fishes indicate differences in growth and maturation rates. Important population traits including reproduction, fecundity, and longevity are governed by the environmental circumstances in a particular habitat. Fish populations in aquatic ecosystems are dispersed over large geographic areas with oceanographic barriers like temperature, salinity, food and predation. These characteristics are closely correlated with the carrying capacity, productivity, and resilience factors of population dynamics (Cole, 1954). For an understanding of population dynamics and an assessment of sustainable harvests, it is thought to be required to identify intraspecific groups with different life history traits. Knowledge of stock structure allows for the development of management techniques that will aid in the preservation of biodiversity associated with various species, subspecies, stocks, and races (Turan et al., 2005). By maintaining distinct stock management, determining the stock-wise population abundance, maintaining biologically viable levels of productivity, assessing the responses of each stock to fishing exploitation and by ecosystem modeling, the goal of fishery stock assessment is achieved. Since every stock responds to exploitation, groups with different growth or reproductive patterns should be addressed and treated separately for the purpose of stock assessment (Cadrin and Silva, 2005). It is possible to separate and manage a distinct population on the basis of phenotypic and genotypic variation among fish populations within a species that emerged as a result of a sufficient level of isolation (Turan, 2004). The whole impact of management efforts, including determining the stock complexity of a fish species, must be taken into account as a fundamental element of stock identification.

Numerous fish stocks have been effectively differentiated using conventional multivariate morphometric analysis, which takes into consideration both size and shape variation (Turan, 1999). Meristic, morphometric, traditional tags, parasites as natural tags, otolith chemistry, and a number of molecular markers (such as protein allozymes, mitochondrial DNA, and microsatellite DNA) have all been proposed as methods for stock identification. However, morphometric traits have proven to be the most widely used and economically expedient approaches (Sajina et al., 2011). In addition, the use of image processing techniques has strengthened conventional procedures that result in better data collection, more accurate shape descriptions, and new analytical tools. The improvement and diversification of morphometric techniques have been made possible by the creation of image analysis systems, which also increases the possibility for employing morphometry as a stock identification tool (Cadrin and Friedland, 1999). According to form variation, truss networks also significantly more effective than manual measurements in identifying intraspecific groups with different life cycle stages (Corti et al., 1988; Strauss and Bookstein, 1982). Populations from various geographic regions exhibit morphological variance, which is thought to be caused by either differing genetic structures or regional environmental factors (Kinsey et al., 1994). Therefore, organisms with similar morphometric traits are frequently thought to make up a stock.

Fish have more diverse morphological differences within and between populations than any other animal (Brraich and Akhter, 2015). All fish species share measurable traits known as morphological features. To determine each unique shape for each fish species, landmarks—a randomly chosen group of places on the body—are used. A landmark is a place on an object that corresponds between and among populations. In addition, meristic measurement is a branch of ichthyology that studies the quantitative traits of fish, including the number of scales or fins. A fish species can be described or a fish species can be determined using a prominent countable attribute of that particular species. After all, meristic and morphology are still frequently employed to distinguish between species and to assess the phenotypic characteristics used in genetic analyses. Several populations including *Trachurus mediterraneus* (Turan, 2004), *Megalaspis cordyla* (Sajina et al., 2011), *Alosa pseudoharengus* (Cronin-Fine et al., 2013), and *Acanthocybium solandri* (Zischke et al., 2013) have been identified using morphometric analysis. To date, several methods of morphological analysis have been developed for differentiating stocks, including univariate comparison, bivariate growth pattern analysis, and multivariate analysis (Cadrin, 2000). For specific acknowledgment of morphological features in stock identification, multivariate analysis using principal component analysis (PCA), discriminate function analysis (DFA), or canonical discriminate function analysis has recently gained popularity (Specziar et al., 2009; Yakubu and Okunsebor, 2011; Cronin fine et al., 2013).

The Truss network system is a landmark-based method of geometric morphometric, which has no restriction on the directions of variation and localization of shape changes and is very useful in capturing information about the shape of an organism (Cavalcanti et al., 1999). This technique has been utilized more and more to differentiate between population shape differences and within-population allometry (Tarun, 1999). Because of this, understanding a species' stock structure is crucial. The truss-network system is utilized as a tool for fisheries management since it can quickly analyze a large number of samples. The truss system can be used to explore stock separation within a species with the same set of measurements, which enables, over time, a better and more direct comparison of the morphological history of stocks. Landmark-based truss network approach has been applied to differentiate among four different *Macrognathus aculeatus* fish population (Sarower et al., 2021). In addition, a landmark-based truss network study also applied in differentiating stock and species of *Lepidocephalichthys guntea* (Sarower et al., 2019), *Rhinomugil corsula* (Hossain et al., 2015), *Cobitis keyvani* and *Cobitis faridpaki* (Mousavi-Sabet and Anvarifar, 2013), *Sillaginopsis panijus* (Siddik et al., 2016), *Labeo calbasu* (Hossain et al., 2010) and many other species.

The vast and enormous water resources of Bangladesh including ponds, ditches, lakes, tiny canals, major rivers, and estuaries cover an estimated 4.34 million hectares. Bangladesh has 714 km of coastline along the Bay of Bengal having a coastal area of 2.30 million hectares, which is home to a substantial coastal and artisanal fishery. There are approximately 795 species (including 12 exotic species) of fish and 61 species of shrimp and prawns available in the freshwater and marine waters of Bangladesh (Mazid, 2002). Moreover, the share of GDP in the fishing sector slightly increased to 3.57 percent in FY 2020−21 from 3.56 percent in FY 2019−20 (Bangladesh Economic Review, 2021). Among them, in Bangladesh marine water, there are 20 species of croaker under 12 genera have been reported.

Croakers, also known as Jewfish or Poa fish locally, are one of the most extensively fished species in the Bay of Bengal waters. Croakers are members of the Sciaenidae family and are classified as Perciformes. The species, *Johnius* are the most abundant, and seven other croakers are very important and play a vital role in the national economy of Bangladesh. Croakers have made a demandable market both locally and internationally, especially in Saudi Arabia, Kuwait, Qatar, and Middle-East countries in fresh and dry conditions. Due to the high demand for croakers and other fisheries, there is increasing fishing pressure in the coastal waters of Bangladesh. Additionally, the stock of both pelagic and demersal fisheries in this area is being hampered by the unselective operation of set bag nets (SBN) and other fishing gear in coastal seas. Because of heavy fishing pressure, the croaker has been overfished for many years. The Pama croaker (Poa) constitutes a major portion of the total catch, which is the 3rd most available fish in Cox's Bazar (DoF, 2022). They are found mainly in the estuaries, and the Bay of Bengal and they enter rivers as far as the tide extends (Rahman, 2005). Within several types of poa fish, *Johnius borneensis, Johnius argentatus*, and *Johnius belangerii* are more available and demandable. However, there are just a few studies on the length-weight relationship, growth patterns, and mortality of a few croaker species, and there is no comprehensive data on the state of the croaker stock in the Bay of Bengal, Bangladesh. Although being commercially harvested off the coast of the Bay of Bengal, croakers are difficult to distinguish from one another. In Bangladesh, landmark-based truss networking and morphometric analysis on croaker fish from the Bay of Bengal, Bangladesh, is not performed. As a result, the present investigation employs multivariate analysis of morphometric, meristic, and landmark-based truss networking to examine the morphometric and form variations among three species of *Johnius* croaker (*Johnius borneensis*, *Johnius argentatus*, and *Johnius belangerii*). So, this study is designed to develop a morphometric, meristic, and landmark-based truss networking description of three commercially important croaker species from the Bay of Bengal.

