VARIATION OF HILSA SHAD, *Tenualosa ilisha* IN BANGLADESH INFERRED FROM GEOMETRIC MORPHOMETRIC AND BIOCHEMICAL ANALYSIS

A Thesis

By

MD. ABU BAKKAR SIDDIK

Examination Roll No: 1605519

Session: 2016-2017

Semester: January-June, 2018

MASTER OF SCIENCE
IN
FISHERIES BIOLOGY AND GENETICS

DEPARTMENT OF FISHERIES BIOLOGY AND GENETICS

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

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June, 2018 ***The Author***

DECLARATION

I hereby declare that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any other degree.

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ABSTRACT

Hilsa shad, *Tenualosa ilisha* is a species of the Family Clupeidae locally known as ilish in Bangladesh. This study investigated the variation among the Hilsa of four wild populations through landmark-based geometric morphometrics and analysis of biochemical composition. To cover the broader zone of the Hilsa availability in Bangladesh, a total of 100 samples of Hilsa 15 females and 10 male of each population were collected from four rivers, The Padma, Meghna, Rupsa and Karnaphuli. After collection, image of each sample were taken for geometric morphometric analyses and muscle tissues were used for biochemical composition analysis. Sixteen landmarks points on the fish's body were digitized using TPS series software and analyses by using PAST software (version 3.0). The overall body shape variations were found in principal component one (PC1: 75.52%) among four populations indicates that there was significant body shape variation of *T. ilisha*. The results of scatter plot and UPGMA cluster analysis showed that there were overlapping between Barisal and Khulna populations and some were Chittagong and Khulna populations, but Barisal and Pabna population were distinctly separated. The biochemical composition of crude protein, lipid and Ash contents were carried out following the methods of AOAC (1980). The highest protein contents were found in Barisal populations (15.27%) and lipid contents were found in Khulna population (16.43%) and Ash content was found in Chittagong population (0.98%). The findings of biochemical composition of Hilsa showed significant variation. These body shape variation and biochemical composition differentiation may be related to geographically isolated regions or habitat or feeding habits or other environmental conditions. This result will be helpful for the proper Hilsa stock management as well as for the consumers, fish processors and the researchers. For clear differentiation among populations, more stocks and individuals of each stock are needed to investigate through morphometric and molecular markers analyses.

Keywords: *Tenualosa ilisha*, Geometic morphometric, PCA, Biochemical composition

ABBREVIATIONS

AOAC	Association of Official Agricultural Chemists
ANOVA	Analysis of Variance
DNA	Deoxyribonucleic acid
DSLR	Digital single-lens reflex
DoF	Department of Fisheries
FAO	Food and Agriculture organization
FASTA	Fast Adaptive Shrinkage Thresholding Algorithm
GDP	Gross Domestic Product
HSTU	Hajee Mohammad Danesh Science and Technology University
RW	Relative Warp
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction fragment length polymorphisms
mtDNA	mitochondrial Deoxyribonucleic Acid
MT	Metric ton
PAST	Paleontological Statistics Software Package for Education and Data Analysis
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PUFA	Polyunsaturated Fatty Acid
SNP	Single Nucleotide Polymorphism
SIS	Small Indigenous Species
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
TPS	Thin Plate Splines
TVC	Total Viable Count
UPGMA	Unweighted Pair Group Method with Arithmetic Mean

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Annex a. Eigenvalues and percent variance of overall population

SL NO.	Eigenvalue (PC1)	%Variance (PC1)	SL NO.	Eigenvalue (PC1)	%Variance (PC1)
1	37.8476	75.525	17	0.0104104	0.020774
2	8.50253	16.967	18	0.0083036	0.01657
3	1.80521	3.6023	19	0.00603034	0.012034
4	1.07889	2.1529	20	0.00540462	0.010785
5	0.302754	0.60415	21	0.00477534	0.0095293
6	0.170873	0.34098	22	0.00449397	0.0089678
7	0.0773884	0.15443	23	0.0035228	0.0070298
8	0.0558208	0.11139	24	0.00341032	0.0068053
9	0.0446006	0.089001	25	0.00293021	0.0058473
10	0.0428697	0.085547	26	0.00196644	0.0039241
11	0.030796	0.061454	27	0.00188387	0.0037593
12	0.0281485	0.056171	28	0.00115061	0.0022961
13	0.0234941	0.046883	29	0.000871074	0.0017382
14	0.0179662	0.035852	30	0.000662657	0.0013223
15	0.0143773	0.02869	31	0.000488348	0.00097451
16	0.0125235	0.024991	32	0.000234107	0.00046716

Annex b. Eigenvalues and percent variance of Barishal and Chattogram population

SL NO.	Eigenvalue (PC1)	%Variance (PC1)	SL NO.	Eigenvalue (PC1)	%Variance (PC1)
1	34.5109	79.005	17	0.00745955	0.017077
2	6.19198	14.175	18	0.00572596	0.013108
3	1.76229	4.0344	19	0.00445617	0.010201
4	0.485019	1.1103	20	0.00337574	0.007728
5	0.283206	0.64834	21	0.00305138	0.0069855
6	0.0837104	0.19164	22	0.00255292	0.0058444
7	0.0753798	0.17257	23	0.00229744	0.0052595
8	0.0697206	0.15961	24	0.0017745	0.0040623
9	0.0495684	0.11348	25	0.00136145	0.0031168
10	0.0295578	0.067666	26	0.00126933	0.0029059
11	0.0242791	0.055582	27	0.0009658	0.002211
12	0.0233385	0.053429	28	0.000769504	0.0017616
13	0.0195168	0.04468	29	0.000633671	0.0014507
14	0.0145068	0.03321	30	0.000258454	0.00059168
15	0.013411	0.030702	31	0.000233398	0.00053431
16	0.00916901	0.02099	32	9.0869E-05	0.00020802

Annex c. Eigenvalues and percent variance of Barishal and Khulna population

SL NO.	Eigenvalue (PC1)	%Variance (PC1)	SL NO.	Eigenvalue (PC1)	%Variance (PC1)
1	21.6064	71.341	17	0.0074135	0.024478
2	5.62872	18.585	18	0.0069258	0.022868
3	1.64531	5.4326	19	0.0057303	0.018921
4	0.567281	1.8731	20	0.00481733	0.015906
5	0.255843	0.84476	21	0.00445236	0.014701
6	0.199166	0.65762	22	0.00305391	0.010084
7	0.080754	0.26664	23	0.00285961	0.009442
8	0.0640694	0.21155	24	0.00219978	0.0072634
9	0.0452215	0.14932	25	0.00132553	0.0043767
10	0.0377179	0.12454	26	0.000955476	0.0031548
11	0.0330868	0.10925	27	0.000827856	0.0027335
12	0.0222449	0.073449	28	0.00070048	0.0023129
13	0.0184493	0.060917	29	0.000591812	0.0019541
14	0.0180375	0.059557	30	0.000327135	0.0010802
15	0.0112492	0.037143	31	0.000135438	0.0004472
16	0.0100225	0.033093	32	9.80639E-05	0.00032379

Annex d. Eigenvalues and percent variance of Barishal and Pabna population

SL NO.	Eigenvalue (PC1)	%Variance (PC1)	SL NO.	Eigenvalue (PC1)	%Variance (PC1)
1	52.8915	85.335	17	0.00645584	0.010416
2	5.93628	9.5776	18	0.00528969	0.0085344
3	1.30187	2.1004	19	0.00496468	0.00801
4	1.12916	1.8218	20	0.00373442	0.0060251
5	0.278374	0.44913	21	0.00316257	0.0051025
6	0.104586	0.16874	22	0.00277082	0.0044705
7	0.0646767	0.10435	23	0.00237583	0.0038332
8	0.0508123	0.081981	24	0.00183366	0.0029584
9	0.0406852	0.065642	25	0.00142947	0.0023063
10	0.0382602	0.061729	26	0.0012618	0.0020358
11	0.0317331	0.051198	27	0.000918208	0.0014814
12	0.0231274	0.037314	28	0.000723328	0.001167
13	0.0159018	0.025656	29	0.000448744	0.00072401
14	0.015276	0.024646	30	0.000385413	0.00062183

15	0.012899	0.020811	31	0.000211403	0.00034108
16	0.00943314	0.015219	32	0.000165305	0.0002667

Annex e. Eigenvalues and percent variance of Chattogram and Khulna population

SL NO.	Eigenvalue (PC1)	%Variance (PC1)	SL NO.	Eigenvalue (PC1)	%Variance (PC1)
1	23.8206	63.242	17	0.00685495	0.018199
2	11.1204	29.524	18	0.00570067	0.015135
3	1.53008	4.0622	19	0.00440627	0.011698
4	0.560953	1.4893	20	0.00379103	0.010065
5	0.23299	0.61857	21	0.0029713	0.0078886
6	0.120026	0.31866	22	0.00220528	0.0058548
7	0.0562098	0.14923	23	0.00192553	0.0051121
8	0.0479711	0.12736	24	0.00137738	0.0036568
9	0.0437508	0.11615	25	0.00115243	0.0030596
10	0.0241021	0.063989	26	0.000937697	0.0024895
11	0.0191142	0.050746	27	0.000831694	0.0022081
12	0.0173807	0.046144	28	0.000497821	0.0013217
13	0.0125503	0.03332	29	0.000288356	0.00076556
14	0.0104696	0.027796	30	0.000172183	0.00045713
15	0.00899163	0.023872	31	0.000118461	0.0003145
16	0.00708665	0.018814	32	9.09104E-05	0.00024136

Annex f. Eigenvalues and percent variance of Chattogram and Pabna population

SL NO.	Eigenvalue (PC1)	%Variance (PC1)	SL NO.	Eigenvalue (PC1)	%Variance (PC1)
1	29.0035	79.615	17	0.0064241	0.017634
2	3.88321	10.659	18	0.00566898	0.015561
3	1.75988	4.8309	19	0.00461096	0.012657
4	1.1147	3.0599	20	0.00406762	0.011166
5	0.268208	0.73623	21	0.00252913	0.0069425
6	0.107088	0.29396	22	0.00200697	0.0055092
7	0.0617466	0.1695	23	0.00182249	0.0050028
8	0.0499095	0.137	24	0.0012186	0.0033451
9	0.0340427	0.093448	25	0.00110297	0.0030277
10	0.0313612	0.086087	26	0.000969356	0.0026609
11	0.021192	0.058172	27	0.000637157	0.001749
12	0.0163476	0.044874	28	0.000531859	0.00146

13	0.0145485	0.039936	29	0.000389691	0.0010697
14	0.0119166	0.032711	30	0.000250131	0.00068661
15	0.0116622	0.032013	31	0.000183574	0.00050391
16	0.00786635	0.021593	32	9.75712E-05	0.00026783

Annex g. Eigenvalues and percent variance of Khulna and Pabna population

SL NO.	Eigenvalue (PC1)	%Variance (PC1)	SL NO.	Eigenvalue (PC1)	%Variance (PC1)
1	39.2501	74.147	17	0.00676985	0.012789
2	10.154	19.182	18	0.00577519	0.01091
3	1.92672	3.6397	19	0.00531208	0.010035
4	0.899114	1.6985	20	0.00438074	0.0082756
5	0.280644	0.53016	21	0.00313185	0.0059163
6	0.132354	0.25003	22	0.00265853	0.0050222
7	0.0623603	0.1178	23	0.0025076	0.0047371
8	0.0404176	0.076352	24	0.00205152	0.0038755
9	0.0305439	0.0577	25	0.00122434	0.0023129
10	0.0276294	0.052194	26	0.000769858	0.0014543
11	0.0244584	0.046204	27	0.000625046	0.0011808
12	0.0226507	0.042789	28	0.000460177	0.00086931
13	0.0160177	0.030259	29	0.000343978	0.0006498
14	0.0127227	0.024034	30	0.000237396	0.00044846
15	0.0117476	0.022192	31	0.000211847	0.0004002
16	0.00771646	0.014577	32	7.94222E-05	0.00015004

CHAPTER 1

INTRODUCTION

1.1 General information

Fisheries is one of the most productive sectors in Bangladesh. This sector has been playing an increasingly important role in the economy over the last few decades. Bangladesh has achieved remarkable progress in fisheries sector since its independence in 1971. Fisheries have high potential for economic development of the country. Fisheries sector contributes 3.65% to our national GDP and 23.81% to the agricultural GDP (DoF, 2017). This sector is contributing significantly in food security by providing animal protein. Fish is one of the main dietary ingredients to the people of Bangladesh that provides about 60% of our daily animal protein intake (Sarker *et al.*, 2016). Bangladesh is one of the leading fish producing countries in the world. In Bangladesh, about 11% of total populations are dependent on fisheries for their livelihoods. The country earns a considerable amount of foreign currencies by exporting fish, shrimps and other fisheries products.