1.2 Aim and Objectives of the study

The objectives of the proposed research are as follows:

1) To determine the morphometric, meristic and land-mark distances among *Johnius borneensis*, *Johnius argentatus* and *Johnius belangerii* from the Bay of Bengal.
2) To differentiate three croaker species more precisely based on their morphological features.

Chapter-02

Review of Literature

2.1 Genus- *Johnius*

A genus of marine ray-fined fish called *Johnius* is a member of the Sciaenidae family, which includes both drums and croakers. They can also be referred to as croakers because of their ability to produce croaking, knocking, and purring sounds. The sounds are primarily made at night and are assumed to be either used for courting or protection (Lin et al., 2007). Marcus Bloch devised the genus name in 1793 based on a specimen named *Johnius carutta* that was obtained from Reverend Christoph Samuel John in Tranquebar. The genus has 35 species, all of which are found in the Indo-West Pacific seas (Chao et al., 2019).

2.2 Taxonomy and characteristics

Within genus *Johnius*, there are two recognized subgenera. The nominate subgenus has an inferior mouth and uniformly sized lower jaw teeth, while there may be a few molar-like teeth in the back of the jaw. Additionally, the teeth on the back of the upper jaw are very closely spaced. In contrast to the subgenus *Johnieops*, which has an inner row of larger teeth on the lower jaw and a terminal or subterminal mouth, the outer teeth on the upper jaw are typically widely spaced out (Sasaki, 2022).

Johnius are normally smaller than 30 cm (12 in) in length. They have a swim bladder that resembles a hammer and has 12 to 20 pairs of dendritic appendages on each side (Sasaki, 2022). The dorsal corner of the gill hole is reached by the first lateral appendage. Large triangular paired sagittal otoliths are seen. The majority of these fishes have a tiny mouth that is inferior to sub-terminal. Most species lack a barbel on the chin, while a few species have a small barbel there. *J. dorsalis* and *J. dussumieri* are the largest species and can both grow to a maximum known total length of 40 cm (Chao et al., 2019).

2.3 Morphometric and meristic characters of croakers

A relatively deep body is a distinguishing feature of the bearded croaker (*Johnius amblycephalus*). A stiff and blunt barbell is present on the chin, and the steep snout, bluntly shaped, and projects in front of the upper jaw. Total length ranges between 216 to 230 mm. The third and second spines are somewhat extended. The second section of the dorsal fin has one spine and 24 rays after 10 spines and a notch. Two spines and seven rays make up the anal fin. Caudal fin has a rhombic shape. There were three to four gill rakers on the upper limb and eight on the lower limb of the first gill arch. In another species, five dark bars that slant from the back to the lower portion of the flanks on the blotched croaker (*Nibea maculata*) help to identify it. Snout extending past the upper jaw. Lacking barbels, the mouth is inferior. Between 200 to 210 mm in total length. The tail had a large, curving tip that was sharply edged. The posterior portion of the dorsal fin has one spine followed by 22–23 rays, whereas the anterior portion has 10–11 spines. Seven rays and a two-spined anal fin follow. Five to six gill rakers on the top limb and ten on the lower limb of the first gill arch (Al-Faisal and Mutlak, 2018).

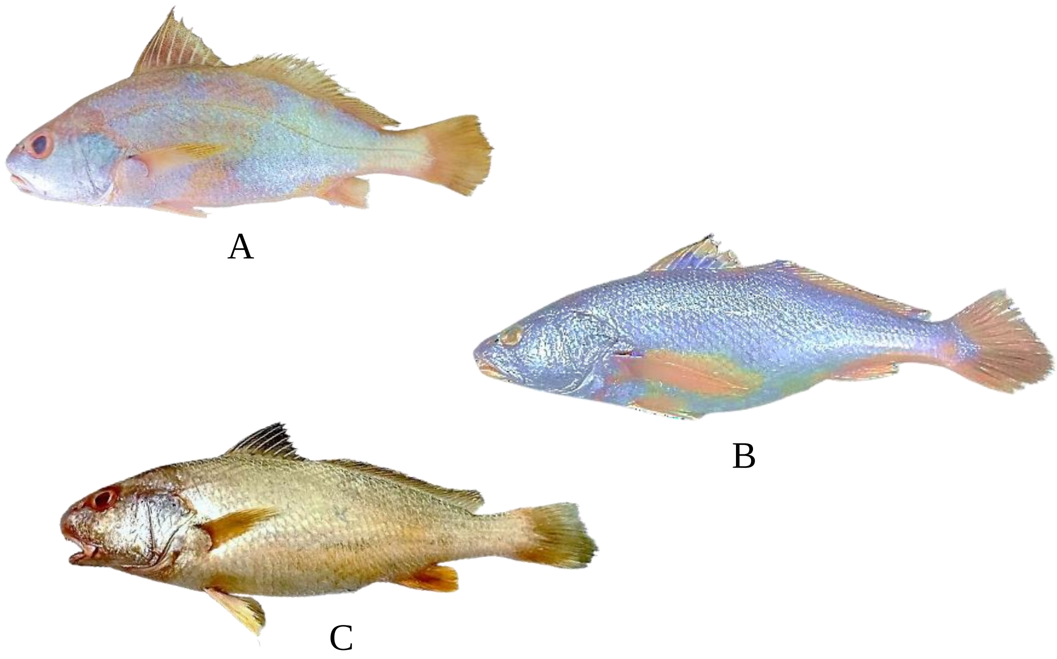

Plate-1: (A) *Johnius borneensis,* **(B)** *Johnius argentatus,* **(C)** *Johnius belangerii*

The lengths *Otolithes ruber* varying from 11.2 cm to 45.2 cm. The largest specimen ever caught was about 45 cm during April on the Thoothukudi coast (Santhoshkumar et al., 2014). In India, *J. borneensis* reaches a total length (TL) of 14−16 cm and an average size of 24 cm and discriminant function analysis (DFA) revealed an overall classification accuracy of 80%. Individual phenotypic variances may be determined by environmental factors such as salinity, temperature, food availability, or persistent swimming at a given place (Sibinamol et al., 2020).

Johnius is the most diversified sciaenid genus with verified monophyly. However, because of their external morphological similarities and overlapping meristic counts, accurate identification is difficult. Hence, *J. borneensis* and *J. belangerii* resolved as polyphyletic groups here, they represent the two more problematic species within the genus. Regretfully, researchers were unable to determine whether this was a species misidentification because the sequence of this taxon was acquired from GenBank (Sasaki et al., 2001).

Otolithes cuvieri and *Otolithes ruber* species were overlapping in terms of head length, orbit diameter, snout length, body depth, caudal depth, and caudal peduncle length, inter orbital distance, and second anal spine length to the standard length or head length. Based on the number of gill-rakers on the lower limb of the first-gill arch and the number of arborescent appendages present on the swim bladder, *O. cuvieri* and *O. ruber* can be distinguished. Trewavas (1977) detected 29−32 dorsal soft rays for *O. cuvieri* and 27−30 for *O. ruber*. A similar observation was also done by FAO (Kumari et al., 2021). The total number of lateral line scales in *O. pama* was 48–52 while Talwar (1995) have reported only 44 – 48. Environmental factors like temperature, oxygen, salinity, pH, food availability, and growth rate may all play a role in these variations, which have been observed in other species (Bhakta et al., 2020).

Johnius borneensis also referred to as the sharp teeth hammer croaker or the sharp nose hammer croaker—is one of the significant Sciaenids that contribute to the fishing in India. The species is typically evaluated alongside other Sciaenids such as *Pennahia anea, Nibea maculata, Johnius sina, J. glaucus, Otolithes ruber, O. cuvieri*, and *Otolithoides biauritus*. According to research conducted in India, *J. borneensis* matures at a total length (TL) of 14 to 16 cm and grows to an average size of 240 mm TL in two years (Sasaki et al., 2001).

The *J. borneensis* samples were selected at random from four commercial marine fish landing centers in India: Mumbai (Versova) in Maharashtra, Veraval (Mangrol) in Gujarat, and Chennai (Kasimedu) in Tamil Nadu on the east coast and Kakinada in Andhra Pradesh on the west coast. The first and second canonical variables' Eigen values (values >1) in the canonical discriminant analysis were found to be 3.7847 and 1.1658, respectively, accounting for 73% and 22.5% of the variation across the samples analyzed from various locations. The location-based bivariate plots showed a clear distinction between the stocks in Chennai and Kakinada, whereas considerable mixing was seen between the stocks in Mumbai and Veraval. In contrast to stocks from other places, the Kakinada stock demonstrated a distinct vertical separation. Additionally, substantial differences between the various stocks were revealed by the multivariate test statistics and pairwise Mahalanobis distances across stocks (Sibinamol et al., 2020). A study combined the outcomes of morphological and genetic methods, verifying seven distinct *Johnius* species in Taiwan waters. The fact that all *Johnius* specimens were monophyletic and form a single phylogenetic cluster as well as being morphologically and genetically similar supported this statement. In the fundamental group clade, their results were consistent *(J. borneensis, J. amblycephalus*, and *J. distinctus*), with the exception of *J. grypotus, J. taiwanensis, J. belangerii,* and *J. trewavasae* had different phylogenetic positions. These variances could be explained by the diverse sample sites and species found. *Johnius* species vary in their physical characteristics, such as their gill rakers and mouth forms. This may be the result of eco-phenotypic factors that affect sympatric species' jaw lengths, gill rakers and anal spine lengths in order to compete for coastal habitat (Norhafiz et al., 2022).

To date, very little is known about the morphological features about the *Johnius* species from the Bay of Bengal, Bangladesh. Though there are few research conducted on the food and feeding habits, reproductive biology, stock assessment and some other life history traits, very few has been known about the comparative morphological differences among three species of croaker (*Johnius borneensis*, *Johnius argentatus*, and *Johnius belangerii*) from the Bay of Bengal. Therefore, this study is undertaken to know the morphometric variations among three species of croaker using truss-network distances among *Johnius borneensis*, *Johnius argentatus*, and *Johnius belangerii* from the Bay of Bengal.

Chapter-3

Materials and Methods

3.1 Ethics statement

The study was conducted in a field location that is not privately owned or protected. There was no endangered or protected species involved in the study. Thus, the ethical issue was not necessary for the described study in Bangladesh.

3.2 Sample collection

A total of ninety (thirty individuals for each species) individuals of poa fish *(Johnius borneensis, J. argentatus,* and *J. belangerii)* were collected from the marine fisheries landing center, Baro-bazar fish market, Baharchora fish market of Cox's Bazar (plate-2). Apparently healthy, fresh and disease-free specimens were collected from September to November, 2021 and were considered for the morphometric study. On field identification of the target species was done by observing the external phenotypic traits. Visual assessment of genital organ and external sexual features was used to determine fish sex. Immediately after collection, the collected species were kept in the bags and ice boxes before being taken to the lab at Coastal Biodiversity, Marine Fisheries and Wildlife Research Center, Cox's Bazar of Chattogram Veterinary and Animal Sciences University (CVASU).

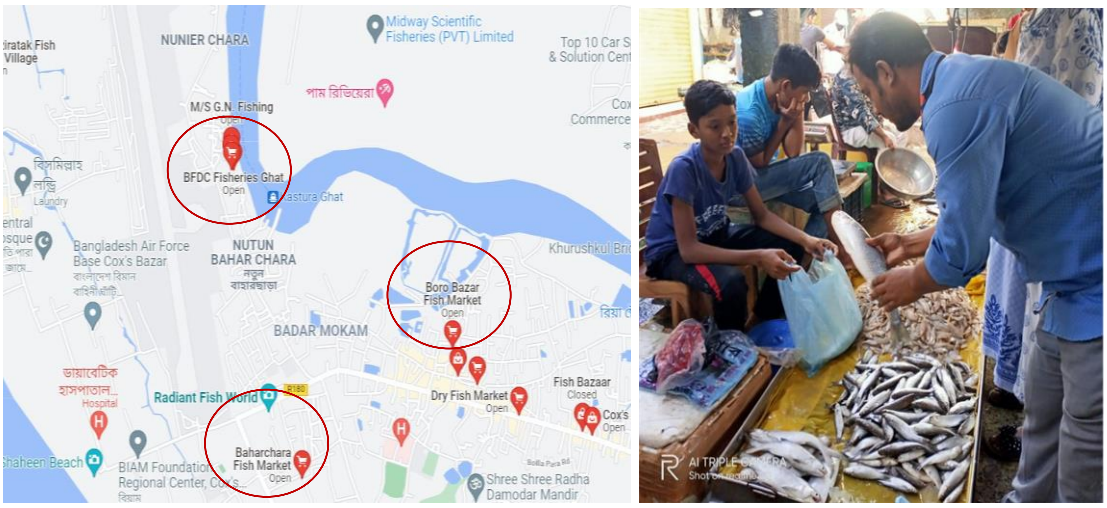

Plate 2: Sample collection

3.3 Measurement of morphometric and meristic characteristics

Generally, morphological properties of fish were measured according to the standard procedures given by Hubbs and Lagler (1958) (Plate 3−5 and table-1). A vernier calipers and a metric scale were employed for measuring 18 morphological lengths in three different species of jewfish. Meristic features such as dorsal fin rays (DFR), pectoral fin rays (PcFR), pelvic fin rays (PvFR), anal fin rays (AFR), caudal fin rays (CFR), Branchiostegal rays number and scales on the lateral line of each samples were counted (Table-2).