1.2 Clupeiformes fishes in the world

Clupeiformes is the order of ray-finned fish that includes about 405 species under seven Families including: Denticipitidae (denticle herring), Engraulidae (anchovies), Spratelloididae, Pristigasteridae (longfin herrings), Chirocentridae (wolf herrings) Dussumieriidae (round herrings), and Clupeidae (herrings, sardines, shads, and menhaden). The clupeid is one of the most important food fishes in the world, with particular interest for production of fish oil and fish meal. Clupeids are mainly marine fish but some of the species are found in fresh water. Most of fishes have a body protected with shiny cycloid scales, a single dorsal fin, with a fusiform body. No species has scales on the head, and some are entirely scale less. The lateral line is short or absent, and the teeth are unusually small.

Clupeids mostly feed on plankton at a size range from 2 to 75 cm. Clupeids spawn up to 200,000 eggs near the surface of the water. After hatching, the larvae live among the plankton until they develop a swim bladder and transform into adults. These eggs and fry are not protected by parents. The adults typically live in large shoals, seeking protection from piscivorous predators such as birds, sharks and other predatory fish. The Indian shad, *Tenualosa ilisha* (Hamilton 1822) belongs to the sub-family Alosinae of Family Clupeidae. This species is one of the most important commercial fishes of the Indo-Pacific region. It has a wide range of distribution and mainly occurs in marine, estuarine and riverine environments.

Table-1.1 List of species under genus *Tenualosa* available in the world

In this genus, there are currently five recognized species.

Sl. No	Common name	Scientific name	Distribution
1	Hilsa shad	*Tenualosa ilisha*	Indian ocean, Bay of Bengal (mainly Bangladesh & India), Gulf of Tonkin, Vietnam, Southern Iran and Myanmar.
2	Long tail shad	*Tenualosa macrura*	Western central pacific; Malaysia, Indonesia & Thailand.
3	Reeves' shad	*Tenualosa reevesii*	South China sea, Eastern Indian ocean, Phuket Island & Andaman sea.
4	Laotian shad	*Tenualosa thibaudeaui*	Cambodia, Thailand & Vietnam.
5	Toli shad	*Tenualosa toli*	Indian ocean, Bay of Bengal, Java sea & South China sea.

In this five species, only two species *Tenualosa ilisha* and *Tenualosa toli* are found in Bangladesh water bodies and large amount of *T. ilisha* are caught in Bangladesh. For this research, selected species is *T. ilisha*.

1.3 General information about Hilsa (*Tenualosa ilisha*)

Taxonomic position

Phylum: Chordata

Class: Actinopterygii

Order: Clupeiformes

Family: Clupeidae

Subfamily: Alosinae

Genus: *Tenualosa*

Species: *T. ilisha* (Hamilton, 1822)

Figure-1.1: Sample collected from Padma River (Ishwardi, Pabna).

1.3.1 Contribution of *T. ilisha* to Bangladesh economy

Tenualosa ilisha is the national fish of Bangladesh. *It* is the largest and single most valuable fishery in Bangladesh. This species is a popular food fish in South Asia. The annual fish production was 41.34 lakh MT in 2016-2017 fiscal years and the production of *T. ilisha* was

4.96 lakh MT which contributes 12% to the total fish production and 1% to the national GDP of the country. The production of *T. ilisha* has been increasing at the rate of 8% to 10% every year in Bangladesh due to the government's effective management strategies such as banning of hilsha fishing during spawning period etc. Bangladesh is the leading country reported from 75% in *T. ilish* production, 15% from Myanmar, 5% from India and 5% from other countries such as Thailand and Iran (Miah, 2015).

1.3.2 Distribution

Tenualosa ilisha is mainly found in the Bay of Bengal, Persian Gulf, Red Sea, Arabian Sea, Vietnam Sea and China Sea. The riverine habitat covers the Satil Arab and the Tigris and Euphrates of Iran and Iraq, the Indus and Pakistan, the Irrawaddy in Myanmar, the rivers of eastern and western India *viz.,* the Ganga, Bhagirathi, Hooghly, Rupnarayan, Brahmaputra, Godavari, Narmada, Tapti and the Padma, Jamuna, Meghna, Karnafuly and other coastal rivers of Bangladesh (Roomiani *et al.,* 2014). *T. ilisha* is a diadromous fish and largest single fishery in both the river and marine waters of Bangladesh. In Bangladesh, *T. ilisha* fish is mainly caught in the Padma (lower Ganges), Meghna (lower Brahmaputra), and Jamuna rivers. There are numerous fishing gears used for catching *T. ilisha* in both rivers and sea in Bangladesh and India. Some types of gears are illegal such as small meshed nets, which indiscriminately kill juvenile known as jatka. *Tenualosa ilisha* is mainly a marine species and reported as anadromous, adult migrate into estuarine and fresh waters from the sea for spawning purposes. During the peak breeding season the adult fish run in shoals and migrate upstream as much as 1200 km (usual run 50-100 km) to the estuaries and the rivers for spawning. In feeding and growth period spend in River channels and estuaries then back to sea for adult (Bhaumik, 2015).

1.3.3 Morphological Description

T. ilisha has a dark blotch behind the gill opening, followed by a series of small spots along the flank in juveniles. The fish has no dorsal spines but 18 - 21 dorsal soft rays and anal soft rays. The belly has 30 to 33 scutes. There is a distinct median notch in upper jaw. Gill rakers fine and numerous and the fins are hyaline. The species filter feeds on plankton and by grubbing muddy bottoms. The fish schools in coastal waters and ascends up the rivers (anadromous) for around 50 - 100 km to spawn during the South West monsoons (June to September) and also in January to April. April is the most fertile month for breeding of *T. ilisha*. The young fish returning to the sea are known in Bangladesh as jatka, which includes any Ilish fish up to 9 inches long. It can grow up to 60 cm in length with weights of up to 3 kg.

1.4 Geometric morphometric analysis

Geometric morphometric is one of the powerful techniques for detecting differences among groups and to differentiate between species of similar shape. This technique can capture differences in structures that are not easily identified through traditional morphometric measurements. Using the landmark-based geometric morphometrics, shapes of fish species that differs due to environmental variability can be detected. Landmarks are selected some arbitrarily points on a fish's body, and these points can be helped to be analyzed the shape of individual. In other words, a landmark point is important that matches between and within population. The body shapes variation of three populations of *Sardinella fimbriata* (Valenciennes, 1847) were determined by using relative warp analysis to distinguish and describe body shape variations existing within and between populations of *S. fimbriata* (Luceño *et al.,* 2013). Geometric morphometric analysis also proved to be successful body shape variation within population of Ibis fish, (*Ambassis interrupta),* are fluctuating asymmetry higher compared to individual variation in females (Medrano and Jumawan, 2016).

1.5 Biochemical composition analysis

Proximate composition or biochemical composition of fish meat mainly composed of protein, fat, carbohydrate, ash, and moisture. Fish meat is basically composed of water (66-81%), protein (16-21%), carbohydrates (<0.5%), lipids (0.2-25%) and ash (1.2-1.5%) (FAO,1999). The biochemical composition of hilsa such as protein (17.61%), lipid (9.37%), ash (0.82%) and moisture (71.78%) were found in jatka fishes (Dewan *et al.,* 2015). The nutritional composition of fish varies greatly from one species to another species and individual to individual, depending on maturity, feed ration, physical activity, and sexual changes related to spawning environment and season (Huss, 1995). The proximate composition such as moisture, protein, fat and ash percentage was determined and showed that Fat content of the Hilsha fish was much higher than many other freshwater fish species (Minar *et al.*, 2012).

The biochemical composition of fish samples were analyzed in triplicate following standard procedures of AOAC: crude protein content (N × 6.25) by the Kjeldahl method using a modified Kjeldahl System, crude fat were determined by Soxhlet method using petroleum ether (60- 80°C) as solvent, Ash content were determined in a Muffle furnace at 500-550° C for 16 h.

1.6 Rationale of the study

The main objectives is to investigate the different body shape variations within and between the four different populations of *Tenualosa ilisha* through the use of landmark based geometric morphometric analysis. The selected four sampling sites (Barishal, Khulna, Chattogram, & Pabna) where *T. ilisha* is widely fished, and probably overexploited. This method is important because body shape gives an idea to the different adaptations developed in a particular environment and stock assessment.

Early studies of population structure of hilsha in Bangladesh used morphometric and meristic traits, allozyme, RAPD analyses and PCR-RFLP of the mtDNA analysis are contradictory. Shifat *et al.* (2003) investigated the genetic variation of two populations (Padma and Meghna rivers) of Hilsa shad, (*Tenualosa ilisha*) by RAPD-PCR method and showed two clusters of Padma and Meghna at different spawning populations with different stocks. Salini *et al.* (2004) studied population structure of hilsa (*Tenualosa ilisha)* though allozymes and morphometric analysis and showed that hilsa vary greatly in shape due to geographic range.

The genetic variability and divergence *of T. ilisha* were investigate by using PCR-RFLP of the mtDNA D-loop region in two riverine (the Jamuna and the Meghna), two estuarine (Kuakata and Sundarbans) and one marine (Cox’s Bazar) populations and observed significant differentiations of haplotype and gene diversity among the populations of *T. ilisha* (Mazumder and Alam, 2009). More recently, studies with length-weight relationship and food and feeding ecology of hilsha (*Tenualosa ilisha*) (Flura *et al.,* 2015 and Hasan *et al.,* 2015).

The biochemical composition, nutritional value, fatty acid and mineral content of hilsha shad (*Tenualosa ilisha*) were studied in various regions in Bangladesh. Dewan *et al.,* (2015) studied the proximate composition of Hilsha at four different size groups (Jatka, Sub- adult, Adult, and Brood) and body parts of Chandpur region and showed that the nutrient content varied greatly with age and size of the fish of same species. The nutritional value of Hilsa (*Tenualosa ilisha*) from the six selected regions (Barishal, Patuakhali, Bhola, Cox’s Bazar, Chandpur and Shariatpur) were determined and suggest that the proximate composition of hilsa fish greatly varies due to physiological reasons and environmental conditions (Begum *et al.,* 2016).

In this study analyzed biochemical composition of Hilsa (*Tenualosa ilisha*) mainly protein, lipid and ash from the four selected rivers (Rupsa, Karnaphuli, Meghna and Padma) and also determined the body shape variation among the populations in Hilsa shad based on landmark based geometric methods. This study is important to know the variation in biochemical composition of selected four populations (Khulna, Chattogram, Barishal, and Pabna) and suggest that where located of *T. ilisha* is best for nutrition and also important to know the body shape variation and differentiate among the populations of *T. ilisha* of specific region.

1.7 Objectives of this study

The goal of this study is to know the level of body shape variation and biochemical composition accumulated due to the distribution of different regional conditions.

- To show the body shape variation and differentiation among the four populations of *Tenualosa ilisha* by Geometric morphometric methods; and
- To know the biochemical composition value of selected four populations of *T. ilisha*

and compare this values among the populations.

Chapter 2

REVIEW OF LITERATURE

In this study, body shape variation of four populations by landmark based geometric methods and proximate composition analysis among the population of Hilsa, *Tenualosa ilisha* were depicted. Literature related to the present study were collected on landmark based geometric, morphometric, biological, genetics and also some biochemical composition aspects of Hilsa, *T. ilisha* and related species. These reviews of literature are given below:

2.1 Geometric morphometric studies

Cavalcanti *et al.* (1999) studied morphological differentiation of Serranid fishes by geometric morphometric method. A total of 52 individuals from 6 species of Serranidae (*Dules auriga*, *Diplectrum formosum*, *D. radiale*, *Epinephelus marginatus*, *Mycteroperca acutirostris*, and *M. bonaci)* were collected from the southeast coast of Brazil. Significant differences were found among species on the basis of body height and caudal peduncle length and changes in body shape were clearly differentiating the species of Serraninae. These shape changes are related to habitat and feeding habits among the species.

Pakkasmaa and Piironen (2001) studied morphological differentiation among three trout (*Salmo truttu)* forms (sea-run trout, lake-run brown trout, and resident brown trout) that were obtained from ten populations in Finland. Morphometric characters were measured from each individual fish that were reared under similar environmental condition. The study showed that morphological differentiation was observed and the differences between populations were greater than differences between forms. This result suggests that the differences have a genetic basis and adaptation in the native habitat.

Shifat *et al.* (2003) investigated the genetic variation of two populations of Hilsa shad, (*Tenualosa ilisha*) by RAPD-PCR that were collected from two rivers (Padma and Meghna) in Bangladesh. A total of fifteen primers were selected and used 34 individuals from two sampling sites for DNA fragments. Variations were found between and within the populations by comparing RAPD banding patterns. A dendrogram was constructed for genetic distance analysis and showed two clusters of Padma and Meghna at different spawning populations with different stocks.

Silva (2003) studied morphometric variation among sardine (*Sardina pilchardus*) populations and collected 14 samples spanning from the northeastern Atlantic and the western Mediterranean regions and analyses morphometric data by multivariate and geometric methods. The analyses explored the homogeneity of sardine shape within the area of that adjacent and distant population. The two groups of sardine are significantly separated by discriminant analysis.

Salini *et al.* (2004) studied population structure of Hilsa (*Tenualosa ilisha)* though allozymes and morphometric analysis. The sample were collected from nine sites within Bangladesh and compared with four other countries (India, Indonesia, Kuwait and Myanmar). The results showed that Hilsa vary greatly in shape due to geographic range. They suggest that morphological results reflect local environmental conditions and allozyme is substantial gene flow between groups of Hilsa within the Bay of Bengal.

Narejo *et al.* (2008) studied morphological differences between two types of Palla, *Tenualosa ilisha* and collected 280 specimens from River Indus, Pakistan. The study revealed significant intertype differences in six morphometric measurements and seven meristic characters. This study showed that Length-weight relationship, condition factor values and GSI values were also different in summer and winter.

Mazumder and Alam (2009) investigated genetic variability and divergence *of T. ilisha* by using PCR-RFLP of the mtDNA D-loop region in two riverine (the Jamuna and the Meghna), two estuarine (Kuakata and Sundarbans) and one marine (Cox's Bazar) populations. In that study, they observed significant differentiations of haplotype and gene diversity among, populations of *T. ilisha*. The study suggested that RFLP of mtDNA D-loop region used as molecular markers for detecting two differentiated populations of *T. ilisha*.

Abed *et al.* (2012) studied morphometric and meristic characteristics of young Hilsa (*Tenualosa ilisha*) and collected 67 specimens from three sampling sites (Dair, Ab-Alkaseeb and Fao) in the Shatt Al-Arab River. The statistical analysis reveals insignificant differences among the investigated sites except for ratio of caudal peduncle depth to standard length, and ratio of inter-orbital distance, head width to its length.

Hossain *et al.* (2010) studied the population status of the endangered carp, kalibaus *Labeo calbasu*, by Landmark-based morphometric methods that are collected from 2 isolated rivers (the Jamuna and Halda) and a hatchery. Significant differences were observed in four of 12 morphometric measurements, two of 9 meristic counts, and four of 22 truss network measurements among the stocks. The dendrogram based on morphometric and truss showed that the distance between the Halda and hatchery populations was the highest.

Luceño *et al.* (2013) studied the Body shapes variation of three populations of *Sardinella fimbriata* by geometric morphometrics analysis. This method is used to distinguish and describe body shape variations existing within and between populations of *S. fimbriata.* They suggested that genetic study is needed to understand the diversity of this species which contribute to mananagement strategies of stocks or populations of *S. fimbriata.*

Thirumaraiselvi *et al.* (2013) studied morphometric and genetic variation in three populations of Indian salmon (*Polydactylus plebeius*) and used RAPD to discriminate and ratify the population status. A total of 1077 scorable bands were produced and used ten arbitrary primers in three populations. In this study showed that all the three populations were significantly higher inter-population genetic distance than the intra-population.

Brahmane *et al.* (2014) investigated mitochondrial DNA cytochrome *b* based on genetic diversity and population differentiation of Hilsa (*Tenualosa ilisha*) from Ganga and Hooghly rivers. Six different haplotypes of 240 samples with a single dominant haplotype present in both rivers. They suggested that the whole population existence of a single population, migrating to Ganga and Hooghly rivers for spawning and breeding purpose.

Mohamed *et al.* (2014) studied stock assessment and management of Hilsa shad (*Tenualosa ilisha*) by using FiSAT II software that were collected from Iraqi marine waters, northwest Arabian Gulf. They observed a bimodal recruitment pattern of unequal strength and showed that *T. ilisha* is over exploited. In this study suggest that some management policy should be taken to the stock in inlands waters.

Luceño *et al.* (2014) studied the Body shapes of three populations of *Sardinella lemuru* (Bleeker, 1853) by using geometric morphometric analysis that was collected from Mindanao Island, Philippines. They collected 30 males and 30 females from each sampling site were digitized and using 18 landmark points in the fish's body. The result showed that landmark-based geometric morphometric method is an effective tool in describing body shape variations that exist within and between populations of *S. lemuru*.

Giducos *et al.* (2015) studied the body shape of *Sardinella lemuru* from Sarangani Bay, Philippines using the landmark based geometric morphometric analysis. They collected 30 males and 30 females from each sampling site were digitized and using 18 landmark points in the fish's body. The body shape variation was obtained by the boxplots of the relative warp (RW) scores along with the extremely positive and negative warps.

Flura *et al.* (2015) studied length-weight relationship of hilsa, *Tenualosa ilisha* (Hamilton, 1822) fishes using monthly samples over a calendar year from the river Meghna flowing through Chandpur district in Bangladesh. A total of 517 specimens (171 male and 346 female) of hilsa were collected. The study revealed that all length-weight relationships were highly correlated. This study was helpful for fishery managers to impose adequate regulations for sustain.

Hasan *et al.* (2016) studied food and feeding ecology of hilsa (*Tenualosa ilisha*) in Meghna River basin by using 'electivity index'. They analysis gut contents and identified a range of phytoplankton and zooplankton genera and also established the hilsa's food preferences at various stages in the life cycle. This study was essential to provide information for policy making and the effective management of the hilsa fishery.

Medrano and Jumawan (2016) studied body shape variation within population of Ibis fish, (*Ambassis interrupta*) using landmark-based geometric morphometric analysis. A total of 100 fish samples are collected from Masao River, Butuan City, Philippines and digitized using sixteen landmark points. Principal Component Analysis (PCA) revealed that fluctuating asymmetry interactions in female fishes are higher compared to fluctuating asymmetry in males.

E-mahfuj *et al.* (2017) studied morphological variations of *Labeo bata* populations based on morphometric and landmark to know the population status in six rivers of Bangladesh. They analyzed 22 truss measurements and 15 morphometric characters revealed a significant inter stock variation among all the samples. This study supported that 'truss network' is an effective tool for determining the population status and baseline information of *L. bata.*

The geometric morphometric studies of three *Anabas testudineus* populations indigenous koi, Thai koi and Vietnamese koi showed considerable variation (Mahajebin, 2017). The overall variation among 3 populations variance was of 94.208% in principal component 1. This variation was depicted in scatter plot. The population specific positions of each individual were observed and some overlapping of plotting between Thai and Vietnamese populations were found.

The geometric morphometric studies of three *Barilius* species, *B. barila, B. barna,* and *B. bendelisis* showed considerable variation (Rekha, 2017). In geometric study, the overall variation among the 3 species, showed the Percent variance (91.282%) and the Eigenvalue (89.0673) in Principal Component 1(PC-1). The results of principal component analysis, scatter plot and UPGMA cluster analysis showed that *B. bendelisis* was clearly distinct from other species

Jearranaiprepame (2017) studied morphological differentiation of *Channa gachua* (Hamilton, 1822) using truss network analysis to know the variability of body shape among distinct populations. A total of 98 fish individuals were collected and 26 truss variables were measured for univariate and multivariate statistical analyses. These statistical analyses are highly significant differences between populations. This result indicate that the presence of three phenotypic stocks of *C. gachua* regarding their habitat across the isolated geographical locations.

2.2 Biochemical composition studies

Sutharshiny and Sivashanthini (2011) determined the flesh quality of three fish species (*Scomberoides lysan, S. tol* and *S. commersonianus)* from Sri Lankan Waters. They estimated major nutrient compositions of raw muscle like protein, lipid, moisture, carbohydrate and ash and showed that biochemical compositions were varied among the species. The results revealed that the highest protein content (21.68%) was recorded in *S. commersonianus* whereas the lowest lipid content (0.59%) was reported in *S. tol*. The results suggested that *S. commersonianus* is an ideal dietetic food among the three fish analyzed.

Begum *et al.* (2012) determined proximate biochemical composition of fresh aquarium pangus (*Pangasianodon hypophthalmus*) fish in Laboratory Condition in Bangladesh. The proximate compositions of raw pangas muscles were determined through determination of moisture, Protein, Fat and Ash of the edible portion. The results indicates that the moisture, protein, fat and ash percentage (%) were 78.29 ±0.22, 12.78 ±0.16, 16.55 ± 1.52 and 1.78 ±0.19, respectively and play a significant role to fulfill the nutrient demand of the people in Bangladesh.

Begum *et al.* (2012) studied body composition of six small indigenous species (SIS) (*Ailiichthys punctata, Clupisoma psendeutropius atherinoides, Puntius sarana, Gudusia chapra, Corica soborna),* One Hilsa fish (*Tenualosa ilisha*) and two shellfishes (*Macrobrachium and Penaeus spp*) that are commonly available in Bangladesh. They determined mainly biochemical composition through moisture, protein, ash and fat and observed considerable variations in the proximate composition of different fish species. In this comparative study suggest that body composition of all fish contain higher nutrient value and helps to fulfill the nutrient demand of our country people.

Kumaran *et al.* (2012) investigated biochemical composition of Mullet (*Mugil cephalus*) in the Parangipettai coastal waters, Southeast Coast of India. The biochemical composition such as moisture, lipid, protein and crude ash (75.27, 2.42, 17.56 and 1.15) were determined and showed that the muscle of flesh of *Mugil cephalus* contains important w-3 and w-6 fatty acids and it’s suitable for human consumption. They also detected ten essential amino acids and eight nutrient elements and all are required daily by human for good health.

Minar *et al.* (2012) studied the biochemical composition of Hilsa *(Tenualosa ilisha)* in laboratory condition. The biochemical composition such as moisture, protein, fat and ash percentage was determined and showed that Fat content of the Hilsha fish was much higher than many other freshwater fish species. This result helps the consumers, fish processors and the researchers providing information on biochemical composition.

Begum *et al.* (2013) studied biochemical composition on monthly sampling of fresh gonia (*Labeo gonius*) that were collected from Chamtaghat area, Mymensingh Bangladesh. In this comparative study to determine several parameter and showed that monthly variation from the point of nutritional aspect. The result found that the highest value of crude protein and moisture was found in May and lipids, carbohydrates, ash and crude fiber were in July and August. The protein and fat content was lower in August due to the breeding season gonia.

Ondo-azi *et al.* (2013) investigated proximate composition (protein, lipid, ash and moisture), energy and microbiological analysis in five marine fish species (*Pseudotolithus typus*, *Pomadasys peroteti*, *Galeoides decadactylus*, *Carassius auratus* and *Pimelodus grosskopfii*) consumed in Gabon. In this five species protein content was highest in *Carassius auratus* (20.78%). Microbiological analysis showed that the Total Viable Count (TVC) and found *Staphylococcus* sp. bacteria were observed in all five species and fungi were not found in these five samples.

Hossain *et al.* (2014) investigate the proximate composition, fatty acid and mineral content of hilsa (*Tenualosa ilisha*) from the Bay of Bengal and Arabian Gulf. The egg protein contents (22.27–23.08%) of hilsa were significantly ($p<0.05$) higher than those of the respective muscle proteins (18.16–19.13%). In the study the high lipid content of the hilsa eggs (14.20–24.23%) than the muscle tissues (11.20–19.94%). They suggest that Hilsa from the Bay of Bengal could be a better source of n-3 PUFA, due to higher lipid content compared to the Arabian Gulf.