Plate-3: Length Measurement

Plate-4: Meristic Count

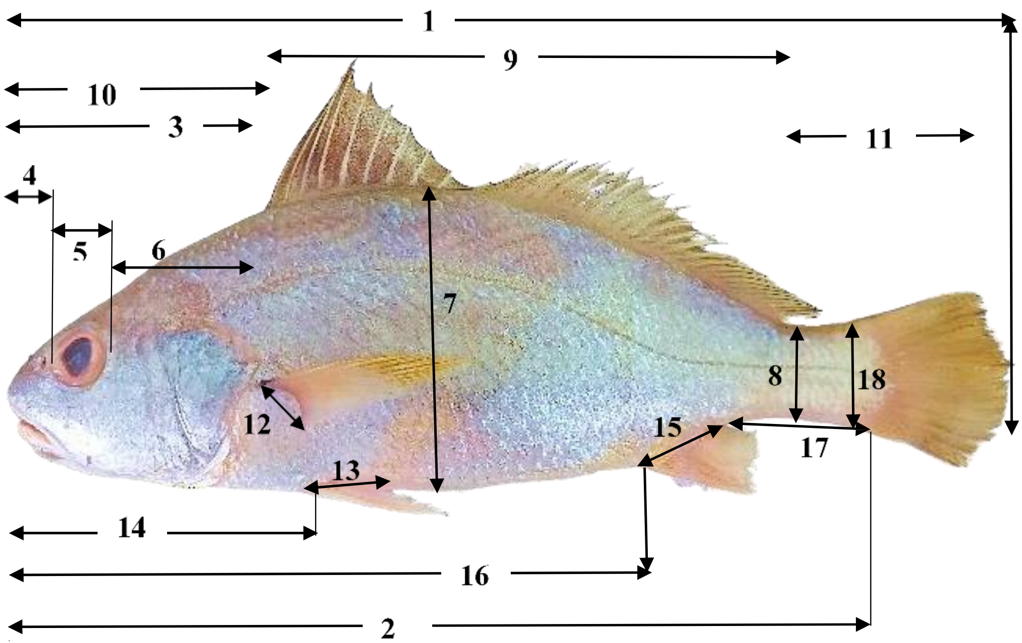

Plate-5: Overview of different morphometric indices of pama croaker (**1**. TL= total length, **2**. SL= standard length, **3**. HL= head length, **4**. PrOL= pre-orbital length, **5**. ED= Eye diameter, **6.** PoOL= post orbital length, **7**. HBD= highest body depth, **8**. LBD= lowest body depth, **9**. DFL= dorsal fin length, **10**. PrDL= pre dorsal length, **11**. PoDL= post dorsal length, **12**. PcFL= pectoral fin length, **13**. PvFL= pelvic fin length, **14**. PrPvFL= pre pelvic fin length, **15**.AFL= anal fin length, **16**. PrAFL= pre anal fin length, **17.**CPL=caudal peduncle length, **18.**CFL=caudal fin length**.**

Table-1: General Morphometric characteristics and their descriptions applied for the differentiating among three species of Jew fish

Sl. No.	Character	Description
01	Total length (TL)	Distance from the tip of the snout of fish to the longest caudal fin ray
02	Standard length (SL)	Distance from the tip of the snout of fish to the end of the vertebral column
03	Head length (HL)	From tip of the snout to posterior margin of operculum
04	Pre-orbital length (PrOL)	From tip of the snout to anterior margin of eye
05	Eye diameter	Diameter of eye
06	Post orbital length (PoOL)	From posterior margin of eye to end of operculum
07	Highest body depth (HBD)	Vertical range of anterior part of first dorsal fin and ventral part of the fish body
08	Lowest body depth (LBD))	Vertical distance at the end of vertebrae
09	Dorsal fin length (DFL)	From base of first dorsal spine to base of last dorsal ray
10	Pre-dorsal fin length (PrDFL)	From tip of the snout to origin of dorsal fin
11	Post–dorsal Fin Length (PoDFL)	End of dorsal fin to longest caudal fin ray
12	Pectoral fin length (PcFL)	Base length of pectoral fin
13	Pelvic fin length (PvFL)	Base length of pelvic fin
14	Pre-pelvic fin length (PrPvFL)	From tip of the snout to the origin of pelvic fin
15	Anal fin length (AFL)	Length of base of anal fin length
16	Pre-anal fin length (PrAFL)	From tip of the snout to origin of anal fin
17	Caudal peduncle length (CFL)	From base of anal fin to base of caudal fin
18	Caudal fin length (CFL)	Base length of caudal fin

Table-2: List of meristic characters used in this species to differentiate among three species of Jew fish

SL. No	Characters
1	Dorsal fin spine (DFS)
2	Dorsal fin soft rays (DFSR)
3	Anal fin spine (AFS)
4	Anal fin soft rays (AFSR)
5	Caudal fin rays (CFR)
6	Pectoral fin rays (PcFR)
7	Pelvic fin rays (PvFR)
8	Scales on the lateral line (LLS)
9	Scales on the lateral transverse (TrLS)
10	Number of branchiostegal rays

3.4 Measurements of land-mark distances

At the frame of the fish, twelve (12) randomly selected land-marks points with twenty-three (23) land-mark distances were chosen and measured to achieve homogeneity of total body plane coverage among the three species of Jew fish (Plate- 7, Table- 3) on the basis of Strauss and Bookstein (1982). To enable for the accurate and consistent measurements, each landmark obtained by laying the fish sample on a square of paper and detecting the landmarks using a color pointer. Finally, the cm scale was used to calculate the distances (Plate-6).

Plate-6: Landmark based length measurement

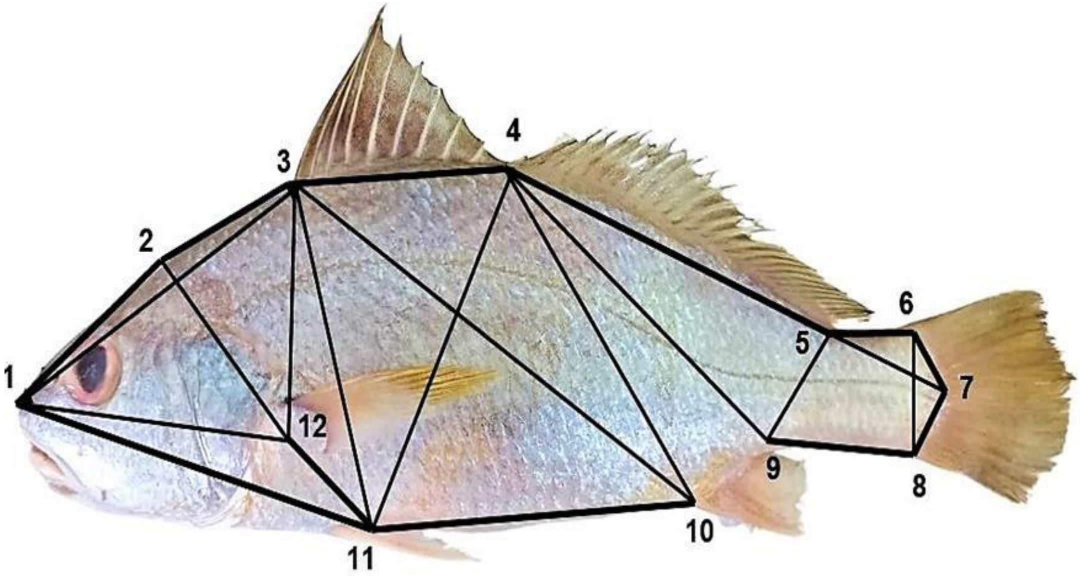

Plate 7: Randomly selected landmark points and made a network on the fish body which was used in the study. The 12 landmark points refer to point 1: anterior tip snout of upper jaw, point-2: cranium, point-3: origin of first dorsal fin, point-4: end of spine dorsal fin, point-5: end of soft dorsal fin, point-6: origin of dorsal caudal fin, point-7: mid-point of caudal fin, point-8: origin of ventral caudal fin, point-9: end of anal fin, point-10: origin of anal fin, point-11: mid-point of pelvic fin, point-12: mid-point of pectoral fin.

Table-3: Description of truss-network characters used for the differentiating among three species of Jew fish

SL No	Character codes	Land-mark Points	Description of characters
1	A1	1−2	Anterior tip of snout to the cranium
2	B1	2−3	Cranium to the origin of dorsal fin base
3	C1	3−4	Origin of dorsal fin base to end of spine dorsal fin
4	D1	4−5	End of spine dorsal to lower end of soft dorsal fin
5	E1	5−6	End of soft dorsal fin to end of the caudal fin base
6	F1	6−7	Origin of upper caudal fin base to mid-point of caudal fin base
7	G1	7−8	Mid-point of caudal fin to lower caudal fin base
8	H1	8−9	Lower caudal fin to end of anal fin
9	J1	10−11	Origin of anal fin base to mid-point of pelvic fin

10	K1	11−1	Mid-point of pelvic fin to anterior tip of snout
11	A2	1−12	Anterior tip of snout to mid-point of pectoral fin base
12	A3	1−3	Anterior tip of snout to origin of dorsal fin base
13	C2	3−10	Origin of dorsal fin base to origin of anal fin base
14	C3	3−11	Origin of dorsal fin base to mid-point of pelvic fin
15	C4	3−12	Origin of dorsal fin base to mid-point of pectoral fin base
16	D2	4−9	End of spine dorsal fin to end of anal fin
17	D3	4−10	End of spine dorsal fin to origin of anal fin base
18	D4	4−11	End of spine dorsal fin to mid-point of pelvic fin
19	E2	5−7	End of soft dorsal fin to mid-point of caudal fin base
20	E3	5−9	End of soft dorsal fin to end of anal fin
21	F2	6−8	Origin of upper caudal fin base to lower caudal fin base
22	B2	2−11	Cranium to mid-point of pectoral fin base
23	K2	11−12	Mid-point of pelvic fin to pectoral fin base

3.5 Data adjustment

Since, differences in morphometric characters should not be correlated to the relative size of fish but rather to differences in body shapes (Reist, 1985). So that, before the analytical analyses, the size effects from the data set were eliminated using a slightly modified version of the algometric formula provided by Elliott et al. (1995).

$$M_{adj} = M\ (Ls/Lo)^{b}$$

Where, M_{adj}: size adjusted measurement,

M: original measurement,

Ls: overall mean of total length for all fish from all samples in each analysis

Lo: total length of fish

With all fish in all groups, the slope of the regression of log M on log Lo was used to estimate parameter "b" for each character from the observed data. TL and converted data were then connected with the effectiveness of the size adjusted values. To determine whether the size relations had been removed or not, the standardized data were evaluated using a bivariate plot in compared to the standard length.

3.6 Statistical analysis

We compared the morphological variation among the collected samples in the first stage of analysis of *Johnius borneensis, J. argentatus, and J. belangerii*. In the second level, we then compared the landmark distances among the J. borneensis, J. argentatus, and J. belangerii. Kruskal-Wallis was calculated to compare means and medians among different species. Based on morphological data (size-adjusted) and landmark distance data, a univariate analysis of variance (ANOVA) was done to determine the significance of each morphological difference ($p < 0.01$). A Discriminant Functional Analysis (DFA) was applied to all morphological data (size-adjusted) and the data of landmark distances. Individual specimens were classified using functions generated from DFA, and the success rate of DFA classification was determined based on the proportion of individuals that were correctly classified into the original samples. We applied principal component analysis (PCA) to identify the morphometric or land-mark distance that most successfully distinguishes between the three species of jewfish. A dendrogram was also constructed based on the Euclidean distances using Unweighted Pair Group Method (UPGMA) with arithmetical average cluster analysis (CA) for investigating the phenotypic relationships among populations, (Sneath and Sokal, 1973). Microsoft Office Excel 2010, R programming, and statistical tools for social sciences (SPSS version 16.0) were used for all statistical studies.

Chapter-4

Results

The descriptive data of length and weight of the collected fish species showing the mean values, minimum and maximum range, and standard deviations are presented in Table In this study, we did not observe any significant correlation (p > 0.05) between standardize truss measurement and the standard length, indicating that the size effect was successfully removed with allometric transformations. The transformed data were then used for the further discriminant functions analysis (DFA) and principal component analysis (PCA).

Table-4: A descriptive data of three *Johnius* species from the Bay of Bengal, Bangladesh-

Sl. no	Species name	No. of fish Samples	Total Length (min-max) cm	Total length (mean ± SD)
1	*Johnius borneensis*	30	20.5−25.3	22.5 ±1.15
2	*Johnius argentatus*	30	23.4−30.2	26.7 ±1.85
3	*Johnius belangerii*	30	20.2−25.7	23.3 ± 1.43

4.1 Meristic counts

Most of the meristic characters of *Johnius borneensis*, *Johnius argentatus* and *Johnius belangerii* were overlapped among the three species of the genus *Johnius* (Table 5). However, the major distinguishable meristic characters are- less number (one) of dorsal fin ray's spine in *J. belangerii*, one short anal fin rays in *J. borneensis* and highest number of scales on lateral line on *J. argentatus* in compared with the other species.

Table 5: Counting of meristic characteristics of three species of the genus *Johnius* (*Johnius borneensis*, *Johnius argentatus* and *Johnius belangerii*) **from the Bay of Bengal, Bangladesh-**

Meristic characters	*Johnius borneensis*	*Johnius argentatus*	*Johnius belangerii*
Dorsal fin ray's spine	X-XI	X-XI	IX-XI
Dorsal fin rays soft	26-28	26-29	26-28

Caudal fin rays	16-18	17-20	17-18
Anal fin rays	I/7	II/7	II/7
Pectoral fin rays	17-19	17-20	17-18
Pelvic fin rays	6-7	6-7	6-7
Lateral line scale	52-54	57-61	49-53
Transverse lateral scale	7,11	7,11	6,10
Branchiostegal rays	VII	VII	VII

4.2 Analysis of Morphometric Measurement

Univariate analysis of variance (ANOVA) showed that, fifteen [Total length (TL), head length (HL), pre-orbital length (PreOL), eye diameter (ED), highest body depth (HBD), least body depth (LBD), dorsal fin length (DFL), pre-dorsal fin length (pre-DL), post-dorsal fin length (PoDFL), pectoral fin length (PcFL), pelvic fin length (PvFL), pre-pelvic fin length (pre-PvFL), anal fin length (AFL), caudal peduncle length (CPL), and caudal fin length (CFL)] out of nineteen morphometric measurements were significantly different ($p < 0.05$) among means of three different species (Table-6).

Table-6: Univariate statistics (ANOVA) among samples of *J. borneensis, J. argentatus* and *J. belangerii* from 19 morphometric measurements. Here, degree of significance was presented as *p < 0.05, **p < 0.01, ***p < 0.001.

Tests of Equality of Group Means					
	Wilks' Lambda	**F**	**Df1**	**Df2**	**Sig.**
TL	0.400	65.357	2	87	<.001
SL	0.939	2.830	2	87	.064
HL	0.824	9.320	2	87	<.001
PrOL	0.556	34.672	2	87	<.001
ED	0.273	116.082	2	87	<.001
PoOL	0.935	2.999	2	87	.055
HBD	0.588	30.419	2	87	<.001
LBD	0.459	51.210	2	87	<.001
DFL	0.885	5.657	2	87	.005
PrDL	0.936	2.972	2	87	.056
PoDL	0.688	19.728	2	87	<.001

PcFL	0.853	7.508	2	87	<.001
PvFL	0.776	12.568	2	87	<.001
PrPvFL	0.638	24.649	2	87	<.001
AFL	0.838	8.391	2	87	<.001
PrAFL	0.859	7.143	2	87	.001
CPL	0.894	5.185	2	87	.007
CFL	0.251	129.729	2	87	<.001
MG	0.939	2.805	2	87	.066

Discriminant function analysis (DFA) resolved that first and second DFA accounted for 80.6%, and 19.4% respectively of among group variability and together they explained 100% of the total variability for morphometric measurements (Table-7). Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions revealed that among the nineteen morphometric measurements, twelve (TL, HL, PrOL, ED, HBD, LBD, PrDFL, PoDFL, PcFL, PrPvFL, PrAFL and CFL) were dominantly contributed to the first DF, while the rest seven were contributed to the second DF (Figure 1).