Ullah *et al.* (2014) studied body composition of two fish species, Common Carp (*Cyprinus carpio*) and Silver Carp (*Hypophthalmichthys molitrix*) that are available in fish market and hotels at Timergara, Pakistan. This study was designed to determine the body composition on dry and wet basis mainly water content, protein, Fat, Organic content, and Ash of these two Chinese carps. The result of this study revealed that there is a significant difference between body composition and variations also exist of all constituents of the studied fish species.

Vijayakuma *et al.* (2014) examined proximate composition of Clupeidae (*Sardinella longiceps*, *S. gibbosa*, *Nematalosa nasus*, *Ilisha melastoma*) and Engraulidae (*Stolephorus indicus*, *S. commersonni*, *Thryssa mystax* and *T. malabarica*) that are collected from Thengaithittu estuary, south east coast of India. The result of this study found that the average moisture content ranged from 67 to 73% and protein contents between 14 to 21% and highest protein content was 21.3% in *T. mystax*. Lipid contents between 2.4 to 6.3% and highest lipid content (6.3%) in *S. longiceps*.

Dewan *et al.* (2015) studied the proximate composition of Hilsa at four different size groups (Jatka, Sub- adult, Adult and Brood) and body parts of Chandpur region. In this study, showed that the nutrient content varied greatly with age and size of the fish of same species. The proximate composition such as protein and ash content to be more or less same but inverse relationship between the lipid and moisture content in different size groups. The protein content was highest value in adult and the lowest in Jatka and the moisture content was highest in jatka and lowest in brood. In this study also found that the protein content was highest in white muscle and lipid content was highest in dark muscle.

Mohanty *et al.* (2015) analyzed the biochemical composition on seasonal basis of three economically important marine fishes (*Tenualosa ilisha, Sillago sihama* and *Mugil cephalus)* in Chandipur, Bay of Bengal, India. The study found that there are significant differences between *S. sihama* and *T. ilisha* and showed that moisture was highest in *S. sihama* whereas protein and lipid was highest in *T. ilisha*. Ash content was not dependent on season basis. No significant differences were observed between *T. ilisha* and *M. cephalus.*

Begum *et al.* (2016) studied the nutritional value of Hilsa fish (*Tenualosa ilisha*) from the six selected regions (Barisal, Patuakhali, Bhola, Cox's Bazar, Chandpur and Shariatpur) in Bangladesh. In this study, they determined the proximate composition such as moisture, protein, fat, carbohydrate and energy value that are regarded useful reference for consumer based on their nutritional contents. These results suggest that the proximate composition of hilsa fish greatly varies due to physiological reasons and environmental conditions.

Majumder *et al.* (2016) studied proximate composition of Paradise threadfin (*Polynemus paradiseus*) that was collected from the Sonapur fish market of Noakhali district of Bangladesh. The proximate composition mainly crude protein is the main component of the species and showed variation of crude protein and lipid (oil) of large and small size fishes. Protein content was higher in large fish (58.24%) than in smaller fish (56.96%) on dry matter basis. The studies conclude that nutritional value are variable according to their size and play a significant role to fulfill the nutrient demand.

Chapter 3

MATERIALS AND METHODS

To achieve the goal of the study, the entire research work were divided into two experiments

Experiment-I

Geometric morphometric variation of Hilsa, *Tenualosa ilisha* from four different rivers of geographically isolated regions of Bangladesh.

Experiment-II

Biochemical composition variation of Hilsa, *T. ilisha* from four different rivers of geographically isolated regions of Bangladesh.

3.1 Experimental period and station

Experiments on geometric morphometric analysis and biochemical composition analysis were done from July to December, 2017. Geometric morphometric analyses were done in Fisheries Biology & Genetics laboratory, Faculty of Fisheries and Biochemical composition analysis was done in the Laboratory of Agricultural Chemistry, Faculty of Agriculture, HSTU, Dinajpur.

3.2 Collection of samples

To cover the broader zone of the hilsha availability in Bangladesh, samples were collected from geographically isolated four rivers of the country. Twenty five samples of Hilsa (15 females and 10 male) from each sources were collected in ice preserved condition during July-September, 2017. Sexes were determined through the examination of the fish’s gonads. The sample collection and collected sites including the name of rivers, zone of the country and longitude-latitude were given in Figure-3.1, 3.2 and Table-3.1.

1.Pabna at Padma river

2.Khulna at Rupsa river

3.Barishal at lower Meghna river

4.Chattogram at Karnaphuli river

Figure-3.1: The sample collection sites including the name of rivers, zone of Bangladesh.

Table-3.1 Collection of samples of *Tenualosa ilisha* from four different Rivers.

Name of the river	Zones of the country	Longitude and Latitude
Rupsa	Khulna	22°50'45.9"N 89°33'26.8"E
Karnaphuli	Chattogram	22°23'33.0"N 91°53'16.2"E
Lower Meghna	Barishal	22°44'26.0"N 90°39'45.2"E
Padma	Pabna	24°06'00.1"N 89°01'30.3"E

Figure-3.2: Sample collected from Karnaphuli River at Chattogram and Meghna River at Barishal

3.3 Experiment-I Geometric morphometric variation of Hilsa, *Tenualosa ilisha* from four different rivers of geographically isolated regions of Bangladesh.

3.3.1 Image acquisition and digitization

A DSLR camera was used to capture the image of all the specimens. Only undamaged fish samples were imaged. Geometric morphometric analysis is used to determine the morphological variations by a phenotypic standpoint. The images were converted in TPS(Thin Plate Splines) file format by using tpsUtil (Version 1.74) program then the TPS(Thin Plate Splines) file were opened in TpsDig2(version 2.29) program. For each individual 16 landmarks were selected According to Bookstein (1991) and the landmark data were digitized using TpsDig2 (version 2.29) software. There were selected 16 homologous anatomical landmarks points that used to analyses the body shape was shown in Table-3.2and digitized image was shown in Figure-3.3

Table-3.2 Total 16 Landmark points that were plotted on the images:

Landmark point	Description
LM1	Upper point of mouth
LM2	Center of the eye (Dorsal side)
LM3	End point of operculum(dorsal side)
LM4	End point of frontal bone
LM5	Origin of dorsal fin
LM6	End point of dorsal fin
LM7	End point of anal fin (dorsal side)
LM8	Start point of caudal fin
LM9	Middle point of caudal fin
LM10	Origin of caudal fin (ventral side)
LM11	End point of anal fin
LM12	Origin of anal fin
LM13	Origin of pelvic fin

LM14	Start point of operculum(ventral side)
LM15	Middle point of operculum
LM16	Lower point of mouth

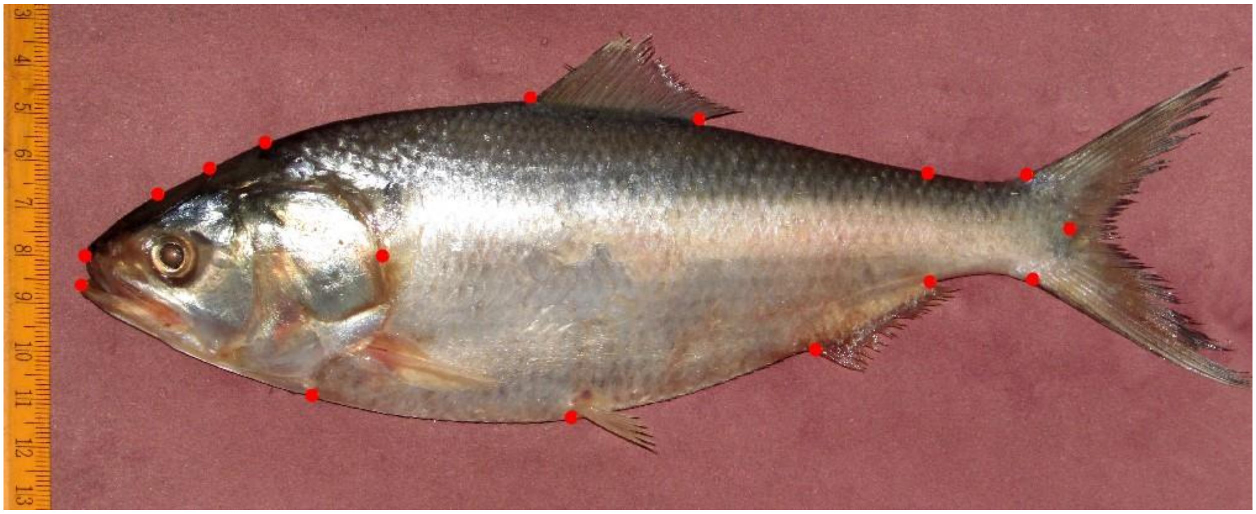

Figure-3.3(a): Red dots represent landmarks those used to analyze body shape.

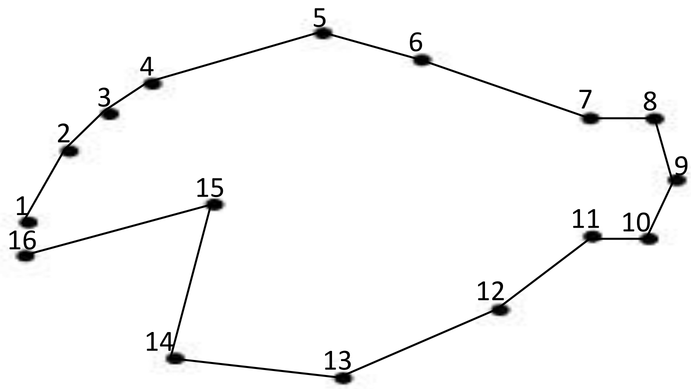

Figure-3.3(b): Landmarks coordinate those used to analyze body shape.

3.3.2 Geometric morphometric analysis using PAST

The landmark coordinates of four populations of Hilsa (*Tenualosa ilisha*) were analyzed with the help of computer software packages TPS series and Past (Version 3.0). Paleontological Statistics (PAST) software version 3.0 was generated to determine body shape variations exhibited by *T. ilisha*. PAST was used to analyses the variations among species and the geographical locations from which the fish were collected. The analysis was involved Principal component analysis (PCA), Scatter plot and clustering. Principal component analysis (PCA) was done to emphasize variation and make data easier to explore and visualize. In the scatter plot for all four populations was represent and showed overlapping of plotting, some were mixing or distinctly separated. Cluster analysis showed the major cluster formation of Barishal, Chattogram, Khulna and Pabna populations or cluster formation between populations to know the populations were separated or mixing cluster formation.

3.4 Biochemical composition analysis

3.4.1 Preparation of sample for biochemical study

The collected fish samples were washed properly with fresh water to remove all dirts, slime, and unnecessary particles. Then the fishes were measured length and weight and all of the collected fish sample were categorized into Jatka (Length: <23cm, Weight: 120 to 210 gm). Then each category fish was cut by sharp knife and ground in a mechanical grinder to make a paste. This ground sample was used for biochemical composition analysis. Total 12 samples were used that were collected in four different Rivers (Rupsa, Karnaphuli, Meghna and Padma). For each source samples of 3 individuals were taken for biochemical analysis.

3.4.2 Estimation of Biochemical composition

This experiment measured the biochemical composition of crude protein, ash, and lipid contents were carried out following the methods of AOAC (1980).

3.4.2.1 Estimation of protein

Protein was estimated by using kjeldahl method. The process was given below**:**

A) Digestion

A total of 10g blended fish sample was taken in a digestion flask. Then 10g potassium sulphate (K_2SO_4), 0.1g copper sulphate ($CuSO_4$),1g selenium powder and 25ml conc.H_2SO_4 was added to it and heated until the solution became clear. Then the flask was cooled.

B) Distillation

After digestion about 300ml distilled water and 125ml 40% NaOH solution was added to it. Then the flask was attached quickly to the distillation set and heated the flask continuously. In the meantime a 250ml conical flask (containing 25ml of 4% boric acid and 4-5 drops of mixed indicator) was placed at the set of the condenser. About 150 ml distillate was collected and was titrated with 0.2 N H_2SO_4 solutions.