Table-7: Summary of Canonical Discriminate Functions for morphometric analysis

Eigenvalues Morphometric Character				
Function	**Eigenvalue**	**% of Variance**	**Cumulative %**	**Canonical correlation**
1	7.152	80.6	80.6	.937
2	1.721	19.4	100.0	.795

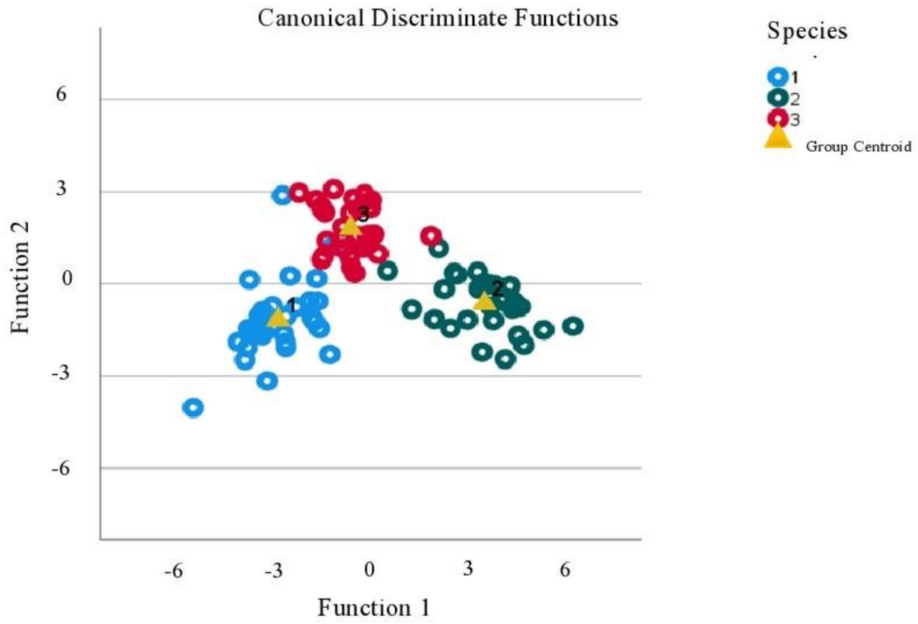

Figure-1: Scatter plot of two Canonical Discriminate Function of morphometric characters for pama croaker (Where, **sp1**= *J. borneensis*, **sp2**= *J. argentatus*, **sp3**= *J. belangerii*) collected from the Bay of Bengal, Bangladesh.

PCA for the morphometric measurement of *J. borneensis, J. argentatus* and *J. belangerii* showed that, the value of KMO for the overall matrix was 0.821 and the Bartlett's Test of Sphericity was significant (p < 0.01). The result of KMO and Bertlett's suggest that the sampled data was appropriate to proceed with a factor analysis procedure (Table 8).

Table-8: KMO and Bartlett's Test of Morphometric Characters

KMO and Bartlett's Test		
Kaiser-Meyer-Olkin Measure of Sampling Adequacy		.821
Bartlett's Test of Sphericity	Approx. Chi-square	765.325
	Df	136
	Sig.	<.001

The PCA based on 14 morphometric measurements retained five components with Eigen values >1, explaining 70.76% of the total variance. The first (PC1) and second (PC2) principal components accounted for 33.8% and 12.8% of total variance respectively. Scatter plots of the specimens relating to the first and second principal component analysis revealed a visual definition of the group as also revealed by the canonical discriminate function analysis (Figure 2). PCA dispersion showed a vast divergence in the SL, PoOL, PrDL, CPL, and MG in *J. argentatus* compared to the *J. borneensis* and *J. belangerii* indicating that *J. argentatus* is morphologically very dissimilar with other two species.

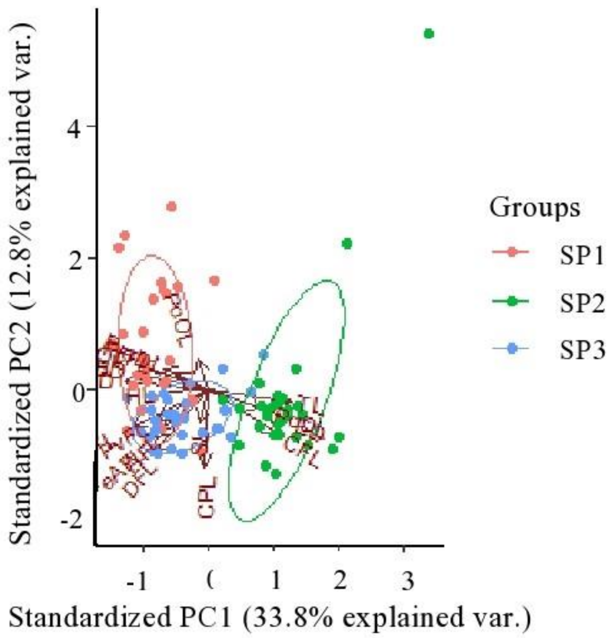

Figure- 2: Principal component analysis of morphometric characters for pama croaker (Where, **sp1=** *J. borneensis*, **sp2**= *J. argentatus*, **sp3=** *J. belangerii*) collected from the Bay of Bengal, Bangladesh

4.3 Analysis of Land-mark distance measurements

Univariate analysis among the *J. borneensis, J. argentatus* and *J. belangerii* population using land-mark distances showed that, twelve distances (D1, E1, C3, C4, D2, D3, D4, E2, E3, F2, B2, K2) out of twenty-three truss measurements were significantly different among population in varying degrees ($p < 0.05$, $p < 0.01$, and/or $p < 0.001$) (Table 9).

Table-9: Univariate statistics (ANOVA) showing the difference among measurement of 23 truss networking (*p < 0.05, **p < 0.01, and ***p < 0.001) of *J. borneensis, J. argentatus* and *J. belangerii* from the Bay of Bengal, Bangladesh

	Tests of Equality of Group Means				
	Wilk's Lambda	**F**	**Df1**	**Df2**	**Sig.**
A1	.981	.838	2	87	.436
B1	.998	.104	2	87	.901
C1	.943	2.651	2	87	.076
D1	.860	7.101	2	87	.001
E1	.926	3.476	2	87	.035
F1	.968	1.441	2	87	.242
G1	.990	.427	2	87	.654
H1	.990	.440	2	87	.654
J1	.990	.458	2	87	.634
K1	.989	.481	2	87	.620
A2	.988	.511	2	87	.602
A3	.956	1.999	2	87	.142
C2	.957	1.966	2	87	.146
C3	.856	7.306	2	87	.001
C4	.909	4.372	2	87	.016
D2	.588	30.505	2	87	<.001
D3	.405	63.834	2	87	<.001
D4	.724	16.543	2	87	<.001
E2	.861	7.000	2	87	.002
E3	.910	4.278	2	87	.017
F2	.598	29.269	2	87	<.001

B2	.631	25.406	2	87	<.001
K2	.389	68.439	2	87	<.001

The first DFA resolved 81.1% and the second DFA accounted for 18.9% respectively of among group variability and together they explained 100% of the total variability for landmark measurements (Table 10). Among the twenty-three measurements, nine measurements (D1, E3, C3, A1, A2, K1, J1, A1, and G1) dominantly contributed to the second DF, while the remaining fourteen measurements contributed to the first DF. By summary of the canonical discriminate function analysis, it can easily identify that, *J. borneensis* and *J. belangerii* were remained closely compared to the *J. argentatus* (Figure- 3).