Calculation:

$$\% \text{ Nitrogen} = \frac{Titrate\ value \times N \times 0.014 \times 100}{Weight\ of\ sample}$$

% Protein = %Nitrogen × 6.25

3.4.2.2Determination of Lipid

The lipid was determined by using soxhlet extraction method. Firstly, all glassware were rinsed with petroleum spirit, drained, and dried in an oven at 102 º C for 30 min. and cooled in a desiccator. Then a piece of cotton wool was placed at the bottom of a 100 mL beaker and put a plug of cotton wool in the bottom of an extraction thimble and stand the thimble in the beaker. Accurately weighted 5 g of sample were taken into the thimble and 1 - 1.5 g of sand was mixed with a glass rod. Then a piece of cotton wool was taken from the bottom of the beaker and placed it the thimble in a Soxhlet liquid/solid extractor. Accurately weighted a clean, dried 150mL round bottom flash and put about 90 mL of petroleum spirit into the flask. Then the solvent was heated in the flask until it boiled and continued the extraction for 6 hours. The extraction unit was removed from the heat source and detaches the extractor and condenser. The flask was replaced on the heat source and evaporates off the solvent. Then the flask was placed in an oven at 102ºC and dried the contents until a constant weighted is reached (1-2hours). The flask was cooled in a desiccator and weighted the flask.

Calculation:

Weight of empty flask (g) = W_1

Weight of flask and extracted fat (g) = W_2

Weight of sample = S

$$\% \text{ Crude fat} = \frac{W_2 - W_1}{S}$$

3.4.2.3 Determination of Ash

Total ash content was determined adopting by AOAC method (1980). The experiments were induced. A clean crucible was dried in oven then cooled and weighed. Then 3-5g sample was taken in the crucible and the crucible was placed in a muffle furnace and burnt the sample at 550-600^0 C for 4-6 hrs. Then removed the crucible from muffle furnace and cooled in desiccators and weighed.

Weight of ash

$$\%\text{Ash} = \frac{(Weight\ of\ ash)}{Weight\ of\ Sample} \times 100$$

3.4.3 Statistical Analysis

Statistical Analysis was done by using Statistical Package for the Social Sciences (SPSS) software. Here one-way analysis of variance (one-way ANOVA) was used to compare the means of protein, lipid and ash of four populations. The simplest ANOVA involved the analysis of data sampled from four populations with four treatments to compare the mean value of biochemical composition among the population. Further, Tukey's test was conducted an analysis of variance and represent differences in treatments mean or treatment different among the four populations. The results of ANOVA were positive; there were significant differences among the population.

Chapter 4

RESULTS

4.1. Geometric morphometric variation of four *Tenualosa ilisha* populations

In geometric morphometric method the principal component analyses, scatter plot and cluster analysis were conducted to depict the variation of *T. ilisha* population collected from 4 rivers of isolated geographic regions of Bangladesh. The details of each result are given in the following sections-

4.1.1 Principal Component Analysis

The geometric morphometric of four population of *T. ilisha* analyzed with the help of computer software packages TPS series and PAST (Version 3.0) are given in Table-4.1

4.1.1.1. Overall Principal Component Analysis of four *T. ilisha* populations

The overall variation among 4 populations of *T. ilisha* having both male and female individuals were estimated for a total of 32 principal components analysis (Annex-a), where the highest Eigenvalue (37.847) and percent variance (75.525%) were found in principal component 1 (Table-4.1).

Table-4.1 Geometric morphometric variation found in four populations of *Tenualosa ilisha*

SL.NO	Population pair/ Combination	Variation type	Eigenvalues				%Variance			
			PC-1	PC-2	PC-3	PC-4	PC-1	PC-2	PC-3	PC-4
1.	Overall	Among all the population	37.847	8.502	1.805	1.078	75.525	16.967	3.602	2.152
2.	Female of four population	Among four female	29.204	4.422	1.696	1.278	77.852	11.79	4.522	3.408
3.	Male of four population	Among four male	51.756	14.314	1.906	0.746	74.658	20.648	2.750	1.076
4.	Barishal female vs. male	Sexual dimorphism	18.622	3.467	1.731	0.569	74.359	13.847	6.912	2.275
5.	Chattogram female vs. male		21.032	3.559	1.615	0.436	77.499	13.116	5.953	1.609
6.	Khulna female vs. male		19.825	7.703	1.037	0.530	66.972	26.025	3.504	1.790
7.	Pabna female vs. male		35.603	3.602	1.189	0.770	85.369	8.639	2.851	1.848
8.	Barisal vs. Chattogram	Between population	34.510	6.191	1.762	0.485	79.005	14.175	4.034	1.110
9.	Barishal vs. Khulna		21.606	5.628	1.645	0.567	71.341	18.585	5.432	1.873
10.	Barishal vs. Pabna		52.891	5.936	1.301	1.129	85.335	9.577	2.100	1.821
11.	Chattogram vs. khulna		23.820	11.120	1.530	0.560	63.242	29.524	4.062	1.489
12.	Chattogram vs. Pabna		29.003	3.883	1.759	1.114	79.615	10.659	4.830	3.059
13.	Khulna vs. Pabna		39.250	11.154	1.926	0.899	74.147	19.182	3.639	1.698

4.1.1.2. Pairwise Principal Component Analysis of *T. ilisha* population

To depict the variation between population, we analyzed 6 pairs; Barishal vs. Chattogram population, Barishal vs. Khulna population, Barishal vs. Pabna population, Chattogram vs. Khulna population, Chattogram vs. Pabna population and Khulna vs. Pabna population. In pairwise variation study, the highest Eigenvalue (52.891) and Percent Variance (85.335%) were found between Barishal and Pabna population followed by between Barishal and Chattogram (34.510 and 79.005%), Barishal and Khulna (21.606 and 71.341%), Chattogram and Khulna (23.820and 63.242%), Chattogram and Pabna (29.003 and 79.615%) and Khulna and Pabna population (39.250 and 74.147%) respectively (Table-4.1).

4.1.2. Overall variaiton of four *T. ilisha* population by scatter plot analysis

Among four populations, the analyses identified 32 principal components (Annex-a) where the highest Eigenvalue (37.847) and percent variance (75.525%) were found in principal component (PC) 1 (Table-4.1). The population specific position of each individual were observed though there were some overlapping of plotting between Barishal and Khulna populations and some were Chattogram and Khulna populations.

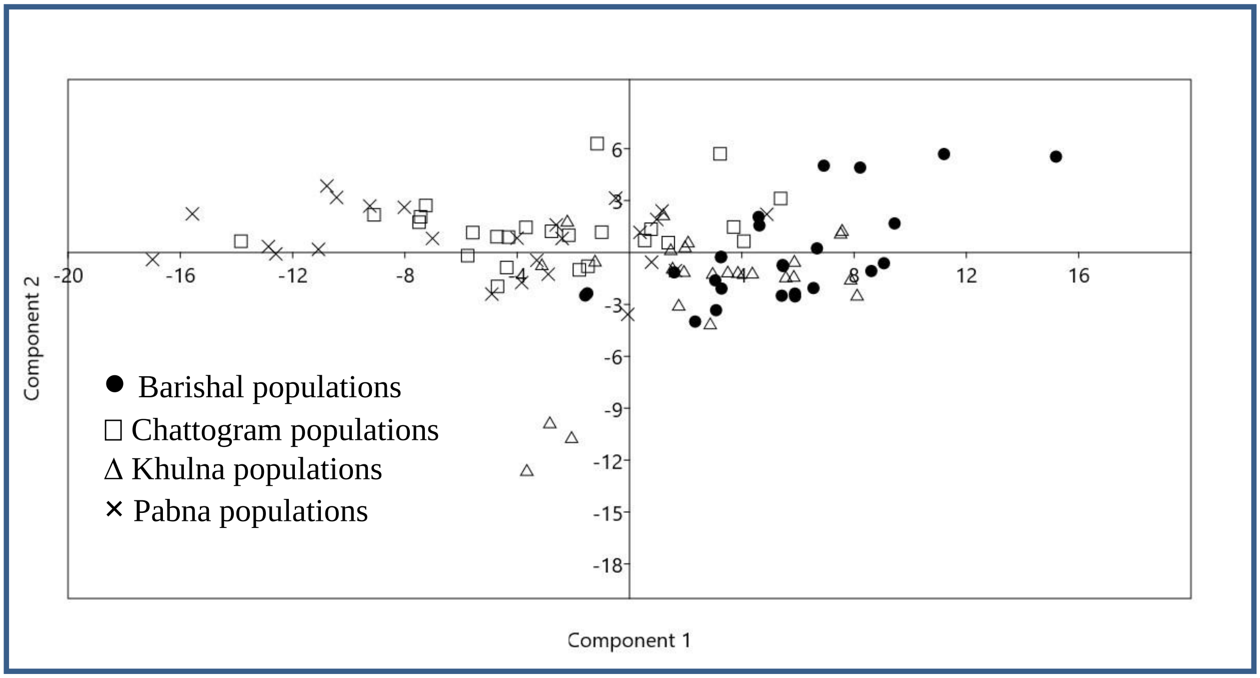

Figure-4.1 The scatter plot of 4 populations of *T. ilisha*;. Symbols refer to individuals in Barishal populations-Dot●; Chattogram populations-Square□; Khulna populations-TriangleΔ; Pabna populations-Cross×.

4.1.3. Pairwise variaiton of four *T. ilisha* populations by scatter plot analysis

To depict the variation between populations, we analyzed 6 pairs; Barishal and Chattogram populations, Barishal and Khulna populations, Barishal and Pabna populations, Chattogram and Khulna populations, Chattogram and Pabna populations and Khulna and Pabna populations.

4.1.3.1: Variation between two populations of Barishal and Chattogram populations

In between Barishal and Chattogram population 32 principal components (Annex-b) were identified where the highest Eigenvalue (34.510) was found in PC 1 with a variance of 79.005% (Table-4.1). The Barishal and Chattogram *T. ilisha* populations were distinctly placed from each other except mixing few individual

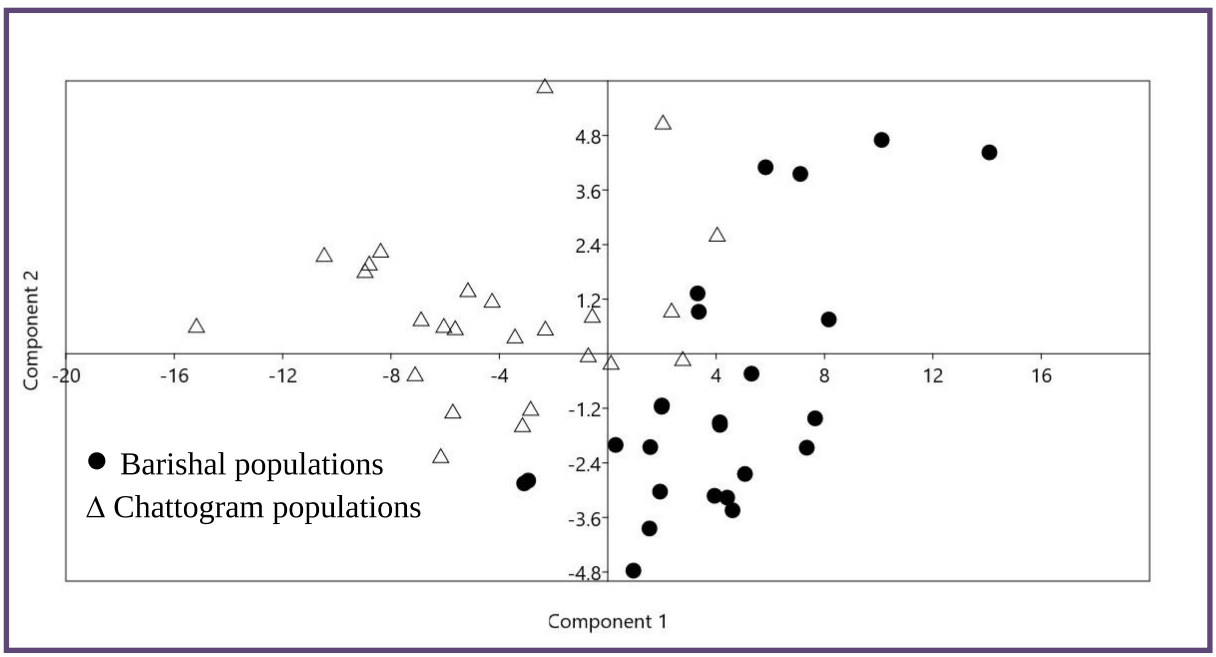

Figure-4.2 Scatter plot between Barishal and Chattogram *T. ilisha* populations; Symbols refer to individuals in Barishal populations-Dot●; Chattogram populations-TriangleΔ.

4.1.3.2: Variation between two populations of Barishal and Khulna populations

In between Barishal and Khulna population 32 principal components (Annex-c) were identified where the highest eigenvalue (21.606) was found in PC 1 with a variance of 71.341% (Table-4.1). The Barishal and Khulna *T. ilisha* populations were overlapping from each other except mixing few individual (Figure-4.3).

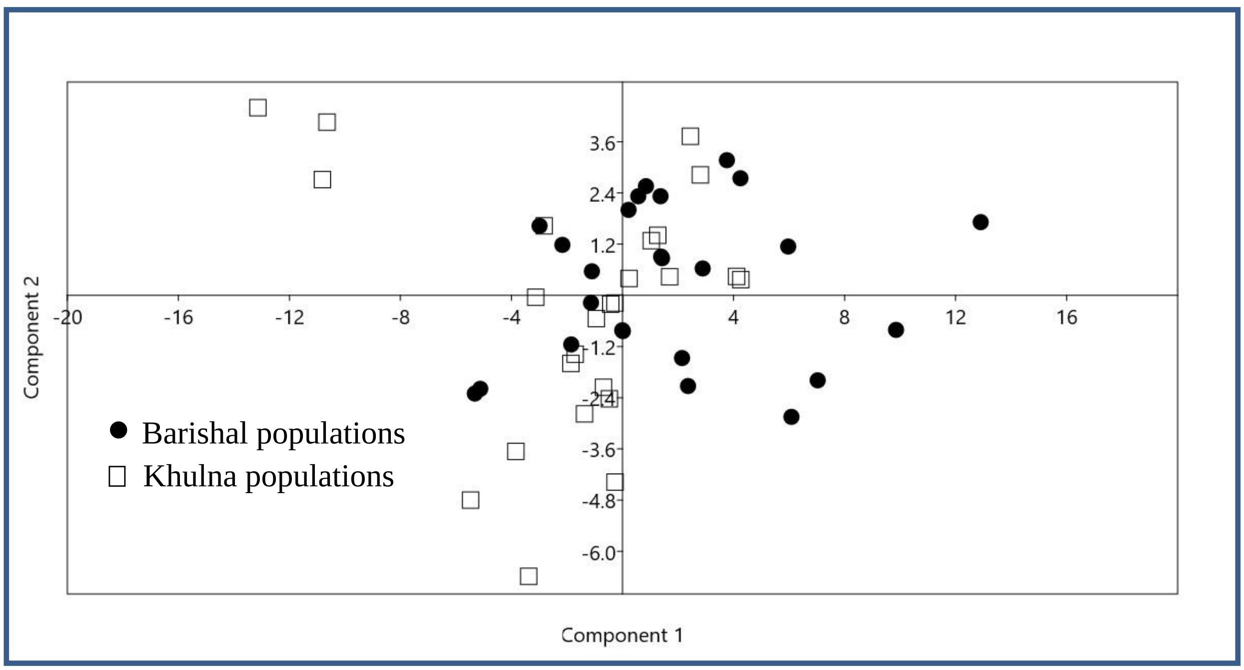

Figure-4.3 Scatter plot between Barishal and Khulna *T. ilisha* populations; Symbols refer to individuals in Barishal populations-Dot●; Khulna populations-Square□.

4.1.3.3: Variation between two populations of Barishal and Pabna populations

In pairwise population study, Barishal and Pabna populations showed 32 principal components (Annex-d) were identified where the highest Eigenvalue (52.891) was found in PC 1 with a variance of 85.335% (Table-4.1). The Barishal and Pabna *T. ilisha* populations were distinctly placed from each other except mixing few individual (Figure-4.4).

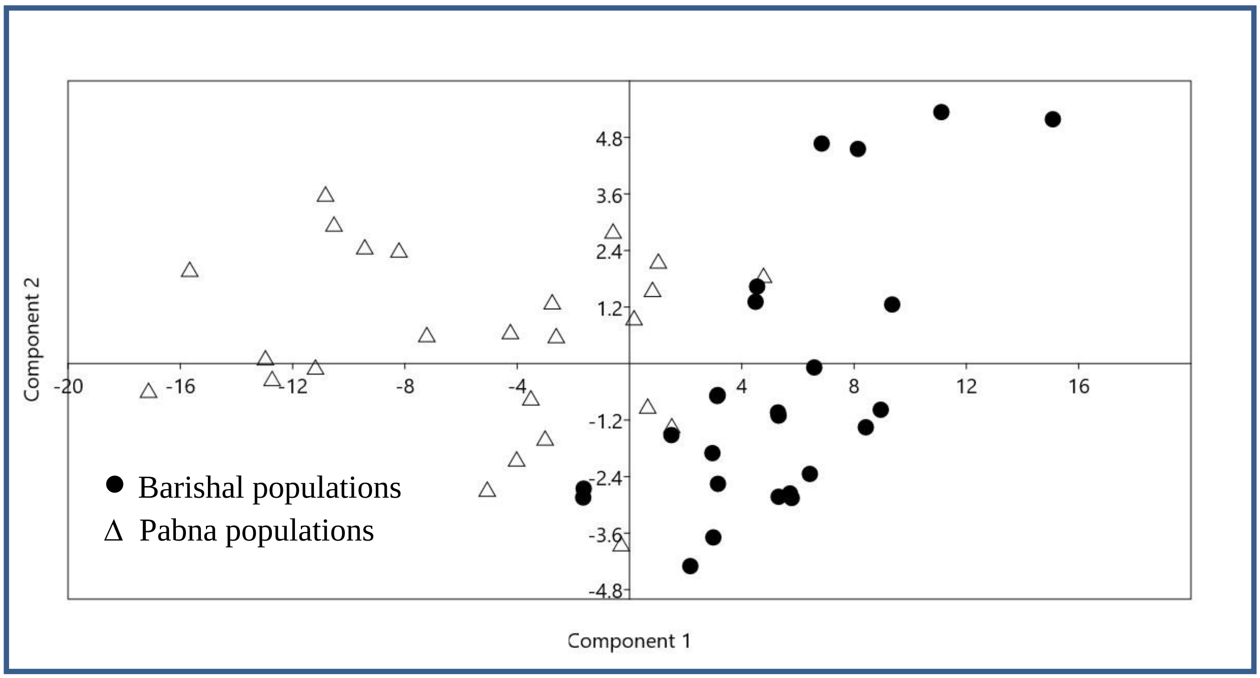

Figure-4.4 Scatter plot between Barishal and Pabna populations; Symbols refer to individuals in Barishal populations-Dot● Pabna populations-Triangle∆.

4.1.3.4: Variation between two populations of Chattogram and Khulna populations

In between Chattogram and Khulna populations 32 principal components (Annex-e) were identified where the highest eigenvalue (23.820) was found in PC 1 with a variance of 63.242% (Table-4.1). The Chattogram and Khulna *T. ilisha* populations were overlapping from each other except mixing few individual (Figure-4.5).

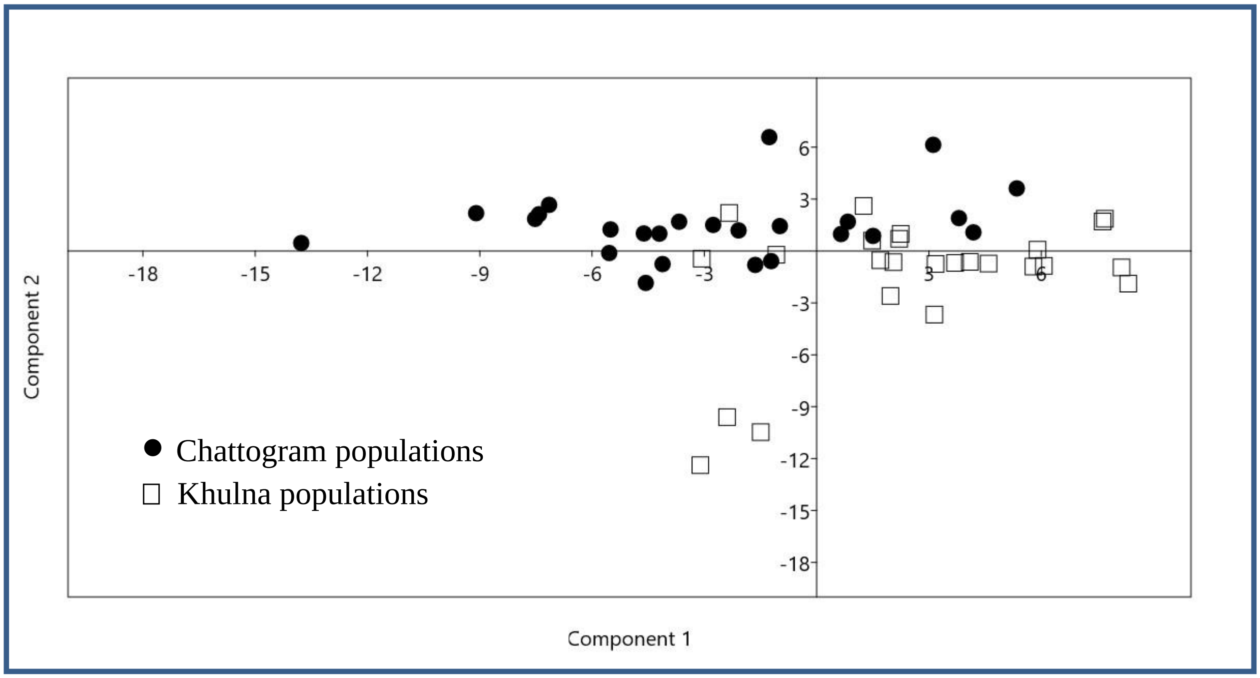

Figure-4.5 Scatter plot between Chattogram and Khulna populations; Symbols refer to individuals in Chattogram populations-Dot●; Khulna ppulations-Square□.

4.1.3.5: Variation between two populations of Chattogram and Pabna populations

In pairwise population study, Chattogram and Pabna populations showed 32 principal components (Annex-f) were identified where the highest Eigenvalue (29.003) was found in

PC 1 with a variance of 79.615% (Table-4.1). The Chattogram and Pabna *T. ilisha* populations were overlapping from each other except mixing few individual (Figure-4.6).

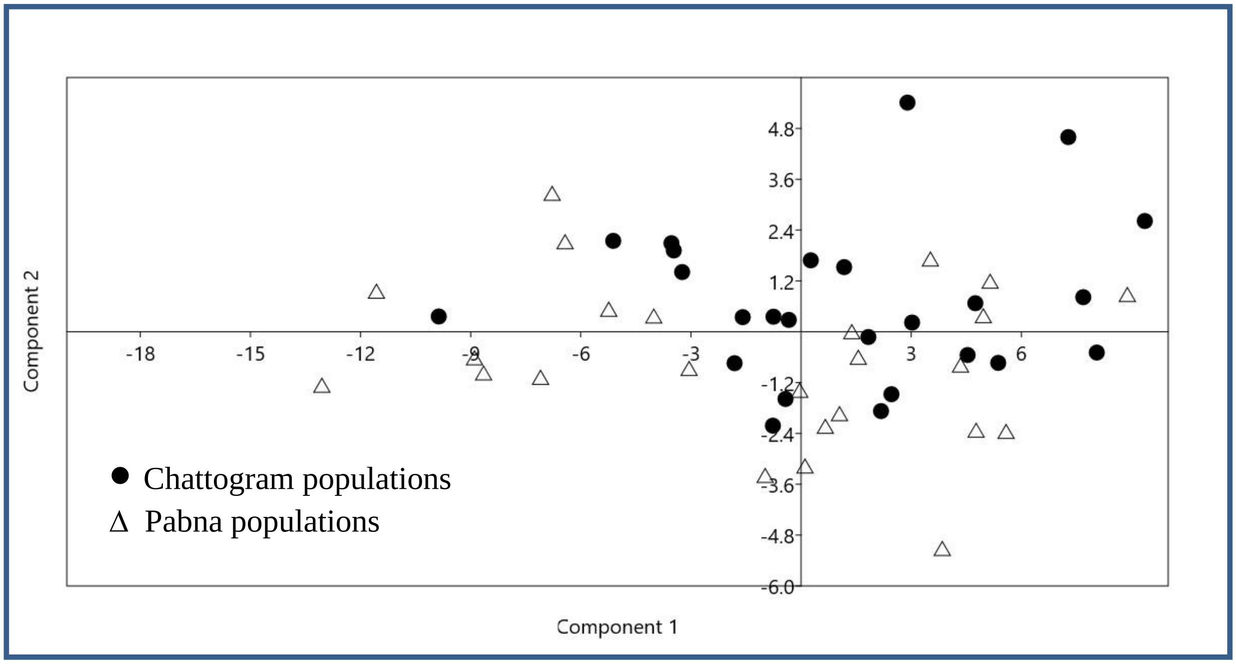

Figure-4.6 Scatter plot of Chattogram and Pabna populations; Symbols refer to individuals in Chattogram populations-Dot●; Pabna populations-TraingleΔ.