Table-10: Summary of Canonical Discriminant Functions for truss networking

Eigen values				
Function	**Eigen value**	**% of Variance**	**Cumulative**	**Canonical Correlation**
1	4.204	81.1	81.1	.899
2	.977	18.9	100.0	.703

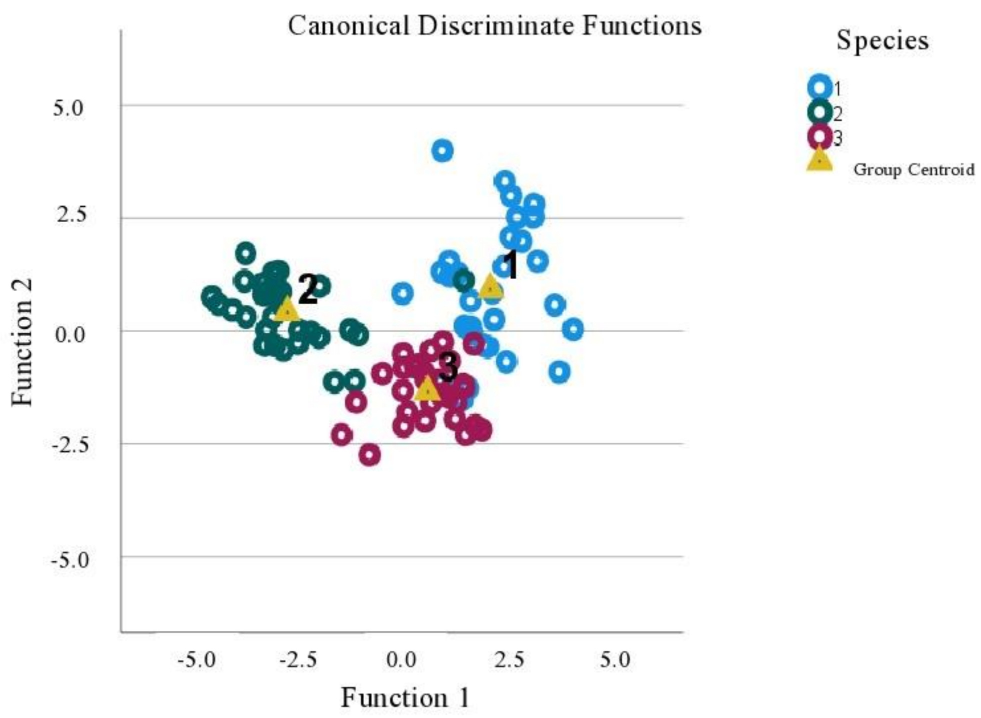

Figure-3: Scatter plot of two Canonical discriminant functions of truss networking for pama croaker (Where, **sp1=** *J. borneensis*, **sp2**= *J. argentatus*, **sp3=** *J. belangerii*) collected from the Bay of Bengal, Bangladesh.

PCA was performed on the relevant attribute identified by univariate analysis (twelve truss network measurement). The truss distances having an Eigen value greater than 1 were included for PCA. Truss distances among the *J. borneensis*, *J. argentatus* and *J. belangerii* showed that, the value of KMO for overall matrix is 0.857 and the Bartlett's Test of Sphericity is significant ($p < 0.01$). The result of KMO and Bertlett's suggest that the sampled data was appropriate to proceed with a factor analysis procedure (Table 10).

Table-11: KMO and Bartlett's Test of Truss Networking

KMO and Bartlett's Test		
Kaiser-Meyer-Olkin Measure of Sampling Adequacy		.857
Bartlett's Test of Sphericity	Approx. Chi-square	516.482
	Df	66
	Sig.	<.001

The PCA based on 12 morphometric measurements retained three components with Eigen value >1 explaining 64.49% of total variance. The first (PC1) and second (PC2) principal component accounted for 40.7% and 11.5 % of total variance respectively. By summary of Principal Component Analysis, we can conclude that, *J. borneensis* and *J. belangerii* are closely related species and *J. argentatus* is distinct species (Figure-4).

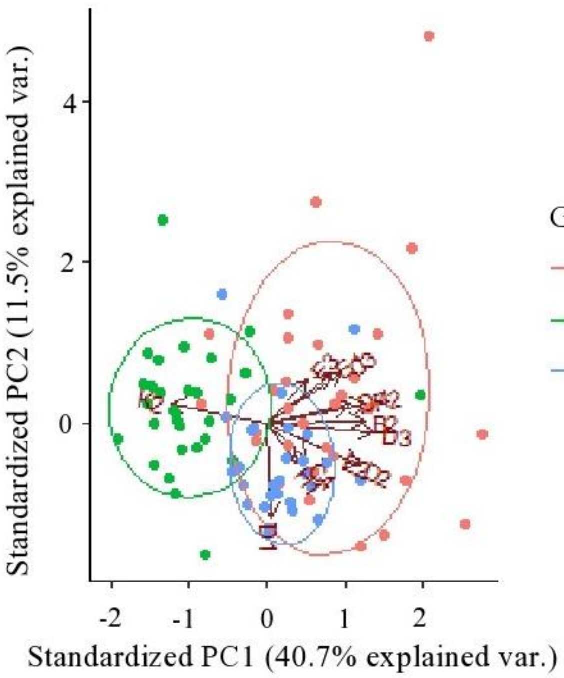

Figure- 4: Principal component analysis of truss network for pama croaker (Where, **sp1=** *J. borneensis*, **sp2**= *J. argentatus*, **sp3=** *J. belangerii*) from the Bay of Bengal, Bangladesh.

4.4 Hierarchical cluster analysis

Using the centroid cluster approach, cluster analysis consolidation steps were carried out based on landmark distances. A dendrogram was build consisting of different truss networking distances and found three or two different clusters based on Squared Euclidean distance measurement indicating that all three species are morphologically dissimilar. Cluster distances are rescaled so that they range from 0 to 25 in this plot (Figure- 14). Here, in cluster-1 (Green), show similarity within the cases (D1, D3, and D4) and in cluster-2 (Red), the cases (B2, E1, C3, C4, E3, K2, E2, and C1) refer resemblance within the cases. Cluster-3 (Yellow), D2 indicates dissimilarity. The clusters are different from each other based on its cases. So, we can conclude that, the three clusters separated the three species of fish.