4.1.3.6: Variation between two populations of Khulna and Pabna populations

In between Khulna and Pabna populations 32 principal components (Annex-g) were identified where the highest eigenvalue (39.250) was found in PC 1 with a variance of 74.147% (Table-4.1). The Khulna and Pabna *T. ilisha* populations were seperated from each other except mixing few individual (Figure-4.7).

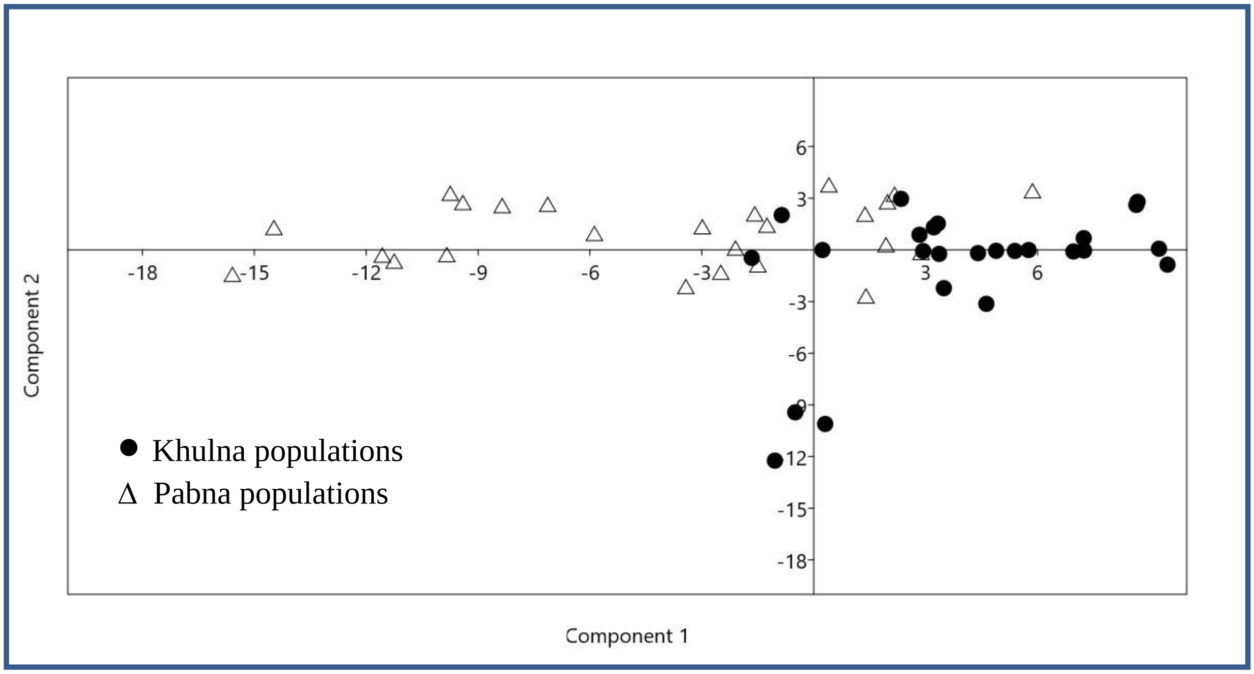

Figure 4.7 Scatter plot of Khulna and Pabna *T. ilisha* populations; Symbols refer to individuals in Khulna populations-Dot●; Pabna populations-TriangleΔ.

4.1.4: Cluster analysis of total four populations

Paired group (UPGMA) using the Euclidean cluster analysis showed the major cluster formation of Barishal, Chattogram, Khulna and Pabna populations (Figure-4.8). Among all the populations were not completly separated but Barishal and Pabna populatios were seperated and Most of the populations were mixing cluster formation.

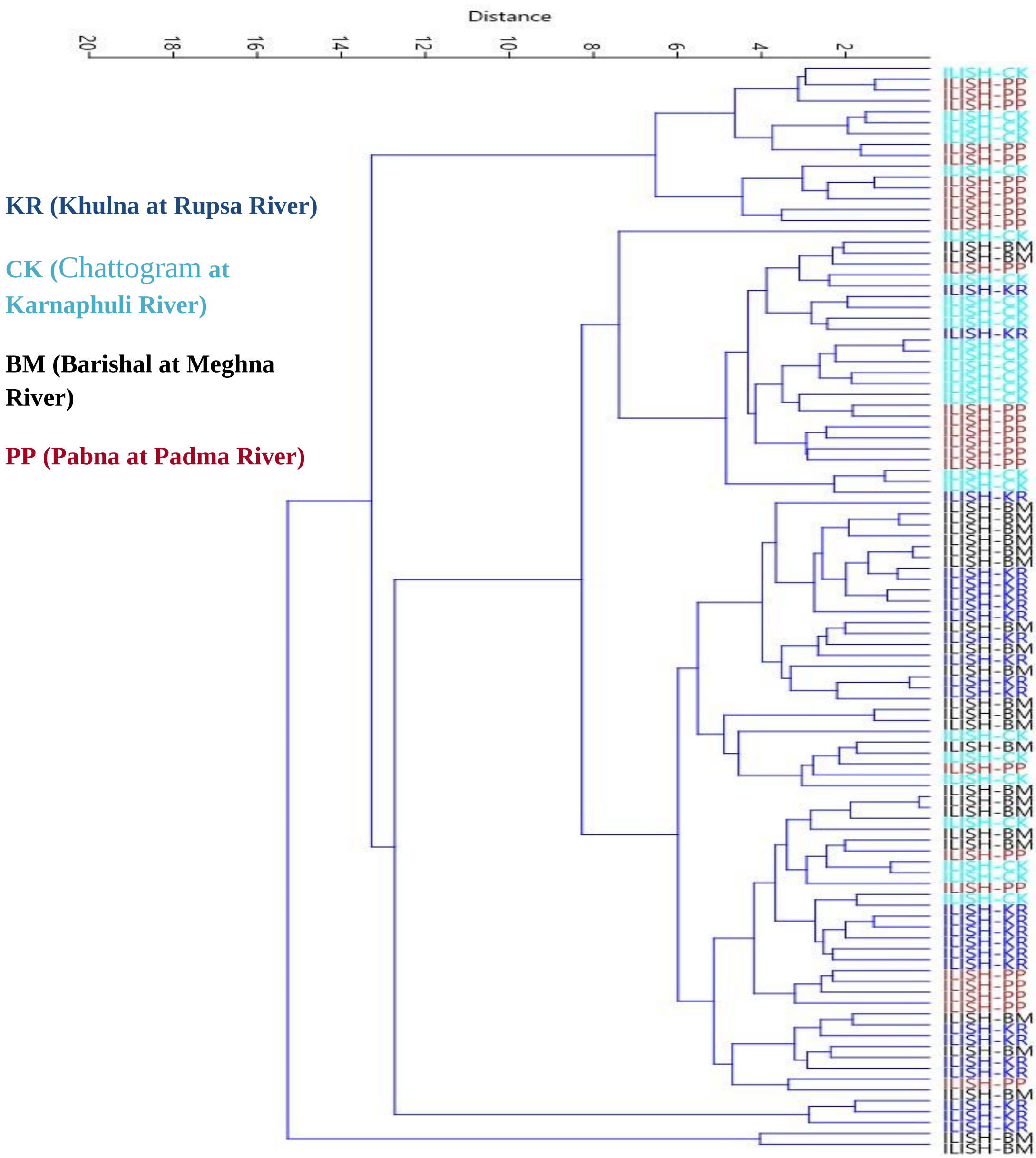

Figure-4.8 Cluster analysis among four population of *T. ilisha* by using UPGMA method.

4.2 Biochemical composition variation of four *T. ilisha* populations

Table-4.2: Biochemical composition of *T. ilisha* among four populations

Total	Source	Sample	%Protein	Avg.	%Lipid	Avg.	%Ash	Avg.

sample		No.						
1		1	12.76%		16.59%		0.78%	
2	Khulna	2	13.32%	13.01%	15.98%	16.43%	0.78%	0.77%
3		3	12.97%		16.74%		0.76%	
4		1	10.76%		13.95%		0.98%	
5	Chattogram	2	11.73%	11.34%	14.07%	14.04%	0.99%	0.98%
6		3	11.55%		14.10%		o.98%	
7		1	15.60%		13.26%		0.94%	
8	Barishal	2	15.23%	15.27%	13.69%	13.44%	0.97%	0.96%
9		3	14.98%		13.38%		o.98%	
10		1	14.95%		14.21%		o.96%	
11	Pabna	2	15.21%	15.01%	13.35%	13.88%	0.89%	0.91%
12		3	14.87%		14.09%		0.87%	

4.2.1 Variation of *T. ilisha* based on their biochemical analysis

Results of biochemical analysis of hilsha at four different populations (Khulna, Chattogram, Barishal and Pabna) were presented in table-4.2. The total protein content in *T. ilisha* ranging from 10.76 to 15.60% with the highest protein content in Barishal population and the lowest in Chattogram population. Lipid content ranged from 13.26 to 16.74% with the highest value in Khulna and the lowest in Barishal. Ash content ranged from 0.76 to 0.99% with the highest range in Chattogram and the lowest in Khulna population.

Table-4.3: Statistical test including One way ANOVA to find significant variation in biochemical composition among the populations.

Treatment	Protein (Mean±SD)	Lipid (Mean±SD)	Ash (Mean±SD)
T_1 (Khulna)	$13.01±0.28^{b}$	$16.43±0.39^{b}$	$0.77±0.01^{a}$
T_2 (Chittagong)	$11.34±0.51^{a}$	$14.04±0.07^{a}$	$0.98±0.01^{c}$
T_3 (Barisal)	$15.27±0.31^{c}$	$13.44±0.22^{a}$	$0.96±0.02^{bc}$
T_4 (Pabna)	$15.01±0.17^{c}$	$13.88±0.46^{a}$	$0.90±0.04^{b}$

- Values (mean ± SD) in coloum with different superscripts are significantly different (Turkey Multiple Range Test, $p<0.05$).

Different subset for alpha in the same column indicates the significance variation based on Turkey Multiple Range Test (0.05% level of significance)

4.2.1.1 Variation of *T. ilisha* based on Protein Percent variation

The average (Mean±SD) protein contents in this four populations (Khulna, Chattogram, Barishal and Pabna) were found ($13.01±0.28^{b}$, $11.34±0.51^{a}$, $15.27±0.31^{c}$, $15.01±0.17^{c}$) respectively (Table-4.3). The highest proteins contents were found in Barishal population (15.27% and 15.01%) and lowest in Chattogram population (11.34%). *T. ilisha* of Khulna and Chattogram were significantly varied from two other populations. There were no significance variations in protein percentage (%) of Barishal and Khulna populations.

4.2.1.2 Variation of *T. ilisha* based on Lipid Percent variation

The average (Mean±SD) lipid contents in this four populations (Khulna, Chattogram, Barishal and Pabna) were found (16.43 ± 0.39^{b} , 14.04 ± 0.07^{a} , 13.44 ± 0.22^{a} , 13.88 ± 0.46^{a}) respectively (Table-4.3). The highest lipid contents were found in Khulna population (16.43%) and lowest in Barishal population (13.44%). *T. ilisha* of Khulna was significantly varied from three other populations. There were no significant variations in lipid percentage (%) among the Chattogram, Barishal and Pabna populations.