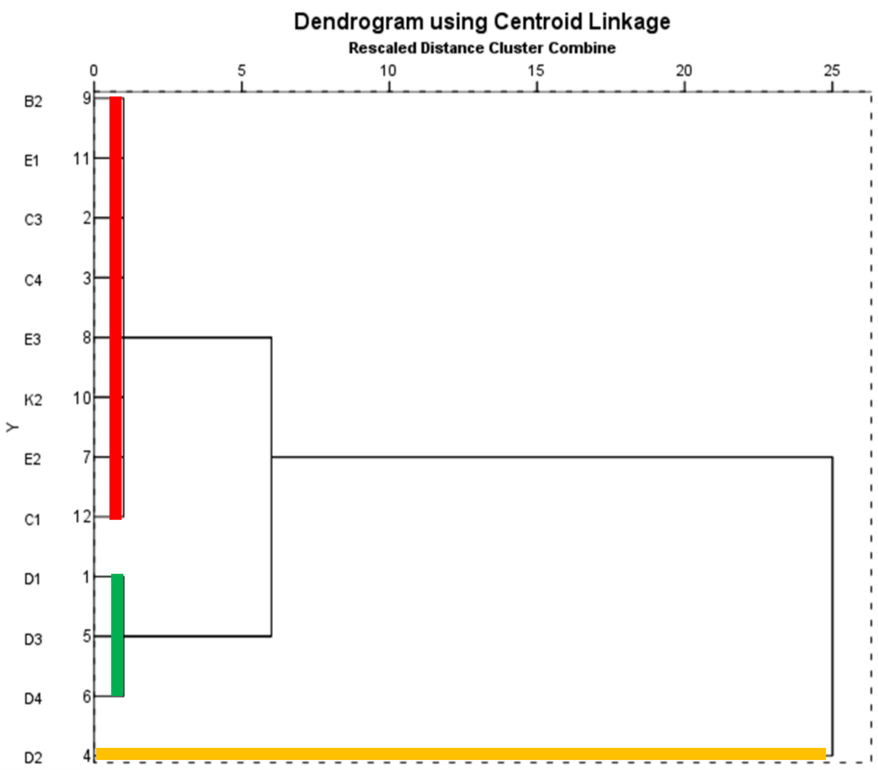

Figure-5: Dendrogram presents the dissimilarity of landmark distances of pama croakers through three clusters

Chapter-5

Discussion

In the present study, morphometric differences among three species of croaker (*J. borneensis, J. argentatus*, and *J. belangerii*) were studied using land-mark-based truss-networking analysis from the Bay of Bengal, Bangladesh. The findings of the present study indicate that there is a high degree of similarity between *Johnius bornensis* and *J. belangerii* while *J. argentatus* is morphologically different. This study is the first report regarding the morphometric variations among three species of croaker from the Bay of Bengal which may further help in stock identification, conservation, and management plan for these species in the Bay of Bengal.

Fish have a very high degree of phenotypic plasticity (Hossain et al., 2010, Ihssen et al. 1981) when morphometric and meristic investigations can be used to identify fish populations. In the present study, the potential differentiation among the *J. borneensis*, *J. argentatus*, and *J. belangerii* populations from the Bay of Bengal, Bangladesh, has been examined using morphometric and meristic features with land-mark-based techniques. This study applied a truss network system as a powerful tool for identifying fish stocks (Turan, 2004). Truss analysis was utilized as a taxonomic method for separating morphologically similar species as well as a stock identification tool (Cadrin and Friedland, 1999). As the area of multivariate morphometric expanded, a set of multivariate techniques was used to examine variation in development and forms among stocks (Cadrin, 2000). Bhakta et al. (2020) reported that the number of scales in the lateral line in *J. argentatus* was 48−52, but Talwar (1995) reported 44−48 in the lateral line. In the present study, the maximum number of meristic characters was found similar to the earlier studies with little difference in the number of gills rakers and the total number of lateral lines. Environmental factors like temperature, salinity, oxygen, pH, food availability, and growth rate may all play a role in these variations, which have been observed in other species.

The truss networking system is a more feasible and effective strategy for explaining shapes, provides a better method of data collection, and allows the data to be used in a variety of ways of analysis to differentiate between phenotypic stocks than the traditional morphometric method. This is because of the set of the created landmarks encloses the entire fish body without affecting the detailed body plan (Dwivedi and Dubey, 2012). Previous studies on the horse mackerel *Trachurus trachurus* (Murta et al., 2008); Indian major carps (Hossain et al., 2010); mullet (Hossain et al., 2015); catfish (Parvej et al., 2014, Rahman et al., 2014); and gobies (Hossain et al., 2014) have all been effectively differentiated and identified using ANOVA (Analysis of variance), DFA (Discriminant function analysis) and PCA (Principal component analysis) (Sabet and Anvarifer, 2013). Furthermore, PCA based on morphometric measurements of *J. borneensis, J. argentatus,* and *J. belangerii* revealed that two *Johnius* species are closely related and *J. argentatus* is different from them. In the case of meristic counts, most of the characters were similar, but during taking the morphometric measurements, relatively significant variances were found. Out of nineteen morphometric characters, seventeen characters showed significant differences in univariate analysis among the population *of J. borneensis, J. argentatus,* and *J. belangerii* in varying degrees ($p <$ or $p < 0.01$, $p < 0.001$). It was described in their (Norhafiz et al., 2022) research that *Johnius* is the most diversified Sciaenid genus with verified monophyly. However, because of their external morphological similarities and overlapping meristic counts, accurate identification is difficult. Hence, *J. borneensis* and *J. belangerii* resolved as polyphyletic groups here, they represent the two more problematic species within the genus.

In the present study, univariate analysis among the *J. borneensis, J. argentatus,* and *J. belangerii* populations using land-mark distances, it was found that twelve, out of twenty-three characters in various degrees were significantly distinct ($p < 0.05$ or $p < 0.01$, $p < 0.001$). The DFA segregation was partly confirmed by PCA, where according to the graphs of PCA1 and PCA2 scores for each sample used in the morphometric analysis, there was higher overlap between species *J. borneensis* and *J. belangerii* whereas, in the case of truss networking more overlapping was observed between *J. borneensis* and *J. belangerii*, and slight interlinking points were overlapped between *J. argentatus* and other two *Johnius* species. Molecular characterization of the cytochrome c oxidase subunit I (COI) gene could finally determine the genetic variations among these three species from other croakers in the Bay of Bengal.

Chapter-6

Conclusion

Fisheries is one of the most productive and dynamic industries with great potential for the future development of the agricultural economy of Bangladesh. At present, marine fish production is 6.75 lac MT, but the marine fish species is decreasing day by day. The fishing sector is under threat from overfishing, pollution, habitat destruction, improper use of agrochemicals, foreign species introduction, a lack of favorable habitat, low fertility, and other factors. The stock, meanwhile, is progressively losing value. The findings of the present study will be used as primary data for stock management, allowing for efficient management techniques for the various fish stocks with a view to making sustainable fisheries and adequate conservation programs in the near future and also will be useful for fisheries management and conservation as well as helpful to fisheries scientists, biologists, and taxonomists who are interested in these three unique species.

Chapter-7

Recommendation and Future Perspectives

According to this research work, the following recommendations should be done.

- ✓ As these three species of croakers have a high market value, so overfishing should be limited.
- ✓ The genetic information of these species must be preserved for the future.
- ✓ During the breeding season of these species, catching should also be more limited.
- ✓ Communication between fisheries scientists and policymakers is needed to develop policies for proper catching.
- ✓ Reducing the catch of brood and fingerlings of these species should be done as part of a larger attempt to survive in the future.
- ✓ Molecular characterization of the cytochrome c oxidase subunit I (COI) gene could finally determine the genetic variations among these three species from other croakers in the Bay of Bengal.

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Brief Biography of the Author

This is Sonjoy Kumar Modak, son of Mr. Khokon Chandra Modak and Archona Rani Modak born at a village named Uchakhila under Ishwarganj Upazila, Mymensingh. He passed his Secondary School Certificate from Uchakhila Higher Secondary School in 2012 and Higher Secondary Certificate from Agricultural University College, Mymensingh in 2014 with brilliant result. Then, he completed his B.Sc. Fisheries (Hons.) degree from Chattogram Veterinary and Animal Sciences University (CVASU). Now he is a candidate for the degree of MS in Fish Biology and Biotechnology under the department of Fish Biology & Biotechnology, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. He is interested in the field of marine fish breeding and modern hatchery development.