4.2.1.3 Variation of *T. ilisha* based on Ash Percent variation

The average (Mean±SD) Ash contents in these four populations (Khulna, Chattogram, Barishal and Pabna) were found (0.77 ± 0.01^{a}, 0.98 ± 0.01^{c}, 0.96 ± 0.02^{bc}, 0.90 ± 0.04^{b}) respectively (Table-4.3). The highest Ash contents were found in Chattogram population (0.98%) and lowest in Khulna population (0.77%). There were significant differences in ash

Chapter 5
DISCUSSION

In this study, the results of my study are discussed and compared with the similar results of the previous studies.

5.1: Shape analysis among four populations of *Tenualosa ilisha* by using landmark based geometric morphometrics

The geometric morphometric assessment of four *T. ilisha* populations showed considerable variation. The overall variation among four populations showed the highest Eigenvalue (37.84) and variance (75.52%) were found in principal component (PC) 1. This variation was presented in scatter plot. In this study, the overall body shape variation (75.52%) in principal component (PC) 1 among four populations indicates that there was significant body shape variation of Hilsa *T. ilisha* among four populations. These shape variation may be related to geographically isolated locations or habitat or feeding habits among the species. The population specific position of each individual were observed though there were some overlapping of plotting between Barishal and Khulna populations and some were Chattogram and Khulna populations. In a study of population structure of hilsa (*T. ilisha)* though allozymes and morphometric analysis and showed that hilsa vary greatly in shape due to geographic range (Salini *et al.,* 2004). Brahmane *et al.*, (2014) investigated mitochondrial DNA cytochrome *b* based on genetic diversity and population differentiation of Hilsa (*Tenualosa ilisha*) from Ganga and Hooghly rivers. They suggested that the whole population existence of a single population, migrating to Ganga and Hooghly rivers for spawning and breeding purpose.

A study was conducted to morphological variation in Atlantic salmon *salmo salar* sampled from wild northern, middle and southern Norway of populations was examined by using principal component analysis and showed largest differences to be in head characters, notably eye diameter and jawbone, with the smallest diameter and head size among the northernmost populations (Solem and Berg 2011). A study was conducted to morphological differentiation of six serranid fishes by geometric morphometric method and found significant differences among species on the basis of body height and caudal peduncle length and changes in body shape were clearly differentiating the species of Serraninae. These shape changes are related to habitat and feeding habits among the species (Cavalcanti *et al.*, 1999)

For clear distinction of the variation, further analyses were performed by different combinations of the populations that include: variation among only 4 female population, variation among only 4 male population of *T. ilisha* and pairwise variation of four populations. Among four female population percent variance (77.85%) were found in principal component 1 and four male populations percent variance (74.66%) were found in principal component 1. In a study of body shape variation within population of Ibis fish, (*Ambassis interrupta*) using landmark-based geometric morphometric analysis that a total of 71.46% of the variation exist in female fish and a total of 62.04% is observed in the fluctuating asymmetry interaction in male fish. The results however indicate that fluctuating asymmetry are higher compared to individual variation in females, but individual variation is higher compared to fluctuating asymmetry in males (Medrano and Jumawan, 2016). In a study of body shape variation of three populations of *Sardinella fimbriata* by geometric morphometrics analysis and showed a more curvature variation in the body compared to the males but the male however showed greater variation in the distension in the mid-section of the body (Luceño *et al.*, 2013).

In pairwise geometric variation, the highest level of percent variance was observed between Barishal and pabna population 85.33%, and lowest level of percent variance 63.24% were observed in between Chattogram and Khulna population. This variation was presented in scatter plot. In pairwise scatter plot analysis some overlapping of plotting between Barishal and Khulna populations and some were Chattogram and Khulna populations and Barishal and Pabna population were distinctly separated and few were mixing between Barishal and Chattogram population. The geometric morphometric assessment findings of three *Anabas testudineus* populations indigenous koi, Thai koi and Vietnamese koi showed overall variation among 3 populations was of 94.21% in principal component 1. The population specific position of each individual was observed and some overlapping of plotting was found between Thai and Vietnamese populations (Mahajebin, 2017). The geometric morphometric studies of three *Barilius* species, *B. barila, B. barna,* and *B. bendelisis* showed the Percent variance (91.282%) and the Eigenvalue (89.0673) in Principal Component 1(PC-1). The results of principal component analysis, scatter plot and UPGMA cluster analysis showed that *B. bendelisis* was clearly distinct from other species (Rekha, 2017).

Cluster analysis showed the major cluster formation of Barishal, Chattogram, Khulna and Pabna populations. Among all the populations were not completely separated and they were mixing cluster formation of four types of population. Similar to this study, Cavalcanti *et al.* (1999) conducted cluster analysis using selected species of serranid fishes and found close relationship between *Diplectrum formosum*, *D. radiale*, *Mycteroperca acutirostris*, and *M. bonaci*. High distance was found between *Dules auriga* and *Epinephelus marginatus*. The genetic variation of two populations of Hilsa shad (*Tenualosa ilisha*) by RAPD-PCR and dendrogram was constructed for genetic distance analysis and showed two clusters of Padma and Meghna at different spawning populations with different stocks (Shifat *et al.*, 2003). Similar to my study, the population status of the endangered carp, kalibaus *Labeo calbasu* by

Landmark-based morphometric and dendrogram based on morphometric and truss network showed that the distance between the Halda and hatchery populations was the highest (Hossain *et al.,* 2010).

5.2 Biochemical analysis of hilsha among the four populations

Biochemical composition mainly protein, lipid, and ash of hilsa (*Tenualosa ilisha)* were determined that were collected from four different rivers (Rupsa, Karnaphuli, Meghna and Padma) and compare this value among this geographically isolated regions of Bangladesh. The average protein contents of Hilsa collected from Khulna, Chattogram, Barishal and Pabna were 13.01%, 11.34%, 15.27% and 15.01% respectively. The highest proteins contents were found in Barishal population (15.27%) and lowest in Chattogram (11.34%). Here Khulna and Chattogram value were significantly varied from Barishal and Pabna. The average lipid contents in this four populations (Khulna, Chattogram, Barishal and Pabna) of Hilsa were found (16.43%, 14.04%, 13.44%, 13.88%) respectively. The highest lipid contents were found in Khulna population (16.43%) and lowest in Barishal (13.44%). Here Khulna lipid values were significantly differences from another three regions. The average Ash contents in this four populations (Khulna, Chattogram, Barishal and Pabna) of Hilsa were found (0.77%, 0.98%, 0.96%, 0.90%) respectively. The highest Ash contents were found in Chattogram population (0.98%) and lowest in Khulna (0.77%) and these values were significantly varied among the populations. In this study, the biochemical compositions of significance variation of hilsa were found among the four populations that may be physiological reasons or different environmental conditions.

Similar to this study, Begum *et al.,* (2016) studied the nutritional value of hilsha fish (*Tenualosa ilisha*) from the six selected regions (Barisal, Patuakhali, Bhola, Cox's Bazar, Chandpur and Shariatpur) in Bangladesh. The total Protein content of six selected regions in hilsa fish ranged from 18.95 to 20.56% respectively. The lipid content of six selected regions in hilsa ranged from 4.97 to 8.21% respectively and Ash content of hilsa ranged from 3.08 to 4.84% respectively. Finally, the mean value of protein, fat and ash was 19.44%, 6.78% and 3.72% respectively. These results suggest that the proximate composition of hilsa fish greatly varies due to physiological reasons and environmental conditions. i.e., spawning, migration, and starvation or heavy feeding (Begum *et al.,* 2016). The protein percentage value of Hilsa at four different size groups (Jatka, Sub- adult, Adult, and Brood) were found (17.61, 17.96, 18.55 and 18.20) respectively and lipid contents were found (9.37, 12.43, 14.44 and 16.15) and Ash contents were found (0.82, 0.87, 0.81 and 0.83) percent respectively (Dewan *et al.*, 2015). They were found protein content was highest value in adult and the lowest in Jatka and the protein content was highest in white muscle and lipid content was highest in dark muscle. The nutrient content varied greatly with age and size of the fish of same species (Dewan *et al.*, 2015). The biochemical composition on seasonal basis of three economically important marine fishes (*Tenualosa ilisha*, *Sillago sihama* and *Mugil cephalus)* were analyzed in Chandipur, Bay of Bengal, India and found that there were significant differences between *S. sihama* and *H. ilisha* and showed that moisture was highest in *S. sihama* whereas protein and lipid was highest in *H. ilisha* (Mohanty *et al.,* 2015). The protein contents of hilsa (*Tenualosa ilisha*) from the Bay of Bengal and Arabian Gulf were found (18.16% and 19.13%) and lipid contents (19.94% and 11.22%) and Ash contents (1.34 % and 1.50%) respectively. The lipid contents of muscle and egg tissues were highly significant from the Bay of Bengal than those of the Arabian Gulf (Hossain *et al.,* 2014). The biochemical composition of Hilsa *(Tenualosa ilisha)* in laboratory condition was determined and showed that Fat content of the Hilsha fish was much higher than many other freshwater fish species (Minar *et al.,* 2012).

Chapter 6

CONCLUSION AND RECOMMENDATION

Hilsa shad, *Tenualosa ilisha* is a species of the Family Clupeidae locally known as Ilish, which is regarded as “national fish” in Bangladesh. *T. ilisha* is considered to be the most economic and important fish because of its high market demand, having good nutritional value, delicious taste and flavor. As it is an anadromous fish, mirgrate from Bay of Bengal to the rivers of southern part of the country due to spawning purpose. After spawning, the larvae and juvenile grow mostly in the southern rivers. Hence in this study attempted were taken to know the variation of Hilsa in four rivers of Padma, Meghna, Rupsa and Karnaphuli. The geometric morphometrics and biochemical composition among the four populations of geographically isolated regions of hilsa *T. ilisha* were studied to know the body shape variation among the population and differentiate among them and also know the biochemical composition value and compare this value among the population. Initially, the body shape variation and differentiation of four populations were done by landmark based geometric morphometric analysis and the biochemical composition of crude protein, lipid and Ash contents were carried out following the methods of AOAC (1980). The geometric morphometric findings of four *T. ilisha* populations showed considerable variation. In this study, the overall body shape variation (75.52%) in principal component (PC) 1 among four populations indicates that there was significant body shape variation of Hilsa *T. ilisha* among four populations. In scatter plot analysis, there were few overlapping of plotting between Barishal and Khulna populations and some were in between Chattogram and Khulna populations. For clear understanding of the body shape variation, further analyses were performed by pairwise variation of four populations and showed that the highest level of percent variance was observed between Barishal and Pabna population 85.33% and this variation was presented in scatter plot. In scatter plot analyses, Barishal and Pabna population were clearly separated and some overlapping of plotting between Barishal and Khulna populations and few were mixing between Barishal and Chattogram population.

These body shape variation may be related to geographically isolated regions or habitat or feeding habits or other environmental conditions among the species. Cluster analysis showed that they were not completely separated and few were mixing cluster formation of four types of population.

Biochemical composition mainly protein, lipid, and ash of Hilsa, were determined and compared these value among this geographically isolated regions of Bangladesh. The highest proteins contents were found in Barishal populations (15.27%) and Khulna and Chattogram population protein values were significantly varied from Barishal and Pabna populations. The highest lipid content was found in Khulna population (16.43%) and Khulna population lipid value was significantly differed from another three populations. The highest Ash content was found in Chattogram population (0.98%) and these values were significantly varied among the populations. In this study, the findings of biochemical composition of Hilsa showed significant variation among the four populations that may be physiological reasons or different environmental conditions. This result helps to provide information on the consumers, fish processors and the researchers.

RECOMMENDATION

Currently the government has emphasized to enhance the production of Hilsa by creating new rules and its implications. Mostly the government has taken initiatives to stop capture of undersized Hilsa (Jatka) and ready to spawn breeders by putting periodic ban on harvest. In this regards knowing the ideal stock of Hilsha is highly required. The present study has shown the body shape variation and biochemical composition variation of Hilsa of different regions of Bangladesh. Detailed study by involving more stocks and individuals of each stock are needed to investigate through morphometric and molecular markers analyses. These sorts of studies are highly required prior to management of the stocks which could contribute to management strategies of populations of *T. ilisha*.

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